

SCIENTIFIC PROGRAM

MONDAY, SEPTEMBER 23, 2019

08:00-09:00 Registration

09:00-09:15 Greetings and Opening, Israel A

Chairs:

Mira Barda-Saad -President of Israel Immunological Society (IIS)

Angel Porgador- President of Israel Society for Cancer Research (ISCR)

09:15-09:45 Keynote A, Israel A

09:15

The New Era of Precision Immunotherapy

Elizabeth Jaffee

Oncology, Johns Hopkins University, USA

09:45-10:15 Keynote B, Israel A

09:45

New insights into the cell biology of human natural killer cells and cytotoxic function

Jordan Orange

Pediatrics, Columbia University, USA

10:15-10:45 Coffee Break and Visit the Exhibition

10:45-12:30 Session 1 A: Visualizing the Immune Response, Immunology Hall, Israel C/D

Chairs: **Leah Gheber** (BGU), **Amnon Peled** (Hadassah - HUJI)

10:45

Bridging the Gap: Modulatory roles of the Grb2-family adaptor, Gads, in cellular and allergic immune responses

Debbie Yablonski, Sigalit Sukenik, Shira Labin, Rose Shalah, Enas Hallumi, Naama Klopstock
Faculty of Medicine, Technion - Israel Institute of Technology, Israel

11:10

Game of clones within immunological niches

Ziv Shulman

Immunology, Weizmann Institute of Science, Israel

11:35

Keep calm and IL-10, a story of gut and brain macrophages

Biana Bernshtein, Anat Shemer, Zhana Haimon, Jung-Seok Kim, **Steffen Jung**

Immunology, Weizmann Institute of Science, Israel

12:00

Resolving mechanisms of T cell activation at the single molecule level

Eilon Sherman

Racah Institute of Physics, The Hebrew University of Jerusalem, Israel

12:15

Conserved mechanisms underlying loss of tolerance to allogeneic tissues in *Botryllus schlosseri* chimeras and HSC transplantation

Benyamin Rosental^{1,2}, Mark Kowarsky³, Daniel M. Corey², Garry P. Nolan⁴, Stephen R. Quake³, Irving L. Weissman^{2,3}, Ayelet Voskoboynik^{2,3}

¹*The Shraga Segal Department of Microbiology, Immunology, and Genetics, and French Associates Institute for Agriculture and Biotechnology of Drylands, Ben Gurion University of the Negev, Israel*

²*Institute for Stem Cell Biology and Regenerative Medicine, Ludwig Center, and Hopkins Marine Station, Stanford University, USA*

³*Chan Zuckerberg Biohub, Stanford University, USA*

⁴*Microbiology and Immunology, Stanford University, USA*

10:45-12:30 Session 1 B: Cancer, Inflammation and Immunity - Friends or Foes? Israel A

Chairs: Dalit Barkan (UoH), Raanan Berger (Sheba)

10:45

Pro and Anti tumorigenic functions of tertiary lymphoid structures

Eli Pikarsky

The Lautenberg Center for Immunology and Cancer Research, Hebrew University of Jerusalem, Israel

11:10

Hallmarks of Neutrophil Anti Tumor Cytotoxicity

Zvika Granot¹, Maya Gershkovitz¹, Sandra Vols¹, Olga Yajuk¹, Ronit Sionov¹, Merav Shaul², Zvi Fridlender²

¹*Developmental Biology and Cancer Research, IMRIC, Faculty of Medicine, Hebrew University of Jerusalem, Israel*

²*Institute of Pulmonary Medicine, Hadassah - Hebrew University Medical Center, Israel*

11:35

Notch-mediated processes promoting inflammation-driven mechanisms in breast cancer progression

Adit Ben-Baruch¹, Yulia Liubomirski¹, Shalom Lerrer¹, Tsipi Meshel¹, Dina Morein¹, Linor Rubinstein-Achiasaf¹, David Sprinzak², Stefan Wiemann³, Cindy Korner³, Marcelo Ehrlich¹

¹School of Molecular Cell Biology and Biotechnology, Tel Aviv University, Israel

²School of Neurobiology, Biochemistry & Biophysics, Tel Aviv University, Israel

³German Cancer Research Center (DKFZ), Division of Molecular Genome Analysis, Germany

12:00

Necroptosis induced by anti-EMMPRIN antibody and complement shifts macrophage polarization

Miki Rahat^{1,3}, Max Ledersnaider¹, Nizar Hijaze^{1,2}, Elina Simanovich¹, Sameer Kassem^{2,3}

¹Immunotherapy Lab, Carmel Medical Center, Israel

²Internal Medicine A, Carmel Medical Center, Israel

³Ruth and Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Israel

12:15

Activated Eosinophil Subsets are an Integral Part of the Tumor Microenvironment in Lung Metastasis, Displaying Anti-tumorigenic Activities

Sharon Grisaru¹, Michal Itan¹, Perri Rosenberg¹, Julie Caldwell², Marc Rothenberg², Ariel Munitz¹

¹Department of Clinical Microbiology and Immunology, Tel-Aviv University, Israel

²Division of Allergy and Immunology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, USA

10:45-12:30 Session 1 C: Cancer Metabolism, Signaling and DNA Damage Response, Oncology Hall, Israel C/D

Chairs: **Haim Cohen (BIU), Esther Priel (BGU)**

10:45

TBC

Gideon Rechavi

Sackler School of Medicine, Sheba Medical Center and Tel-Aviv University, Israel

11:10

Rewiring cellular metabolism: a novel connection between ESR1 activating mutations and aggressiveness of breast cancer

Ido Wolf

Tel Aviv Medical Center, Israel

11:35

Current Trends and Our Own Efforts in Cancer Therapy

Yosef Yarden

Department of Biological Regulation, Weizmann Institute of Science, Israel

12:00

Tumor dependence on cytosolic one-carbon metabolism is determined by cellular capacity to retain folates

Tomer Shlomi

Lokey Center, Cancer Metabolism and Systems Biology, Technion-Israel Institute of Technology, Israel

12:15

Macrophage-Assisted DNA damage response

Jacob Rachmilewitz, Avital Guedj, Yael Volman, Anat Geiger-Maor, Eithan Galun

Goldyne Savad Institute of Gene Therapy, The Hebrew University of Jerusalem - Hadassah Medical Center, Israel

12:30-13:30 Lunch and Poster Presentation

13:30-14:30 Cutting Edge Technologies +, Israel A

13:30

A bridge between cell specific isolation and functional studies using laser microdissection and fast fluorescence lifetime confocal imaging

Daniel Smeets

Dover

13:50

Morphology-Driven High-Plex Spatial Analysis of Tissue Microenvironments with the GeoMx™ Digital Spatial Profiling

Alexandre Darmoise

Eldan

14:10

Multi-modal Kinetic Profiling of T cell Activation and Tumor Cell Interaction Using the xCELLigence eSight System

Yama Abassi

Lumitron

14:30-16:30 Session 2 A: Inflammation and Innate Immunity, Immunology Hall, Israel C/D

Chairs: **Shamgar Ben-Eliyahu (TAU), Marcelo Ehrlich (TAU)**

14:30

Emerging Roles For Eosinophils in the Tumor Microenvironment

Ariel Munitz

Clinical Microbiology and Immunology, Sackler School of Medicine, Tel Aviv University, Israel

14:55

IFN β is a Novel Effector Cytokine in Resolving Inflammation

Amiram Ariel

Human Biology, University of Haifa, Israel

15:20

A distinct subset of Th1 cells express the high-affinity Fc γ receptor and exert antibody-mediated cytotoxic activity in solid tumors

Yaron Carmi

Pathology, Sackler Faculty of Medicine, Tel Aviv University, Israel

15:45

Neutrophils as modulators of the immune tumor microenvironment

Zvi Fridlender

Institute of Pulmonary Medicine, The Hebrew University of Jerusalem - Hadassah Medical Center, Israel

16:00

STING Promotes Macrophage-Mediated Resolution of Inflammation through IFN β Production

Sergei Butenko, Sagie Schif-Zuck, Amiram Ariel

Human Biology, University of Haifa, Israel

16:15

Brain-mediated regulation of oral tolerance

Maria Krot^{1,2}, Tamar Koren^{1,2}, Eden Avishai^{1,2}, Maya Schiller^{1,2}, Nadia Thawho Boshnak^{1,2}, Haitham Hajjo^{1,2}, Maryam Amer^{1,2}, Hedva Haykin^{1,2}, Tamar Ben Shaanan³, Hilla Azulay-Debby^{1,2}, Kobi Rosenblum⁴, Asya Rolls^{1,2}

¹*Immunology, Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Israel*

²*Neuroscience, Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Israel*

³*Microbiology and Immunology, University of California, USA*

⁴*Neurobiology and Ethology, University of Haifa, Israel*

14:30-16:30 Session 2 B: Check Point Pathways, Cancer and Immunotherapy, Israel A

Chairs: **Alona Zer (TAU), Albert Grinshpun (HUJI)**

14:30

Reversed Personalized Immuno-Oncology

Gal Markel

Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Israel

14:55

Shaping the Inflammatory Niche: Cancer-Associated Fibroblasts Facilitate Breast Cancer Metastasis

Ophir Shani¹, Yael Raz¹, Or Megides², Noam Cohen¹, Lea Monteran¹, Tsarfaty Ilan², **Neta Erez**¹

¹Department of Pathology, Sackler Faculty of Medicine, Israel

²Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Israel

15:20

UVB-induced tumor heterogeneity directs immune response in melanoma

Yardena Samuels

Molecular Cell Biology, Weizmann Institute of Science, Israel

15:45

Alternative Splicing Of The Receptor SLAMF6 Reveals a Novel Regulatory Mechanism Of T Cell Activation And Can Be Used For Cancer Immunotherapy

Emma Hajaj^{1,2}, Galit Eisenberg², Shiri Klein², Shoshana Frankenburg², Sharon Merims², Jonathan Cohen², Tamar Peretz², Michal Lotem²

¹Medical School, The Hebrew University of Jerusalem, Israel

²Oncology, Hadassah Medical Organization, Israel

16:00

The pioneer round of translation and MHC-I peptides presented to cytotoxic T lymphocytes

Hadas Weinstein^{1,2,3}, Liron Hendel^{1,2}, Efrat Avigad Laron^{1,2}, Adi Sharabi-Nov², Alon Margalit^{1,2}, Gideon Gross^{1,2}

¹Biotechnology, Immunology, MIGAL - Galilee Research Institute, Israel

²Biotechnology, Immunology, Tel-Hai College, Israel

³Department of Medicine, Hadassah University Hospital, Faculty of Medicine, Hebrew University, Israel

16:15

Induction and transcriptional regulation of the co-inhibitory gene module in T cells

Asaf Madi

Pathology, Tel Aviv University, Israel

14:30-16:30 Session 2 C: Solid Cancers: from Experimental Models to Treatment, Oncology Hall, Israel C/D

Chairs: **Michal Besser (Sheba), Elena Voronov (BGU)**

14:30

p53 deregulation in cancer: cell-autonomous and non-autonomous implications

Moshe Oren¹, Yael Aylon¹, Sharathchandra Arandkar¹, Noa Furth¹, Martino Maddalena¹, Ori Hassin¹, Giuseppe Mallel¹, Gal Benor², Eytan Domany²

¹Molecular Cell Biology, Weizmann Institute of Science, Israel

²Physics of Complex Systems, Weizmann Institute of Science, Israel



14:55

Fer and FerT sustain the metabolic plasticity of metastatic non-small cell lung cancer cells

Uri Nir, Linoy Mehazri, Sally Shpungin

The Mina and Everard Faculty of Life-Sciences, Bar-Ilan University, Israel

15:20

Cancer-associated fibroblast heterogeneity in breast cancer

Ruth Scherz-Shouval

Department of Biomolecular Sciences, Weizmann Institute of Science, Israel

15:45

The Future of Automating and Commercializing Autologous Cell Therapies

Eytan Abraham

Personalized Medicine, Lonza, Israel

16:00

CARTIV - a novel platform for regulation of gene expression under the control of inflammation-induced promoters

Yariv Greenshpan, Angel Porgador, Roi Gazit

Microbiology and Immunology, Ben-Gurion University of the Negev, Israel

16:15

A single-cell atlas of metastatic breast cancer charting oncogenic transcriptional programs in malignant cells and the tumor microenvironment

Ofir Cohen^{1,2}, Daniel Abravanel², Michal Slyper¹, Johanna Klughammer¹, Judit Jane-Valbuena¹, Sebastien Vigneau², Jingyi Wu², Karla Helvie², Laura Dellostretto², Asaf Rotem², Orit Rozenblatt-Rosen¹, Bruce Johnson², Investigators KCO¹, Investigators CCPM², Aviv Regev¹, Nikhil Wagle^{1,2}

¹Cancer Program, Broad Institute of MIT and Harvard, USA

²Department of Medical Oncology, Dana-Farber Cancer Institute, USA

16:30-17:00 Coffee Break and Visit the Exhibition

17:00-18:10 Session 3 A: Inflammation and Host-Pathogen Interaction, Immunology Hall, Israel C/D

Chairs: Yossi Mekori (TAU), Zvi Fridlender (Hadassah)

17:00

Sex-related perturbations in schizophrenia and bipolar disorder brains reflect microRNA-mediated cholinergic/neurokine interactions

Sebastian Lobentanzer¹, Geula Hanin^{2,3}, Jochen Klein¹, Hermona Soreq²

¹Department of Pharmacology, College of Pharmacy, Goethe University, Germany

²The Edmond and Lily Safra Center for Brain Science and the Life Sciences Institute, The Hebrew University of Jerusalem, Israel

³Department of Genetics, University of Cambridge, UK

17:25

Exploiting an Achilles' Heel of Cancer with a Novel Oncolytic Virus

Marcelo Ehrlich

School of Molecular Cell Biology and Biotechnology, Tel Aviv University, Israel

17:40

Synergism between human apoptotic cell infusion and human chimeric antigen receptor (CAR)-T therapy in fighting solid human tumor in SCID Bg mice

Dror Mevorach

Medicine, The Hebrew University of Jerusalem, Israel

17:55

The Ebola-Glycoprotein Modulates the Function of Natural Killer Cells

Avishay Edri^{1,2}, Avishai Shemesh^{1,2}, Muhammed Iraqi^{1,2}, Omri Matalon³, Michael Brusilovsky^{1,2}, Uzi Hadad^{1,2}, Olga Radinsky^{1,2}, Orly Gershoni-Yahalom^{1,2}, John M. Dye⁴, Ofer Mandelboim⁵, Mira Barda-Saad³, Leslie Lobel^{1,6}, Angel Porgador^{1,2}

¹*The Shraga Segal Department of Microbiology, Immunology and Genetics, Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel*

²*National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Israel*

³*The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Israel*

⁴*U.S. Army Medical Research Institute of Infectious Diseases, U.S. Army, USA*

⁵*The Lautenberg Center for General and Tumor Immunology, The BioMedical Research Institute Israel Canada of the Faculty of Medicine (IMRIC), The Hebrew University Hadassah Medical School, Israel*

⁶*Department of Emerging and Reemerging Diseases and Special Pathogens, Uganda Virus Research Institute (UVRI), Uganda*

17:00-18:10 Session 3 B: Microenvironment and Immuno-Oncology - I, Israel A

Chairs: **Isaac Witz (TAU), Gilad Bachrach (HUJI)**

17:00

Are we mature enough to apply anti-IL-1beta therapy in cancer patients?

Irena Kaplanov, Sapir Maudi-Boker, Elena Voronov, **Ron N. Apte**

The Shraga Segal Department of Microbiology, Immunology and Genetics, Faculty of Health Sciences Ben-Gurion University of the Negev, Israel

17:25

Heparanase2 (Hpa2) Promotes a Higher Degree of Cell Differentiation by Inducing Sox2 Expression in Head and Neck Carcinoma

Miriam Gross-Cohen, Yifat Yanku, Ofra Kessler, Neta Ilan, Israel Vlodavsky

Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Technion Integrated Cancer Center (TICC), Israel

17:40

An Anti-Metastatic Role for Macrophage-Derived PROS1

Avi Maimon, Amit Halpern, Victor Yahid, Shivam Priya, Gabriel Mizraji, **Tal Burstyn-Cohen**

Institute for Dental Science, Faculty of Dental Medicine, Israel

17:55

Pre-Metastatic Niche: In Vivo Tissue Changes in Mechano-Structure by Chemotherapy-Induced Tumor-Derived Microparticles

Daphne Weihs¹, Tamar Barenholz-Cohen¹, Yulia Merkher¹, Dvir Shechter², Jozafina Haj², Yuval Shaked²

¹*Faculty of Biomedical Engineering, Technion-Israel Institute of Technology, Israel*

²*Faculty of Medicine, Technion-Israel Institute of Technology, Israel*

17:00-18:10 Session 3 C: Cancer Metastasis, Oncology Hall, Israel C/D

Chairs: **Lea Eisenbach (WIS), Rotem Karni (HUJI)**

17:00

The role of the CXCL10-CXCR3 axis in directing the biological function of anti-tumor CD8+ T cells

Nathan Karin, Ghada Jarrous, Hila Razon

Immunology, Rappaport Faculty of Medicine, Technion - Israel Institute of Technology, Israel

17:25

FAK Family Kinases: The Yin and Yang of Breast Cancer Metastasis

Hava Gil-Henn¹, Alessandro Genna^{1,2}, Stefanie Lapetina¹, Shams Twaфра¹, Tomer Meirson¹, George Karagiannis², Ved Sharma², Yarong Wang², David Entenberg, David Entenberg², John Condeelis²

¹*Faculty of Medicine, Bar-Ilan University, Israel*

²*Anatomy and Structural Biology, Albert Einstein College of Medicine, USA*

17:40

Improving biomarkers of metastasis in colorectal and breast cancer patients through perioperative blockade of inflammatory-stress responses in two phase-II clinical trials

Shamgar Ben-Eliyahu¹, Lee Shaashua¹, Rita Haldar¹, Tanir Alweis², Eran Sharaon³, Steve Cole⁴, Oded Zmora⁵

¹*Sagol School of Neuroscience and School of Psychological Sciences, Tel Aviv University, Israel*

²*Department of Surgery, Kaplan Medical Center, Israel*

³*Department of Pathology, Rabin Medical Center, Israel*

⁴*Departments of Medicine and Psychiatry, David Geffen School of Medicine at UCLA, USA*

⁵*Department of Surgery and Transplantation, Sheba Medical Center, Israel*

17:55

Converting Invasive Breast Cancer Cells Into Adipocytes Inhibits Cancer Metastasis

Dana Ishay-Ronen^{1,2}, Maren Diepenbruck¹, Nami Sugiyama¹, Ravi KR Kalathur¹, Robert Ivanek¹,
Glenn Bantug¹, Marco Morini¹, Christoph Hess¹, Gerhard Christofori¹

¹Department of Biomedicine, University of Basel, Switzerland

²Oncology Institute, Chaim Sheba Medical Center, Tel-Hashomer, Israel

18:10-18:40 Keynote C, Israel A

18:10

Characterization of TCR-activated Signaling Complexes and Microclusters

Lawrence Samelson

Laboratory of Cellular and Molecular Biology, Center for Cancer Research, National Cancer Institute,
NIH, USA

18:50-21:00 Welcome Reception

TUESDAY, SEPTEMBER 24, 2019

09:00-09:30 **Morning Gathering, Foyer**

09:30-10:00 **Keynote D, Israel A**

09:30

Mechanical Control of T cell Function by the Actin Cytoskeleton

Janis Burkhardt

*Pathology and Laboratory Medicine, Children's Hospital of Philadelphia Research Institute,
University of Pennsylvania, USA*

10:00-10:30 **Coffee Break and Visit the Exhibition**

10:30-12:15 **Session 4 A: Mechanotransduction in the Immune System, Immunology Hall,
Israel C/D**

Chairs: **Amit Tzur (BIU), Benyamin Rosental (BGU)**

10:30

Nuclear Checkpoints for Melanoma and Breast Cancer Lung Metastasis

Ronen Alon¹, Francesco Roncato¹, Ofer Regev¹, Nehora Levi², Sara Feigelson¹, Gabi Gerlitz²

¹Department of Immunology, Weizmann Institute of Science, Israel

²Department of Molecular Biology, Ariel University, Israel

10:55

Mechanotransduction as a novel immune checkpoint in NK cell cytotoxicity

Mira Barda-Saad

The Mina and Everard Goodman Life Sciences Faculty, Bar-Ilan University, Israel

11:20

Synthetic immune niche (sin) for enhancement of T-cell therapies

Benjamin Geiger¹, Shimrit Adutler-Lieber¹, Nir Friedman²

¹Department of Molecular Cell Biology, Weizmann Institute of Science, Israel

²Department of Immunology, Weizmann Institute of Science, Israel

11:45

Nanodevices for the Study of Immune Cell Function

Mark Schwartzman

Materials Engineering, Ben-Gurion University of the Negev, Israel

12:00

Integrin-mediated cell-matrix adhesions at the crossroad between microtubules and the actomyosin cytoskeleton

Alexander Bershadsky

Mechanobiology Institute, National University of Singapore, Singapore

Department of Molecular Cell Biology, Weizmann Institute of Science, Israel

10:30-12:15 Session 4 B: Immuno-Oncology and Microbiome, Israel A

Chairs: Tomer Hertz (BGU), Ilana Livyatan (WIS)

10:30

TIGIT and Its bacterial and cellular ligands

Ofer Mandelboim

Lautenberg Center, The Hebrew University of Jerusalem, Israel

10:55

Paneth cells secrete lysozyme via secretory autophagy during bacterial infection of the intestine

Shai Bel

Medicine, Bar-Ilan University, Israel

11:20

Thinking outside the mouse: ex-vivo dissections of host-microbiome cross talks

Nissan Yissachar

The Mina and Everard Goodman Faculty of Life Sciences, and the Bar-Ilan Institute of Nanotechnology and Advanced Materials (BINA), Bar-Ilan University, Israel

11:45

Activation of the Siglec-7 inhibitory receptor: A novel approach for fighting cancer

Ilan Zaffran¹, Nadine Landolina¹, Ofer Mandelboim², Francesca Levi Schaffer¹

¹*Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem - International, Israel*

²*The Lautenberg Center for Immunology and Cancer Research, Faculty of Medicine, The Hebrew University of Jerusalem - International, Israel*

12:00

Complex phospho-regulation pathways of a cancer related mitotic motor protein

Alina Goldstein-Levitin¹, Nurit Siegler¹, Darya Goldman¹, Mary Popov¹, Himanshu Pandey¹, Ervin Valk², Mart Loog², Liam Holt³, Larisa Gheber¹

¹*Department of Chemistry and Ilse Katz Institute for Nanoscale Science and Technology, Ben-Gurion University of the Negev, Israel*

²*Institute of Technology, University of Tartu, Estonia*

³*Institute for Systems Genetics, New York University Langone Health, USA*



10:30-12:20 Session 4 C: Bioinformatics, Big Data and Cancer, Oncology Hall, Israel C/D

Chairs: Ramit Mehr (BIU), Tal Shay (BGU)

10:30

Decreased A-to-I RNA editing activate dsRNA sensing by innate immunity

Erez Levanon

Faculty of Life sciences, Bar-Ilan University, Israel

10:55

Dissecting the glioma ecosystem by single cell RNA-seq

Itay Tirosh

Molecular Cell Biology, Weizmann Institute of Science, Israel

11:20

Multibodies – Attacking multiple pathways with a single, computationally-designed, antibody

Yanay Ofran

Laboratory of Systems Biology and Functional Genomics, Institute of Nanotechnology and Advanced Materials, Bar Ilan University, Israel

11:40

Targeting Signaling in Cancer – lessons from acute lymphoblastic leukemia

Shai Izraeli

The Rina Zaizov Division of Pediatric Hematology Oncology, Schneider Children's Medical Center, Israel

Sackler Faculty of Medicine, Tel Aviv University, Israel

12:05

RNA Sequence Analysis Reveals Macroscopic Somatic Clonal Expansion Across Normal Tissues

Keren Yizhak¹, Francois Aguet¹, Jaegil Kim¹, Julian Hess¹, Kirsten Kubler^{1,2,3}, Jonna Grimsby¹,
Ruslana Frazer¹, Ayelet Segre^{1,4}, Paz Polak^{1,5}, Kristin Ardlie¹, Gad Getz^{1,2,3,6}

¹Cancer Genome Analysis, The Broad Institute of MIT and Harvard, USA

²Center for Cancer Research, Massachusetts General Hospital, USA

³Pathology, Harvard Medical School, USA

⁴Department of Ophthalmology, Massachusetts Eye and Ear, USA

⁵Oncological Sciences, Mount Sinai Health System, USA

⁶Pathology, Massachusetts General Hospital, USA

12:20-13:30 Lunch and Poster Presentation

13:30-14:30 Flash Talks: Immunology I, Immuno-Oncology I, Oncology I



14:30-16:30 Session 5 A: Immunopathologies and Precision Medicine - I, Immunology Hall, Israel C/D

Chairs: **Sol Efroni (BIU), Esti Yeger-Lotem (BGU)**

14:30

Refining Immune Checkpoints for Natural Killer-based immunotherapy

Angel Porgador

Faculty of Health Sciences, The Shraga Segal Department of Microbiology, Immunology and Genetics, Ben-Gurion University of the Negev, Israel

14:55

The Immunotherapeutic Properties of Novel Tellurium Compounds: Mechanism of Action and Clinical Results

Benjamin Sredni

Life Sciences, Bar-Ilan University, Israel

15:20

A Tissue Atlas of Human B-Cell Receptor Populations Reveals Two Separate Immune Networks in the Gut and in the Blood

Uri Hershberg

*Human Biology, University of Haifa, Israel
School of Biomedical Engineering, Drexel University, USA*

15:45

TCR Repertoires of Tumor Infiltrating T Cells in Metastatic Breast Cancer

Erez Greenstein¹, Leticia De Mattos-Arruda^{2,3}, Carlos Caldas^{2,4}, Nir Friedman¹

¹*Department of Immunology, Weizmann Institute of Science, Israel*

²*Department of Oncology and Cancer Research, University of Cambridge, UK*

³*Vall d'Hebron Institute of Oncology, Vall d'Hebron University Hospital, Spain*

⁴*Breast Cancer Programme, Cancer Research UK Cambridge Cancer Centre, UK*

16:00

Hematopoietic stem cell transplantation in the era of next generation sequencing

Polina Stepensky

Department of Bone Marrow Transplantation and Cancer Immunotherapy, Hadassah Medical Center, Israel

16:15

TP73-AS1 Promotes Chemotherapy resistance in Glioblastoma cancer stem cells

Gal Mazor, Barak Rotblat

Life Science, Ben-Gurion University of the Negev, Israel

14:30-16:30 Session 5 B: CAR and CTL Therapy in Cancer, Israel A

Chairs: Gideon Gross (Migal), Shimon Slavin (Biotherapy International)

14:30

Engineering Immune Effector Molecules and Cells for Immunotherapy of Cancer and Autoimmunity

Yoram Reiter

Biology, Technion - Israel Institute of Technology, Israel

14:55

Jacob Shechter

15:20

Co-stimulatory Switch Receptors – deriving benefit from immunosuppression to enhance T-cell function

Cyrille Cohen

Head, Laboratory of Tumor Immunology and Immunotherapy, Bar-Ilan University, Israel

15:45

Dual CAR T-Cells to Treat Multiple Myeloma

Anat Globerson Levin¹, Moran Rawet Slobodkin¹, Tova Waks¹, Boris Tartakovsky², Ella Naparstek², Yael Cohen², Irit Avivi², Zelig Eshhar¹

¹*Immunology, Tel Aviv Sourasky Medical Center, Israel*

²*Hematology, Tel Aviv Sourasky Medical Center, Israel*

16:00

CAR-T cell therapy in Israel – Clinical results of the first 90 patients treated with on-side produced CD19 CAR T cells

Michal Besser^{1,2}, Orit Itzhaki¹, Abraham Avigdor⁴, Amos Toren³, Arnon Nagler⁴, Jacob Schachter¹, Elad Jacoby³

¹*Ella Lemelbaum Institute for Immuno-Oncology, Sheba Medical Center, Israel*

²*Department of Clinical Microbiology and Immunology, Tel Aviv University, Israel*

³*Division of Pediatric Hematology and Oncology, Sheba Medical Center, Israel*

⁴*Division of Hematology and Bone Marrow Transplantation, Sheba Medical Center, Israel*

16:15

Microvilli: The ERM Dependent Activation Hubs of T-Cells

Shirsendu Ghosh¹, Vincenzo Di Bartolo³, Liron Tubul¹, Eyal Shimoni², Elena Kartvelishvily², Tali Dadosh², Sara W. Feigelson⁴, Ronen Alon⁴, Andres Alcover³, Gilad Haran¹

¹*Department of Chemical Physics, Weizmann Institute of Science, Israel*

²*Chemical Research Support, Weizmann Institute of Science, Israel*

³*Lymphocyte Cell Biology Unit, INSERM U1221, Department of Immunology, Institut Pasteur, Paris, France*

⁴*Department of Immunology, Weizmann Institute of Science, Israel*

14:30-16:30 Session 5 C: State-of-the-Art Methodologies in Cancer Research, Oncology Hall, Israel C/D

Chairs: Alexander Bershadsky (WIS; MIS, Singapore), Oren Parnas (HUJI)

14:30

CRISPR Gene Correction: A Potential New Class of Medicines for Primary Immunodeficiency Diseases

Ayal Hendel

Life Sciences, The Mina and Everard Goodman Faculty of Life Sciences and Advanced Materials and Nanotechnology Institute, Bar-Ilan University, Israel

14:55

Proteomics of Melanoma Response to Immunotherapy Reveals Dependence on Mitochondrial Function

Michal Harel¹, Rona Ortenberg², Ettai Markovits², Erez Baruch², Mariya Mardamshina¹, May Arama¹, Gal Markel², **Tamar Geiger**¹

¹*Human Molecular Genetics and Biochemistry, Tel Aviv University, Israel*

²*Ella Lemelbaum Institute of Immuno-Oncology, Sheba Medical Center, Israel*

15:20

Gold nano rods and diffusion reflection imaging for mapping tumor margins

Dror Fixler

Faculty of Engineering, Director of the Nano Institute, Bar-Ilan University, Israel

15:45

Noninvasive sensor technologies for disease detection via breath Volatolomics

Hossam Haick, **Yoav Broza**

The Department of Chemical Engineering and Russell Berrie Nanotechnology Institute, Technion - Israel Institute of Technology, Israel

16:00

Proteasome Profiling of Non-Small Cell Lung Carcinoma

Aaron Javitt¹, Merav Shmueli¹, Avital Eisenberg-Lerner¹, Assaf Kacen¹, Hila Wolf-Levy², Inbal Zigdon¹, Yishai Levin², Nir Friedman¹, Yifat Merbl¹

¹*Department of Immunology, Weizmann Institute of Science, Israel*

²*De Botton Institute for Protein Profiling, The Nancy and Stephen Grand Israel National Center for Personalized Medicine, Weizmann Institute of Science, Israel*

16:15

Topological-Proteomics of Breast Cancer Intra-Tumor Heterogeneity Reveals Diversity within Single Tumors

Mariya Mardamshina¹, Daniela Necula², Kateryna Krol², Irina Marin², Iris Barshack², Tamar Geiger¹

¹*Department of Human Molecular Genetics and Biochemistry, Tel Aviv University, Israel*

²*Pathology Institute, Sheba Medical Center, Israel*

16:30-17:00 Coffee Break and Visit the Exhibition

17:00-18:10 Session 6 A: Metabolism and Immunity, Immunology Hall, Israel C/D

Chairs: Barak Rotblat (BGU), Tsaffrir Zor (TAU)

17:00

Matrix-localized substrate-level-phosphorylation is critical for mitochondrial remodeling during CD8⁺ T cell priming

Michael Berger

The Lautenberg Center of Immunology and Cancer Research, The Hebrew University of Jerusalem, Israel

17:25

The metabolic liability of T cell activation

Noga Ron-Harel¹, Jonathan Ghergurovich², Giulia Notarangelo³, Martin Lafleur⁴, Arlene H. Sharpe⁴, Joshua D. Rabinowitz², Marcia C. Haigis³

¹*Biology, Technion-Israel Institute of Technology, Israel*

²*The Lewis-Sigler Institute for Integrative Genomics, Princeton University, USA*

³*Cell Biology, Harvard Medical School, USA*

⁴*Immunology, Harvard Medical School, USA*

17:40

Glucose dependent insulinotropic polypeptide (GIP) immune cell interactions control body weight during obesity via modulation of energy expenditure

Irina Efimova^{1,2}, Fernanda Mantelmacher^{1,2}, Isabel Zvibel¹, Keren Cohen^{1,2}, Alona Epshtein¹, Thomas Vogl⁴, Daniel Drucker³, Sigal Fishman¹, **Chen Varol^{1,2}**

¹*The Research Center for Digestive Tract and Liver Diseases, Tel Aviv Sourasky Medical Center, Israel*

²*Clinical Microbiology and Immunology, Tel-Aviv University, Israel*

³*The Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, University of Toronto, Canada*

⁴*Institute of Immunology, University of Münster, Germany*

17:55

Lactate Release by Inflammatory Bone Marrow Neutrophils Induces Their Mobilization Via Endothelial GPR81 Signaling

Eman Khatib-Massalha¹, Suditi Bhattacharya¹, Hassan Massalha², Karin Golan¹, Orit Kollet¹, Anju Kumari¹, Stefan Offermanns⁴, Ronen Alon¹, Amiram Ariel³, Tsvee Lapidot¹

¹*Immunology, Weizmann Institute of Science, Israel*

²*Molecular Cell Biology, Weizmann Institute of Science, Israel*

³*Human Biology, University of Haifa, Israel*

⁴*Pharmacology, Max-Planck-Institute for Heart and Lung Research, Germany*



17:00-18:10 Session 6 B: Inflammation and Immunotherapy, Israel A

Chairs: **Amir Onn (TAU), Yishai Ofran (Rambam)**

17:00

The potential of immune checkpoint blockade for fighting against Alzheimer`s disease

Michal Schwartz

Neurobiology, Weizmann Institute of Science, Israel

17:25

Mutant p53 governs microenvironmental dynamics via exosomes and outer membrane vesicles

Ishai Luz¹, Ioannis Pateras², Vassilis Gorgoulis², Curtis Harris³, **Tomer Cooks**¹

¹*Genetics, Microbiology and Immunology, Ben-Gurion University of the Negev, Israel*

²*Molecular Carcinogenics Group, School of Medicine, National Kapodistrian University of Athens, Greece*

³*Laboratory of Human Carcinogenesis, National Institutes of Health, USA*

17:40

Engineering B Cells as an Evolving Drug to Fight HIV

Alessio Nahmad¹, Tal Akriv¹, Daniel Nataf¹, Miriam Fried¹, Yariv Wine², Itai Benhar², Iris Dotan¹, **Adi Barzel**¹

¹*Biochemistry and Molecular Biology, Tel Aviv University, Israel*

²*Molecular Cell Biology and Biotechnology, Tel Aviv University, Israel*

17:55

Aging promotes reorganization of the CD4 T-cell landscape toward extreme phenotypes implicated in age-related diseases

Alon Monsonego

Microbiology, Immunology and Genetics, Ben-Gurion University of the Negev, Israel

17:00-18:10 Session 6 C: Cancer Therapy: Advances in Drug Design and Delivery, Oncology Hall, Israel C/D

Chairs: **Vered Padler-Karavani (TAU), Tomer Kalisky (BIU)**

17:00

Ibrutinib-disabled immunosuppressive microenvironment sensitizes melanoma to PD-1/OX40 immune checkpoint modulators following immunization with dendritic cell-targeted nano-vaccines

João Conriot⁴, Anna Scomparin¹, Eilam Yeini¹, Sabina Pozzi¹, Ron Kleiner¹, Hila Doron², Neta Erez², Steffen Jung³, Helena F Florindo⁴, **Ronit Satchi-Fainaro**¹

¹*Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, Israel*

²*Department of Pathology, Sackler Faculty of Medicine, Tel Aviv University, Israel*

³*Department of Immunology, Weizmann Institute of Science, Israel*

⁴*Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal*

17:25

Engineering Affinity, Specificity and Stability in Protein Therapeutics

Niv Papo

Biotechnology Engineering, Ben-Gurion University of the Negev, Israel

17:40

Hypoxia Targeted Infectious Knockdown for the Treatment of Solid Tumors

Shahar Frenkel¹, Maria Gimelshein^{1,2}, Hanna Voropaev^{1,2}, Shahar Luaski^{1,2}, Alik Honigman²

¹*Ophthalmology, Hadassah-Hebrew University Medical Center, Israel*

²*Biochemistry and Molecular Biology, IMRIC Faculty of Medicine Hebrew University, Jerusalem, Israel*

17:55

When do colorectal tumors die following therapy?

Albert Grinshpun¹, Anatoli Kustanovich¹, Ruth Schwartz¹, Daniel Neiman², Myriam Maoz¹, Esther Tahover⁴, Ruth Shemer², Yuval Dor², Ayala Hubert¹, Eli Sapir³

¹*Sharet Institute of Oncology, The Hebrew University of Jerusalem - Hadassah Medical Center, Israel*

²*Department of Developmental Biology and Cancer Research, Institute for Medical Research Israel-Canada, the Hebrew University-Hadassah Medical School, Israel*

³*Radiation Oncology Center, Assuta Ashdod University Hospital, Israel*

⁴*Oncology Institute, Shaare Zedek Medical Center, Israel*

18:10-18:40 Keynote E, Israel A

18:10

Mechanical control of T cell cytotoxicity

Morgan Huse

Memorial Sloan Kettering Cancer Center, USA

WEDNESDAY, SEPTEMBER 25, 2019

09:00-09:30 *Morning Gathering*

09:30-10:00 **Keynote F, Israel A**

09:30

Using Super-Resolution Microscopy to Watch Immune Cells Kill

Daniel M. Davis

Manchester Collaborative Center for Inflammation Research, University of Manchester, UK

10:00-10:30 *Coffee Break and Visit the Exhibition*

10:30-12:15 **Session 7 A: Lymphocyte Activation and Exhaustion, Immunology Hall, Israel C/D**

Chairs: **Alex Braiman (BGU), Asaf Madi (TAU)**

10:30

B Cell Homeostasis under Control Of microRNAs

Doron Melamed

Immunology, Technion - Israel Institute of Technology, Israel

10:55

The Role of Fc Receptors in the Therapeutic anti-tumor Activity of Checkpoint Antibodies

Rony Dahan

Immunology, Weizmann Institute of Science, Israel

11:20

The molecular mechanisms regulating the cross talk between CLL cells and their microenvironment

Idit Shachar

Immunology, Weizmann Institute of Science, Israel

11:45

Elucidating Molecular Mechanisms Suppressing the NK Cell Killer Instinct

Aviad Ben-Shmuel, Batel Sabag, Omri Matalon, Noah Joseph, Danielle Keizer, Mira Barda-Saad
The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Israel

12:00

T cell regulation in pancreatic ductal adenocarcinoma

Enas Abu-Shah^{1,3}, Shivan Sivakumar^{1,2}, David Ahern¹, Nagina Mangal⁴, Srikanth Reddy⁴, Aniko Rendek⁵, Zahir Soonawalla⁴, Michael Silva⁶, Mark Middleton², Michael Dustin¹

¹Kennedy Institute of Rheumatology, University of Oxford, UK

²Department of Oncology, University of Oxford, UK

³Sir William Dunn School of Pathology, University of Oxford, UK

⁴Nuffield Department of Surgical Sciences, University of Oxford, UK

⁵Department of Cellular Pathology, Oxford University Hospitals, UK

⁶Cancer Services, Oxford University Hospitals, UK

10:30-12:15 Session 7 B: Tumor Microenvironment and Immuno-Oncology - II, Israel A

Chairs: Eitan Yefe Nof (HUJI), Tal Burstyn-Cohen (HU)

10:30

Myeloid Derived Suppressor Cells as Plastic Sensors and Outcome Orchestrators During Chronic Inflammation

Michal Baniyash, Yaron Meirow, Hadas Ashkenazi, Kerem Ben Meir, Nira Twaik, Ivan Mikula, Or Reuven, Leonor Daniel

The Lautenberg Center for Immunology and Cancer Research, Israel-Canada Medical Research Institute, Faculty of Medicine, The Hebrew University of Jerusalem, Israel

10:55

New players in melanoma micro environment

Carmit Levy

Genetics, Tel Aviv University, Israel

11:20

Immune surveillance of senescent cells- from embryonic development to aging

Valery Krizhanovsky, **Hilah Gal**

Molecular Cell Biology, Weizmann Institute of Science, Israel

11:45

Mapping Stromal And Immune Cell Dynamics During Breast Cancer Metastasis By scRNA-seq

Ido Yofe¹, Noam Cohen², Liat Stoler-Barak¹, Amir Giladi¹, Adam Yalin¹, Lea Monteran², Ziv Shulman¹, Neta Erez², Ido Amit¹

¹Department of Immunology, Weizmann Institute of Science, Israel

²Department of Pathology, Tel Aviv University, Israel

12:00

Activation of CD45 Reverses Tumor Suppression of Src Family Tyrosine Kinases in Leukocytes.

Annat Raiter¹, Julia Lipovetski¹, Shany Mugami¹, Oran Zlotnik³, Eran Sharon³, Rinat Yerushalmi^{1,2}

¹Felsenstein Medical Research Center, Felsenstein Medical Research Center, Tel Aviv University, Sackler School of Medicine, Rabin Medical Center, Israel

²Davidoff Cancer Center, Rabin Medical Center, Israel

³Surgery Department, Rabin Medical Center, Israel

10:30-12:15 Session 7 C: Bio-Markers and Cancer Theranostics, Oncology Hall, Israel C/D

Chairs: **Doron Ginsberg (BIU), Iris Barshack (TAU)**

10:30

Global views of degradation in cancer

Yifat Merbl

Immunology, Weizmann Institute of Science, Israel

10:55

Gold nanoparticles for cancer immunotherapy: Imaging, diagnosis and stratified medicine

Rachela Popovtzer, Oshra Betzer, Rinat Meir, Tamar Sadan, Menachem Motiei

Bar-Ilan University, Israel

11:20

TBC

Hovav Nechushtan

Medical Center Sharett Institute of Oncology, Hadassah Hebrew University, Israel

11:45

Analysis of the HLA peptidome for development of personalized cancer immunotherapy

Arie Admon¹, Nataly Rijensky¹, Michal Hayun³, Yishai Ofra²

¹Biology, Technion - Israel Institute of Technology, Israel

²Hematology and bone marrow transplantation, Rambam Health Care Campus, Israel

³Leukemia and signaling research, Rambam Health Care Campus, Israel

12:00

ARTS and ARTS mimetics promote cancer cell killing by degrading anti-apoptotic proteins

Sarit Larisch, Dana Mamriev, Ruqaiya Abbas, Juliana Kagan

Human Biology, University of Haifa, Israel

12:15-13:30 Lunch and Poster Presentation

13:30-14:30 Flash Talks: Immunology II, Immuno-Oncology II, Oncology II

14:30-16:30 Session 8 A: Microbial Infections, Resistance & Immunity, Immunology Hall, Israel C/D

Chairs: Zvi Bentwich (BGU), Sarah Feigelson (WIS)

14:30

The intestinal microbiota programs DNA methylation to control tissue homeostasis and inflammation

Yehudit Bergman^{1,5}, Ihab Ansari¹, Günter Raddatz², Julian Gutekunst², Meshi Ridnik¹, Daphne Cohen¹, Monther Abu-Remaileh¹, Hagit Shapiro³, Eli Pikarsky⁴, Eran Elinav³, Frank Lyko^{2,5}

¹Department of Developmental Biology and Cancer Research, Institute for Medical Research Israel-Canada, Hebrew University Medical School, Israel

²Division of Epigenetics, DKFZ-ZMBH Alliance, German Cancer Research Center, Germany

³Department of Immunology, The Weizmann Institute of Science, Israel

⁴Hebrew University Medical School, The Lautenberg Center for Immunology, Institute for Medical Research Israel-Canada, Israel

⁵Co-senior, author

14:55

The gut microbiota as modulators of the immune system and their potential in cancer therapy

Naama Geva-Zatorsky, Noa Mandelbaum, Nadav Ben-Assa, Dana Kadosh, Neerupma Bhardwaj, Michal Brunwasser-Meirom, Lillie Beck, Shaqed Carasso, Rawi Naddaf, Tal Gefen

Technion Integrated Cancer Center, Faculty of Medicine,, Technion - Israel Institute of Technology, Israel

15:20

Exploring the link between the oral microbe Porphyromonas gingivalis and pancreatic cancer

JebaMercy Gnanasekaran¹, Adi Binder Gallimidi^{1,2}, Luba Eli Berchoer¹, Esther Hermano², Sarah Angabo¹, Hasna'a Makkawi¹, Arin Khashan¹, Alaa Daoud¹, Michael Elkin², **Gabriel Nussbaum**¹

¹The Institute of Dental Sciences, The Hebrew University of Jerusalem - Hadassah Faculty of Dental Medicine, Israel

²Sharett Oncology Institute, The Hebrew University of Jerusalem - Hadassah Medical Center, Israel

15:35

Ezh2 regulates early stages of T-helper cell differentiation

Orly Avni, Moran Titelbaum, Boris Brant, Yiftah Barsheshet

Azrieli Faculty of Medicine, Bar Ilan University, Israel

15:50

Exclusive Temporal Stimulation of IL-10 Expression in LPS-Stimulated Mouse Macrophages by cAMP Inducers and Type I Interferons

Tsaffrir Zor¹, Yifat Glucksam-Galnoy¹, Muhammad Athamna^{1,2}, Iris Ben-Dror¹, Yair Glick³, Doron Gerber³, Bibek Bhatta¹, Hadar Ben-Arosh¹, Galit Levy-Rimler¹, Orna Ernst¹

¹School of Neurobiology, biochemistry & Biophysics, Tel Aviv University, Israel

²Department of Biochemistry, Triangle Regional Research and Development Center, Israel

³The Nanotechnology Institute, Bar-Ilan University, Israel



16:05

Small RNA and RNA modification affecting motility and infectivity of trypanosomatids parasites

Shulamit Michaeli

Faculty of Life Sciences, Bar Ilan University, Israel

14:30-16:30 Session 8 B: Controversies in Clinical Immuno-Oncology, Israel A

Chairs: **Israel Vlodavski (TECH), Laila Roisman (Soroka)**

14:30

Cancer Immunotherapy: What we have achieved and where we are going

Jonathan Cohen

Sharett Institute of Oncology and The Wohl Institute for Translational Medicine, Hadassah Hebrew University Medical Center, Israel

14:55

Untangling the mechanisms behind SLAMF6 switchy nature

Michal Lotem

Center for Melanoma and Cancer Immunotherapy, Hadassah Hebrew University Medical Center, Israel

15:20

Oncogenic Drivers Shape the Glioma Immune Landscape

*Prerna Magod, Liat Rousoo-Noori, Lilach Agemy, Dinorah Friedmann-Morvinski
Biochemistry and Molecular Biology, Tel Aviv University, Israel*

15:45

Mesencephalic astrocyte-derived neurotrophic factor, a potential immunomodulator, is secreted from interferon- γ –stimulated tumor cells through ER calcium depletion

Michael Peled¹, Tali H Bar-Lev¹, Jair Bar², Iris Kamer², Adam Mor³, Amir Onn¹

¹*Institute of Pulmonary Medicine, Sheba Medical Center, Israel*

²*Thoracic Oncology Unit, Institute of Oncology, Sheba Medical Center, Israel*

³*Columbia Center for Translational Immunology, Columbia University Medical Center, USA*

16:00

Diagnostics of Glioma Tumor by Non-Invasive Liquid Biopsy using Circulating Tumor DNA in Plasma

Milana Frenkel-Morgenstern, Vikrant Palande

Azieli Faculty of Medicine, Bar-Ilan University, Israel



16:15

The AP-1 complex regulates AXL expression and determines sensitivity to PI3Ka inhibition in esophagus and head and neck squamous cell carcinoma

Limor Cohen¹, Mai Bdarny¹, Manu Prasad¹, Noa Balaban¹, Ben-Zion Joshua², Anat Bahat Dinur², Reidar Grénman³, Moshe Elkabets¹

¹Microbiology Immunology and Genetics, Ben-Gurion University of the Negev, Israel

²Department of Otolaryngology-Head & Neck Surgery, Soroka Medical Center, Israel

³Department of Otorhinolaryngology – Head and Neck Surgery, Turku University and Turku University Hospital, Finland

14:30-16:30 Session 8 C: Genomic Instability, Cancer Signaling and Cancer Secretome, Oncology Hall, Israel C/D

Chairs: **Zvi Livneh (WIS), Chaya Brodie (BIU)**

14:30

Establishment of cancer stem cells is mutant p53 dependent

Varda Rotter

Department of Molecular Cell Biology, The Weizmann Institute of Science, Israel

14:55

Stem-Cells to Immune Cells and Back Again

Roi Gazit

The Shraga Segal Dept. of Microbiology Immunology and Genetics; BGU Center for Regenerative Medicine and Stem Cells; National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Israel

15:20

Exosomes as Circulating Biomarkers and Mediators of the Cross-Talk of Metastatic Brain Tumor Cancer Stem Cells with Microglia

Xin Hong², Simona Cazacu², Hodaya Fruchter¹, Steve Kalkanis², **Chaya Brodie**¹

¹Faculty of Life Sciences, Bar-Ilan University, Israel

²Neurosurgery, Henry Ford Health System, USA

15:45

CD8 T cells enhance the anti-tumor efficacy of Trametinib in head and neck cancer

Moshe Elkabets, Manu Parsad, Sankar Jagadeeshan, Sapir Tzadok, Limor Cohen

The Shraga Segal Department of Microbiology, Immunology and Genetics, Ben-Gurion University of the Negev, Israel



16:10

LATS1 and LATS2 Suppress Breast Cancer Progression by Maintaining p53 activity, Cell Identity and Metabolic state

Yael Aylon¹, Noa Furth¹, Ioannis Pateras², Ron Rotkopf⁵, Ina Schmitt³, Randy Johnson⁴, Vassilis Gorgoulis², Moshe Oren¹

¹Department of Molecular Cell Biology, Weizmann Institute of Science, Israel

²Laboratory of Histology and Embryology Medical School, University of Athens, Greece

³Systems Biology of Signal Transduction, DKFZ, Germany

⁴Department of Cancer Biology, MD Anderson Cancer Center, USA

⁵Department of Life Sciences Core Facilities, The Weizmann Institute of Science, Israel

16:30-17:00 Coffee Break and Visit the Exhibition

17:00-18:10 Session 9 A: Immunopathologies and Precision Medicine - II, Immunology Hall, Israel C/D

Chairs: **Lior Carmon (Biotech), Jacob Gopas (BGU)**

17:15

Probing the Role of Microglia in Relapsing-Remitting EAE

Zhana Haimon, Louise Chappell-Maor, Steffen Jung

Immunology, Weizmann Institute of Science, Israel

17:15

B Cell Engineering for a Regulated, Potent and Evolving Response to HIV

Alessio Nahmad, Tal Akriv, Miri Fried, Daniel Nataf, Iris Dotan, Yariv Wine, Itai Benhar, Adi Barzel

Biochemistry and Molecular Biology, Tel Aviv University, Israel

17:30

Multi-Kinase Inhibitors Targeting CK1 α and CDK7/9 are Novel Anti Leukemic Compounds with a Strong Immuno-Modulating Activity

Avner Fink, Eric Hung, Waleed Minzel, Avanthika Venkatachalam, Amitai Rivlin, Indranil Singh, Irit Snir-Alkalay, Yinon Ben-Neriah

Lautenberg Center for Immunology and Cancer Reserach, The Hebrew University of Jerusalem, Israel

17:00-18:10 Session 9 B: Modulation of Tumor Immunity, Israel A

Chairs: **Nurit Hollander (TAU), Chen Varol (Sourasky- TAU)**

17:00

Mutant p53 enhances the signal of hepatocyte growth factor (HGF) to endow cancer cells with drug resistance

Yan Stein, Adi Berger, Naomi Goldfinger, Ravid Straussman, Varda Rotter

Department of Molecular Cell Biology, Weizmann Institute of Science, Israel



17:15

The immunomodulatory properties of Cannabinoids- lessons from murine models of Bone Marrow Transplantation.

Osnat Almogi-Hazan, Iman Khuja, Zhanna Yekhtin, Reuven Or
Laboratory of Immunotherapy and Bone Marrow Transplantation, Hadassah Medical Center, Hebrew University of Jerusalem, Israel

17:30

Neuronal regulation of anti-tumor immunity

Asya Rolls

HHMI-Wellcome International Scholar, Rappaport Institute for Medical Research, Technion-Israel Institute of Technology, Israel

17:00-18:10 Session 9 C: Leukemia: From Stem Cell to Therapy, Oncology Hall, Israel C/D

Chairs: **Avigdor Avraham** (Sheba), **Michael Danilenko** (BGU)

17:00

uORF-encoded Peptides as Novel Regulators of Protein Kinase Activity: New Opportunities for Cancer Therapy

Etta Livneh, Divya Ram Jarayam¹, Sigal Frost¹, Chanan Argov², Assaf Ben-Ari¹, Amitha Muraleedharan¹, Rosa Sinay¹, Esti Yeger-Lotem²

¹*The Sharaga Segal Department of Immunology, Microbiology and Genetics, Ben Gurion University of the Negev, Israel*

²*Clinical Biochemistry & Pharmacology, Ben Gurion University of the Negev, Israel*

17:15

Identifying a malignant B-cell Lymphoma clone in the peripheral blood using Immunoglobulin high-throughput sequencing and lineage tree analysis

Meirav Kedmi^{1,2,3}, Hadas Neuman³, Guy Bitansky², Meital Nagar¹, Gaelle Scheinert-Shenhav¹, Ninette Amariglio^{1,3}, Iris Barshack^{2,4}, Ginette Schiby^{2,4}, Hilla Tabibian-Keissar⁴, Arnon Nagler^{1,2}, Abraham Avigdor^{1,2}, Ramit Mehr³

¹*Division of Hematology and Bone Marrow Transplantation, Chaim Sheba Medical Center, Tel-Hashomer, Israel*

²*Sackler School of Medicine, Tel-Aviv University,, Israel*

³*The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Israel*

⁴*Department of Pathology, Chaim Sheba Medical Center, Tel-Hashomer, Israel*

17:30

Recurrent pre-leukemic deletions are the result of microhomology-mediated end joining DNA repair

Liran Shlush, Tzah Feldman

Immunology, Weizmann Institute of Science, Israel

18:10-18:15 Concluding remarks and Poster Awards, Israel A

The joint meeting of the Israeli Immunological Society (IIS)
and the Israeli Society for Cancer Research (ISCR)

SEPTEMBER 23-25, 2019 DAN PANORAMA, TEL-AVIV



Israel Immunological Society

FLASH TALKS

TUESDAY, SEPTEMBER 24, 2019

13:30-14:30 Flash Talk: Immunology I, Immunology Hall, Israel C/D

13:30

Interleukin-1 α Induced Expression of Steroidogenic Acute Regulatory Protein (StAR) Facilitates Fibroblasts Survival Following Myocardial Infarction

Talya Razin¹, Naomi Melamed-Book², Jasmin Argaman¹, Iris Galin¹, Eli Anuka¹, Nili Naftali-Shani³, Michal Kandel-Kfir⁴, Yehuda Kamari⁴, Jonathan Leor³, Joseph Orly¹

¹Department of Biological Chemistry, The Alexander Silberman Institute of Life Sciences, the Hebrew University of Jerusalem, Israel

²Bio-Imaging Unit, The Alexander Silberman Institute of Life Sciences, the Hebrew University of Jerusalem, Israel

³Tamman Cardiovascular Research Institute, Sheba Center of Regenerative Medicine, Stem Cells and Tissue Engineering, Sheba Medical Center and Neufeld Cardiac Research Institute, Sackler Faculty of Medicine, Tel-Aviv University, Israel

⁴Bert W. Strassburger Lipid Center, Sheba Medical Center, Tel Hashomer, Sackler Faculty of Medicine, Tel-Aviv University, Israel

13:35

Novel Immune History-Based Correlates of Risk and Protection for Influenza

Ayelet Shagal¹, Lilach Friedman¹, Joshua Petrie², Emily Martin², Arnold Monto², Tomer Hertz^{1,3}

¹Department of Microbiology, Immunology and Genetics, NIBN, Ben-Gurion University of the Negev, Israel

²Department of Epidemiology, School of Public Health, University of Michigan, USA

³Vaccine and Infectious Disease Division, Fred Hutch Cancer Research Center, USA

13:40

High-Throughput Fluorescence Polarization Screen for Inhibitors of VICKZ1 RNA Binding

Nadav Wallis

Department of Developmental Biology and Cancer Research, Hebrew University of Jerusalem, Israel

13:45

Profiling the Effect of Obesity on the Influenza Vaccine-Induced Antibody Repertoire Using Antigen Microarrays

Marwa Abd Alhadi¹, Erik Karlsson³, Lilach M. Friedman¹, Melinda Beck⁴, Tomer Hertz^{1,2}

¹The Shraga Segal Department of Microbiology, Immunology and Genetics, Ben Gurion University of the Negev, Israel

²Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, USA

³Virology Institut Pasteur du Cambodge, Institut Pasteur du Cambodge, Cambodia

⁴Department of Nutrition, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, USA

The joint meeting of the Israeli Immunological Society (IIS)
and the Israeli Society for Cancer Research (ISCR)

SEPTEMBER 23-25, 2019 DAN PANORAMA, TEL-AVIV



Israel Immunological Society

13:50

Study of antigen presentation and antigen-specific immunomodulation in multiple sclerosis by T cell receptor-like antibodies

Alona Goor, Inbar Arman, Efrat Altman, Maya Haus-Cohen, Yoram Reiter

Laboratory of Molecular Immunology, Faculty of Biology, Technion-Israel Institute of Technology, Israel

13:55

COMMD10 Promotes Resolution Of Acetaminophen-Induced Liver Injury By Immunoregulation Of Monocyte And Macrophage Activity

Keren Cohen^{1,2}, Odelia Mouhadab^{1,2}, Daniel Keidar^{1,3}, Nathan Gluck¹, Chen Varol^{1,2}

¹The research center for digestive tract and liver diseases, Sourasky medical center and Tel-Aviv University, Israel

²Department of clinical microbiology and immunology, Sackler faculty of medicine, Tel-Aviv University, Israel

³Department of Plastic and Reconstructive Surgery, Sourasky medical center, Tel-Aviv University, Israel

14:00

Tellurium-based immunomodulating compound SAS inhibits the progression of early-onset Alzheimer`s disease in the 5xFAD mouse model.

Julia Manoim^{2,4}, Tomer Illouz^{1,3}, Eitan Okun^{1,3}, **Benjamin Sredni**^{2,4}

¹The Leslie and Susan Gonda Multidisciplinary Brain Research Center, Bar Ilan University, Israel

²the Mina and Everard Goodman Faculty of Life-Sciences, Bar Ilan University, Israel

³The Paul Feder Laboratory for Alzheimer`s Disease Research, Bar-Ilan University, Bar Ilan University, Israel

⁴C.A.I.R. Institute, the Safdié Aids and Immunology Research Center, Bar Ilan University, Israel

14:05

Chemotherapy treated-metastatic cells harness macrophages to support metastatic outgrowth

Shira Michaeli-Ashkenasi

University of Haifa, Israel

14:10

Probing functional contributions of microglia and non-parenchymal CNS macrophages in physiology and pathophysiology

Jung-Seok Kim, Yuan Xia, Louise Chappell-Maor, Masha Kolesnikov, Zhana Haimon, Anat Shemer, Sigalit Boura-Halfon, Steffen Jung

Department of Immunology, Weizmann Institute of Science, Israel

14:15

A Genome-Wide Screen Identifies a Critical Role for Mitochondrial NDP Kinases in Inflammasome Activation

Orna Ernst Rabinovich¹, Jing Sun¹, Bin Lin¹, Christopher Rice⁵, Balaji Banoth³, Samuel Katz^{1,4}, Michael Dorrington¹, Jonathan Liang^{1,4}, Nadia Slepushkina², Daniel McVicar⁵, Clare Bryant⁴, Fayyaz Sutterwala³, Scott Martin², Madhu Lal-Nag², Iain Fraser¹

¹Laboratory of Immune System Biology, NIAID, NIH, USA

²NCATS, NIH, USA

³Infectious and Immunologic Diseases Research Center, Cedars-Sinai Medical Center, USA

⁴Department of Veterinary Medicine, University of Cambridge, UK

⁵NCI, NIH, USA

13:30-14:30 Flash Talk: Immuno-Oncology I, Israel A

13:30

Association Between Immune-Related Adverse Events During Anti-PD-1 Therapy and Tumor Mutational Burden

David Bomze¹, Omar Hasan Ali¹, Andrew Bate², Lukas Flatz¹

¹Institute of Immunobiology, Kantonsspital St Gallen, Switzerland

²Division of Translational Medicine, NYU School of Medicine, USA

13:35

Heterogeneous landscape of CD4 T cells develops with age and comprises unique subsets with distinct phenotypic properties

Yehezqel Elyahu¹, Idan hekselman², Inbal Eizenberg-Magar³, Omer Berner¹, Itai Strominger¹, Maya Schiller³, Kritika Mittal¹, Anna Nemirovsky¹, Ekaterina Eremenko¹, Assaf Vital², Eyal Simonovsky², Vered Chalifa-Caspi², Nir Friedman³, Esti Yeger-Lotem², Alon Monsonego¹

¹Immunology and Microbiology, Ben Gurion University of The Negev, Israel

²Biochemistry, Ben Gurion University of The Negev, Israel

³Immunology, Weizmann Institute of Science, Israel

13:40

Successful Immunotherapy For Melanomas Requires Tumor-Infiltrating Monocyte-Derived Dendritic Cells

Nadine Santana Magal, Leen Farhat, Diana Rasoulouniriana, Amit Gutwillig, Lior Tal, Annette Gleiberman, Peleg Rider, Yaron Carmi

Pathology, Tel Aviv University, Israel

The joint meeting of the Israeli Immunological Society (IIS)
and the Israeli Society for Cancer Research (ISCR)

SEPTEMBER 23-25, 2019 DAN PANORAMA, TEL-AVIV



Israel Immunological Society

13:45

The PD-L1/PD-1 Axis Blocks Neutrophil Cytotoxicity in Cancer

Olga Yajuk, Maya Gershkovitz, Tanya Fainsod-Levy, Zvi Granot

Developmental Biology and Cancer Research, The Hebrew University-Hadassah Medical School, Israel

13:50

Targeting the tumor microenvironment by IL-1 neutralization

Sapir Maudi-Boker, Irena Kaplanov, Elena Voronov, Ron N. Apte

Immunology and Genetics, Faculty of Health Sciences Ben-Gurion University of the Negev, The Shraga Segal Department of Microbiology, Israel

13:55

Differential Neutrophil Extracellular Traps Release (NETosis) In Cancer-Related Neutrophils

Ludovica Arpinati, Merav E. Shaul, Naomi Kaiser-Iluz, Shira Mali, Zvi G. Fridlender

Institute of Pulmonary Medicine, Hadassah-Hebrew University Medical Center, Israel

14:00

Repression of AXL expression by AP-1/JNK blockage overcomes resistance to PI3Ka therapy

Mai Badarni¹, Mai Badarni¹, Limor Cohen¹, Manu Parsad¹, Noa Balaban¹, Ben-Zion Joshua², Anat Bahat Dinur², Moshe Elkabets¹

¹*Microbiology, Immunology and Genetics, BGU, Israel*

²*The Department of Ear, Nose and Throat, Soroka Medical Center, Israel*

13:30-14:30 Flash Talk: Oncology I, Oncology Hall, Israel C/D

13:30

Enhancement of the immune response induced by Alpha Radiation- Based Brachytherapy can Inhibit Triple Negative Breast Cancer tumor development and Cure Colon Tumors in Mice

Vered Domankevich-Bachar^{1,2}, Adi Cohen¹, Margalit Efrati¹, Michael Schmidt², Itzhak Kelson^{2,3}, Yona Keisari^{1,2}

¹*Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Israel*

²*Biomedical R&D, Alpha Tau Medical, Israel*

³*Sackler Faculty of Exact Sciences, School of Physics and Astronomy, Tel Aviv University, Israel*

13:35

Formation of a fibrotic pre-metastatic niche is mediated by systemic Activin A during pulmonary metastasis of breast cancer

Noam Cohen

Tel Aviv University, Israel

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and the Israeli Society for Cancer Research (ISCR)

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Israel Immunological Society

13:40

Reciprocal Interactions between Melanoma and Microglia Cells Reprogram Melanoma Malignancy Phenotype

Sivan Izraely¹, Shlomit Ben-Menachem¹, Orit Sagi-Assif¹, Tsipi Meshel¹, Sapir Malka¹, Marcelo Ehrlich¹, Matthew P. Salomon², Metsada Pasmanik-Chor³, Dave S.B. Hoon², Isaac P. Witz¹

¹School of Molecular Cell Biology and Biotechnology, Tel Aviv University, Israel

²Department of Translational Molecular Medicine, John Wayne Cancer Institute at Providence Saint John's Health Center, USA

³Bioinformatics Unit, Tel Aviv University, Israel

13:45

The Non-Cell Autonomous Role of YAP Affects Blood Vessel Integrity and Immune Response in Tumors

Anat Gershoni, Yael Aylon, Moshe Oren

Molecular cell biology, Weizmann Institute for science, Israel

13:50

Ligand Binding Domain Activating Mutations of ESR1 Rewire Cellular Metabolism of Breast Cancer Cells

Lotem Zinger^{1,2}, Keren Merenbakh-Lamin^{1,2}, Anat Klein Klein^{1,2}, Adi Elazar^{1,2}, Shani Journo^{1,2}, Tomer Boldes^{1,2}, Metsada Pasmanik-Chor³, Avishay Spitzer¹, Tami Rubinek^{1,2}, Ido Wolf^{1,2}

¹Institute of Oncology, Tel Aviv Sourasky Medical Center, Israel

²Sackler Faculty of Medicine, Tel Aviv University, Israel

³Bioinformatics Unit, Faculty of Life Sciences, Tel Aviv University, Israel

13:55

Adaptation Of Colon Cancer Cells To The Brain Microenvironment: The Role Of IRS2

Inbal Greenberg^{1,2}, Anat Klein¹, Rachel Grossman³, Ethan Sokol⁴, Tami Rubinek^{1,2}, Ido Wolf^{1,2}

¹Oncology, Tel Aviv Sourasky Medical Center, Israel, Israel

²Sackler Faculty of Medicine, Tel Aviv University, Israel, Israel

³Neurosurgery, Tel Aviv Sourasky Medical Center, Israel, Israel

⁴Foundation Medicine, Massachusetts Institute of Technology, USA, Israel

14:00

Metastasis Prediction: Mechanobiology-Based Early Determination of Metastatic Risk in Pancreatic Tumors

Daphne Weihs¹, Yulia Merkher¹, Offir Ben-Ishay², Yoram Kluger^{2,3}

¹Faculty of Biomedical Engineering, Technion-Israel Institute of Technology, Israel

²Department of General Surgery, Rambam Health Care Campus, Israel

³Faculty of Medicine, Technion-Israel Institute of Technology, Israel



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14:05

Development and Characterization of the Metastatic Niche in Ovarian Carcinoma

Hadil Onallah¹, Avi Ben-Shushan², Reuven Reich¹

¹The Institute for Drug Research, School of Pharmacy, Faculty of medicine, The Hebrew University of Jerusalem, Israel

²Department of Gynecology, Hadassah Medical Organization, Israel

14:10

Role STIM2 in Human Breast Cancer

Ruslana Militsin, Raz Palty, Sunny Singh, Elia Zumot, Hadas Achildiev

Department of Biochemistry, Rappaport Faculty of Medicine, Technion, Israel

WEDNESDAY, SEPTEMBER 25, 2019

13:30-14:30 Flash Talk: Immunology II, Immunology Hall, Israel C/D

13:30

Development of multivirus-specific T (VST) cells for the prophylaxis and treatment of viral infections following hematopoietic stem cell transplantation

Nathalie Asherie

The Department of Bone Marrow Transplantation and Cancer Immunotherapy, Hadassah Medical Center, Israel

13:35

Daily Rhythms Of Neutrophil Activation Programs Host Response To LPS-Induced Infection

Suditi Bhattacharya¹, Eman Khatib-Massalha¹, Karin Golan¹, Orit Kollet¹, Sergei Butenko², Anoop Babu-Vasandan¹, Lizeth Alejandra Ordonez Moreno¹, Amiram Ariel², Tsvee Lapidot¹

¹Department of Immunology, Weizmann Institute of Science, Israel

²The Department of Human Biology, University of Haifa, Israel

13:40

A Novel Subset of CD4+ T Cell Expressing the High Affinity Fcγ Receptor Links Antibody and T Cell Immunity

Diana Rasoulouniriana, Nadine Santana-Magal, Loir Tal, Amit Gutwillig, Leen Farhat, Peleg Rider, Yaron Carmi

Pathology, Sackler Faculty of Medicine, Tel Aviv University, Israel



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13:45

Blocking of TGF β Signaling Using a Novel Platform for Neutrophil Specific Targeting Prevents Metastasis

Sandra Voels¹, Arik Ryvkin², Haim Ashkenazy², Meital Ben-Naim¹, Naomi Kaisar Iluz¹, Jonathan M. Gershoni², Zvi G. Fridlender¹, Zvi Granot¹

¹Department of Developmental Biology and Cancer Research, Hebrew University Medical School, Israel

²Department of Cell Research and Immunology, Tel Aviv University, Israel

13:50

IgA Clonal Lineage Analysis Reveals Class Switch Dynamics in Human Gut

Hadas Neuman¹, Giuliana Magri^{2,3}, Andrea Cerutti^{2,3}, Ramit Mehr¹

¹The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Israel

²Program for Inflammatory and Cardiovascular Disorders, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Spain

³Division of Clinical Immunology, Department of Medicine, Catalan Institute for Research and Advanced Studies (ICREA), Spain

13:55

κ -helix and the helical lock and key model: A pivotal way of looking at polyproline II

Tomer Meirson¹, David Bomze², Avraham Samson¹

¹Drug Discovery Laboratory, The Azrieli Faculty of Medicine, Bar Ilan University, Israel

²Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel., Israel

14:00

Structure-based Optimized Antibody for Targeting Pancreatic Cancer

Aliza Borenstein-Katz¹, Ron Amon³, Shira Warszawski², Hai Yu⁴, Xi Chen⁴, Sarel Fleishman², Vered Padler-Karavani³, Ron Diskin¹

¹Structural Biology, Weizmann Institute

²Biomolecular Sciences, Weizmann Institute

³Cell research and immunology, Tel Aviv University

⁴Chemistry, UC Davis

14:05

Pan-Cancer Single Cell RNA-Seq Uncovers Recurring Programs of Cellular Heterogeneity

Alissa Greenwald, Gabriela Kinker¹, Rotem Tal¹, Zhanna Orlova¹, Mike Cuoco², James McFarland², Allie Warren², Aviv Regev², Itay Tirosh¹

¹Molecular Cell Biology, Weizmann Institute of Science, Israel

²Broad Institute, Broad Institute, USA



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14:10

Prolongation of IL-2 half-life, by addition of highly-glycosylated sequences, has the added benefits of promoting anti-inflammation immune response

Aner Ottolenghi, Priyanka (Equal) Bolel, Angel Porgador

Immunology, Microbiology and Genetics, Ben Gurion University of the Negev, Israel

13:30-14:30 Flash Talk: Immuno-Oncology II, Israel A

13:30

Expression- and immune-profiling of neuroblastoma-associated Opsoclonus Myoclonus Ataxia Syndrome (OMAS) identifies features of auto- and tumor-immunity

Miriam Rosenberg¹, Jessica Panzer², Erez Greenstein³, Reut Timmor⁴, Dan Reshef³, Martin Buchkovich⁵, Victor Weigman⁵, Gur Yaari⁴, Nir Friedman³, John Maris⁶

¹EEB, Hebrew University of Jerusalem, Israel

²Neurology, Children's Hospital of Philadelphia, USA

³Department of Immunology, Weizmann Institute of Science, Israel

⁴Department of Engineering, Bar Ilan University, Israel

⁵R&D, Q2 Solutions, USA

⁶Department of Oncology, Children's Hospital of Philadelphia, USA

13:35

CX3CR1 Expressing Macrophages Infiltrate the Tumor Microenvironment and Promote Radiation Resistance in a Mouse Model of Lung Cancer

Tammy Ben-Mordechai¹, Yaacov R. Lawrence^{1,2}, Zvi Symon^{1,2}, Ariel Shimoni-Sebag¹, Sarit Appel¹, Uri Amit^{1,3}

¹Department of Radiation Oncology, Sheba Medical Center, Tel-Hashomer, Israel

²Sackler faculty of Medicine, Tel-Aviv University, Israel

³The Dr. Pinchas Borenstein Talpiot Medical Leadership Program, Sheba Medical Center, Tel-Hashomer, Israel

13:40

CD40 costimulation enhances CAR-T cell activation

Ofir Levin-Piaeda, Noam Levin, Hadas Weinstein-Marom, Gideon Gross
Biotechnology, Tel-Hai College, MIGAL - Galilee Research Institute, Israel

13:45

The Effect of Maternal Age On the Ovarian Immune Milieu

Tal Ben Yaakov, Tanya Wasserman, Yonatan Savir

Faculty of Medicine, Dept. of Biophysics and Systems Biology, Technion, Israel



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13:50

Effective Targeting of Multiple Tumours by Combining CRISPR-based Genome Editing and CAR/TCR-engineering in Human T Cells

Vasyl Eisenberg¹, Shiran Hoogi¹, Jenny Shapiro¹, Tilda Barliya¹, Angel Porgador², Ayal Hendel¹, Cyrille Cohen¹

¹Life Sciences, Bar Ilan, Israel

²Life Sciences, Ben-Gurion University, Israel

13:55

The bilateral interplay between cancer immunotherapies and neutrophils` phenotypes and sub-populations

Naomi Kaisar Iluz, Ludovica Arpinati, Merav E Shaul, Zvi G Fridlender
Pulmonology, Hadassah-Hebrew University Medical Center, Israel

14:00

Decoupling epithelial-to-mesenchymal transitions from stromal profiles by integrative analysis

Michael Tyler, Itay Tirosh
Molecular Cell Biology, Weizmann Institute of Science, Israel

13:30-14:30 Flash Talk: Oncology II, Oncology Hall, Israel C/D

13:30

MET activation confers resistance to cetuximab, and prevents HER2 and HER3 upregulation in head and neck cancer

Ofra Z Novoplansky¹, Matthew Fury², Manu Prasad¹, Ksenia Yegodayev¹, Jonathan Zorea¹, Limor Cohen¹, Raphael Pelossof³, Liz Cohen¹, Nora Katabi⁴, Fabiola Cecchi⁵, Ben-Zion Joshua⁶, Aron Popovtzer⁷, Jose Baselga⁸, Maurizio Scaltriti⁹, Moshe Elkabetz¹

¹The Shraga Segal Department of Microbiology, Immunology and Genetics, Ben-Gurion University of the Negev, Israel

²Department of Medicine, Memorial Sloan Kettering Cancer Center, USA

³Computational Biology Program, Memorial Sloan Kettering Cancer Center, USA

⁴Department of Pathology, Memorial Sloan Kettering Cancer Center, USA

⁵Medical Center Drive, NantOmics , , MD, 20850, USA

⁶Department of Otolaryngology-Head & Neck Surgery, Soroka University Medical Center, Israel

⁷Head and Neck Cancer Radiation Clinic, Davidoff Cancer Center,, Institute of Oncology Rabin Medical Center, Israel

⁸(VHIO), Vall d'Hebron Institute of Oncology, Spain

⁹Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, USA

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13:35

Cellular Evolution Upon Glioblastoma Recurrence At Single-Cell Resolution

Avishay Spitzer¹, Masashi Nomura², Mario Suva², Itay Tirosh¹

¹Molecular Cell Biology, Weizmann Institute of Science, Israel

²Department of Pathology and Center for Cancer Research, Massachusetts General Hospital and Harvard Medical School, USA

13:40

Patient-derived 3D Models as a Novel Tool for Drug Developing and Testing

Sarah Hofmann, Raichel Cohen Harazi, Igor Koman

Institute for Personalized and Translational Medicine, Ariel University, Israel

13:45

Developing Nanobodies and Small Molecule Conjugates Targeting Prostate Specific Membrane Antigen Targeting for Prostate Cancer Imaging and Therapy

Lior Rosenfeld

Biotechnology Engineering, Ben Gurion University of the Negev, Israel

13:50

ARTS Mimetic (AM) Small Molecules Enhance The Killing Effect Of BH₃ Mimetic ABT-199 (Venclexta[®])

Nir Shahar, Juliana Kagan, Sarit Larisch

Human Biology, University of Haifa, Israel

13:55

Targeting Points of Vulnerability in individual Melanoma Tumors for Reducing Acquired Drug Resistance

Naama Dekel¹, Roni Oren³, Orit Itzhaki², Tomer Meir Salame⁴, Michal Besser², Adi Kimchi¹

¹Molecular Genetics, Weizmann Institute of Science, Israel

²Ella Institute, Sheba Medical Center, Israel

³Veterinary Resources, Weizmann Institute of Science, Israel

⁴Department of Life Sciences Core Facilities, Weizmann Institute of Science, Israel

14:00

Phenotypic and Mechanistic Characterization of Drug-Tolerant Persisters

Adi Jacob Berger¹, Elinor Gigi¹, Zohar Meir^{2,3}, Amos Tanay^{2,3}, Amir Pri-Or⁵, Yishai Levin⁵, Shlomit Gilad⁵, Yitzhak Pilpel⁴, Ravid Straussman¹

¹Department of Molecular Cell Biology, Weizmann Institute of Science, Israel

²Department of Biological Regulation, Weizmann Institute of Science, Israel

³Department of Computer Science and Applied Mathematics, Weizmann Institute of Science, Israel

⁴Department of Molecular Genetics, Weizmann Institute of Science, Israel

⁵The Nancy and Stephen Grand Israel National Center for Personalized Medicine (INCPM), Weizmann Institute of Science, Israel

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14:05

Constructing a novel Dynamic Three Dimensional *in Vitro* Model for Investigation of Ovarian Carcinoma Progression at the Different Anatomic Sites of the Disease

Aharon Baskin^{1,2}, Ben Davidson³, Reuven Reich¹, Tali Tavor Reem²

¹School of Pharmacy, Hebrew University, Jerusalem., Israel

²Pharmaceutical Engineering, Azrieli College of Engineering, Israel

³The Medical Faculty, University of Oslo, Norway

14:10

Elucidating the role of PROS1 in glioma cell plasticity

Divsha Sher¹, Liat Rousso-Noori¹, Tal Burstyn-Cohen², Dinorah Friedmann-Morvinski^{1,3}

¹Department of Biochemistry and Molecular Biology, The George S. Wise Faculty of Life Sciences, Tel Aviv University

²Faculty of Dental Medicine, Institute for Dental Sciences, Hadassah Medical School, Hebrew University of Jerusalem, Israel

³Sagol School of Neurosciences, Tel Aviv University, Israel, Israel

14:15

Cyclosporine H Improves Lentiviral Transduction of Murine Hematopoietic Stem and Progenitor Cells

Leonid Olender, Nir Bujanover, Oron Goldstein, Roi Gazit

The Shraga Segal Department of Microbiology and Immunology, Faculty of Health Sciences, Ben Gurion University of the Negev, Israel



ABSTRACTS

Cancer Metastasis

Cyclin Dependent Kinase Inhibitors 2A and 2B Loss Drive Pancreatic Ductal Adenocarcinoma Liver Metastasis

Shani Journo^{1,2}

¹Institute of Oncology, Tel Aviv Sourasky Medical Center, Israel

²Sackler Faculty of Medicine, Tel Aviv University, Israel

Introduction: Pancreatic Ductal Adenocarcinoma (PDAC) metastasizes mostly to the liver. A critical step of metastasis formation is the ability of cancer cells to colonize in foreign microenvironment. Yet, no genomic driver of liver metastasis (LM) in PDAC was identified. To identify mutations that prone PDAC cells to colonize in the liver and decipher mechanisms enabling the process.

Material and method: NGS of 312 cancer-related genes in 1741 PDAC primary and 1765 metastasis samples at different sites was conducted by FoundationOne. Proliferation, migration and invasion were assessed using methylene-blue, transwell, and wound-healing assays. Three-dimensional PDAC spheres were generated using InSphero assay. Mouse primary hepatocyte-conditioned media (PH-CM) was generated.

Results and discussion: FoundationOne database showed increased prevalence of frameshift mutations, resulting in deletion, in cyclin dependent kinase inhibitors 2A/2B (CDKN2A/B) in PDAC LM relative to primary tumor and other metastatic sites. High mRNA and protein levels of CDKN2A/B were noted in WT-CDKN2A/B COLO-357 cells while no expression was detected in mutated-CDKN2A/B MIA-PaCa-2 and PANC-1 cells. Overexpression of these genes in mutated-CDKN2A/B cells inhibited their proliferation while co-silencing in WT-CDKN2A/B COLO-357 increased proliferation and aggressive phenotype. The ability of CDKN2A/B loss to promote LM was examined. Overexpressing CDKN2A/B in mutated cells inhibited their proliferation in PH-CM compared to control. Whereas co-silencing of CDKN2A/B in WT COLO-357 cells increased proliferation and sphere formation in PH-CM compared to control.

Conclusion: This data indicate, for the first time, unique genomic profile of liver PDAC metastasis and indicate loss of p15 and p16 as promoting liver metastases.

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Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment

Tributyltin and triphenyltin isothiocyanates down-regulate immune check-point receptors, but not HLA G in human triple-negative breast carcinoma MDA-MB-231 cell line.

Luba Hunakova¹

BMC, Cancer Research Institute, University Science Park for Biomedicine, Slovak Academy of Sciences, Slovakia

Introduction

Programmed Death 1 receptor (PD-1) is expressed in activated T-lymphocytes, B-lymphocytes, mononuclear cells, NK cells and some dendritic cells. As a consequence of PD-L1 ligand binding to PD-1 is down-regulation of T-cell activity. Human leukocyte antigen G (HLA-G) is an immunotolerant nonclassical major histocompatibility complex Class Ib molecule, overexpressed in tumors and involved in cancer immune evasion.

We combined anticancer/genotoxic properties of two chemically different types of molecules, triorganotins and isothiocyanate, into tributyltin isothiocyanate (TBT-ITC) and triphenyltin isothiocyanate (TPT-ITC) and recently have shown their genotoxic effects in human breast carcinoma MCF 7 and MDA-MB-231 cell lines. In this study, we demonstrate immunomodulatory properties of their non-genotoxic concentrations in triple negative human breast cancer cell line MDA-MB-231.

Material and Method

To select the optimal compounds concentrations that do not affect cell viability, the MTT test has been used. Conventional comet assay and its modification – incubation of cells with styrene oxide (StO) were used for the detection of DNA damage as DNA breakage as well as DNA crosslinks formation. PDL-1 and HLA G expression were determined by flow cytometry.

Results and Discussion

100 nM concentration of studied compounds has been selected for further *in vitro* experiments based on MTT test for 48 h. This concentration kept the viability of cells over 93%. Comet assay did not reveal the presence of DNA breaks or crosslinks in MDA-MB-231 cells after 24 h treatment with both ITC derivatives, indicating their non genotoxic potential at low 100 nM concentrations. Immune-regulatory properties of tested derivatives were demonstrated by flow cytometry and displayed down-regulation of PD-L1 molecule after 24 hour incubation with 100 nM concentration of both triorganotin isothiocyanates. However, these compounds did not modulate HLA G expression.

Conclusion

Serious interests in novel organotin compounds are undoubtedly increasing due to their possible use in clinical oncology. Here, we demonstrated down-regulation of an immune regulatory molecule that suppresses the anticancer tumor PD-L1 response on the surface of MDA-MB-231 cells after TBT-ITC and TPT-ITC treatment. These triorganotin compounds did not alter HLA G expression.



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Bio-Markers and Cancer Theranostics, State-of-the-Art Methodologies in Research, Precision in Personalized Cancer Immunotherapy

Diagnosis of Glioma Tumors Using Circulating Cell-Free DNA

Vikrant Palande

The Azrieli Faculty of Medicine, Bar-Ilan University, Israel

Introduction

Gliomas are the most frequent brain tumors, making up about 30% of all brain and central nervous system tumors, and 80% of all malignant brain tumors. Existing standard diagnostic technique for glioma tumor includes tissue biopsy, which is a highly invasive and hence a risky technique for the patient's survival. 'Liquid biopsy' is a new and recently developed non-invasive cancer diagnostic technique, which includes use of circulating cell-free DNA (cfDNA) fragments for tracing tumor markers. CfDNA fragments are one of those molecular bits that are released into the bloodstream after rapid apoptosis or necrosis of the tumor cells in the cancer patients.

Our goal is to do comprehensive study between distinct types of glioma cancer tumors and cfDNA of the respective patients, to elucidate the scope of cfDNA in liquid biopsy technique for glioma diagnosis.

Methods

We collected 8 different glioma patient's tumor tissue and plasma samples and then isolated tumor DNA from glioma tumor tissue and circulating cell-free DNA(cfDNA) from the respective glioma patient's plasma. Isolated tumor DNA and cfDNA then deeply sequenced on Illumina HiSeq 2500 and then NGS data was analyzed to find out single nucleotide variants (SNVs) as well as structural variants on both cfDNA and tumor gDNA.

Results

We have successfully detected glioma specific mutations such as *IDH1*, *IDH2*, *PDGFRA*, *NOTCH1*, *PIK3R1* and *TP53*, from cfDNA isolated from the plasma of glioma patients and could relate this mutations to the different tumor grades of glioma. We are also studying the dynamics of these mutations in response to glioma drug treatment by collecting blood samples at different time intervals.

Discussion

This study may help in developing liquid biopsy technique for glioma tumor diagnosis and in its prognosis for monitoring the glioma treatment by non-invasive approach, and will eventually help physicians to decide the right treatment on appropriate time while bypassing the existing 'wait-and-see' approach of treatment monitoring.

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Bio-Markers and Cancer Theranostics

Predicting and affecting response to anti-cancer therapy based on pathway-level biomarkers

Rotem Ben-Hamo Deutsch^{1,2}

¹*Department of Molecular Cell Biology, Weizmann Institute of Science, Israel*

²*Cancer program, Broad Institute of Massachusetts Institute of Technology (MIT) and Harvard, USA*

The identification of robust, patient-specific, predictive biomarkers is a major obstacle in precision oncology. Their reproducibility is key requirement in the process of becoming clinically relevant. This crucial step is also needed to identify new personalized synergistic combination approaches. To address these problems and optimize patient-specific therapeutic strategies, we utilized pathway knowledge with drug sensitivity, RNAi, and CRISPR-Cas9 high-throughput screens of hundreds of cell lines from 9 tumor types. We found that pathway activity levels significantly predicted the essentiality of 15 genes that have the potential to act as therapeutic targets. Furthermore, we identified four signaling pathways that can act as strong and robust predictive biomarkers for BCL2-family, BRAF, and MEK inhibitors as well as microtubule inhibitors. Finally, this work demonstrates for the first time that pathway activity level modulation can sensitize NSCLC cells and human lung cancer tumors to microtubule inhibition therapy.

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Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment, Cancer Therapy: Advances in Drug Design and Delivery

Optimized PD-1 Variants as non-antibody Biologic Drug

Maayan Assor^{1,2}

¹Biotechnology, TEL HAI, Israel

²Biotechnology, Migal, Israel

Immune-checkpoint receptors are a set of signal transduction proteins that can stimulate or inhibit specific anti-tumor response. The `programmed cell death 1` (PD1) protein is an immune checkpoint receptor expressed on the surface of activated T cells where it is known to modulate T-cells response during events such as pregnancy, tissue allografts, and autoimmune diseases. In cancer, PD-1 ligands (PDL-1/2) are over expressed on tumor cells and bind to PD-1 leading an immune-suppression. In this way, tumor cells evade antitumor response and facilitate their survival. Several mAbs were approved for the treatment of various cancer by targeting PD-1. However, there are several disadvantages that are associated to antibody drugs, such as production cost, stability, high molecular weight, Immunogenicity and Toxicity. The present study aims to interfere with the interactions between PD-1 to PD-L1 by designing an optimized PD-1 protein variant, using directed-evolution. For that purpose, the use of *E. coli* as the host organism is robust and cost-effective choice. In addition, it enables large flexibility to address different structure-function aspects regarding the target protein. We used a simple ELISA-based screening system that given a pair of interacting proteins, enable the selection of variant with better solubility and affinity. The system is based on the expression of PD-1 variants library in *E.coli* conjugated to GST-tag and on the binding strength of the variants to the ligand, PDL-1. Within only two screening rounds, the most active variant showed a 5-fold higher affinity and 2.4-fold enhanced cellular activity as compared to the wild type protein. These results thus highlight the potential of those PD-1 variants as a small, non-antibody therapeutics for enhanced cancer immunotherapy and immune diagnostics.



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Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment, Cancer Metastasis, State-of-the-Art Methodologies in Research, Cancer Therapy: Advances in Drug Design and Delivery, Leukemia: From Stem Cell to Therapy

Targeting Hematopoietic Malignancies by using Small Molecule Compounds

Guy Biber

The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Israel

Hematopoietic malignancies are extremely challenging to treat due to their highly metastatic nature which allows them to spread throughout the human body. Therefore, hematopoietic cancers remain associated with high mortality rates in patients even with new treatment options. Current treatments for hematopoietic malignancies still mainly rely on chemo- and radiotherapies which are non-specific and accompanied by severe side effects. New immune-based and biological therapies have recently emerged in the clinic, however a large proportion of patients demonstrate primary and acquired resistance to these new treatments, or suffer from severe auto-immune side effects. Cancer cells depend on actin cytoskeleton rearrangement in order to carry out hallmark malignant cellular functions including cellular activation, proliferation, migration, and invasiveness. In this study, we performed in silico screening in order to find novel small molecule compounds (SMCs) that would downregulate cytoskeletal rearrangement in malignant hematopoietic cells. This protein-specific approach results in the inhibition of actin-dependent processes which regulate the cellular functions of hematopoietic malignant cells without affecting healthy hematopoietic or non-hematopoietic cells. This novel therapeutic approach might serve as an effective strategy to treat hematopoietic malignancies in a safe and specific manner.



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Metabolism and the DNA-Damage Response, Cancer Metastasis, Genomic Instability, Cancer Signaling and Cancer Secretome

The potential role of the autoimmune regulator (Aire) in tumorigenesis

Yael Gropper

Department of Immunology, The Weizmann Institute of Science, Israel

Autoimmune regulator (Aire) is a unique transcriptional regulator that induces promiscuous expression of thousands of tissue restricted antigens (TRAs) in medullary thymic epithelial cells (mTECs), a step critical for induction of immunological self-tolerance.

On the molecular level Aire doesn't function as a classical transcription factor, as it does not bind specific DNA sequences, instead it primarily localizes to transcription start sites (TSS) and/or enhancer regions. Results from our lab demonstrate that Aire utilizes a rather unconventional mechanism for transcriptional activation, which involves activation of the DNA damage response (Chuprin et al; submitted). Specifically, Aire induces formation of DNA damage foci in mTECs, characterized by phosphorylation of histone H2AX (e.g. γ H2AX), and physically localizes to these DNA damage foci. Given that DNA damage is one of the key mediators of tumorigenesis, and that previous studies showed that Aire is also expressed in several types of human cancers such as skin tumor keratinocytes (Hobbs et al; Nature Genetic. 2015), breast cancer (Bianchi et al; Cell Cycle. 2016) and osteosarcoma (Matsuda et al; Clinical & Experimental Metastasis. 2018), the above data raise a question whether Aire may possess oncogenic activity.

To test this hypothesis, we generated a transgenic mouse model with inducible and ubiquitous expression of Aire and a mutation (R172H) in the main tumor suppressor gene - p53. These transgenic mice were injected once a month from age of 8 weeks either with sub-lethal dose of doxycycline (4ug/ml) to induce Aire expression or a control vehicle and followed for ~30 weeks. Interestingly while several mice with ectopic-Aire expression developed sarcoma tumors in the bladder and abdomen at 30 weeks, all mice with no ectopic expression of Aire were tumor free. As accumulating and non-resolved DNA damage is one of the key triggers for cancer development, these results suggests that Aire may possess potential oncogene activity and that ectopic expression of Aire in tissues other than the thymus may lead to cancer development.

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Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment, Precision in Personalized Cancer Immunotherapy, Bioinformatics, Big Data and Cancer

Analysis of Broad Melanoma Proteomic Dataset Associates Prior Treatments with Response to Immunotherapy.

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Introduction: Melanoma is one of the most immunogenic tumors, due to its high mutational burden. The most prevalent mutation in melanoma is an activating mutation in BRAF, which can be targeted therapeutically by selective kinase inhibitors. Despite the high initial response to BRAF inhibitors, its durability is limited due to acquired resistance. The combination of tumor aggressiveness, high mortality, BRAF inhibitor resistance and high immunogenicity made melanoma a prime target for immunotherapy. Despite the great success of immunotherapy, still only a subset of patients responds to these treatments. Aiming to understand resistance mechanisms, we performed a proteomic analysis of responders and non-responders to immunotherapy, in order to associate the proteomic profiles with patient clinical parameters and BRAF status.

Materials and methods: The proteomic data is based on a former dataset of formalin-fixed paraffin-embedded (FFPE) tissues from 185 metastatic melanoma patients, before treatment with tumor infiltrating lymphocytes (TIL), anti-CTLA4 or anti-PD1. Quantitative proteomic analysis was performed by high-resolution LC-MS/MS analysis followed by computational analysis.

Results and discussion: Based on the clinical data, we examined the differences between tumor samples from patients with mutant BRAF which previously received treatment with RAF/MEK inhibitors, in comparison to samples with mutant BRAF from patients that did not receive this treatment and Wild type (WT) samples. We saw that the BRAF mutant untreated group has a significantly higher response to immunotherapy compared to WT or to prior treated samples. In sequential proteomic analysis, we found a significant elevation in mitochondria-related annotations, such as OXPHOS and TCA cycle pathways in the treated group. In the untreated group, we saw higher levels of proteins involved in cell movement and ECM, and in proteins connected to the innate and adaptive immune system.

Conclusion: Altogether, our results revealed an association between prior treatments and immunotherapy response and suggests mechanisms of treatment resistance.



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Israel Immunological Society

Lymphocyte Activation & Exhaustion

Homeostatic Mechanisms Controlling B Lymphopoiesis in Aging

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Generally, the number of peripheral B cells does not change significantly throughout life as generation of new B cells in the Bone Marrow (BM) is balanced by death of B cells in the periphery, a process referred to as cellular homeostasis. This balance between cell input and output within the B cell compartment is important for the integrity of the organism and for mounting an effective immune response. Yet, cellular homeostasis adapts to physiological changes. A good example for such adaptation is the dramatic changes in the B cell compartment that occur with aging, where B cell production declines and long-lived memory B cells accumulate in the periphery. However, these changes in B cell homeostasis are associated with reduced responsiveness to vaccination and increased morbidity.

So far, the mechanism by which cellular homeostasis is established is still unknown. Studies in our lab have demonstrated a feedback mechanism by which peripheral B cells suppress B lymphopoiesis in aging. In these studies we showed that removal of peripheral B cells reactivates B lymphopoiesis in the BM and rejuvenates the peripheral compartment in old mice and humans.

In the present study we aim to identify molecular mechanisms and/or molecules that mediate this cross-talk mechanism. To test this, we used an *in vitro* bone marrow culture system to grow B lineage cells from progenitors. We found that B cells purified from old mice suppressed B lymphopoiesis when cultured in direct contact or in transwell system. To support these findings in an *in vivo* setting, we transferred old splenic B cells into young hCD20Tg mice that were treated for B cell depletion. Bone marrow analysis for B lymphopoiesis revealed a significant suppression relative to control hCD20Tg mice that were injected with young splenic B cells. Furthermore, we found that B lymphopoiesis is effectively suppressed in the presence of sera from old mice. These findings confirm that old B cells inhibit B lymphopoiesis and that this inhibition is mediated by a secreted soluble factor. Applying proteomics approach to analyze soluble factors in serum we identified Insulin-like Growth Factor 1 (IGF-1) as a potential molecule whose reduced level in aging may mediate this suppression effect. Preliminary studies supported this possibility.

Collectively, our results suggest that in aged individuals B lymphopoiesis is subjected to homeostatic feedback mechanisms imposed by mature B cells in the peripheral compartment.

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Lymphocyte Activation & Exhaustion

A Causal Role Of Dicer During Activation-Induced Cell Death

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During B cells development in the bone marrow, autoreactive cells encountering receptor-self-antigen interactions are eliminated by an active form of apoptosis called activation-induced cell death (AICD). Recent studies in our lab have been focused on the role of microRNAs (miRNAs) in regulating AICD. A global approach to study miRNA-regulated mechanisms is to ablate or suppress expression of Dicer ribonuclease, which is a central enzyme in the processing of miRNAs, double strand RNA (dsRNA) and other small RNAs. To do so we used shRNA to knockdown Dicer expression in B cells and found that loss of Dicer suppresses biogenesis of miRNAs and enhances sensitivity to AICD imposed by anti-B cell receptor stimulation. Moreover, in a conditional mouse model enabling inducible ablation of Dicer ($Dicer^{flox/flox}$ Mx-Cre) we show that resistance to AICD of mature splenic B cells is lost upon ablation of Dicer. Further, using wt and Dicer-deficient HEK cells we show that apoptosis induced by TNF α is enhanced in the absence of Dicer. These studies suggest that through controlling biogenesis of miRNAs Dicer plays a central regulator in determination of cell fate.

Yet, in recent years cleavage of Dicer was suggested to play an active direct role in the process of apoptosis. In agreement with this, we find that Dicer is specifically cleaved in B lymphocytes undergoing AICD and that this cleavage is correlated with elevation in active caspase 3. Our plans are to test whether cleaved Dicer functions as DNAs and to study whether cleavage-resistant form of Dicer will enhance resistance to AICD. We think this study may uncover novel mechanism for activation-induced apoptosis.

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CAR and CTL Therapy in Cancer, Cancer Therapy: Advances in Drug Design and Delivery

A Novel Universal CAR T Cells Immunotherapy For The Treatment Of Solid Tumors

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Recently, remarkable clinical responses were observed in patients treated with engineered T cells, establishing immunotherapy as one of the most promising clinical approaches for cancer. Nonetheless, for unknown reasons, therapies using engineered T cells are currently effective only with hematological malignancies, and treated patient often suffer a relapse. Furthermore, the lack of identified tumor-specific antigens limits the clinical use of this therapy and often results in on-target, off-site toxicity. Therefore, an urgent need remains to develop treatments capable of eradicating solid tumors, which feature a higher safety profile and do not depend exclusively on the host T-cell repertoire.

In an unpublished study, we have recently discovered a novel subset of CD4⁺ T cells that directly kill tumor cells coated with antibodies. These cells express the high affinity Fcγ receptor (FcγRI), which enables them to bind coated tumor cells and secrete lytic granules resulting in remarkable tumor lysis. Recognition of the tumor also involves engagement of their T cell receptor (TCR), thus providing another layer of specificity. Importantly, we were able to recapitulate the cytotoxic capacities of that population in conventional CD8⁺ T cells by co-infecting them with FcγRI and the FcR common gamma chain. Indeed, these FcγRI engineered T cells exerted remarkable killing capabilities of solid tumors, upon incubation with antibodies. Unlike chimeric-antigen receptor T (CAR-T) cells, which require chaining the targeting receptor with each tumor, this technology can be applied to treat a wide range of cancers by using clinically-approved antibodies, or antibodies with a high safety profile that have failed in phase III clinical trials.

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Host-Pathogen Interaction

Transcriptomics Of Monocyte-Derived Satiated Macrophages

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Introduction: Monocyte-derived macrophages are readily differentiating cells that adapt their gene expression profile to environmental cues and functional needs. Engulfment of apoptotic leukocytes (efferocytosis) by macrophages during the resolution of inflammation is essential for tissue homeostasis and results in macrophage reprogramming/immune-silencing. During the resolution of inflammation, monocytes initially differentiate into reparative phagocytic macrophages and later into pro-resolving satiased macrophages that produce high levels of IFN β to boost resolutive events.

Materials and methods: Transcriptomic analysis of phagocytic and satiased macrophages was performed to reveal their unique gene expression in comparison to resident peritoneal macrophages (RPM) and monocytes, and identify gene clusters that distinguish between these phenotypes and probably confer distinct functions to these resolution-associated myeloid cells.

Results and discussion: Our analysis reveals phagocytic and satiased resolution phase macrophages express similar gene signatures that distinct them from other myeloid cells. Moreover, we confirm these macrophages express closer transcriptomes to monocytes than to RPM. A comparison between these subsets indicated satiased macrophages down-regulate gene clusters associated with excessive tissue repair and fibrosis, ROS and NO synthesis, glycolysis, and blood vessel morphogenesis. On the other hand, satiased macrophages enhance the expression of genes associated with migration, oxidative phosphorylation, and mitochondrial fission as well as anti-viral responses when compared to phagocytic macrophages. Notably, conversion from phagocytic to satiased macrophages is associated with a reduction in the expression of extracellular matrix constituents that were demonstrated to be associated with Idiopathic Pulmonary Fibrosis.

Conclusion: Macrophage satiased during the resolution of inflammation seems to bring about a transcriptomic transition that resists tissue fibrosis and oxidative damage while promoting the restoration of tissue homeostasis to complete the resolution of inflammation.

Visualizing the Immune Response

The function of iASPP in Cardiomyocytes

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Introduction: Dilated cardiomyopathy (DCM) is a life-threatening disorder whose genetic basis is heterogeneous and mostly unknown. In our recent published work in collaboration with Prof. Tzipora C. Falik-Zaccai, five Arab-Christian-infants, ages 4-30 months from four families were diagnosed with DCM associated with mild skin, teeth and hair abnormalities. All passed away before age 3. A homozygous sequence variation in *PPP1R13L* encoding the iASPP protein, was identified in three infants, and heterozygous in the mother of the other two. The patients' fibroblasts and *PPP1R13L*-knocked down human fibroblasts presented higher expression levels of pro-inflammatory cytokine genes in response to Lipopolysaccharide (LPS), as well as *ppp1r13l*-knocked down murine cardiomyocytes and hearts of *ppp1r13l*-deficient mice. The hypersensitivity to LPS was NF- κ B-dependent, and NF- κ B-inducible binding activity to promoters of pro-inflammatory cytokine genes was elevated in patients' fibroblasts. RNA-sequencing of *ppp1r13l*-knocked down murine cardiomyocytes and of hearts derived from different stages of DCM development in *ppp1r13l*-deficient (*Wa3*) mice revealed the crucial role of iASPP in dampening cardiac inflammatory response. Altogether, these results demonstrated *PPP1R13L* as the gene underlying a novel autosomal recessive cardiocutaneous syndrome (CCS) in humans, and strongly suggest that the fatal DCM during infancy is a consequence of failure to regulate transcriptional pathways necessary for tuning cardiac threshold response to common inflammatory stressors. However, the mechanisms underlying the function of iASPP in regulating NF- κ B activity and cardiac response are unknown.

Material and method: Heart tissues were purified, and cardiomyocytes were harvested from homozygous BALB/c *wa3* mice and their littermate WT mice and were processed for confocal microscopy imaging and molecular assays.

Results and discussion: Our preliminary results suggest that iASPP regulates cellular translocations of NF- κ B.

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Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment

Antibody-mediated Recruitment of Viral-specific Effector Cells via Recombinant Antibody-MHC T cells Engagers

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Our lab has developed a novel T cell engager (TCE) format whereby a Recombinant antibody fragment specific for a tumor-associated antigen of choice is genetically fused to HLA-A2/peptide complex that presents a viral T cell epitope. The HLA-A2/peptide complex is designed to engage the TCR of a CD8⁺ T cell while the scFv or Fab redirects specificity towards a tumor associated antigen (TAA), thus recruiting potent antiviral memory CTLs to attack tumor cells. We used the highly immunogenic cytomegalovirus (CMV) pp65-derived peptide as a target peptide for CTLs, taking into consideration the CMV prevalence of seropositive humans. Mesothelin was used as a TAA.

We monitored the binding of TCEs to target cells demonstrating that their binding is dependent exclusively on the interaction of the targeting domain (scFv/Fab) of the fusion molecule with mesothelin, and is dose-dependent. Potency of TCEs was determined *in vitro* using co-cultures of CMV-specific CTLs and mesothelin-expressing human tumor cell lines. The TCEs specifically and potently mediated the killing of mesothelin-positive cells in TCE-dose-dependent matter. Mesothelin-negative control cells were not affected. Moreover, a significant increase in CD25 expression and dose-dependent secretion of IL2 and IFN γ were observed in CTLs exposed to mesothelin-positive cells. *In-vivo* studies using mice models are planned.

The results represent a powerful new approach for immunotherapy. The MHC-based TCEs can mediate specific and efficient recruitment of viral-specific CTLs to kill tumor cells, adding an approach by which specific population of T cells can be engaged and recruited to the tumor site in a controllable manner in contrast to current CD3-based T cells engagers that cannot selectively target the specificity of interest. Such treatments can be very potent but with decreased toxicities involved, such as cytokine storm.



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Visualizing the Immune Response, Immuno-Oncology and the Microbiome

Deciphering the role of the gut microbiome in the development of chemotherapy-induced mucositis, using a novel gut organ culture system

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The main side effect of anti-cancer chemotherapy is the development of mucositis, a severe inflammation of the mucosal epithelial lining throughout the GI tract, which affects 80% of the patients undergoing high-dose chemotherapy. The molecular mechanisms underlying mucositis development and the role of microbiome are largely unknown. We developed a novel experimental system for gut organ cultures that provides absolute control over the drug dosage, luminal flow rate and exposure time (Yissachar *et al.*, *Cell*. 2017). In our laboratory, we utilize the versatility of this unique system to dissect the immediate-early intestinal responses to chemotherapy in the presence of endogenous microbiota, or chemotherapy-disrupted microbiota, which might be associated with long-term development of gut inflammation. We used transcriptional profiling of whole gut tissues, and their sub-cellular components, following *ex-vivo* introduction of low and high dose of cytarabine and we identified a number of significant changes in neuronal factors and immune pathways that must be further investigated. Moreover, intestinal permeability increases after chemotherapy treatment, and has been shown to be one of the hallmarks of the third and fourth phases of mucositis (Sonis *et al.*, *Nat Rev Cancer*. 2004). Therefore, we took advantage of the gut organ culture to develop an innovative on chip permeability assay, that allows us to test the effect of chemotherapy and chemotherapy-derived microbiome on gut barrier function. Interestingly we found that certain chemotherapy-disrupted microbiota induced an increase in gut permeability, while others didn't have a significant effect. Those results were confirmed by a well established *in vivo* permeability assay, using fluorescent-labeled dextrans. Characterizing the bacterial populations that might directly affect gut permeability will allow us to narrow down the molecular mechanism associated with this phenomenon and promote new therapeutic approaches to restore the balance between gut bacteria and the immune system during chemotherapy, ameliorate intestinal inflammation, and boost anti-cancer therapy.

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Lymphocyte Activation & Exhaustion

Dual function of Polycomb group proteins in T-helper (Th) cells

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Aim & Background: The immune system distinguishes between self and non-self but also between different types of non-self, such as bacteria, viruses and worms. Th cells have a fundamental role in that challenge. Following antigen recognition, naive Th cells can differentiate toward one of the several effector lineages, each expressing a distinctive transcriptional profile of cytokines and other lineage specific genes, which eventually instruct the strategy of the immune response. In our lab, we are interested in understanding the mechanisms underlying differentiation and stimulation of these cells. My work is especially focused on exploring in a genome wide manner the binding activity of- and the epigenetic regulation by the polycomb group (PcG) proteins such as the Ezh2 in differentiated Th cells.

Methods: We performed ChIP-Seq and RNA-Seq of *in vitro* differentiated Th1 and Th2 cells derived from normal and Ezh2-conditionally deficient mice.

Results& Conclusion: We demonstrated that Ezh2 has a dual function as a positive and a negative transcriptional regulator in Th cells; Ezh2 is required for robust expression of the signature transcriptional programs of differentiated Th1 and Th2 cells. We further revealed that Ezh2 possesses a differentiation- and stimulation-dependent binding activity in Th cells, and its binding is correlated with Th1 and Th2 specific transcription factor motifs. We found that Ezh2 is associated also with nascent RNA, and we currently studying the potential

Cancer Metastasis

Non-melanoma skin tumors: Rapid prediction of invasiveness using mechanobiology

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Introduction

Skin cancer is the most common human malignancy, more people have had skin cancer than all other cancers combined. The main cause of cancer morbidity, including in skin cancers, is formation of metastases and recurrence; those require cell invasion into surrounding tissue. Invasive subpopulations of cells mechanically interact with their environment, thus we have developed a rapid (2hr) mechanobiology-based assay to quantify their metastatic risk. We have shown that the mechanically invasive subset of cancer cells, seeded on an impenetrable, physiological-stiffness, synthetic gel, will forcefully push into and indent the gel; non-invasive, benign or normal cells do not considerably indent. We have recently shown that larger mechanically invasive subsets agree with increased *in vitro* metastatic potential and also with grim clinical prognosis, by the histopathology and patient outcomes, e.g. in pancreatic cancer. Here, we demonstrate ability to rapidly identify non-melanoma invasive skin cancer samples.

Materials and Methods

Suspected non-melanoma skin tumor were excised and a small non-edge sample was taken for invasiveness determination. Enzymatic degradation was used to extract cells, which are then seeded on polyacrylamide gels (1-2 kPa stiffness). After 1hr cell-attachment the percentage of indenting cells and their attained depths on the gel were determined by microscopy. The bulk of the tumor-samples were sent for detailed histopathological examination, including tumor-sizing, maximal depth, Clark's level, presence of desmoplasia/acantholysis, special histopathologic subtypes (basosquamous etc.), and mitotic index. Clinical follow-up of patients over 6 months showed no recurrence.

Results and Discussion

Non-invasive basal and squamous cell carcinomas (i.e. BCC and SCC) exhibit below 18% indenting cells, as compared to 30% invasive SCC and desmoplastic (fibrotic) BCC samples; depths were 2-6mm, control-scar was similar to non-invasive. Our mechanical invasiveness measure directly agrees with the clinical histopathology. Thus, using small amounts of cells (100k/ml), we can separate invasive/non-invasive non-melanoma with high sensitivity.

Conclusions

Mechanical invasiveness can rapidly (~2hr) distinguish BCC and SCC and reveal secondary processes e.g. desmoplasia (neoplasm-associated fibrosis) and risk of local-invasiveness or metastasis. Combined with histopathology it can provide a rapid diagnosis and prediction of clinical metastatic risk.

Microenvironment and Immuno-Oncology

The role of stromal cells in resistance to cetuximab treatment in head and neck cancer

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Introduction: Resistance to cetuximab, an antibody that blocks the epithelial growth factor receptor (EGFR), is a significant problem in the treatment of head and neck cancer (HNC) patients. Cetuximab is effective in a fraction of HNC patients, who eventually all develop resistance that leads to disease relapse. The resistance to cetuximab is known to be mediated, in part, by cells surrounding the tumor cells, stromal cells. However, the mechanisms underlying the migration, accumulation, and differentiation of these stromal cells are still unclear.

Material and method: We used HNC patient-derived xenografts (PDXs) models to investigate the interactions between tumor cells and their stroma. Five different PDXs were implanted in NOD.SCID mice and the response to cetuximab was measured. Molecular and pathological analyses of tumors using RNA sequencing (for three PDXs) and immunohistochemistry (IHC) (for all PDXs), respectively, were performed to gain insight on the changes that occur in the tumor and their stroma during cetuximab treatment. Specifically, staining of fibroblasts (αSMA), endothelial cells (CD31), tumor cells (KRT14), epithelial to mesenchymal markers (E-cadherin and Vimentin), and proliferation (KI67) were tested in PDXs treated with vehicle or cetuximab.

Results and discussion: All five PDXs responded to cetuximab indicated by a significant tumor growth inhibition. However, two of the five PDXs started to regrow and progress after a stable disease of 20 days, while in the other three, high sensitivity to cetuximab, we detected tumor shrinkage and even tumor elimination. RNA sequencing of 2 sensitive PDXs and 1 progressed PDX reveals that in the stromal compartment of the progressed PDX TGF beta signaling pathway was upregulated after cetuximab treatment, while in the sensitive PDXs, TGF beta pathway was downregulated. Furthermore, in the two sensitive PDXs, an upregulation of natural killer (NK) cell mediated cytotoxicity signature was detected. In the human compartment, the expression of several chemokines were altered after treatment, among them CXCL1 was the only chemokine that was highly expressed in the progressed tumor and its expression was altered in sensitive tumors after treatment with cetuximab. In addition, using IHC we observed a unique rearrangement of the cancer-associated fibroblast (CAFs) that surround the proliferating tumor cells after cetuximab treatment, while in the untreated tumors, CAFs were located between tumor cells. This phenomenon was highly prominent in the progressed PDXs.

Conclusion: Cetuximab treatment affects the plasticity and heterogeneity of tumor cells and their microenvironment (TME). Progression to cetuximab is associated with high levels of CXCL1 and upregulation of TGF beta signaling by the TME. In contrast, sensitivity to cetuximab is associated with low CXCL1 expression by tumor cells, downregulation of TGF-beta and upregulation of NK cells signatures.

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Inflammation and Immunity – Friends or Foes?

The anti-inflammatory properties of marine microalgae in LPS-induced macrophages

Or Rozen^{1,2}

Inflammatory diseases such as inflammatory bowel disease (IBD) have become one of the leading causes of health issue throughout the world, having a considerable influence on healthcare costs. Moreover, patients with IBD are at higher risk for developing colorectal cancer than the general population. Until date, there is no known cure for IBD. Existing treatments are not effective and emphasize the need for a new biologic approach to improve IBD symptoms. Inflammation is mediated by cytokines produced by stimulated immune cells such as macrophages. There are both pro-inflammatory cytokines such as Tumor necrosis factor- α (TNF α) and anti-inflammatory cytokines such as Interleukin (IL)-10. Targeting cytokines often reduce the disease processes by influencing immune cells, tissue healing and inflammatory aspects of the diseases.

With the emerging developments in natural product, notable success has been achieved in discovering natural products and their synthetic structural analogs with anti-inflammatory activity. Marine microalgae have been identified as an underexplored reservoir of unique anti-inflammatory compounds. These include polyphenols, sulfated polysaccharides, terpenes, fatty acids and proteins. Consumption of these marine algae could provide defense against the pathophysiology of many chronic inflammatory diseases such as IBD. With further investigation, microalgae have the potential to be used as therapeutics with profound anti-inflammatory activity with reduced side effects.

In this study, the anti-inflammatory potential of microalgae was assessed in lipopolysaccharide (LPS)-stimulated murine RAW264.7 macrophages. Extraction from the microalgae was prepared using solid-liquid of ethyl acetate or Ethanol:H₂O (70:30%). We have found that the ethyl acetate extract was the most effective in reducing inflammation. From this extract, we isolated and identified the active fraction using a series of chromatographic steps and various instrumentations analytical methods.

The results showed that pretreatment of RAW 264.7 cells with the crude extract from microalgae significantly inhibited the secretion of TNF α by 50-60% (p 0.001). Furthermore, the fractions extracts effectively inhibited the LPS-induced TNF α similar to the effect obtained by the crude extract. Interestingly, some fraction contained omega 3 and omega 7 that are known to have anti-inflammatory properties.

Our findings suggest that the microalgae might become a natural source for new anti-inflammatory treatment and lead to a promising route of treatment for chronic inflammation-linked diseases. Further research will be required in order to understand the mechanism of action of these compounds.

Inflammation and Immunity – Friends or Foes?, Metabolism and the DNA-Damage Response

Glucose dependent insulintrophic polypeptide (GIP) immune cell interactions control body weight during obesity via modulation of energy expenditure

Irena Efimova^{1,2}

Incretin peptides, mainly glucagon like peptide-1 (GLP-1) and glucose-dependent insulintropic peptide (GIP) are gut derived hormones, which are secreted upon cues from ingested food and regulate glucose concentration, eating behavior and energy expenditure to maintain body weight. As a result, a myriad of therapeutics for metabolic diseases based on the actions of incretins, particularly GLP-1, are currently under clinical use and development. Nevertheless, the biology of GIP in the immune system remains overlooked, plagued by contradictions and unanswered questions. Our lab has previously shown that the long-acting GIP analogue [d-Ala²]GIP reduces innate and adaptive inflammatory responses in the epididymal adipose tissue (epiWAT) of high-fat diet (HFD) fed mice, but the direct immunometabolic roles of GIP remained unknown. Here, we show that mice with GIP receptor (GIPR)-deficiency targeted to immune cells display increased weight gain, insulin resistance, hepatic steatosis, myelopoiesis, impaired energy expenditure and impaired white adipose tissue (WAT) beiging under HFD. These effects were mediated by the unrestrained activity of the alarmin S100A8/A9 in GIPR-deficient myeloid immune cells. Recent studies have outlined a pivotal role for type 2 immune cell networks in maintaining metabolic homeostasis and energy balance. In alignment with their impaired energy expenditure, HFD fed mice with GIPR-deficiency in immune cells exhibited significant alterations in WAT type 2 inflammatory circuits, and co-deletion of S100A8/A9 was sufficient to restore it. Finally, GIP augmentation facilitated inguinal WAT beiging and induced the expression of the beiging supportive type 2 cytokines in lean mice under cold conditions. Collectively, our results identify an immune–GIPR–S100A8/A9 signaling axis coupling nutrient signals to the control of inflammation and adaptive thermogenesis.

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CAR and CTL Therapy in Cancer

A TIGIT-based chimeric co-stimulatory switch receptor improves T-cell anti-tumor function

Shiran Hoogi

Tumors can employ different mechanisms to evade immune surveillance and function. Overexpression of co-inhibitory ligands that bind to checkpoint molecules on the surface of T-cells can greatly impair the function of latter. TIGIT (T cell immunoreceptor with Ig and ITIM domains) is such a co-inhibitory receptor expressed by T and NK cells which, upon binding to its ligand (e.g., CD155), can diminish cytokine production and effector function. Additionally, the absence of positive co-stimulation at the tumor site can further dampen T-cell response. As T-cell genetic engineering has become clinically-relevant in the recent years, we devised herein a strategy aimed at enhancing T-cell anti-tumor function by diverting T-cell coinhibitory signals into positive ones using a chimeric costimulatory switch receptor (CSR) composed of the TIGIT exodomain fused to the signaling domain of CD28.

After selecting an optimized TIGIT-28 CSR, we co-transduced it along with tumor-specific TCR or CAR into human T-cells. TIGIT-28-equipped T-cells exhibited enhanced cytokine secretion and upregulation of activation markers upon co-culture with tumor cells. TIGIT-28 enhancing capability was also demonstrated in an original *in vitro* model of T-cell exhaustion we developed. Finally, we tested the function of this molecule in the context of a xenograft model of established human melanoma tumors and showed that TIGIT-28-engineered human T-cells demonstrated superior anti-tumor function. Overall, we propose that TIGIT-based CSR can substantially enhance engineered T-cells function and thus lead to the improvement of T-cell immunotherapy.

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Inflammation and Immunity – Friends or Foes?

PD-L1 and PD-L2 Regulation in triple negative breast cancer

Tamir Baram¹

Triple-negative breast cancer (TNBC) is a subtype of breast cancer which is very aggressive, highly metastatic and often a recurrent type of disease. Conventional chemotherapy is currently the only, yet not always successful, systemic treatment option available for these patients. Due to their relatively high mutation rate, TNBC tumors are expected to be more immunogenic than other breast cancer subtypes. However, the ability of immune infiltrates to exert an anti-tumor immunological response is often suppressed by inhibitory molecules expressed by the tumor cells, such as PD-L1/PD-L2.

Although PD-L1-targeted therapy proved to be successful in treating several cancer types, not much is known about its regulation in TNBC. Here, we investigated the control of PD-L1/PD-L2 by pro-inflammatory mediators that prevail in TNBC and are connected to poor clinical outcome. Our results indicate that TNBC, but not luminal-A breast cancer cells (that have a better prognosis) express endogenous levels of PD-L1 and PD-L2. Approximately 50-70% of the cells express both molecules. Joint stimulation by two pro-inflammatory cytokines has led to significant up-regulation of PD-L1 and PD-L2 by TNBC cells, and the cytokines acted in a cooperative manner. To follow up on induction of NF- κ B and STAT1 activation in the cells, using CRISPR-cas9 we found that the signals transmitted by p65 are channeled to STAT1 activation, which is the main regulator of PD-L1 and PD-L2 up-regulation.

Our findings indicate that pro-inflammatory signals induce the expression of immune checkpoints by TNBC cells, possibly potentiating their ability to escape anti-tumor immune activities. Our initial molecular analyses on the mechanisms up-regulating PD-L1/PD-L2 expression are now being followed by additional studies of the implications of these findings in TNBC tumor progression.

Lymphocyte Activation & Exhaustion

A miRNA switch in regulation of PI3K activity controls B cell maturation and survival

Ofer Harel

Introduction:

PI3K-AKT is the main pathway which controls B-cell development and survival. During development in the bone marrow, the regulation of PI3K is dependent on miR17-92 cluster through PTEN phosphatase. However, with maturation expression of miR17-92 decreases, while PI3K activity increases. Hence, we hypothesize that B cell maturation is accompanied with miRNA switch controlling of PI3K. We show that regulation of PI3K switches from miR17-92 in developing cells to miR29 in mature cells, and this is critical for B cell maturation and survival.

Material and methods:

Mice: Dicer F/F and PTEN F/F mice crossed with Mx-CRE transgenic mice.

Apoptosis quantification: cells were stained for 7AAD.

Luciferase assay: used to quantify binding of miR29 to PTEN 3'UTR.

qPCR analysis: using Taqman probes.

Results and discussion:

- Using mice where Dicer can be inducibly ablated, we show that miRNAs are critical to regulate PI3K activity and mature B cell survival. Loss of one PTEN allele restores PI3K activity in Dicer ablated cells and promotes their survival.
- Analysis of miRNA in B cells suggests miR-29 is preferentially expressed in mature B cells. We validated this by qPCR of B cell subsets and demonstrated its binding to PTEN 3'UTR using luciferase.
- To test the interrelationship between miR17-92 and miR29, we applied overexpression and knockdown in-vitro. Splenic B cells treated with miR29a antisense oligos expressed decreased levels of miR19b, whereas overexpression of miR29a in WEHI cells resulted in a decrease in miR17 and miR19. These two findings indicate a control loop between miR29 and miR17-92 cluster, whose mechanism is still unknown.
- Using miR29 deficient mice we are able to show that loss of miR29 results in impaired maturation of splenic B cells, increased apoptosis, increased PTEN levels and reduced PI3K activity.

Conclusion: These findings suggest that PI3K regulation by miRNAs is developmentally regulated and guided by a specific switch from miR17-92 during development to miR29 in mature. Since PI3K activity is critical for B cell survival, we speculate that this miRNA switch may have major role in B cell pathologies. Further studies exploring this are now conducted in the lab.

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Israel Immunological Society

Microenvironment and Immuno-Oncology, Cancer Metastasis

Uncovering the role of tumor-derived LCN2 (NGAL), a systemic mediator of astrogliosis, in facilitating melanoma brain metastasis

OMER ADLER¹

Introduction: Malignant melanoma is the deadliest skin cancer with rising incidence worldwide. Melanoma frequently metastasizes to the lungs, bone, liver and brain. Although the development of targeted therapies and immune checkpoint inhibitors has dramatically improved patient overall survival, brain metastases still pose an unmet clinical challenge. The microenvironment plays a crucial role in facilitating metastasis by promoting survival, colonization and proliferation of disseminated tumor cells to distant organs. Therefore, understanding the early changes in the brain microenvironment that precede metastasis is of great clinical importance. Astrocytes are key components of the brain microenvironment. Neuroinflammation is a prominent feature of reactive astrocytes, characterized by the release of pro-inflammatory cytokines and chemokines, increased blood-brain barrier permeability and immune cell infiltration, and is also a hallmark of brain metastatic niche formation. Current models of metastasis indicate that specific organs are predisposed for metastases formation following active modifications by both the primary tumor and reactive stromal cells at the metastatic site.

Lipocalin 2 (LCN2), is a 25 kDa secreted glycoprotein known for sequestering iron as a physiological response of fighting bacterial infections. LCN2 was also shown to be a pro-inflammatory factor, overexpressed in various malignancies. More importantly, LCN2 is a known activator of astrocytes, implicated in numerous CNS pathologies. However, the role of LCN2 in melanoma is largely unexplored.

M&M: Utilizing a transplantable model of spontaneous melanoma brain metastasis in immune-competent mice, recently established in our lab, we compare the metastatic capacity of brain-tropic cell lines to that of the parental cell line. We analyze plasma levels of LCN2 in both human and mouse samples using ELISA, as well as expression levels using qRT-PCR.

Results: We show that plasma levels of LCN2 increase in melanoma-bearing mice and in human patients with melanoma brain metastasis. Moreover, LCN2 is overexpressed in brain-tropic melanoma cell lines both *in vitro* and in primary tumors of injected mice *in vivo*, suggesting that melanoma-derived LCN2 facilitates brain tropism and metastases formation by activating astrocytes and instigating neuroinflammation.

Conclusion: Uncovering this systemic signaling pathway may be beneficial for the design of novel therapeutic approaches to prevent early changes in the brain microenvironment, rendering it less susceptible to metastasis.

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Microenvironment and Immuno-Oncology, Cancer Metastasis

CysC and GM-CSF Participate in Shaping the Malignancy Phenotype of Melanoma Cells in the Brain

Orit Sagi-Assif¹

Introduction: Melanoma brain metastasis (MBM) is common in patients with malignant melanoma. We have shown that *in vitro* stroke-like conditions as well as treatment with melanoma-conditioned medium (MCM) increased Cystatin C (CysC) and Granulocyte-macrophages colony-stimulating factor (GM-CSF) secretion by brain microenvironmental cells. The present study is aimed to elucidate the role of CysC and GM-CSF in MBM progression.

Methods: Infection with virions containing shCysC and shGM-CSF was used to downregulate the expression of these genes. Wound healing and transmigration assays were performed to determine the effect of CysC and GM-CSF on cell migration or BEC penetration. NGS mice were subjected to ischemic stroke. MBM were generated in mice 7 days after stroke. Non-stroked mice were inoculated similarly. Control group was neither stroked nor inoculated. IC inoculation of melanoma cells into nude mice and human-specific RT-qPCR were performed to determine GM-CSF effect on MBM formation.

Results and Discussion: Microglia-conditioned medium (MGCM) upregulated CysC secretion from melanoma cells. Reciprocally, MCM upregulated CysC secretion from microglia. Whereas CysC enhanced melanoma migration and penetration through a layer of brain endothelial cells (BEC), it inhibited the migration of microglia cells toward melanoma cells. CysC promoted the formation of melanoma 3D structures. IHC revealed increased expression levels of CysC in the brains of mice bearing xenografted human MBM compared to the brains of control mice. Increased expression levels of CysC were detected in the regenerating brains of mice after stroke. Post-stroke brains with MBM showed an even stronger expression of CysC. Put together these results demonstrated that CysC may promote but also inhibit MBM formation. MCM also stimulated GM-CSF secretion from BEC and astrocytes. GM-CSF was found to enhance melanoma transendothelial migration, effecting only the BEC and not melanoma cells. MGCM inhibited GM-CSF secretion from melanoma cells. TNF- α and IL-1A, secreted by microglia in its pro-inflammatory (anti-tumor) state, enhanced GM-CSF secretion by melanoma. Upregulation of GM-CSF secretion by melanoma cells was inhibited when melanoma cells were treated with TNF- α or IL-1 in combination with MGCM, indicating that microglia-derived factors either inhibit or enhance GM-CSF secretion by melanoma. All these results demonstrate the complexity of melanoma-microglia interactions. IC inoculation of melanoma cells knocked down to GM-CSF resulted in an increase in MBM formation compared to control cells, showing that despite increasing BEC penetration, GM-CSF is an anti-metastatic factor in the context of MBM.

Conclusion: Cells and molecules in the brain microenvironment exert yin yang functions either supporting or inhibiting tumor progression. The overall balance between the two determines the metastatic fate.

Microenvironment and Immuno-Oncology

Molecular Events Driving Luminal-A Breast Cancer Progression: Interactions Of Tumor Cells With An Integrative Tumor Microenvironment Stimulus

Hagar Etzion¹

Luminal-A is the most prevalent breast cancer subtype. Although most Luminal-A patients initially have a favorable outcome, some of them become resistant to endocrine therapy and develop metastases. Our previous studies indicate that the tumor microenvironment (TME) is a major driver of this violent phenotype. To further investigate the effects of the TME, we have developed a stimulus of Luminal-A cells by factors that integrate three arms of the TME (called herein “**TME-Stimulation**”): hormonal, inflammatory and growth-stimulating. Following three days of stimulation by estrogen (representative of the hormonal arm), TNF α (pro-inflammatory) and EGF (growth-stimulating), human Luminal-A cells acquired a metastatic phenotype, evident both by *in-vitro* assays and *in-vivo* studies.

In addition to the invasive characteristics acquired by the Luminal-A breast tumor cells following TME-Stimulation, this stimulus has led to enrichment of CD44⁺/CD24^{low/-} cells in the population. While in unstimulated cells this sub-population was negligent, in TME-stimulated cells it increased significantly. CD44⁺/CD24^{low/-} are markers of cancer stem cells (CSCs) in breast cancer, having the potential to generate a heterogeneous population of tumor cells, increase therapy resistance and have an EMT-related phenotype; they are considered to constitute the tumor sub-population that initiates metastases, and therefore are also called tumor-initiating cells.

In our previous studies we have shown that the sub-population CD44⁺/CD24^{low/-} cells, that was enriched by TME-Stimulation of Luminal-A cells, indeed carried typical characteristics of CSCs (observations were made with two human Luminal-A cells, MCF-7 and T47D). We also demonstrated that this sub-population stood in the basis of metastasis formation *in-vivo*. Currently, our goal is to identify the molecular mechanisms through which TME-Stimulation leads to reprogramming of Luminal-A cells to CSCs. Understanding these processes will allow us to characterize key targets that may be relevant for therapy of Luminal-A patients.

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Microenvironment and Immuno-Oncology

Pro-Inflammatory Control Of The Phenotype And Functional Properties Of Triple Negative Breast Cancer Cells

Dina Morein

School of Molecular Cell Biology and Biotechnology, Tel Aviv University, Israel

The triple negative subtype of breast cancer (TNBC) is a most aggressive disease type, demonstrating high relapse rate and poor survival. This subtype is defined by the lack of expression of three receptors: estrogen receptors, progesterone receptors and epidermal growth factor receptor 2 (HER2); thus, receptor-targeting therapies cannot be applied in this type of disease and chemotherapy is the only treatment option possible for TNBC patients.

Breast tumors include a complex tumor microenvironment (TME) that consists of different cell types and soluble factors, and has an important role in determining the aggressiveness and the progression of the disease. TNF α and IL-1 β are enriched in the TME of TNBC tumors, where they promote cancer progression and are related to poor prognosis. In many patients, the tumor cells are exposed continuously to pro-inflammatory cytokines from the time of malignant transformation and on, throughout disease progression.

In our study we stimulated human TNBC cells with the pro-inflammatory cytokines TNF α and IL-1 β for prolonged periods, and determined cytokine impacts on TNBC tumor-promoting characteristics via live cell imaging, XTT, chemotaxis and RNAseq. We found that in response to the cytokines, human TNBC cells acquired morphological changes that are connected to increased migration and higher motility. These traits were accompanied by extensive changes in gene expression and modified metabolic phenotypes. We observed increased expression of genes involved in oxidative phosphorylation in the mitochondria, and also detected those elevated abilities in functional assays. A screen assay that we have developed is now being used as a basis for the identification of inhibitors that will prevent the cytokine-induced changes in TNBC cells. Those molecules that will be able to interfere with the process may lead to development of new therapies, and also will allow us to track the molecular mechanisms that are related to long-term cytokine stimulation.

Overall, we identify in our studies inflammation-driven effects on the TNBC characteristics that may promote their aggressiveness and metastatic potential.



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Microenvironment and Immuno-Oncology, Bioinformatics, Big Data and Cancer

Inflammation-Driven Plasticity of Stromal Cells in Luminal-A Breast Cancer

Linor Rubinstein¹

Luminal-A is the most common subtype of breast cancer, strongly affected by factors of the tumor microenvironment (TME). Between others, the TME of breast tumors is populated by stromal cells. In most research systems, stromal cells have been shown to promote angiogenesis, tumor growth and metastasis. In luminal-A breast cancer, the TME is also enriched with pro-inflammatory factors, including cytokines. The cytokines TNF α and IL-1 β are continuously co-expressed in the majority of patient breast tumors and have causative roles in promoting the malignancy process, by acting on the tumor cells themselves and on cells of the TME.

Our hypothesis in this study is that exposure of stromal cells to pro-inflammatory mediators, such as TNF α and IL-1 β , enhance breast cancer progression.

To determine the impact of the pro-inflammatory stimulation on stromal cells and on progression of luminal-A tumors thereafter, and to identify the mechanisms involved, we stimulated the cells with the pro-inflammatory cytokines TNF α + IL-1 β . RNAseq profiling revealed extensive changes in gene expression in the stromal cells following prolonged TNF α + IL-1 β stimulation, including genes that are related to dynamic changes in fibroblasts; moreover, cytokine-stimulated stromal cells have expressed higher levels of genes that may lead to their pro-tumorigenic effects on tumor cells.

Additional analyses indicated that factors secreted by the TNF α + IL-1 β -stimulated stromal cells induced epithelial-to-mesenchymal transition (EMT)-related morphological changes in luminal-A breast tumor cells. Moreover, luminal-A tumor cells have acquired functions that are related to increased aggressiveness, such as scattering out of tumor spheroids, spontaneous migration and migration in response to serum factors.

Overall, our results suggest that the pro-inflammatory conditions in the TME induce a pro-metastatic phenotype in stromal cells, leading to elevated aggressiveness in luminal-A tumors. Determination of the molecular mechanisms involved in the process may enable the identification of potential targets that could be inhibited and used as potential therapies in luminal-A breast cancer.

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Visualizing the Immune Response

Characterization of Gads N Terminal SH₃ Domain Role on Modulating TCR+ CD28 Co-Stimulation Responsiveness

Enas Hallumi

GRB2-related adaptor downstream of Shc (GADS) is a member of the Grb2-family adaptor proteins, composed of a central SH₂ domain and a proline-rich linker flanked by two SH₃ domains. Via its different domains, Gads can mediate diverse interactions with key signaling proteins. These interactions may amplify or attenuate the intensity of T cell receptor signals, or change the balance of downstream pathways, leading to different cellular responses. Early studies defined the role of Gads SH₂ and C terminal SH₃ domains, which interact with two adaptor proteins LAT and SLP76 respectively, thereby bridging these two proteins to create a large complex that activates the enzyme-PLC γ and contributes to T cell responses.

The N terminal SH₃ domain (N-SH₃) of Gads has no known function or binding partners, yet it is extremely conserved.

We uncovered a newly phosphorylation site pY45 in the Gads N terminal SH₃ domain. Our data show that this site is phosphorylated by the tec family kinase ITK in vitro and in intact cells .

Functional comparison of a Gads-deficient T cell line (dG32), reconstituted with wild type Gads, or Gads muted to Y45F on its N-SH₃ domain, revealed that the Y45F mutation abrogates activation of the RE/AP composite element which is dependent on CD28 signaling, while the CD69 activation marker and the calcium flux were not affected.

Based on these preliminary results, Gads Y45 phosphorylation may be involved in uncovered pathway leading to the activation of the RE/AP transcriptional element via a co-stimulation dependent manner. In conclusion , we aim to explore the Y45 phosphorylation mechanism using in-vitro and in-vivo models to facilitate a better understanding of the TCR+ CD28 Co-stimulation signaling responsiveness.

Cancer Metastasis, Precision in Personalized Cancer Immunotherapy, Immunopathologies and Precision Medicine

PD-L1 and PD-1 Mechanism in Triple Negative Breast Cancer Tumors

Nofar Erlichman

Introduction/ Background

Triple negative breast cancer (TNBC) is characterized by the absence of estrogen receptor, progesterone receptor and HER 2 amplification. TNBC is a very aggressive and highly metastatic cancer which does not respond to hormone therapies. The tumor microenvironment is the cellular environment in which the tumor exists which contains many components including inflammatory mediators and immune checkpoints.

Methods/ Materials

Not much is known about the mechanism of PD-L1 in TNBC so in this study, we studied the effect of PD-L1 overexpression in co-culture with PD-1 overexpression. Three cell lines were involved: MDA-MB-231, BT 549 and HEK 293 cells. Co-cultures were done in order to look at the proliferation and morphology changes between the cell lines. FACS of both co-cultures was done to look at the transfer of cherry from MDA-MB-231 cells (or BT 549) to HEK 293 cells. MDA-MB-231 was grown with recombinant PD-1 (rPD-1) to compare with the results in the co-cultures.

Results

We grew four combinations for the co-cultures and our results indicate that the combination that has both PD-L1 and PD-1 being overexpressed has higher proliferation and more drastic changes in morphology (such as elongated and clustered cells). FACS test indicated that the HEK 293 cells did indeed take some cherry and became more positive. When we tested rPD-1 on the cell lines with the same combination, we saw the same results in the combination that has both PD-L1 and PD-1 being overexpressed.

Conclusion

The findings of this study indicate that PD-L1 expression in combination with PD-1 is prevalent in breast cancer and does affect both the morphology and the proliferation of the cell lines involved in this study. The initial experiments on the mechanism of PD-L1 with PD-1 is being followed up with subsequent experiments on the effect of this mechanism on triple negative breast cancer tumor progression.

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Lymphocyte Activation & Exhaustion, CAR and CTL Therapy in Cancer

Effector CD4+ T cells, rather than CD8+, increase the efficacy of antibody-driven tumor immunity

Amit Gutwillig

Most studies and therapeutic strategies in tumor immunology have focused on CD8+ T cells and their cytotoxic activity, with relatively little emphasis has been placed on understanding and harnessing the CD4+ T cell response. In a recent study, aimed to dissect the changes in T cell compartment across the body of tumor-bearing mice following antibody-mediated immunotherapy, we found that antigen-experienced CD4+ and CD8+ T cells are highly associated with tumor regression. Here, we further tested which cell subset better potentiate antibody-mediated tumor immunity. Initially, we isolated effector CD8+ and CD4+ T cells from tumor-bearing mice, expand them ex vivo and inject them intravenously to mice bearing established melanoma tumors along with tumor-binding antibodies. We found that adoptive transfer of effector CD4+ T cell induced a longer, more potent tumor-protecting immunity compared to transfer of effector CD8+ T cells. Deep sequence analysis of CD4+ T cell receptors (TCR) in tumor-bearing mice revealed that T cell in different organs, and especially in tumors, have different TCR. Therefore, we sought to elucidate which organ contains the most effective tumor-reactive CD4+ T cells. To this end, we isolated effector CD4+ T cells from various organs of tumor-bearing mice, expand them ex vivo and adoptively transferred them to mice bearing large and established melanoma. We found that CD4+ T cells from tumors and draining lymph nodes, but not from blood or non-draining lymph nodes, induce regression of large established tumors. Overall, this study highlights the therapeutic capacities of CD4+ T cells and their synergism with antibody-driven immunotherapies, and provides a rationale for deeper studies aimed at leveraging CD4+ T cell responses.

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Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment

The Tellurium compound SAS decreases PD-L1 protein expression on melanoma cells and in mice models: mechanisms of action and clinical

Tal Lantner

SAS is an organotellurium compound with multifaceted immunoregulatory properties that is remarkable for its lack of toxicity. The therapeutic effect of SAS had been tested in our lab on murine B-16 melanoma cells and C57BL male mice.

Among the inhibitory immune mediators that play an important role in the maintenance of peripheral tolerance are PD-1 receptor (programmed death 1) and its ligand PDL-1.

Some cancer cells may benefit from the PD-1:PDL-1 pathway interaction -

PDL-1 expressed on tumor cell surface interacts with PD-1 receptor expressed on CD8+ T cells leading to T cell anergy preventing their functions against tumor cells. In addition, some studies revealing a potential linkage between chemotherapy and immunoresistance through activation of ERK pathway that leads to overexpression of PDL-1.

Therefore inhibition of one or more of these immune checkpoints may play an important role in the future of immunotherapy.

In this work we show how the tellurium compound SAS inhibits PD-L1 activity via the inactivation of the VLA-4/ p-Akt/ NFκB axis. In our results in B-16 melanoma cells we show that the compound also has the ability of inhibiting intracellular expression and secretion of the anti-inflammatory cytokine IL-10 (Interleukin 10). Moreover, we found that SAS mediates B16 cells death caused by CD8+ T cells in a significant death rate of about 75% of cells using FACS analysis.

In VIVO treatment with SAS in a mouse model of B16 melanoma, resulted in tumor shrinkage due to PD-L1 inhibition. Furthermore, combination therapy with SAS and anti PD-1 resulted in an additive effect or even synergistic.

The results suggest that the tellurium compound SAS could be useful as a therapeutic approach for immunotherapy in melanoma.

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Microenvironment and Immuno-Oncology, Bioinformatics, Big Data and Cancer

Exploring Glioblastoma intra-tumor heterogeneity through microenvironment cell-cell interactions

Rony Chanoch¹

Introduction

Glioblastoma (GBM) is the most aggressive and common brain cancer, known for its various sources of heterogeneity. The intra-tumor heterogeneity of GBM can be described by the existence of a limited set of meta-programs, or expression states, that occur in the malignant cells of GBMs. The frequency of each state in a given GBM is influenced by a plasticity between the states and by different genetic drivers. However, the mechanism by which this plasticity occurs remains mostly unknown.

Materials and methods

We profiled several recently-derived GBM cell lines and a mouse model of GBM with scRNA-seq in order to find model systems that recapitulate the diversity of the cellular states observed in our cohort of GBM patient samples. Marker genes for each state enable us to isolate distinct state populations from these models by flow cytometry, in order to explore the cellular states' plasticity experimentally.

Results and discussion

We show that macrophages can influence the cellular state distributions in our GBM model systems both *in vitro* and *in vivo* and identify a specific macrophage-secreted cytokine that mediates this interaction. To further understand the interaction between the GBM states and macrophages, we used computational analyses of scRNA-seq data of human GBM, through which we identified a unique macrophage phenotype associated with GBM.

Conclusion

The cellular states and the transitions between them are crucial to understanding the intra-tumor heterogeneity of Glioblastoma. By combining computational analyses of scRNA-seq data from GBM patient samples with experimental methods in model systems of GBM, we explore the effect of microenvironment cell-cell interactions on the GBM state plasticity. Our findings identify macrophages as an important factor on the cellular state distribution of GBM and set us on a path that can improve the therapeutic outcome of this fatal disease.

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Inflammation and Immunity – Friends or Foes?, Microenvironment and Immuno-Oncology, Cancer Metastasis

Spontaneous Breast Cancer Bone Metastasis is Associated with Mesenchymal Phenotype and Immune Suppression

Lea Monteran¹

Introduction:

Mortality from breast cancer is almost exclusively due to metastasis. Bone metastasis occur in up to 85% of patients with advanced breast cancer, and are associated with morbidity and mortality. Breast cancer-induced bone metastases are characterized by changes in the bone microenvironment and frequently osteolytic lesions. These alterations disrupt the homeostasis of the immune cell populations within the bone marrow, presumably affecting the entire immune balance of the body.

Materials and methods:

To characterize the changes at the metastatic microenvironment we generated bone-seeking variants of 4T1 cells by two rounds of *in vivo* selection (4T1.1 and 4T1.2). We calibrated the timeline for the formation of a pre-metastatic niche, before the formation of macro-metastases, and mapped the early changes of various immune cell populations in multiple organs, as well as the transcriptome of bone-tropic cells compared with the parental cells.

Results and discussion:

We found that the bone-metastasizing 4T1.2 cells had a more mesenchymal phenotype compared to the parental 4T1, suggesting that bone metastasizing cells undergo epithelial to mesenchymal transition in order to invade the bone. In addition, the bone seeking cells exhibit "osteomimicry" behavior, reflected by increased expression of bone-specific genes (ALP, BSP, OCN), suggesting that adapting to the specific metastatic microenvironment is required for metastatic seeding. Moreover, analysis of cell populations in BM of bone metastases-bearing mice revealed an immune-suppressed microenvironment, including an increase in the population of granulocytic BMDCs and a decrease in T cells. Importantly, the immune cell changes in bone, blood, spleen and lungs preceded the formation of spontaneous bone metastases, suggesting that formation of an immune suppressed niche facilitates organ colonization, in agreement with the notion that breast cancer is a systemic disease.

Conclusions:

In this work, we established and characterized a spontaneous model of breast cancer bone metastasis, and demonstrated that alterations in the bone microenvironment occur prior to metastases detection. Our findings may provide the basis for identifying new targets that could prevent bone metastatic relapse.

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Immunopathologies and Precision Medicine

Immunomodulation of EAE using CAR-T Cells that Target Autoreactive Epitopes

Inbar Arman

Autoimmune diseases are often mediated by abnormal activation of autoreactive CD4+ T cells. These cells interact with antigen presenting cells (APCs) that express disease associated autoantigens on MHC class II molecules in a pro-inflammatory context, thereby inducing an inflammatory response against self. Multiple sclerosis (MS) is a CD4+ mediated demyelinating autoimmune disease that is associated with HLA-DR2 MHC class II allele. Presentation of myelin associated epitopes, such as myelin oligodendrocyte glycoprotein (MOG) on HLA-DR2 by APCs in a pro-inflammatory context, results in autoreactive T cell activation and consequently nerve cells damage. Current MS treatments are based on immune response suppression and therefore development of antigen-specific treatments is highly desirable. The APC:T cell interactions are the core drivers of immune responses and also exhibit a specific check-point in the inflammatory process. Thus, we suggest that neutralization of myelin-associated epitopes presentation by APCs should decrease myelin-specific CD4+ T cell activation and consequently reduce inflammation during MS. Accordingly; we have isolated a T cell receptor like (TCRL) antibody that specifically targets and constructed second-generation chimeric antigen receptor (CAR) molecules based on its variable regions. specific CAR-CD8+ T cells successfully induced APCs death *in vitro*. We are currently conducting *in-vivo* experiments in experimental autoimmune encephalomyelitis diseased HLA-DR2 transgenic mice to assess the function of CAR-CD8+ T cells. These data may provide a proof of concept for the notion that elimination of disease-specific APCs by TCRL-based CAR-CD8+ T cells may serve as an antigen-specific therapeutic approach for autoimmune disease.

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Inflammation and Immunity – Friends or Foes?, Microenvironment and Immuno-Oncology, Cancer Therapy: Advances in Drug Design and Delivery

Optimizing The Anti-tumoral Efficacy Of Micro-particles Loaded With Innate Inducers.

Amir Goren^{1,2}

The immune system can attack abnormal cells such as cancer cells. Immunotherapy, which can be used to treat Cancer is challenging due to the cellular diversity and imposed immune tolerance in the tumor microenvironment (TME). Using innate activators loaded onto micro-particles to induce inflammatory reaction in the TME may present a solution for this problem. Previous studies showed that treating mice with a combination of innate activators loaded onto micro-particles (MP) induced acute inflammatory cytokine and chemokine activity *in vitro* and *in vivo*. This study suggested that establishing high quality quantification methods will help to improve the inflammatory reaction in the TME to delay tumor growth. Quantification methods were established for the inducers loaded onto particles. Two inducers; Complement component 5a (C5a) and *Lipopolysaccharide* (LPS) were tested and optimized. These inducers were loaded on MP, and their effect on the immune activation was evaluated, *in vitro* on mice splenocytes and *in vivo* on B16 F10 subcutaneous induced melanoma in C57BL6 mice. LPS quantification was established by using *Endosafe PTS Endotoxin Testing System* and C5a peptide quantification was established using Q-TOF mass spectrometry. MP loaded with LPS (2.4% of total loading space) and C5a peptide (97.6% of total loading space) showed over 65% reduction in tumor growth rate as compared to non-loaded MP. The results suggest that combining inducers of distinct innate immune activation pathways in certain concentrations holds promise for successful redirection of TME-residing innate immune cells toward anti-tumoral activation.



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Inflammation and Immunity – Friends or Foes?, Cancer Metastasis, Cancer Therapy: Advances in Drug Design and Delivery

Evaluating Treatment Of Mouse Tumors With Natural Oncolytic Viruses

Daria Oren Aharon^{1,2}

Oncolytic viruses infect and kill cancer cells while leaving normal cells intact and may act on the tumor as well as metastases. The main obstacle of virotherapy is neutralization of the treatment by the patient own immune system. The ability to switch and use a new strain of virus for treatment after the rise of an immunological response, enable to overcome this major obstacle and allow prolonged treatment. Such a treatment will overcome an immune response against the virus and prevent escape of the tumor thus, enabling treatment of a range of cancers originated from various mutations. In this study we developed a method of screening for oncolytic potential of veterinary viruses. The source of the virus may be avian or mammalian source thus decreasing the potential danger to humans. The ability of those viruses to kill murine tumor cells was evaluated *in vitro*. There was no apparent correlation between the virus virulence and its capability to destruct malignant cells. We characterized all candidate viruses by TCID₅₀ /ml assay in VERO cell line. Then, we performed MTT tests on 6 different murine cancer cell lines to determine the activity of the viruses. There were differences in the effect of viruses on the various cell lines. Based on the results of the tests we focused on 3 distinct viruses for *in vivo* tests in mice. The study evaluated the potential of sequentially administered oncolytic veterinary viruses, which we have characterized, to treat cancer in an immunocompetent murine model. The variety of potential oncolytic viruses in arsenal and the possibility to use combinations of viruses (as cocktail or one by one) may allow repeated treatments, overcoming the immune response on one hand and attacking the tumor by more than one mechanism on the other hand.

Cancer Metastasis

Activating mutations of the estrogen receptor confer chemotherapy resistance in breast cancer through the activation of JNK pathway

Marwa Taya^{1,2}

Introduction: Endocrine therapy is the mainstay treatment for estrogen receptor- α (ER) + metastatic breast cancer (BC) patients. However, all patients eventually develop resistance to these therapies. Recently, our lab discovered activating mutations, Y537S and D538G, in the ER ligand-binding domain (LBD) that confer resistance in nearly 40% of patients. We and others noted that the disease in LBD-ER patients is characterized by increased liver metastasis and a worse prognosis. Patients who develop endocrine resistance are treated with chemotherapy, and chemo-resistance is a major obstacle in these patients. We hypothesized that LBD-ER mutations confer resistance to chemotherapy, leading to worse prognosis. Therefore, our aim was to elucidate whether LBD mutations confer resistance to chemotherapy, growth advantage in liver microenvironment and elucidate the mechanism of action.

Methods: MCF-7 cells stably-expressing WT or the LBD mutations were used. Cell proliferation and viability were determined using MTT, methylene blue and colony assays. mRNA expression was performed using qRT-PCR, protein expression by Western-blot and transcriptional activity by a gene-reporter assay. Apoptosis studied by cleaved-PARP and Annexin/ DAPI analysis. Tumor growth was evaluated in an orthotopic model of BC.

Results:chemotherapeutic drugs doxorubicin and paclitaxel induced less apoptosis in LBD- cells, proliferated better, compared to WT-ER cells. In vivo, we found that the D538G-ER cells exhibited liver tropism while Y537S-ER cells mainly metastasized to the lungs and lymph nodes. Recent studies suggest that the JNK pathway may confer resistance to chemotherapy and that c-Jun overexpression is linked to breast cancer liver metastasis. Indeed, D538G-cells exhibited elevated JNK pathway activity evidenced by p-JNK and down-stream transcriptional activity, and activity further increased following doxorubicin treatment. In accordance, JNK inhibition further elevated apoptosis in D538G cells.

Conclusion: Our results indicate a new role to LBD-ER, beyond resistance to endocrine therapy, namely, resistance to chemotherapy. We revealed that in D538G-ER the resistance is mediated through the activation of the JNK pathway. Importantly, D538G confers predilection to liver metastasis. These results may aid in overcoming resistance in these D538G-patients by targeting the JNK pathway.



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Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment

Elucidating the role of human anti-PD-L1 antibodies Fc-dependent signaling in tumor therapy

Noy Cohen Saban

Checkpoint inhibitor immunotherapies (checkpoint blocking antibodies) have helped many, but unfortunately, not all patients overcome advanced cancer. To help the remaining patients, we need a better understanding of how these therapeutic antibodies work and what aids their effectiveness. Our proposed study aims to do just that, by focusing on the modes of action of anti-PD-L1 checkpoint blocking antibodies. We have recently found that while the “active ends” of these drugs target the pro-tumor PD-1 pathway, their “back ends” also improve their effectiveness. Driven by this observation made for mouse antibodies, here we will advance our study to focus on the impact of the “back ends” of human anti-PD-L1 drugs. We will use advanced mouse genetic, microscopy, and molecular biology approaches to decipher the therapeutic mechanism mediated by of the “back ends” of these immunotherapies. This study may pave the way for the development of new ways of designing anti-PD-L1 checkpoint blocking antibodies to further improve their anti-cancer activity.

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CAR and CTL Therapy in Cancer

Improvement of The Anti-Tumor Activity of T-Cells by Modifying the 1G4 TCR

Astar Shamul

Introduction:

T-cell based immunotherapy for cancer is based on the corollary that tumors express defined antigens which expression may help discriminate between normal and cancerous tissues. One of the most attractive tumor antigens to target is New York Esophageal Squamous Cell Carcinoma-1 (NY-ESO-1) which has been reported to be expressed in a wide range of tumor types, including neuroblastoma, myeloma, metastatic melanoma, synovial sarcoma, etc. It is possible to retarget T-cell specificity by virally transducing them to express a T-cell receptor for a tumor epitope. 1G4 TCR, is an NY-ESO₁₅₄₋₁₆₂ / HLA-A*0201 specific TCR with relatively low/medium anti-tumor reactivity.

Methods:

Based on our previous work aimed at improving TCR stability to enhance its function, we developed in this project a novel 1G4 TCR derivative that we termed- 1G4s, in which we optimized the primary structure, enhanced the stability of the transmembrane region and included selected mutations in the CDR₃.

Results:

We examined the influence of improving modifications on the 1G4s-TCR function. We observed increased functionality manifested by higher levels of cytokine secretion (IFN γ , TNF α and IL2), following co-cultures of T-cells engineered to express either the 1G4s TCR compared to those which expressed the wt 1G4-TCR. Furthermore, T-lymphocytes expressing 1G4s-TCR facilitated increased surface expression levels of T-cell activation markers OX40 and 41BB, when compared to the WT. 1G4s also manifested higher functional avidity in the detecting concentration of epitopes in the 10⁻⁹-10⁻¹⁰M range.

Conclusion:

Overall, we conclude that it possible to manipulate the primary structure of TCRs at several levels to enhance their function. As such, the 1G4s demonstrated a better functional potential than its wt counterpart, leading us to pursue its development as a therapeutic reagent to treat patients with NY-ESO1/HLA-A*0201 positive tumors.

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CAR and CTL Therapy in Cancer

Optimization Of Anti-BCMA CAR By Manipulating Domain Structure

Ortal Harush

CAR T-cell based immunotherapy has become a promising treatment mainly for hematological malignancies. Following the major success of CD19-targeted CAR, new potential targets for other malignancies such as multiple myeloma are being designed, mainly based on targeting tumor associated antigens such as BCMA. Being a crucial determinant for its function, CAR composition and structure is generally optimized empirically. Thus, we aimed at assessing the function of different BCMA CARs based on the same targeting moiety but with a different hinge and co-stimulatory domain. We compared their function to that of a previously published BCMA CAR used in clinical trials. All constructs were expressed at high levels by primary human T-cells and could trigger cytokine production and cytotoxicity upon co-culture with multiple myeloma targets. Nonetheless, critical differences were observed in off-target activation, PD1 expression and *in vivo* activity mediated by these different constructs, facilitating the identification of an optimal BCMA-specific CAR. Overall, this work strengthens the need to evaluate empirically CARs in order to determine optimal configuration and presents an ideal composition for a BCMA CAR that demonstrated long-term *in vivo* protection.

Visualizing the Immune Response

Comparing antibody profiles to upper respiratory tract viruses in elite combat soldiers during training and their support staff

Shosh Skorniakov^{1,2}

Introduction: During the first year of combat training, soldiers of elite units are under intense physical and mental stress. The antibody response to influenza vaccination or exposure has been reported to be enhanced in individuals that are seropositive for cytomegalovirus (CMV). To investigate the effect of stress and extensive physical training on the immune system, and the interplay between antibodies to different viruses, we longitudinally profiled changes in antibodies to upper respiratory tract viruses and cytokine profiles in a cohort of IDF elite soldiers during their first year of training and headquarter soldiers from the same base.

Methods: An antigen microarray, spotted with recombinant glycoproteins and whole inactivated viruses of common upper respiratory tract infections, was used to profile antibodies in serum and saliva samples collected from two groups of soldiers: (1) combat soldiers from an IDF elite unit during their first year of training (recruits; n=51), and (2) support staff from the unit's headquarters (n=34). In both groups some of the soldiers were vaccinated with the seasonal influenza vaccine a few months before the trial began. Our microarrays included antigens of 54 influenza strains, to compare the response to vaccination in the groups. Serum and saliva samples were collected from each soldier at four different time points over a period of 15 months (T1-T4).

Results: Among the unvaccinated recruits, a significant increase in the median magnitude of anti-influenza antibodies at T2 suggested influenza infections in this group. Such increase in the median levels of anti-influenza antibodies was not observed in the vaccinated recruits or in support soldiers.

We also found that CMV seropositivity was associated with development of higher levels of IgG antibodies to whole influenza viruses but lower levels of IgG to recombinant influenza hemagglutinin (HA) proteins following influenza vaccination.

Discussion and conclusions: These findings suggest that vaccination of recruits is important for preventing influenza infections. In addition, CMV infection may affect not only the level but also the repertoire of antibodies developed following influenza vaccination.

Microenvironment and Immuno-Oncology

Elucidating mechanisms that regulate the expression of the heparanase homolog, Hpa2

Ibrahim Knani¹

Introduction. Heparanase activity is strongly implicated in tumor angiogenesis and metastasis attributed to remodeling of the subepithelial and subendothelial basement membranes. Augmented level of heparanase was documented in an increasing number of human carcinomas and hematological malignancies. In many cases, heparanase induction correlated with increased tumor metastasis, vascular density, and shorter survival rate post-operation, thus providing strong clinical support for the pro-tumorigenic function of the enzyme, making it an attractive target for the development of anti-cancer drugs. Heparanase homolog termed heparanase 2 (Hpa2) was cloned based on sequence similarity. Unlike heparanase, Hpa2 lacks intrinsic heparan sulfate (HS)-degrading activity, the hallmark of heparanase, but retains the capacity to bind heparin/HS. In fact, Hpa2 exhibits even higher affinity towards heparin/HS than heparanase, suggesting that Hpa2 may inhibit heparanase activity by competition for the HS substrate. Clinically, Hpa2 expression was markedly elevated in head and neck carcinoma patients, correlating with prolonged time to disease recurrence and inversely correlating with tumor cell dissemination to regional lymph nodes, suggesting that Hpa2 functions as a tumor suppressor. Similarly, Hpa2 was not detected in normal ducts of the pancreas but appeared to be expressed at high levels by some pancreatic ductal carcinomas. We hypothesized that Hpa2 induction in various tumors is due to stress conditions that tumor cells often experience in a fast-growing tumor lesion.

Methods. Pancreatic (Panc-O1, ASPC), head and neck (FaDu) and ovarian (Hey, SKOV-3) cells were exposed to ER-stress (by Thapsigargin), hypoxia or both and Hpa2 expression was evaluated by qPCR. We also examined Hpa2 expression in a panel of gastric and ovarian carcinoma cell lines in correlation with p53 status (wild-type vs mutant). We constructed a reporter gene composed of Hpa2 promoter fused with luciferase coding sequence that will enable the identification of regions and sequences that mediate Hpa2 gene regulation.

Results. ER stress and hypoxia each elicits modest, 3-5 fold increase in Hpa2 expression. However, combining ER stress and hypoxia resulted in a synergistic effect with an over 100-fold increase in Hpa2 expression. Moreover, we found that cells in which p53 is mutated are endowed with a much lower Hpa2 expression than cells carrying wild-type p53. Indeed, treatment with Nutlin that dissociates p53 from MDM2 and increases its activity resulted in increased Hpa2 expression.

Conclusions. We have identified, for the first time, mechanisms that regulate Hpa2 expression and will thereby assist in deciphering the function of Hpa2 in tumor growth.

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Precision in Personalized Cancer Immunotherapy

The New Era of Precision Immunotherapy

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Immune checkpoint inhibitors (ICIs) are providing durable clinical responses in about 20% of cancer patients. But these agents have minimal effect in cancers without intratumoral T cells. Approaches that turn currently unresponsive cancers into ones that are more “antigenic” are needed to sensitize tumors to ICIs. Tumor genome mutations can express mutant proteins that are tumor-specific and not expressed on normal cells (neoantigens), and cancers with the highest mutational burdens are more likely to respond to single agent ICIs. However, most cancers have lower mutational loads resulting in lower antigenicity, weaker endogenous T cell repertoires, and T cells in the tumor. The best example of a high mutation load cancer is mismatch repair deficient (MMRd) cancers; these cancers often have 1000 mutations/exome and have a 50% response to anti-PD-1 ICIs. But cancers including pancreatic ductal adenocarcinoma (PDA) and microsatellite stable colorectal carcinoma (MSS CRC) have on average only 50-70 expressed mutations per exome and do not respond to single agent ICIs. Emerging data suggest that it should be possible to develop precision immunology approaches that combine a neo-antigen targeting vaccine to activate and expand the limited repertoire of T cells specific for the expressed neo-antigens found in low mutation cancers, with ICIs to induce clinically relevant anti-tumor responses. But challenges to successful immunization include knowledge about the repertoire and functional state of pre-existing anti-tumor T cells, identification of the best adjuvants, and approaches that more precisely predict which expressed neo-antigens are the best T cell targets for immunization. In addition, we now appreciate that there are many different immune regulatory signals on T cells, monocytes and other tumor microenvironment cell populations that are likely regulated by the genetic alterations specific to a given patient’s tumor. This talk will discuss current knowledge of precision immune oncology and novel clinical trial approaches under development.



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Visualizing the Immune Response

New insights into the cell biology of human natural killer cells and cytotoxic function

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Natural Killer (NK) cells are the main cytotoxic lymphocytes of the innate immune system. They utilize lysosome-related organelles (called lytic granules) loaded with perforin and granzymes to mediate the destruction of diseased cells. The process by which they kill is a highly coordinated series of cell biological steps in which the lytic granules are mobilized, polarized and then carefully provided access to the NK cell membrane so that their contents can be secreted. Our laboratory has been studying the cell biological regulation of the mechanics of this process and has also attempted to gain insights from genetic diseases of immunity. An overview as well as some new insights into the cell biological process of cytolytic function of NK cells will be presented as will be some lessons taught by novel and known genetic disease. As the cytolytic defenses mediated by NK cells are highly relevant to host defense against and even in treatment of cancer a better understanding of how NK cells kill will hopefully allow us to better harness their activities therapeutically.

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Visualizing the Immune Response, Lymphocyte Activation & Exhaustion

Bridging the Gap: Modulatory roles of the Grb2-family adaptor, Gads, in cellular and allergic immune responses

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Antigen receptor signaling pathways are organized by adaptor proteins, including three hematopoietic adaptors: LAT, Gads and SLP-76, that are required for antigen responsiveness in T cells and in mast cells. Upon antigen recognition by the TCR or by the FCεRI, the Grb2-family adaptor, Gads, bridges the recruitment of the cytoplasmic adaptor, SLP-76, to the membrane-bound adaptor, LAT. Cooperative binding events contribute to the formation of large LAT-nucleated signaling complexes that are competent to mediate downstream responsiveness; however, the molecular basis for cooperativity was not known. We demonstrated an SH2-intrinsic mechanism of cooperativity, based on Gads SH2 dimerization. Gads dimerization promotes its preferentially paired binding to dual-phosphorylated LAT molecules, and facilitates discrimination between dual and singly phosphorylated LAT molecules. We developed a rapid and sensitive method for measuring cooperativity at LAT. Using this method, we are able to define the regions of Gads and of LAT that stabilize their cooperative interaction. Mutational inactivation of the Gads dimerization interface reduced cooperativity, and abrogated Gads signaling functions in T cells and in mast cells. Dimerization-dependent, cooperative binding of Gads to LAT may increase antigen receptor sensitivity, by reducing signalosome formation at incompletely phosphorylated LAT molecules, thereby prioritizing the formation of stoichiometrically complete signalosomes.

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Visualizing the Immune Response

Game of clones within immunological niches

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Coordinated communication among different types of immune cells within specialized niches is essential for mounting a protective immune response against harmful microorganisms. Within these niches immune cells exchange signals that are essential for activation of cells bearing various antigen-specific receptors that mediate pathogen clearance. Our studies focus on the humoral immune response and how different adaptive immune cells, primarily B and T lymphocytes cooperate for the generation of pathogen-specific high-affinity antibodies and establishment of long-lasting immunity. For this, we use intravital two-photon laser scanning microscopy that allows visualization of immune cell dynamics deep within tissues of living mice. In addition, we examine the distribution of various immune cells within multiple niches and compartments using whole organ imaging by light sheet fluorescence microscopy. This imaging approach allows us to visualize all the immune cells as they recognize the pathogen and generate a complete picture of the immune response. These techniques allow us to visualize immune cell niches in various organs including the intestinal tract.

Gut-derived antigens trigger immunoglobulin A (IgA) immune responses that are initiated by cognate B cells in the Peyer's patch (PP) within specialized niches. These cells colonize the subepithelial domes (SEDs) of the PP and subsequently infiltrate into special type of niches known as germinal centers (GCs). We defined the pre-GC events and the niches at which affinity-based B cell selection occurred in PPs. Using whole-organ imaging, we showed that the affinity of the B cell antigen receptor (BCR) regulated infiltration of antigen-specific B cells into GCs, but not clonal competition in the SED. Follicular helper-like T cells resided in the SED and promoted its B cell colonization, independently of the magnitude of BCR affinity. Imaging and immunoglobulin sequencing indicated that selective clonal-expansion ensued during infiltration into GCs. Thus, in PPs, in contrast to draining lymph nodes and spleen, T cells predominantly promoted the expansion of B cells without clonal selection during pre-GC events. Understanding immune cell communications within specialized niches can lead to the design of improved vaccines against infectious pathogens. Our studies may also lead to the discovery of novel checkpoints that can be targeted in autoimmune diseases and prevent the formation of pathogenic anti-self antibodies.

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Inflammation and Immunity – Friends or Foes?, Immunopathologies and Precision Medicine

Keep calm and IL-10, a story of gut and brain macrophages

Steffen Jung

The recent past has seen major advances in our understanding of macrophage contributions to physiology and pathophysiology. Intra-vital imaging, fate mapping, cell ablation and targeted mutagenesis in mouse models, complemented by advanced transcriptome, translatoome, and epigenome profiling have provided insights of unprecedented depth. Collectively, these studies highlight that macrophages need to be studied in physiological context since their identities and functions are defined by the local tissue environment. Here I will discuss our efforts to probe tissue macrophages in gut and CNS. Specifically, we focus on the IL10 / IL10 receptor axis and its critical role in ensuring macrophage quiescence following respective physiological environmental challenge in the intestine and the brain.eed

In the colon, macrophages are critical for gut homeostasis. In a murine IBD model based on a macrophage-restricted Interleukin 10 (IL-10) receptor deficiency, pro-inflammatory mutant gut macrophages cause severe spontaneous colitis resembling the condition of children carrying IL10R mutations (Zigmond et al., 2014). We now established that macrophage-derived IL-23 is the driving factor of this pathology. Specifically, we report that $Cx3cr1^{Cre}: Il10ra^{fl/fl}: Il23a^{fl/fl}$ mice harboring macrophages deficient for both IL-10R and IL-23 are protected from colitis (Bernshtein et al., SI 2019). Furthermore, by analyzing the epithelial response to pro-inflammatory macrophages, we provide evidence that T cells of colitic animals produce deleterious IL-22 that induces epithelial chemokine expression and detrimental neutrophil recruitment. Collectively, this defines critical cell-type-specific contributions to the induction and effector mechanism of macrophage-driven colitis, as manifested in mice harboring IL-10R deficiencies and human IBD pathologies.

Also all microglia, the parenchymal brain macrophages, prominently express IL10 receptor. However, analysis of Tamoxifen-treated $Cx3cr1^{CreER}: Il10ra^{fl/fl}$ mice suggests that IL-10 sensing is dispensable to maintain brain homeostasis (Shemer et al. in preparation). Rather, we will report data establishing that IL-10 is required to rapidly and robustly restore microglia quiescence following peripheral endotoxin challenge.



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Visualizing the Immune Response, Lymphocyte Activation & Exhaustion

Resolving mechanisms of T cell activation at the single molecule level

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T cells mount an immune response to foreign pathogens by searching the surface of antigen presenting cells for cognate antigens, and their specific recognition by the T cell receptor (TCR). First engagement between these cells occurs via dynamic T cell protrusions, e.g. microvilli, before forming a tight immune synapse. We used single molecule localization microscopy (SMLM) in live cells to resolve TCR-dependent signaling at tight cell contacts. We show that early contacts are sufficient for robust TCR triggering and its rapid signal amplification. We further show dynamic nanoscale molecular patterning of the TCR and related signaling and structural proteins at the tight contacts that facilitates T cell activation. Thus, early and tight T cell contacts function as both sensing and decision-making entities in T cell activation.

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Visualizing the Immune Response, Inflammation and Immunity – Friends or Foes?

Conserved mechanisms underlying loss of tolerance to allogeneic tissues in *Botryllus schlosseri* chimeras and HSC transplantation

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The mechanisms that sustain immunological non-reactivity are the basis for understanding the maintenance of tissue in syngeneic and allogeneic settings. While most transplantation rejection occurs due to the adaptive immune response, the pro-inflammatory response of innate immunity is necessary for the activation of adaptive immunity - both in syngeneic and allogeneic settings. We study a unique chordate model, *Botryllus schlosseri*, that lacks a classic adaptive immune system, yet has the ability to reject allogeneic individuals or form chimeras with compatible animals. This organism demonstrates three major innate immunity responses: non-inflammatory program cell removal, acute rejection (between non-compatible animals) and allogeneic resorption (between compatible colonies that formed chimeras).

Using flow cytometry, whole-transcriptome sequencing of defined cell populations and tissues, and diverse functional assays, we isolated 34 *B. schlosseri* cell populations, identified hematopoietic stem cell (HSC), progenitors, immune-effector cells, and the HSC niche. Completing a full model for HSC transplantation. Furthermore, we identified a *B. schlosseri* cytotoxic cell population originating from large granular lymphocyte-like cells and demonstrated their function in acute and chronic rejection processes. Studying the molecular and cellular framework underlying loss of tolerance to allogeneic tissues within the *B. schlosseri* chimera, we found that developmental cell death programs license cytotoxic cells to eliminate histocompatible partners. This study demonstrates that interactions between pro-inflammatory and damaged tissue removal, lead to robust cytotoxic and phagocytic clearance programs within the allogeneic microenvironment.

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Inflammation and Immunity – Friends or Foes?

Pro and Anti tumorigenic functions of tertiary lymphoid structures

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The different roles of the adaptive immune system in cancer are beginning to unfold. The dramatic responses to immune check point drugs in some tumors generated an accelerated need for understanding the complex set of interactions between tumor and immune cells. In view of the major pathophysiological role of immune cells in hepatocellular carcinoma it is not surprising that malignant hepatocytes interact extensively with adaptive immune cells, resulting in both pro-tumor immunopathology and anti-tumor protective immunity. Identifying potential responders to drugs that target the adaptive immune system, monitoring their immune response to the tumor and devising the best treatment combinations depends on understanding the complex set of interactions taking place within the tumor and in the adjacent hepatic parenchyma. Cellular infiltration usually entails a diffuse influx of immune cells, scattered throughout the inflamed tissue. However, infiltrating leukocytes often form ectopic lymphoid aggregates or even more complex structures that histologically resemble lymphoid organs. These structures direct various B and T cell responses and are referred to as ectopic lymphoid-like structures (ELSs). ELSs often develop at sites of chronic inflammation where they can influence the course disease. In many cancers, the presence of ELSs correlates with a better prognosis and they may coordinate endogenous antitumor immune responses. Surprisingly, in the liver – ELSs have diverse roles – while intratumoral ELS are associated with a good prognosis, the presence of ELSs in the liver parenchyma is positively correlated with HCC. We are currently dissecting the potential role of immunosuppression in ELS protumorigenic function and aiming to identify ways to restore functional immunity upon ELSs.

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Inflammation and Immunity – Friends or Foes?

Hallmarks of Neutrophil Anti Tumor Cytotoxicity

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The role neutrophils play in tumor growth and metastatic progression has been a matter of debate as they were shown to possess both tumor promoting and tumor inhibitory traits. The capacity of neutrophils to limit tumor growth and metastatic progression is largely attributed to their ability to selectively kill tumor cells. Whereas the process is highly specific it is also very complex. Neutrophils are expected to migrate towards tumor cells, identify them as a target and induced tumor cell apoptosis. We have previously shown that tumor-secreted factors (G-CSF, GM-CSF) mobilize and activate (CCL2 and others) neutrophils. These neutrophils are attracted to tumor-secreted chemokines such as CXCL2 and identify surface molecules such as RAGE to target and eliminate tumor cells. Neutrophils eliminate tumor cells in an H₂O₂-dependent mechanism. Unexpectedly, we found that rather than inducing apoptosis via oxidative stress, neutrophil secreted H₂O₂ induces a transient but lethal increase in free intracellular Ca²⁺. Furthermore, we found that H₂O₂ induces an influx of Ca²⁺ from the extracellular milieu rather than from intracellular stores suggesting the involvement of an H₂O₂ dependent Ca²⁺ channel. Indeed, we found that neutrophil secreted H₂O₂ activates TRPM2, a ubiquitously expressed Ca²⁺ channel which is frequently upregulated in tumors. High levels of TRPM2 render tumor cells more susceptible to neutrophil cytotoxicity whereas low TRPM2 levels render tumor cells neutrophil resistant. Taken together these observations provide insight into key processes that mediate neutrophil anti-tumor cytotoxicity. However, neutrophils' anti-tumor potential is rarely evident at the primary tumor due to high levels of TGFβ – a potent immunosuppressive factor. While this limits neutrophils' contribution to tumor eradications it concomitantly highlights potential targets that may be utilized for future, neutrophil-based anti-tumor immunotherapies.

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Microenvironment and Immuno-Oncology

Notch-mediated processes promoting inflammation-driven mechanisms in breast cancer progression

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Introduction: The triple-negative subtype of breast cancer (TNBC) accounts is a most aggressive disease subtype, emphasizing the need for improved understanding of the mechanisms leading to its progression. Stromal cells and pro-inflammatory cytokines play key roles in promoting the aggressiveness of TNBC tumors. **Materials, methods and results:** We took an integrative approach and determined the impact of tumor-stroma-inflammation networks on pro-metastatic phenotypes in TNBC, focusing on TNF α and IL-1 β as representatives of the pro-inflammatory TME. Stimulation of TNBC:stroma co-cultures by these two cytokines has led to increased pro-metastatic activities at multiple levels, including: expression levels of the chemokines CXCL8, CCL2 and CCL5, angiogenesis, cancer cell morphology towards and EMT-phenotype, tumor cell migration and tumor cell invasion. Importantly, we found that CXCL8 was a key regulator of the pro-metastatic activities that came into play in the TNBC-stroma-inflammation networks, including angiogenesis, metastasis-related morphology, tumor cell migration and invasion of TNBC cells. Moreover, the tumor-stroma-inflammation network has led to elevated metastasis *in vivo*. To identify the mechanisms mediating the interactions between TNBC cells and stromal cells in the context of pro-inflammatory signals, we explored the roles of Notch receptors in regulating such cross-talks. We found that the Notch pathway was a prime regulator of tumor cell migration and invasion in the tumor-stroma-inflammation network. Also, our findings indicated that Notch1 was a key mediator of CXCL8 up-regulation, and that p65 (the NF-kB pathway) induced the expression of CXCL8 and of Notch1 activation. These findings indicate that when TNBC cells interact with stromal cells, further exposure to pro-inflammatory signals gives rise to p65 activation, leading to Notch1 activation and consequently to up-regulation of CXCL8. Then, CXCL8 promotes angiogenesis, as well as tumor cell migration and invasion. These processes eventually lead to a higher aggressiveness phenotype in TNBC *in vivo*. **Conclusions:** Our data point at complex control mechanisms that are governed by the NF-kB and Notch pathways and by CXCL8 in the setting of TNBC-stroma-inflammation triage that promotes TNBC progression. These findings propose that combined targeting of the Notch pathway and of the pro-inflammatory tumor microenvironment could be considered a relevant therapy option in TNBC.

Microenvironment and Immuno-Oncology

Necroptosis induced by anti-EMMPRIN antibody and complement shifts macrophage polarization

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Tumors grow and metastasize by reprogramming the immune system to secrete mediators that promote angiogenesis and suppress the anti-tumoral functions of existing and recruited immune cells. Thus, disrupting this reprogramming and restoring the ability of the immune system to attack the tumor is a major therapeutic goal. We have previously identified a novel epitope in EMMPRIN, a pro-angiogenic protein, and used a polyclonal rabbit-anti-mouse antibody (161-pAb) to attack it in three different mouse tumor models. 161-pAb significantly reduced tumor growth and number of metastatic foci, reduced angiogenesis, alleviated immunosuppression in the tumor microenvironment (TME) by reducing TGF β levels, and increased macrophage and CD8 T cell infiltration into the tumors. Investigating the mechanisms involved, we now show that 161-pAb together with complement, induced necroptotic cell death *in vitro* in both mouse (CT26, RENCA) and human (Skov3, A498) cell lines. This was corroborated by the co-existence of necrotic (release of LDH) and apoptotic (reduced caspase 3 activity) features, as well as unique necroptosis features such as reduced caspase-8 activity, increased phosphorylation of MLKL, and release of dsRNA. Incubating the mouse RAW 264.7 macrophages or the human U937 monocytic-like cells with supernatants obtained from tumor cells subjected to the antibody and complement, resulted in a significant elevation in IL-10 (which stimulate CD8 T cell cytotoxicity), IL-1 β , and TNF α levels, that did not occur in cells subjected to necrosis (H₂O₂) or apoptosis (doxorubicin or etoposide). Thus, necroptosis initiates the re-polarization of macrophages, which may lead to the alleviation of immune suppression in the TME.

Microenvironment and Immuno-Oncology

Activated Eosinophil Subsets are an Integral Part of the Tumor Microenvironment in Lung Metastasis, Displaying Anti-tumorigenic Activities

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Cancer-associated mortality is nearly exclusively a result of tumor metastasis, and the lung is a main metastatic site for multiple tumors. Eosinophils are granulocytes, which reside in mucosal tissues such as the lungs. Although eosinophils are highly equipped to shape the tumor microenvironment, their roles and phenotypes in lung metastasis are understudied.

Intravenous injection of PyMT (breast cancer), B16-F10 (malignant melanoma) and MC38 (colorectal cancer) cells resulted in experimental lung metastasis, which was associated with increased recruitment of eosinophils. At least two eosinophil subsets were identified within the tumor microenvironment as defined by the expression of Siglec-F and CD125. Immunohistochemical staining of the metastatic lungs revealed distinct location for eosinophils (i.e. within tumors and in the lung parenchyma). Recruitment of eosinophils into the metastatic lung was independent of the hallmark CCR3-eotaxin chemokine axis since eosinophils were readily detected in the lungs of tumor-colonized *Ccr3*^{-/-} mice. Consistent with our *in vivo* findings, PyMT, B16-F10 and MC38 cells did not express eotaxins, yet tumor-secreted factors, present in their culture media induced rapid and marked eosinophil migration *in vitro* and following intraperitoneal injections *in vivo*. Adoptive transfer of eosinophils to eosinophil-deficient mice (i.e. $\Delta dbfGATA$ mice) undergoing breast cancer associated lung metastasis resulted in preferential and rapid homing of eosinophils displaying high Siglec-F into the metastatic lung compare to naïve lung. In addition, tumor-secreted factors prolonged eosinophil survival. Similarly, conditioned media of human colorectal, melanoma and breast cancer cells, prolonged human eosinophil survival *in vitro*. Intravenous injection of PyMT and B16-F10 cells to wild type and $\Delta dbfGATA$ mice, resulted in markedly increased tumor burden in $\Delta dbfGATA$ mice. In support of these findings, co-culture of eosinophils with PyMT or B16-F10 cells, resulted in significant eosinophil-driven cytotoxicity towards the tumor cells. Notably, the antitumorigenic activities of eosinophils towards PyMT cells were lung-specific since no difference was observed between wild type and $\Delta dbfGATA$ mice, in a model of primary breast cancer.

We demonstrate potent recruitment and anti-tumorigenic activities for eosinophils in the lungs in response to several tumor cell types. These data highlight eosinophils as a novel cellular target for immunotherapy in lung metastasis.



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Israel Immunological Society

Metabolism and the DNA-Damage Response

Rewiring cellular metabolism: a novel connection between ESR1 activating mutations and aggressiveness of breast cancer

Ido Wolf

PURPOSE:/h4

Mutations in the ligand-binding domain (LBD) of estrogen receptor α (ER) confer constitutive transcriptional activity and resistance to endocrine therapies in patients with breast cancer. Accumulating clinical data suggest adverse outcome for patients harboring tumors expressing these mutations. We aimed to elucidate mechanisms conferring this aggressive phenotype.

EXPERIMENTAL DESIGN:/h4

Cells constitutively expressing physiologic levels of ER-harboring activating LBD mutations were generated and characterized for viability, invasiveness, and tumor formation *in vivo*. Gene expression profile was studied using microarray and RNAseq technologies. Metabolic properties of the cells were assessed using global metabolite screen and direct measurement of metabolic activity.

RESULTS:/h4

Cells expressing mutated ER showed increased proliferation, migration, and *in vivo* tumorigenicity compared with cells expressing the wild-type ER (WT-ER), even in the presence of estrogen. Expression of the mutated ER was associated with upregulation of genes involved in invasion and metastases, as well as elevation of genes associated with tumor cell metabolism. Indeed, a metabolic examination revealed four distinct metabolic profiles: WT-ER-expressing cells either untreated or estrogen treated and mutated ER-expressing cells either untreated or estrogen treated. Pathway analyses indicated elevated tricarboxylic acid cycle activity of 537S-ER-expressing cells. Thus, while WT-ER cells were mostly glucose-dependent, 537S-ER were not addicted to glucose and were able to utilize glutamine as an alternative carbon source.

CONCLUSIONS:/h4

Taken together, these data indicate estrogen-independent rewiring of breast cancer cell metabolism by LBD-activating mutations. These unique metabolic activities may serve as a potential vulnerability and aid in the development of novel treatment strategies to overcome endocrine resistance.



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Precision in Personalized Cancer Immunotherapy, Cancer Therapy: Advances in Drug Design and Delivery

Current Trends and Our Own Efforts in Cancer Therapy

Yosef Yarden

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The addiction of many tumors to their driver mutations (i.e., oncogenes) exposes critical vulnerabilities, which might be exploited by pharmacological strategies. In addition to ‘oncogene addiction’, cancer drugs may target non-oncogene addictions, primarily survival pathways characteristic to low-grade, hormone-, or growth factor-dependent tumors. Examples include the estrogen receptor in breast cancer and EGFR in colorectal cancer.

The tumor microenvironment (TME), a collection of non-tumor cells embedded in the vicinity extracellular matrix and blood vessels, has provided additional opportunities. Unlike tumor cells, which constantly acquire new mutations and they display clonal heterogeneity, the TME is genetically stable and its clonal structure might be simpler. Agents targeting the endothelium and suppressing angiogenesis, as well as antibodies targeting immune checkpoints exemplify the potential of TME-directed drugs.

The currently available armamentarium of medical oncologists includes 100 clinically approved drugs (excluding . Because more than one third comprises kinase inhibitors and the other third contains monoclonal antibodies, it is logical assuming that combinations of antibodies and kinase inhibitors will become a mainstay in oncology. Our own studies focus on EGFR-specific kinase inhibitors: three generations of quite effective inhibitors have been approved for the treatment of non-small lung cancer, but emergence of resistance preempts further treatment with third generation drugs. I will describe an alternative approach that combines antibodies and kinase inhibitors, and effectively inhibits all mutant forms of EGFR.

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Tumor dependence on cytosolic one-carbon metabolism is determined by cellular capacity to retain folates

Tomer Shlomi

Folate metabolism supplies one-carbon (1C) units for nucleotide biosynthesis. Mitochondrial serine catabolism is considered the sole contributor of 1C units in proliferating cancer cells, facilitated via the shuttling of mitochondria produced formate to cytosol. Here, we show that the concentration of folate in cell culture media determines the relative contribution of the cytosolic versus mitochondrial folate pathway to 1C unit production. We find that under physiological folate levels, the cytosolic pathway is the predominant source for 1C units in a variety of cancers. Tumor specific reliance on cytosolic 1C flux is determined by the capacity of cancer cells to retain high intracellular folate pools, which is determined by the expression of the Reduced Folate Carrier (RFC). We show that inhibition of the cytosolic serine hydroxymethyltransferase (SHMT1) in cells with low RFC expression impairs pyrimidine biosynthesis and tumor growth. Our findings reveal major diversity in the utilization of the cytosolic versus the mitochondrial folate cycle across cancers and RFC as a marker for induced dependence on SHMT1.

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Metabolism and the DNA-Damage Response, Microbial Infections, Resistance & Immunity

Macrophage-Assisted DNA damage response

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The DNA-damage response (DDR) is a comprehensive and complex network of phosphorylation-mediated signaling pathways that endogenously originates from the DNA lesion. Depending on the extent of DNA damage, the response leads to alternative cellular outcomes, ranging from DNA repair and normal replication, or in the face of incomplete repair to cell cycle arrest, senescence or apoptosis.

We recently provided evidence for a macrophage-dependent mechanism that regulates the response to DNA damage and cell fate decision. In this study we demonstrated that macrophages through the proteolytic release of macrophage-derived HB-EGF, enhance DDR in neighboring cells suffering from DNA damage. Consequently, HB-EGF-treated cells exhibit higher double-strand break (DSB) rejoining and display lower levels of residual DSBs. *In vivo*, macrophage depletion or blocking HB-EGF activity in diethylnitrosamine (DEN)-treated mouse livers resulted in higher levels of non-repairable DSBs.

This study establishes for the first time that macrophages, acting through the activation of the EGF:EGFR cascade, constitutes an important and novel extrinsic, cell-non-autonomous component of the DDR. Hence, in addition to the autonomous mechanism of DNA damage response and repair that transpires inside the cell, there are cell-extrinsic or cell non-autonomous systemic mechanisms influencing the response to DNA damage. Such systemic regulation of the DNA damage response, which impacts a wide range of cells, is likely to contribute to a range of diseases and physiological events, such as cancer and **aging**.

Indeed, we further demonstrated that while HB-EGF enhanced DNA double strand break repair, chronic age-dependent secretion of pro-inflammatory cytokines (specifically TNF α and IL-1 β), induced by gut microbiota, impaired the beneficial effect of EGFR signaling on DDR. Consequently, this process accounts for age-dependent decline in DNA damage repair capacity.

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Morphology-Driven High-Plex Spatial Analysis of Tissue Microenvironments with the GeoMx™ Digital Spatial Profiling

Alexandre Darmoise

Historically, immunohistochemistry and immunofluorescence have been used to assess spatial heterogeneity of proteins and nucleic acids in tissue specimens. However, these techniques are of limited utility due to restricted dynamic range, difficult quantification and limited multiplexing capability.

NanoString's GeoMx™ Digital Spatial Profiling (DSP) is a novel, non-destructive, highly multiplexed assay for the digital characterization of protein and RNA expression from spatially discrete regions of interest (ROI) in FFPE tissue sections. GeoMx™ DSP can simultaneously quantify 96 protein targets and 1,000s of transcripts in multiple ROI and for up to 20 slides per day. Current applications include biomarker discovery, mechanism of action studies, and rare cell characterization.



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Inflammation and Immunity – Friends or Foes?

Emerging Roles for Eosinophils in the Tumor Microenvironment

Ariel Munitz

Immunotherapies targeting T lymphocytes are revolutionizing cancer therapy yet they only benefit a subset of patients, especially in colorectal cancer (CRC). Thus, knowledge regarding additional cells in the tumor microenvironment (TME) is urgently required. Eosinophils are bone marrow-derived cells that have been largely studied in the context of allergic diseases and parasite infections. Despite the fact that tumor-associated eosinophilia has been described in various solid tumors including CRC, fundamental knowledge is still missing regarding their activities and even the basic question of whether the TME promotes eosinophil recruitment without additional manipulation (e.g. immunotherapy) is unclear.

Herein, we report that eosinophils are swiftly recruited into developing tumors during induction of inflammation-induced CRC and in *Apc^{min/+}* mice, which develop spontaneous intestinal adenomas. Using adoptive transfer and cytokine neutralization experiments, we demonstrate that the TME supported prolonged eosinophil survival independent of IL-5, a key eosinophil survival cytokine. Tumor-infiltrating eosinophils consisted of degranulating eosinophils and were essential for tumor rejection independently of CD8⁺T cells. Transcriptome and proteomic analysis revealed a functionally distinct IFN- γ -linked signature for intratumoral eosinophils that was noticeably different from that of macrophages. Our data establish key anti-tumorigenic roles for eosinophils in CRC. These findings may facilitate the development of new pharmacological treatments unleashing anti-tumor responses by eosinophils especially in CRC patients displaying eosinophilia.



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Host-Pathogen Interaction

IFN β is a Novel Effector Cytokine in Resolving Inflammation

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The engulfment of apoptotic leukocytes (efferocytosis) by macrophages during the resolution of inflammation is essential for tissue homeostasis and results in macrophage reprogramming/immune-silencing. However, a distinct subset of resolution phase macrophages loss their phagocytic capacity following intense efferocytosis, and hence were termed satiated macrophages. Here, we show using an unbiased RNA-Seq analysis that satiated macrophages express a distinct IFN- β -related signature. Consequently, we determined IFN- β is a bona fide pro-resolving cytokine that exerts various inflammation-terminating functions *in vivo* and *ex vivo*, such as enhancing PMN apoptosis and promoting macrophage efferocytosis as well as their reprogramming to pro-resolving phenotypes. Thus, IFN- β is a novel multi-pronged effector cytokine in resolving inflammation.



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Visualizing the Immune Response

A distinct subset of Th1 cells express the high-affinity Fc γ receptor and exert antibody-mediated cytotoxic activity in solid tumors

Yaron Carmi

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While a high frequency of T helper 1 (Th1) cells in tumors is associated with improved cancer prognosis, this benefit has been attributed to supporting the cytotoxic activity of CD8⁺ T cells and macrophages. In studies of the effect of T cells on antibody-driven immunity, we found a remarkable synergy between CD4⁺ T cells and tumor-binding antibodies. This surprising synergy was mediated by a small subset of tumor-infiltrating CD4⁺ T cells that express the high-affinity Fc γ R for IgG (Fc γ RI) in both mouse and human patients. These cells efficiently lyse tumor cells coated with antibodies through concomitant crosslinking of their T cell receptor (TCR) and Fc γ RI. By expressing Fc γ RI and its signaling chain in conventional CD4⁺ T cells, we successfully employed this mechanism to treat established solid cancers. Overall, this discovery sheds new light on the biology of this previously unknown T cell subset, their function during tumor immunity and their potential use in immunotherapy.

Microenvironment and Immuno-Oncology

Neutrophils as modulators of the immune tumor microenvironment

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Tumor-associated neutrophils (TANs) make up a significant portion of the immune cell infiltrate in many cancers, and the exact mechanisms by which these cells affect tumor progression are gradually discovered. It is now well accepted that neutrophils also play a key role in multiple aspects of cancer biology. We have studied the many different ways by which TANs impact other tumor-infiltrating immune cells. TANs actively secrete cytokines and chemokines, modifying the recruitment and polarization of various immune cells in the tumor microenvironment, acting as regulators of the immune system.

In several studies we have done, we isolated TANs from lung adenocarcinoma primary tumors, and evaluated their capacity to recruit, activate or inhibit immune cells *in vitro*, i.e. on cells isolated from spleens of tumor-bearing mice. We further assessed the *in vivo* effects of TANs, e.g. by using neutralizing antibodies to TANs or to specific chemokines/cytokines.

In previous work we demonstrated the active secretion of CCL17 by TANs followed by active recruitment of CD4⁺ T-regulatory cells (T-regs) into the tumor microenvironment. Depletion of tumor neutrophils strongly reduced the chemoattraction of T-regs. Recruiting T-regs to the tumor site induces immunologic self-tolerance and impairs immune response to tumor cells. In another study TANs isolated from murine tumors promote immunosuppression by strongly inducing CD8 T-cell apoptosis. The TNF α pathway in TANs is critical for the induction of apoptosis, involving the production of NO, but not ROS. Interestingly, TANs were found to have contradictory effects on CD8 T-cells, capable of activating these cells, but specifically induce apoptosis only of non-activated CD8⁺CD69⁻ cells.

In our recent unpublished work, TANs isolated from primary tumors in LLC tumor-bearing mice, were found to attract significant amounts of monocytes (CD11b⁺Ly6C⁺), dendritic cells (CD11b⁺CD11c⁺) and B cells (CD45⁺C19⁺B220⁺). Neutrophil depletion significantly reduces the amount of tumor-infiltrating B cells. Our data suggest that TNF α , but not CXCL12, CXCL13 or CXCL9 play a major role in B cell recruitment by TANs. Accordingly, TANs isolated from TNF α -KO mice attracted significantly less B cells *in vitro*. We further identified subsets of B cells infiltrating the tumors and attracted specifically by TANs. We found tumor-infiltrating B cells to be mainly composed of B220⁺CD1d⁺CD23⁻ and B220⁺CD1d^{dim}CD23⁺. Isolated TANs attracted splenic B220⁺CD1d⁺CD23⁻ cells but also appear to have the capability to activate splenic B cells, increasing significantly their expression of CD1d. Deeper characterization of these B cells subsets is currently being completed. Mounting evidences have recently documented a role for B cells in modulating the immune response to tumors. Our data suggests an active role of TANs in the differential recruitment and activation of B-cells in the tumor microenvironment.

Our studies elucidate the chemotactic forces played by neutrophils affecting immune-cells infiltration into the tumor and their activation, promoting or inhibiting the establishment of a permissive tumor microenvironment. These important mechanisms provide us with a deeper understanding of the ways these cells support or fight cancer, ultimately helping develop new strategies to direct the immune system against the tumor.

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Host-Pathogen Interaction

STING Promotes Macrophage-Mediated Resolution of Inflammation Through IFN β Production

Sergei Butenko

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Introduction: The engulfment of apoptotic leukocytes (efferocytosis) by macrophages during the resolution of inflammation is essential for tissue homeostasis and results in macrophage reprogramming/immune-silencing. Previously, a distinct subtype of resolution-phase macrophages characterized by decreased expression of CD11b, arrest of efferocytosis (satiation) and enhanced reprogramming into pro-resolving and anti-fibrotic phenotypes was identified. These satiated macrophages display increased production and secretion of the immunomodulatory cytokine IFN β .

Materials and Methods: To elucidate the role of the intracellular adaptor protein stimulator of IFN genes (STING) in macrophage production of IFN β and its consequences, satiated macrophages were sorted from zymosan-A induced peritonitis and the activation of the STING pathway in these macrophages was examined. In addition, resolution phase macrophages were recovered from WT and STING^{-/-} mice, and analyzed for their cytokine production, efferocytosis, and reprogrammed phenotype using flow cytometry, WB, ELISA and fluorescent microscopy.

Results and Discussion: Here, we show that satiated macrophages display increased activation of STING, Tank binding kinase (TBK) 1, and IRF3 concomitantly with increased expression of IFN β and ISG15. However, IFN β levels were reduced in peritoneal exudates from STING^{-/-} mice. Moreover, activation of the STING-IFN β pathway, macrophage efferocytosis, reprogramming and responsiveness to apoptotic cells were all diminished in STING^{-/-} resolution phase macrophages, and rescued, at least in part, by exogenous IFN β .

Conclusions: Thus, our findings indicate that STING is an essential mediator in driving IFN β expression and secretion by satiated macrophages and consequently in shaping macrophage function and phenotype changes during resolving inflammation.

Host-Pathogen Interaction, Lymphocyte Activation & Exhaustion, Microbial Infections, Resistance & Immunity

Brain-mediated regulation of oral tolerance

Maria Krot^{1,2}

Introduction: Immune cells in the gut are exposed daily to a range of foreign antigens that are mostly innocuous (e.g. dietary antigens). Accordingly, inflammatory immune reactions towards orally consumed antigens are prevented by an active process named oral tolerance. This process requires the generation of a local immunosuppressive environment in the gut, conditioned by cytokines, such as transforming growth factor- β (TGF- β) and interleukin-10 (IL-10). Although the mechanisms of oral tolerance were extensively studied, the factors that regulate the formation of a tolerogenic state in the gut (e.g. TGF- β secretion) remain unknown. Here, we introduce the involvement of the gut-brain axis in the regulation of the intestinal environment and oral tolerance establishment. Our hypothesis is based on the fact that whenever we consume food, the brain receives sensory information (e.g. taste, odor) that can predict whether it is safe or harmful (e.g. sour taste can indicate fermented food). Thus, the brain can offer valuable information required for the formation of an adequate immune response.

Materials & methods: In this study, we examined whether food-related sensory information encoded by the insular cortex in the brain can affect the intestinal immune response towards ingested antigens. Using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) that allow us to control neural activity, we were able to manipulate neurons in the insular cortex and evaluate the impact on the ensuing immune response in the gut and the development of oral tolerance.

Results & discussion: We show that neurons in the mid-posterior region of the insular cortex (mpIC), an area known to integrate sensory and visceral information, respond to oral consumption of a novel antigen. Inhibition of this neural activity affects the intestinal immune response towards the antigen and attenuates oral tolerance evaluated by the delayed-type hypersensitivity model. In contrast, activation of the mpIC promoted tolerogenic conditions in the gut by increasing the proportion of TGF β -expressing immune cells.

Conclusion: Collectively, our results suggest that although oral tolerance is generated in the gut, it can be regulated centrally, specifically by the insular cortex in the brain. This offers new mechanistic insight to understand pathologies in which oral tolerance is disrupted (e.g. food allergies) and novel potential therapeutic interventions by targeting the brain.



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Microenvironment and Immuno-Oncology

Shaping the Inflammatory Niche: Cancer-Associated Fibroblasts Facilitate Breast Cancer Metastasis

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Mortality from breast cancer is almost exclusively a result of tumor metastasis. Formation of a hospitable microenvironment in the target distant organ is required for the establishment of metastases. Cancer-associated fibroblasts (CAFs) are prominent players in the microenvironment of many primary tumors, including breast cancer. However, the role of CAFs in the formation of a permissive metastatic niche is still largely unresolved. To characterize the co-evolution of CAFs during the formation of lung metastases, we isolated lung fibroblasts in an unbiased manner from normal mice, or from mice with micro- or macro-metastases and profiled their transcriptome by RNA-Seq. Data analysis revealed that fibroblasts in the lung metastatic niche are transcriptionally dynamic and plastic. Characterization of the most prominent transcriptional programs indicated that the main tasks operative in metastases-associated fibroblasts include extracellular matrix remodeling, stress response and shaping the immune milieu at the metastatic niche.



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Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment, Precision in Personalized Cancer Immunotherapy

UVB-induced tumor heterogeneity directs immune response in melanoma

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Clonal neoantigen burden is associated with improved response to immune-therapy, however, the underlying functional basis for this relationship in melanoma remains unclear. Here we study this question in a novel, controlled experimental UVB mouse melanoma model that enables one to study the effects of intra-tumor heterogeneity on tumor aggressiveness and immune response, independently of tumor mutational burden (TMB). The induction of UVB-derived mutations in parental melanoma cell-lines gives rise to high TMB tumors that are highly aggressive accompanied by decreased anti-tumor activity of tumor infiltrating lymphocytes (TILs). However, strikingly, UVB single-cell derived melanoma clones with high TMB levels but reduced Intratumor heterogeneity (ITH) are swiftly rejected. Their rejection is accompanied by increased TILs reactivity, increased CD8+ T cell core infiltration and a less suppressive microenvironment. Using phylogenetic tree analyses and mixing experiments of 20 single cell UVB clones that lie along the phylogenetic tree, we further systematically tease apart two main characteristics of tumor ITH: we show that tumor rejection is inversely associated with the number of clones forming the tumor and their diversity. Notably, these results are recapitulated and reinforced in the analysis of melanoma patient data, both in terms of their survival with or without immune check point therapy. Taken together, our results highlight the central importance of clonal mutations in robust immune surveillance and the need to quantify the heterogeneity of patient tumors, a central determinant of their survival and response to checkpoint blockade.

Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment

Alternative Splicing Of The Receptor SLAMF6 Reveals a Novel Regulatory Mechanism Of T Cell Activation And Can Be Used For Cancer Immunotherapy

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Introduction: SLAMF6 is a homotypic receptor abundantly expressed on CD8+ T-lymphocytes and thus of interest for its role in anti-tumor response. Recently we evaluated two isoforms of the *SLAMF6* gene: The 'canonical' sequence, and *SLAMF6*^{Δ17-65}, missing part of exon-2. In this work we set to evaluate the immune modulatory role of the long and the short isoforms of SLAMF6 and test their effect on anti-tumor immunity.

Materials: SLAMF6 splice isoforms were identified by RT-PCR and in RNA-seq databases from human donors and lymphoid cell lines. Melanoma lines aberrantly expressing each isoform were produced to generate a model of trans-activation of T cells via SLAMF6. In parallel, selective expression of *SLAMF6*^{Δ17-65} in lymphocytes was achieved in Jurkat cells using CRISPR-Cas9 genome editing. To identify transcript levels of SLAMF6 isoforms in both healthy individuals and cancer patients receiving PD1-inhibitors, samples were collected from patients before and during anti-PD1 therapy, cells were sorted to CD8+ subsets and qPCR was performed. Finally, to shift the splicing, antisense-oligonucleotides (ASO) were transfected into Jurkat cells using electroporation.

Results: Using existing databases and our-own human-derived T cell samples, we showed that all SLAMF6 isoforms are constitutively apparent on T-cells. However, different isoform ratios were found in CD8+ subsets, determined by their differentiation states. In melanoma patients receiving PD-1 inhibitory antibody, a transition was noted in isoforms ratio, favoring a rise in the shorter isoform, which was most emphasized in patients with auto-immune side effects (n=7).

Melanoma cells aberrantly expressing canonical SALMF6 had distinct inhibitory effect on cognate TILs. However, to our surprise the opposite was observed with *SLAMF6*^{Δ17-65}. Melanoma expressing this isoform enhanced IFN-γ production by cognate TILs significantly and reproducibly.

In line with this, the exclusive expression of the short isoform in Jurkat cells was associated with a three-fold increase in IL-2 secretion.

Lastly, using ASO designed to target SLAMF6 splice junctions, we managed to shift the splicing in Jurkat cells, increasing the short isoform on the account of the canonical isoform. As an outcome, cells expressing higher levels of *SLAMF6*^{Δ17-6} had a significantly improved function.

Conclusion: We showed that *SLAMF6*^{Δ17-65} isoform has a strong agonistic effect on T cell activation while its canonical isoform is an inhibitor. The change in isoform ratio observed during anti-PD-1 therapy may suggest a new regulatory mechanism that T cells adopt along their activation. The agonistic effect achieved by splice-diverting ASO may be exploited in the future for cancer immunotherapy.



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CAR and CTL Therapy in Cancer

The pioneer round of translation and MHC-I peptides presented to cytotoxic T lymphocytes

Hadas Weinstein^{1,2,3}

Cytotoxic T lymphocytes (CTLs) detect disease-associated antigens through the presence of unique peptides displayed at the cell surface by MHC-I molecules. To allow immune surveillance, the entire pool of the cell's proteins is constantly subjected to the MHC-I processing and presentation machinery. Perhaps the earliest protein products of most cellular genes are synthesized during the pioneer round of translation (PRT), a key step in nonsense-mediated mRNA decay (NMD) which allows scanning of new transcripts for the presence of a premature termination codon (PTC). It has been demonstrated that at least some PRT degradation products can be targeted to MHC-I presentation thus T cell activation.

To gain new insight into this putative PRT-to-MHC-I route we established an experimental system based on two pairs of reporter genes, where the two genes in each pair encode an identical fusion protein between a model antigenic peptide and EGFP, one of which harbors a PTC. We expressed these genes in different mouse and human cell lines and confirmed enhanced NMD activity for the PTC(+) gene in each pair by monitoring the effect of cycloheximide on the level of the respective mRNA. We then exploited several strategies for establishing the ratio between level of peptide presentation and total amount of protein product. We consistently observed significantly higher ratios for the PTC(+) mRNAs compared to the PTC(-) ones, pointing to correlation between the turnover of otherwise identical proteins and the fate of their template mRNA. Using confocal microscopy we showed a clear link between NMD, the presence of misfolded EGFP polypeptides and enhanced MHC-I peptide presentation. Our research provides, for the first time, strong evidence that peptides derived from degraded PRT products that are not defective, can still be efficiently directed to the MHC-I processing and presentation pathway for T cells. As the PRT does not discriminate between defective and correct transcripts and all newly synthesized mRNAs in the cell are potential substrates for scanning, our work suggests that the PRT can be an important source for MHC-I peptides which represent all cellular proteins.

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Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment,
Lymphocyte Activation & Exhaustion

Induction and transcriptional regulation of the co-inhibitory gene module in T cells

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Expression of co-inhibitory receptors, such as CTLA-4 and PD-1, on effector T cells is a key mechanism for ensuring immune homeostasis. Dysregulated co-inhibitory receptor expression on CD4⁺ T cells promotes autoimmunity while sustained overexpression on CD8⁺ T cells promotes T cell dysfunction or exhaustion, leading to impaired ability to clear chronic viral infections and cancer. Here, we used RNA and protein expression profiling at single-cell resolution to identify a module of co-inhibitory receptors that includes not only several known co-inhibitory receptors (PD-1, Tim-3, Lag-3, and TIGIT), but also a number of novel surface receptors. We functionally validated two novel co-inhibitory receptors, Activated protein C receptor (Procr) and Podoplanin (Pdpn). The module of co-inhibitory receptors is co-expressed in both CD4⁺ and CD8⁺ T cells and is part of a larger co-inhibitory gene program that is shared by non-responsive T cells in multiple physiological contexts and is driven by the immunoregulatory cytokine IL-27. Computational analysis identified the transcription factors Prdm1 and c-Maf as cooperative regulators of the co-inhibitory module, which we validated experimentally. This molecular circuit underlies the co-expression of co-inhibitory receptors in T cells and identifies novel regulators of T cell function with the potential to regulate autoimmunity and tumor immunity.



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Genomic Instability, Cancer Signaling and Cancer Secretome

p53 deregulation in cancer: cell-autonomous and non-autonomous implications

Moshe Oren¹

The TP53 gene, encoding the p53 tumor suppressor, is the most frequently mutated gene in human cancer: TP53 mutations occur in about half of all cancer cases. Often, these are missense mutations, associated with accumulation of large amounts of the mutant p53 protein within the cancer cells. In addition to abrogating p53's tumor suppressor activities, such mutations can also endow the mutant p53 proteins with novel oncogenic gain-of-function (GOF) activities. p53 has a variety of cell autonomous activities, which presumably prevent the cell from becoming cancerous, and which are subverted when the TP53 gene acquires mutations. In addition, p53 can also exert a variety of non-cell autonomous effects. Thus, analysis of TCGA data from a number of different cancer types suggests that p53 mutations, either through loss of wild type p53 activity or/and oncogenic GOF, may quench the anti-tumoral immune response, making the tumors more "immune cold" and probably favoring their escape from immune attack. Additionally, we found that p53 can act as a non-cell autonomous tumor suppressor in normal stromal fibroblasts, restricting the ability of those fibroblasts to support tumor growth. Interestingly, in cancer-associated fibroblasts p53 is rewired, and instead of acting as a tumor suppressor it becomes a non-cell autonomous tumor promoter – without undergoing any mutation. Computational analysis suggests that such non-mutational conversion of wild type p53 into a "pseudomutant" state occurs also in a fraction of human tumors that retain non-mutated p53, and contributes to tumor aggressiveness. Thus, p53 can act through both cell autonomous and non-cell autonomous mechanisms, both in the cancer cells and in the cancer microenvironment.



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Metabolism and the DNA-Damage Response

Fer and FerT sustain the metabolic plasticity of metastatic non-small cell lung cancer cells

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Metabolic reprogramming and acquired metabolic plasticity are hallmarks of the ability of metastatic cancer cells to survive and disseminate under hostile and stressful growth conditions. Therefore, deciphering these processes is of great importance and bears potential translational and therapeutic implications. The intracellular tyrosine kinases Fer and its sperm and cancer specific variant, FerT, accumulate in various subcellular compartments and associate with complex I (Comp. I) of the mitochondrial electron transport chain (ETC) in sperm and cancer cells. Here, we show that metastatic non-small cell lung cancer (NSCLC) cells (H358) devoid of Fer and FerT (H358 Δ Fer/ Δ FerT), are strictly dependent on glucose supplementation and exhibit an elevated glycolytic flux. Unlike their parental cells, H358 Δ Fer/ Δ FerT cells fail to rely on glutamine for their growth, and in the absence of glucose they demonstrate increased ROS production and induction of a DNA damage response. This response was accompanied by onset of apoptosis and attenuation of cell-cycle progression, thereby leading to a severely impaired growth. Selective knock-out of Fer while maintaining the expression FerT, restored the ability of the cells to rely on glutamine supplementation, but impaired their capacity to upregulate compensatory glycolysis under hypoxic conditions. Notably, cells lacking Fer and FerT failed to upregulate Hif1/2 alpha under hypoxia. Strikingly, while absence of Fer and FerT severely attenuated the progression of H358 tumors in-vivo, the presence of FerT without Fer eliminated the growth of NSCLC xenografts in mice. Thus, unbalanced expression of Fer and FerT impedes the development of metastatic NSCLC tumors.



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Microenvironment and Immuno-Oncology

Cancer-associated fibroblast heterogeneity in breast cancer

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Tumors are evolving ecosystems, in which malignant and non-malignant cells engage in complex interactions. An overarching challenge in the field of tumor microenvironment is to understand how stromal cells are rewired to become protumorigenic. Recently we have shown that a stress response driven by the master transcriptional regulator heat shock factor 1 (HSF1) plays a major role in this evolution, enabling the rewiring of fibroblasts into protumorigenic cancer-associated fibroblasts (CAFs). Here we explore the heterogeneity of CAF rewiring and the role of stress responses in enabling this heterogeneity.

We demonstrate by single-cell transcriptomics of mouse tumors that CAF rewiring is highly heterogeneous, and that distinct subtypes of CAFs exist in breast cancer. These cluster into two major subtypes, the relative abundance of which changes with tumor progression and metastasis. Using an evolutionary theory to infer tasks performed by these CAF subtypes, we find a division of labor between CAFs performing tasks such as ECM remodeling, biosynthesis, catabolism and growth factor signaling. We further explore the effect of CAF rewiring on cancer phenotypes by targeted dissection of specific stress responses, and unravel a striking effect of stromal HSF1 on extracellular matrix (ECM) assembly. Moreover, we find crosstalk between the ER stress response and the stromal HSF1 program. These findings provide important mechanistic insights into the altered homeostasis of the tumor ecosystem and the complexity of tumor heterogeneity.



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CAR and CTL Therapy in Cancer, Precision in Personalized Cancer Immunotherapy

CARTIV - a novel platform for regulation of gene expression under the control of inflammation-induced promoters

Yariv Greenshpan

Harnessing the immune system to eradicate cancer is becoming a reality in recent years. The ability of immune cells to identify and destruct cancerous cells within the body is showing superior potency; however, such great power also possesses a practical hazard. For example, the usage of engineered immune cells, such as Chimeric-Antigen-Receptor T-cells (CAR-T), is facing the danger of an overt life-threatening immune response. Critically, several patients had already died following adoptive transfer of CAR-T cells. We hypothesize that engineering CAR-T cells to express the activating-receptor within Tumor Microenvironment (TME), but not in normal tissues, will bring a novel safety mechanism. Improved control of the expression of the engineered chimeric receptor is needed to reduce the risks of the life-threatening hazard. This may open the therapeutic window for many tumor-antigens that has low expression on normal cells. We have developed a novel platform for regulation of gene expression under the control of inflammation-induced promoters, together with a Tet-On response circuit. Several promoters were tested in human cells, demonstrating functional abilities to respond to major inflammatory cytokines, and to their combinations, representing TME. Few promoter which showed superior activity were tested in human primary T cells and one was able to activate human primary T cells by controlling the expression of a Herceptin based CAR. This novel platform can improve the various types of CAR-T cells, as well as other engineered immune cells that will provide improved focused activities against their targets within the body.

Microenvironment and Immuno-Oncology, Cancer Metastasis, State-of-the-Art Methodologies in Research, Bioinformatics, Big Data and Cancer

A single-cell atlas of metastatic breast cancer charting oncogenic transcriptional programs in malignant cells and the tumor microenvironment

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Metastatic breast cancer (MBC) remains a leading cause of cancer-related death among women due, to the ubiquitous evolution of drug-resistance. While recent studies have begun to elucidate the genomics of metastatic breast cancer (MBC), the transcriptional programs that drive the drug-resistant phenotype remain poorly understood.

We prospectively collected biopsies from patients with MBC augmented by detailed clinicopathologic features, including treatment and response characteristics. We profiled 29 biopsies by single-cell RNA seq, as well as bulk RNA-seq and whole-exome sequencing on an additional 207 biopsies (ongoing biopsies collection). We analyzed these data to generate an atlas, delineating the cell-types, cell-states, and transcriptional programs.

We profiled 100,470 single-cell transcriptomes and generated a comprehensive MBC atlas of the tumor and tumor microenvironment (TME). We next inferred cell types and programs associated with clinicopathologic characteristics. For example, we found significant differences in the TME of liver metastases compared to other sites, consistent with immunosuppression in the hepatic space. In particular, liver metastases were depleted in activated B-cells with lower expression of CXC chemokine receptors, and several activation-related chemokines, and their infiltrating T-cells expressed lower levels of effector and cytotoxicity markers (Odds-ratio (OR)=4.07, p-value (p)= 7.29e-07, PMID: 28052254) including CD8, CD3, beta chemokines, perforin, granulysin, and granzymes, and in addition - exceptionally low expression of TCR signaling genes and antigen processing genes including B2M and several HLA genes (OR=32.1, p=7.26e-07, PMID: 28052254).

To increase our power to make clinically relevant associations, we performed a joint analysis of the single-cell and bulk RNA-Seq data, to identify malignant programs related to specific oncogenic mutations, with implications for metastatic and drug-resistance phenotypes. For example, we characterized the oncogenic prog associated with activating estrogen receptor (ESR1) mutation (ESR-mut). As expected, ESR1-mut prog overlaps with many known ER and luminal B markers (OR=5.8, p=2.3e-11, PMID: 11823860). ESR1-mut prog also included specific Interferon-stimulated genes (ISGs) – IFI6, ISG15, IFIT1, STAT1, which are associated with tamoxifen resistance (OR=10.36, p=0.00036, PMID 17016442) and extracellular matrix-mediated regulation of apoptosis (OR=8.24, p=0.0028, PMID 17016442). These ISGs are predictive of poor prognosis among endocrine-treated patients (HR=1.69, p=1e-04, n=929, kmplotter). The ESR1-mut prog also included genes associated with cell-migration (SOX9, AGR2, TXNIP, and several S100 genes). This suggests a role for ESR1 mutation in pathogenicity, beyond ligand-independent activation of ER signaling. We similarly recovered additional mutation-specific oncogenic programs, including for RB1, TP53, GATA3, FOXA1, HER2, and FGFR mutants, forming a compendium of in-vivo oncogenic signatures.

To the best of our knowledge these data represent of the first integration of single-cell and bulk RNA-seq data in MBC, resulting in a comprehensive single-cell-resolution transcriptional atlas, and a catalog of drug-resistance oncogenic programs with implications for immunotherapy and precision-oncology.

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Cancer Metastasis

Sex-related perturbations in schizophrenia and bipolar disorder brains reflect microRNA-mediated cholinergic/neurokine interactions

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Recent reports revealed massive overlaps between the molecular pathologies of schizophrenia (SCZ) and bipolar disorder (BD), but did not address the prominent clinical differences between men and women patients with these syndromes and the corresponding neurotransmitter and transcript regulators of their biological features. Here, we report sex-specific divergence of central cholinergic transcript networks in large patient brain datasets. Connectome analyses based on single cell- and small RNA-sequencing highlighted the gp130-family neurokine pathway controllers of immune functions, the LHX6 and LHX8 transcription factors that can drive neuronal cholinergic differentiation, the circadian regulators CLOCK and RORA, and the microRNA hsa-miR-125, -520, and -1275 families. Moreover, exposing the female human originated LA-N-2 neuronal cells to the neurokine `ciliary neurotrophic factor` induced the transcriptional network surrounding acetylcholine (ACh) function, including the choline acetyltransferase (ChAT) and its intronic vesicular transporter (vAChT), the high affinity choline transporter (HACU, aka SLC5A7), muscarinic and nicotinic ACh receptors and the ACh hydrolyzing enzyme acetylcholinesterase (AChE), which we showed to be suppressed by miR-125b-5p. Independent patient datasets implicated miR-regulated cholinergic-neurokine interactions in SCZ and BD in the sex-related and circadian control of cholinergic neurons, opening new venues for seeking sex-related biomarkers and therapeutic targets, also in other transmitter systems and diseases.



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Host-Pathogen Interaction

Exploiting an Achilles' Heel of Cancer with a Novel Oncolytic Virus

Marcelo Ehrlich

Selective pressure applied by immune surveillance mechanisms may modify expression or function of regulators of cell autonomous immunity in cancer cells. Such modified expression alters interactions between cancer cells and the immune component of the tumor microenvironment, and weakens antiviral responses of cancer cells. However, the prevalence of such modifications, their molecular underpinnings, the degree of coordination in altered expression of different genes, and their effect on susceptibility of tumors to viral oncolysis, are uncharacterized. In this study, we analyzed gene expression and promoter methylation employing The Cancer Genome Atlas skin melanoma database, and identified and coordinated alterations in expression of STAT1-related genes, including regulators and effectors of antiviral responses. To probe if such changes affect the susceptibility of melanoma cells to viral oncolysis, we employed B16F10 murine melanoma as tumor model, immortalized mouse skin fibroblasts as control, and the oncolytic Epizootic Hemorrhagic Disease Virus-Tel Aviv University (EHDV-TAU). B16F10 cells were responsive to interferon stimuli but failed to elicit interferon responses upon challenge with EHDV-TAU, resulting in productive viral infection and oncolysis. Antiviral signaling in B16F10 was restored via pre-incubation with interferon, which elevated expression of RNA viral sensors. In mice, intra-tumoral injection of EHDV-TAU reduced tumor growth in treated and distal tumors and enhanced infiltration of effector immune cells; both in accord with its ability to induce tumor cell oncolysis and stimulation of anti-tumor immunity.

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Inflammation and Immunity – Friends or Foes?

Synergism between human apoptotic cell infusion and human chimeric antigen receptor (CAR)-T therapy in fighting solid human tumor in SCID Bg mice

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Robust results from treatment of hematological malignancies with CAR-T were not replicated to date in solid tumors. We present a new mechanism of action which increases CAR-T efficacy in solid tumors. HeLa-CD19 was stably transduced with pLenti-PGK-V5-Luc-Neo and CAR was prepared using 3rd generation CD19-CAR plasmids. SCID-Bg mice were injected intra-peritoneally with human HeLa-CD19-luciferase cells, apoptotic cells or vehicle, and CD19-CAR T cells or mock T cells. Mice survived 30±5 days, and mock treatment non significantly ameliorated their survival to 34±4 days. CAR T cell therapy significantly ameliorated their survival to 55±11 days. Single-cell analysis confirmed by flow cytometry revealed that macrophages that were associated with anti-tumor activity, completely disappeared during tumor progression, and reappeared during successful CAR T therapy. Apoptotic cells injected during tumor progression were able to stabilize the presence of macrophages as confirmed by single cell and flow cytometry analysis, and synergize with the anti-tumor CAR-T cell effect, resulting in significantly increased anti-tumor macrophage population and increased survival to 75±10 days (p<0.05).



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Host-Pathogen Interaction, Microbial Infections, Resistance & Immunity

The Ebola-Glycoprotein Modulates the Function of Natural Killer Cells

Avishay Edri^{1,2}

The Ebola virus uses evasion mechanisms that directly interfere with host T-cell antiviral responses. By steric shielding of HLA-I, the Ebola glycoprotein (GP) blocks interaction with T-cell receptors (TCRs), thus rendering T cells unable to attack virus-infected cells. It is likely that this mechanism could promote increased natural killer (NK) cell activity against GP-expressing cells by preventing the engagement of NK inhibitory receptors; however, we found that primary human NK cells were less reactive to GP-expressing HEK293T cells. This was manifested as reduced cytokine secretion, a reduction in NK degranulation and decreased lysis of GP-expressing target cells. We also demonstrated reduced recognition of GP-expressing cells by recombinant NKG2D and NKp30 receptors. In accordance, we showed a reduced mAb-based staining of NKG2D and NKp30 ligands on GP-expressing target cells. Trypsin digestion of the membrane-associated GP led to a recovery of the recognition of membrane-associated NKG2D and NKp30 ligands. We further showed that membrane-associated GP did not shield recognition by KIR2DL receptors; in accordance, GP expression by target cells significantly perturbed signal transduction through activating, but not through inhibitory, receptors. Our results suggest a novel evasion mechanism employed by the Ebola virus to specifically avoid the NK cell immune response.

Microenvironment and Immuno-Oncology

Are we mature enough to apply anti-IL-1 β therapy in cancer patients?

Ron N. Apte

Introduction- Targeting the tumor microenvironment, except of immune checkpoint blockade, is not yet widespread. Interleukin-1 (IL-1) is an abundant cytokine in tumor sites and it controls the inflammatory pro-invasive and immunosuppressive nature of the microenvironment. It activates the microenvironment and also affects the malignant cells. We have studies how the secreted form of IL-1, i.e., IL-1 β affects the microenvironments of primary breast tumors and lung metastases and whether its neutralization will affect invasiveness.

Materials and Methods- We have used the model of murine 4T1 breast cancer cells, which represents the equivalent of human triple negative breast cancer (TNBC). Upon orthotopic injection of 4T1 cells, local primary tumors develop followed by spontaneous metastasis to the lungs.

Results and Discussion- In wild-type (WT) mice, progressive tumors developed and induced spontaneous lung metastasis, while in IL-1 β knockout (KO) mice, we observed initial tumor growth followed by regression, no formation of lung metastases and development of resistance to a challenge with the malignant cells. We further studied the role of microenvironment IL-1 β on inflammation/immunity, emphasizing early events (days 10-14 after inoculation), which are critical for the outcome of the malignant process. In WT mice, early IL-1 β -induced and CCL2-mediated recruitment of inflammatory monocytes was potent, however, impaired in IL-1 β KO mice. Also, IL-1 β -induced *in-situ* differentiation of inflammatory monocytes into IL-10-secreting immunosuppressive TAMs, by CSF-1 and other mediators, was potent in WT mice and marginal in IL-1 β KO mice. The relative abundance of inflammatory monocyte-derived IL-12 producing DCs was much higher in tumor sites in IL-1 β KO mice, as compared to WT mice. In IL-1 β KO mice, activated CD8⁺ in tumor cell deposits were abundant and resulted in its regression. IL-1 β neutralization induced only partial anti-tumor effects, but its combination with anti-PD-1 antibodies, completely inhibited tumor growth. In WT mice with large tumors, in which the primary tumor was resected, and were subsequently treated with anti-IL-1 β and anti-PD-1 antibodies, lung metastasis was significantly reduced.

Conclusion- Treatment of minimal residual disease (MRD), after tumor debulking, enables targeting of the microenvironment, also with anti-IL-1 β anti-PD-1 antibodies, can be effective for the prevention of tumor recurrence and metastasis.

Microenvironment and Immuno-Oncology, Cancer Metastasis

Heparanase2 (Hpa2) Promotes a Higher Degree of Cell Differentiation by Inducing Sox2 Expression in Head and Neck Carcinoma

Miriam Gross-Cohen

Introduction. Heparanase is an endoglycosidase that cleaves heparan sulfate (HS) side chains of proteoglycans, activity that is highly implicated in tumor metastasis and angiogenesis. Heparanase2 (Hpa2) is a close homolog of heparanase that lacks intrinsic HS-degrading activity but retains the capacity to bind HS with high affinity, possibly leading to inhibition of heparanase activity. In head and neck cancer patients, Hpa2 expression is markedly elevated, correlating with prolonged time to disease recurrence and inversely correlating with tumor cell dissemination to regional lymph nodes, suggesting that Hpa2 functions as a tumor suppressor. The molecular mechanism associated with favorable prognosis following Hpa2 induction is unclear. We found that Hpa2 regulates selected genes that affect tumor vascularity, tumor fibrosis, ER-stress, and apoptosis, together resulting in tumor suppression. Furthermore, Hpa2 overexpression induces cellular differentiation thereby maintaining a more normal epithelial phenotype correlated with low tumor grade and better prognosis.

Materials and Methods. In order to better elucidate the mode of Hpa2 action as a tumor suppressor in head and neck cancer, we applied gene array, gene silencing, CRISPR/Cas9 and RNA seq methodologies.

Results and discussion. RNA was extracted from control (Vo) and Hpa2-overexpressing FaDu cells and gene array was utilized to assess differences in gene transcription. This system was preferred because tumor growth was markedly attenuated in FaDu cells overexpressing Hpa2 (Gross-Cohen et al, Cancer Res, 76: 2791-801, 2016). Among other genes, we found and validated that the expression of sex-determining region Y-box 2 (Sox2) was markedly increased in cells overexpressing Hpa2. Moreover, Hpa2 gene editing (CRISPR/Cas9) was associated with reduced Sox2 levels, further supporting the notion that Hpa2 regulates Sox2 expression.

To reveal the significance of Sox2 for Hpa2-mediated tumor attenuation, we applied si-, and shRNA to silence Sox2 expression. Interestingly, Sox2 gene silencing was associated with reduced levels of cytokeratins 13 and 15 shown to be induced in FaDu cells overexpressing Hpa2. This suggests that the pro-differentiation function of Hpa2 is mediated, at least in part, by Sox2. Furthermore, Sox2 overexpression decreased while Sox2 silencing increased xenograft growth by 2.

Although Sox2 may have proto-oncogenic roles in various malignancies, several studies indicate that Sox2 is down-regulated in some malignancies and is often associated with better prognosis of head and neck cancer patients.

Conclusions. Our results further elucidate the mechanism by which Hpa2 functions, apparently involving the regulation of selected genes that affect tumor vascularity, tumor fibrosis, and cell differentiation, in part via up-regulation of Sox2, thus expanding the repertoire of Hpa2 functions as a tumor suppressor.

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Microenvironment and Immuno-Oncology

An Anti-Metastatic Role for Macrophage-Derived PROS1

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Introduction

Host macrophages were shown to support primary tumor growth and contribute to increased metastasis through various secreted molecules, which modulate inflammation. Protein S (PROS1) expressed by immune cells, activates the TAM family of receptor tyrosine kinases, comprising of TYRO3, AXL and MERTK, to suppress inflammation in immune cells. Therefore, their inactivation is thought to mount a significant anti-tumor immune response, and subsequent inhibition of tumor growth. Here, we investigate the role of the TAM agonist PROS1 in macrophages as a mediator of inflammation, and its impact on tumor growth and metastasis.

Materials and Methods

We genetically deleted PROS1 expression in myeloid cells (Pros1-cKO) by crossing Pros1^{fl/fl} mice to LysM-Cre⁺ mice. Pros1-cKO mice and littermate controls were challenged with syngeneic Lewis Lung Carcinoma (LLC) or orthotopic mammary breast AT3 tumor cells. Three weeks later, we assessed primary tumor growth and lung metastasis, and performed histological and molecular analysis for gene expression by RT-qPCR. The impact of PROS1 on inflammatory gene expression by bone marrow-derived macrophages (BMDMs) was evaluated by RT-qPCR and ELISA. The effect of BMDM-derived PROS1 on BMDM-secreted factors and on tumor cells was assessed both in-vitro and in-vivo following incubation of conditioned medium with cancer cells.

Results and Discussion

Primary tumor size in control and in Pros1-cKO mice was similar, By contrast, lung metastasis was significantly enhanced in Pros1-cKO mice using both lung and breast cancer models, pointing to an anti-metastatic effect of PROS1. Deletion of PROS1 in the myeloid compartment alone was sufficient to induce inflammation in the lungs of Pros1-cKO mice, with increased levels of pro-inflammatory cytokines. Moreover, PROS1-deficient macrophages increase tumor cell aggressiveness and lung colonization through secreted factors, and induce oncogenic pathways in both lung and mammary tumor cells. Finally, supplementation of exogenous PROS1 rescues this phenotype both in-vitro and in-vivo.

Conclusion

PROS1 in the host macrophages attenuates inflammation and is a potent anti-metastatic factor, implying its possible implementation in inhibiting metastasis.

Microenvironment and Immuno-Oncology

Pre-Metastatic Niche: *In Vivo* Tissue Changes in Mechano-Structure by Chemotherapy-Induced Tumor-Derived Microparticles

Daphne Weihs¹

Introduction/h3

The mechanics of the tumor microenvironment greatly affects the cell and whole tumor growth rate, the tumor cells' invasive capacity, and their survival following treatment. Following chemotherapy, tumor cells respond by shedding increased numbers of tumor-derived microparticles (TMPs), i.e. 0.1-1 μm extracellular vesicles. The TMPs support tumor growth, e.g. by contributing to rapid mobilization of bone marrow derived proangiogenic cells (BMDCs) to the tumor site and likely also to potential metastatic sites. The BMDCs, with other local cells, can modify the microenvironment, making it more favorable to tumor cell seeding. Here, we evaluate effects of TMPs and TMPs from paclitaxel-treated breast cancer cells on physical and mechanical structure of potential pre-metastatic sites (i.e. lung, liver) in cancer-free mice.

Materials and methods/h3

We collected TMPs from growth media of murine 4T1 breast cancer cells, exposed to Paclitaxel or untreated controls. Taxol or untreated TMPs are injected into a mouse's tail vein and PBS injection is used as a negative-control mouse-group. After several weeks, lungs and liver are harvested from the mice and 100 μm thick slices are cut by microtome. We measure the tissue-slice mechanics (e.g. elasticity and stiffness) of the three groups using shear rheometry.

Results and Discussion/h3

Mouse lungs and liver are viscoelastic with combined liquid- and solid-like responses. The elasticity and viscosity is Taxol-TMP untreated-TMP control, and reduces under strain such that all samples reach similar mechanics under high (physiological) strain; Taxol-TMPs samples were significantly more uniform across slices and mice. We note that strain-induced changes in mechanics are mostly reversible, indicating that samples are unbroken, yet can potentially restructure differently; differences are smaller in the Taxol-TMP samples. Reduced resistance and increased reversibility under mechanical strain suggests changed ECM organization and structure under TMP-Taxol, which we also establish by changes in ECM composition and structure (e.g., collagen, fibronectin).

Conclusion/h3

TMPs and especially chemotherapy induced TMPs induce mechano-structural changes in tissue (with no cancer in the body). Those changes may prepare tumor-favorable pre-metastatic niches, as escape-sites for tumor cells; thus, chemotherapy may potentially promote metastasis.



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Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment

The role of the CXCL10-CXCR3 axis in directing the biological function of anti-tumor CD8+ T cells

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Chemokines are small (~8-14 kDa) secreted proteins, structurally similar to cytokines. They regulate cell trafficking through interactions with a subset of seven-transmembrane G protein-coupled receptors (GPCRs). Almost 20 years ago in attempt to comprehend what makes few chemokines, and not many others, key targets for neutralization in inflammatory- autoimmunity we raised the hypothesis that few chemokines should be considered as “Driver chemokines” as aside for their chemotactic properties they also direct the biological function of immune cells. Our major study focused on CXCR3 and its ligands. CXCR3 is a chemokine receptor with three known ligands CXCL9, CXCL10 and CXCL11. Seventeen years ago, we have reported that CXCL10 polarizes and potentiates effector CD4+ T cells, thus its neutralization suppresses autoimmunity. Later we observed that CXCL11 induces FOXP3-negative T cells to restrain inflammation and thus the CXCL10/CXCL11 balance regulates the dynamic of adaptive immunity. Focusing on CXCL10 we further examined its role in cancer diseases. While vast majority of chemokines that are produced by cancer cells support tumor development either directly or by recruiting myeloid derived suppressor cells, and tumor associated macrophages to support tumor progression, CXCL10 suppresses tumor growth. On the mechanistic basis we found that this includes direct suppression of tumor growth, and potentiation of anti-tumor CD8+ T cells.

Cancer Metastasis

FAK Family Kinases: The Yin and Yang of Breast Cancer Metastasis

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Introduction

Metastatic dissemination of cancer cells from the primary tumor and their spread to distant sites in the body is the leading cause of mortality in breast cancer patients. Invasive cancer cells penetrate through the basement membrane and into blood vessels using invadopodia, F-actin rich protrusions with matrix-degrading activity. Focal adhesion kinase (FAK) and proline-rich tyrosine kinase 2 (Pyk2) define a distinct family of non-receptor tyrosine kinases that are highly expressed in invasive cancers, but the molecular mechanism by which these proteins regulate invasion of breast cancer cells to neighboring tissues and their subsequent metastatic dissemination was unclear until recently.

Materials and Methods

To identify potential substrates and interactors of Pyk2 in invadopodia, we used high-throughput protein array screening followed by bioinformatic analysis. The role of Pyk2 in invadopodia formation and activation was examined by invadopodium precursor formation, matrix degradation, and barbed end formation assays. 2D and 3D motility assays were used to compare the roles of Pyk2 and FAK in cell migration and invasion. *In vivo* metastasis assays and intravital imaging of single tumor cells within the primary tumor of a live mouse were used to follow the behavior of cancer cells in their natural microenvironment, which was validated by transcriptomics and network analysis. Patient database analysis was used to determine correlation of gene expression to metastasis.

Results and discussion

Using high-throughput screening, we identified cortactin as a substrate and interactor of Pyk2 in invadopodia. Pyk2 colocalizes with cortactin to invadopodia of invasive breast cancer cells, where it mediates EGF-induced cortactin tyrosine phosphorylation both directly and indirectly via Src-mediated Abl-related gene (Arg) activation, leading to actin polymerization in invadopodia, ECM degradation, and tumor cell invasion. While Pyk2 regulates tumor cell invasion by controlling invadopodium-mediated functions, FAK controls invasiveness of tumor cells by regulating focal adhesion-mediated motility and functions. These observations were validated by tumor transcriptome and network analysis, revealing the *in vivo* molecular mechanisms and signaling pathways by which FAK family proteins coordinate the regulation of breast cancer metastasis. Further breast cancer patient database analysis revealed that increased mRNA co-expression of Pyk2, Arg and cortactin, or high expression of both Pyk2 and FAK, is associated with significantly lower distant metastasis free-survival.

Conclusion

Our data suggest that Pyk2 and FAK may be used to predict the probability of distant metastasis and that inhibition of either or both kinases may potentially reduce invadopodia-dependent functions and consequent breast cancer dissemination.

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Cancer Metastasis

Improving biomarkers of metastasis in colorectal and breast cancer patients through perioperative blockade of inflammatory-stress responses in two phase-II clinical trials

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Introduction: Excess release of prostaglandins and/or catecholamines was suggested to mediate pro-metastatic effects of stress and surgery. In our previous work in six tumor models we showed that a perioperative combined blockade of prostaglandins and catecholamines improves postoperative resistance to tumor metastasis and/or overall long-term survival rates.

Material and method: Here, in two recent biomarker clinical trials (randomized and placebo-controlled) in colorectal (CRC, n=34) and in breast cancer patients (BC, n=38), we tested the combined perioperative use of the COX2-inhibitor, etodolac, and the β -blocker, propranolol, scheduled for 20 perioperative days, starting 5 days before surgery. Excised tumor tissue was subjected to histological analyses, whole genome mRNA profiling, and transcriptional control pathways analyses. In BC patients, repeated blood samples were assessed for cytokine levels and immune indices.

Results and discussion: In both studies, (i) drugs were well tolerated and (ii) whole genome mRNA profiling of excised tumors showed decreased epithelial-to-mesenchymal transition (EMT); down-regulation of the transcriptional activity of CREB, NF κ B, GATA family, and STAT3; reduced presence of tumor-associated monocytes; and increased presence of NK cells (CRC) and B cells (BC). In blood samples of BC patients, treatment reduced serum IL-6 and CRP levels (starting before surgery) without affecting anti-inflammatory soluble factors (cortisol and IL-10), improved cytotoxicity markers in NK cells, and enhanced induced production of IFN γ and IL-12. The tumor proliferation marker Ki67 was tested and significantly reduced by drug treatment in BC patients; and in CRC patients three-year follow-up showed large but statistically insignificant improvement in disease free survival (DFS) (1/15 vs 5/19, treated vs placebo groups), suggesting the long-term safety and efficacy of the paradigm. Overall, these findings suggest a metastatic-reducing impact of this novel treatment, which should be tested in larger clinical trials.

Conclusion: Such an inflammatory-stress-reducing approach may enable us to exploit the critical perioperative period to reduce cancer recurrence, potentially leading to improved long-term survival rates.

Cancer Metastasis

Converting Invasive Breast Cancer Cells Into Adipocytes Inhibits Cancer Metastasis

Dana Ishay-Ronen^{1,2}

Introduction

Cancer cell plasticity facilitates the development of therapy resistance and malignant progression. Dedifferentiation processes such as epithelial-to-mesenchymal transition (EMT) enhance cellular plasticity facilitating in drug resistance and metastasis formation. Here, we hypothesize that a moment of cellular plasticity during cancer dissemination can be exploited therapeutically by forcing the trans-differentiation of breast cancer cells into post-mitotic adipocytes to inhibit cancer metastasis.

Material and method

Using established EMT models of murine mammary cancer cells, we studied the adipogenesis trans-differentiation potential of EMT-derived cancer cells versus their epithelial ancestors. We utilized established methods from adipogenesis studies to morphologically and functionally characterize cancer-derived adipocytes *in vitro*. The kinetics and gene expression regulation during cancer cell adipogenesis were analyzed based on RNA sequencing. Analysis of TGF β signaling pathway activation during adipogenesis of cancer cells revealed clinically relevant targets for the induction of adipogenesis *in vivo*.

Delineation of the molecular pathways underlying such trans-differentiation has motivated a combination therapy with a MEK inhibitor and a PPAR γ ligand in various mouse models of murine and human breast cancer *in vivo*. Direct cancer cell- adipogenesis, primary tumour invasiveness and metastasis formation were analyzed. To test the effect of adipogenic trans-differentiation as therapeutic option for breast cancer, we used a patient-derived xenograft (PDX) mouse model of human breast cancer in preclinical settings.

Results and discussion

Our results emphasize that cancer cells undergoing EMT gain trans-differentiation potential and can undergo cellular conversion into post-mitotic and functional adipocytes. Epithelial cancer cells lack this potential, supporting the notion that an EMT coincides with increased cell plasticity. In various preclinical mouse models, a combination therapy of FDA-approved drugs provoked the conversion of invasive and disseminating cancer cells into post-mitotic adipocytes leading to the repression of primary tumor invasion and metastasis formation.

Conclusion

The results underscore the pivotal role of cancer cell plasticity in malignant tumor progression and reveal the therapeutic potential that lies in the inhibition of cellular plasticity by forcing post-mitotic adipogenesis.



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Visualizing the Immune Response, Lymphocyte Activation & Exhaustion

Characterization of TCR-activated Signaling Complexes and Microclusters

Lawrence Samelson

Laboratory of Cellular and Molecular Biology, Center for Cancer Research, National Cancer Institute, NIH, USA

Engagement of the T cell receptor (TCR) results in the formation of microclusters containing many signaling molecules making protein:protein interactions to form signaling complexes. Subsequent signaling events lead to structural rearrangements that produce an immune synapse between the T cell and antigen presenting cell. Microclusters form within seconds of TCR engagement and are the basic signaling units required for T cell activation. However, the key sequence of events by which T lymphocytes establish signaling microclusters remains unclear. To understand the key events that lead to microcluster formation, we imaged the TCR, the transmembrane signaling molecule LAT and many other signaling molecules using super-resolution microscopy. We were able to describe the kinetics of microcluster structure formation. We have extended this work to study the mechanisms by which microcluster formation occurs. In complementary work we have spent many years reconstituting signaling complexes in vitro and have performed biophysical studies to define protein interactions at a molecular level.



Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment, Cancer Metastasis, Precision in Personalized Cancer Immunotherapy

An Exceptional Response to Immunotherapy Doublet in Combined Hepatocellular Carcinoma-Cholangiocarcinoma

Esther Tahover

We present here a case of a previously healthy 67-year-old male. He presented with severe abdominal pain and weight loss of 25%. Liver function tests, alpha-feto protein (AFP) and Carbohydrate antigen 19-9 (CA19-9) were within normal limits, and CA125 was 5 times upper normal limit (ULN). Computed tomography showed liver masses and enlarged retroperitoneal lymph nodes. Biopsy from a liver mass showed Combined Hepatocellular Carcinoma (HCC)-Cholangiocarcinoma (CC) (CHC).

This a rare tumor, with an incidence of less than 10% of primary liver tumors. In whole exome analysis, there were numerous ubiquitous mutations shared by HCC and CC that suggest the monoclonal (bipotent cell) origin. Mutated genes identified were speculated to contribute to distinct differentiation of HCC and CA. EpCAM was highly expressed, implying the stemness.

There are no guidelines or randomized trials regarding treatment. In an analysis of 36 patients who were treated with chemotherapy, the progression-free survival was 2.8 months, with an overall response rate of 5.6%. Targeted agents had minimal effect on survival.

In our case, extensive testing was performed. No genomic alterations were identified, tumor mutational burden was low and microsatellite status was stable. 7 of 9 immune checkpoint genes were overexpressed. Also, there was a variant in CDK12 which was shown to be associated with elevated neoantigen burden and may predict benefit from immune checkpoint therapy.

The patient began immunotherapy with ipilimumab and nivolumab for 4 treatments followed by nivolumab, which he is continuing reaching 14 months from diagnosis. No treatment side effects have been noted otherwise than hypothyroidism and Addison`s disease which are being treated with hormonal replacements.

His clinical response was dramatic, he regained all the lost weight, and discontinued high dose opiate treatment. ECOG performance status improved from 2 to 0. Repeated PET-CT showed all tumor sites decreased in size and uptake, ca125 decreased from 6 times ULN to normal, alkaline phosphatase from 3 times ULN to normal, LDH which was 8 times ULN decreased by 75%.

To summarize, we present here an exceptional case of a rare tumor, where the patient had a clinical, laboratory and radiological response and a significant improvement in quality of life, suggesting that these tumors are sensitive to immunotherapy. To our knowledge no published cases have been treated with immunotherapy doublet.

	Before immunotherapy	Last test (after 11m)	Normal range
Alk phos	456	83	38-150
GGT	588	72	15-73
Ca125	210	21	2-35



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Bioinformatics, Big Data and Cancer

Identifying drug combinations for the treatment of resistant acute myeloid leukemia patients

Robert Hanes

Introduction: AML is the most common form of acute leukemia in adults and is classified into 14 different groups depending on its genetic makeup. The heterogeneity of AML is defined by a diverse genetic landscape and is one of the major challenges in finding an effective treatment option for patients that do not respond to current standard treatment, which remained unchanged for the past 20 years and is applied across all 14 different groups not considering the genetic diversity of this disease.

Material and method: Therefore, we aim to identify the characteristic properties behind the response and resistance of individual patients, who might not only develop resistance to standard treatment, but also to targeted therapy. The ability to predict the potential risk of resistance to a treatment and identify strategic and personalized treatment options for a group of individual patients is of substantial significance. We have screened a group of patients by assessing the sensitivity of primary patient-derived cancer cells *ex vivo* from individual AML patients and healthy donors to a panel of anticancer drugs.

Results and discussion: We observed that only a group of patient-derived cancer cells showed response to a number of drugs. However, the other group did not show any therapeutically relevant response to the most effective drugs indicating the potential of resistance in an eventual treatment. We are approaching this therapeutic issue through a systematic screening of synergizing drug combinations. We have been able to develop computational methods for personalized single and/or combinatorial drug-sensitivity screens from high-throughput experiments including randomized dispensing and automated deconvolution of big data for further qualitative downstream analysis.

Conclusion: We further aim to map potential genetic alterations and immunological phenotypes to drug response or resistance and to identify potential biomarkers through multidimensional data together with the implementation of automated high-throughput screening methods in the hope not only to predict response and resistance, but also identify strategic treatment options for individual AML patients.

Bio-Markers and Cancer Theranostics

The role of IgG Fc glycan in cancer

Inbal Farkash Paskal¹

Introduction

IgGs are structurally composed of variable fragment antigen-binding (Fab) domains that confer their binding specificity to the antigen, and an Fc domain that determine their effector function. The effector functions mediated by IgG rely on their interactions with Fcγ receptors (FcγRs) that are expressed on various immune cells. While the Fc portion is traditionally considered the invariant region of an IgG molecule, this domain displays considerable heterogeneity. This heterogeneity arises from the differences between the subclasses, allotypes and the composition of the attached glycan complex at the CH₂ domain. These variations result in selective engagement of particular classes of FcγRs with distinct effector activities.

While much progress has been made in our understanding the role of specific *human* IgG Fc glycans in mediating diverse immune responses, there is lack of knowledge regarding the heterogeneity of the *mouse* IgG Fc glycans, and their impact on the mouse antibody-mediated immune responses in general and during cancer development. The aim of this study is to decipher the mouse Fc fingerprint in naïve mice and in mice inflicted with different kinds of tumors. We will characterize how this affects the IgG Fc interaction with different FcγRs, and evaluate the overall degree of homology between mouse and human IgG glycosylation pathways and their immunological role.

Material and methods

We will initially perform a *mass spectrometry-based discovery* step characterizing the murine Fc N-linked glycoform repertoire. We will isolate IgG from serum of naïve mice and mice under different physiological conditions (vaccinated mice, tumor-bearing mice, autoimmunity models, etc.) and will pool them together for the discovery step. The discovery will identify the Fc glycans repertoire and will set the database to identify glycans in the following experiments.

We will next compare the Fc fingerprint (total IgG and relevant antigen-specific IgG) in healthy vs. tumor bearing mice and at different time points and assess whether it can serve as a diagnostic, prognostic or any other marker.

o Preliminary Results and discussion

Following the discovery step we have established the glycan repertoire that will serve for the future targeted analysis of our experimental samples. We have several glycan fingerprints of naïve mice that were analyzed following this discovery step. Interestingly, each IgG subclass displayed a unique Fc fingerprint, suggesting this process is a well regulated one. After establishing this step, we plan to analyze samples from tumor bearing mice and assess their significance.

o Conclusion

Fc glycoforms modulate the structure of the Fc to alternate between different FcγR types binding conformations. We saw that each IgG subclass has a unique Fc fingerprint, suggesting that different factors contribute to the final IgG and this is probably a tightly regulated process. We plan to further elucidate these factors and whether they can modulate or predict tumor progression.



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Microenvironment and Immuno-Oncology

The Effects Of Cellular Senescence In Endothelial Cells On Breast Cancer Development

Yael Gabai

During early stages of tumorigenic transformation, cells are exposed to various types of stress. In response, cells can undergo senescence to avoid uncontrolled cell divisions and tumor development. Senescent cells are thought to be non-dividing, yet their presence in the tumor lesion, or in the stroma, may influence cells in their environment through cytokine secretion or other means, and thereby exert either tumor-promoting or tumor-suppressing effects. Endothelial cells are an important component of the tumor stroma. These cells affect their environment by delivery of oxygen and nutrients, and also by secretion of growth factors and by immune cell recruitment. Modification of tumor microvasculature function can thereby affect cancer development. Here, we aim to uncover the consequences of senescence in endothelial cells within tumors. To do this, we generated transgenic mice that allow the induction of p16^{Ink4A}, a main activator of the senescence program, in the vasculature of mice that develop breast cancer. Strikingly, we found that p16^{Ink4A} activation in blood vessels of developing tumors leads to decreased numbers of proliferating cells in the lesions, without affecting vessel numbers. This suggests that endothelial senescence causes reduced support of tumor growth by the vasculature, either by influencing vessel structure and perfusion or by altering endothelial secretion of supportive angiocrine factors. Furthermore, we observed an elevation in interferon response markers in p16^{Ink4A}-induced mice, which implicate immune system involvement. We are currently studying this involvement to better understand the mechanisms by which senescent tumor endothelial cells affect tumor growth and progression.

Bio-Markers and Cancer Theranostics

The Role Of IgG Fc Fingerprint In Cancer

Inbal Farkash Paskal¹

Introduction

IgGs are structurally composed of variable fragment antigen-binding (Fab) domains that confer their binding specificity to the antigen, and a fragment crystallizable (Fc) domain that determine their effector function. The effector functions mediated by IgG rely on their interactions with Fcγ receptors (FcγRs) that are expressed mainly on immune cells. While the Fc portion is traditionally considered the invariant region of an IgG molecule, this domain displays considerable heterogeneity. This heterogeneity arises from the differences between the subclasses, allotypes and the composition of the attached glycan complex at the CH₂ domain of the Fc region. These variations result in selective engagement of particular classes of FcγRs with distinct effector activities.

While much progress has been made in our understanding the role of specific *human* IgG Fc glycans in mediating diverse immune responses, there is lack of knowledge regarding the heterogeneity of the *mouse* IgG Fc glycans, and their impact on the mouse antibody-mediated immune responses in general and during cancer development. The aim of this study is to decipher the mouse Fc fingerprint in naïve mice and in mice inflicted with different kinds of tumors, allowing for a more in depth mechanistic understanding of this fingerprint. We will characterize how this affects the IgG Fc interaction with different FcγRs, and evaluate the overall degree of homology between mouse and human IgG glycosylation pathways and their immunological role.

Material and methods

We initially performed a *mass spectrometry-based discovery* step characterizing the murine Fc N-linked glycoform repertoire. We isolated IgG from serum of naïve mice and mice under different immunological conditions (vaccinated mice, tumor-bearing mice etc.) and pooled them together for the discovery step. We have identified the Fc glycan repertoire which will be used as a reference library to identify glycans in the following experiments.

We plan to compare the Fc fingerprint (total IgG and relevant antigen-specific IgG) in healthy vs. tumor bearing mice, at different time points during disease progression and upon immunotherapy in order to assess whether it can serve as a diagnostic or prognostic marker.

Preliminary Results and discussion

Following the discovery step we have established the glycan repertoire and identified several Fc fingerprints that will serve for the future targeted analysis of our experimental samples. Interestingly, each IgG subclass displayed a unique Fc fingerprint, suggesting this process is well-regulated. Following, we plan to analyze samples from tumor bearing mice and assess the significance of Fc fingerprint in this context.

Conclusion

Fc glycoforms modulate the structure of the Fc moiety to accommodate different binding affinities to the FcγRs. We have demonstrated that in mice each IgG subclass has a unique Fc fingerprint, suggesting that different factors contribute to the final IgG functions and that this is probably a tightly regulated process. We plan to further elucidate these factors and whether they can modulate or predict tumor progression.

Inflammation and Immunity – Friends or Foes?

Defining cell-type specific Stat3 enhancers, their role in IBD development, and monocyte differentiation into colonic and ileal macrophages

Serkalem Ayanaw¹

Introduction

Macrophages are long-lived, tissue-resident phagocytes that, in most organs, are established prenatally. In the gut and other barrier tissues, however, macrophages display continuous replenishment by blood monocytes. To investigate what governs this steady state differentiation of monocytes into intestinal macrophages we employed a combination of cell ablation and precursor engraftment. We have identified factors associated with gradual adaption of monocytes to tissue residency in the ileum and colon. In the colon, monocyte-derived gut macrophages must sense IL-10 to maintain intestinal homeostasis. Mice with a macrophage-specific *Il10ra* deficiency develop severe colitis, as do children harboring *IL10RA* loss-of-function (LOF) mutations (Zigmond *et al.*, 2014, Glocker *et al.* 2009). Mechanistically, IL-10Ra deficient macrophages produce IL-23, which activates T_H17 cells to secrete IL-22, which is sensed by epithelial cells exacerbating intestinal inflammation (Bernshtein *et al.*, 2019). Interestingly, all the above cytokine responses require STAT3 for signaling and indeed Stat3B loci-associated single nucleotide polymorphisms (SNPs) were identified by GWAS as inflammatory bowel disorders (IBD) risk factors. In contrast, patients carrying these LOF mutations do not display IBD, but suffer from an autosomal dominant Hyper-IgE syndrome (AD-HIES). To understand, the underlying logic for this observation, we are in the process to define enhancer elements driving Stat3 expression in man and mice.

Material and methods

STAT3 regulatory elements and SNPs were retrieved from the GeneHancer (Fishilevich *et al* 2007) and UCSC genome browser. CRISPR/Cas9 technology is being used to generate mutant human cell lines and knock-out mice. To study monocyte differentiation into macrophages, monocytes were transferred into macrophage-depleted mice. Engrafted macrophages were then sorted and subjected to transcriptome profiling on various time points after transfer.

Results and discussion

Three out of 4 IBD-associated STAT3 SNPs were found to be located in regulatory elements, one of which (rs6503695) is predicted to be solely active in macrophages. Investigating monocyte differentiation, we identified a total of 634 and 539 genes, including transcription factors, involved in adaption to the colonic and ileal microenvironments, respectively.

Conclusion

Understanding cell-type specific Stat3 enhancers, particularly in myeloid cells, and differentiation of monocytes into gut macrophages might reveal critical pathways in the development of IBD. This will potentially allow the development of novel therapeutics.

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Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment, Microenvironment and Immuno-Oncology, Cancer Metastasis, Cancer Therapy: Advances in Drug Design and Delivery, Immuno-Oncology and the Microbiome

Stimulation of antineoplastic potency of targeted therapeutic drugs in combination with NKp44-derived peptide studied in tumor ex-vivo model

Muhammed Iraqi

Muhammed Iraqi[†], Priyanka Bolel[†], Nir Peled, Moshe Elkabets and Angel Porgador^{*}

Lung adenocarcinoma is one of the most common cancer worldwide with less than 30% of survival rate of nearly 5 years. In recent years, increasing understanding and characterization of cancer microenvironment resulted in the development of numerous cancer-specific targeted drugs as personalized medicine. Unfortunately, the lung cancer patient's survival rate remains low. This work will strongly prove the concept of combinatorial treatment of PCNA inhibitor peptide and anti-cancer targeted drugs using the Tumor Ex VIVO Analysis (TEVA) model, which can predict patient-specific drug response. Four Patient-derived xenografts (PDXs) were employed for TEVA. PDX's were cut into 2 x 2 x 2 mm³, explanted and treated with in-house designed PCNA targeting peptide, R11-NLS-pep8 (PEP8), combined with clinically resistant targeted therapeutic drugs based on genomics sequences for 24h. Results were analyzed by calculating TEVA Score based on immunohistochemistry staining of TUNEL and Ki67 (apoptosis and proliferation markers); showing that two patients out of four responded to the combination of PEP8 with targeted therapeutic drugs comparing to single treated explants. Response score for combinatorial treatment was 6 and 10 times more compared to the control whereas single treatment did not show a significant difference. In-Vivo experiments for LSE-Pt#1 resulted in suppression of the tumor growth after treating with PEP8 and targeted therapeutic drugs in combination. Overall our data provide new evidence that PCNA inhibitor peptide, PEP8, can enhance the efficiency of targeted therapeutic drugs for some patients.

Immunopathologies and Precision Medicine

Adoptive Approach to Cell-Supportive Therapy Based on Chimeric Receptors

Zoe Taylor

Type 1 diabetes mellitus (T1DM) is an immune-mediated disease resulting in the damage and destruction of pancreatic β -cells and eventual complete loss of endogenous insulin supply. It predominately develops at a young age and accounts for 5-10 % of the diabetic population. Regardless of the advancements in diabetes therapeutics and technologies, the majority of the cohort with T1DM do not achieve recommended glycemic goals and remain at high risk of developing microvascular complications, such as neuropathy, nephropathy and, retinopathy.

In healthy individuals, the incretin effect is responsible for approximately 70% of insulin secretion. Preliminary results have shown a possible defect in the incretin effect in patients with T1DM. The incretin hormone, glucagon-like peptide (GLP)-1, has the potential to provide a therapeutic therapy affecting the underlying pathophysiology and improving glycemic control in T1DM. GLP-1 agonists may be beneficial in both longstanding and new onset T1DM patients by reducing the insulin requirements in longstanding T1DM and perhaps even delaying the absolute dependence upon insulin administration in new onset T1DM. One of the main limitations of GLP-1 agonists is their short biological half-life.

Our study focuses on employing chimeric receptors to generate primary T-cell cultures, overexpressing various GLP-1R-agonistic vectors, known as TRAMMICS cells, with the ability to bind to and activate pancreatic islet β -cells resulting in the release of insulin and expectantly the proliferation of the pancreatic β -cells. The advantages of using T-cells for this concept include, their long retention time in the body, their ability to penetrate various tissues when activated, possible increased receptor cross-linking efficiency compared to soluble ligands and less side effects owing to it being a more targeted system.

In order to test our hypothesis *in-vitro* we generated target and effector cell lines overexpressing the GLP-1R and various GLP-1R-agonistic vectors such as, α -GLP-1R or double exendin-4. To date, we have demonstrated our effector BW-TRAMMICS cells have the capability to activate the GLP-1R on various targets expressing the GLP-1R by the release of insulin and mouse IL-2. We have also shown that our target cell lines are able to activate the effector TRAMMICS cells upon interaction, shown by the secretion of mouse IL-2.

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CAR and CTL Therapy in Cancer

Targeting Glycosylated Antigens on Cancer Cells Using Siglec-7/9 Based CAR T-Cells

Sara Meril

Chimeric antigen receptor (CAR) T-cells treatments demonstrate the increasing and powerful potential of immunotherapeutic strategies. Chimeric antigen receptor (CAR) treatment represents a promising treatment for hematological malignancies using genetically engineered T-cells. Still, more efforts are needed to develop efficient CAR-T cell approaches for the treatment of a broader spectrum of tumors. Indeed, cancer cells develop strategies to evade immune response such as the expression of inhibitory ligands such as hypersialylated proteins (sialoglycans) on their surface. These may be recognized by Sialic acid-binding immunoglobulin-type lectins (Siglecs) which are surface receptors found primarily on immune cells. For example, Siglec-7 and -9 are found on immune cells such as NK cells and dendritic cells and they can promote immune suppression binding to sialic acids expressed on target cells.

In the present study, we hypothesized that it is possible to use genetically engineered T-cells expressing Siglec-based CARs, enabling them to recognize and eliminate tumor cells, in a non MHC restricted way. Thus, we genetically modified human T-cells with different chimeric receptors based on the exodomain of human Siglec-7 and -9 molecules and selected optimal receptors. We then assessed their antitumor activity in vitro demonstrating the recognition of cell lines from different histologies. These results were confirmed in a tumor xenograft model in vivo exemplifying the potential of the present approach. Overall, this study demonstrates the benefit of targeting cancer-associated glycosylation patterns using immune cells receptors when expressed in human primary T-cells.

Cancer Metastasis, State-of-the-Art Methodologies in Research, Genomic Instability, Cancer Signaling and Cancer Secretome

Genetic Heterogeneity in Oropharyngeal Cancer Revealed by Single-Cell RNA Sequencing

Michael Mints¹

Introduction

Oropharyngeal squamous cell carcinoma (OPSCC) is a heterogenous tumour type due to the high mutation rate in HPV- and the process of viral integration in HPV+ tumours. This heterogeneity contributes to drug resistance and tumour progression. Single-cell RNA sequencing (scRNASeq) allows analysis of tumours with an unprecedented resolution, enabling identification of small but biologically significant populations of cancer and stromal cells that have an impact on prognosis and drug sensitivity.

Our aim was to prove the feasibility of large-scale scRNASeq in OPSCC through characterising these subpopulations and their functions in a large number of patients with the end goal of improved patient stratification to avoid overtreatment as well as identifying new treatment targets.

Materials and Methods

16 patients with OPSCC, 3 HPV- and 13 HPV16+, undergoing curative surgery 2018-19 were included in the study. Fresh tumour samples (and adjacent normal samples in three patients) were dissociated into a single-cell suspension. Cells were barcoded using the Chromium 10x system, followed by Illumina sequencing. The generated sequences were aligned to the human transcriptome as well as the transcriptomes of HPV 16, 18, 31, 33 and 35.

Cells were clustered and their gene expression patterns examined to define the major cell types. Cancer cells were defined by the presence of copy number aberrations (CNA) inferred through mRNA expression across chromosomal regions. Gene expression patterns recurring in cell populations from multiple patients were combined into metaprograms representing biological functions.

Results and Discussion

More than 60000 cells were identified and could be classified according to cell type. In cancer cells, metaprograms representing senescence, proliferation, epithelial-mesenchymal transition and stemness were found. Clonal tumour evolution could be traced through identifying cells with differing CNA in the same tumour.

Particularly interesting were the facts that one tumour classified as HPV+ by pathologists due to p16 expression could be reclassified as HPV- due to absence of HPV transcripts, while another tumour showed two highly distinct CNA patterns, suggesting two biologically unrelated tumours in the same location. We also found both HPV transcripts and CNA in epithelial cells from a pathologically normal sample taken beyond the surgical margin, suggesting cancer spread beyond the margin.

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Conclusion

This is, to date, the largest single-cell transcriptomic study of head and neck cancer. We provide a comprehensive map of the tumour ecosystem and identify distinct subpopulations of biological significance in all the major cell types that make up the tumour. Our re-classification of a p16-positive tumour, tumour evolution tracing and identification of cancer cell populations with different biological functions, as well as finding cancer cells beyond the surgical margins all highlight the potential for single-cell technology to be used in pathology for improved patient stratification and treatment selection.

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Cancer Therapy: Advances in Drug Design and Delivery

A Small Molecule ARTS Mimetics Degrade Both Major Anti-Apoptotic Proteins XIAP And Bcl-2 And Preferentially Kills Breast, Ovary and Kidney Cancer Cell Lines

Ruqaia Abbas

ARTS (Sept4_{i2}) is a pro-apoptotic and tumor suppressor protein. ARTS promotes apoptosis by directly binding to both major anti-apoptotic proteins XIAP (X-Linked Inhibitor of apoptosis protein) and Bcl-2 (B-cell lymphoma 2), leading to their degradation by the Ubiquitin proteasome system (UPS). Studies in mice and human patient samples show that ARTS expression is lost in various types of cancers. In addition, many types of cancers escape cell death by overexpressing XIAP and Bcl-2. Thus, these two proteins have become major targets for developing anti-cancer therapeutics. Here we describe the identification of a small molecule, AM (ARTS Mimetic), that specifically binds to XIAP, but not cIAP1. AM initiates UPS-mediated degradation of both XIAP and Bcl-2, resulting in caspase activation and apoptosis. Significantly, treating Sept4/ARTS-null MEFs with AM successfully decreased the levels of XIAP and Bcl-2, suggesting that AM indeed mimics the main function of ARTS in promoting cell death. In addition, overexpression of XIAP rescued HeLa cells from AM-induced apoptosis. This confirms that AM indeed acts through binding and degrading XIAP. Collectively, our results describe the first ARTS mimetic small molecule that provides a novel approach for developing anti-cancer drugs that act by dual degradation of both XIAP and Bcl-2.

Inflammation and Immunity – Friends or Foes?

IFN- β mediates drug-induced blockade of macrophage differentiation to osteoclasts

Gal Cohen^{1,2}

Introduction: Bone fractures are the biggest problem facing most of the ageing people with bone disease, especially those with osteoporosis. Bisphosphonates (BPs) is the most common drug prescribed to treat osteoporosis and to prevent the occurrence of bone fractures. However, such treatments can lead to Medication Related Osteonecrosis of the Jaw (MRONJ), that is defined as prolonged exposure of bone in the maxillofacial region following anti-RANKL and anti-VEGF therapy and appears following combined therapy, like with the BP zoledronic acid (ZOL) and dexamethasone (DEX). In healthy patients, wound healing is associated with secretion of pro-resolving cytokines, as IL-10 and TGF- β , that induce migration/generation of macrophage that promote injured tissue recovery and homeostasis. Recently, we found IFN- β to be a macrophage-derived effector cytokine in resolving inflammation that promotes macrophage reprogramming to anti-inflammatory phenotypes. In addition, IFN- β is a well-established inhibitor of osteoclast differentiation.

Material and method: RAW 264.7 or peritoneal macrophages were treated with ZOL/DEX for 4-24 hrs, and IFN- β expression was examined by Real Time polymerase chain reaction (RT-PCR) and Western blotting. In addition, the RANKL-induced differentiation of RAW 264.7 macrophages to osteoclasts was evaluated by TRAP assay following treatment with ZOL/DEX, IFN- β , anti-IFN- β , and STAT1/3 inhibition.

Results: Here, we found ZOL+DEX increased IFN- β expression by RAW 264.7 macrophages when treated with or without RANKL. Moreover, these drugs and IFN- β blocked RANKL-induced osteoclast differentiation, and IFN- β neutralization by antibodies reversed the ZOL+DEX effect. In addition, we found that early STAT1 or STAT3 inhibition did not affect ZOL+DEX blockade of osteoclastogenesis, while inhibition of STAT1, but not STAT3, partially restored osteoclastogenesis in IFN- β -treated macrophages. Finally, we found ZOL+DEX also induced IFN- β expression in peritoneal resolution phase macrophages, suggesting they might be used to resolve acute inflammation.

Conclusions: Altogether, our findings suggest MRONJ-inducing drugs block macrophage differentiation to osteoclasts through the induction of high IFN- β expression in macrophages. The induction of IFN- β expression is STAT-independent, while the IFN- β -mediated inhibition of osteoclastogenesis is mediated, at least in part by STAT1. Thus, anti-IFN- β agents could serve as therapeutic strategies for MRONJ disease.

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Immuno-Oncology and the Microbiome

The FcγRs-Dependent IgG-Mediated ADCC/ADCP Effector Functions Are Influenced by a Microbiome Modulation

Samuel Ovadia

Introduction: Monoclonal antibody (mAb)-based immunotherapies revolutionized cancer treatment over the last two decades. Based on their mode of action, we can classify these therapies into two groups: mAbs directly targeting the tumor, and those targeting immune checkpoint. The cellular receptor of antibodies, Fc-gamma receptors (FcγRs), play important role in mediating the therapeutic activity of mAbs from these two types. Recent studies demonstrated that the gut microbiota tightly regulates immunotherapies efficacy and toxicity. Here, we set out to decipher the role of the microbiome in the FcγRs-mediated anti-tumor function. We aim to correlate a microbiome disruption with a modulation of the FcγR-mediated IgG functions, in different immunological organs and immune context.

Material and Method: The gut microbiota was depleted via a broad cocktail of antibiotics diluted in the drinking water during either 2 or 4 week long. We analyzed by flow cytometry the FcγRs expression profile in spleen and blood, as well as in the B16F10 tumor microenvironment (TME).

Results and Discussion: In the spleen, microbiome depletion induces a significant downregulation of all activating-FcγRs and the FcγRIII density, at the Red-Pulp macrophages and neutrophils cell surface, respectively. These cells are known to mediate Antibody-dependent cell cytotoxicity/phagocytosis (ADCC/ADCP) effector functions in the spleen. Therefore, our molecular data suggest an impairment of these functions. We are currently addressing it by relevant in vivo experiments. In the blood, only the FcγRI density on monocytes is negatively impaired by the microbiome disruption, conversely to the FcγRIII that is upregulated at 2 week of treatment. Although the monocyte population is well described as effector of the IgG-mediated ADCC/ADCP, in contrast to the FcγRIII, the FcγRI seems barely involved in those functions. Finally, in the skin-B16F10 TME, we observed an increased density of all activating-FcγRs on the tumor-associated macrophages consequently to the microbiome depletion. We hypothesize that this may potentiate the FcγRs-dependent IgG activity within the TME.

Conclusion: Here, by linking the FcγRs expression pattern and the microbiome integrity, we gathered preliminary data suggesting a subsequent mAb-mediated effector function modulation. Our data imply on novel mAb's anti-tumor and toxic mechanisms, and may pave a way to optimize anti-cancer mAb-based immunotherapies.

Host-Pathogen Interaction

Constitutive Impaired Expression of Antiviral Effectors Sensitizes Melanoma Cells Viral Oncolysis

Hamutal Ben-Dov

Introduction:

Immunoediting of malignant cells commonly dampens the function of interferon (IFN) antiviral responses and the expression of IFN stimulated genes (ISGs), making cancer cells potentially susceptible for viral attacks. Oncolytic virotherapy exploits such characteristics to specifically infect and kill cancer cells and stimulate anti-tumor immunity. In recent years, the Ehrlich and Bacharach labs at the Tel Aviv University have developed a novel oncolytic virus, the Epizootic Hemorrhagic Disease Virus-Tel Aviv University (EHDV-TAU). Starting from the Ibaraki strain of EHDV2, and employing an *in vitro* evolution process in IFN-defective human prostate cancer cells (LNCaP), we have selected the EHDV-TAU clone. Initial characterization of EHDV-TAU showed a million-fold increased replication efficiency in LNCaP cells, selectivity towards cancer cells (vs. normal cells), and the ability to induce different forms of cancer cell death (including apoptosis and necroptosis).^{1,2} In the present study, we expand on published studies and probe for the efficiency and safety of EHDV-TAU with the B16F10 murine melanoma in immunocompetent C57BL6 mice.

Methods:

EHDV-TAU-mediated oncolysis of B16F10 was characterized *in vitro* and *in vivo*. The former line of experimentation, which employed immortalized skin fibroblasts (ISFs) as control, comprised measurements of viral RNA and proteins, production of infectious virions, and assessments of cell death. Moreover we also assessed the expression and function of mediators of innate immune antiviral responses, prior to, and following infection with EHDV-TAU. *In vivo* oncolysis assessed EHDV-TAU-mediated effects on tumor growth, mouse survival, and immune activation.

Results and Discussion:

The susceptibility of B16F10 melanoma cells to EHDV-TAU infection and oncolysis was markedly higher than that of ISF cells, with high cell death rate and increased formation of infectious particles. The increased susceptibility correlated with near-absent constitutive expression of viral sensors and ISGs, and with the inability to mount JAK/STAT-based antiviral responses upon exposure to EHDV-TAU. Preliminary analysis with inhibitors of DNA methylation and histone deacetylation suggest the role of epigenetic silencing of antiviral genes in B16F10 cells. Notably, infected B16F10 cells retained the ability to secrete immune-attractants such as CXCL1, CXCL2 and CXCL10, suggesting their ability to activate immune cells. Indeed, *in vivo*, EHDV-TAU markedly reduced tumor volume, enhanced survival and promoted intratumoral infiltration of effector immune cells.

Conclusions:

Our results suggest that EHDV-TAU may function as a novel and effective anti-tumor agent. Moreover, we propose that epigenetic silencing of antiviral genes may serve as a molecular mechanism for the acquired Achilles' Heel of cancer cells when challenged with oncolytic viruses.

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Host-Pathogen Interaction, Microenvironment and Immuno-Oncology, Precision in Personalized Cancer Immunotherapy

Intricate Interplay Between Androgen Receptor Signaling, Viral Infection and The Functionality of JAK/STAT and NF-kB Pathways

Lilach Barer

Introduction. In prostate cancer, tumor-promoting acquisition of androgen-independency may influence immunoediting via modulation of cell autonomous immune responses. Such responses, in which JAK/STAT/NF-kB signaling play central regulatory roles, concomitantly mediate the interaction of the cancer cell with the immune components of the microenvironment and determine its susceptibility to oncolytic viral infection. However, the mode, extent and consequence of crosstalk between androgen receptor (AR) signaling and inflammatory/antiviral responses is not known, and may determine the susceptibility of prostate cancer cells of different phenotype to oncolytic viruses.

Methods. Here, to dissect the mutual influence of AR signaling, antiviral responses and viral composition on oncolysis of prostate cancer cells we employed PC-3 metastatic prostate cancer cells subtypes that differ in AR expression, thus, mimicking the natural processes of prostate cell oncogenesis. Such cells, under different regimens of cytokine stimulation and/or activation state of AR and antiviral signaling pathways, were challenged with wild type and oncolytic Vesicular Stomatitis Virus (VSV) or with a novel oncolytic virus: the Epizootic hemorrhagic disease virus-Tel Aviv University (EHDV-TAU). Multiple parameters of infection, oncolysis, activation of signaling responses and regulation of gene were assessed.

Results. Overexpression of AR increased the constitutive expression of inflammation-related and interferon-stimulated genes (ISGs) in uninfected cells, indicative of crosstalk between AR and JAK/STAT/NF-kB pathways. Moreover, and in line with the increased ISGs expression levels, infection levels and signaling events that the infection evokes, were reduced in cells expressing higher levels of AR. Furthermore, and independently of AR expression status, while EHDV-TAU infection was markedly increased (as expected) by inhibition of JAK/STAT signaling, it was decreased upon NF-kB inhibition, revealing an unexpected dependency of virus replication on NF-kB signaling.

Conclusions and future directions. Intricate interplay between AR signaling, viral infection and the functionality of JAK/STAT and NF-kB pathways exist in prostate cancer cells. Consequences of such interactions should be taken into consideration while choosing virus-cell combinations and combination therapy settings.

Microenvironment and Immuno-Oncology, Cancer Metastasis

Elucidating the role of Heparanase-2 (Hpa2) in pancreatic cancer

Yasmin Kayal

Introduction. Heparanase is an endoglycosidase that specifically cleaves heparan sulfate (HS) side chains of proteoglycans that are abundantly present in the extracellular matrix (ECM) and on the cell surface. Heparanase activity is strongly implicated in tumor angiogenesis and metastasis attributed to remodeling of the subepithelial and subendothelial basement membranes. Heparanase 2 (Hpa2) is a close homolog of heparanase that lacks intrinsic HS-degrading activity but retains the capacity to bind HS with high affinity, thus competing for HS binding and inhibiting heparanase enzymatic activity. In head and neck cancer patients, Hpa2 expression was markedly elevated, correlating with prolonged time to disease recurrence and inversely correlating with tumor cell dissemination to regional lymph nodes, suggesting that Hpa2 functions as a tumor suppressor. The molecular mechanism associated with favorable prognosis following Hpa2 induction is unclear. Hpa2 nonetheless appears to down-regulate the expression pro-angiogenic genes (i.e., VEGF-A, VEGF-C) and to promote cell differentiation (i.e., E-cadherin, cytokeratins). Here, we examine the role of Hpa2 in pancreatic cancer.

Materials and methods. Human pancreatic tumor biopsies were subjected to immunostaining for Hpa2 and the immunostaining intensity was correlated to the clinical record of patient. Control (Vo) and Hpa2 over-expressing pancreatic carcinoma cell lines (i.e., Panc01, MiaPaca, Capan, AsPC, BxPC3), were examined for tumorigenic capacity. Luciferase-labelled cells were inoculated sub-cutaneously, intra-peritoneally, and orthotopically and tumor growth was followed by IVIS. We further examined Hpa2 mode of action as a tumor suppressor in pancreatic cancer, focusing on angiogenesis, lymph-angiogenesis, tumor fibrosis and ER-stress.

Results. Hpa2 was not detected in normal pancreatic ducts was its expression was noticeably increased in some pancreatic ductal adenocarcinoma. Notably, patients stained positively for Hpa2 survived longer than Hpa2-negative patients. Hpa2 over expression inhibited pancreatic tumor growth in different in-vivo models, and the Hpa2 over expressing tumors showed an increased level of ER-stress (i.e., Bip, CHOP, pPERK, pEIF2), likely leading to apoptotic cell death. Interestingly, ER-stress and hypoxic, each alone and in combination induced Hpa2 expression in several pancreatic cancer cell lines. Taken together, a feed-back cycle seems to persist, leading to continuous cell stress, leading to reduced tumor growth.

Conclusions. Different in-vivo models results suggest that Hpa2 functions as a tumor suppressor. In addition, both in-vivo and in-vitro experiments indicate that Hpa2 elicit ER-stress, thus providing a novel mechanism by which Hpa2 restrain tumor growth.



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Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment

Exploiting breakdown of self-tolerance to tissue-specific antigens for cancer immunotherapy

Ayelet Avin

The immune system is delicately balanced between its response to millions of diverse pathogens and its tolerance towards thousands of self-antigens. For this end, T lymphocytes are ‘educated’ in the thymus, a primary lymphoid organ, and undergo a process of selection by a rare population termed thymic epithelial cells (TECs). Specifically, medullary thymic epithelial cells (mTECs) play a key role in inducing central tolerance, by their unique capacity to express and present almost all self-antigens, including thousands of peripheral tissue antigens (PTAs). This ability was shown to be largely dependent on a single molecular factor - the autoimmune regulator (Aire). It is well established that Aire-deficient mice on NOD genetic background develop multi-organ autoimmunity, characterized by generation of autoantibodies against various tissue-specific self-antigens. In this regard, mTECs represent an interesting paradigm for the field of cancer immunology, as mTEC-driven induction of central tolerance to self-antigens and immune response against tumor-associated self-antigens could be viewed as opposite sides of the same coin. By harnessing targeted breakdown of self-tolerance to various tissue-specific antigens, as seen in the Aire-deficient mice, we aim to isolate highly specific autoantibodies, which could potentially be exploited as the “magic bullet” to ultimately defeat human tumors. This could present a novel immune strategy for diagnosing and treating cancer.

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Inflammation and Immunity – Friends or Foes?

Inhibition of IL-1 β in combination with conventional chemotherapy improves the outcome of CRC treatment

Mirel Bitton

The tumor microenvironment consists of tumor, immune, stromal, and inflammatory cells, which produce cytokines, growth factors and adhesion molecules that promote tumor progression and metastasis. IL-1 is an upstream cytokine that controls inflammation by inducing cytokine/chemokine networks and promoting immunosuppression in the tumor microenvironment, thus leading to tumor progression. We found that in mice deficient in IL-1 β , colorectal cancer develops more slowly than in control mice. In IL-1 β KO mice, we noted decreased angiogenesis and an increase in the immune response, such as recruitment of CD8-positive cells into the tumor site. Based on these results, we applied therapeutic approaches using anti-IL-1 β antibodies in mice that were injected orthotopically with murine colon cancer cells into the cecum. Antibodies were used alone or in combination with standard chemotherapy protocols. We found that anti-IL-1 β alone did not inhibit tumor growth but chemotherapy (FolFox) or a combination of FolFox with anti-IL-1 β antibodies led to a reduction in the size of the local tumor. Nevertheless, histological examination revealed a high number of micrometastases in livers of mice treated with only FolFox. Fewer micrometastases were found in untreated mice or in mice that received only anti-IL-1 β antibodies. Interestingly, there were dramatically fewer micrometastases in mice receiving the combination therapy. The appearance of liver macrometastases was observed only in mice treated with FolFox alone. To elucidate the mechanisms of chemotherapy-induced liver metastasis in our model, we assessed the expression of various pro-inflammatory cytokines in local tumors and at the metastatic site. We observed inhibition of IL-1 α and IL-1 β after administration of chemotherapy alone or in combination with anti-IL-1 β antibodies in local tumors. In contrast, in liver tissue, treatment with only FolFox induced increased expression and secretion of both IL-1 agonistic molecules and other pro-inflammatory molecules. On the other hand, combination treatment led to a decrease in local and systemic inflammation. It is clear that anti-tumor therapy is complex and varies depending on differences in the microenvironment of local tumors and metastasis. Addition of anti-IL-1 β antibodies to standard chemotherapy protocols may be a promising approach in patients with colorectal cancer for prevention of metastases development.



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Inflammation and Immunity – Friends or Foes?, Genomic Instability, Cancer Signaling and Cancer Secretome

Understanding the role of HSF1 in pro tumorigenic reprogramming of macrophages in cancer

Shimrit Mayer

The tumor microenvironment (TME) is a complex biological system of cells and extracellular proteins that support tumor formation and progression. Tumor associated macrophages (TAMs) are prominent components of the immune tumor microenvironment. Their presence is associated with poor prognosis as they were found to have pro-tumoral functions such as angiogenesis, remodeling of the extracellular matrix to aid invasion, motility, intravasation and immunosuppression. In the tumor, most macrophages display an anti-inflammatory (M₂-like) polarization state, suggesting that signals from the TME inflict a switch process that promotes a tumor supporting phenotype in these cells. This tumor supporting reprogramming process occurs in most TME cell types and the pathways responsible for it are not yet fully understood. We have recently shown that transcriptional reprogramming of fibroblasts into cancer-associated fibroblasts is mediated by the heat shock factor 1 (HSF1) transcription factor. HSF1 is a proteotoxic stress master regulator, and was shown to be an important player in the support of cancer cell survival and malignancy. Here we hypothesize that HSF1 may also play a complementary tumor-promoting role in tumor associated macrophages (TAMs). Using invitro and in-vivo models, we found different gene signatures between HSF1 WT macrophages and HSF1 null macrophages. Consequently, HSF1 might have an important role in macrophages polarization towards a tumor supporting state.

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Inflammation and Immunity – Friends or Foes?

Tumor-Stroma-Inflammation Networks Promote Aggressiveness in TNBC by Activating the Notch Pathway

Yulia Liubomirski¹

Triple-negative breast cancer (TNBC) is an aggressive disease with poor prognosis and high recurrence rate. The lack of known targetable molecules for this specific disease subtype emphasizes the great need for improved understanding of the mechanisms driving TNBC progression. Components of the tumor microenvironment play key roles in promoting disease progression in TNBC; thus, here, we took an integrative approach in which we investigated the combined effects of tumor-stroma-inflammation cross-talks on the pro-metastatic phenotype of TNBC cells. Major focus was put on tumor-promoting functions and molecular events exerted by TNBC cells in the presence of mesenchymal stem cells (MSCs) or patient-derived cancer-associated fibroblasts (CAFs), and their regulation by the pro-inflammatory cytokines tumor necrosis factor α (TNF α) and interleukin 1β (IL1 β) that are enriched in TNBC tumors.

Our findings indicate that co-cultures of TNBC:stroma cells (MSCs, CAFs) stimulated by TNF α , expressed exacerbated levels of the pro-metastatic chemokines CXCL8, CCL2 and CCL5, largely as a result of direct physical contacts between the tumor and stromal cells; similar findings were obtained in IL-1 β -stimulated TNBC:stroma co-cultures. Functionally, TNBC-MSC-TNF α networks increased endothelial cell migration and sprouting, and enhanced invasive and migratory properties of the tumor cells. Furthermore, these elevated pro-metastatic activities were reversible and largely depended on CXCL8-induced signaling, as indicated by using CXCL8 siRNA. Importantly, TNF α stimulation of TNBC:MSC co-cultures has increased the aggressiveness of TNBC cells in vivo, leading to higher incidence of mice with lung metastases .

Mechanistically, we found that activation of the Notch pathway has driven forward the migratory and invasive properties gained by TNBC cells that formed physical contacts with stromal cells in the presence of TNF α . Moreover, by using siRNA/CRISPR approaches, we demonstrated that TNF α has induced p65 activation in TNBC:MSC “Contact” co-cultures, which then has led to Notch1 activation; in turn, Notch1 up-regulated the expression of CXCL8 by the tumor-stroma-inflammation network. Consequently, CXCL8 promoted angiogenesis, as well as TNBC cell migration and invasion.

Overall, our findings identify novel findings on p65-Notch1-mediated tumor-stroma-inflammation interactions that promote pro-metastatic characteristics in TNBC; these findings pose the pro-inflammatory elements CXCL8/p65 and Notch1 as candidate targets whose combined inhibition may prevent metastasis-promoting activities of the most aggressive subtype of breast cancer.



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Visualizing the Immune Response

Mechanical Control of T cell Function by the Actin Cytoskeleton

Janis Burkhardt

The T cell actin cytoskeleton serves as an essential intermediate during multiple phases of the T cell response. The actin network binds to or corrals many signaling molecules, and since it functions as a mechanical unit, it can promote crosstalk among even spatially segregated receptors, e.g. TCR and integrins. For example, during T cell activation, TCR engagement induces actin polymerization, exerting forces that activate integrins. Since integrins also interact with actin, this creates positive and negative feedback loops that modulate TCR signaling. During T cell trafficking, engagement of β_2 integrins induces actin polymerization, which triggers downstream signaling via mechano-sensitive signaling intermediates. This process produces the polarized cell morphology needed for upstream migration under shear flow conditions, and generates the protrusive forces needed for transendothelial migration. Disruption of these events can be used to tune T cell trafficking in immunotherapy settings, to allow graft-vs-leukemia responses while ameliorating GvHD.

Cancer Metastasis

Nuclear Checkpoints for Melanoma and Breast Cancer Lung Metastasis

Ronen Alon¹

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Introduction

Metastasis involves cancer cell intravasation and extravasation from blood vessels at target organs. The mechanisms by which the nuclei of metastatic tumor cells squeeze through different vascular and epithelial barriers are poorly understood. The nuclear lamina attaches chromatin domains to the nuclear periphery and controls the mechanical properties of the nucleus and its crosstalk with the cell cytoskeleton. A-type lamins, lamin A and its splice variant lamin C, are key nuclear lamina proteins that control nucleus stiffness and regulate chromatin conformation. Reduced lamin A was reported in some aggressive cancers.

Results and Discussion

Using a new in vitro transendothelial migration (TEM) assay we found that melanoma and breast cancer cells slowly squeeze their nuclei and complete TEM through endothelial junctions irrespective of their relative lamin A/C levels, suggesting that lamin A/C dependent nuclear stiffening is not a rate-limiting step for tumor squeezing through endothelial cells. We currently explore whether reduced lamin A/C expression enhances the emigration of murine melanoma cells and breast cancer cells across lung capillaries and alveolar epithelial barriers of syngeneic mice in vivo. Emigration analysis is performed by new 3D imaging of fluorescently labeled cancer cells with high and low lamin A/C levels. This imaging is based on light sheet microscopy of lipid cleared lungs of recipient mice in situ labeled with anti-vascular CD31 mAb. We have recently found that reduced lamin A/C levels affect the constitutive heterochromatin of melanoma cells and accelerate cell cycle progression and tumor growth in vitro. Hence, we are currently testing if reduced levels of transcriptionally repressed chromatin also affect the growth of tumor cells with variable lamin A/C levels after they cross the lung vasculature and enter the lung parenchyma. Since reduced levels of lamin A/C in the nuclear lamina may also enhance nuclear rupture in tumor cells squeezing through confined and rigid spaces, we also assess if lamin A/C silencing in melanoma and breast cancer cells reduce their survival inside the lung parenchyma.

Conclusions

Our results suggest that subsets of tumor cells may benefit from reducing their nuclear content of lamin A/C both at the level of barrier crossing and at the level of post extravasation proliferation. These subsets may, however, need to harness machineries to repair their ruptured nuclear envelopes. How alterations in lamin A/C content contribute to epigenetic tumor heterogeneity and metastatic potential remains an open question.

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Mechanotransduction in the Immune System

Mechanotransduction as a novel immune checkpoint in NK cell cytotoxicity

Mira Barda-Saad

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Natural killer (NK) cells are a powerful weapon against viral infections and tumor growth. Although the actin-myosin (actomyosin) cytoskeleton is crucial for a variety of cellular processes, the role of mechanotransduction, the conversion of actomyosin mechanical forces into signaling cascades, was never explored in NK cells. Here, we demonstrate that actomyosin retrograde flow (ARF) controls the immune response of primary human NK cells through a novel interaction between β -actin and the SH2-domain containing protein tyrosine phosphatase-1 (SHP-1), converting its conformation state, and thereby regulating NK-cell cytotoxicity. Our results identify ARF as a master regulator of the NK cell immune response. Since actin dynamics occur in multiple cellular processes, this mechanism might also regulate the activity of SHP-1 in additional cellular systems.

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Mechanotransduction in the Immune System

Synthetic immune niche (sin) for enhancement of T-cell therapies

Benjamin Geiger¹

Introduction: Adoptive immunotherapy is based on ex vivo expansion and stimulation of T-cells, followed by their transfer into patients. The ex vivo culturing step provides an opportunity for modulating the properties of transferred T-cells, enhancing their antitumor abilities, and increasing their expansion.

Materials and Methods: We have developed synthetic surfaces coated with the chemokine CCL21 together with the adhesion molecule intercellular adhesion molecule 1 (ICAM1) for increasing the proliferation and functionality of mouse and human CD8⁺T-cells, activated by antigen-loaded dendritic cells or activation microbeads.

Results and discussion: We first evaluated the effect of various molecular components of the lymph node, alone or in combination, for their capacity to mimic the physiological microenvironment encountered by T-cells during their activation and expansion in the lymph node. We found that substrates coated with a combination of CCL21+ICAM1 enhance the proliferation of ovalbumin-specific murine CD4⁺ and CD8⁺T-cells. The latter cell population, cultured on this substrate also displayed augmented cytotoxic activity toward ovalbumin-expressing melanoma cells, both in-vitro and in-vivo. This increase in specific cytotoxic activity was associated with a major increase in the cellular levels of the killing-mediator granzyme B. Initial experiments, carried out with microbead-activated human cells are currently in progress.

Conclusions: Our results suggest that that synthetic lymph-node mimetic surfaces may be used for enhancing T-cell expansion and generation of T-cells with augmented cytotoxic function, for use in cancer immunotherapy.

Cancer Therapy: Advances in Drug Design and Delivery

Nanodevices for the Study of Immune Cell Function

Mark Schwartzman

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Immune cells recognize tumorous and viral cells by binding using receptors that bind to ligands (antigens) on the membrane of target cells. Although this recognition and following immune activation are extensively studied today, their exact mechanism is barely understood. Here, applied a concept of controlled nanoscale assembly^[1] to engineer a biochip, which can be used as an “artificial antigen presenting cell” for study of role of ligand arrangement in function of Natural Killer cells – lymphocytes of the innate immune system. The chip contained regular arrays of sub-10 nm metallic dots patterned by ultra-high resolution nanoimprint lithograph, and biofunctionalized with antigens. By studying the NK cell immune response to the matrix geometry, we discovered the minimal ligand distribution needed to stimulate the cell activation^[2].

Remarkably, in-vivo function of NK cells is regulated by the signaling balance of activating and inhibitory receptors. To explore the role of the receptor spatial arrangement in this signaling crosstalk, we engineered a more complex multifunctional biochip that simultaneously regulates both receptors. The chip contains mixed nanodots of different metals, selectively functionalized with activating and inhibitory ligands. We fabricated the chip using novel nanoimprint lithography and sequential angle evaporation, combined with our recently developed orthogonal biofunctionalization^[3].

Finally, we explored the nanoscale mechanical sensitivity of Natural Killer cells, by interfacing them with vertical ligand-functionalized nanowires^[4]. We indicated mechanical forces applied by the cells via enhanced cell contraction and the nanowire bending. Furthermore, we found that while either nanowires or ligand presence alone was insufficient to stimulate cell immune response, their combination substantially boosted NK cell degranulation. In this sense, NK are analogous to Boolean “AND gate” with independent mechanical and chemical logic inputs. Our findings provide an important insight into the mechanism of NK cell function, and demonstrate a novel toolbox for the study of the cell immune activation with an unprecedented spatial and mechanical resolution.

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Visualizing the Immune Response

Integrin-mediated cell-matrix adhesions at the crossroad between microtubules and the actomyosin cytoskeleton

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Actomyosin cytoskeleton and cell-matrix adhesions are the key elements determining cell morphogenesis. Peripheral domains of the actin cytoskeleton associated with the clusters of integrin transmembrane receptors, comprise several types of mechanosensing cell-matrix adhesions, such as focal adhesions and podosomes. Myosin-IIA filaments assemble into superstructures (“stacks”) organizing and remodeling actin filament networks, including the cell-matrix adhesions. The myosin-IIA filaments affect the adhesion structures in a differential manner, promoting the integrity and growth of focal adhesions but disrupting the podosomes. A feedback response from the integrin adhesions to the myosin IIA filaments is in part mediated by another essential cytoskeletal system, microtubules. Focal adhesions and podosomes capture microtubules through KANK family proteins, which connect the integrin-binding protein talin with the cortical microtubule-stabilizing complexes (CMSCs). Capturing of microtubules by integrin adhesions suppresses, while detachment - promotes the myosin-IIA filament formation. The mechanism underlying these effects depends on Rho activation by guanine nucleotide exchange factor GEF-H1, which is trapped by microtubules when they are coupled with integrin adhesions via KANK proteins. Microtubule uncoupling from the integrin adhesions triggers a release of GEF-H1 from microtubules, activation of Rho and Rho-associated kinase (ROCK), and consequently the assembly of myosin-IIA filaments. Thus, microtubule capturing by integrin-mediated adhesions modulates the effect of microtubules on the actomyosin cytoskeleton. The myosin-IIA filaments then remodel the focal adhesions and podosomes, closing the regulatory loop.

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Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment

TIGIT and Its bacterial and cellular ligands

Ofer Mandelboim

The use of checkpoint inhibitors antibodies has revolutionized cancer therapy. Unfortunately, these therapies often cause immune-related adverse effects, largely due to a lack of tumor specificity. We examined tumor-markers for checkpoint activity and found that Nectin4 is a novel ligand of TIGIT, a powerful inhibitory receptor, and is the only Nectin family member that interacts with TIGIT alone. We show that the TIGIT-Nectin4 interaction inhibits natural killer cell activity, a critical part of the tumor immune response. We developed blocking Nectin4 antibodies and demonstrate that they enhance tumor killing. Thus, our Nectin4-blocking antibodies represent a unique synergy between pure inhibitory effect, cancer specificity, and immune checkpoint activity, which may prove effective and safe as cancer immunotherapy.

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Host-Pathogen Interaction

Paneth cells secrete lysozyme via secretory autophagy during bacterial infection of the intestine

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Intestinal Paneth cells limit bacterial invasion by secreting antimicrobial proteins, including lysozyme. However, invasive pathogens can disrupt the Golgi apparatus, interfering with secretion and compromising intestinal antimicrobial defense. Here we show that during bacterial infection, lysozyme is rerouted via secretory autophagy, an autophagy-based alternative secretion pathway. Secretory autophagy was triggered in Paneth cells by bacteria-induced endoplasmic reticulum (ER) stress, required extrinsic signals from innate lymphoid cells, and limited bacterial dissemination. Secretory autophagy was disrupted in Paneth cells of mice harboring a mutation in autophagy gene *Atg16L1* that confers increased risk for Crohn's disease in humans. Our findings identify a role for secretory autophagy in intestinal defense and suggest why Crohn's disease is associated with genetic mutations that affect both the ER stress response and autophagy.

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Immuno-Oncology and the Microbiome

Thinking outside the mouse: ex-vivo dissections of host-microbiome cross talks

Nissan Yissachar

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Investigations of host-environment interactions in the gut would greatly benefit from a culture system that preserves cellular architecture yet allows tight experimental control. We have devised a microfabricated organ culture system that viably preserves the multicellular architecture of the mouse intestine, with luminal flow to control environmental parameters and permit experimental perturbation with microbes, drugs or nutrients. Using this system, we have recently identified differential involvement of the enteric nervous system in bifurcating pro- or anti-inflammatory responses to the gut microbiota (Yissachar et al., Cell 2017). We now utilize this system to study the potential role of the gut microbiota in chemotherapy-induced gut inflammation (mucositis). We show that chemotherapy administration disrupts the gut microbial community and that chemotherapy-disrupted microbiome rapidly alters gut permeability, independently of the drug. We thus suggest that chemotherapy-induced microbial dysbiosis may contribute to the initiation or maintenance of gastrointestinal mucositis. Further investigations of the underlying mechanisms may eventually facilitate the use of microbial-based perturbations to ameliorate mucositis in cancer patients.



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Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment

Activation of the Siglec-7 inhibitory receptor: A novel approach for fighting cancer

Ilan Zaffran¹

Advanced systemic mastocytosis (SM) is a rare and still untreatable disease. Blocking antibodies against inhibitory receptors (IRs), also known as "immune checkpoints", have revolutionized anti-cancer treatment. IRs are expressed not only on normal immune cells, including mast cells (MCs) but also on neoplastic cells. Whether activation of IRs through monoclonal antibodies (mAbs) can lead to tumor growth inhibition remains mostly unknown. Here we show that the IR Siglec-7 is expressed by primary neoplastic MCs in patients with systemic mastocytosis and by MC leukemia (MCL) cell lines. Activation of Siglec-7 by anti-Siglec-7 mAb caused phosphorylation of Src homology region 2 domain-containing phosphatase-1 (SHP-1), reduced phosphorylation of KIT and of downstream signaling molecules, and induced growth inhibition in MCL cell lines. In SCID-beige mice injected with either the human MCL cell line HMC-1.1 and HMC-1.2 or with Siglec-7 transduced B cell lymphoma cells, anti-Siglec-7 mAb reduced tumor growth by a mechanism involving Siglec-7 cytoplasmic domains in "preventive" and "treatment" settings. Finally, we showed that anti-Siglec-7 affect also other cancer cells expressing Siglec-7, and reduces the cell growth of colon cancer carcinoma cell (LOVO) both *in vitro* and *in vivo*. These data demonstrate that activation of Siglec-7 on MCL cell lines can inhibit their growth *in vitro* and *in vivo*. This might pave the way to additional treatment strategies for mastocytosis and also to other cancer type expressing Siglec-7 as well.

Cancer Therapy: Advances in Drug Design and Delivery

Complex phospho-regulation pathways of a cancer related mitotic motor protein

Alina Goldstein-Levitin¹

Introduction: Kinesin-5 motor proteins play pivotal roles in mitotic cell division since they are involved in essential functions of mitotic spindle dynamics. These motor proteins are attractive targets for anti-cancer treatment by arresting mitosis progression and since high expression of kinesin-5 motors is correlated with poor prognosis. Few such drugs were previously investigated and tested in clinical trials, however, attempts to finalize an effective anti-cancer drug were futile. In order to investigate regulation pathways of kinesin-5 motor proteins we use the *S. cerevisiae* yeast model which express two kinesin-5 homologues, Cin8 and Kip1. Recently it was found that Cin8 is phospho-regulated by Cdk1, which governs its localization to the mitotic spindle during mitosis. Although it was demonstrated that phospho-regulation of Cin8 at its motor domain by Cdk1 regulates its localization, the contribution of each of the three Cdk1 sites was never explored.

Materials and methods: We first established the role of each of the three Cdk1 sites in the motor domain by characterizing phospho-deficient and phospho-mimic mutants of Cin8 by several *in vivo* methods such as viability assay and live cell imaging. We then combined these results to analysis by *in vitro* methods such as quantitative phosphorylation assay and single molecule *in vitro* motility assay conducted on purified mutants of Cin8.

Results and discussion: We found that each of the sites serve different role in phospho-regulating Cin8; S277, in the motor domain of Cin8, is the major regulator of localization and functionality *in vivo*. However, the mostly conserved site at position S493 is responsible for regulating Cin8 motile properties. In addition, we tested the rigidity of phospho-regulation of Cin8, and examined whether phosphorylation at newly created Cdk1 sites can mimic the known phospho-regulation or create new phenotypes. For this purpose, we generated phospho-deficient mutant of Cin8 and introduced novel Cdk1 sites by single amino acid replacement. We found that out of 29 novel sites, only two sites resulted in phospho-regulation of Cin8, although they were not able to fully recapitulate the original phospho-regulation of Cin8. Position 276 which is in high proximity to the native site S277, and position 148, which was sampled in evolution in different homologues and is located in α -helix 1 which has unknown contribution to functionality to kinesin-5 motor proteins.

Conclusions: Our results indicate that phospho-regulation of Cin8 by Cdk1 is rigid and highly dependent on the structural context and that phospho-regulation pathways of kinesin-5 motor proteins are much more complex than initially anticipated. This complex regulation suggests that the activity of kinesin-5 motor proteins is highly susceptible to regulation by phosphorylation.



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Host-Pathogen Interaction, Bioinformatics, Big Data and Cancer

Decreased A-to-I RNA editing activate dsRNA sensing by innate immunity

Erez Levanon

Faculty of Life sciences, Bar-Ilan University, Israel

Modifications of RNA affect its function and stability. RNA editing is unique among these modifications because it not only alters the cellular fate of RNA molecules but also alters their sequence relative to the genome. The most common type of RNA editing is A-to-I editing by double-stranded RNA-specific adenosine deaminase (ADAR) enzymes. Recent transcriptomic studies have identified a number of 'recoding' sites at which A-to-I editing results in non-synonymous substitutions in protein-coding sequences. Many of these recoding sites are conserved within (but not usually across) lineages, are under positive selection and have functional and evolutionary importance. However, systematic mapping of the editome across the animal kingdom has revealed that most A-to-I editing sites are located within mobile elements in non-coding parts of the genome. Editing of these non-coding sites is thought to have a critical role in protecting against activation of innate immunity by self-transcripts. Both recoding and non-coding events have implications for genome evolution and, when deregulated, may lead to disease. Specifically, loss of editing activity in tumors improves response to immunotherapy and overcomes therapeutic resistance.



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Bioinformatics, Big Data and Cancer

Dissecting the glioma ecosystem by single cell RNA-seq

Itay Tirosh

Molecular Cell Biology, Weizmann Institute of Science, Israel

Glioblastoma is an incurable malignancy that remains poorly understood. Heterogeneous genetic, epigenetic and developmental programs are thought to drive glioblastoma, but their precise characterization remains challenging. Here we apply an integrative approach to dissect glioblastoma, combining single-cell RNA-sequencing of 28 tumors with bulk genetic and expression analysis of 401 specimens from the TCGA. We find that malignant cells in glioblastoma exist in four main cellular states that recapitulate distinct steps of neural development, are influenced by the tumor micro-environment, and exhibit plasticity. Importantly, we show that while each glioblastoma contains similar cellular states, their relative frequency varies between tumors and is influenced by chromosomal aberrations that each influence a particular state. By providing a roadmap of the cellular programs of malignant cells in glioblastoma and their modulation by genetic drivers, our work proposes a unifying model for glioblastoma.



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Multibodies – Attacking multiple pathways with a single, computationally-designed, antibody

Yanay Ofran

Because of the complex and ever-changing natures of both cancer and the immune system, therapeutic approaches that focus on one protein rarely work for an extended period of time. This is why most clinical trials in immuno-oncology today explore combinations of two drugs. However, combination therapies present tremendous challenges, including the need to clinically validate each molecule on its own before exploring the effect of the combination. This makes clinical development even longer and more expensive than it usually is. It also hinders the study of truly synergistic combinations that may be toxic or ineffective on their own. We present an approach for the design of Multibodies, human antibodies that can precisely manipulate multiple pathway by specifically binding functional epitopes on more than one target. This approach may allow for the execution of novel and sophisticated molecular strategies.



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Bioinformatics, Big Data and Cancer

Targeting Signaling in Cancer – lessons from acute lymphoblastic leukemia

Shai Izraeli

Most of the novel targeted cancer therapies block signaling kinases. Here I shall discuss the opportunities and challenges as presented by kinase driven acute lymphoblastic leukemia (Herein ALL) Somatic activation of JAK/STAT signaling in B cell precursor ALL is most commonly caused by aberrant expression of CRLF2 that by heterodimerization with interleukin 7 receptor alpha (IL7R) creates the receptor to Thymic Stromal Lymphopoietin (TSLP). This receptor signals through JAK1 and JAK2. Often additional activating mutations in JAK enzymes or CRLF2 or IL7R cause constitutive activation of this pathway. These aberrations characterize 50% of the ALLs in children with Down Syndrome and 5-10% of ALLs in children and young adults without Down Syndrome. The prognosis of these leukemias is poor. I will describe our unpublished experiments demonstrating the importance of CRLF2/IL7R in generating ALL in primary human hematopoietic progenitors. Our detailed genomic studies of diagnostic and relapse CRLF2/JAK mutated ALLs demonstrate the significant therapeutic challenges posed by these leukemias. Importantly, we discovered that Ruxolitinib, the JAK1 and JAK2 inhibitor can act as a double edge sword. Low dose Ruxolitinib paradoxically enhances survival JAK driven B-ALL while high dose eliminates the leukemic cells. This observation may represent a general phenomenon of B-ALL cells that could complicate treatment with signaling inhibitors.

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Bioinformatics, Big Data and Cancer

RNA Sequence Analysis Reveals Macroscopic Somatic Clonal Expansion Across Normal Tissues

Keren Yizhak¹

Introduction: Cancer genome studies have significantly contributed to the analysis and discovery of somatic mutations that drive cancer growth. However, studying the genetic makeup of a tumor when it is already fully developed limits our ability to uncover how and which somatic mutations accumulate in normal tissues in the stages preceding cancer initiation. Although efforts have begun to collect and analyze DNA from normal tissues, we still lack a comprehensive catalog of genetic events and clonal properties across a large number of tissues and individuals. By analyzing the information-rich content in RNA now available from recent advances in RNA-sequencing methods, we may be able to significantly expand the scope and scale of these studies.

Materials and Methods: Some mutations found in the DNA can be detected in the corresponding RNA, depending on the mutation allele fraction and sequence coverage. We therefore hypothesized that a careful analysis of RNA sequences from normal bulk tissues could uncover somatic mutations reflecting macroscopic clones within the samples. To this end, we developed a new method called RNA-MuTect, to identify somatic mutations using a tissue-derived RNA sample and its matched-normal DNA. RNA-MuTect is designed to remove RNA-specific false positive variants that are result of sequencing and alignment noise.

Results: We validated RNA-MuTect on both tumor-adjacent and cancer samples from The Cancer Genome Atlas, wherein DNA and RNA were co-extracted from the same samples, and show high sensitivity and precision levels. When applied to the GTEx dataset of normal tissues, including over 6,500 samples from ~500 individuals, and spanning across 30 different normal tissues, multiple somatic mutations were detected in almost all individuals and tissues studied here, including in known cancer genes. The three tissues with the largest number of somatic mutations were sun-exposed skin, esophagus mucosa, and lung, suggesting that environmental exposure can promote somatic mosaicism. Both the individuals' age and tissue-specific proliferation rate were found to be associated with the number of detected mutations.

Conclusions: Genetic clones carrying somatic mutations are detected to differing extents across normal tissues, and these differences depend on factors such as the tissue's exposure to environmental mutagens, natural architecture, proliferation rate, and the microenvironment. Some of these clones may be the result of genetic drift. Others, however, may develop due to positive selection driven by certain somatic events, thus potentially representing the earliest stages of tumorigenesis. Higher-resolution studies of normal tissues and pre-cancerous lesions are required for advancing our understanding of both aging and early cancer development.

RNA sequence analysis reveals macroscopic somatic clonal expansion across normal tissues. *Science* (80-). 2019;364: eaaw0726. doi:10.1126/science.aaw0726

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Inflammation and Immunity – Friends or Foes?, Bioinformatics, Big Data and Cancer

Association Between Immune-Related Adverse Events During Anti-PD-1 Therapy and Tumor Mutational Burden

David Bomze¹

Importance: The clinical use of anti-PD-1 therapy is curbed by immune-related adverse events (irAEs). There is yet a lack of validated biomarkers that allows clinicians to predict the development of an irAE prior to therapy initiation.

Objective: To determine whether tumor mutational burden (TMB), which has been identified to be a potential biomarker for response to ICI therapy, is associated also with irAEs.

Design, Setting and Participants: A database study of adverse event reports that have been submitted to the FDA Adverse Event Reporting System (FAERS), ranging from July 2014 to March 2019.

Main Outcomes and Measures: Reporting odds ratio for any irAEs that developed during anti-PD-1 monotherapy (nivolumab or pembrolizumab) for any type of cancer.

Results: We identified 16,397 patients treated with anti-PD-1 monotherapy, who developed an adverse event. For 19 different cancer types there were at least 100 reports available. The most common therapeutic indications were melanoma, non-small cell lung carcinoma (NSCLC), renal cell carcinoma, head and neck cancer, and urothelial cancer. Out of all patients, 3,661 reported any irAE (22.3%; 95% CI, 21.7-23.0). The cancers with the highest proportion of reported irAEs were melanoma, Hodgkin's lymphoma, NSCLC of the adenocarcinoma subtype, and NSCLC of the squamous cell carcinoma subtype. Pharmacovigilance analysis revealed a significant correlation between the odds of reporting an irAE and TMB across multiple cancer types treated with anti-PD-1 therapy (Pearson's $R = 0.704$; $P = 0.001$).

Conclusion and Relevance: In this observational study, we show that irAEs are associated with TMB across multiple cancer types. Thus, TMB may be a useful biomarker to not only indicate therapy response, but also the risk of developing an irAE during anti-PD-1 therapy.

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Inflammation and Immunity – Friends or Foes?, Lymphocyte Activation & Exhaustion

Heterogeneous landscape of CD4 T cells develops with age and comprises unique subsets with distinct phenotypic properties

Yehezqel Elyahu¹

Introduction:

One of the key hallmark of aging is the deterioration of the immune system, a phenomenon which prone to increased susceptibility of elderly individuals to infection, chronic inflammatory disorders and vaccination failure. A significant change observed in aging is a dynamic variation in the composition and functionality of CD4 T cells along with the accumulation of dysregulated T cells. However, a detailed, high resolution, characterization of CD4 T-cell phenotypes, which may explain these dysregulated functional properties, is lacking.

Materials and methods:

We used scRNA-seq and flow cytometry data, together with functional assays which allowed us in-depth characterization of CD4 T-cell subsets in aging. We profiled thousands of CD4 T cells obtained from young and old mice in order to explore the differences in population structure at different stages of aging.

Results and discussion:

We found that the cell composition of CD4 T-cell subsets in old mice is markedly different from young mice. Three cell populations were identified in this study: exhausted, MHCII-restricted cytotoxic, and activated regulatory (aTregs) CD4 T cells. These subsets appear rarely in young mice and gradually accumulate in old mice, reaching about 30% of the CD4 T-cell lineage. Moreover, the cytotoxic CD4 T cells and aTregs presented high variability at different stages of aging.

Conclusion:

In the current study, we aimed to comprehensively describe how the CD4 T-cell compartment is sculptured during the process of aging. We identified a gradual reorganization of the CD4 T-cell compartment, where regulatory, exhausted and cytotoxic phenotypes co-emerge with age. These findings provide a thorough view of the dynamic reorganization of the CD4 T-cell milieu with age and illuminate dominant cell subsets associated with declined immunity and chronic inflammation.

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Inflammation and Immunity – Friends or Foes?, Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment

Successful Immunotherapy For Melanomas Requires Tumor-Infiltrating

Monocyte-Derived Dendritic Cells

Nadine Santana Magal

The recent success of checkpoint blockade therapies has established immunotherapy as one of the most promising treatments for melanoma. Nonetheless, a complete curative response following immunotherapy is observed only in a fraction of the patients. To understand the factors that limit the efficacy of immunotherapies, we established mouse models, which cease to respond to immunotherapies once tumors reach a certain stage. We compared the changes within immune cell populations in the tumor, draining lymph node (DLN) and blood, in responding and non-responding melanoma tumor-bearing mice. Analysis of their immune system revealed that the numbers of tumor-infiltrating dendritic cells (TIDC) drastically decrease with time. We further found that in contrast to the current paradigm, once melanoma is established, TIDC do not migrate into sentinel lymph nodes. Instead, these dendritic cells (DC) undergo local cell death due to excessive phagocytosis of melanoma-derived exosomes. Importantly, we found that TIDC are required to license the cytotoxic activity of tumor CD8⁺ T cells *in situ*, and in their absence, T cells will not lyse melanoma cells. Overall, this research provides a new insight into the roles of TIDC in tumor, and the means to rescue them from exosome-induced apoptosis, thereby increasing the efficacy of checkpoint blockades, adoptive T cell transfer, as well as other T cell-based immunotherapies.

Microenvironment and Immuno-Oncology

The PD-L1/PD-1 Axis Blocks Neutrophil Cytotoxicity in Cancer

Olga Yajuk

Introduction

Blocking PD-L1 and/or PD-1 is therapeutically utilized for maintaining the anti-tumor functions of the adaptive immune system. However, the consequences of blocking the PD-L1/PD-1 axis on innate immune responses remains largely unexplored. In cancer, neutrophils were shown to consist of different subpopulations, which possess either pro- and anti-tumor properties. We found that PD-L1 expression is not limited to the tumor promoting neutrophil subset but is also evident in the anti-tumor neutrophil subset. In this study we evaluated the role played by PD-L1 in regulating anti-tumor neutrophil cytotoxicity.

Materials and methods

The expression of PD-1/PD-L1 on tumor cells and neutrophils respectively was evaluated by flow cytometry. The consequences of manipulating the PD-1/PD-L1 axis on neutrophil cytotoxicity were evaluated in a tumor cell-neutrophil co-culture setting. The effect of manipulating neutrophil PD-1/PD-L1 interactions with tumor cells in vivo was evaluated in NOD-SCID mice which lack adaptive immunity.

Results and discussion

We show that neutrophil cytotoxicity is efficiently blocked by tumor cell expressed PD-1. Furthermore, blocking of either neutrophil PD-L1 or tumor cell PD-1 maintains neutrophil cytotoxicity and enhances tumor cell apoptosis. Importantly, we show that high tumor cell PD-1 levels block neutrophil cytotoxicity and promotes tumor growth regardless the adaptive immune system. Taken together, these findings highlight the therapeutic potential of enhancing anti-tumor innate immune responses via blocking of the PD-L1/PD-1 axis.

Conclusion

In summary, our study provides novel insight into the interaction of neutrophil PD-L1 and tumor cell PD-1. We demonstrate that tumor cell PD-1 blocks neutrophil cytotoxicity and has a dramatic impact on the anti-metastatic function of neutrophils.

Microenvironment and Immuno-Oncology

Targeting the tumor microenvironment by IL-1 neutralization

Sapir Maudi-Boker

Introduction- Targeting the tumor microenvironment, except of immune checkpoint blockade, is not yet widespread. Interleukin-1 (IL-1) is an abundant cytokine in tumor sites and it controls the inflammatory pro-invasive and immunosuppressive nature of the microenvironment. It activates the microenvironment and also affects the malignant cells. We have studied how the secreted form of IL-1, i.e., IL-1 β affects the microenvironments of primary breast tumors and lung metastases and whether its neutralization will affect invasiveness.

Materials and Methods- We have used the model of murine 4T1 breast cancer cells, which represents the equivalent of human triple negative breast cancer (TNBC). Upon orthotopic injection of 4T1 cells, local primary tumors develop followed by spontaneous metastasis to the lungs.

Results and Discussion- In wild-type (WT) mice, progressive tumors developed and induced spontaneous lung metastasis, while in IL-1 β knockout (KO) mice, we observed initial tumor growth followed by regression, no formation of lung metastases and development of resistance to a challenge with the malignant cells. We further studied the role of microenvironment IL-1 β on inflammation/immunity, emphasizing early events (days 10-14 after inoculation), which are critical for the outcome of the malignant process. In WT mice, early IL-1 β -induced and CCL2-mediated recruitment of inflammatory monocytes was potent, however, impaired in IL-1 β KO mice. Also, IL-1 β -induced *in-situ* differentiation of inflammatory monocytes into IL-10-secreting immunosuppressive TAMs, by CSF-1 and other mediators, was potent in WT mice and marginal in IL-1 β KO mice. The relative abundance of inflammatory monocyte-derived IL-12 producing DCs was much higher in tumor sites in IL-1 β KO mice, as compared to WT mice. In IL-1 β KO mice, activated CD8 $^{+}$ in tumor cell deposits were abundant and resulted in its regression. IL-1 β neutralization induced only partial anti-tumor effects, but its combination with anti-PD-1 antibodies, completely inhibited tumor growth. In WT mice with large tumors, in which the primary tumor was resected, and were subsequently treated with anti-IL-1 β and anti-PD-1 antibodies, lung metastasis was significantly reduced.

Conclusion- Treatment of minimal residual disease (MRD), after tumor debulking, enables targeting of the microenvironment, also with anti-IL-1 β anti-PD-1 antibodies, can be effective for the prevention of tumor recurrence and metastasis.

Microenvironment and Immuno-Oncology

Differential Neutrophil Extracellular Traps Release (NETosis) In Cancer-Related Neutrophils

Ludovica Arpinati

Introduction: Neutrophils play a major role in tumor biology. At least three distinct populations of circulating neutrophils have been described in the context of cancer, including the mature high-density neutrophils (HDN) as well as mature and immature low-density neutrophils (LDN), with pro- and anti-tumor characteristics. Among other functions, cancer-related neutrophils were shown to release upon activation Neutrophil-extracellular traps (NETs), mesh-like structures of decondensed chromatin fibers, in a process named NETosis. In this work, we aimed to characterize the cellular mechanisms leading to NETosis in murine and human cancer-related neutrophils and the differential ability of neutrophil subpopulations in releasing NETs.

Methods: Mice were injected with AB12 (mesothelioma) or LLC (carcinoma) cell lines. Circulating neutrophils were isolated from tumor bearing mice and from peripheral blood of Lung Cancer patients using a density gradient centrifugation. Following stimulation with Ionomycin or PMA, cells were fixed and stained for Myeloperoxidase, citrullinated-Histones₃ and DAPI. NET formation was determined as the percentage of cells identified positive for MPO and CitH₃ signal. Data were acquired using a Nikon Confocal microscope and analyzed by ImageJ.

Results: We find that HDN and LDN show comparable NETotic potential following activation. As suggested by previous studies, we find that circulating neutrophils isolated from tumor bearing mice display higher ability to release NETs than healthy controls. Surprisingly, HDN isolated from mice bearing G-CSF-enriched tumors do not display higher NETotic ability compared to neutrophils isolated from control mice. However, the addition of G-CSF to the media strongly induced the release of NETs. Moreover, we find that neutrophils' expression of TNF α is important for the release of NETs by LDNs, but not by HDN. Human HDN present significantly higher release of NETs compared to murine neutrophils (~15% vs 3% respectively). Similarly to murine neutrophils, G-CSF stimulation enhanced NETosis in human HDN.

Conclusions: Our findings demonstrate that NETosis is preponderant in cancer-related neutrophils, both in mice and humans. Our data suggest that the constitutive stimulation by G-CSF is required to maintain the enhanced susceptibility of neutrophils to form NETs independently of the cancer-induced predisposition.

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Cancer Therapy: Advances in Drug Design and Delivery

Repression of AXL expression by AP-1/JNK blockage overcomes resistance to PI3Ka therapy

Mai Badarni¹

Background: The phosphatidylinositol-4, 5-bisphosphate 3-kinase (PI3K) pathway, which regulates cell proliferation and survival, is hyper-activated or mutated in over 50% of HPV-related head and neck squamous cell carcinoma (HNSCC). Blocking the isoform specific of the PI3K, p110a, using BYL719 showed a promising anti-tumor activity in PIK3CA-mutated HNSCC. However, intrinsic or acquired-resistance was observed in pre-clinical models and in-patients. We previously showed that upregulation of receptor tyrosine kinase AXL induced resistance to BYL719 in vitro, in vivo and in patients.

Objectives: To uncover the molecular machinery leading to AXL up-regulation following BYL719.

Methods: Western blot analysis was performed to determine the correlation between AXL expression and transcription factors like c-JUN in HPV-related HNSCC cell lines. Cell proliferation assay was performed to determine the role of AXL and c-JUN in the response to BYL719 in vitro. Immunohistochemistry was used to measure AXL and c-JUN expression in tissue sections.

Results: A good correlation between AXL and c-JUN expression in HPV-related HNSCC cell lines was observed. Knockdown of c-JUN using siRNA was sufficient to re-sensitize HPV-related HNSCC tumor cells to BYL719 in vitro. In vitro, inhibition of BYL719 and JNK inhibitors (inhibits c-JUN) showed a synergistic anti-tumor effect. Mechanistically, inhibition of the JNK pathway prevents AXL expression following BYL719.

Conclusions: AXL/c-JUN levels determine sensitivity to PI3Ka inhibition. AXL expression levels are regulated by c-JUN. Blocking c-JUN using JNK inhibitors re-sensitized tumor cells to BYL719.

Microenvironment and Immuno-Oncology

Enhancement of the immune response induced by Alpha Radiation- Based Brachytherapy can Inhibit Triple Negative Breast Cancer tumor development and Cure Colon Tumors in Mice

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Introduction

Alpha radiation efficiently kills tumor cells by creating bulky double strand breaks, thereby releasing tumor antigens and DAMP signals. *Diffusing Alpha emitters Radiation Therapy* (DaRT), employs Radium-224 loaded sources (DaRT seeds), which are inserted into the tumor and release, by recoil, short-lived alpha-particle emitting atoms, 2-3 mm from the source. It was previously shown that *in situ* ablation by DaRT induces a systemic antitumor immune response, as a monotherapy. Here strategies to boost this response by TLR agonists and inhibition of immune suppressor cells were investigated. In addition, the specificity of the response was demonstrated.

Materials and methods

4T1 and CT26 bearing mice were treated with DaRT in combination with TLR3/9 agonists. In the CT26 model DaRT and TLR3 agonist were combined with Tregs/MDSC inhibition achieved by low dose cyclophosphamide and Sildenafil, respectively. The specificity of the antitumor immune response was investigated by challenge and Winn assays.

Results and discussion

It was observed that in a non-immunogenic triple negative breast cancer tumor model, 4T1, DaRT combined with intratumoral administration of the TLR3 agonist polyIC significantly retarded tumor growth compared to DaRT alone. In colon cancer mouse model, CT26, DaRT in combination with TLR3,9 agonists retarded tumor progression and prolonged overall survival. Adding Tregs/MDSC inhibition to DaRT and the TLR9 agonist, CpG, resulted in the cure of 51% of the mice, compared to only 6% cure of mice treated with non-radioactive seeds and the immune enhancers. This effect was mediated by a specific immune memory against tumor antigens, demonstrated by 100% resistance only to CT26 tumor challenge and high percentage of protection by splenocytes from cured mice specifically against CT26 inoculation in naïve mice.

Conclusion

The current results indicate that ablation by DaRT is required to enhance a specific antitumor immune response, which vaccinate the host against tumor antigens. The concurrent use of DaRT, Tregs/MDSCs inhibitors and immunoadjuvants, resulting in the cure of tumor bearing mice, synergize probably through activation of antigen presenting cells and creation of immune memory against specific tumor antigens.

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Microenvironment and Immuno-Oncology

Formation of a fibrotic pre-metastatic niche is mediated by systemic Activin A during pulmonary metastasis of breast cancer

Noam Cohen

Introduction: Advanced metastatic cancers are mostly incurable and available therapies can only prolong life to a limited extent. It is increasingly appreciated that the metastatic microenvironment is crucial in supporting metastases formation. Previous studies demonstrated that changes in the composition and function of the ECM play a major role in shaping the microenvironment at the metastatic site. Since fibroblasts are central modulators of ECM composition, we set out to study the role of fibroblasts in facilitating pulmonary metastases of breast cancer.

M&M: Utilizing multiple mouse models of breast cancer metastasis, we performed different histology stainings for collagen fibers, including Masson Trichrome, Sirius Red and SHG. For RNA expression analysis of specific gene signatures, we used qRT-PCR. Proteomic analysis was performed with Ray Biotech® protein array or ELISA.

Results and discussion: We show that enhanced collagen deposition is a relatively early event during the formation of the pre-metastatic niche in lungs, which precedes metastases formation. Moreover, we found that tumor-derived secreted factors induce fibroblast activation and upregulation of pro-fibrogenic signaling in fibroblasts. Proteomic analysis of serum from normal and tumor-bearing mice revealed significant changes between the two groups. One of the proteins that were highly upregulated in the serum of tumor bearing mice was Activin A (ActA), known to enhance the expression of pro-fibrogenic genes. We demonstrated that ActA was sufficient to activate normal lung fibroblasts, and its inhibition attenuated this pro-fibrogenic activation. Moreover, we show that systemic ActA levels are gradually upregulated in the circulation during tumor progression in two different mouse models of breast cancer. Importantly, the expression of ActA is upregulated in human breast cancer and in multiple other human cancers.

Conclusion: Our findings suggest a functional role for ActA in driving collagen deposition in the lungs and in facilitating lung metastatic relapse in breast cancer.

Microenvironment and Immuno-Oncology, Cancer Metastasis

Reciprocal Interactions between Melanoma and Microglia Cells Reprogram Melanoma Malignancy Phenotype

Sivan Izraely¹

Introduction: Melanoma has one of the highest propensities to metastasize to the brain. Once disseminated in the brain, melanoma cells communicate with microglia (MG), the main immunocompetent cells in the CNS. The present study is aimed to elucidate the role of MG in the formation and survival of melanoma brain metastasis (MBM).

Methods: Human and mouse brain specimens bearing MBM were stained by IHC for the MG marker Iba1. Melanoma cells sorted from melanoma-MG co-cultures as well as naïve melanoma cells were inoculated subcutaneously into nude mice to determine the effect of MG on melanoma tumorigenicity. Human MBM cells from different patients were exposed to human MG cells or MG-derived soluble factors. Human MG cells were similarly exposed to MBM cells or their soluble factors. RNA-seq, RPPA, cytokine arrays and phospho-protein arrays were used to determine the alterations in each of these cell types following the exposure to the counter cell type. Treated cells were tested for alterations in migration ability, matrix metalloproteinase-2 (MMP2) activity, proliferation, morphology, and spheroid formation. Melanoma cells were also tested for transendothelial migration. Similar in vitro assays were performed using melanoma cells with high or low Aldolase C (ALDOC) expression to demonstrate its role in the formation and maintenance of MBM.

Results and discussion: IHC demonstrated infiltration of MG cells to MBM lesions and a close spatial distance between the two cell types. Subcutaneous inoculation of melanoma cells sorted from melanoma-MG co-cultures resulted in increased tumor size, compared to naïve melanoma cells. Reprogramming of gene expression, cell signaling and cytokine secretion occurred in MBM and MG cells following the exposure of each cell type to supernatants of the counter cell type. Melanoma cells exerted morphological changes on MG cells, enhanced their proliferation, MMP2 activity and migration. MG cells enhanced melanoma malignant phenotype: increased melanoma proliferation, migration, MMP2 activity, brain endothelial penetration and spheroid formation. One of the consequences of melanoma-MG interactions was ALDOC upregulation in melanoma cells. Melanoma cells overexpressing ALDOC demonstrated enhanced migration, adhesion to brain endothelial cells, brain endothelial penetration, viability and spontaneous brain micrometastasis formation. Soluble factors from ALDOC overexpressing melanoma cells increased MG proliferation and migration, which may induce MG involvement in the metastatic process and act as a positive feedback loop, further enhancing MBM progression. Inter-tumor heterogeneity was demonstrated with respect to the effect of ALDOC on melanoma malignancy.

Conclusion: Melanoma and MG cells interact in a bi-directional, reciprocal manner. Such interactions may promote melanoma progression by several mechanisms.



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Microenvironment and Immuno-Oncology

The Non-Cell Autonomous Role of YAP Affects Blood Vessel Integrity and Immune Response in Tumors

Anat Gershoni

Breast cancers are a family of malignancies arising from mammary epithelial cells. 4T1 cells are a triple negative breast cancer epithelial cell line, which metastasizes spontaneously *in-vivo* from a primary tumor in the mammary fat pad to multiple distant sites including lymph nodes, liver, lung and bone. In syngeneic, immune competent balb/c mice, the progressive spread of 4T1 metastases to the draining lymph nodes and other organs is similar to that of human mammary cancer and thus represents an accurate model for human breast cancer. Hippo signaling has been known to assume different roles in different cell contexts and microenvironments, due to its diversified interplay with many signaling transduction cascades. This is illustrated by reports demonstrating both tumor suppressive and oncogenic roles of YAP in breast cancer. We knocked-out *Yap* in 4T1 cells, using CRISPR/Cas9, to investigate YAP -dependent transcriptional programs and their effect on the *in vivo* tumor microenvironment. Surprisingly, the 4T1 *Yap* knock-out cells, generated larger tumors compared to wild type cells, with more mature blood vessels and reduced immune response. This suggests that tumors expressing YAP may be vulnerable to immune surveillance due to the formation of more permeable blood vessels. We plan to explore the impact of YAP on maintaining a tumor suppressive microenvironment during breast tumorigenesis.

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Metabolism and the DNA-Damage Response

Ligand Binding Domain Activating Mutations of ESR1 Rewire Cellular Metabolism of Breast Cancer Cells

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Introduction: Mutations in the ligand binding domain (LBD) of estrogen receptor α (ER) confer constitutive transcriptional activity and resistance to endocrine therapies in breast cancer patients. Accumulating clinical data suggest adverse outcome for patients harboring tumors expressing these mutations. We aimed to elucidate mechanisms conferring LBD-ER aggressive phenotype.

Materials and methods: Cells constitutively expressing physiologic levels of ER harboring activating LBD mutations were generated and characterized for viability, invasiveness and tumor formation in vivo. Gene expression profile was studied using microarray and RNAseq technologies. Metabolic properties of the cells were assessed using global metabolite screen and direct measurement of metabolic activity.

Results and discussion: Cells expressing mutated ER showed increased proliferation, migration and in vivo tumorigenicity compared to cells expressing the WT-ER, even in the presence of estrogen. Expression of the mutated ER was associated with upregulation of genes involved in invasion and metastases, as well as elevation of genes associated with tumor cell metabolism. Indeed, a metabolic examination revealed four distinct metabolic profiles: WT-ER expressing cells either untreated or estrogen-treated and Mutated-ER expressing cells either untreated or estrogen-treated. Pathway analyses indicated elevated TCA cycle activity of 537S-ER expressing cells. Thus, while WT-ER cells were mostly glucose-dependent, 537S-ER were not addicted to glucose and were able to utilize glutamine as an alternative carbon source.

Conclusions: Taken together, these data indicate estrogen-independent rewiring of breast cancer cell metabolism by LBD-activating mutations. These unique metabolic activities may serve as a potential vulnerability and aid in the development of novel treatment strategies to overcome endocrine resistance.

Cancer Metastasis

Adaptation Of Colon Cancer Cells To The Brain Microenvironment: The Role Of IRS2

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Introduction: Development of brain metastases (BMs) requires adaptation of cancer cells to the hostile brain environment (BE). BMs from colorectal cancer (CRC) are the fourths leading cause of BMs. Yet, mechanisms mediating the formation of CRC BMs are currently unknown. A possible candidate is insulin receptor substrate-2 (IRS-2), a cytoplasmic molecule mediating insulin and IGF-1 effects by acting as an adaptor between their receptors and downstream effectors.

We aimed to explore genomic drivers enabling tropism and adaptation of CRC cells to the BE and decipher mechanisms enabling the process.

Material and method: Analysis of FoundationOne database including 115 CRC BMs and 2935 CRC liver metastases (LMs). CRC cell line used was HCT-116. Proliferation was assessed using methylene-blue; migration using transwell and wound-healing assays. Three-dimensional model that recapitulates the BE was generated using InSphero assay. Conditioned media (CM) was prepared from brain and activated-human astrocytes (aHA). IRS2 expression was studied using IHC, qRT-PCR, and western-blot.

Results and discussion: Increased prevalence of IRS2 gene amplification was observed in 13% of BMs compared to 3% in LMs ($p=0.0001$). Furthermore, IHC of human clinical samples showed increased expression of IRS2 in BMs compared to LMs. Mimicking BE (hypoxia and brain or aHA CM) increased IRS2 expression in CRC cells. IRS2-overexpressed CRC cells had increased proliferation, migration and colony formation capacity. Finally, IRS2-overexpressed HCT-116 three-dimensional spheroids survived better in aHA-CM and had enhanced proliferation in co-culture with HA.

Conclusion: These data indicate, for the first time, a unique genomic profile of CRC BMs and implies IRS2 role in promoting BMs. Understanding the mechanisms enabling CRC cells to colonize and grow in the brain may help to reveal novel therapeutic targets to inhibit development of BMs.

Cancer Metastasis

Metastasis Prediction: Mechanobiology-Based Early Determination of Metastatic Risk in Pancreatic Tumors

Daphne Weihs¹

Introduction

The main cause of cancer-related deaths is metastasis. A critical step in metastases formation is invasion of cancer cells through tissue. Invading metastatic cells are dynamic, rapidly changing morphology and applying forces to their surroundings. Evaluating forceful interactions of cells on an impenetrable, synthetic gel, we have previously shown that subsets of invasive breast-cancer cells indent custom gels while benign/non-invasive cells do not. Specifically, we rapidly and quantitatively evaluate the cells' invasive capacity by the amounts of indenting cells and their attained depths; the mechanical invasiveness reliably separates cells with high and low metastatic potential (MP). Here, we show that the mechanical invasiveness of fresh, human, primary-site pancreatic tumors agrees with the clinical histopathology and matches results in established cell lines.

Materials and methods

We have seeded cells from 6 pancreatic cell lines (ATCC, Manassas, VA) or from fresh, human pancreatic tissue samples (enzymatically degraded) on physiological-stiffness (2.4 kPa) polyacrylamide gels. Within 1-hr of seeding, the fraction of invasive cells indent the gels, their amounts and depths are determined by fluorescence microscopy and provide the mechanical invasiveness. In cell lines, we compare to amount of cells trespassing 8 μ m pores of trans-well migration assay within 72 hrs. For tumor samples, clinical histopathology and patient follow-up are used as gold-standard.

Results and discussion

The mechanical invasiveness of pancreatic cell-lines with high- and low-MP differed in a statistically significant manner, respectively, with 83-85% and 46-53% percent indenting cells and attained depths of 4.7-5 μ m and of 2.9-4.1 μ m; results agree with the trans-well migration assay. We show agreement with fresh, human tumor samples, where invasive samples (e.g. metastatic adenocarcinoma) exhibit 40% indenting cells and benign/non-invasive samples (e.g. non-invasive fibrotic tumors or pre-cancerous lesions) are at 20%.

Conclusions

Our unique mechanobiology-based approach provides rapid (2hr) cancer identification and prediction of metastatic likelihood of a tumor, already during the first diagnosis, which agrees with the clinical histopathology and outcome in patients. Early prognosis can critically affect choice of patient-specific treatment protocols and increase life expectancy.

Cancer Metastasis

Development and Characterization of the Metastatic Niche in Ovarian Carcinoma

Hadil Onallah¹

Ovarian carcinoma has a unique pattern of metastasis, it remains largely restricted to the abdominal cavity, where the omentum; a large fat pad in front of the bowel, is a major site of the metastasis. The development and progression of cancer is closely associated with the tumor microenvironment. Tumor cells exist in a microenvironment composed of fibroblasts, epithelial cells, leukocytes, lymphocytes, extracellular matrices, etc. Interaction between the disseminating tumor cells and the local microenvironment at that site, the pre-metastatic niche, makes it a major site for metastasis.

The metastatic niche is composed of a variety of cells including immune cells, endothelial cells and activated fibroblasts and adipocytes; the Cancer Associate Fibroblasts (CAFs). CAFs contribute to tumor cell proliferation, invasion and progression. They are characterized with the expression of alpha-smooth muscle actin (α -SMA) and fibroblast activation protein-alpha (FAP- α). There are two major sites for CAFs in ovarian cancer, both the tumor stroma and the omentum. In ovarian cancer, it was recently reported that several lncRNA have been shown to be overexpressed in CAFs and contribute to their ability to promote metastasis. To further explore the role of CAFs in the metastatic niche, we conducted a study to characterize them. For isolation of primary CAFs, fresh material from primary omentum of ovarian cancer were obtained from patients undergoing surgery at Hadassah Medical Organization (n = 18). Normal fibroblasts (NF) were isolated from patients undergoing non-cancerous prophylactic surgery (n=11). The study was approved by the local ethics committee and informed consent was obtained from the patients. Both sets of fibroblasts were cultured and mRNA levels were tested for several lncRNAs using qPCR. CAFs and NFs were characterized by the expression of FAP-1 α , α -SMA and Periostin; potential markers of activated fibroblasts. Remarkably, various lncRNAs were found to be differentially expressed in CAFs and NFs. GAS5, known as a potential tumor suppressor, was upregulated in NFs samples compared to CAFs, while FLJ22447, a yet uncharacterized lncRNA, was found to be upregulated in CAFs compared to NFs. Migration of the CAF or NF samples towards OVCAR3-an ovarian cancer cell line, was tested using cell culture chamber slide and the results revealed that CAFs show an increased motility towards ovarian cancer cells compared to NFs. Furthermore, we found that MRC-5, a NF cell line, can be activated and transformed to CAF, when exposed to CAFs conditioned media. These results demonstrate that targeting the activation of CAFs in the omentum of ovarian cancer patients could serve a novel appealing approach to inhibit the metastatic niche formation.

Cancer Metastasis

Role STIM2 in Human Breast Cancer

Ruslana Militsin

Background: Metastasis is a multistep process that remains a major cause of death for most oncologic patients. However, the molecular mechanisms which underlie the metastatic process are still poorly understood.

Store-Operated Calcium (Ca^{2+}) Entry (SOCE), a major mechanism for Ca^{2+} permeation across membranes of cells, is essential for various cellular processes including cell migration. SOCE depends on stromal interaction molecules (STIM) and Orai channels. Mammalian cells express two STIM isoforms, STIM1 and STIM2. Ablation of STIM1 expression has been shown to reduce the cellular migration, invasion and focal adhesion turnover rate and STIM1 has been implicated in epithelial-to-mesenchymal transition of breast cancer cells. In contrast, however, much less is known about the contribution of STIM2 to cancerous processes.

Materials and methods: The role of STIM2 in metastasis was studied in high metastatic human breast cancer cell line LM24 cells. We ablated the expression of STIM2 in LM24 cells by using CRISPR-Cas9 gene editing and determined STIM2 knockout (KO) clones by using DNA sequencing and Western Blot analyses. Ca^{2+} imaging was used to determine the activity of STIM2 KO cells in SOCE. Boyden chamber-based migration and invasion assays were used for testing the mobility of KO cells. Immuno-labeling and live cell-edge dynamics were used for assessing cell-extracellular matrix (ECM) adhesions. Tumor development was examined *in vivo* following subcutaneous injection of wild type (WT) cells or STIM1 KO or STIM2 KO cells into flanks of 8-weeks old SCID male mice.

Results and discussion: While knockout of STIM1 abolished SOCE in LM24 cells, knockout of STIM2 only mildly attenuated SOCE. Despite the mild effect of STIM2 KO on SOCE, assays of invasion and migration demonstrated a significant reduction in motility of STIM2 KO cells compared to WT indicating an essential function of STIM2 in cell motility. Assessment of cellular adhesion showed deficiency of STIM2 KO cells compared to WT cells indicating the important contribution of STIM2 to cell adhesion. Finally, we investigated tumor formation *in vivo*. While WT cells formed tumors, STIM2 KO or STIM1 KO cells did not, suggesting STIM2 may constitute an important marker for tumor progression and prognosis.

Conclusion: In our study, we characterized the role of STIM2 in highly metastatic human breast cancer. We found that while STIM2 is not essential for SOCE, it plays an important role in cellular migration and invasion. STIM2 cells showed deficiency in interaction with ECM and failed to form tumors *in vivo*. Thus, we suggest that by regulating cellular adhesion, STIM2 contributes to tumor formation and metastatic.

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Israel Immunological Society

Visualizing the Immune Response, Inflammation and Immunity – Friends or Foes?

Interleukin-1 α Induced Expression of Steroidogenic Acute Regulatory Protein (StAR) Facilitates Fibroblasts Survival Following Myocardial Infarction

Talya Razin¹

Introduction: Release of proinflammatory interleukin-1 α (IL-1 α) from necrotic myocytes injured by myocardial infarction (MI) is the proximal alarming signal that triggers post-ischemic innate inflammation by activating resident fibroblasts in the infarct border zone (BZ) tissue. Recent studies have shown that while the inflammatory phase is in progress, resident fibroblasts of the infarct BZ undergo proliferation, populate the infarct vacant space and differentiate into active myofibroblasts. The myofibroblasts drive tissue remodeling by deposition of extracellular matrix to form fibrotic scar replacing lost cardiomyocytes and thereby prevent left ventricular wall rupture.

Important questions remained unaddressed are how the tissue remodeling BZ fibroblasts survive during the inflammatory response known to locally generate proapoptotic stress milieu. We suggest that a key player for understanding the BZ fibroblasts robustness is their unexpected ability to express the steroidogenic acute regulatory protein (StAR), a long known protein indispensable for steroid hormones biosynthesis. We have shown that cardiac StAR is not associated with steroidogenesis. We therefore hypothesized that the cardiac StAR should have a new alternative activity.

Methods and Results: Biochemical and histochemical approaches showed that StAR expression is transient and lasts during the inflammatory response after MI. IHC evidenced searching for co-expression of StAR with cell markers such as PDGFR α and periostin, revealed that StAR appears in interstitial and adventitial fibroblasts/myofibroblasts residents of the infarct BZ. Work with primary culture of rat cardiac fibroblasts revealed that *Star* gene products are specifically upregulated by the proinflammatory IL-1 α , a notion further confirmed after MI in IL-1 α deficient mouse model (IL-1 α KO). Furthermore, IL-1 α treatment of the cardiac fibroblasts in culture turns the cells markedly resistant to induced apoptosis by a strong apoptogen, cisplatin. This anti-apoptotic impact of IL-1 α activation of the fibroblasts is strictly dependent on IL-1 α induction of StAR expression. *In vivo*, myocardial infarction in IL-1 α deficient mice resulted in 65-70% reduction of Postn, PDGFR α and proliferating cells nuclear antigen (PCNA) expression, which suggests a 70% decline of replicating fibroblasts in the LV free wall. Further experiments are expected to unravel if inflammation related fibroblasts cell death underlies the observed loss of these cells in the IL-1 α /StAR deprived KO mice.

Conclusions: These findings identify a new mechanism by which IL-1 α confers anti-apoptotic protection mediated by StAR expression in fibroblasts/myofibroblasts expected to survive during the inflammatory response in order to ensure proper tissue remodeling after MI.

Visualizing the Immune Response

Novel Immune History-Based Correlates of Risk and Protection for Influenza

Ayelet Shagal¹

Introduction

Vaccination, the most cost-effective public health intervention, stimulates the immune system to generate protective memory response. Seasonal influenza vaccines effectiveness varies by year and wanes over time. A variety of factors contribute to high heterogeneity in vaccine induced immune responses, such as age, gender and individual's 'immune history' – the memory antibody repertoire of previously encountered pathogens and vaccines. The current gold standard correlate of protection is the hemagglutinin inhibition assay, which has limited prediction power and is time and sample consuming. Here, we designed a novel Antigen Microarray (AM) assay to study the immune history of influenza IgG and IgA antibody repertoires and to identify novel correlates of protection based on these measurements.

Material and Methods

To study the role of immune-history of previous influenza infections, we have developed a novel influenza antigen microarray spotted with whole- inactivated influenza viruses, recombinant surface glycoproteins and their respective overlapping peptides. Viruses from influenza A H₃N₂, H1N1 and B subtypes were included on the array.

We used serum samples from FluVacs – a randomized double-blind placebo-controlled influenza vaccine efficacy trial comparing the vaccine efficacy of the inactivated (IIV) and live-attenuated (LAIV) vaccines in adults aged 18-65, conducted in 2007-2008.

We analyzed serum samples from a case-control set of 162 individuals including all participants that were infected with influenza during the trial. Samples were collected at 3 timepoints for each subject: pre-vaccination baseline (d₀), post vaccination (d₂₁) and at the end of the season (d₉₀). IgG and IgA antibody profiles at all timepoints were captured using our novel antigen array.

Results and Discussion

We observed a high heterogeneity of responses to historical influenza strains at baseline and divided them to high, medium and low responders. We compared the antibody profiles at baseline and post-vaccination of all subjects who subsequently became infected to those who did not. Low responders in both the placebo and vaccine arms had infections rates 80%. In contrast, high responders had infections rates of 22% in placebos and 0% in IIV vaccinated subjects. We further found that stratifying individuals based on their post-vaccination responses allowed to identify additional vaccinated subjects that were at high or low risk to acquire infection.

Conclusion

Our data suggests that serum IgA immune history profiles are novel correlates of risk and protection from influenza infection. They further suggest that individuals can be stratified at baseline to identify a sub-population of low responders who are at high risk of acquiring influenza infection.

Visualizing the Immune Response, Cancer Therapy: Advances in Drug Design and Delivery

High-Throughput Fluorescence Polarization Screen for Inhibitors of VICKZ1 RNA Binding

Nadav Wallis

Introduction VICKZ (Igf2bp) protein family consists of RNA binding proteins (RBPs) that have important roles during development in many embryonic cell types. Three VICKZ paralogs have been identified in mammals; after birth, VICKZ1&3 is dramatically down-regulated and is almost non-detectable in adults. In many types of cancers, VICKZ1&3 is re-expressed and has been correlated with many pro-oncogenic processes. VICKZ1 has been shown to promote proliferation, invasion and chemo-resistance in malignancies by regulating post-transcriptional processes of RNA in the cell, VICKZ1 binds a number of specific mRNAs. One such mRNA is Kras.

Material and method Fluorescence Polarization This assay is based on changes in fluorescence polarization as a result of binding of a protein to a labeled RNA. When polarized light excites a fluorophore, such as fluorescein-labeled K-ras RNA, the relatively small fRNA usually undergoes rotational diffusion more rapidly than the time required for light emission. Therefore, the position of the fRNA at the time of light emission is largely randomized, resulting in depolarization of the emitted light. In contrast, when protein, such as VICKZ1 binds to the fRNA, the larger size and volume of the protein-fRNA complex causes rotation to be slower, increasing the likelihood that the protein-fRNA complex will be in the same plane at the time of light emission as it was at the time of excitation. Therefore, the emitted light remains highly polarized.

Results and discussion In collaboration with the Israel National Center for Personalized Medicine (INCPM), I have performed a high-throughput Fluorescence polarization (FP) screen for small molecule inhibitors of VICKZ1 RNA binding. By comparing 5' fluorescently-labeled fragments of Kras mRNA, we identified by FP a 200nt sequence in its 3'UTR that binds VICKZ1. Using this fragment as a probe, we scanned over 100,000 compounds for those that would prevent VICKZ1 binding. This highly robust assay has yielded approximately 7 reproducible hits whose inhibition is dose-dependent (IC₅₀ in the tens of μ M) and specific for VICKZ1 (no effect on a control RBP - La protein - binding to its target - Bcl RNA).

These hits are currently being validated with orthogonal assays such as electrophoretic mobility shift assay (EMSA), Microscale thermophoresis (MST), and isothermal titration calorimetry (ITC). Binding of IGF2BP1 to Kras RNA was inhibited 8 - 10 fold at high concentrations (25-50 μ M) of the lead compound. Incubation of cells with the lead compound demonstrated dose-dependent inhibition of wound healing, reduction of VICKZ1 target RNAs as determined by real time PCR, and downregulation of ERK phosphorylation (a readout of K-ras signaling). No toxicity was observed (cell proliferation unaffected) in any of the cell lines tested.

Conclusion We have performed a screen for small molecule inhibitors of VICKZ1 binding to Kras mRNA,, we have compounds that inhibit binding in a FP. The lead compound was confirmed and demonstrated biological effectiveness with no toxicity. Analogs of the confirmed hit are currently being screened to select molecules that can work at even lower concentrations. These analogs will be tested in in vivo models.

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Visualizing the Immune Response

Profiling the Effect of Obesity on the Influenza Vaccine-Induced Antibody Repertoire Using Antigen Microarrays

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Introduction: Influenza viruses cause up to 500,000 deaths per year worldwide. Vaccination is the most effective strategy for preventing influenza infection and is a key component for pandemic preparedness. However, vaccines fail to provide optimal protection in high-risk groups such as obese and the elderly. Obesity is a risk factor for developing severe influenza infection making vaccination of utmost importance for this high-risk population.

Methods: To study the effects of obesity on the anti-influenza antibody repertoire before and following influenza vaccination we used serum samples that were collected from healthy weight (Body Mass Index: $18.5 \leq \text{BMI} \leq 24.9$) and obese ($\text{BMI} \geq 30 \text{ kg/m}^2$) patients at baseline and 30 days following vaccination with the 2010-2011 trivalent inactivated influenza vaccine (TIV). We developed a novel antigen microarray (AM), spotted with BPL-inactivated influenza A and B viruses, partially overlapping 20mer peptides of the hemagglutinin (HA) and neuraminidase (NA) surface proteins of the Cal2009 H1N1 vaccine strain (Calog).

Results: We found that both healthy-weight and obese subjects generated antibodies against the vaccine strains as well as cross-reactive antibodies against other influenza viruses from the same subtypes. However, binding of serum antibodies to both peptides and whole virus antigens demonstrated significant differences between obese and healthy-weight humans. Healthy-weight subjects generated stronger and broader antibody responses to the Calog vaccine strain, both pre- and post-vaccination. While both groups demonstrated a significant rise in the antibody titers to whole influenza viruses post-vaccination, this rise was higher and more significant in healthy-weight than in obese individuals.

Discussion: These findings suggest that healthy weight and obese individuals generate different repertoires of antibodies following TIV, and demonstrate that AMs can be used to identify population specific antibody responses that may be potential correlates of protection of influenza vaccines.

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Immunopathologies and Precision Medicine

Study of antigen presentation and antigen-specific immunomodulation in multiple sclerosis by T cell receptor-like antibodies

Alona Goor

Introduction. Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) attacking approximately 2.5 million people worldwide. Symptoms include blurred vision, nausea and numbness and can deteriorate to complete loss of mobility and death. MS is characterized by distraction of the myelin tissue by auto-reactive CD4+ T cells: activation of CNS-infiltrating auto-reactive CD4+ T cells is triggered by the recognition of myelin-specific peptides presented on MHCII by antigen presenting cells (APCs), resulting in massive inflammation and consequently tissue damage.

Inhibition of inflammatory processes during MS is frequently achieved by non-specific down regulation of the immune system, which may lead to severe and potentially life-threatening side effects. Thus, targeted and specific inhibition of autoreactive CD4+ T cells is highly desirable as a treatment for MS and autoimmune diseases in general. We suggest that during inflammatory processes in MS, APCs can present several myelin derived epitopes (e.g. MOG and MBP) simultaneously and as a consequence activate a variety of autoreactive T cells. We therefore suggest that targeting one of these epitopes with a T cell receptor-like (TCRL) antibody (Ab) could result in the elimination of specific APCs, thus prevent epitope spreading, decrease myelin-specific T cells activation, affect differentiated T cell populations and most importantly decrease disease exacerbation.

Materials and methods. We have isolated and characterized TCRL Abs directed against MOG (35-55) or MBP (85-99)/ HLA-DR2 epitopes. The potential mode of action of these antigen-specific TCRL Abs can be exerted via two mechanisms: (i) blocking the interactions between pathogenic T cells and their peptide-MHC ligands presented on APCs and (ii) specifically eliminate APCs presenting MS-associated autoantigens.

Results. The isolated TCRL Abs exhibit specific inhibition of T cell proliferation *in vitro* and *in vivo*. Moreover, treatment with a TCRL Ab of established EAE in HLA-DR2 Tg mice model significantly attenuate disease progression and abolish EAE associated symptoms. Additionally, administration of the TCRL prevents EAE development in these mice.

Conclusions. Our data demonstrate that targeting the core driving force of the immune response, the TCR-MHC axis, in an antigen-specific manner may lead to a new approach for immunotherapy of autoimmune diseases and other inflammatory disorders.

Inflammation and Immunity – Friends or Foes?

COMMD10 Promotes Resolution Of Acetaminophen-Induced Liver Injury By Immunoregulation Of Monocyte And Macrophage Activity

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Introduction: Drug-induced liver injury is a challenge for drug development. Hepatocyte necrosis initiates an innate inflammatory response that facilitates the recovery from injury. Using a murine model of acetaminophen-induced liver injury (AILI) we have previously demonstrated that liver resident Kupffer cells (KCs) and infiltrating monocyte-derived macrophages (MoMFs) play an important role in liver resolution. Their local inflammatory response has to be critically regulated to avoid collateral damage and ensure proper liver regeneration, yet, the immunoregulatory mechanisms remain unknown. COMMD family proteins, and specifically COMMD10, are emerging as key regulators of signaling and protein trafficking during inflammation. Here we investigated the cell-specific immunoregulatory circuits orchestrated by COMMD10 in effector Ly6C^{hi} monocytes, their MoMF descendants and KCs in AILI. **Methods:** We established a conditional COMMD10-deficient mouse (*Commd10^{fl/fl}*). COMMD10-deficiency was targeted to liver Ly6C^{hi} monocytes, MoMFs and KCs using the *Lyz2^{cre/+}* mice (*LysM^{ΔCommd10}*), and specifically to KCs using the *Cx3cr1^{cre/+}* mice (*Cx3cr1^{ΔCommd10}*). **Results and discussion:** *LysM^{ΔCommd10}* and *Cx3cr1^{ΔCommd10}* mice exhibited significantly exacerbated hepatic damage and increased infiltration of pro-inflammatory Ly6C^{hi} monocytes and neutrophils. Our results outline a pivotal role for COMMD10 in restraining inflammasome activity in Ly6C^{hi} monocytes. Moreover, COMMD10-deficiency in Ly6C^{hi} monocytes prominently abolished expression of type I interferon induced genes, which are upregulated upon their differentiation into pro-restorative MoMFs. With respect to resident KCs, COMMD10-deficiency was associated with diminished expression of the apoptotic cell clearance receptors TIM4 and MERTK resulting in their impaired ability to clear damaged hepatocytes and neutrophils. **Conclusion:** COMMD10 plays a pivotal pro-restorative role in AILI via its restraining of inflammasome activity in Ly6C^{hi} monocytes and promotion of apoptotic cell clearance by KCs.

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Inflammation and Immunity – Friends or Foes?

Tellurium-based immunomodulating compound SAS inhibits the progression of early-onset Alzheimer`s disease in the 5xFAD mouse model.

Benjamin Sredni^{2,4}

Objectives. Alzheimer`s disease (AD) is a progressive incurable neurodegenerative disorder. To date, therapies suggested to be beneficial for AD, are clinically tested at an advanced age, after disease onset. AD is characterized by an overload of amyloid-beta ($A\beta$) plaque in the central nervous system (CNS); the functional decline correlates with the deposition of $A\beta$ peptide containing plaques, hence clearance of $A\beta$ from the brain is an important therapeutic strategy for Alzheimer`s disease. Previous studies in our lab showed the ability of tellurium compounds to inhibit PD-L1 expression, IL-10, caspase-1, and caspase-3 activities, which were associated with decreased neuronal death, inhibition of IL-1 β and up-regulation of GDNF. These results led us to examine the ability of the small organotellurium compound octa-O-bis-(R, R)-tartrate ditelluride (SAS) to reduce $A\beta$ deposits and ameliorate cognitive deficits.

Methods. 5xFAD mice were administered with SAS every other day. $A\beta$ plaques were quantified using immunohistochemistry, cognitive and behavioral abilities were assessed using behavioral paradigms.

Results. Administration of SAS has shown a significant effect of $A\beta$ clearance in the cortex of 5xFAD mice in 5 and 9m of age. 5xFAD/SAS mice exhibit normalized exploratory behavior, along with the alleviation of short-term memory deficits compared with non-treated controls 5xFAD/PBS. This results suggesting that early treatment with SAS may inhibit the progression of AD pathology. We hypothesized that possible mechanism of action is by inhibition of PD-L1 expression and pro-inflammatory cytokine secretion, a process that eventually leads to elevation of neuroprotective factors, nowadays we try to find a possible mechanism of action.

Conclusions. SAS confers long-term beneficial effects in mice, possibly by downregulation of inflammatory cytokines and elevation of neuroprotective factors, resulting in $A\beta$ -clearance and amelioration of cognitive deficits.

Inflammation and Immunity – Friends or Foes?

Chemotherapy treated-metastatic cells harness macrophages to support metastatic outgrowth

Shira Michaeli-Ashkenasi

Metastatic breast cancer can appear years after mastectomy and chemotherapy treatments and represents the principal cause of breast cancer related deaths. These recurring lesions arise from disseminated tumor cells (DTCs) that lay dormant (quiescent) and resist chemotherapy treatments. Recent studies demonstrate that chemotherapeutic drugs can induce pro-metastatic activities at distant sites leading to metastatic recurrence. However, the mechanisms underlying this pro-metastatic activity are largely unknown. We hypothesized that different chemotherapeutic treatments of metastatic breast cancer cells will harness macrophages ($M\phi$) to promote or repress the outgrowth of dormant DTCs. To test our hypothesis, we utilized $M\phi$ cell line; RAW 264.7 and D2A1 mammary cancer cell line that display a transient dormant phase in a 3D BME system that model's tumor dormancy and outgrowth and *in vivo*. D2A1 cells were treated with either Doxorubicin (DOX) or Paclitaxel (PTX) to induce their apoptosis. Our results demonstrate no significant difference in the efferocytosis capacity of the macrophages engulfing PTX/DOX treated D2A1 cells. However, macrophages engulfing PTX/DOX treated D2A1 cells displayed distinct gene signature determined by RNA-seq and NMDS analysis. Furthermore, Go-term enrichment analysis among the differentially expressed genes displayed enrichment for genes related to defense and immune response in $M\phi$ engulfing DOX-treated D2A1 cells opposed to $M\phi$ engulfing PTX-treated D2A1 cells. Furthermore, enrichment in genes related to fibroblasts proliferation and wound healing was evident in $M\phi$ engulfing PTX-treated D2A1 cells. These results were further corroborated by our findings demonstrating that soluble mediators present in the conditioned media of $M\phi$ engulfing PTX-treated D2A1 cells promoted the formation of a fibrotic-like niche *in vitro*, whereas conditioned media of $M\phi$ engulfing DOX treated-D2A1 cells prevented the formation of a fibrotic-like niche. Notably, formation of a fibrotic-like niche was previously shown to induce the outgrowth of dormant DTCs. These findings suggest that macrophages engulfing chemotherapy-eradicated metastatic cells will be reprogrammed to distinct entity that may promote or repress the permissive microenvironment of dormant DTCs. The reprogramming of the macrophages is depended on the chemotherapy induced apoptotic signal. We are currently elucidating the identity and mechanism of action of the pro-fibrotic mediators and exploring whether these mediators will promote dormant DTCs outgrowth.

Significance: Identification and characterization of the pro-metastatic mediators secreted by these newly reprogrammed $M\phi$ may serve as a 1) surrogate marker to follow potential recurrence of the disease after chemotherapy treatment 2) potential novel therapeutic target(s) to prevent the pro-metastatic effect of the chemotherapy treatment. This may pave the way to design complimentary treatments to the chemotherapy treatments to prevent breast cancer from ever recurring.

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Inflammation and Immunity – Friends or Foes?

Probing functional contributions of microglia and non-parenchymal CNS macrophages in physiology and pathophysiology

Jung-Seok Kim

Introduction

Brain macrophages have emerged as major players in central nerve system (CNS) physiology and pathophysiology. Much of the recent insight derives from fate mapping, intra-vital imaging, cell ablation and targeted mutagenesis using respective Cre / loxP system-based mouse models. In parallel, advances in flow cytometry and single cell transcriptomics have highlighted the complexity of the brain macrophage compartment^{1,2}. Specifically, the latter comprises parenchymal microglia and non-parenchymal, CNS border-associated macrophages (BAM) located in perivascular and meningeal niches, as well as the choroid plexus. In depth understanding of specific functional contributions of these distinct CNS macrophage populations will require the development of novel binary transgenic Cre approaches that allow the study in physiological context.

Material and Methods

Here, we developed novel binary transgenic mouse models relying on the co-expression of split Cre fragments to target specific CNS macrophage populations. Following crossing to animals harboring conditional reporter alleles or a 'Ribo-tag' allele that allows transcriptome profiling³ we confirmed differential targeting of microglia and BAM.

Results and discussion

According to imaging, flow cytometry analysis, and mRNA expression profiling results, a combination of $Cx3cr1^{C-Cre}$ and $Sall1^{N-Cre}$ transgenes allowed for specific targeting of microglia, unlike previously established $Cx3cr1^{CreER}$ and $Sall1^{CreER}$ transgenes (Chappell-Maor et al, submitted). In contrast, $Cx3cr1^{C-Cre}; Lyve1^{N-Cre}$ transgenic mice were found to specifically target non-parenchymal macrophages located in meningeal and perivascular regions. We are currently using these animals in combination with the RiboTag approach to define distinct functional contributions of microglia and BAM to CNS pathologies.

Conclusion

Advanced sequencing studies highlight the transcriptomic heterogeneity of macrophage populations in CNS. Taking advantage of a binary transgenic split Cre system, we have started to dissect the functional heterogeneity of macrophage populations in their physiological brain context.

Metabolism and the DNA-Damage Response, Host-Pathogen Interaction

A Genome-Wide Screen Identifies a Critical Role For Mitochondrial NDP Kinases in Inflammasome Activation

Orna Ernst Rabinovich¹

Introduction:

While there has been a remarkable progress in understanding the mechanisms of cytosolic LPS sensing by caspase11, the cellular processes regulating non-canonical inflammasome activation are less clearly understood.

Methods:

In an effort to address this, we have conducted a genome-scale siRNA screen for the non-canonical inflammasome response to cytosolic LPS. We have used a screening-optimized HTRF assay for secreted IL-1 α in RAW264.7 mouse macrophage cells. Through a combination of LDH and TNF α measurements we have filtered and prioritized hits in a secondary screen. We used siRNA knockdown in primary BMDM and CRISPR/Cas9 knock out of hit genes both in macrophage cell lines and mice to further validate and study novel mechanisms of inflammasome activation.

Results:

Among the top screen hits we identified numerous expected genes, including *Myd88*, *Irak4*, *Irak2*, *Casp4*, *Gsdmd* and *Il1 α* , and also discovered numerous novel regulators. Significant mitochondrial-associated gene enrichment supported an important role for the mitochondria and cellular metabolism in inflammasome activation. Specifically, we investigated the role of NDP kinase in inflammasome activation and find NDPK^{-/-} macrophages have a strongly diminished IL-1 α response to cytosolic-LPS, show defective ROS- and cardiolipin-dependent mitochondrial licensing, and have markedly dysregulated inflammasome priming and triggering. Metabolic analysis suggests NDPK is critical to priming-induced glycolytic commitment, however we observe normal NF- κ B and MAPK activation in primed NDPK^{-/-}, suggesting the mitochondrial and metabolic contribution to inflammasome priming occurs independently of these signaling responses. We also find that NDPK deficient mice show substantial resistance to LPS-induced endotoxic shock.

Conclusions:

We found an unexpected role for the NDPK family of mitochondrial dinucleotide kinases in the effective priming of the inflammasome response by TLR ligands, thus establishing a link between the cellular metabolic state of the macrophage and the LPS-driven inflammatory response. Our data delineate the mitochondrial and metabolic processes critical to inflammasome activation and suggest that a critical mitochondrial fitness signal is required to induce inflammasome activation.

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Israel Immunological Society

Immunopathologies and Precision Medicine

Refining Immune Checkpoints for Natural Killer-based immunotherapy

Angel Porgador

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The cancer-associated immunome, particularly within the cancer microenvironment, is associated with cancer prognosis. The presence and phenotype of intratumoral natural killer (NK) cells in the cancer microenvironment is tightly associated with cancer prognosis. NK cell activity is a balance between signals delivered by inhibitory and activating receptors. The issue of altered expression of activating/inhibitory isoforms of immune-associated genes needs will be presented; in particular, the splicing-enabled paradoxical role of the NK receptor, NKp44/NCR2. The NKp44-PCNA immune checkpoint will be detailed with references to cancer and pregnancy. Monoclonal antibody based blocking of immune checkpoints involving the CTLA4-B7 and the PD1-PDL1 inhibitory axes enhance T cell-based adaptive responses in cancer patients. Similarly, anti-tumor responses by Natural Killer (NK) cells can also be enhanced by checkpoint blocking mAb against Proliferating Cell Nuclear Antigen (PCNA) which is expressed on the surface of cancer cells and acts as an inhibitory ligand for the NK cell receptor, NKp44-isoform1. Hybridoma technology followed by FACS- and ELISA-based screenings were done to generate the specific mAb, 14-25-9. FACS and ImageStream based staining of cell lines and immunohistochemistry of human cancer FFPE tissues were done to test for cytoplasmic and membrane-associated PCNA. NK functions were measured using ELISA-based IFN- γ secretion assays and FACS-based killing assays. In vivo efficacy was evaluated on patient-derived xenografts (PDX)-bearing NSG mice. The 14-25-9 mAb effectively inhibits binding of chimeric NKp44 receptor to PCNA and stains mostly the cytoplasm and membrane of tumor cells, whereas commercial antibody (clone PC10) stains nuclear PCNA. The NK92-NKp44-1 cell line and primary human NK cells showed increased IFN- γ release upon co-incubation with mAb 14-25-9 and various solid tumor cell lines and leukemia. Treatment with 14-25-9 also increased the NK cytotoxic activity. In PDX-bearing mice, intravenous administration of mAb 14-25-9 increased degranulation (CD107a expression) of intratumorally-injected patient-autologous or allogeneic NK cells as well as inhibited tumor growth when treated long term. This study represents a novel mAb against the NKp44-PCNA innate immune-checkpoint, which can enhance NK cell anti-tumor activity both *in vitro* and *in vivo*.

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Precision in Personalized Cancer Immunotherapy

The Immunotherapeutic Properties of Novel Tellurium Compounds: Mechanism of Action and Clinical Results

Benjamin Sredni

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Tellurium is a rare element, which has been regarded as a non-essential trace element; its biological role, if any, has not been clearly established to date. The investigation of therapeutic activities of tellurium compounds is rather limited in the literature, despite the relative abundance of tellurium in the human body. Nevertheless, the varied activities of tellurium agents in both malignant and normal cells are extremely exciting, though very complex. Not surprisingly, an increased interest in tellurium among biological chemists and pharmacists has fuelled the search for more and more diverse tellurium compounds. We will focus on two small inorganic tellurium complexes, ammonium trichloro(dioxoethylene-O,O')tellurate (AS101) and Octa-O-bis-(R,R)-tartarate ditellurane (SAS), thoroughly investigated by us, converging at their clinical activities, and elucidating their mechanism of action. AS101 is probably the most extensively studied synthetic tellurium compound from the standpoint of its biological activity. It is a potent immunomodulator with a variety of potential therapeutic applications. It is probably the only tellurium compound to be tested in phase I/II clinical studies including Cancer patients and aging Macula degeneration (AMD). The effects of AS101 and SAS are primarily caused by their specific Te(IV) redox-modulating activities enabling the inactivation of the Integrin VLA-4. All of these have profound consequences regarding clinical activity including sensitization of tumors to chemotherapy and anti-angiogenic activities. These properties, coupled with the excellent safety profile of the compounds, suggest promising clinical therapeutic potential for tellurium compounds such as AS101 or SAS.



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Visualizing the Immune Response, Bioinformatics, Big Data and Cancer

A Tissue Atlas of Human B-Cell Receptor Populations Reveals Two Separate Immune Networks in the Gut and in the Blood

Uri Hershberg

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The function of the immune system cannot be reduced to the action of a single immune cell, rather it is the result of a cooperative interaction of multiple immune cells, each with its own context of interaction and environmental feedback and each with a repertoire of antibodies that have different binding capabilities. For instance, while immune receptors with some level of affinity to one's self are often observed, only some individuals suffer from autoimmunity. A key element in the comparison of the behavior of immune cells and repertoires is the definition of individual clonotypes (sets of cells that are derived from a common progenitor cell with a common B-cell receptor, or BCR). Clonotypes comprise the basic building blocks of repertoires and their response.

In our study published in Nature Biotechnology we analyzed in depth the repertoires of 6 individual organ donors in 8 different tissues, from one of which we analyzed over 50 samples per tissue and identified ~50 million unique sequences and ~1,000,000 different clonotypes. Due to this extreme depth of sequencing and our exact assessment of sampling sufficiency and levels of overlap, we could determine that the human immune repertoire is divided into two nearly orthogonal clone networks. These networks—one in the gut and the other in the blood/lung/spleen—show different levels of mixing internally amongst themselves and do not mix between each other. These findings have great implications regarding how we monitor and treat diseases in the different organs and are a first step in the science of tissue specific immune manipulation.



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Cancer Metastasis, State-of-the-Art Methodologies in Research, Bioinformatics, Big Data and Cancer

TCR Repertoires of Tumor Infiltrating T Cells in Metastatic Breast Cancer

Erez Greenstein¹

Department of Immunology, Weizmann Institute of Science, Israel

Breast cancer is the most prevalent cancer in women around the world, accounting for 30% of all new cancer cases in women in the US. In this study, we examine genetic and immunological data from eight breast cancer patients who had died from metastatic breast cancer, focusing on the T cell response to the metastases. This response is mediated by tumor infiltrating lymphocytes (TILs). We capture a sample of the T cell repertoire in the metastases by sequencing the alpha and beta chains of T cell receptors, found on TILs inside the different metastases, using high-throughput sequencing (TCRSeq).

We start by studying classic notions of TCR repertoires like sharing and expansion and show the role they play in shaping the metastatic repertoire. Next, by using unsupervised learning techniques we are able to show that the T cell response has a distinct organ specific signature, i.e., it is more similar in metastases in the same organ than in metastases in different organs. We also show that there is a high correlation between the organization of the T cell response, and the mutational landscape of breast cancer as captured by a phylogenetic model of the evolution of patient's metastases.

This work has interesting consequences for designing new T cell based immune-therapies. Especially, it shows that any therapy of this kind should take into account the different immune signature of metastases in different organs.

These findings were published recently (May 2019) in Cell Reports: "The genomic and immune landscapes of lethal metastatic breast cancer"



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Leukemia: From Stem Cell to Therapy

Hematopoietic stem cell transplantation in the era of next generation sequencing

Polina Stepensky

Primary immune deficiency (PID) refers to a large heterogeneous group of disorders that result from defects in immune system development and/or function leading to increased susceptibility to infections, disorders of immune regulation and malignancies. PID prevalence is estimated at 2-5:100.000. The incorporation of next generation sequencing (NGS) into routine immunological practice has enabled the identification of novel inborn errors of disease, helped define new categories of immune deficiency and extended the clinical spectrum associated with many long-recognised diseases. Indeed, sixteen years after the completion of the human genome project, the use of whole exome sequencing (WES) has turned into a primary diagnostic tool in cases of PIDs. Between 2012 and 2019, hundreds of WES were conducted at Hadassah Medical Center on pediatric patients with suspected PID. In about 60% of cases, a precise genetic diagnosis was found. A major strength of our translational work derives from the fact that our clinical practice comprises a large number of patients born to consanguineous families. Indeed, in collaboration with community physicians from Israel and the Palestinian Authority, we have identified new forms of inherited immune deficiency. The first disorders were identified by homozygosity mapping (*ITK* and the *SP110* genes) and additional disorders were later discovered by whole exome sequencing (the *CARD11*, *IKK β* , *VPS45*, *EFL1*, *TPP2* and *LAT* deficiencies). The use of WES in suspected PID displays several advantages. First, WES enables the early diagnosis of affected, yet asymptomatic, family members, often before the onset of severe disease features, which significantly reduces the successful rate of hematopoietic stem cell transplantation (HSCT) if required. Characterization of disease-causing genes, together with a detailed immunological workup, both carried out in our institution, contribute to the understanding of novel mechanisms of immune disease and influence the clinical decision-making: the knowledge that these defects affect the hematopoietic system gave the confidence to proceed with HSCT and subsequently cure numerous patients of their disease. In the near future, the deep understanding of the biological basis of PIDs could lead to the development of novel therapeutic avenues.



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Metabolism and the DNA-Damage Response, Bio-Markers and Cancer Theranostics

TP73-AS1 Promotes Chemotherapy resistance in Glioblastoma cancer stem cells

Gal Mazor

Glioblastoma multiform (GBM) is the most common brain tumour characterized by a dismal prognosis. GBM cancer stem cells (gCSC) or tumour-initiating cells are the cell population within the tumour driving therapy resistance and recurrence. While temozolomide (TMZ), an alkylating agent, constitutes the first-line chemotherapeutic significantly improving survival in GBM patients, resistance against this compound commonly leads to GBM recurrence and treatment failure. Although the roles of protein-coding transcripts, proteins and microRNA in gCSC and therapy resistance have been comprehensively investigated, very little is known about the role of long non-coding RNAs (lncRNAs) in this context. Using non-overlapping, independent RNA sequencing and gene expression profiling datasets, we reveal that *TP73-AS1* constitutes a clinically-relevant lncRNA in GBM. Specifically, we demonstrate significant overexpression of *TP73-AS1* in primary GBM samples, which is particularly increased in the gCSC. More importantly, we demonstrate that *TP73-AS1* comprises a prognostic biomarker in glioma and in GBM with high expression identifying patients with particularly poor prognosis. Using CRISPRi to downregulate our candidate lncRNA in gCSC, we demonstrate that *TP73-AS1* promotes TMZ resistance in gCSC and is linked to regulation of expression of metabolism related genes and *ALDH1A1*, a protein known to be expressed in cancer stem cell marker and protect gCSC from TMZ treatment. Taken together, our results reveal that high *TP73-AS1* predicts poor prognosis in primary GBM cohorts and that this lncRNA promotes tumour aggressiveness and TMZ resistance in gCSC.

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Immunopathologies and Precision Medicine

Engineering Immune Effector Molecules and Cells for Immunotherapy of Cancer and Autoimmunity

Yoram Reiter

Biology, Technion - Israel Institute of Technology, Israel

Antibody and protein engineering approaches are used in our laboratory to develop new cancer immunotherapy strategies which combine the advantage of the well-established tumor targeting capabilities of high affinity recombinant fragments of antibodies with the known efficient, specific, and potent killing ability and unique specificity of CD8 T lymphocytes directed against highly antigenic MHC/peptide complexes or other effector functions.

Two approaches have been developed by our research team. First, is a new class of recombinant chimerical molecules created by the genetic fusion of scFv antibody fragments, specific for tumor cell surface antigens, to monomeric single-chain HLA-A2 complexes containing immunodominant tumor or viral-specific peptides. Second, are unique recombinant antibodies that mimic the fine specificity of the T cell receptor and recognize tumor and viral specific peptide-MHC complexes.

The molecular feature of these molecules/approaches and their in vitro and in vivo activities will be described. The future development of these approaches as new modalities to immunotherapy, bridging antibody and T lymphocyte attack on cancer cells, will be discussed in the context of their development path to clinical trials humans.

The use of these novel molecules to study basic questions of tolerance will be described as well demonstrating the bridge between basic and translational immunological research.



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Co-stimulatory Switch Receptors – deriving benefit from immunosuppression to enhance T-cell function

Cyrille Cohen

Head, Laboratory of Tumor Immunology and Immunotherapy, Bar-Ilan University, Israel

Adoptive cell transfer of tumor specific lymphocytes, whether naturally occurring in patients or genetically engineered can lead to impressive objective response in terminally patients. Tumors can employ different mechanisms to evade immune surveillance and function. Overexpression of co-inhibitory ligands that bind to checkpoint molecules on the surface of T-cells can greatly impair the function of latter. PD1 and TIGIT (T cell immunoreceptor with Ig and ITIM domains) are such a co-inhibitory receptors expressed by T and/or NK cells which, upon binding to their ligand on tumor cells can diminish cytokine production and effector function. Additionally, the absence of positive co-stimulation at the tumor site can further dampen T-cell response.

As T-cell genetic engineering has become clinically-relevant in the recent years, we devised herein a strategy aimed at enhancing T-cell anti-tumor function by diverting T-cell coinhibitory signals into positive ones using a chimeric costimulatory switch receptor (CSR) composed of either the PD1 or TIGIT exodomain fused to the signaling domain of CD28.

After selecting an optimized TIGIT-28 CSR, we co-transduced it along with tumor-specific TCR or CAR into human T-cells. TIGIT-28-equipped T-cells exhibited enhanced cytokine secretion and upregulation of activation markers upon co-culture with tumor cells. TIGIT-28 enhancing capability was also demonstrated in an original in vitro model of T-cell of hypofunction induction upon repetitive antigen exposure. Finally, we tested the function of this molecule in the context of a xenograft model of established human melanoma tumors and showed that TIGIT-28-engineered human T-cells demonstrated superior anti-tumor function. Overall, we propose that TIGIT-based CSR can substantially enhance T-cell function and thus contribute to the improvement of engineered T cell-based immunotherapy.

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CAR and CTL Therapy in Cancer

Dual CAR T-Cells to Treat Multiple Myeloma

Anat Globerson Levin¹

Immunology, Tel Aviv Sourasky Medical Center, Israel

Multiple Myeloma (MM) is a clonal malignancy of plasma cells. Patients with standard-risk myeloma have a median overall survival of 6–7 years, while those with high-risk disease have a median survival of less than 2–3 years. Despite recent progress in drug development, MM remains incurable mostly due to the development of recurrent disease that resists available therapeutic agent(s). Here, we show the construction of chimeric, antibody-based receptors (CAR) to redirect T cells for adoptive cell treatment of MM. CAR T cell therapy, pioneered in our lab, is a powerful tool for cancer treatment. This approach has proven very effective in clinical trials in leukemia and lymphoma patients and has recently gained FDA approval to treat certain types of large B-cell lymphomas.

Today, the major challenge in the CAR T cell field is to prevent `off-tumor on-target` toxicity, namely, the risk of damage to the patient`s healthy tissues which also express the target antigen of the selected CAR. We took advantage of the surface expression of several antigens that are widely expressed on MM cells and are poorly expressed by hematopoietic stem cells, generating CAR T cells with dual specificity expressing two complementary CARs (dual CARs) for the specific and effective treatment of MM. The complementary chimeric receptors transmit a full T cell activation signal only upon dual engagement with tumor-associated antigens. Here we show the killing effect *in vitro*, and the superior survival curve of mice treated with dual CAR T cells without unwanted effects against healthy tissue.

CAR and CTL Therapy in Cancer

CAR-T cell therapy in Israel – Clinical results of the first 90 patients treated with on-site produced CD19 CAR T cells

Michal Besser^{1,2}

Introduction

Autologous CD19 chimeric antigen receptor (CAR) T cells demonstrate outstanding remission rates in pediatric and adult patients with relapsed and refractory acute lymphoblastic leukemia (ALL) and Non-Hodgkin lymphoma (NHL). Two CD19 CAR T cell products received recently FDA approval.

Patients and method

In October 2017, the Sheba Medical Center initiated the first phase 1b/2 study with on-site produced CD19 CAR T cells in Israel. Gamma-retrovirus encoding for a CD19 CAR based on an FMC63 derived scFv, CD28 costimulatory domain and CD3-zeta signaling domain was used for the study. CAR-T cells were produced from patients' PBMC within 9-10 days.

Results and discussion

Until June 2019, 95 patients (half ALL and half NHL) were enrolled to the trial and 90 (95%) patients were treated. CAR T cells were successfully produced for 94 of 95 (99%) patients. Four patients dropped out of the study due to clinical deterioration. The median age of patients was 34 years (range 22 months – 71 years). Patients had a median of 4 prior regimens, including other CD19-based therapies and stem-cell transplantations. The overall response rate of evaluated patients was 70% (81% in ALL and 56% in NHL), including complete remission in 53%. The 1-year overall survival was 61%.

Conclusion

The clinical response rate and overall survival in refractory patients treated with on-site produced CAR T is remarkable and comparable with commercial CAR T products. The extremely fast turn-around time from PBMC collection to CAR T infusion of only 9-10 days, in contrast to 30-60 days with commercial CAR T products, and the high production success (99%) enabled the treatment of 95% of enrolled patients. We are currently aiming to transfer the production process into an automated system and make the therapy available to even more patients.

Visualizing the Immune Response

Microvilli: The ERM Dependent Activation Hubs of T-Cells

Shirsendu Ghosh¹

Introduction:

When T cells encounter cognate peptide-MHC complexes on antigen presenting cells, they respond within seconds. How such a fast response is orchestrated in very short periods of time has long been disputed. Interestingly, T-cell surfaces are covered with microvilli, actin-rich and flexible protrusions. Surprisingly, the role of the microvilli in the activation process of T-cells has long been underrated. Here we probe the localization of key surface molecules involved in the initial immune response, and demonstrate their enrichment on microvilli, implication these structures as T-cell activation hubs.

Materials and methods: Variable-angle total internal reflection microscopy was conducted on pre-fixed human T-cells cells residing on a glass surface. The fluorescence intensity measured at each point in an image was converted into the distance of that point from the glass, which permitted us to plot the 3D topography of the membrane surface. We then recorded stochastic localization nanoscopy images of antibody-labelled membrane proteins from the same cells, which allowed us to determine the positions of individual molecules with an accuracy well below the diffraction limit. By superimposing the super-resolved membrane protein map on the 3D membrane topographical map, we were able to characterize the distribution of each protein molecule in relation to microvilli.

Results and discussion: We show that 90% T-cell receptor (TCR) complex molecules $TCR\alpha\beta$ and $TCR\zeta$, as well as the co-receptor CD4 and the co-stimulatory molecule CD2 reside on microvilli on the T cell surfaces. Furthermore, TCR proximal signaling molecules involved in the initial stages of the immune response, such as the protein tyrosine kinase Lck and the key adaptor molecule LAT, are also enriched on microvilli. Phosphorylated proteins of the ERM (ezrin, radixin, moesin) family colocalize with $TCR\alpha\beta$ heterodimers as well as with actin filaments within the microvilli of resting T cells. This finding implies a role for one or more phosphorylated ERMs in linking the TCR complex to the actin cytoskeleton within microvilli. Indeed, expression of a dominant-negative ezrin fragment effectively redistributes TCR molecules over the whole T cell surface.

Conclusion:

Our results establish microvilli as key signaling hubs, on which the TCR complex and its proximal signaling molecules and adaptors are pre-assembled prior to TCR activation. The preformed organization of these actin-binding TCR assemblies on individual microvilli can facilitate the local transmission of TCR signals seconds after TCR occupancy and might also impact the slower subsequent events that lead to the assembly of immunological synapses.

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Immunopathologies and Precision Medicine

CRISPR Gene Correction: A Potential New Class of Medicines for Primary Immunodeficiency Diseases

Ayal Hendel

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The severe combined immunodeficiencies (SCIDs) are a set of life threatening genetic diseases in which patients are born with mutations in single genes and are unable to develop functional immune systems. While allogeneic bone marrow transplantation can be curative for these diseases, there remain significant limitations to this approach. Gene therapy using viral vectors containing a corrective transgene is being developed for some of these disorders; however, for other SCID disorders, such as those caused by genetic mutations in *RAG1* and *RAG2*, the transgene needs to be expressed in a precise, developmental and lineage specific manner to achieve functional gene correction and to avoid the risks of cellular transformation. In contrast to using viral vectors to deliver transgenes in an uncontrolled fashion, we are working towards developing CRISPR genome editing to correct the *RAGs* disease-causing mutations by precisely modifying the genome. CRISPR genome editing requires delivery of both the Cas9 nuclease and the targeting guide RNA (gRNA). The gRNA component can be generated in multiple ways, each with advantages and disadvantages. Here we compare the efficiency of editing, the on- and off-target repair profiles of *RAG1* and *RAG2* gRNAs delivered as a chemically-synthesized sgRNA and a chemically-synthesized bipartite complex (crRNA + tracrRNA). Our results show that the chemically-modified sgRNAs and the Alt-R® bipartite crRNA + tracrRNA complex, delivered as a ribonucleoprotein (RNP) complex, enable the highest genome editing in human primary CD34+ hematopoietic stem and progenitor cells (HSPCs) with lowest toxicity. Additionally, we show that we can use the combination of CRISPR-Cas9 RNP, chemically modified gRNAs, and recombinant adeno-associated viral vector (rAAV) donor transduction to effectively target functional *RAG2* cDNA into the endogenous locus in human primary CD34+HSPCs. We will also present a summary of a comprehensive analysis of the off-target events associated with the delivery of the synthetic *RAG1* and *RAG2* gRNA forms. The off-target profiles for each class of gRNA will be compared using the unbiased GUIDE-seq approach and quantified using rhAmpSeq™, a multiplexed, amplification-based, target enrichment next-generation sequencing (NGS) approach and finally the implication of our findings for therapeutic genome editing will be discussed.



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Microenvironment and Immuno-Oncology, Precision in Personalized Cancer Immunotherapy,
Bioinformatics, Big Data and Cancer

Proteomics of Melanoma Response to Immunotherapy Reveals Dependence on Mitochondrial Function

Tamar Geiger¹

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Immunotherapy has revolutionized cancer treatment, yet most patients do not respond. Here, we investigated mechanisms of response by deeply profiling the proteome of clinical samples from advanced stage melanoma patients undergoing either tumor infiltrating lymphocytes (TIL)-based or anti-PD1 immunotherapy. Using high-resolution mass spectrometry, we quantified over 10,300 proteins with high accuracy. Statistical analyses revealed higher oxidative phosphorylation and lipid metabolism in responders in both treatments, and identified proteomic signatures for response. Aiming to elucidate the effects of the metabolic state on the immune response, we examined melanoma cells upon metabolic perturbations or Crisp-Cas9 knockouts. These experiments indicated lipid metabolism as a regulatory mechanism that increases melanoma immunogenicity by elevating antigen presentation, thereby affecting the sensitivity to T-cell mediated killing both in-vitro and in-vivo. Altogether, our proteomic analyses revealed novel association between the melanoma metabolic state and the response to immunotherapy, which can be the basis for future improvement of therapeutic response.



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State-of-the-Art Methodologies in Research

Gold nano rods and diffusion reflection imaging for mapping tumor margins

Dror Fixler

A critical challenge arising during a surgical procedure for tumor removal is the determination of tumor margins. Gold nanorods (GNRs) conjugated to epidermal growth factor receptors (EGFR) (GNRs-EGFR) have long been used in the detection of cancerous cells as the expression of EGFR dramatically increases once the tissue becomes cancerous. Optical techniques for the identification of these GNRs-EGFR in tumor are intensively developed based on the unique scattering and absorption properties of the GNRs. In this study, we investigate the distribution of the GNRs in tissue sections presenting squamous cell carcinoma (SCC) to evaluate the SCC margins. Air scanning electron microscopy (airSEM), a novel, high resolution microscopy is used, enabling to localize and actually visualize nanoparticles on the tissue. The airSEM pictures presented a gradient of GNRs from the tumor to normal epithelium, spread in an area of 1 mm, suggesting tumor margins of 1 mm. Diffusion reflection (DR) measurements, performed in a resolution of 1 mm, of human oral SCC have shown a clear difference between the DR profiles of the healthy epithelium and the tumor itself.



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State-of-the-Art Methodologies in Research

Noninvasive sensor technologies for disease detection via breath Volatolomics

Yoav Broza

The use of a volatolomic signatures based on volatile organic compounds (VOCs) is a novel and tested approach for diagnosing different diseases. Sensors have a high potential as easy to use point-of-care systems. In this talk we will focus on recent achievements in Gastric cancer study. It was previously shown that GC can be diagnosed from breath. In previous study on GC we classified sensors on a training and validation population of 484 people. In recent work we further extent the latter and use the database of 441 people, (excluding 43 dysplasia and peptic-ulcer samples) as the training classifier for screening a new set of 726 presumed non-cancer volunteers.

State-of-the-Art Methodologies in Research

Proteasome Profiling of Non-Small Cell Lung Carcinoma

Aaron Javitt¹

Department of Immunology, Weizmann Institute of Science, Israel

Introduction:

The mammalian proteasome is estimated to degrade ~70% of all intracellular proteins and plays a key role in antigen processing and MHC presentation. Recently we have developed a system for proteasome footprinting by mass-spectrometry analysis of proteasome-cleaved peptides (MAPP)^[1]. MAPP offers, for the first time, direct analysis of the degradation products. Utilizing this system we have analyzed a clinical cohort of Non-Small Cell Lung Carcinoma (NSCLC) tumor samples and adjacent tissue controls to study the tumor degradome. Analysis by MAPP identified differences in the tumor tissue, some of which were not detected by standard proteomics of the same samples. Further, our analysis revealed altered metabolic pathways in the tumor tissue and offer novel targets for therapeutic intervention in NSCLC.

Methods:

The cohort included lung adenocarcinoma samples with matching adjacent tissue from nine patients, obtained from the Midgam biobank. MAPP was performed as previously described¹ by cellular crosslinking followed by immunopurification of cellular proteasomes with nascent cleaved peptides. Peptides were then eluted from proteasomes and analyzed by MS/MS. In parallel to MAPP, we also performed bottom-up tryptic proteomics from whole cell extract, thereby assessing the abundance of proteins in the same samples as well as their degradation profiles.

Results:

We found that there was a significant increase in the number of proteasome-generated peptides identified in the tumor samples as compared to the adjacent control. This correlates with an increase in proteasome abundance in the tumor. Intriguingly, there were many proteins which had a change in degradation level that was observed across the majority of the patients in the cohort. Key proteins in several pathways, some of which are already known to be altered in NSCLC such as glycolysis and glucose metabolism, were also misdegraded in the samples analyzed. Finally, there were many proteins which were not detected as degraded in the adjacent tissue but were highly degraded in a majority of tumor samples. Importantly, these differentially degraded proteins would have been missed by proteomics from whole cell extracts or RNAseq alone. These proteins are potential targets for cancer vaccines as they are present and degraded only in the tumor tissues.

Conclusion:

Taken together, MAPP offers a broadly applicable method to facilitate the study of cellular degradation in various human pathologies involving changes in proteasomal degradation, including cancer. The ability to use MAPP in clinical settings with small sample quantities promised to push the forefront of proteomics-based personalized medicine, highlighting new drug targets that are crucial to disease pathogenesis.

[1] Wolf-Levy, H., Javitt, A., Eisenberg-Lerner, A. et al. Revealing the Cellular Degradome by Mass Spectrometry Analysis of Proteasome-Cleaved Peptides. *Nature Biotechnology*. in Print (2018)

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State-of-the-Art Methodologies in Research, Bioinformatics, Big Data and Cancer

Topological-Proteomics of Breast Cancer Intra-Tumor Heterogeneity Reveals Diversity within Single Tumors

Mariya Mardamshina¹

Introduction.

One of the major obstacles in breast cancer treatment is its high degree of heterogeneity and ambiguous classification. The molecular and histological subtypes are routinely defined in the clinic to determine treatment modalities; however, these distinct subtypes co-exist in single tumors and therefore lead to therapy resistance. Here we aim to delineate the proteomic landscapes of breast cancer intratumoral diversity based on clinical-pathological parameters using mass spectrometry-based microproteomics.

Materials and methods.

Using histopathological analysis of immunostained formalin-fixed paraffin-embedded (FFPE) tissues, we assembled more than 300 tumor regions comprising of different histopathological and molecular subtypes that originate from 35 patients. Cancer regions were isolated using laser-capture microdissection (LCM). Proteins were solubilized and digested following the SP3 protocol. Peptides were labeled with TMT 10-plex and analyzed on the Q-Exactive HF mass spectrometer.

Results and discussion.

MaxQuant analysis identified a total of more than 8300 proteins. Topological proteomic analysis revealed proteomic portraits that are associated with receptor expression levels and histological characteristics. The main parameter for the segregation of tumor regions was based on the level of heterogeneity. Tumor regions within a homogeneous environment demonstrate elevated immune activity in receptor-negative (TN) and ER-positive/PR-positive tumors. These proteins were previously shown to be associated with immune response, and suggest potential relevance to immunotherapy in breast cancer. Interestingly, tumor regions within a heterogeneous environment show involvement of metabolic reprogramming with elevated TCA cycle, oxidative phosphorylation, and fatty acid metabolism. For instance, TN regions within receptor-positive environment displayed shared features of basal-like and hormone-positive tumors.

Conclusion.

These findings suggest that integrated topo-proteomic landscape of inter- and intratumoral heterogeneity can serve as a platform for deciphering the mechanisms underlying tumorigenesis, distinct profiles of cancer cell subpopulations, cancer evolution, and therapy resistance.

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Israel Immunological Society

Lymphocyte Activation & Exhaustion

Matrix-localized substrate-level-phosphorylation is critical for mitochondrial remodeling during CD8+ T cell priming

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Oxidative phosphorylation (OXPHOS) is thought to be critical to meet the energetic demand of T cells activation. However, why activated T cells still require OXPHOS in spite of a clear "switch" to aerobic glycolysis remains incompletely understood. Here we show the critical role of OXPHOS-coupled matrix substrate-level-phosphorylation in powering mitochondrial remodeling in activated CD8+ T cell. We demonstrate that shortly upon stimuli, T cell cytoplasmic function becomes independent of mitochondrial ATP outflux. In contrast, OXPHOS restriction leads to arrest of ATP dependent mitochondrial functions, which could be then rescued by restimulation of matrix substrate-level-phosphorylation. Thus, following the switch to glycolysis, OXPHOS acts as an electron sink, facilitating matrix substrate-level-phosphorylation, a primary ATP source for mitochondrial remodeling during T cell activation.



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Microbial Infections, Resistance & Immunity

The metabolic liability of T cell activation

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To exit quiescence, T cells rely on environmental nutrients, to simultaneously fuel multiple metabolic pathways, which often share common precursors. Such nutrients include: glucose and the amino acids glutamine, leucine, serine and arginine. Controlling the uptake to these nutrients is a key mechanism for regulating T cell activation. In contrast to these amino acids, which are either essential or require multi-step biosynthetic processes, the amino acid alanine can be made from pyruvate in a single transamination step. Nevertheless, we found that extracellular alanine is essential for T cells to exit quiescence, as they lack expression of alanine aminotransferase, the enzyme that interconverts pyruvate and alanine. Alanine deprivation leads to wide-ranging metabolic changes and functional impairment in T cells. Interestingly, consumed alanine is not catabolized but is instead directly used by T cells for protein synthesis. Taken together, our findings suggest that T cells may be sensitive to changing alanine concentrations during physiological or disease states. Accordingly, interventions that target alanine uptake and synthesis may provide a new therapeutic strategy for modulating T cell function.

Microbial Infections, Resistance & Immunity

Glucose dependent insulintrophic polypeptide (GIP) immune cell interactions control body weight during obesity via modulation of energy expenditure

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Incretin peptides, mainly glucagon like peptide-1 (GLP-1) and glucose-dependent insulintrophic peptide (GIP) are gut derived hormones, which are secreted upon cues from ingested food and regulate glucose concentration, eating behavior and energy expenditure to maintain body weight. As a result, a myriad of therapeutics for metabolic diseases based on the actions of incretins, particularly GLP-1, are currently under clinical use and development. Nevertheless, the biology of GIP in the immune system remains overlooked, plagued by contradictions and unanswered questions. Our lab has previously shown that the long-acting GIP analogue [d-Ala²]GIP reduces innate and adaptive inflammatory responses in the epididymal adipose tissue (epiWAT) of high-fat diet (HFD) fed mice, but the direct immunometabolic roles of GIP remained unknown. Here, we show that mice with GIP receptor (GIPR)-deficiency targeted to immune cells display increased weight gain, insulin resistance, hepatic steatosis, myelopoiesis, impaired energy expenditure and impaired white adipose tissue (WAT) beiging under HFD. These effects were mediated by the unrestrained activity of the alarmin S100A8/A9 in GIPR-deficient myeloid immune cells. Recent studies have outlined a pivotal role for type 2 immune cell networks in maintaining metabolic homeostasis and energy balance. In alignment with their impaired energy expenditure, HFD fed mice with GIPR-deficiency in immune cells exhibited significant alterations in WAT type 2 inflammatory circuits, and co-deletion of S100A8/A9 was sufficient to restore it. Finally, GIP augmentation facilitated inguinal WAT beiging and induced the expression of the beiging supportive type 2 cytokines in lean mice under cold conditions. Collectively, our results identify an immune–GIPR–S100A8/A9 signaling axis coupling nutrient signals to the control of inflammation and adaptive thermogenesis.

Inflammation and Immunity – Friends or Foes?, Microbial Infections, Resistance & Immunity

Lactate Release by Inflammatory Bone Marrow Neutrophils Induces Their Mobilization Via Endothelial GPR81 Signaling

Eman Khatib-Massalha¹

Innate immune neutrophils provide the first line of host defense against bacterial infections, which requires their rapid recruitment from the bone marrow (BM) to the circulation.

The metabolite lactate has long been considered a “waste byproduct” of cell metabolism that accumulates during stress induced inflammation and sepsis. Increased lactate levels in human patients coincide with the presence of neutrophils in the circulation and inflamed tissues, and are used as a marker for sepsis diagnosis. However, the direct effector actions of lactate during acute inflammation and particularly in regulating neutrophil mobilization and function during the onset of inflammation has remained obscure.

Utilizing murine bacterial infection models, we report in this study that neutrophil-derived lactate promotes their rapid mobilization from the bone marrow (BM) into the circulation.

Bacterial lipopolysaccharides (LPS) or *Salmonella Typhimurium* treatment in vivo increase NADPH oxidase-mediated reactive oxygen species and HIF-1 α levels in BM neutrophils, triggering lactate production and release from these cells via its transporter MCT4.

Elucidating the molecular basis of lactate production and function, we found that LPS treatment did not increase metabolic cascades and lactate production in mice deficient in NADPH oxidase (NOX) or in myeloid-specific HIF-1 α . Direct evidence by exogenous lactate administration increased the defective LPS-induced activated neutrophil mobilization in NOX mutated mice. These results further demonstrate the essential role of lactate in optimal neutrophil mobilization during onset of acute inflammation.

Adding mechanistic insights to our observations, we further identified that sinusoidal BM endothelial cells (sBMECs) functionally express the G-protein coupled lactate-receptor GPR81. We show that in response to LPS, lactate-released from BM neutrophils activates GPR81 signaling that in turn downregulates Epac1 and reduces VE-cadherin expression in sBMECs, thereby increasing BM vascular permeability and enhancing neutrophil mobilization to the circulation.

Our study highlights neutrophil-released lactate as a key pro-inflammatory stimulus in the onset of inflammation that rapidly and locally opens the BM vascular barrier to facilitate neutrophil mobilization and recruitment to sites of inflammation.

Targeting this immune-metabolic crosstalk between lactate-producing neutrophils and the BM endothelium could potentially control pathological neutrophil activities during bacterial infections and potentially help rescue immune disorders, thus having a clinical relevance.



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Inflammation and Immunity – Friends or Foes?

The potential of immune checkpoint blockade for fighting against Alzheimer`s disease

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The brain has been considered as an autonomous tissue that performs best without any assistance from the immune system. Now, it is now widely accepted, much through our work, that circulating monocytes and CD4+ T cells are needed for supporting brain repair and functional plasticity. We demonstrated that leukocytes can get access to the brain territory through a unique interface, the epithelial layer that forms the blood-CSF-barrier, the choroid plexus epithelium (CP). We discovered by immunogenomic and by immunohistochemistry that in aging and in Alzheimer`s disease (AD) mice this interface is suppressed with respect to its ability to allow leukocyte trafficking. Transiently reducing systemic immunosuppression by blocking the inhibitory immune checkpoint pathways PD-1/PD-L1, regulatory pathways that maintain systemic immune homeostasis, led to recruitment of monocyte-derived macrophages to sites of brain pathology. Using anti-PD-1 antibodies in several mouse models of AD and age-related dementia was found to be effective in reversing cognitive loss, in reducing brain pathology, and in restoring brain homeostasis as determined by the inflammatory molecular profile. Such an approach is not meant to be directed against any disease-escalating factor within the brain, but rather it empowers the immune system of the individual to drive the process of repair. This, approach by directly targeting the immune system, provides addresses numerous factors that go awry in the brain regardless of the primary cause of the disease or disease etiology.

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Microenvironment and Immuno-Oncology

Mutant p53 governs microenvironmental dynamics via exosomes and outer membrane vesicles

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Background:

Mutations in TP53 are considered one of the most frequent genetic alterations in human cancer. Besides the abrogation of the wild-type (WT) p53-mediated tumor suppression, a distinct set of missense mutations was reported to endow mutant p53 proteins with novel activities termed gain-of-function (GOF). Even though mutations in TP53 are typically thought to arise in the tumor cells rather than in the stroma, the non-cell-autonomous effects of these mutants over the tumor microenvironment are poorly understood.

In the presented studies, focusing on colon cancer as well as on lung cancer microbiome, we investigated intercellular interactions mediated by exosomes and outer membrane vesicles (OMVs) in the context of cancers harboring mutant p53.

Results: In the colon, tumor cells harboring mutp53 were found to exert a non-cell-autonomous effect over macrophages. When exposed to tumor cells harboring mutp53, monocytes became polarized towards a distinguished subset of macrophages characterized by TAMs-related markers. The mutant p53 affected TAM were characterized as TNF- α^{low} / IL-10^{high}, over expressing CD-206 and CD163, with decreased phagocytic ability and increased invasion and matrix degradation potency. Investigating the exosomal transfer from mutp53 tumor cells to macrophages, revealed a mutp53-specific miRs signature led by miR-1246 promoting the TAM phenotype and creating an invasive front together with tumor cells. MiR-1246 was also found to be the top mutp53-associated miR in a cohort of 57 human colorectal resected tumors.

Separately, in two lung cancer cohorts, we identified a signature of microbiome members associated with p53 mutations. *Acidovorax Temperans*, a Gram negative bacterium, was found to be abundant in tumors of patients with mutant p53. We found a significant increase in tumor volume in animals inoculated with *Acidovorax temperans* as compared to Sham treated animals, and increased lung weight as a percent of total body weight. These preliminary data indicate that *Acidovorax temperans* contributes to lung tumorigenesis in the presence of activated K-Ras and mutant p53. OMVs shed by *Acidovorax temperans* promoted inflammatory signaling in lung carcinoma cells and elevated CD47 expression on tumor cells and SIRP α levels on macrophages.

Conclusions: Altogether, these findings are consistent with a microenvironmental role for specific “hot-spot” GOF p53 mutants tightening the interaction between the tumor cell and the immune compartment in colon cancer. In both colon and lung cancer, mutant p53 facilitates cellular interactions within the tumor microenvironment mediated by vesicles.

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Immunopathologies and Precision Medicine

Engineering B Cells as an Evolving Drug to Fight HIV

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HIV viremia can be controlled by chronic antiretroviral therapy. However, treatment tolerability and adherence remain a challenge. Combination therapies of broadly neutralizing antibodies (bNAbs) can suppress viremia, but they may have to be chronically administered at a higher cost. bNAbs may be constitutively expressed from muscle, liver or mucosa following AAV transduction, but the antibody is of a single isotype, emergence of resistant strains is probable in lack of affinity maturation, and anti-drug antibodies (ADA) may develop due in part to improper glycosylation. In contrast, bNAbs integrated at the IgH locus in transgenic mice and human B cells were shown capable of undergoing class switch recombination (CSR) and somatic hyper mutation (SHM) and may be less prone to ADA.

Here, we develop IgH engineering in B cells as a therapeutic approach for fighting HIV infections. We use CRISPR/Cas9 and AAV to introduce the anti-HIV bNAb 3BNC117. In particular, we target the intronic sequence downstream to the variable region and upstream to the IgM switch region. In an immunocompetent mouse model, we demonstrate antigen-induced B cell activation leading to germinal center occupancy and differentiation into memory B cells and plasma cells. Antibody secretion is further increased upon boost immunization and is accompanied by class switch recombination. By recoding 3BNC117 to reconstitute hotspots for AID, we allow increased rates of somatic hypermutation (SHM) that are concentrated at the CDR2, demonstrating affinity maturation. Importantly, we have also efficiently engineered PBMC-derived human B cells which also show high rates of antigen-induced activation.

Finally, we demonstrate how B cell genome editing could be performed without CRISPR. Instead, we integrate the bNAb coding cassette into the breaks which occur during the natural process of class switch recombination. Obviating the use of nucleases diminishes the associated risks and IP challenges, creating a clear path for a clinical application.

Uniquely, our method enables antigen-induced bNAb secretion that may be further augmented by affinity maturation, class switch recombination, and the retention of immunological memory. B cells could thus be engineered as a living and evolving drug to counteract HIV escape.



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Lymphocyte Activation & Exhaustion

Aging promotes reorganization of the CD4 T-cell landscape toward extreme phenotypes implicated in age-related diseases

Alon Monsonego

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Aging can be considered as a gradual decline in repair processes resulting in accumulating tissue damage and dysfunction that impact life quality and longevity. As life expectancy is continuously on the rise, it becomes essential to elucidate mechanisms of repair failure so that age-related diseases can be better predicted, monitored, prevented and/or treated. Age-associated changes in the functionality of CD4 T cells have been linked to declined immunity and chronic inflammation. However, a detailed characterization of CD4 T-cell phenotypes, which may explain these dysregulated functional properties, is lacking. Here, we used single-cell RNA sequencing and multidimensional protein analyses to profile thousands of CD4 T cells obtained from young and old mice. We found that the landscape of CD4 T-cell subsets is markedly different between young and old mice, such that three cell subsets, including exhausted, cytotoxic, and activated regulatory (aTregs) cells, appear rarely in young mice and gradually accumulate with age. Most unexpected were the cytotoxic CD4 T cells and aTregs exhibiting extreme pro- and anti-inflammatory phenotypes, respectively. These findings provide a comprehensive view of the dynamic reorganization of the CD4 T-cell milieu with age and illuminate dominant cell subsets associated with declined immunity and chronic inflammation, suggesting new therapeutic avenues for age-related diseases.

Precision in Personalized Cancer Immunotherapy

Ibrutinib-disabled immunosuppressive microenvironment sensitizes melanoma to PD-1/OX40 immune checkpoint modulators following immunization with dendritic cell-targeted nano-vaccines

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Introduction

Low response rate, acquired resistance, and severe side effects have limited the clinical outcomes of immune checkpoint therapy. We hypothesized that the combination of cancer nano-vaccines with anti-PD-1 for immunosuppression blockade, and the agonist antibody anti-OX40 for effector T cell stimulation, expansion, and survival, could potentiate the efficacy of melanoma therapy.

Materials and Methods

We developed dendritic cell-targeted mannose-grafted poly(lactic-co-glycolic acid) nano-vaccines containing melan-A/MART-1 peptides and immune potentiators.

Results and Discussion

Both prophylactic and therapeutic combination regimens of mannosylated nano-vaccines with anti-PD-1/anti-OX40 (α PD-1/ α OX40) demonstrated synergism, stimulating T cell infiltration into tumors at early stages of the treatment. The prophylactic regimen inhibited tumor growth to a greater extent compared to the α PD-1/ α OX40 alone, however, treatment at the therapeutic regimen did not result in enhanced inhibition of tumor growth compared to the α PD-1/ α OX40 alone. An increased infiltration of myeloid-derived suppressor cells (MDSC) was observed in tumors of animals treated at the therapeutic regimen with the combination of mannosylated nano-vaccines with α PD-1/ α OX40. In fact, when combining ibrutinib, an MDSC-inhibitor, with the double therapy mannosylated nano-vaccines and α PD-1/ α OX40, a remarkable tumor remission and prolonged survival was achieved in treated melanoma-bearing mice.

Conclusions

The synergy between the mannosylated nano-vaccines, ibrutinib and α PD-1/ α OX40 provides essential insights to devise alternative regimens and combination therapies to improve the efficacy of immune checkpoint modulators in solid tumors, by regulating the endogenous immune response.



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Cancer Therapy: Advances in Drug Design and Delivery

Engineering Affinity, Specificity and Stability in Protein Therapeutics

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Characterizing the binding selectivity landscape of interacting proteins is crucial both for elucidating the underlying mechanisms of their interaction and for developing selective inhibitors. However, current mapping methods are laborious and cannot provide a sufficiently comprehensive description of the landscape. Here, we introduce a novel and efficient strategy for comprehensively mapping the binding landscape of proteins using a combination of experimental multi-target selective library screening and *in silico* next-generation sequencing analysis. We map the binding landscape of a non-selective trypsin inhibitor, the amyloid protein precursor inhibitor (APPI), to each of four human serine proteases (kallikrein-6, mesotrypsin, and anionic and cationic trypsins). We then use this map to dissect and improve the affinity and selectivity of APPI variants toward each of the four proteases. Our strategy can be used as a platform for the development of a new generation of target-selective probes and therapeutic agents based on selective protein–protein interactions.

Cancer Therapy: Advances in Drug Design and Delivery

Hypoxia Targeted Infectious Knockdown for the Treatment of Solid Tumors

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Introduction

Intratumoral hypoxia is a characteristic feature of solid tumors. The management of tumor cells in hypoxic regions is a critical barrier in oncology, as they have a slow metabolism, and are resistant to chemotherapy and radiation. HIF-1 and CREB provide a survival advantage to tumors in the hypoxic tumor microenvironment. We have previously shown that knockdown of either CREB or HIF-1 inhibits tumor progression. To date, there are no effective treatments for metastatic uveal melanoma (UM). The aim of this study is to test the effect of infectious knockdown of CREB and HIF-1 on UM.

Material and method

Uveal melanoma cells expressing luciferase and infected with a MuLV-based replication competent retroviral (RCR) vector expressing shRNA targeting either CREB or HIF-1 were tested for knockdown efficiency *in vitro*. The effect of the armed viruses on subcutaneous tumor growth in mice was monitored weekly via bioluminescence (IVIS). 5 weeks post-implantation, tumors were excised and analyzed.

Results and discussion

Infection with the armed viruses resulted in an efficient knockdown CREB, HIF-1, and downstream genes. The efficient knockdown resulted in an inhibition of cell growth *in vitro*. Subcutaneous xenografts infected with armed viruses had a halted growth rate as opposed to the steady fast growth of the control tumors. In correlation with the non-invasive luciferase-based method, at the end of the experiment, the mean weight of the tumors infected with an armed virus knocking down HIF-1 was only 42% of the mean tumor weight of the control tumors, and the mean weight of the tumors infected with an armed virus knocking down CREB was only 16% of the mean control tumor.

Conclusion

Infectious knockdown via armed viruses targeting the hypoxia regulators CREB and HIF-1 is effective against metastatic uveal melanoma *in vitro* and *in vivo*. These results indicate that armed viruses controlling the cellular response to hypoxia may be the basis of a new treatment modality for solid tumors.

Bio-Markers and Cancer Theranostics

When do colorectal tumors die following therapy?

Albert Grinshpun¹

Introduction

Chemotherapy and radiotherapy are still the mainstay cancer therapies in multiple tumor types. The main tool for evaluation of treatment response is imaging, performed at intervals of several months. A rising promising approach that may revolutionize oncology is the analysis of blood for circulating tumor cell-free DNA (cfDNA). Its level represents nearly `real-time` tumor turnover and has the potential to improve decision making regarding treatment efficacy.

Here, we present the preliminary results of our study aimed to predict treatment response based on changes in cfDNA within short time frames, to facilitate faster response evaluation in colorectal cancer patients.

Methods

We have recruited 2 cohorts of newly diagnosed patients: metastatic colorectal cancer (MCRC) or locally advanced rectal carcinoma (LARC). All patients gave informed consent and were treated with standard protocols (systemic chemotherapy, pre-operative chemo-radiation lasting 5-6 weeks, respectively). Plasma of MCRC patients was drawn at baseline and then daily for 5 days. In the LARC cohort, plasma was collected at baseline, at first and last day of each radiation week. Detection of unique intestinal methylation patterns in cfDNA was performed, enabling the quantification of intestinal-derived cfDNA level. Patients` outcomes were determined at first imaging scan results (MCRC group) or at surgical outcomes (tumor viability status for LARC cohort).

Results

Ten patients were recruited to MCRC group (n=54 samples) and twenty patients into the LARC cohort (n=150 samples). In the MCRC cohort, no correlation between treatment response and variation in intestine-derived cfDNA was detected. Clear cfDNA peak representing tumor death was not seen. Yet, shallow whole-genome-sequencing (x0.5) of cfDNA revealed obvious effects of chemotherapy on cancer-derived cfDNA.

Nonetheless, in the LARC group, 20% of patients achieved maximal response to therapy, defined as no viable tumor cells on surgical specimen following therapy. We have detected a significant difference between intestine-derived cfDNA levels in maximal responders vs. poor responders. The significant difference was evident as early as second week of treatment (lower levels of cfDNA in max. responders, $p=0.007$). Interestingly, in many patients cfDNA levels were higher at end of treatment weeks, in comparison to Sundays, probably representing therapy-induced tumor death.

Conclusions

Intense liquid biopsy approach using colorectal-specific methylation markers is feasible and enables to monitor treatment and predict outcomes in patients with LARC. Additional research may promote this tool to select patients in which rectal surgery may be omitted. In the metastatic setting, we have not clearly identified specific time of treatment-induced tumor death. We assume that predictive tumor response will be evident at later stages of therapy. Further research is undoubtedly required.

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Visualizing the Immune Response

Mechanical control of T cell cytotoxicity

Morgan Huse

Cytotoxic T lymphocytes (CTLs) fight viral infections and cancer by selectively recognizing and destroying infected or cancerous target cells. CTLs kill by forming a specialized interface, known as an immunological synapse, with their target cell, into which they secrete a mixture of toxic proteins. Our laboratory is interested in the cytoskeletal architecture of the immunological synapse and how this architecture contributes to the potency and the specificity of effector responses like target cell killing. To this end, we have developed a multidisciplinary approach that combines single cell biophysical measurements, synthetic chemistry, fluorescence imaging, and functional assays. Our recent studies have focused on the generation of mechanical force at the immunological synapse and the implications of this force exertion for cytotoxicity and intracellular communication.

Visualizing the Immune Response

Using Super-Resolution Microscopy to Watch Immune Cells Kill

Daniel M. Davis

Human Natural Killer (NK) cells can directly kill diseased cells by secretion of cytolytic granules across an immune synapse. The molecular choreography that leads to assembly of the synapse and the secretion of granules has widely been studied. However, a long-standing gap in our understanding of this process is how disassembly of the synapse occurs, allowing immune cells to dissociate from target cells. We report that shedding of an activating receptor increased NK cell motility and facilitated detachment of NK cells from target cells. Disassembly of the immune synapse caused by receptor shedding aided NK cell survival and boosted serial engagement of target cells. Thus, counter-intuitively, shedding of receptors can positively impact immune responses, by allowing immune cells to sequentially move from one cell to another. In a separate line of research, using super-resolution microscopy, we have found that inhibitory Killer Ig-like receptors (KIR) encoded by different genes and alleles organise differently at the surface of primary human NK cells. KIR which are expressed at a low level at the cell surface assemble in smaller clusters than KIR which are highly expressed. Upon receptor triggering, lowly expressed receptors generate more phosphorylated Crk than highly expressed receptors. Thus, genetic variation modulates the nanoscale organisation of inhibitory KIR, which in turn impacts receptor signalling. This identifies a new way in which genetic diversity can impact immune responses. Finally, NK cells contribute to immune surveillance against cancer, but the impact of commonly used treatments on their activity is poorly understood. Radiotherapy, one of the most broadly used treatments for solid tumours, is known to influence many aspects of anti-tumour immunity, but little is still known about its effect on the interaction between NK cells and cancer cells. Surprisingly, treatment with three doses of 8Gy radiation on consecutive days reduced NK cell cytotoxicity dramatically. Moreover, irradiated cancer cells were found to require a 2-4-fold greater concentration of purified perforin protein to induce lysis. Thus, radiotherapy induces a profound reduction in the susceptibility of cancer cells to NK cell cytotoxicity, which is important to consider for cancer patients treated with a combination of radiotherapy and immunotherapy.



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Lymphocyte Activation & Exhaustion

B Cell Homeostasis Under Control Of microRNAs

Doron Melamed

Development of B lineage cells is guided by antigen receptor signaling through positive and negative selection check-points. The PI3K has been shown as the major pathway in regulating positive and negative selection of developing B cells, and survival of mature B cells. Activation of PI3K is balanced by phosphatase and tensin homolog (Pten), the PI3K's main antagonistic phosphatase. Yet, the extent of feedback regulation between PI3K activity and Pten expression during B cell development is unclear. We study the regulation of PI3K by microRNAs and uncovered an autostimulatory axis composed of a transcription factor (c-Myc), a microRNA (miR17-92) and Pten. The mechanism by which this biochemical circuit controls PI3K activity and determines B cell fate decisions in developmental check-points will be discussed,

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Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment

The Role of Fc Receptors in the Therapeutic anti-tumor Activity of Checkpoint Antibodies

Rony Dahan

Immunology, Weizmann Institute of Science, Israel

Immunomodulatory mAbs are revolutionizing cancer treatment due to their clinical effective stimulation of therapeutic anti-cancer immunity. Recent studies demonstrated the importance of the Fc domain of these types of mAbs. Their optimal activity can be critically depended on their ability to engage defined FcgR pathways. I will discuss our recent characterization of these FcgR-dependent mechanisms, and how they can be exploit to design 2nd generation Fc-optimized immunomodulatory mAbs.

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Microenvironment and Immuno-Oncology

The molecular mechanisms regulating the cross talk between CLL cells and their microenvironment”

Idit Shachar

Immunology, Weizmann Institute of Science, Israel

Chronic lymphocytic leukemia (CLL), the most common leukemia in the Western world, is characterized by the progressive accumulation of small, mature CD5+ B lymphocytes in the peripheral blood, lymphoid organs and bone marrow (BM). The hallmark of the disease is mainly decreased apoptosis, resulting in accumulation of these malignant cells. Immunosuppression is a prevailing clinical feature in CLL patients, with many patients demonstrating increased susceptibility to infections, as well as increased failure of an intrinsic anti-tumor immune response. However, little is currently known regarding the precise mechanisms that cause this immunosuppressive phenotype in CLL.

Dynamic interactions between cell-surface molecules orchestrate the immune response. The signaling lymphocyte activation molecule (SLAM) family includes nine receptors that modulate the immune responses by homophilic and heterophilic interactions. CD84 is a member of the SLAM family. It is a cell-surface protein, which forms homophilic dimers by self-association. Our studies have previously characterized a novel survival pathway in CLL regulated by CD84. In addition, we recently showed that CD84 serves as an important bridge mediating the interaction between CLL and the various cells in their microenvironment *in vitro* and *in vivo*.

Furthermore, we show that a cell-cell interaction mediated through human and mouse CD84 upregulates PD-L1 expression on CLL and their microenvironment, and PD-1 expression on T cells. This results in suppression of T cell response and activity *in vitro* and *in vivo*. Thus, our results demonstrate a novel role for CD84 in regulation of immune checkpoints by leukemia cells, and suggest CD84 blockade as a novel therapeutic strategy to reverse tumor-induced immune suppression.



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Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment, Microenvironment and Immuno-Oncology, Lymphocyte Activation & Exhaustion

Elucidating Molecular Mechanisms Suppressing the NK Cell Killer Instinct

Aviad Ben-Shmuel

The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Israel

Natural killer (NK) cells are a powerful tool of immune defense against viral infections and tumor development, operating through the cytotoxicity of target cells and the production of cytokines. NK cells are regulated by a balance between activating and inhibitory signals. Engagement of inhibitory receptors antagonizes activating pathways through the recruitment and activation of the protein tyrosine phosphatase SHP-1 at the NK immunological synapse (NKIS). Until recently, only the signaling molecule VAV1 was identified as a direct substrate of SHP-1 in human NK cells. Since SHP-1 activity is the major mechanism preventing NK cell autoimmune responses, it is of great importance to determine whether additional substrates of SHP-1 exist and whether additional molecular mechanisms downregulate NK cell cytotoxicity. In the present study we demonstrate, using multidisciplinary approaches which include FRET analysis, that in response to KIR receptor engagement, SHP-1 and the E3 ubiquitin ligases c-Cbl and Cbl-b negatively regulate the linker for the activation of T cells (LAT) and phospholipase C γ (PLC γ)_{1/2}. LAT dephosphorylation by SHP-1 abrogated PLC γ _{1/2} recruitment to NKIS, thereby decreasing degranulation and NK cell cytotoxicity. Furthermore, LAT ubiquitylation via c-Cbl and Cbl-b following NK cell inhibition leads to its degradation and to the downregulation of NK cell activation. Expression of an ubiquitylation resistant LAT, or gene silencing of the Cbls, significantly increased NK cell mediated killing of inhibitory target cells, converting the inhibitory NK cell response into an activating one. These mechanisms serve as a key checkpoint in tuning NK cell activation threshold and immune response. This study, in addition to elucidating how NK cells can rapidly tune their activation threshold in the tumor microenvironment, may have far reaching implications for immunotherapy.

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Visualizing the Immune Response, Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment, Microenvironment and Immuno-Oncology

T cell regulation in pancreatic ductal adenocarcinoma

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Background:

Pancreatic cancer has the worst prognosis of any human malignancy. We have shown using transcriptomics and histopathology that immune infiltrate in resection samples from a whipple's operation is predictive of prognosis. In particular, lymphocytes appear to be a major prognostic marker. With promising immunotherapies being proposed for other cancers, there is a need for a deeper understanding of the immune landscape of pancreatic cancer to identify points of intervention.

Methods:

We have developed a 37-marker mass cytometry staining panel to characterise the dominant immune populations within primary pancreatic cancer. Our panel further analyses T-cell subpopulations and their functional status including a host of clinically relevant checkpoint markers and immunosuppressive signatures.

Results:

The degree of immune infiltration we observe is highly variable between patients, but all patients equivocally show a complex immune microenvironment consistent of macrophages, neutrophils and different lymphocytes. The T-cells infiltrating the tumour, both CD4 and CD8 T-cells, appear to be dysfunctional with hardly any activation signature. A highly suppressive phenotype also characterises the regulatory T-cell population. Our data suggest that the microenvironment of pancreatic cancer is extremely suppressive and could be a major driver of poor prognosis. Yet, this work identifies potential therapeutic targets and avenues that should be further investigated and may inspire future clinical trials.

Future Work:

We are planning to investigate the behaviour of T-regs from PDAC using *in-vitro* assays. To that end, we will use a recently developed advanced 3D culture system to visualise their interactions with CD8s and the functional consequences of those interactions on CD8 mediated killing.



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Israel Immunological Society

Inflammation and Immunity – Friends or Foes?, Microenvironment and Immuno-Oncology

Myeloid Derived Suppressor Cells as Plastic Sensors and Outcome Orchestrators During Chronic Inflammation

Michal Baniyash

The Lautenberg Center for Immunology and Cancer Research, Israel-Canada Medical Research Institute, Faculty of Medicine, The Hebrew University of Jerusalem, Israel

In pathologies characterized by chronic inflammation altered myelopoiesis is evident, which is associated with the accumulation of myeloid derived suppressor cells (MDSCs). MDSCs are characterized by diverse phenotypes and functions, they are arrested in their immature state, are polarized towards highly suppressive cells and migrate from the bone marrow to the periphery and sites of inflammation, where they impair effector functions of innate and adaptive immune cells, promote tumor growth, angiogenesis, and tissue damage. When reaching new environments, which exhibit a different array of cytokines, chemokines, and pro-inflammatory mediators, MDSCs sense and adapt to the altered micro-environment by virtue of acquiring different features that involve changing their cell fate, surface receptors, metabolism and intracellular as well as secreted molecules. Based on the plasticity and biological diversity of MDSCs, they have a dual use: 1) As biomarkers for the evaluation of chronic inflammation-induced complications and for the prediction of success rates of immune based therapies, and 2) As targets for treatments aimed at combating them or manipulating their differentiation state or suppressive activity towards achieving recuperated homeostasis and/or improving therapies in various pathologies characterized by chronic inflammation. Examples for MDSCs as plastic sensors and outcome orchestrators will be presented and the clinical implications will be highlighted.

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Microenvironment and Immuno-Oncology

New players in melanoma micro environment

Carmit Levy

Genetics, Tel Aviv University, Israel

Melanoma originates in the epidermis and becomes metastatic after invasion into the dermis. Prior interactions between melanoma cells and dermis are poorly studied. We have previously showed that melanoma cells directly affect the formation of the dermal tumour niche by microRNA trafficking before invasion. Melanocytes, cells of melanoma origin, are specialized in releasing pigment vesicles, termed melanosomes. In melanoma in situ, we found melanosome markers in distal fibroblasts before melanoma invasion. The melanosomes carry microRNAs into primary fibroblasts triggering changes, including increased proliferation, migration and pro-inflammatory gene expression, all known features of cancer-associated fibroblasts (CAFs). Here we show that these melanosomes are up taken by endothelial cell and macrophages, leading to further shaping of the metastatic niche by enhancing angiogenesis and affecting the immune system.



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Inflammation and Immunity – Friends or Foes?, Metabolism and the DNA-Damage Response

Immune surveillance of senescent cells- from embryonic development to aging

Hilah Gal

The placenta is an autonomous organ that maintains fetal growth and development. Its multinucleated syncytiotrophoblast layer, providing fetal nourishment during gestation, exhibits characteristics of cellular senescence. We show that in human placentas from pregnancies with intrauterine growth restriction, these characteristics are decreased. To elucidate the functions of pathways regulating senescence in syncytiotrophoblast, we used dynamic contrast-enhanced MRI in mice with attenuated senescence programs. This approach revealed an altered dynamics in placentas of $p53^{-/-}$, $Cdkn2a^{-/-}$, and $Cdkn2a^{-/-};p53^{-/-}$ mice, accompanied by histopathological changes in placental labyrinths. Human primary syncytiotrophoblast upregulated senescence markers and molecular pathways associated with cell-cycle inhibition and senescence-associated secretory phenotype. The pathways and components of the secretory phenotype were compromised in mouse placentas with attenuated senescence and in human placentas from pregnancies with intrauterine growth restriction. We propose that molecular mediators of senescence regulate placental structure and function, through both cell-autonomous and non-autonomous mechanisms.

Cancer Metastasis

Mapping Stromal And Immune Cell Dynamics During Breast Cancer Metastasis By scRNA-seq

Ido Yofe¹

Mortality from most cancers is almost exclusively a result of tumor metastasis. Since advanced metastatic cancers are incurable, understanding the biology of tumor metastasis is a significant challenge in cancer research today. The microenvironment of tumors has been proven as crucial in supporting tumor growth, however the role of the metastatic microenvironment, and how the immune system is suppressed in it, in supporting the multistage process of tumor metastasis is still unresolved. A major challenge is therefore uncovering the dynamic plasticity of the microenvironment during the early stages of metastasis, which orchestrates the formation of a hospitable metastatic niche.

We used a mouse model of spontaneous lung metastasis following surgical resection of breast cancer primary tumor. We applied our NICHE-seq technology by using photoactivatable-GFP mice, which allowed us to label cells in lung tissues residing in either metastatic sites (MET), areas adjacent to metastatic sites (ADJ), or non-metastatic areas (non-MET). Single-cell RNA-seq profiling of immune and stromal cells in each spatial compartment was performed. Moreover, we examined as a reference whole lung tissues from mice prior to primary tumors resection (pre-MET), and healthy mice (NT).

Analysis of the expression profiles of immune and stromal cells revealed shifts in cell states throughout the metastatic process. First, compared to NT controls, pre-MET lung tissues (from mice still harboring the primary breast tumor) showed a large infiltration of monocyte and neutrophil populations, and reduction of macrophages, NK, T and B lymphocytes, implying that alterations in the lung microenvironment occur very early in the metastatic process. Second, lung from mice that went through primary tumor resection but did not developed lung metastases were by and large comparable to those of NT mice, showing a decrease in macrophages and B lymphocytes, and an increase of neutrophils. Finally, MET and ADJ tissues were highly infiltrated by large amounts of macrophages, including two metastases-specific subsets with unique expression profiles.

Our findings illustrate the diverse transcriptional landscape of immune and stromal subpopulations in spatial compartments of metastatic tissues, shedding a light on the early events that occur during metastases formation, and in the future may assist in the development of effective therapeutic strategies to prevent metastatic relapse.

Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment

Activation of CD45 Reverses Tumor Suppression of Src Family Tyrosine Kinases in Leukocytes.

Annat Raiter¹

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Introduction: Cancer cells apply multiple immunosuppressive mechanisms to evade immune-cell responses. The advent of immune-checkpoint inhibitors is a promising new strategy to treat solid tumors. This breakthrough is mainly based on monoclonal antibodies which block inhibitory molecules like the PD-1/PD-L1 axis to neutralize tumor immune evasion mechanisms. However, attempts to treat breast cancer with immunotherapy drugs have not shown much benefit for the vast majority of patients. The triple negative breast cancer (TNBC) is considered to be amongst the most aggressive subtype, generally with a worse outcome. Much effort is being invested to find a new generation of immune-modulatory strategies to address TNBC in particular.

Material and Methods and Results: We found a novel mechanism via which tumors induce immune suppression. C24D, a 24 amino acid homo-dimer peptide, reverses such tumor immunosuppression by binding and activating the CD45 receptor on leukocytes. Co-cultures of human TNBC cells with human peripheral blood mononuclear cells (PBMCs) resulted in the inhibition of the Src family of tyrosine kinases pathway in leukocytes. Addition of C24D to the immune-suppressed cells, induced Lck and Zap70 phosphorylation, resulting in TCR activation, tumor cell killing and IFN γ secretion. The resulting increase of highly cytotoxic T and NK cell sub-populations: CD8⁺/CD69⁺, CD56⁺/CD69⁺, CD56⁺/CD57⁺ and CD8⁺/CD56⁺ in the C24D treated cultures, confirmed leukocytes activation.

In *in vivo* studies, intravenous treatment of C24D to nude mice engrafted with MDA-MB-231 human TNBC cells and transfused intravenously with human PBMCs resulted in inhibition of tumor growth compared with scrambled peptide treatment. In histological sections of the tumors extracted from the C24D treated mice, we found tumor infiltrated CD8⁺ and human activated CD56⁺ cells. Consequently, we found a significant increase in tumor apoptotic (caspase 3⁺) cells.

We demonstrated that the immune-activation effect of the C24D peptide on PBMCs occurs only in the presence of tumors. In the absence of tumors and in co-cultures with normal breast cells (MCF-10A), C24D does not activate Lck and ZAP70 in leukocytes. Moreover, no IFN γ , TNF α and IL-2 was found in the supernatants of those cultures.

Conclusions: In this study we described a new mechanism of tumor immune suppression based on tumor inhibition of Lck and ZAP70 in T and NK cells. By binding to CD45 on leukocytes previously exposed to tumor cells, C24D peptide reverses tumor-induced immune-suppression, resulting in tumor cell killing. This study sets the stage for a new treatment modality of TNBC.

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Bio-Markers and Cancer Theranostics

Global views of degradation in cancer

Yifat Merbl

The mammalian proteasome is estimated to cleave ~70% of all intracellular proteins and is increasingly recognized as a dynamic complex that modulates cellular function in health and disease. However, the regulatory principles targeting specific substrates to proteasomal degradation and their cleavage products are still poorly understood. Recently, we developed mass spectrometry analysis of proteolytic peptides (MAPP), a method for proteasomal profiling that allows capture, isolation and analysis of proteasome-cleaved peptides. Application of MAPP to a clinical samples of cancer patients reveals novel regulatory principles of proteasomal degradation as well as putative targets for cancer immunotherapy.



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Gold nanoparticles for cancer immunotherapy: Imaging, diagnosis and stratified medicine

Rachela Popovtzer

Immunotherapy has made enormous progress in offering safer and more effective treatments for cancer. However, due to the complexity and heterogeneity of tumors, as well as the diversity in patient response, success rates of current available treatments (whether T cell or immune checkpoint blockage therapy) are extremely varied. Moreover, the efficacy of immunotherapy can be evaluated only several months after start of treatment. Therefore, early identification of potential responders and non-responders to therapy, using noninvasive means, is crucial for improving treatment decisions. In this talk, I will share with you a straightforward approach for fast, image-guided prediction of immunotherapy response, by using gold nanoparticles and CT imaging. This technology can be developed into a powerful tool for early and noninvasive patient stratification.



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Precision in Personalized Cancer Immunotherapy

Analysis of the HLA peptidome for development of personalized cancer immunotherapy

Arie Admon¹

Biology, Technion - Israel Institute of Technology, Israel

The MHC peptidome is the assortment of peptides presented by the Major Histocompatibility Molecules on the surface of most nucleated cells in the body, providing the immune system with information about the 'health state' of the cells and the organism. The MHC molecules (called the Human Leukocyte Antigens in humans, HLA) are the most polymorphic protein/genes in the human genome and each allomorph binds and presents a different HLA peptidome comprising of tens of thousands different peptides. The presentation of disease-related peptides may cause immune reactions that can bring about the elimination of the diseased cells, and therefore, while aiming to develop cancer vaccines, extensive efforts are made to identify those HLA peptides that are derived from cancer-specific proteins that are not expressed by any of the normal/essential cells in the body, so that these may be administered as immunotherapeutics for cancer, without the fear of inducing adverse effects. The most important cancer candidates are Cancer/Testis Antigens, which are proteins that are expressed only in the germline cells or in embryonal tissues, and neoantigens, which are expressed only in the cancer cells since they contain mutated sequences unique to the cancer cells. The approach we take is to combine the commonly used exome analyses of the tumor and normal cells with and transcriptome and HLA peptidome analyses of the tumor cells alone and look among the identified peptides for those that are derived from Cancer/Testis antigens and neoantigens. This approach is advantageous since it can identify from among the thousands of possible candidate peptides, those few that are really produced and presented by the cancer cells and prioritize them for further immunogenicity testing and clinical applications. This is done separately for every patient, and the results are highly personalized cancer vaccines.

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Bio-Markers and Cancer Theranostics

ARTS and ARTS mimetics promote cancer cell killing by degrading anti-apoptotic proteins

Sarit Larisch

Human Biology, University of Haifa, Israel

Many human cancers over-express Inhibitor of Apoptosis (IAP) proteins or Bcl-2 (B-cell lymphoma 2). Thus, they represent favorable therapeutic targets. ARTS (Sept4_i2) binds directly to Bcl-2 and XIAP and promotes apoptosis by stimulating the degradation of both proteins via the Ubiquitin Proteasome System (UPS). ARTS is required for bringing Bcl-2 into close proximity with XIAP forming a complex which promotes auto-ubiquitylation and degradation of the E3-ligase XIAP and of Bcl-2. ARTS binds directly to XIAP-BIR3 domain in a pocket which is distinct from that of SMAC/Diablo. We have used a structure-based computational screen to identify ARTS mimetic small molecules. These molecules were identified based on their specific docking to the unique binding site of ARTS within XIAP-BIR3. MST (Microscale Thermophoresis) binding assays identified one of these compounds which binds to XIAP but not cIAP1. This compound initiates UPS-mediated degradation of XIAP and Bcl-2, resulting in caspase 3 and 9 activation and apoptosis. Significantly, this compound decreases XIAP and Bcl-2 levels in MEFs from Sept4/ARTS deficient mice. Thus, it can substitute the function of ARTS. Furthermore, it also behaved as a functional XIAP antagonist and was able to counteract inhibition of cIAP1 in SKOV-3 cells. Importantly, cancer cells exhibiting high levels of XIAP were significantly more sensitive to treatment with this ARTS mimetic and high XIAP levels correlated with low IC₅₀, efficient killing with this compound, with no effect on non-malignant PBMC (peripheral blood mononuclear cells). This provides a proof-of-concept that the ARTS distinct binding site in XIAP is “druggable”. Furthermore, that this first identified ARTS mimetic, represents a novel class of dual targeting compounds stimulating apoptosis by UPS-induced degradation.

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Inflammation and Immunity – Friends or Foes?

Expression- and immune-profiling of neuroblastoma-associated Opsoclonus Myoclonus Ataxia Syndrome (OMAS) identifies features of auto- and tumor-immunity.

Miriam Rosenberg¹

Introduction: Opsoclonus myoclonus ataxia syndrome (OMAS) is a devastating neuroimmune disease that occurs in 2-3% of children with neuroblastoma (NB), characterized by ataxia, myoclonic jerks, chaotic eye movements, and mood and behavioral disturbances in a previously well child. While high risk (HR) NB has poor survival- approximately 60% of HR patients succumb to the disease- tumor related outcomes in patients with both OMAS and NB are excellent. We hypothesize that defining the OMAS immune response in NB patients may help illuminate mechanisms of effective anti-NB tumor immunity.

Materials and Methods: A large cohort of OMAS NB samples were collected for a COG clinical trial of IVIg as part of OMAS therapy. We used the Illumina RNA Access platform for RNAseq of OMAS NB, alongside low- and high-risk NB without OMAS, to identify differentially expressed or novel antigens that may drive anti-neuronal or anti-tumor immunity. We also inferred HLA types from RNAseq to carry out the first OMAS association study. Finally, we used genomic DNA from tumors to profile T cell receptor beta chain (TCRB) and Ig heavy chain (IgH) repertoires in OMAS and control NBs.

Results and Discussion: While we predicted a clonal, antigen driven TCR response in OMAS patient tumors, instead, we observe extremely diverse T cell (and B cell) receptor repertoires in patients with OMAS, with very little clonality. While all NBs in our cohort exhibited considerable lymphocytic infiltration, OMAS associated NBs are significantly more infiltrated by T cells and B cells than high risk NB, a finding supported both by immune repertoire sequencing and RNAseq data. Immune features dominated differentially expressed gene sets in RNAseq. In spite of their diversity, we identify TCRB and IGH sequences and features shared across OMAS patients. Finally, we identify several MHC Class II alleles whose expression is skewed in OMAS patients compared to non-OMAS NB patients, suggesting a basis for OMAS predisposition.

Conclusion: We conclude that effective anti-tumor activity in OMAS patients is likely shaped by their autoimmunity, which results in tremendous diversity of both TCRs and BCRs that also respond to tumor. Identification of antigens and receptors involved in OMAS etiology will be of interest in development of novel immune therapies for NB.

Inflammation and Immunity – Friends or Foes?, Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment

CX3CR1 Expressing Macrophages Infiltrate the Tumor Microenvironment and Promote Radiation Resistance in a Mouse Model of Lung Cancer

Tammy Ben-Mordechai¹

Department of Radiation Oncology, Sheba Medical Center, Tel-Hashomer, Israel

Introduction: Combining radiation and immunotherapy targeting T lymphocytes is extensively studied in lung cancer treatment. Current evidence suggests that only a subset of patients will benefit, while most tumors will eventually develop resistance and progress. Tumor associated macrophages (TAM) are a significant component of the tumor microenvironment (TME) capable of both suppressing (M1) and promoting (M2) tumor growth based on their functional state. Chemokine receptor, CX3CR1, plays an important role in macrophage homeostasis and effector functions, however its role in the TME following radiation treatment remains unknown. We hypothesized that macrophages expressing the CX3CR1, play a central role in TME after radiation therapy.

Materials and Methods: Mouse lung cancer model was performed by subcutaneously inoculating Lewis Lung Carcinoma (LLC) expressing luciferase (Luc-2) and mCherry cells in CX3CR1^{GFP/GFP} reporter mice and CX3CR1^{DTR/+} mice. Tumor growth was monitored by bioluminescence imaging and caliper. TME inflammatory composition was assessed by flow cytometry. Clonogenic assay was used to assess tumor survival after radiation. LLC cell cycle was analyzed by flow cytometry.

Results and discussion: Ten days after tumor irradiation with 8 Gy, irradiated tumors were smaller than non-treated tumors. In-vivo bioluminescent imaging and flow-cytometry demonstrated a significant influx of CX3CR1 expressing cells into the irradiated TME, notably macrophages (F4/80+ CX3CR1+). To establish the direct effect of CX3CR1 expressing macrophages on tumor growth *in-vitro*, we performed a clonogenic assay, by co-culturing peritoneal macrophages with LLC cells. Eliminating CX3CR1 expressing macrophages from the culture (by negative selection), reduced LLC survival fraction by 25% (P=0.005). Furthermore, co-culture of negative CX3CR1 macrophages with irradiated LLC led to LLC accumulation in the cell cycle S-phase. Finally, to evaluate CX3CR1 depletion effect *in-vivo*, we injected LLC-mCherry-Luc2 cells subcutaneously into CX3CR1^{DTR/+} mice, sensitive to the diphtheria toxin, and C57BL/6J control mice. Two weeks after inoculation, tumors were irradiated with 8Gy, and mice were treated every 3 days with diphtheria toxin, leading to reduction in CX3CR1 expressing cells. Three weeks after radiation, CX3CR1 depleted mice showed reduced tumor growth. Furthermore, flow cytometry analysis showed reduction in pro-tumoral M2 population (F4/80+CD206+), with no difference in T-lymphocyte or programmed cell death-1 expressing cells.

Conclusion: CX3CR1 expressing macrophages invade the TME after radiation therapy and contribute to radiation resistance and lung cancer progression, by promoting tumor survival. Thus, we propose a novel strategy to improve radiation sensitivity by targeting the CX3CR1 expressing macrophages in the TME.

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CAR and CTL Therapy in Cancer

CD40 costimulation enhances CAR-T cell activation

Ofir Levin-Piaeda

The clinical use of chimeric antigenic receptor (CAR)-T cells yields impressive clinical responses in the treatment of B cell leukemias and lymphomas. Yet, clinical benefit in other types of cancer, especially solid tumors, is limited, underscoring the need in improving the functional potency of CAR-T cells. In attempt to overcome these limitations, great effort is put in recent years into optimizing the signaling moieties incorporated into the intracellular portion of 2nd and 3rd generation CARs, which largely govern the clinical outcome of CAR-T cell therapy.

Among the different costimulatory signaling elements capable of augmenting T cell function and persistence explored so far, the most widely used are undoubtedly CD28 and 4-1BB. In a number of publications we have recently reported that different derivatives of the signaling domain of CD40, a member of the TNF receptor family mainly expressed by professional antigen-presenting cells, exert remarkable phenotypic and functional enhancing effects in human T cells. We hypothesized that the incorporation of the CD40 signaling domain as a costimulatory element in 2nd and 3rd CARs can similarly improve the functional properties of antitumor CAR-T cells.

To test our hypothesis we have constructed a series of new anti-HLA-A2 CARs harboring either the CD40 or the 4-1BB element, with or without CD28, and tested these in mRNA-electroporated human CD8 T cells. Indeed, CD40 consistently and reproducibly exhibited marked superiority over 4-1BB in upregulating costimulatory markers and activating the NFkB pathway and in antigen-specific induction of proinflammatory cytokines secretion, and was equally effective in mediating direct target cell killing.

We conclude that new CARs harboring the CD40 signaling element can endow gene-modified T cells with superior functionality compared to current CARs and potentially improve the clinical efficacy of CAR T cell therapy.



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Inflammation and Immunity – Friends or Foes?, Immunopathologies and Precision Medicine

The Effect of Maternal Age On the Ovarian Immune Milieu

Tal Ben Yaakov

Other than its protective role in defending against pathogens, the immune system has a crucial role in maintaining the body's healthy physiology and homeostasis. In particular, immune cells which reside and infiltrate different sites in the female reproductive tract are part of the regulation of the female fertility. These cells take part in maintaining the proper function of processes such as ovulation and pregnancy, as well as in regulating and setting the prognosis of ovarian cancer. However, from a certain age, both the fertility and the general immune function are found at a constant decline and our understanding of the role of maternal age on the interactions between the immune system and the female reproductive system is still lacking.

In this work, we characterized the immune milieu, at the single-cell level, in the ovaries of mice at different ages. We show an age-dependent change in the ovarian immune system which is independent of the cycle stage. Young mice showed macrophages ($CD11b^+ F4/80^+$) abundant ovarian immune system, whereas older ovaries showed a shift in their immune milieu toward a lymphocytes-rich ($CD3^+$) immune system compared to their younger counterparts. These $CD3^+$ lymphocytes contain $TCR\beta^+$ and $TCR\beta^-$ populations, both $CD4^- CD8^-$ (Double negative). Our results suggest a significant organ-specific change in the immune milieu in the ovary throughout age.

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CAR and CTL Therapy in Cancer

Effective Targeting of Multiple Tumours by Combining CRISPR-based Genome Editing and CAR/TCR-engineering in Human T Cells

Vasyl Eisenberg¹

Cancer immunotherapy culminated in the last years FDA-approval of the CAR-T cells directed against haematological malignancies. However, in the field of the solid neoplasm, canonical scFv-based CARs were found to be less effective. Beyond the immunosuppressive nature of solid tumour microenvironment, this lack of functionality was also attributed to the xenogeneic origin of the scFv-based CAR and their potential immunogenicity. In the current work, we have designed an 'autologous' CAR, based on the extracellular domain of the human NCR2/NKp44 receptor. The latter belongs to the NCR family which are receptors derived from NK cells and which was shown to mediate the recognition of cancer-associated ligands. By combining NK tumour recognition pattern with T cell effectiveness, we showed that an optimized NCR2-CAR, namely s4428z, confer T cells with the specific recognition and killing of solid tumor cell lines from different histologies.

Beyond specificity, signals conveyed by immune checkpoints may dampen engineered T-cell function such as those mediated by the CD155/TIGIT immuno-inhibitory pathway. Herein, we explored the benefit of CRISPR-Cas9 technology to knock out the TIGIT receptor in human T-cells and its impact on T-cell response. Finally, we also apply CRISPR-Cas9 technology to knock-out TIGIT and its related receptors in NY-ESO1-specific TCR, namely 1G4 to improve the T cells anti-tumour function.

Overall we demonstrate the feasibility of the combination of CRISPR-Cas9 approach in T cells engineered to express specific anti-tumour receptors. Moreover, we show that knock-out of receptors belonging to the CD155/TIGIT pathway in engineered T cells is beneficial to the anti-tumour response.

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Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment

The bilateral interplay between cancer immunotherapies and neutrophils` phenotypes and sub-populations

Naomi Kaiser Iluz

INTRODUCTION: Immunotherapy has become a leading modality for the treatment of cancer but despite its increasing success, a substantial number of patients do not benefit from it. Cancer-related neutrophils became in recent years a subject of growing interest in cancer research. Excitingly, distinct sub-populations of neutrophils have been identified at advanced stages of cancer, some presenting anti-tumor properties (e.g. high density neutrophils (HDN)), whereas others show pro-tumor phenotype (e.g. low density neutrophils (LDN)), suggesting them as potential targets of therapy. We aim to evaluate in animal models of lung cancer the immunological consequences of manipulating the amount of circulating neutrophil subsets on tumor development and on the efficacy of anti-PD-1 therapy, and assess the impact of immunotherapy on neutrophils` phenotype.

METHODS: C57/129 mice were injected subcutaneously to the flank with LKR-M cells (Lung K-Ras metastatic tumor model). Fifteen days following injection, tumor-bearing mice were injected intraperitoneally with combinations of two different treatments: 1- 5×10^6 HDN previously isolated from LKR-M-tumor-bearing mice for three subsequent days; 2- 150 μ g anti-PD-1 antibody every three days for two weeks with or without HDN. Tumor growth was monitored and the immune composition of the primary tumor and blood was assessed, together with phenotypic markers of neutrophils, using flow cytometry.

RESULTS: Tumor growth in the PD1-treated or HDN-treated mice is reduced compared to control groups. Both treatment types alter tumor composition, including an increase in the infiltration of tumor-associated neutrophils (TANs) and CD8⁺ T Cells. Preliminary results suggest that the expression levels of CCR2 (chemotaxis), 4-1BBL, OX40L (costimulatory molecules), ICAM-1 (Adhesion Molecules), CD62-L (neutrophil aging) and PDL-1 (checkpoint) are modulated in TANs following anti-PD-1 therapy. The impact of an anti- PD-1/HDN combination on tumor growth rate and immune responses is currently being investigated.

CONCLUSION: Together, our results suggest that neutrophils are modulated following anti-PD-1 treatment and could potentially improve the efficacy of immunotherapy. The outcome of this research could help us develop new strategies to direct the immune system against the tumor, and potentially improve the exciting new modality of immunotherapy in cancer.

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Bioinformatics, Big Data and Cancer

Decoupling epithelial-to-mesenchymal transitions from stromal profiles by integrative analysis

Michael Tyler

Introduction

Epithelial-to-mesenchymal transition (EMT) is the most commonly cited mechanism for cancer metastasis, but it is difficult to distinguish from expression profiles of normal stromal cells in the tumour microenvironment. In this study we compared expression of mesenchymal signature genes in cancer cells and stromal cells using single cell RNA-seq data for several tumour types. We then developed a method to deconvolve the mesenchymal signature in bulk expression profiles into stromal and cancer-cell-specific EMT components. We applied this method to bulk RNA-seq data for hundreds of samples from many cancer types, and examined the common properties of the resulting EMT signatures and their association with clinical features.

Materials and Methods

We used single cell RNA-seq data from several published studies to examine expression of mesenchymal signature genes in different cell types. By aggregating samples of single cells from these datasets, we constructed simulations of bulk expression profiles, which we used to test our deconvolution method. We applied this method to bulk RNA-seq data from The Cancer Genome Atlas (TCGA), and tested the resulting EMT signatures for association with prognostic features using the TCGA clinical annotations.

Results and Discussion

We showed that many classical EMT marker genes are more strongly associated with fibroblasts than with cancer cells, indicating that their expression levels in bulk profiles primarily reflects stromal content. Other genes, including several laminins and integrins, proved to be more reliable indicators of EMT in cancer cells. The EMT signatures correlated with metastasis in only a few cancer types, and in some cases they showed association with other clinical features, such as therapy resistance.

Conclusion

Through our pan-cancer deconvolution analysis of bulk expression data, we showed that classical EMT marker genes often primarily reflect stromal content, while our inferred cancer-cell-specific EMT signatures usually do not correlate with metastasis. This study demonstrated the importance of distinguishing 'true' EMT from stromal contributions in order to elucidate the therapeutic relevance of EMT in cancer.

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Genomic Instability, Cancer Signaling and Cancer Secretome

MET activation confers resistance to cetuximab, and prevents HER2 and HER3 upregulation in head and neck cancer

Ofra Z Novoplansky¹

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An understanding of the mechanisms underlying acquired resistance to cetuximab is urgently needed to improve cetuximab efficacy in patients with head and neck squamous cell carcinoma (HNSCC). Here, we present a clinical observation that MET pathway activation constitutes the mechanism of acquired resistance to cetuximab in a patient with HNSCC. Specifically, RNA sequencing and mass spectrometry analysis of cetuximab-sensitive (Cetux^{Sen}) and cetuximab-resistant (Cetux^{Res}) tumors indicated MET amplification and overexpression in the Cetux^{Res} tumor compared to the Cetux^{Sen} lesion. Stimulation of MET in HNSCC cell lines was sufficient to reactivate the MAPK pathway and to confer resistance to cetuximab *in vitro* and *in vivo*. In addition to the direct role of MET in reactivation of the MAPK pathway, MET stimulation abrogates the well-known cetuximab-induced compensatory feedback loop of HER2/HER3 expression. Mechanistically, we showed that the overexpression of HER2 and HER3 following cetuximab treatment is mediated by the ETS homologous transcription factor (EHF), and is suppressed by MET/MAPK pathway activation. Collectively, our findings indicate that evaluation of MET and HER2/HER3 in response to cetuximab in HNSCC patients can provide the rationale of successive line of treatment.



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State-of-the-Art Methodologies in Research, Bioinformatics, Big Data and Cancer

Cellular Evolution Upon Glioblastoma Recurrence At Single-Cell Resolution

Avishay Spitzer¹

Glioblastoma (GBM) is the most aggressive and prevalent type of primary brain cancer and has a poor prognosis with a median survival of approximately 1 year. The disease is managed best with a multimodal approach combining surgical resection, postoperative radiation therapy and adjuvant chemotherapy, however, even with the best medical treatment the majority of patients relapse following initial therapy. Moreover, once the disease recurs it is practically resistant to treatment and both patients and caregivers are left with very few second line treatment options in their arsenal which are only marginally efficient.

Substantial efforts have been made in recent years to characterize the molecular mechanisms governing GBM tumor evolution, including characterization of four GBM genomic subtypes by The Cancer Genome Atlas (TCGA) as well as dissection of the GBM heterogeneous intra-tumor ecosystem at the unprecedented resolution provided by single-cell sequencing technologies. However, despite these breakthroughs that laid the ground to understanding the biological mechanisms underlying this terrible disease and the forces driving it, a comprehensive understanding and characterization of the mechanisms responsible for recurrence and resistance to treatment are still lacking.

Our work tackles this problem at the single-cell resolution and to the best of our knowledge is first of its kind. Using frozen tumor sample pairs obtained from adult and pediatric GBM patients at two different time points - at initial diagnosis (the primary lesion) and at first disease recurrence - we perform single-nuclei RNA sequencing and computationally analyze the transcriptomes of thousands of cells to dissect the intra-tumor composition and uncover the differences between the transcriptomes of these matched samples. This unveils the cellular evolution upon recurrence that is driven by tremendous genetic and epigenetic changes, as well as by changes in the composition of the tumor microenvironment (TME), that these tumors undergo between the different time-points and enables characterizing better the mechanisms that govern resistance to treatment which will ultimately result in improved therapeutic outcomes for patients suffering from this horrible disease.

State-of-the-Art Methodologies in Research

Patient-derived 3D Models as a Novel Tool for Drug Developing and Testing

Sarah Hofmann

o Introduction

The process from drug discovery and development to commercialization and medical application is very long, complex, expensive and inefficient. Only about 8% of the drugs that are reaching clinical phase I trials will be successfully commercialized in the end. Therefore, there is an urgent need to develop assays and methods that can reliably predict the efficiency and usability of a drug under in vivo conditions during clinical trials. Using in vitro systems for drug development and testing has the advantages of avoiding ethical issues, as well as being more cost-effective than in vivo animal models. However, the 2D cell culture models cannot accurately mimic the complex response of an organ to anti-cancer treatments. That is the reason why around 95% of the anti-cancer drugs that showed a promising and potential response in 2D monolayers, failed during the clinical trials. In recent years, many systems have thus been created, and a growing number of research studies have been conducted in order to establish the usage of 3D tissue culture models.

o Material and method

One elegant and promising approach to build patient-derived 3D models is the microtissue culture system from InSphero AG (Switzerland) that provides functional 3D spheroids in a scaffold-free system. These 3D microtissue models present more accurately the physiological conditions in vivo. Here we used biopsy samples from breast (and anal) cancer patients, generated in vitro 3D models, applied a panel of chemotherapy drugs, and measured the cellular viability of the microtissue.

o Results and discussion

We were able to establish a well-working protocol for the generation of patient-derived 3D models in vitro. Due to the higher organization of cells grown in 3D systems (as compared to 2D monolayers), the testing of drugs on the 3D models better reflects the effect of irradiation, physical or chemical treatment on the micro-environment of the tissues regarding e.g. the access to nutrients, oxygen, growth factors, metabolites and paracrine factors.

o Conclusion

3D cell culture models present an ideal tool for drug developing and testing, and for a more accurate and reliable prediction of the therapeutic outcome on tumor growth.

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Cancer Therapy: Advances in Drug Design and Delivery

Developing Nanobodies and Small Molecule Conjugates Targeting Prostate Specific Membrane Antigen
Targeting for Prostate Cancer Imaging and Therapy

Lior Rosenfeld

Prostate cancer is one of the most widespread cancer types throughout the world, and the second most prevalent in males. The current common method for prostate cancer detection results in high false positive and negative rates, leading to an increasing need for an alternative approach for diagnosis. Upon detection, prostate cancer treatment options with cytotoxic agents are limited, with the exceptions of Docetaxel and Cabazitaxel, which are often used as second-line treatment in more aggressive types of prostate cancer. Other chemotherapies were found to cause adverse side effects, partially due to high dosing that is required for efficacy. Prostate specific membrane antigen (PSMA) is an enzyme that is highly overexpressed in prostate cancer cells and the neovasculature of human prostate tumors, and serves as a promising target for prostate cancer identification and targeting. In order to develop both an accurate diagnostic agent and drug carriers for prostate cancer, we developed four single domain antibodies (nanobodies) from camel, with extremely high *in vitro* affinities towards PSMA, starting from K_D of 50 pM. These nanobodies present good and specific binding to PSMA-expressing cell lines, but do not inhibit the enzymatic activity of PSMA. Most importantly, our proteins show accumulation in prostate cancer tumors expressing PSMA but not in tumors lacking it in *in vivo* optical imaging assays. All four variants internalize into PSMA expressing cells. This ability allows the nanobodies to serve as carriers for targeted drug delivery to reduce the necessary dosing amount. The nanobody with the highest *in vitro* affinity, as well as longest clearance time from tumors, was conjugated to a pH-sensitive linker and doxorubicin. This nanobody-drug conjugate internalize specifically into PSMA expressing cells, where the doxorubicin was separated from the protein. Cytotoxic activity was observed *in vitro* in PSMA expressing cells. *In vivo*, similar tumor growth inhibition was observed in animals treated with doxorubicin alone and animals treated with 20-fold less doxorubicin, conjugated to the nanobody. Our data suggests that conjugating a nanobody with high affinity to a cytotoxic drug enables the use of much lower doses, while maintaining similar efficacy. This could allow the use of drugs that were previously shown to cause adverse side effect and expand the possibilities to treat different types of cancer.

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Cancer Therapy: Advances in Drug Design and Delivery

ARTS Mimetic (AM) Small Molecules Enhance The Killing Effect Of BH₃ Mimetic ABT-199 (Venclexta[®])

Nir Shahar

Bcl-2 (B-cell lymphoma 2) protein functions as a potent inhibitor of apoptosis. Bcl-2 is highly expressed in many types of cancers. Therefore, Bcl-2 is a major target for developing anti-cancer drugs. Bcl-2 contains a BH₃ binding domain which enables it to interact and neutralize other pro-apoptotic Bcl-2 family members resulting in inhibition of apoptosis. ABT-199 (Venclexta[®]) is a BH₃ mimetic drug, approved by the FDA for treatment of CLL (Chronic Lymphocytic Leukemia) patients. ABT-199 acts by binding to Bcl-2 and neutralizing its anti-apoptotic effect, leading to death of the treated cancer cells. ARTS is a pro-apoptotic protein that promotes apoptosis by binding and degrading Bcl-2 through Ubiquitin-Proteasome-System (UPS). ARTS brings Bcl-2 into close proximity with X-linked inhibitor of apoptosis protein (XIAP). XIAP acts as an E₃ ligase to ubiquitylate and degrade Bcl-2. Our lab has identified several ARTS-mimetic (AM) small molecules which bind XIAP and promote the degradation of both Bcl-2 and XIAP itself. This leads to killing of cancer cells. We have found that ABT-199 induces upregulation of Bcl-2 and MCL-1 levels in several cancer cell lines. Significantly, the combination of ABT-199 with Bx (an AM) reduces both Bcl-2 and MCL-1 expression. This culminates in a substantial increase in ABT-199 induced cell death. We show that Bx increases the direct binding of ARTS to XIAP which can activate the E₃-ligase function of XIAP on Bcl-2. We hypothesize that Bx enhances the effect of ABT-199 on cancer cells by further degradation of Bcl-2 and XIAP, which amplifies the apoptotic outcome. Increased expression of MCL-1 is the potential cause of resistance to Bcl-2 inhibition by ABT-199. Our results provide an alternative and complementary approach to treatment of cancers that developed resistance to BH₃ mimetics.

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Bio-Markers and Cancer Theranostics

Targeting Points of Vulnerability in individual Melanoma Tumors for Reducing Acquired Drug Resistance

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Targeted drug therapy for melanoma patients carrying the BRAFV600E driver mutation with vemurafenib provides a temporary remission, followed by severe relapses due to the dominance of drug-resistant tumors. Acquired drug resistance can develop from persister tumor cells that maintain their cell viability during prolonged drug treatment. Here we propose a functional approach to circumvent this problem, by identifying the soft-spots in the cell death machinery of patients' metastases, the targeting of which strongly reduces the number of persister cells. This challenge required a smart solution, as there exists huge heterogeneity in the composition of the cell death map among patients' tumors. To this end, we developed a personalized prescreening platform that maps the soft-spots in each tumor carrying the BRAF mutation, **before** patients are treated with the drug. The platform is based on applying siRNA libraries targeting 81 genes of apoptosis, autophagy and necroptosis pathways, one at a time, to tumors derived from Melanoma and identifying the hits that reduce the number of persister cells upon BRAF inhibitor treatment. We have demonstrated the feasibility of this approach in an analysis of a cohort of 12 metastatic melanoma early passage cell cultures carrying BRAF mutation supplied by the Sheba Medical Center. By applying this platform we identified a large heterogeneity in the number and position of the soft spots among patients. In some tumors, the soft spots were spread over the three PCD modules, including clusters of hits targeting autophagic genes that participate in the ubiquitin like conjugation pathways. In other tumors the number of soft spot was low, mapped to one or two of the PCD modules at different positions. After validating the soft spots we were able to replace the siRNAs with small molecule inhibitors where available. Most importantly, by reducing the number of persister cell over long term of vemurafenib treatment, by simultaneously targeting one of the identified soft spots, we could reduce the number of drug resistant clones emerging after 4-5 weeks. We also measured the outcome of double hits either by testing all possible combinations, or in a rationally designed choice of targets by superimposing the soft-spots on the integrated map of programmed cell death that we have delineated in our lab. This approach has the potential to dramatically change the state-of-the-art of **combinatorial drug therapy** in precision cancer treatment, by reducing the number of the persister cells surviving the initial treatment by several orders of magnitude, thereby lessening the odds of developing drug resistance at later stages, and preventing tumor relapses.

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Bio-Markers and Cancer Theranostics, Cancer Therapy: Advances in Drug Design and Delivery, Bioinformatics, Big Data and Cancer, Genomic Instability, Cancer Signaling and Cancer Secretome

Phenotypic and Mechanistic Characterization of Drug-Tolerant Persisters

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Introduction

Drug-tolerant persisters are thought to represent a small subpopulation of cancer cells that can survive drug treatments despite the absence of resistance-mediating genetic alterations. Indeed, upon treatment withdrawal, these cells give rise to a new population with comparable drug sensitivity (as measured by IC₅₀) to that of the treatment-naïve population. As persisters can give rise to genetically resistant cells, understanding the mechanisms that enable them to persist drug treatment is crucial for providing better treatment options for cancer patients.

Material and method

Using cancer cell lines as a model system, we have generated hundreds of single-cell derived colonies from each cell line and tested their persister percentage upon drug treatment. To identify single cell biomarkers of persistence, as well as the underlying molecular mechanisms of survival, we subjected colonies to karyotyping, RNA-Sequencing, proteomics, phospho-proteomics and apoptotic profiling by apoptosis antibody array. High throughput drug screens were used to find modulators of persister percentage in response to anti-cancer drugs.

Results and discussion

We found that persisters are not a well-defined subpopulation, but rather, a continuous trait in which every cell in the population has a specific “Chance to Persist” (CTP) a drug treatment. Clone-specific CTPs were found to be stable over months in culture, and the CTPs of their sub-clones averaged around the CTP of the mother clone with some drift to both directions. We show that there is no correlation between the IC₅₀ of growth inhibition and CTP across clones. Indeed, while persister progenies have the same IC₅₀ values as the treatment naïve population, their percentages of persisters are significantly higher. A detailed study of clones with a wide range of CTP values demonstrated that CTP does not correlate with growth rate, cell cycle status or EMT markers and that clones have a target-specific CTP value. We also found several biomarkers that correlate with CTP. A potential mechanism underlying the CTP of non-small cell lung cancer cells treated with EGFR inhibitors will also be discussed.

Conclusion

Overall, our study demonstrates that the chance to persist anti-cancer drugs does not depend on a pre-existing subpopulation of drug-tolerant persister cells that later give rise to sensitive cells, but rather, is a continuous and inheritable trait. Our findings have multiple clinical implications for cancer patient treatment.

Cancer Metastasis, State-of-the-Art Methodologies in Research

Constructing a novel Dynamic Three Dimensional *in Vitro* Model for Investigation of Ovarian Carcinoma Progression at the Different Anatomic Sites of the Disease

Aharon Baskin^{1,2}

Introduction: Ovarian carcinoma (OC) metastases reported to have a much higher malignancy and therapy resistance compare to the primary tumor. In addition, transition from a solid form of the tumor to a detached cellular spheroid form, in effusion presents a special challenge both in designing effective drug treatment and in understanding cellular processes during disease progression. There is a lack of an appropriate *in vitro* model of the tumor microenvironment mimicking three different sites of OC. Emerging evidence shows the various functions of sphingolipids in cellular trafficking and cell motility. Specifically, Sphingolipid-1-phosphate (S1P) has been implicated as a potent regulator of cancer progression. Herein, our main objective was to examine the main S1P-associated genes from different OC anatomical sites. Based on the acquired data, we aim to establish a three-dimensional (3D) *in vitro* culture system of OC cells, using alginate macroporous-based scaffolds under dynamic conditions to mimic the 3D tumor microenvironment.

Material and method: The basal mRNA levels of S1P receptors' expression and relative proportion were established by RT-PCR analyzing of 250 OC samples. A multi chamber perfusion system was applied, designed for optimal flow conditions, homogeneity of the flow within all scaffolds and control over oxygen level, and thus, better mimicking *in vivo* conditions. 433 or ES2 OC cells were cultured in 4 different culture forms for 72 h: (a) monolayer (b) seeded into alginate porous scaffolds, under static conditions, (c) alginate scaffolds in perfusion bioreactor, (D) cell spheroids. RT-PCR analysis was conducted.

Results: Cultivation of 433 cells within alginate scaffolds, cultured under flow velocity of 50 mL/h resulted in S1P receptor mRNA expression levels similar to those of primary samples from OC patients. Moreover, the relative proportions of each receptor were also similar to those of the primary tumor samples. By contrast, no such similarity was detected for other culture methods: monolayer, spheroid cultures or static 3D alginate scaffolds. Similarly, ES2 culture in bioreactor lead to S1P receptor mRNA expression levels and relative proportions similar to those of effusion samples OC patients, compared to all other culture methods.

Conclusions: A novel *in vitro* model was designed and established for Primary OC, appropriated for experiments with a relatively large number of the samples.



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Genomic Instability, Cancer Signaling and Cancer Secretome

Elucidating the role of PROS1 in glioma cell plasticity

Divsha Sher¹

Glioblastoma (GBM) is a malignant grade IV brain tumor with poor prognosis and high recurrence rates. The difficulty treating GBM arises among other reasons from the heterogenous nature of the cancer cells. The population of glioma stem cells (GSC) is thought to sustain tumor growth and confer its resistance to conventional therapies. Advances in the understanding of the genomic landscape of GBM have given rise to new and promising treatments. As part of the project to better understand the mechanisms driving cancer progression we choose to focus on proteins S (PROS1).

PROS1, an anticoagulant glycoprotein, has been implicated in various biological pathways, including those involved in cancer pathology. These functions have been investigated in several cancers, however the effect PROS1 has on GBM progression has yet to be discovered. We aim to investigate to role of PROS1 expression levels in GBM and the correlation between expression, cell state and aggressiveness of GBM.

To accomplish these goals, two constructs were used for silencing PROS1 expression; shRNA and CRISPR/cas9 (sgRNA). PROS1 KO GBM cells showed increased sensitivity to oxidative stress resulting in slower proliferation of the cells and reduced dedifferentiation abilities. Additionally, PROS1 KD cells showed compromised cell plasticity following cycles of differentiation and dedifferentiation. Tumor response to oxidative stress has been linked to chemotherapy resistance and maintenance of GSCs and these results indicate a role for PROS1 in the responsible pathways.

Shedding light on the processes driving cancer progression will contribute in the search for potential drug targets and novel therapeutic strategies.

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Leukemia: From Stem Cell to Therapy

Cyclosporine H Improves Lentiviral Transduction of Murine Hematopoietic Stem and Progenitor Cells

Leonid Olender

Hematopoietic Stem Cells (HSCs) have the potential for lifetime production of blood and immune cells. Introduction of transgenes into HSCs is important for basic research, as well as for multiple clinical applications thanks to the fact that HSC-transplantation is already established. However, the efficient introduction of a transgene into HSCs had been proven challenging. Recently, a major advancement was reported with the use of Cyclosporine H (CsH), that can significantly enhance lenti-viral (LV) transduction of human Hematopoietic Stem- and Progenitor Cells (HSPCs). In this study we applied CsH for LV transduction of murine HSCs, or defined progenitors, having improved resolution of cell-types. Our data confirm increased efficiencies, in agreement with the published data. We further challenged cells with multi-vector transduction, gaining robust increase with CsH for either Progenitors or pure HSCs. CsH was reported to reduce innate-resistance mechanism against LV, and indeed we found that pre-treatment of cells could increase transduction even better than the original protocol. However, prolonged CsH treatment also inhibited cell proliferation *in vitro*. Therefore, we had transplanted HSCs after multi-vector transduction with or without CsH, to test for their actual abilities to perform Stem Cell function *in vivo* after *ex-vivo* manipulations. Our data suggest that CsH can robustly increase the levels of LV transduction, without perturbing HSC's function for transplantation. This new additive will surely help many studies in animal models and is suggesting for potent option towards clinical utilization.

Leukemia: From Stem Cell to Therapy

Development of multivirus-specific T (VST) cells for the prophylaxis and treatment of viral infections following hematopoietic stem cell transplantation

Nathalie Asherie

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative option for the treatment of some malignant (i.e., leukemia and lymphoma) and non-malignant hematological diseases (i.e., mutation-related primary immune-deficiencies). Post-HSCT patients are severely immunocompromised and are consequently prone to viral infections, which are a major cause of morbidity and mortality. Viral infections may result from the reactivation of endogenous latent viruses such as cytomegalovirus (CMV) and Epstein-Barr Virus (EBV), or from exogenous agents such as Adenovirus (Adv). Although some drugs are available against CMV infection, they are not effective against EBV or Adv. In addition, their efficacy is limited when there is no concomitant antiviral immune reconstitution. Clonal expansion of cytotoxic T lymphocytes (CTLs) specific for CMV, EBV and Adv (tri-VST) can safely prevent or limit infections with these three most common viruses. There is no doubt regarding the efficacy of HSCT donor-derived virus-specific T (VST) cells in the prophylaxis and the treatment of viral infections associated with HSCT. However, the treatment has not yet become a standard of care post-transplantation in Israel, and only few Centers in the world have successfully implemented this preventive/therapeutic platform as a standard of care for post-HSCT patients. Obstacles to that therapy includes the personalized nature of the treatment, the fact that a VST product can be generated only if the donor cells are HLA-matched and only in the case the donor is seropositive for the virus of interest. Furthermore, the manufacture logistic may delay product availability in the case of early post-transplant infection. In attempt to solve some of these issues, the generation of a third party VST cell bank covering a broad range of HLA-restricted HSCT recipients, sounds to be a particularly attractive option as a high percentage of the BMT candidates at Hadassah Center are originated from consanguineous populations, and therefore share conserved HLA types. We have successfully conducted a pilot experiment in which tri-virus specific CD4⁺ and CD8⁺ T cells were specifically enriched in a single culture process, display activation markers (i.e., 4-1BB) and secrete IFN- γ when stimulated with specific viral triggers. We aim to introduce VST cellular products into clinical trials as preventive/standard treatment for post-HSCT viral infections, to extend the VST repertoire to a broad range of post-transplant life-threatening viruses, while shortening the time-consuming manufacturing procedures.

We are confident that once this powerful platform will be robustly implemented, it would significantly improve the patients` resistance to a broad range of viruses, and consequently, increase their chance of survival.

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Inflammation and Immunity – Friends or Foes?, Microbial Infections, Resistance & Immunity

Daily Rhythms Of Neutrophil Activation Programs Host Response To LPS-Induced Infection

Suditi Bhattacharya¹

Background: Neutrophils are the most abundant leukocytes and act to resolve infection by phagocytosis, degranulation or the formation of neutrophil extracellular traps (NETs). Golan et.al. (*Cell Stem Cell*, 2018) showed that murine bone marrow hematopoietic stem cells (HSC) primarily differentiate to mature leukocytes (neutrophils and monocytes) during the day. We suspect that this causes a hyper-inflammatory response in the day, therefore making mice more susceptible to bacterial endotoxins in the day (resting phase) compared to night (active phase).

Moreover, the neutrophil response to infection also involves the activation of thrombotic processes. Together, the immune and coagulation system act to locally compartmentalize the bacteria. However, the circadian regulation of coagulation- particularly by innate immune cells is poorly understood.

Materials and Methods: Wild type (WT) C57 BL/6 Mice were housed under 12:12 hours of light-dark cycle, with lights switched on at 6 AM. Mice were challenged at different time points with LPS (10mg/kg) to mimic bacterial sepsis. Reactive Oxygen Species (ROS) were scavenged using NAC. Bone Marrow (BM), Peripheral Blood (PB) and Liver cells were harvested and analyzed by flow cytometry. The Evans Blue Dye Assay was used to assess Bone Marrow vascular endothelial permeability.

Results: We found that exposure to LPS in the day dramatically elevated ROS production by neutrophils and increased mobilization of neutrophils and monocytes into circulation. This corresponds to a marked increase in the levels of pro-inflammatory cytokines in the blood in the afternoon, which may be lethal. But this is in sharp contrast to mice injected at midnight, where the number of activated neutrophils in PB reduce after LPS treatment- due to a significant reduction in BM vascular permeability, probably due to the high levels of the darkness hormone melatonin at night. The reduced inflammatory response at night is accompanied by a very high recruitment of neutrophils to the inflamed liver- possibly due to higher expression of adhesion molecules in the liver at night (*He et.al, Immunity*, 2018). Inhibiting the differentiation of BM HSC by scavenging available ROS led to a decreased level of neutrophils in the blood after LPS challenge in the afternoon.

Leukocytes also have a pro-coagulant activity by generating thrombin, which can induce further inflammation by activating their G-protein-coupled membrane receptors, protease-activated receptor-1 (PAR-1). We found that PAR-1 deficient mice do not have a higher recruitment of neutrophils to the liver at night. In addition, we established chimeric mice in which hematopoietic cells from the BM of PAR-1 KO mice were transplanted into WT mice and vice versa. Our results show that PAR-1 expression on hematopoietic cells and not in the stromal compartment is responsible for the differences in vascular permeability, as well as neutrophil recruitment.

Conclusion: Here we report our recent efforts to understand daily oscillations in neutrophil phenotype and kinetics, and their role in regulating the coagulation process. Our study could help in designing strategies to better respond and cope with sepsis.



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CAR and CTL Therapy in Cancer

A Novel Subset of CD4⁺ T Cell Expressing the High Affinity Fc γ Receptor Links Antibody and T Cell Immunity

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While CD4⁺ cells are known to associate with improved tumor prognosis, their main role is attributed to supporting the cytotoxic activity of CD8⁺ T cells and macrophages. By attempting to potentiate antibody-driven immunity, we found a remarkable synergy between CD4⁺ T cells and tumor-binding antibodies. This surprising synergy was mediated by a small subset of tumor-infiltrating CD4⁺ T cells express the high affinity Fc γ R for IgG (Fc γ RI) in both mouse and human patients. These cells efficiently lyse tumor cells coated with antibodies through concomitant crosslinking of their T cell receptor (TCR) and Fc γ RI. By infecting conventional CD4⁺ T with Fc γ RI and its signaling chain, we successfully employed this mechanism to treat established solid cancers. Overall, this discovery shed new light on the biology of this previously unknown T cell subset, their function during tumor immunity and open a new venue to utilize their unique killing signals in immunotherapy.

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Microenvironment and Immuno-Oncology, Cancer Metastasis, State-of-the-Art Methodologies in Research, Cancer Therapy: Advances in Drug Design and Delivery, Immuno-Oncology and the Microbiome

Blocking of TGF β Signaling Using a Novel Platform for Neutrophil Specific Targeting Prevents Metastasis

Sandra Voels¹

Introduction

In recent years, our understanding of neutrophil function in health and disease is rapidly expanding. However, neutrophils were never regarded as an attractive therapeutic target due to their short half-life. Here we present a novel in-vivo platform for neutrophil-specific drug delivery which may be exploited therapeutically.

Material and Method

We used a phage display library to screen for short peptides binding specifically to mouse and human neutrophils. The selected peptides were used to decorate PLGA nanoparticles (NP) containing SB431542 (TGF β inhibitor). We then tested the therapeutic effect of neutrophil specific nanoparticles containing SB431542 in a preclinical model of metastatic breast cancer.

Results and Discussion

Using phage display screen combined with next generation sequencing, we identified several peptides specifically binding to mouse and human neutrophils. We identified the binding partner of the peptides as the neutrophil-specific surface protein CD177 and show co-localization of peptide and receptor by super resolution microscopy (STORM). Further, we employ these peptides to decorate biodegradable PLGA NP and achieve highly specific NP uptake by neutrophils *ex vivo* and *in vivo*. We then used SB431542 containing NP to inhibit TGF β signaling in murine neutrophils to preserve their anti-tumor properties in context of cancer. SB431542 containing NP effectively blocked TGF β induced phosphorylation of Smad2 in neutrophils *ex vivo*. Importantly, we show that treatment of tumor-bearing mice with SB431542 containing neutrophil-specific PLGA NP prevents metastatic outgrowth.

Conclusion

We have generated a novel platform for neutrophil-specific drug delivery employing peptide-decorated PLGA NP. These NP can be loaded with any drug of choice and may be used for manipulating neutrophil activity in cancer and other inflammatory diseases. Thus far neutrophils were rarely considered as a valid target for immunotherapy. This novel platform allows the modification of neutrophil function, in a specific manner *in vivo*, and may revolutionize immunotherapy by adding neutrophils to the therapeutic arsenal.

Bioinformatics, Big Data and Cancer

IgA Clonal Lineage Analysis Reveals Class Switch Dynamics in Human Gut

Hadas Neuman¹

Introduction: IgA is the dominant antibody class in the gut, secreted by more than 75,000 IgA-producing plasma cells daily. Human IgA subtypes show distinct anatomical expression patterns, with IgA1 dominating in serum and IgA2 is abundant at sites colonized by a large microbiota, including the distal intestinal tract. IgA1 has a longer hinge region than IgA2, making it more vulnerable to degradation by bacterial proteases that target its hinge region. Direct IgM to IgA2 switching, rather than sequential switching through IgA1, may allow B cells to acquire resistance to BCR degradation. We aimed to estimate the relative frequencies of these two pathways.

Materials and methods: Ig RNA was obtained from cells sampled at the normal terminal ileum and the ascending colon of four patients undergoing right hemicolectomy and sequenced. Sequences were pre-processed using pRESTO (Vander Heiden et al., 2014), assigned for sub-isotype using a custom python script and annotated using IMGT/HighV-QUEST (Brochet, Lefranc, & Giudicelli, 2008). Clones were assigned using Change-O (Gupta et al., 2015) and sampling depth was assessed using rarefaction plots. Lineage trees were constructed using IgTree© (Barak, Zuckerman, Edelman, Unger, & Mehr, 2008) and analyzed using PopTree© and custom R scripts.

Results and discussion: Sampling depth of all the samples was good. More clones were shared between IgA1 and IgA2 than between IgM and IgA2. Lineage tree analysis revealed that the most abundant class switching in the ileum and colon is from IgA1 to IgA2, and that IgM cells tend to change location or population, rather than switching. Finally, direct IgM to IgA2 switching was significantly more abundant than IgM to IgA1; however, IgA1 to IgA2 switching was significantly much more abundant than IgM to IgA2.

Conclusion: Overall, these results support the idea that the majority of the IgA population in the intestine is created early in life. The IgM tendency to switch to IgA2 rather than IgA1, may allow B cells to acquire resistance to BCR degradation by bacterial proteases that target the hinge region of IgA (Cerutti, 2008).

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Bioinformatics, Big Data and Cancer

κ -helix and the helical lock and key model: A pivotal way of looking at polyproline II

Tomer Meirson¹

Motivation

Polyproline II (PPII) is a common conformation, comparable to α -helix and β -sheet and is a candidate for being the most prevalent secondary structure. PPII, recently termed with a more generic name – κ -helix, adopts a left-handed structure with 3-fold rotational symmetry and the structure plays a primary role in immune response and carcinogenesis. Lately, a new type of binding mechanism – the helical lock and key model was introduced in SH3-domain complexes, where the interaction is characterized by a sliding helical pattern. However, whether this binding mechanism is unique only to SH3 domains is unreported.

Results

Here, we show that the helical binding pattern is a universal feature of the κ -helix conformation, present within all the major target families - SH3, WW, profilin, MHC-II, EVH1, and GYF domains. Based on a geometric analysis of 255 experimentally solved structures, we found that they are characterized by a distinctive rotational angle along the helical axis. Furthermore, we found that the range of helical pitch varies between different protein domains or peptide orientations and that the interaction is also represented by helical dynamic motion. The discovery of rotational interactions as a mechanism, reveals a new dimension in the realm of protein-protein interactions, which introduces a new layer of information encoded by the helical conformation. Due to the extensive involvement of the conformation in functional interactions, we anticipate our model to expand the current molecular understanding of the relationship between protein structure and function.



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Bio-Markers and Cancer Theranostics

Structure-based Optimized Antibody for Targeting Pancreatic Cancer

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Diagnosis and staging of pancreatic cancer are commonly assisted by FDA approved *in vitro* assays conducted with monoclonal antibody (mAb) 1116NS19.9. This mAb recognizes an aberrant tetra-glycan carbohydrate antigen 19-9 (CA19-9) also known as Sialyl-Lewis A antigen, which decorates cell-surface proteins as well as proteins that are found in cancer patient sera. Shortcomings of this mAb include a failure to recognize the early stages as well as an insufficient positive predictive value. To address these shortcomings, we structurally characterized the recognition between the 1116NS19.9 mAb and its CA19-9 antigen. The structure revealed high chemical and geometrical complementarities between the mAb and the CA19-9 antigen. Structural analysis suggested very limited options for modifying the CDRs to enhance CA19-9 binding. As an alternative approach, we used our structure and an algorithm, called AbLIFT*, which uses phylogenetic analysis and Rosetta atomistic design to optimize the interface between the antibody variable domain light and heavy chains. Two designs exhibited tenfold improvement in K_D and are currently being further tested in *ex vivo* patient samples. Another well characterized mAb for targeting CA19-9 is mAb 5b1. We solved the structure in the bound form and found that it has a different solution for CA19-9 binding, suggesting a synergistic effect for combining Ab 1116NS19.9 and Ab 5b1 in treatment. Furthermore, our newly improved 1116NS19.9 designs and structural insights offer a potential reagent for earlier diagnosis of pancreatic cancer and immunotherapy.

* Web site of server: <http://ablift.weizmann.ac.il/bin/steps>

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Israel Immunological Society

Bio-Markers and Cancer Theranostics, Genomic Instability, Cancer Signaling and Cancer Secretome, Leukemia: From Stem Cell to Therapy

Pan-Cancer Single Cell RNA-Seq Uncovers Recurring Programs of Cellular Heterogeneity

Alissa Greenwald

Cultured cell lines are the workhorse of cancer research, although they do not model certain aspects of human tumors, such as their microenvironment. It is unclear to what extent cancer cell lines recapitulate the cellular heterogeneity that exists within tumors. Here, we used a multiplexing approach to profile ~200 cancer cell lines by single cell RNA-seq. We uncovered recurrent expression programs primarily driven by epigenetic plasticity that are associated with diverse biological processes. Many of the recurrent expression programs we identified, including senescence and epithelial-to-mesenchymal transitions (EMT), recapitulated in vivo expression programs observed in tumors.

Additionally, we prioritize certain cell lines as model systems of cellular plasticity, and demonstrate the dynamics, regulation and vulnerabilities of a novel cancer senescence program, EpiSen. Unlike classical senescence, EpiSen is dynamic and does not represent complete exit from cell cycle. We demonstrate differential drug sensitivity between the EpiSen high and EpiSen low states in two head and neck cancer cell lines and highlight clinically relevant vulnerabilities. Taken together, our work demonstrates that many cancer cell lines harbor significant transcriptional diversity in the absence of a tumor microenvironment and that an understanding of this diversity can be leveraged in the rational selection of optimal model systems.



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Inflammation and Immunity – Friends or Foes?, Lymphocyte Activation & Exhaustion

Prolongation of IL-2 half-life, by addition of highly-glycosylated sequences, has the added benefits of promoting anti-inflammation immune response

Aner Ottolenghi

IL-2 is the master-regulator cytokine for T-cell dependent response. It is crucial for proliferation and survival of T-cells, and therefore was considered as a possible treatment for cancer. It was quickly discovered, however, that IL-2 has a toxic effect at high doses, and does not encourage an anti-cancerous effect as was hypothesized. Eventually it was discovered that IL-2 also promotes the T-regulatory (T_{Reg}) subset of T-cells which reduces inflammation and act as a pro-cancerous agent. IL-2 fell out of grace as a cancer treatment in high doses, but a rapidly growing body of research points to a possible other use as a treatment in low doses for autoimmune diseases, utilizing the very same T_{Reg} mechanism. IL-2 is still a toxic treatment, and very unstable protein, with short half-life in blood, which makes it less than ideal as a treatment. In this work we show that adding two flanking highly-glycosylated sequences to IL-2, improves the pharmacokinetic properties of IL-2. Furthermore our variant promotes an anti-inflammatory response which is evident from increase in T_{Reg} populations in mice. Additionally, administration of the variant impedes the progress of colitis, in a mouse model, and improves recovery. Our results suggest new avenues for an old treatment mostly abandoned, and a new technique for manipulating instable cytokines, to form stable non-toxic treatments.



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Inflammation and Immunity – Friends or Foes?, Microbial Infections, Resistance & Immunity

The intestinal microbiota programs DNA methylation to control tissue homeostasis and inflammation

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Although much research has been done on the diversity of gut microbiome, little is known about how it influences intestinal homeostasis under normal and pathogenic conditions. Epigenetic mechanisms have recently been suggested to operate at the interface between the microbiota and the intestinal epithelial cells. In this study we present a completely new sequencing-based analysis to demonstrate the impact of commensal bacteria on epigenetic DNA methylation landscapes of mouse intestinal cells under normal and acute inflammatory conditions. We show that exposure to microbiota under **physiological** conditions is associated with extensive alterations in DNA methylation at regulatory elements, which culminates in transcriptional activation of a set of “early sentinel” response genes that play a role in the normal intestinal homeostasis. We further show that exposure to microbiota in **acute inflammation** results in profound changes in DNA methylation and chromatin accessibility at regulatory elements, which culminate in alterations in expression of genes enriched in colitis and cancer. Our studies provide a new mechanistic understanding of the dual role microbiota plays in the intestine. In health, it generates colonic homeostasis via epigenetic programming, while in acute inflammation microbiota impacts a deleterious chromatin programming.



Microbial Infections, Resistance & Immunity

The gut microbiota as modulators of the immune system and their potential in cancer therapy

Naama Geva-Zatorsky

Emerging evidence demonstrates the pivotal role of gut microbes in shaping our immune system. Studies have demonstrated the immunomodulatory capabilities of gut microbes in health conditions and a few diseases¹⁻³. In order to study the mechanistic interactions of gut microbes with the host, we have applied and further developed a metabolic labeling approach that allows fluorescent labeling, visualization and tracking of live gut microbes in real-time, in their natural localization, and in live mice⁴. We are studying the mechanistic interactions of gut microbes with the host immune system by combining immunology, microbiology, chemistry and fluorescent imaging. Recently studies have revealed intriguing potential of microbes in cancer progression and therapy⁵⁻⁷. We are currently focusing on microbes that have potential effects on both cancer induction and on the efficacy of cancer immunotherapy. We see the gut microbiota as a treasure trove of potential therapeutics and believe that deciphering the principles of their interactions with the host will open new avenues for cancer treatment.

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Host-Pathogen Interaction

Exploring the link between the oral microbe *Porphyromonas gingivalis* and pancreatic cancer

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Porphyromonas gingivalis is a member of the dysbiotic oral microbiome associated with oral inflammation. Intriguingly, multiple epidemiological studies link *P. gingivalis* to an increased risk of pancreatic cancer. Given that oral bacteria are detected in PDAC, and both mouse and human pancreata harbor microbiota, we tested the ability of *P. gingivalis* to migrate from the oral cavity to the pancreas, and explored the involvement of *P. gingivalis* in pancreatic tumorigenesis using cell lines and a xenograft model. *P. gingivalis* was detected in the mouse pancreas following oral inoculation. In vitro, *P. gingivalis* induced proliferation of pancreatic cancer cells, however, surprisingly, this effect was independent of Toll-like receptor 2, the innate immune receptor engaged in response to *P. gingivalis* in the oral cavity. Instead, we found that *P. gingivalis* survives inside pancreatic cancer cells, a trait that is selectable and enhanced by hypoxia, a characteristic of pancreatic carcinoma. Intracellular *P. gingivalis* induces markers of cancer aggressiveness and epithelial-to-mesenchymal transformation. Increased tumor cell proliferation was related to the degree of intracellular persistence, and infection of tumor cells with *P. gingivalis* led to enhanced growth in vivo. To the best of our knowledge, this study is the first to demonstrate migration of *P. gingivalis* to the pancreas, and the direct effect of exposure to *P. gingivalis* on pancreatic tumor progression. Our findings shed light on potential mechanisms underlying the PDAC-periodontitis link, and may lead to better diagnosis and care.

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Ezh2 regulates early stages of T-helper cell differentiation

Orly Avni

Azrieli Faculty of Medicine, Bar Ilan University, Israel

Following their first interaction with the antigen, naive T-helper (Th; CD4⁺) cells can differentiate into distinct effector and regulatory lineages characterized by specific profile of cytokines. These cytokines instruct eventually the strategy of the immune response. Previous studies in our lab showed that the polycomb group (PcG) proteins function in Th cells as both negative and positive transcriptional regulators. Our current study delineates a model in which Ezh2 dynamically modulates nuclear actin in a developmental- and lineage-specific manner.

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Inflammation and Immunity – Friends or Foes?

Exclusive Temporal Stimulation of IL-10 Expression in LPS-Stimulated Mouse Macrophages by cAMP Inducers and Type I Interferons

Tsaffrir Zor¹

Introduction: Expression of the key anti-inflammatory cytokine IL-10 in lipopolysaccharide (LPS)-stimulated macrophages is mediated by a delayed autocrine / paracrine loop of type I interferons (IFN) to ensure timely attenuation of inflammation. Anti-inflammatory macrophages, characterized by enhanced IL-10 expression, can be also generated by a combination of LPS and a second signal, such as an IgG immune complex, apoptotic cell remnants, or a cAMP inducer.

Materials and Methods: We examined the mechanism of IL-10 induction by cAMP in LPS-stimulated mouse macrophages at the promoter level, and explored the crosstalk between type I IFN signaling and cAMP, using the β -adrenergic receptor agonist, isoproterenol, as a cAMP inducer. In addition to experiments performed with primary BMDM and with RAW264.7 macrophages, we evaluated the physiological effect of cAMP induction on IL-10 expression in a mouse septic shock model.

Results and Discussion: We show that the cAMP pathway directly up-regulates IL-10 transcription and plays an important permissive and synergistic role in early MyD88-dependent LPS-stimulated IL-10 mRNA and protein expression in mouse macrophages and in a mouse septic shock model. In contrast, the cAMP pathway is unable to amplify the late type I IFN-dependent IL-10 induction in LPS-stimulated macrophages and *in-vivo*. Yet, silencing of the type I IFN receptor enables isoproterenol to synergize with LPS also at the late phase, implying that autocrine type I IFN activity hinders synergistic augmentation of LPS-stimulated IL-10 expression by cAMP at the late phase. Furthermore, IL-10 expression in LPS-stimulated macrophages is exclusively stimulated by either IFN α or isoproterenol. We identified a set of two proximate and inter-dependent cAMP response element (CRE) sites that cooperatively regulate early IL-10 transcription in response to isoproterenol-stimulated CREB and that further synergize with a constitutive Sp1 site. At the late phase, up-regulation of Sp1 activity by LPS-stimulated type I IFN is correlated with loss of function of the CRE sites, suggesting a mechanism for the loss of synergism when LPS-stimulated macrophages switch to type I IFN-dependent IL-10 expression.

Conclusion: This report delineates the molecular mechanism of cAMP-accelerated IL-10 transcription in LPS-stimulated murine macrophages that can limit inflammation at its onset.

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Immuno-Oncology and the Microbiome

Cancer Immunotherapy: What we have achieved and where **we** are going

Jonathan Cohen

Immune-checkpoint inhibitors (ICIs) have revolutionized cancer care. Evidence is now emerging for the long term effects of treatment with unprecedented five-year survival in metastatic melanoma and lung cancers. Single agent anti-CTLA4 has shown prolonged effects after treatment cessation, emerging evidence similarly supports a maintained cancer-directed immune-response after anti-PD1 cessation albeit with higher response rates. Various biomarkers are in use to select for ICI use.

Despite the tremendous advances achieved with ICIs, a large proportion of patients are either primary resistant or acquire resistance to ICIs. Various approaches are being employed to overcome this including combination therapy with novel ICIs as well as T cell engineering with chimeric antigen receptors (CAR) and recombinant TCRs. We are currently developing an NY-ESO1 targeting TCR for clinical use.

The brain is a common site of metastases for melanoma, lung and other tumors. Combination ICI, as opposed to single agent blockade has shown intra-cranial responses similar to extra-cranial response in melanoma. Still, the brain poses unique challenges for cancer immunotherapy. We are exploring the unique characteristics of the brain-tumor immune-response using paired intra- and extra-cranial metastases.

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Controversies in Clinical Immuno-Oncology

Untangling the mechanisms behind SLAMF6 switchy nature

Michal Lotem

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Invited_Lecture

Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment

Deciphering SLAMF6's uncanny mechanism of switch

Michal Lotem

Historically, SLAM family receptors were studied for their part in X-linked lymphoproliferative disease (XLP), a complex genetic immune dysfunction due to a mutation of their adaptor, SAP. For years, it was unclear if loss of SAP converts all SFRs into “super-inhibitory” receptors or the contrary- unleash lymphocyte to proliferate and resist apoptosis. The role of SLAMF6, common to T cells, was held a dual functioning receptor consequent to the interplay between SAP and SHP-1 and 2, protein phosphatases that bind to tyrosines on the cytoplasmic tail of the receptor.

With a new Pmel-1 x SLAMF6 KO mice we found that in its full-length form, SLAMF6 is a strongly negative determinant of tumor immunity. In this talk a form of SLAMF6 will be described, that generates a strong agonistic signal in CD8 T cells, which is opposite to the effect of the parental receptor. Therapeutic models targeting SLAMF6 will be discussed and splicing modulation via regulatory elements will be described as a potential new avenue to modify T cells.

Microenvironment and Immuno-Oncology, Controversies in Clinical Immuno-Oncology

Oncogenic Drivers Shape the Glioma Immune Landscape

Dinorah Friedmann-Morvinski

Dinorah Friedmann-Morvinski^{1,3}

Introduction: Glioblastoma (GBM) is an aggressive, highly invasive primary brain tumor with near total fatality. GBM remains a challenge for prognosis despite intensive therapies. Transduction by oncogenic lentiviral-vectors, irrespective of the initiating cell population, astrocytes, mature neurons or neural stem cells (NSCs) share a common stem-like cancer cell population that can originate from dedifferentiation of mature transformed cells in GBM tumors. We believe that the tumor microenvironment (TME) may contribute to the process of tumor reprogramming. Although most of the infiltrating cells in the tumor are peripheral macrophages and microglia, recent appreciation of the effects of neutrophils in cancer directed our efforts in understanding their role in GBM.

Material and methods: Transgenic-CRE mice were injected with lentivirus carrying different oncogenic drivers (GBM subtypes) or initiated in different cell-of-origin. Tumors were harvested at different time-points, dissociated and enriched for CD45+ cells. The samples were processed for flow cytometry analysis, qPCR and *in-vitro* studies. Neutrophils (NF) were isolated via gradient centrifugation and utilized for functional assays; migration, flow cytometry, neutrophil activation, and cytotoxic effect.

Results and discussion: Flow cytometry analysis revealed differences in the brain TME in both the innate and adaptive immune subpopulations compared to healthy brain tissue. Temporal changes were also observed in spleen and bone marrow even at early stages of GBM development. The NF population varies not only at various time-points but also between the tumor subtypes. *In-vitro* assays showed higher migration and formation of neutrophils extracellular traps (NETs) on exposure to glioma cells condition media. *In vivo* depletion of NF at an early time point resulted in faster tumor development, while depletion at a later stage did not have an effect on tumor development.

Conclusion: Our lentiviral GBM mouse model allowed us to reveal the longitudinal TME immune cell populations, from initiation to endpoint of the disease, and compare between different GBM subtypes (oncogenic drivers). The dynamic changes in the TME and the differences in the expression levels of NF- κ B targets suggest a cross-talk between tumor cells and the TME. Preliminary results hint at neutrophils acquiring a pro-tumorigenic phenotype as tumor progresses.

Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment

Mesencephalic astrocyte-derived neurotrophic factor, a potential immunomodulator, is secreted from interferon- γ –stimulated tumor cells through ER calcium depletion

Michael Peled¹

Introduction: The most successful immunotherapeutic agents are blocking antibodies to either programmed cell death-1 (PD-1), an inhibitory receptor expressed on T lymphocytes, or to its ligand, programmed cell death-ligand 1 (PD-L1). Nevertheless, many patients do not respond, and additional approaches, specifically blocking other inhibitory receptors on T cells, are being explored. Importantly, the ligands for these receptors are often expressed on the surface of the tumor cells. Indeed, cancer cells express high levels of PD-L1 upon stimulation with interferon- γ (IFN- γ), a major cytokine in the tumor microenvironment. The increase in PD-L1 expression serves as a negative feedback towards the immune system, and allows the tumor to evade the attack of immune cells. We hypothesized that additional immunomodulator ligands are present on cancer cells, and similar to PD-L1, their expression is increased in the inflammatory microenvironment. **Methods:** A mass spectrometry-based screen was performed, in which tumor cells were treated with IFN- γ and their membrane associated proteome was analyzed. **Results:** 60 membrane-associated proteins were upregulated upon IFN- γ treatment, of which 17 are known drug targets, while 16 have never been implicated therapeutically in cancer and therefore are possible drug targets. To validate the mass spectrometry results, we focused on one of the proteins that have not been implicated directly in cancer, mesencephalic astrocyte-derived neurotrophic factor (MANF). MANF is known to be secreted upon ER calcium depletion, to bind phospholipids on membranes, and to induce an alternatively activated macrophage phenotype (M₂), which may support tumor growth. We found that both membrane-bound and secreted MANF were increased in IFN- γ -treated cells, demonstrated by western blot analysis and ELISA. IFN- γ induced MANF secretion from diverse cell-lines - melanoma cells, lung cancer cells, colon carcinoma cells and hepatoma cells. Surprisingly, there was no increase in MANF RNA or intracellular protein level upon IFN- γ stimulation. However, IFN- γ induced ER calcium depletion, which can explain MANF secretion. Indeed, Dantrolene, an inhibitor of ER calcium release, prevented MANF secretion. **Conclusion:** MANF is secreted from IFN- γ -stimulated tumor cells; thus, it may serve as an immunomodulator and further studies will be conducted to elucidate its role in tumor immunity.

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State-of-the-Art Methodologies in Research, Bioinformatics, Big Data and Cancer

Diagnostics of Glioma Tumor by Non-Invasive Liquid Biopsy using Circulating Tumor DNA in Plasma

Milana Frenkel-Morgenstern

Dr. Frenkel-Morgenstern, the Azrieli Faculty of Medicine, and Prof. Tali Siegal, from the Neuro-Oncology Department, Rabin Medical Centre, have been collaborating for the last three years in developing a liquid biopsy platform for glioblastoma patients using circulating cell-free DNA (cfDNA), particularly circulating tumor DNA (ctDNA) in blood plasma of patients. Currently, glioblastoma, which is the most dangerous type of brain tumour, is resistant to both radiotherapy and chemotherapy regimens with the average survival of 15 months. The collaborative project has already collected samples from 20 patients, including samples obtained from Midgam (The Israeli National Tissue Bank); and in collaboration with Prof. Rainer Grass, Neurosurgical Research, University Clinics Munich, Germany, and Prof. Michael Synowitz, Department of Neurosurgery, UKSH, Campus Kiel, Germany. The liquid biopsy platform, which has been developed by the PhD student, Vikrant Palande and by Dr. Dorith Raviv-Shay, the lab manager at the Frenkel-Morgenstern's lab, has been able to distinguish sensitively between cancer patients and normal controls (95% specificity, 80% sensitivity). Moreover, unique pathogenic mutations identified in ctDNA corresponded to the mutations appearing in the tumour biopsies (80% sensitivity). Interestingly, one "suspected" patient who was tested by three independent liquid biopsy tests showed unexpectedly low amounts of ctDNA in their plasma. From our experience, we suggested that this patient might not have glioblastoma. After three months, this patient was finally confirmed as non-cancerous, by means of multiple MRI images, surgery and clinical workup. Today, this patient's condition has improved and he is considered healthy. This case and others suggest high potential of the sensitivity of our liquid biopsy platform for brain tumors.

Precision in Personalized Cancer Immunotherapy

The AP-1 complex regulates AXL expression and determines sensitivity to PI3Ka inhibition in esophagus and head and neck squamous cell carcinoma

Limor Cohen¹

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Introduction

AXL overexpression is a common resistance mechanism to anti-cancer therapies, including the resistance to BYL719 (Alpelisib) – the p110a isoform specific inhibitor of phosphoinositide 3-kinase (PI3K) – in esophagus and head and neck squamous cell carcinoma (ESCC, HNSCC respectively). However, the mechanisms underlying AXL overexpression in resistance to BYL719 remain elusive.

Materials and methods:

To identify the transcription factors correlated with AXL overexpression in resistance to BYL719 we used RNA sequencing, western blot analysis (WB), immunohistochemistry (IHC), and quantitative real-time PCR methods. To study the role of c-JUN and c-FOS transcription factors in BYL719 resistance we performed gene silencing using siRNA, proliferation assays using specific inhibitors, and in-vivo experiments for efficacy of drug combinatiois.

Results and discussion:

Here we demonstrated that the AP-1 transcription factors, c-JUN and c-FOS, regulate AXL overexpression in HNSCC and ESCC. The expression of AXL was correlated with that of c-JUN both in HNSCC patients and in HNSCC and ESCC cell lines. Silencing of c-JUN and c-FOS expression in tumor cells downregulated AXL expression and enhanced the sensitivity tumor cells to BYL719 in vitro. Blocking of the c-JUN N-terminal kinase (JNK) using SP600125 in combination with BYL719 showed a synergistic anti-proliferative effect in vitro, which was accompanied by AXL downregulation and potent inhibition of the mTOR pathway. In vivo, the BYL719–SP600125 drug combination led to the arrest of tumor growth in cell line-derived and patient-derived xenograft models, and in syngeneic head and neck murine cancer models.

Conclusion:

Collectively, our data suggests that JNK inhibition in combination with anti-PI3K therapy is a new therapeutic strategy that should be tested in HNSCC and ESCC patients.

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Genomic Instability, Cancer Signaling and Cancer Secretome

Establishment of cancer stem cells is mutant p53 dependent

Varda Rotter

Mutations in the tumor suppressor p53 are the most frequent alterations in human cancer. These mutations include p53-inactivating mutations as well as oncogenic gain-of-function (GOF) mutations that endow p53 with capabilities to promote tumor progression. A primary challenge in cancer therapy is targeting stemness features and cancer stem cells (CSC) that account for tumor initiation, metastasis, and cancer relapse. Here we show that in vitro cultivation of tumors derived from mutant p53 murine bone marrow (BM) mesenchymal stem cells (MSC) gives rise to aggressive tumor lines (TL). These MSC-TL exhibited CSC features as displayed by their augmented oncogenicity and high expression of CSC markers. Comparative analyses between MSC-TL with their parental mutant p53 MSC allowed for identification of the molecular events underlying their tumorigenic properties, including an embryonic stem cell (ESC) gene signature specifically expressed in MSC-TL. Knockout of mutant p53 led to a reduction in tumor development and tumorigenic cell frequency, which was accompanied by reduced expression of CSC markers and the ESC MSC-TL signature. In human cancer, MSC-TL ESC signature-derived genes correlated with poor patient survival and were highly expressed in human tumors harboring p53 hotspot mutations. These data indicate that the ESC gene signature-derived genes may serve as new stemness-based prognostic biomarkers as well as novel cancer therapeutic targets.



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Stem-Cells to Immune Cells and Back Again

Roi Gazit

Immune cells are continuously exchanged throughout healthy life, and even more so during disease. Hematopoietic Stem Cells (HSCs) are able to give rise to all types of blood and immune cells, they also enable bone-marrow transplant that is leading stem-cell's clinical usage. Surprisingly, relatively little is known about HSCs during acute infection. In addition, obtaining of compatible primary HSCs is still limiting the treatment of patients. We have generated and analyzed an extensive set of expression-profiles covering most of the murine hematopoietic system, to identify HSC-specific genes. Following previous success of reprogramming, we cloned few dozens of HSC transcription-factors. Transient overexpression of these factors in committed progenitors endowed them with extended self-renewal and multipotency. We have further identified 6 "core-factors" that can reprogram adult mouse blood cells back into transplantable "induced-HSCs". This novel direct reprogramming of adult cells into adult stem-cells is being translated into human, and already provides new insights into HSC's regulation during normal and malignant states. In parallel, we have generated an HSC reporter mouse by targeting a fluorescent protein into the *Fgd5* locus. The *Fgd5*-mCherry proved highly specific, enabling identification and isolation of functional HSCs solely by this endogenous reporter. While previous studies have reported a paradox of reduced transplantation-activity of the bone-marrow upon immune-stimulation with an increase of immune-phenotype HSCs, we do observe a clear reduction of the frequencies, and total numbers, of *Fgd5*-mCherry⁺ HSCs upon either pIC or LPS stimulation. Understanding the dynamics of activated stem-cells will shed new light on the progressive immune system in action.

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Microenvironment and Immuno-Oncology, Cancer Metastasis, Bio-Markers and Cancer Theranostics

Exosomes as Circulating Biomarkers and Mediators of the Cross-Talk of Metastatic Brain Tumor Cancer Stem Cells with Microglia

Chaya Brodie¹

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Brain metastases are the most common secondary brain tumors in adults. Despite their high frequency and patient poor prognosis, very little research has been performed on lung tumor-derived brain metastases, mainly due to the lack of appropriate experimental models. In this study, we isolated cancer stem cells (CSCs) from fresh specimens of lung-tumor derived brain metastases. The CSCs were analyzed for sphere formation and limiting dilution analyses, stemness markers, ability to generate xenografts and for their interaction with microglia cells. We found that CSCs derived from brain metastases had a high sphere forming capacity and self-renewal ability comparable to that of glioma stem cells. The CSCs expressed the stemness markers, CD133, Sox2, Klf4, Aldh2a, CD44 and the lung tumor markers, cytokeratin 7 and CD166. Transplantation of the CSCs or organoids generated from these cells formed xenografts that recapitulated the parental tumors. These xenografts were infiltrated by a large number of amoeboid microglia that expressed high levels of M2 markers. Using co-culture experiments, we further found that CSCs derived from brain metastasis induced the polarization of microglia to M2 phenotype via secreted exosomes and M2 microglia cells increased the self-renewal and stemness of the CSCs. RNA sequencing analysis identified specific miRNAs and lncRNAs that were associated with the CSC-microglia interactions. Using specific reporters, antagomiRs and CRISPR/Cas9 we demonstrated that miR-21, miR-1246 and the lncRNA TALNEC2 played a major role in the M2 polarization of microglia cells both in vitro and in vivo. Importantly, significantly higher levels of these non-coding RNAs were also identified in serum exosomes of patients with brain metastases compared with healthy controls. In conclusion, we generated CSCs and organoids from lung tumor-derived brain metastases that can serve as valuable in vitro and in vivo models for analyzing mechanisms involved in brain metastasis, tumor-microenvironment interactions and for personalized screening of therapeutic targets. Moreover, we identified exosomal novel non-coding RNAs that mediate the cross-talk of CSCs with microglia and represent potential circulating biomarkers for these tumors.

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Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment, Microenvironment and Immuno-Oncology, Precision in Personalized Cancer Immunotherapy, Cancer Therapy: Advances in Drug Design and Delivery

CD8 T cells enhance the anti-tumor efficacy of Trametinib in head and neck cancer

Moshe Elkabets

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Acquisition of resistance to anti-cancer therapies is associated with increase expression of immunosuppressor modulators that enable tumor cells to escape from the anti-tumor immunity machinery. To understand the mechanisms of immune-escape from MEK inhibitor in head and neck cancer (HNC), we have developed two MAPK pathway-driven cancers in immunocompetent mice. Specifically, 4NQO (Tobacco surrogate) -carcinogenesis induced HNC model, and novel KRAS_{G12}-driven HNC models using CRISPR technology. Both of these HNC models are sensitive to MEK1/2 inhibitor, Trametinib, however, over time resistance was developed and tumor progression was detected. Analysis of the tumor site during the acquisition of resistance indicated that the tumor cells and the tumor ecosystem were altered. At the initial response we detected a massive infiltration of CD8 T cells, however upon progression tumor site was enriched with exhausted CD8 T cells that express high levels of PD-1. Depletion of CD8 T cells reduced Trametinib efficacy, and blocking the immune-escape mechanisms using anti PD-1/PD-L1 drugs together with Trametinib induced tumor eradication and most mice were cured. Mechanistically, we found that chronic treatment with Trametinib induces both an upregulation of PD-L1 expression by tumor cells, and an accumulation of CD11c cells that express PD-L1. Overall, our findings suggest that simultaneous administration of blockers of the PD1/PD-L1 axis may enhance the clinical activity of MAPK-targeted drugs and delay the appearance of resistance.

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Israel Immunological Society

Genomic Instability, Cancer Signaling and Cancer Secretome

LATS1 and LATS2 Suppress Breast Cancer Progression by Maintaining p53 activity, Cell Identity and Metabolic state

Yael Aylon¹

Introduction:

Hippo signaling inhibits tumorigenesis by regulating proliferation, differentiation and epithelial–mesenchymal transition (EMT). At the core of the Hippo pathway are the LATS1/2 (LATS) tumor suppressors, which inhibit the downstream effectors YAP and TAZ. Beyond their function in Hippo signaling, LATS kinases engage in cross-talk with the tumor suppressor p53.

Material and methods:

Bioinformatic analysis (TCGA), MMTV-PyMT mouse model, human IHC, gene expression analysis (RNA-seq), metabolic analysis (Sea Horse).

Results and discussion:

We examined the consequences of downregulation of LATS1 and LATS2 in breast cancer. Consistent with their proposed tumor suppressive roles, expression of both paralogs is significantly downregulated in human breast cancer, and loss of either paralog accelerates mammary tumorigenesis in mice. Together, LATS function to maintain p53 in a tumor suppressive conformation. However, LATS1 and LATS2 also exert distinct impacts on breast cancer. LATS2 depletion in luminal B tumors results in metabolic rewiring, with increased glycolysis and reduced PPAR γ signaling. Furthermore, pharmacological activation of PPAR γ elicits LATS2-dependent death in luminal B-derived cells. In contrast, LATS1 depletion augments cancer cell plasticity, skewing luminal B tumors towards increased expression of basal-like features, in association with increased resistance to hormone therapy. Hence, these two closely related paralogs play common and distinct roles in protection against breast cancer.

Conclusion:

LATS-compromised tumors may harbor wild-type p53 with reduced tumor suppressive function (“pseudomutant p53”). Reduced expression of LATS1 or LATS2 in breast cancer may rewire distinct signaling networks resulting in differential susceptibilities to anti-cancer treatments.

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Inflammation and Immunity – Friends or Foes?

Probing the Role of Microglia in Relapsing-Remitting EAE

Zhana Haimon

Microglia are the specialized phagocytes of the brain parenchyma. In multiple sclerosis (MS), an autoimmune disease targeted at the CNS, microglia phagocytose cell debris and upregulate immune-related transcripts. However, it is not clear whether microglia play a beneficial or detrimental role, whether they interact with the infiltrating immune cells and whether or not these cells mediate recovery. Here, we used the Relapsing-Remitting Experimental Autoimmune Encephalomyelitis (RR-EAE) mouse model for MS on SJL*B6 F1 hybrids to study the role of microglia in different stages of the disease, focusing on their contributions to recovery and relapse. Using the RiboTag approach in *Cx3cr1^{CreER};Rpl22^{HA}* mice ¹, we revealed that microglia actively translate inhibitory molecules, such as Lag3, PDL1 and IL18bp. Moreover, microglia depletion experiments indicate a delayed recovery accompanied by T cell accumulation in the brains. Collectively, our results suggest that the presence and immune competence of microglia might be important for the recovery phase in RR-EAE. Inhibitory molecules expressed by microglia could mediate recovery, however the precise molecular mechanism by which this takes place remains under investigation.

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Inflammation and Immunity – Friends or Foes?, Host-Pathogen Interaction, Lymphocyte Activation & Exhaustion

B Cell Engineering for a Regulated, Potent and Evolving Response to HIV

Alessio Nahmad

Biochemistry and Molecular Biology, Tel Aviv University, Israel

Broadly Neutralizing antibodies (bNAbs) are an effective class of antibodies against HIV and their administration to infected individuals is associated with good clinical outcomes. However, high cost of treatment and repeated injections are predictable. Moreover, **viral escape limits the therapeutic effect of injected bNAbs.**

To tackle these issues, we engineer B cells to be bNAbs expressers in an endogenously regulated manner. These cells would undergo differentiation to germinal center B cells, enhancing the response by Class Switch Recombination (CSR) and Somatic Hypermutation (SHM), facilitating affinity maturation and countering pathogen escape. The cells would then differentiate to plasma cells or memory B cells, allowing long term potency and systemic control.

Using CRISPR/Cas9 and Adeno Associated Vectors (AAV), we introduce the anti-HIV broadly neutralizing antibody 3BNC117 at the immunoglobulin heavy chain (IgH) locus of B cells. The introduced cassette is regulated by a derivative of a murine IgH promoter that is active only upon on-target integration in proximity to the endogenous enhancers, as we first demonstrated using a GFP reporter gene.

For therapeutic cassettes, the light chain is encoded in full followed by the variable segment of the heavy chain, separated by a 2A peptide. Downstream of the variable heavy chain, we inserted a splice donor, which allows splicing with the endogenous constant segment. Flow cytometry and ELISA showed bNAb expression as a B cell receptor and as secreted immunoglobulins, respectively, in both cell culture and activated splenic B lymphocytes.

Importantly, adoptive transfer of the engineered cells into syngeneic mice allowed antigen-induced activation upon immunization. Donor specific cells were tracked with the CD45.1 cell surface marker in CD45.2 recipient mice. **In germinal centers (GC) following immunization, all donor specific cells were binding to the HIV antigen gp120, demonstrating the strong selection for the specific humoral response.** Strikingly, most of the gp120 binding cells in GCs were donor derived, demonstrating immunodominance of the engineered cells over the endogenous natural response. We followed the serological response using an anti-idiotypic antibody to quantify antibody titers. Interestingly, the bNAb would undergo CSR *in-vivo*, as demonstrated by IgA isotype in serum and in GCs. The total antibody response **rose much further following boost immunization implying immunological memory and affinity maturation.**

Uniquely, our method enables antigen-induced bNAb secretion that may be further enhanced by affinity maturation, class switch recombination, and the retention of immunological memory. B cells could thus be engineered as a living and evolving drug to counteract pathogen escape.



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Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment, Cancer Therapy: Advances in Drug Design and Delivery, Leukemia: From Stem Cell to Therapy

Multi-Kinase Inhibitors Targeting CK1 α and CDK7/9 are Novel Anti Leukemic Compounds with a Strong Immuno-Modulating Activity

Avner Fink

Casein Kinase 1 alpha (CK1 α) is a major regulator of cellular proliferation. It destabilizes β -catenin and p53 and its ablation hyperactivates the Wnt and p53 pathways. As CK1 α affects hematopoietic stem cell survival, we set to explore the effect of CK1 α on leukemic stem cells (LSCs). We developed a series of kinase inhibitors that target CK1 α and activate p53 and showed that these compounds are highly active against leukemic cells, including LSCs which are generally more resistant to treatment. Further studies showed that these compounds target also CDK7/9, allowing for a robust anti-leukemic response with high specificity. Long term treatment of leukemic mice (MLL-AF9 model) with a selected compound showed prolonged survival and probably cure for 40-50% of the treated mice. Our data further suggests that to achieve long lasting protection and cure, mice need to have a complete immune system, as Rag1^{-/-} mice fail to show such protection upon treatment with our lead compound A51. Ongoing studies suggest that T cells are involved in this protection and the cellular mechanisms underlying this protection are being actively explored. Finally, we are able to show that transplanted splenocytes from successfully-treated, likely cured mice in combination with A51 treatment, can confer long term protection to Rag1^{-/-} mice, suggesting the presence of leukemia specific memory T cells in the rescued mice. As our lead compound A51 is currently moving into clinical trials, understanding the immune regulation exerted by A51 can allow for further combinations with other drugs, to optimize the anti-leukemic effect of our compound.

Genomic Instability, Cancer Signaling and Cancer Secretome

Mutant p53 enhances the signal of hepatocyte growth factor (HGF) to endow cancer cells with drug resistance

Yan Stein

Background

Approximately 50% of human cancers harbor p53 mutations, which often endow cancer cells with novel oncogenic functions, such as promoting resistance to various chemotherapeutic drugs. It was recently demonstrated that secreted molecules from the microenvironment can promote drug resistance as well. However, whether cancer cell determinants, such as mutant p53, can act cooperatively with stromal-derived factors, to promote tumorigenesis at large and drug resistance in particular, has not been comprehensively studied yet. In the current study, we were interested to investigate whether mutant p53 can act in a cooperative manner with various secreted molecules to promote resistance to pathway-targeted therapy.

Materials and Methods

We utilized the human lung adenocarcinoma cell-line PC9, which harbors an EGFR hyperactivating mutation and an endogenous “hotspot” mutation in p53 DNA binding domain, R248Q. To study mutant p53-dependent effects, we created PC9 sub-lines which stably express turbo-RFP (tRFP) and either an shRNA targeting mutant p53 (PC9 shp53) or control shRNA (PC9 Mut-p53). We then subjected both PC9 sub-lines to either gefitinib, an EGFR inhibitor, or DMSO as control, together with a library of 298 recombinant secreted molecules. We then followed the growth of each of the sub-lines in the different conditions by monitoring changes in their tRFP signal for another 6 days.

Results

We did not observe significant mutant p53-dependent resistance to gefitinib in the absence of secreted molecules. However, we identified several such molecules that endowed the Mut-p53 sub-line with increased survival compared to shp53 sub-line when treated by gefitinib. Interestingly, the secreted factor that exhibited the most significant mutant p53-dependent rescue from gefitinib was hepatocyte growth factor (HGF). We were able to demonstrate a similar rescuing effect of mutant p53 and HGF in colon cancer cells treated with a MEK inhibitor. We further observed that mutant p53 downregulation attenuated the activation of the HGF receptor MET and the reactivation of downstream pro-survival ERK pathway, which corroborates the results of our proliferation assay. Furthermore, we were able to demonstrate the synergistic effect of HGF and mutant p53 on gefitinib resistance, as well as on ERK and MET activation, by administering conditioned medium derived of HGF-secreting fibroblasts. Finally, in a mass-spectrometry analysis, we were able to identify potential binding partners of mutant p53 which could mediate this mutant p53 and HGF dependent effect on gefitinib resistance.

Conclusion

In all, we describe here a novel mechanism for mutant p53-mediated drug resistance, which is not solely dependent on cell-autonomous factors, but rather on a synergistic effect between mutant p53 and stromal-secreted HGF. In a broad sense, we demonstrate how a molecular determinant in the cancer cell modulates a stromal signal to promote a pro-oncogenic phenotype.

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Check Point Pathways, Cancer and Immunotherapy from Experimental Models to Treatment

The immunomodulatory properties of Cannabinoids- lessons from murine models of Bone Marrow Transplantation.

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Introduction- In recent years, there has been a rapid increase in the medical use of cannabis. While cannabis is not registered as a drug or a medical product, the potential of cannabinoid-based medicines for the treatment of various conditions has led many countries around the world to authorize the use of such treatments. Cannabinoids, the biologically active constituents of Cannabis, have potent neuronal and immunological effects. However, the basic and medical research dedicated to medical cannabis and cannabinoids is limited. Hematopoietic stem cell transplantation (HSCT) is a well-established treatment for malignant and non-malignant hematological diseases. Allogeneic HSCT can cause the inflammatory condition, Graft versus Host Disease (GVHD), a major cause of morbidity and mortality in HSCT patients. In addition, slow, impaired or dysregulated reconstitution of donor derived immune cell populations cause susceptibility to both common and rare infections. The influence of cannabinoid-based treatments on hematologic reconstitution and on the development of GVHD after HSCT is largely unknown.

Material and methods- In our research, we compared the consequences of treatment with THC and CBD in vitro and in murine BMT models. Since it has been suggested that the combination of cannabinoids with other active molecules in the plant may achieve better clinical results than pure cannabinoids (known as the entourage effect), we also examined the differences between the effects of the pure cannabinoids and high THC/high CBD cannabis extracts.

Results and discussion- Our in vitro results demonstrate that the cannabinoid-based treatments decrease activated lymphocyte proliferation and affect cytokine secretion. We also discovered that CBD and THC utilize different receptors to mediate these effects. In vivo, in a syngeneic transplantation model, we demonstrate that all treatments inhibit lymphocyte reconstitution and show the inhibitory role of the cannabinoid receptor type 2 (CB₂) on lymphocyte recovery. Although pure cannabinoids exhibited a superior effect in vitro, in an allogeneic (C57BL/6 to BALB/c) BMT mouse model, THC-high and CBD-high cannabis extracts treatment reduced the severity of GVHD and improved survival significantly better than the pure cannabinoids.

Conclusions- Cannabinoids have significant immunomodulatory properties, which should be considered in clinical use. Our results highlight the complexity of using cannabinoid-based drugs and the need for additional comparative scientific results.



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Neuronal regulation of anti-tumor immunity

Asya Rolls

Epidemiological studies reveal a connection between the patient's mental state and cancer survival. Nevertheless, the underlying mechanisms are still largely unknown. Our group studies how specific brain activity affects tumor growth and specifically, anti-tumor immunity. We found that in tumor-bearing mice (Lewis lung carcinoma (LLC) and B16 melanoma) activation of the brain's reward system, a key circuitry in emotional processes attenuated tumor growth. This effect was mediated via the sympathetic nervous system (SNS), resulting in an attenuated noradrenergic input to a major immunological site, the bone marrow. Moreover, solid tumors develop in innervated tissues, and neurons invade the growing tumor itself. Thus, understanding how these local neuronal innervations affect the local anti-tumor immune response is expected to reveal potential new therapeutic avenues for immunotherapy. In this talk, I will discuss our findings with targeted brain manipulations as well as an optogenetic manipulation of neurons innervating the tumor site.



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Genomic Instability, Cancer Signaling and Cancer Secretome

uORF-encoded Peptides as Novel Regulators of Protein Kinase Activity: New Opportunities for Cancer Therapy

Etta Livneh

Introduction. Approximately 40% of human mRNAs contain upstream open reading frames (uORFs) in their 5' untranslated regions. Many of these uORF sequences, thought to impact translation or degradation of the primary ORF, were recently shown to be translated, but the function of encoded peptides remained unknown. We have previously published the presence of uORFs in PKCs. PKCs are involved in cell proliferation, differentiation, and apoptosis, among other processes impaired in cancer. Here we show for the first time that PKCeta, a signaling and anti-apoptotic stress kinase of PKC family, encodes for a uORF peptide with novel kinase inhibitory functions and therapeutic potential in cancer.

Materials and Methods. Bio-informatics analysis was used to identify uORFs in PKCs of different species and their conservation. The translational probability was determined by ribosome profiling data analysis and by generating uORF-luciferase fusion plasmids with point mutations in their respective initiation and stop codons. The ability of the peptides to inhibit PKCs kinase activity was investigated using in vitro kinase assays. Effects on cell viability and synergy with chemotherapy were studied as well as effects on cell migration and response to DNA damage.

Results and Discussion. We previously identified two uORFs upstream PKCeta that regulate its translation under normal growth conditions and upon stress. Here, we demonstrate that one of these uORFs possesses the typical pseudosubstrate motif present in all PKCs, auto-inhibiting their kinase activity. We show that this uORF-encoded peptide (uPEP₂) inhibits the kinase activity of PKCeta and of other members of the novel PKC sub-family, implying for network regulation in this family of kinases. Functionally, the peptide inhibits proliferation and migration of different aggressive tumors including breast cancers and leukemia cells. uPEP₂ synergizes with a chemotherapeutic agent by interfering with the response to DNA damage by interfering with gH2AX phosphorylation that marks DNA double strand breaks for repair. We show that uPEP₂ interferes with DNA repair processes upstream of H2AX phosphorylation, resulting in enhanced cell death.

Conclusion. Our studies introduce uORFs as new players in protein networks regulation, adding another layer of complexity to eukaryotic protein control mechanisms. Furthermore, these novel peptides may provide potential therapeutic agents for cancer treatment.

Identifying a malignant B-cell Lymphoma clone in the peripheral blood using Immunoglobulin high-throughput sequencing and lineage tree analysis

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Background:

Roughly 80% of aggressive B-cell lymphoma patients respond to frontline therapy but only 50%-60% are cured, necessitating longitudinal monitoring. Post-therapy surveillance PET-CT scans have not been shown to improve the outcome of patients. Identifying the malignant B-cell clone using Immunoglobulin (Ig) high-throughput sequencing (HTS) from plasma DNA was shown to be feasible as a method to detect minimal residual disease (MRD), but has not been standardized for MRD detection in lymphomas.

Aims:

We aimed to follow malignant B-cell clones using HTS of original lymph node (LN) biopsies, bone marrow (BM) samples collected during screening and serial blood samples from patients treated for relapsed/refractory aggressive B-cell lymphoma in a phase II clinical trial with ibrutinib, bendamustine and rituximab (NCT02747732).

Methods:

Genomic DNA was extracted and IgV libraries were produced and sequenced. Sequences were pre-processed using pRESTO and annotated using IMGT/HighV-QUEST. Clones were assigned using Change-O. Lineage trees were constructed using IgTree© and analyzed using PopTree© and custom R scripts.

Results:

Analyzing 10 samples from one patient, 98 of the 24,328 clones included the same VJ as the malignant clone; two of them included sequences from both the original LN biopsy and other blood samples. Without lineage tree analysis, we would have not been able to track our sequences to their correct ancestor in the malignant lymph node.

Conclusions:

Thus, using HTS combined with lineage tree analysis it is possible to reconstruct and follow a malignant B cell clone with blood sampling. We therefore suggest to use these methods for MRD tracking in B-cell malignancies.

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Bioinformatics, Big Data and Cancer, Leukemia: From Stem Cell to Therapy

Recurrent pre-leukemic deletions are the result of microhomology-mediated end joining DNA repair

Tzah Feldman

The mechanisms underlying myeloid malignancies deletions are not well understood, nor is it clear why specific genomic hotspots are predisposed to particular deletions. Inspecting the genomic regions around recurrent deletions in myeloid malignancies, we identified microhomology-mediated end-joining (MMEJ) signatures in recurrent pre-leukemic deletions. Introducing CRISPR Cas9 double-strand breaks (DSBs) into exon 12 of *ASXL1*, we successfully generated recurrent *ASXL1* deletion in human hematopoietic stem and progenitor cells (HSPCs). Our further analyses show that deletions with MMEJ signature enrich myeloid malignancies and can be detected in multipotent HSCs. Gene expression analysis in single human adult bone marrow HSPCs exposed differences between myeloid and lymphoid biased progenitors. Overall we provide evidence that somatic deletions in pre-leukemic HSCs are associated with specific DSBs followed by MMEJ repair. A better understanding of the source of these DSBs and the regulation of the HSC MMEJ repair pathway might aid with preventing recurrent deletions in human pre-leukemia.