The Naked Mole-Rat Epigenome: Unraveling the Secret for Exceptional Longevity

Huda Adwan [1], Rotem Vered [1], Danielle Gutman [1], Lital Sharvit [1], Rochelle Buffenstein[3], James Nelson[4], Gil Atzmon [1,2]

[1] Department of Human biology , University of Haifa, Israel; [2] Medicine and Genetics Department, Albert Einstein College of Medicine, New York, USA; [3]Barshop Institute for Aging and Longevity Studies, *[4]Department of physiology, University of Texas Health Science Center, San Antonio, USA.*

http://gilatzmon.wix.com/aging-and-longevity-

Introduction

Aging

Aging is generally accompanied by age-associated chronic diseases such as **Alzheimer's disease**[1], **type 2 diabetes**[2], **cardiovascular disease (CVD)**[3], and **many forms of cancer**[4-6] affecting both the quality of life and lifespan.

Longevity

A term that can be defined as an individual's ability to reach longer lifespan under ideal and proximal conditions[7].

The Naked Mole-Rat

- Small rodent (~28 grams)
- Subterranean lifestyle
- Eusocial colonies
- Extraordinary longevity up to 28 years (8-10 times longer than any other rodent).
- Anti-aging mechanisms[8-10]:
  - Cancer free good health in stressful environment
  - Unchanged physiological functions
  - Sustained reproductive capacity
  - Negligible senescence

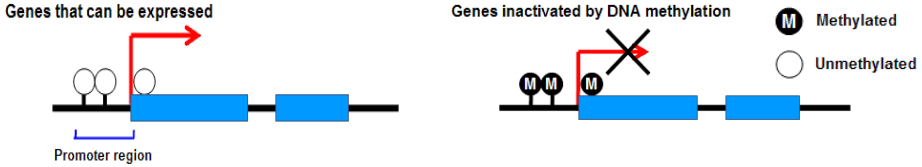
The Naked Mole-Rat is an excellent candidate model to examine mechanisms leading to "successful" aging and longevity.

Epigenetics

- Epigenetic changes refer to gene expression alteration that arise from chromosomal changes without DNA sequence modification.

The mother's diet during pregnancy affect the fur color of the litters[11]

- DNA methylation: addition of a methyl group to a cytosine (CpG).



- Methylation changes at specific gene regions have been shown to be associated with age associated diseases[12-16].

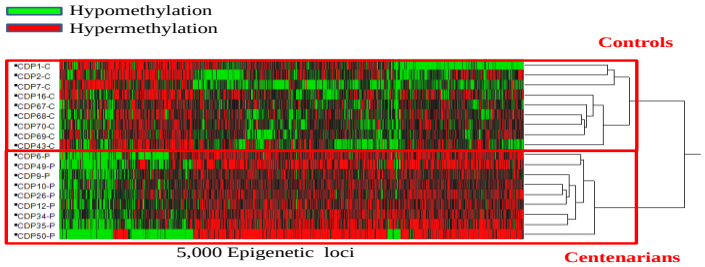
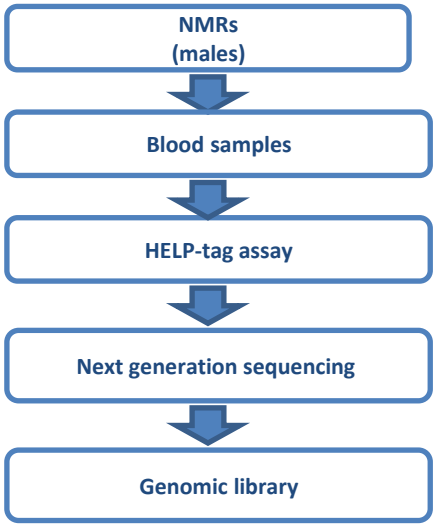


Figure 1. Unsupervised clustering of HELP data from CD34+ cells shows difference in methylation between Centenarians and Controls. The heat map shows more methylated loci in red, less methylated in green.

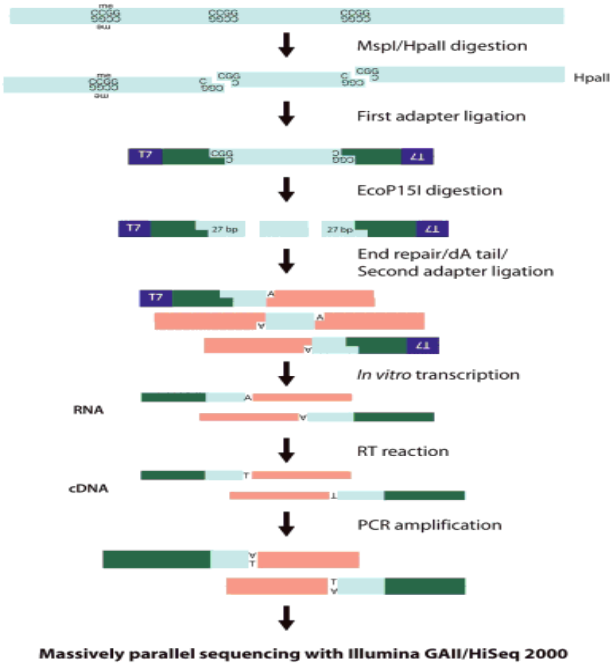
Hypotheses

- Epigenetic changes associated with aging In NMRs could serve as markers for healthy lifespan.
- Epigenetic changes may represent one of the central mechanisms of gene regulation by which many age-related diseases are buffered.
- Older NMRs will exhibit differential epigenetic changes at sites distinct from younger NMRs.

Methods



HELP-tag assay[17]

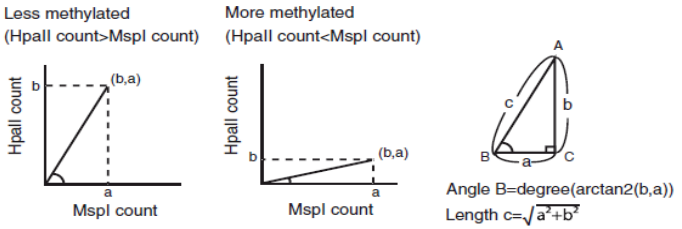


- **NMRs Colony at UTHSCSA is the largest colony in the USA and contains the longest-lived of theses animals, age reaching older than 32 years**

- **DNA extraction from blood samples**

HELP-tag assay:

- **Two lines from each DNA sample**
  - **Each is treated with a different restriction enzyme**
  - **HpaII-** methylation sensitive
  - **MspI-** methylation insensitive
- **Building two cDNA libraries**
- **Next generation sequencing (NGS)**
- **Sequence Data**
- **Methylation score (linear scaling of angle value) between 0 and 100 (% of unmethylation at that locus) and confidence score.**



- **Alignments and calculation of the methylation fraction[18]**

Preliminary Results

Table1. Genome size and CpG quantity			
	Genome length (bp)	CG found	% of CG
Human	3095694110	28104642	0.91
NMR	2015372038	13192873	0.65

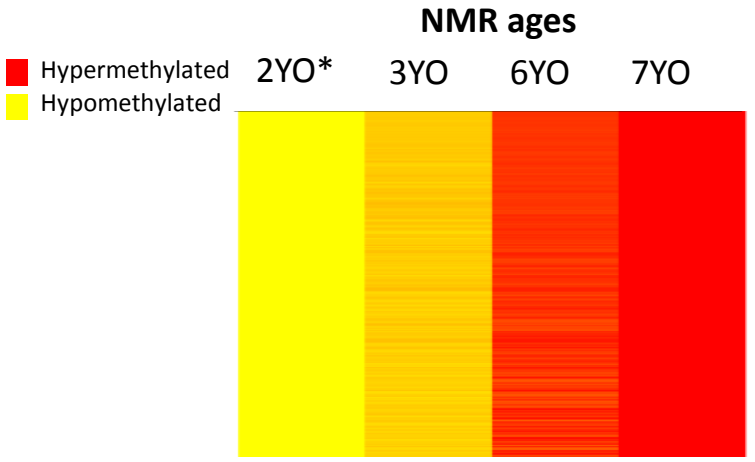


Figure 3. Unsupervised clustering of HELPtag data (3400 loci-horizontal lines) from blood cells shows differences in methylation between 4 NMRs. The heat map shows more methylated loci in red, less methylated in yellow. Distinct patterns are obviousbetween 2YO **(*set as reference)** to the 7 YO animal.

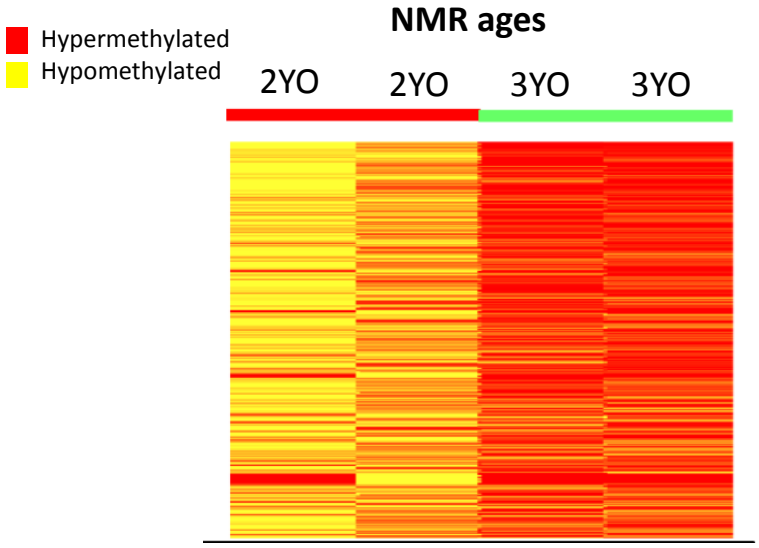


Figure 2. Unsupervised clustering of HELPtag data from blood cells. The heat map shows more methylated loci in red, less methylated in yellow. Distinct patterns are obvious between 2YO to the 3YO groups.

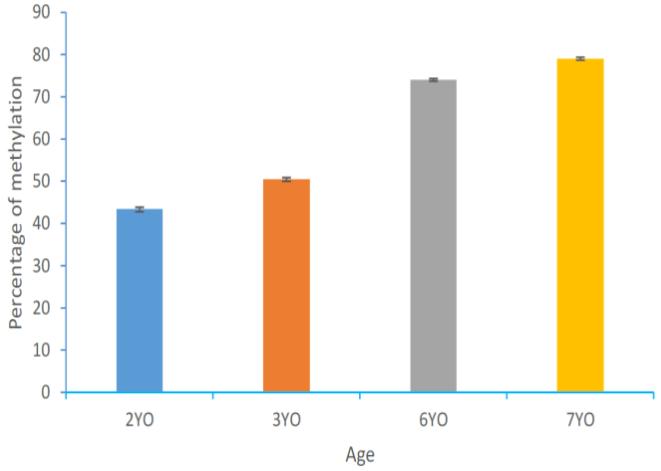


Figure 4. Animals' average and SE percentage of methylation (3400 loci) demonstrates increase methylation with age.

Conclusions

- Profiling the epigenome of NMRs, especially methylation patterns, is feasible.
- Subset of loci that characterizes these groups' epigenetic aging features can provide insights into their exceptional healthy life.
- These epigenetic elements can be translated to human and will provide a candidate loci for manipulation either environmentally or by drug design.
- Similar to humans, NMRs demonstrate differential epigenetic profiling within ages. One year difference in NMR life can result with traceable epigenetic changes.

Future directions:

- Building the epigenomic aging profile for NMR
- EWAS
- Testing for candidate loci in human tissue/cell culture in different environments
- Translation of the results from in vitro to Human

Thank You

References

1. 1. G. S. Zubenko, H. B. Hughes, 3rd, J. S. Stiffler, D10S1423 identifies a susceptibility locus for Alzheimer's disease in a prospective, longitudinal, double-blind study of asymptomatic individuals. *Mol Psychiatry* **6**, 413-419 (2001).
2. 2. H. E. Resnick, M. I. Harris, D. B. Brock, T. B. Harris, American Diabetes Association diabetes diagnostic criteria, advancing age, and cardiovascular disease risk profiles: results from the Third National Health and Nutrition Examination Survey. *Diabetes care* **23**, 176-180 (2000).
3. 3. S. S. Najjar, A. Scuteri, E. G. Lakatta, Arterial aging: is it an immutable cardiovascular risk factor? *Hypertension* **46**, 454-462 (2005).
4. 4. M. Lahn *et al.*, Protein kinase C-alpha in prostate cancer. *BJU Int* **93**, 1076-1081 (2004).
5. 5. G. R. Newell, M. R. Spitz, J. G. Sider, Cancer and age. *Seminars in oncology* **16**, 3-9 (1989).
6. 6. S. J. Rulyak, A. B. Lowenfels, P. Maisonneuve, T. A. Brentnall, Risk factors for the development of pancreatic cancer in familial pancreatic cancer kindreds. *Gastroenterology* **124**, 1292-1299 (2003)
7. 7. Atzmon G. (Ed.). Longevity Genes A Blueprint for Aging, Series: Advances in Experimental Medicine and Biology, Vol. 847. Springer. 2015
8. 8. K. M. Grimes, A. K. Reddy, M. L. Lindsey, R. Buffenstein, And the beat goes on: maintained cardiovascular function during aging in the longest-lived rodent, the naked mole-rat. *American journal of physiology. Heart and circulatory physiology*, (2014).
9. 9. R. Buffenstein, Negligible senescence in the longest living rodent, the naked mole-rat: insights from a successfully aging species. *Journal of comparative physiology. B, Biochemical, systemic, and environmental physiology* **178**, 439-445 (2008).
10. 10. Y. H. Edrey, T. J. Park, H. Kang, A. Biney, R. Buffenstein, Endocrine function and neurobiology of the longest-living rodent, the naked mole-rat. *Exp Gerontol* **46**, 116-123 (2011).
11. H. D. Morgan, H. G. E. Sutherland, D. I. K. Martin, E. Whitelaw, Epigenetic inheritance at the agouti locus in the mouse. *Nature Genetics* 23, 314 - 318 (1999)
12. 11. J. Pasquier, J. Hoarau-Vechot, K. Fakhro, A. Rafii, C. Abi Khalil, Epigenetics and Cardiovascular Disease in Diabetes. *Curr Diab Rep* **15**, 108 (2015).
13. 12. T. Hamidi, A. K. Singh, T. Chen, Genetic alterations of DNA methylation machinery in human diseases. *Epigenomics* **7**, 247-265 (2015).
14. 13. Y. Cao *et al.*, Impact of epigenetics in the management of cardiovascular disease: a review. *Eur Rev Med Pharmacol Sci* **18**, 3097-3104 (2014).
15. 14. M. A. Jeffries, A. H. Sawalha, Autoimmune disease in the epigenetic era: how has epigenetics changed our understanding of disease and how can we expect the field to evolve? *Expert Rev Clin Immunol* **11**, 45-58 (2015).
16. 15. D. Ben-Avraham, Epigenetics of aging. *Adv Exp Med Biol* **847**, 179-191 (2015).
17. 16. B. Khulan *et al.*, Comparative isoschizomer profiling of cytosine methylation: the HELP assay. *Genome Res* **16**, 1046-1055 (2006).
18. M. Suzuki, Q. Jing, D. Lia, M. Pascual, A. McLellan, J. M. Greally, Optimized design and data analysis of tag-based cytosine methylation assays. *Genome biology* 2010, **11**:R36