Cancer and Aging: Biological and Phenotypic Measures of Aging

A WEBINAR TRIBUTE TO DR. ARTI HURRIA

Luigi Ferrucci, M.D., Ph.D.
Morgan Levine, Ph.D.
Cancer & Aging Research Group
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Today’s speakers:

Luigi Ferrucci, M.D., Ph.D.
Geriatrician and Epidemiologist
Scientific Director
National Institute on Aging

Morgan Levine, Ph.D.
Assistant Professor
Department of Pathology
Yale School of Medicine
“Connecting the biological and phenotypic manifestations of aging: the case of muscle aging”.

Luigi Ferrucci - National Institute on Aging
The Metrics of Aging

**Functional Aging** (impact on daily life)
- Cognitive Function
- Physical Function
- Mood
- Mental Health

** Phenotypic Aging** (phenotypes that change)
- Body Composition
- Energetics
- Homeostatic Mechanisms
- Brain health

**Biological Aging** (root mechanisms)
- Molecular damage
- Defective repair
- Energy exhaustion
- Signal/noise reduction

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**Frailty**
- Sleep fragmented
- Mobility
disability
- Poor performance
- Depression
- Fatigability

**Sarcopenia**
- Poor muscle quality

**Immunosenescence**
- Dysautonomia
- High RMR
- Insulin resistance
- Osteoporosis

**Sensory impairment**
- Lack of coordination
- Loss of autonomy
- Exhaustration

**Comprehensive Geriatric Assessment**
- Mobility disability
- Poor performance
- Faulty decision making
- Confusion

**Cellular senescence**
- Mitochondria attrition
- Stem cell exhaustion
- Genomic instability
- Deregulated nutrient sensing

**Epigenetic alteration**
- Telomere attrition
- Nutrient sensing

**Sensory impairment**
- Bad memory
- Fatigue
- Low fertility
- Low NCV
- Kidney damage
Genomic Instability
The Accumulation of Somatic Mutations with Aging

Cellular Senescence
Trade-off Between Cancer and Aging

Epigenetics (methylation)
The “Epigenetic Clock”

Mitochondrial Dysfunction
The Power Plant

Proteostasis (autophagy)
Repair, Recycle or Trash?

Telomere Length
Protecting the DNA During Replication

Stem Cell Exhaustion
Templates for Cells Restoration

Cell to Cell Communication
Accuracy and Context in the Flow of Information
Genomic Instability
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The Hallmarks of Aging
Carlos Lopez-Otin et al.
Muscle
Strength/ Mass Ratio in BLSA Participants 60-70 yrs Old

Percent Change Compared to the Average for the Younger Age Group

-25% versus <25
-20% versus 25-34
-15% versus 35-44
-10% versus 45-54
-5% versus 55-64
0% versus 65-74
5% versus 75-84
10% versus 85+

Muscle CSA (Mid-Thigh)
Knee Extension Torque
Mid-thigh T1w MRI Images (Men; GESTALT)

Age 23 Years
Age 26 Years
Age 28 Years
Age 31 Years
Age 31 Years
Age 42 Years
Age 45 Years
Age 45 Years
Age 51 Years
Age 52 Years
Age 57 Years
Age 60 Years
Age 67 Years
Age 72 Years
Age 81 Years
Age 83 Years
Selection of 79 pairs of cases (low muscle quality) and controls (high muscle quality), matched by age (±2.5 years), sex, and height (±1.5cm). Muscle quality defined as knee extension torque/mid-thigh muscle cross-sectional area.

NESTED CASE-CONTROL STUDY in BLSA

126 Metabolites according to down-regulation or up-regulation in cases (low muscle quality) compared to controls (high muscle quality)


Metabolomics on Muscle Biopsies
BCAAs stimulates energy production and protein synthesis

BCAAs extracellular

SNAT2  LAT1
Protein Breakdown

BCAAs intracellular

Gln

SIRT-1

PGC-1α
HIF-1α

mTOR (TORC1)

4E-BPs

eIF4E

P70 S6 Kinase

Protein Synthesis Cascade

Myocyte

BCAT = Branched chain aminotransferase
BCKDH = Branched-chain alpha-keto acid dehydrogenase

BCAAs

BCKDH

Active

Inactive

BCKDH Kinase

BCKDH Phosphatase

R-CoA

(IB-CoA, MB-CoA, IV-CoA)

Succinyl-CoA

Acetyl-CoA

Acetoacetate

TCA Cycle

Muscle Protein Synthesis

Muscle Protein
Synthesis

BCAT = Branched chain aminotransferase
BCKDH = Branched-chain alpha-keto acid dehydrogenase

BCAAs extracellular

SNAT2  LAT1
Protein Breakdown

BCAAs intracellular

Gln

SIRT-1

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TCA Cycle

Muscle Protein Synthesis

BCAT = Branched chain aminotransferase
BCKDH = Branched-chain alpha-keto acid dehydrogenase
Figure 1. Skeletal muscle oxidative capacity, a proxy marker of mitochondrial function, declines with aging both in men (n=400) and women (n=331). BLSA 2020 limited to first measures.
Muscle Strength Mediates the Effect of Mitochondrial Function on Walking Performance.

**Mitochondrial Function** (KPCr) → **Muscle Strength**

- **6m Usual Pace**: 0.102 (-16%)
- **6m Fast Pace**: 0.074 (-29%)
- **150s Usual Pace**: 0.110 (-27%)
- **400m Fast Pace**: 0.160 (-23%)*

Walking Speed

- Beta Coefficient for KPCr on Walking Speed
  - **6m usual**: 0.10
  - **6m fast**: 0.05*
  - **150s usual**: 0.15*
  - **400m fast**: 0.20*

* Sobel Test (mediation): P<.05

All models adjusted for sex, height, weight, and %PCr depletion; * P<.05; ** P<.01

Zane A et al. Aging Cell 2016
Classification of Age-associated Proteins In Skeletal Muscle
Proteins associated with Aging

Ubaida-Mohien C et al. eLife 2019
Classification of Age-associated Proteins In Skeletal Muscle

Proteins associated with Aging

Proteins associated with Physical Activity

Ubaida-Mohien C et al. eLife 2019

Ubaida-Mohien C et al. Frontiers Physiol 2019
Hallmarks of Aging

Age Associated Protein Categories
Adjusted for Gender, PA, Race, Fiber ratio, BMI and Batch effects

Abundance Decreased With Age (361)
Abundance Increased With Age (904)

Physical Activity Associated Protein Categories
Adjusted for Gender, Age, Race, Fiber ratio, BMI and Batch effects

Abundance Decreased With PA (695)
Abundance Increased With PA (324)

Mitochondrion
Splicing
Others
(Immunity/Inflammation, NMJ, Proteostasis, Senescence, Transcription Regulation, Epigenetic Regulations, Genomic Maintenance, Ribosomal, Unknown)
Figure 3 Functional Decline of Mitochondrial Proteins with Age

A. Bio Energics
- Glycolysis: 26 [31]
- TCA Cycle: 22 [24]
- Respiratory Chain: 125 [170]
- Electron Transport: 69 [109]

B. Localization
- Inner Membrane: 181 [263]
- Membrane Space: 35 [49]
- Mitochondron Matrix: 117 [178]
- Outer Membrane: 65 [124]
- Other Mitochondron: 666 [1150]

C. CD antigen CD73 (NT5E)
- Protein Abundance (Log2)
- Age Beta Coefficient

D. NAM N-methyltransferase (NNMT)
- Protein Abundance (Log2)

E. NNAD(P) transhydrogenase (NNT)
- Protein Abundance (Log2)

F. Succinate dehydrogenase assembly factor 2 (SDHAF2)
- Protein Abundance (Log2)

G. Nicotinamide riboside kinase 1 (NMRK1)
- Protein Abundance (Log2)

H. Poly [ADP-ribose] polymerase 1 (PARP1)
- Protein Abundance (Log2)
Figure 4: Implications of Proteins that Modulate Transcription and Splicing

A. Kelch-like protein 31 (KLHL31)
- r² = 0.11, p = 0.01269

B. Myocyte-specific enhancer factor 2D (MEF2D)
- r² = 0.39, p = 0.00318

C. Transcriptional repressor CTCF
- r² = 0.12, p = 0.10867
Discovery proteomics on muscle biopsies quantified using TMT and LC-MS methods. Overall, 132 spliceosome pathway proteins were quantified. Of these, 122 were underrepresented in master athletes compared to controls, and for 22 of them the difference was significant.
Adjusting for Age and Physical Activity, Up-Regulation of the Splicing Machinery is Associated with Better Mitochondrial Function

Post-exercise phosphocreatine (PCr) recovery time ($\tau_{PCr}$) measured by $^{31}$P MRS; reflects in vivo oxidative capacity of skeletal muscle.

Skeletal muscle protein association with ($\tau_{PCr}$)

Network representation of the 253 proteins associated with better muscle oxidative capacity
3D Reconstructions of FIB-SEM images in 3 different age groups. Mitochondria are pink, Z-bands are cyan, and voided areas are gray.
Geroscience anticipates secondary prevention

U.S., 2011 Medicare Current Beneficiary Survey

% Prevalence of Mobility Limitation

Men  Women
65-74 75-84 85+  65-74 75-84 85+

34.0 47.0 68.9  37.4 55.7 78.7
DNA Methylation Landscapes in Aging & Disease

Morgan Levine
Assistant Professor
Department of Pathology
Yale University School of Medicine
What is the biggest risk factor for lung cancer?

Smoking increases lung cancer incidence and death by 15 to 30 fold.

1 in 200k chance for ages 25-29, nearly 400 in 100k chance ages 75-79.
The aging process is thought to play a causal role in the etiology of most major chronic diseases.
Cancer Risk & Age

Is aging causal or consequential in cancer?

The more times you roll the dice (function of chronological time) the more likely your chances?
Is aging causal or consequential in cancer?

Adaptive Oncogenesis Model
Dr. James DeGregori

Roughly half of all mutations occur before full body maturation

Context matters!
System-level dynamics that change with age alter the fitness landscape
Biomarkers of Aging

Useful proxies that estimate aging (or agedness) of a sample.

**Should Answer:**
Biologically, what differentiates the average 20 year old from the average 80 year old?

What differentiates a healthy 80 years old from an unhealthy 80 year old?
Epigenetics: Molecular OS

DNA Methylation (DNAm)
Involved in cell proliferation/differentiation, transcriptional repression, genomic imprinting, organization of chromatin.
Epigenetic Clocks

Because of the precise age changes, we can use machine learning to predict “the age” of a sample based on its DNAm levels.
Epigenetic Clocks

1st Generation: Age Predictors
Supervised machine learning to predict age from hundreds of CpGs
Epigenetic Clocks

2nd Generation: Aging Outcome Predictors
Supervised machine learning to predict age-related outcomes from hundreds of CpGs
Epigenetic Clocks

- Correlation 0.96, p<1e-200
- Correlation 0.8, p<1e-200
- Correlation 0.68, p<1e-200

DNA methylation (DNAm) age vs. chronological age for different tissues and conditions:

- Whole Blood
- Breast (Normal)
- Buccal
- Frontal Cortex
- Temporal Cortex
- Epidermis
- Fetal (Multi Tissue)
- Colon (Normal)
- Cord Blood
- Dermis
- Fibroblasts
- DLPFC
- Glia (Occipital Cortex)
- Neurons (Occipital Cortex)
Epigenetic Clocks

Horvath1 p = 0.21

Hannum p = 0.23

Levine p = 0.0023

Lin p = 0.88

Horvath2 p = 0.19

Yang p = 0.01
Epigenetic Clocks

DNAm can capture a lot of cellular/molecular changes.

What are the core signals (parts) being captured by the clocks?

Are there shared signals across aging phenomena and/or tissues?
Shared DNAm Signals

DNA Methylation

PCA in 9 Datasets
- Whole blood
- Adult Brain
- Developmental Brain
- Dermis
- Epidermis
- Senescence
- iPSC/Reprogramming
- Tumor/Normal
Shared DNAm Signals

PCA in 9 Datasets
- Whole blood
- Adult Brain
- Developmental Brain
- Dermis
- Epidermis
- Senescence
- iPSC/Reprogramming
- Tumor/Normal

Estimate top 10 PCs in all datasets (n=90 variables in each dataset)
10 of the 90 PCs had consistent age and/or aging-outcome associations across all 9 datasets.
Shared DNAm Signals

Blood bicor=0.42, p=2e-29

DLPFC bicor=0.71, p=1.4e-104

Dermis bicor=0.35, p=0.027

Epidermis bicor=0.6, p=6.8e-05

Colon bicor=0.38, p=1.8e-06

Sen p = 0.0085

iPSC p = 0.0062

iPSC p = 0.017

Tumor p = 2.2e-05
Shared DNAm Signals

Blood bicor=0.37, p=1e-22

DLPFC bicor=0.48, p=3.4e-40

Dermis bicor=0.59, p=6.2e-05

Epidermis bicor=0.28, p=0.089

Colon bicor=0.32, p=6.9e-05

Sen p = 0.0057

iPSC p = 0.0062

iPSC p = 0.13

Tumor p = 3.5e-07
Conclusions

Does biological aging in a tissue predispose it to tumorigenesis?

• Aging/Cancer: probability with time vs. causal driver

Preliminary Evidence for Aging → Cancer

1. One can estimate “aging” in various tissues using DNAm.
2. For most clocks, tissues show different rates of aging.
3. DNAmAge can differentiate tumor versus normal tissue (acceleration in cancer).
4. DNAmAge can differentiate normal breast tissue in women with history of breast cancer versus controls.
5. DNAm patterns in cancer apply to other tissues.
   1. Correlate with age in blood, brain, skin, colon
   2. Accelerated in skin exposed to sun
   3. Accelerated in senescent cells (oncogene induced and replicative)
Acknowledgements

Laboratory for AGING IN LIVING SYSTEMS

Current Members
Kyra Thrush (PhD Student Computational Biology)
Albert Higgens-Chen (Post Doc)
John Gonzalez (PhD Student in Molecular Medicine)
Margarita Meer (Assistant Research Scientist)
Diana Leung (PhD Student Computational Biology)
Chris Minteer (PhD Student in Molecular Medicine)

Former Members
Zuyun Liu (Former Post Doc)
Q&A
Save the date for the next webinar:

• September 14, 1-2 p.m. ET
  • Speakers: Dr. Hyman Muss and Dr. Grant Williams

• Send speaker suggestions and other feedback to:
  NCIDCCPSagingwebinar@mail.nih.gov