Aging and Arterial Senescence: Putative Role of Telomere Function

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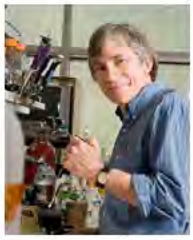
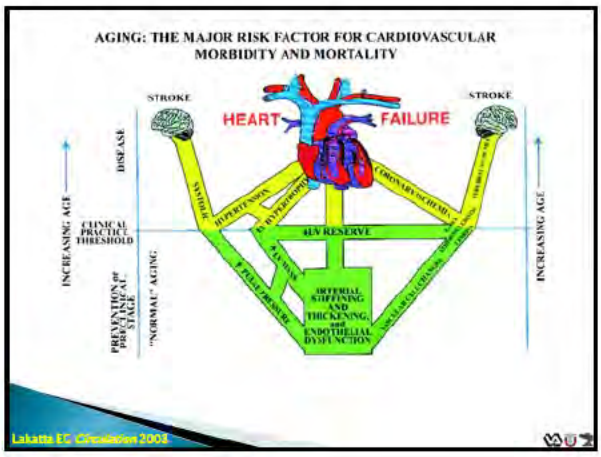
CENTER ON AGING THE UNIVERSITY OF UTAH

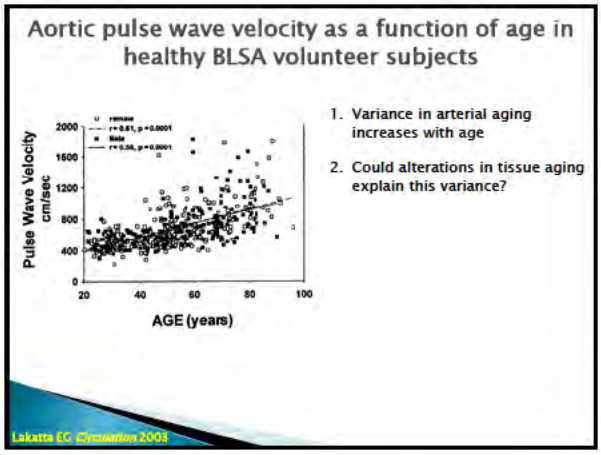
The University of Utah Division of Geriatrics

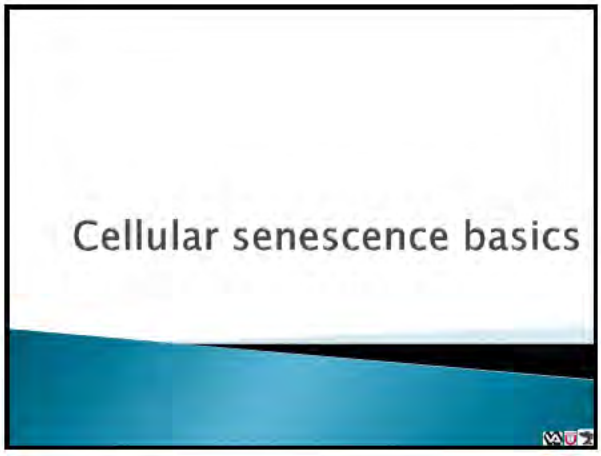
Center on Aging Collaborators



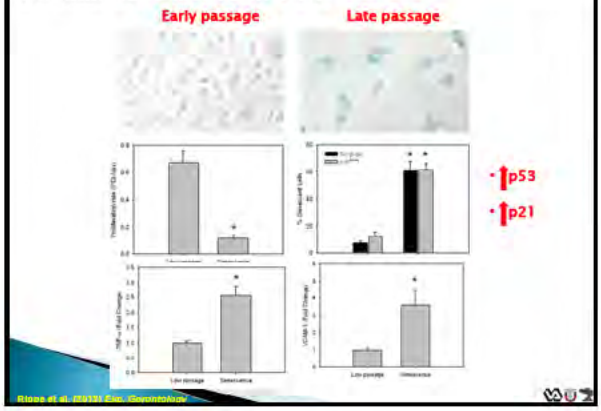
Lots of thanks to R. Garrett Morgan


AGING: THE MAJOR RISK FACTOR FOR CARDIOVASCULAR MORBIDITY AND MORTALITY
STROKE
HEART
FAILURE
STROKE
INCREASING AGE
DISEASE
CLINICAL PRACTICE THRESHOLD
PREVENTION OF PRECLINICAL STAGE
"NORMAL" AGING
INCREASING AGE
HYPERTENSION
DIASTOLIC DYSFUNCTION
CORONARY ISCHEMIA
LVH
4LV RESERVE
ARTERIAL STIFFENING AND THICKENING; and ENDOTHELIAL DYSFUNCTION
Lakatta EG. Circulation 2003

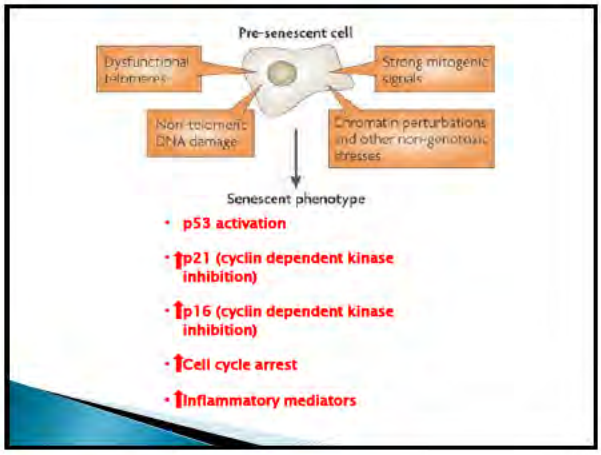

Aortic pulse wave velocity as a function of age in healthy BLSA volunteer subjects
Pulse Wave Velocity cm/sec
2000
1600
1200
800
400
0
20
40
60
80
100
AGE (years)
1. Variance in arterial aging increases with age
2. Could alterations in tissue aging explain this variance?
Lakatta EG. Circulation 2003


Cellular senescence basics

Cellular senescence *in vitro*



Rizos et al. (2010) Exp. Gerontology



Cellular senescence *in vivo*

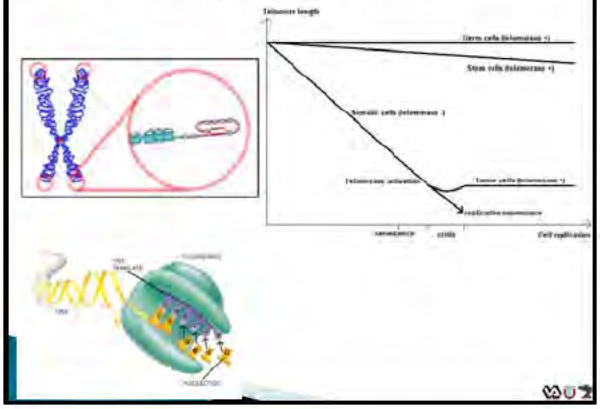
LETTER

Clearance of p16^{Ink4a}-positive senescent cells delays ageing-associated disorders

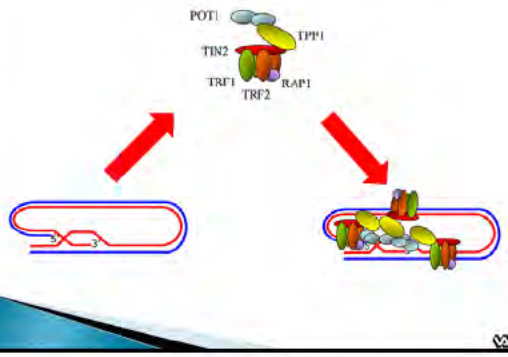
1. Improved treadmill time
2. Decreased cataract formation
3. Augmented BMD
4. Improved adipose tissue

Telomere basics

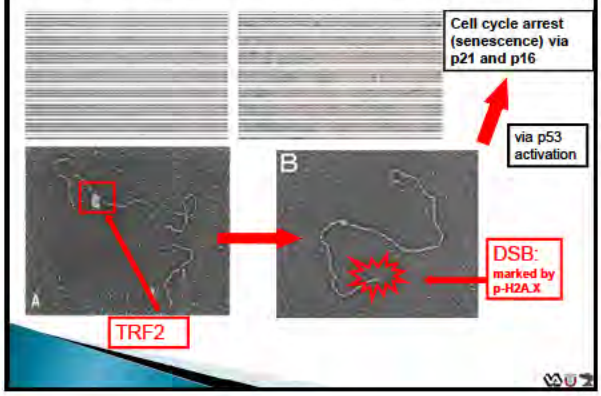
Telomeres, T-loops and Telomerase



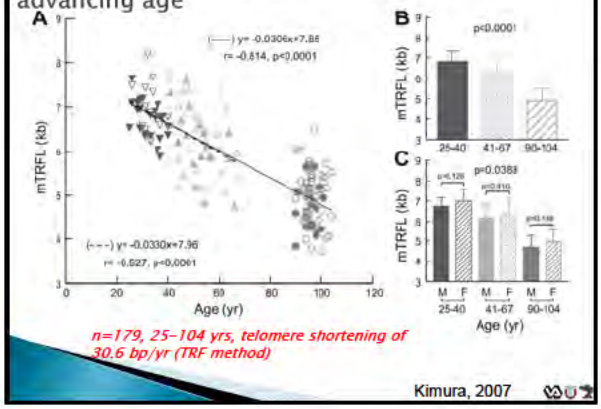
Telomere binding proteins and the T-loop



Telomere shortening leads to T-loop uncapping



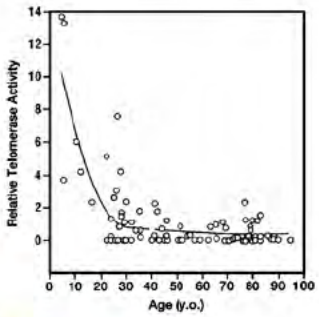
Telomere shortening in leukocytes with advancing age



n=179, 25–104 yrs, telomere shortening of 30.6 bp/yr (TRF method)

Kimura, 2007

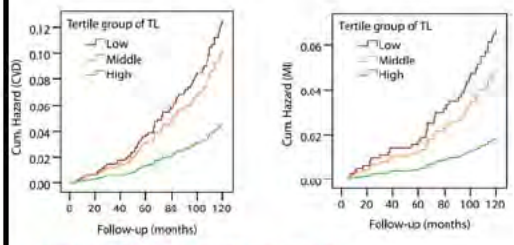
Telomerase activity in leukocytes with advancing age (*as assessed by TRAP assay*)



Iwama et al., 1998

Putative role of telomere shortening in CVD

Age and sex-adjusted cumulative hazard curves for composite CVD and MI predicted by leukocyte telomere length tertiles



n=88, 45-84 yrs, telomere shortening of 45.5 bp/yr (Cawthon qPCR method)

Willeit et al., 2010

Working hypothesis

- Age-related differences in arterial telomere structure/function, will lead to senescence signaling and is associated with age-related differences in arterial inflammation in human arteries.

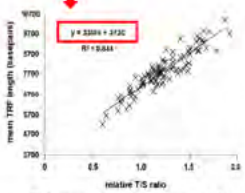
Experimental approach

- General procedures
  - *Subjects and General Procedures:* 97 subjects across various ages who underwent a lymph node biopsy at the University of Utah & Huntsman Cancer Center donated arterial tissue for the study.
  - ***Medical conditions and medications will be assessed, and subjects with overt cardiovascular disease or metastatic cancer (melanoma has spread to other tissues) will be excluded.***

High-throughout telomere length measurement by qPCR

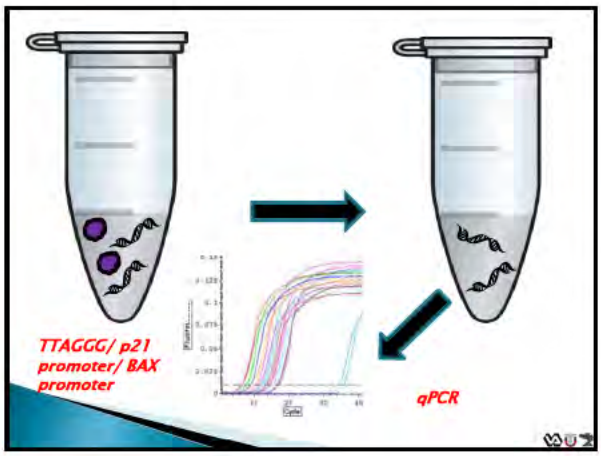

Telomere length measurement by a novel monochrome multiplex quantitative PCR method

**Convert T/S ratio to telomere length (bp)*

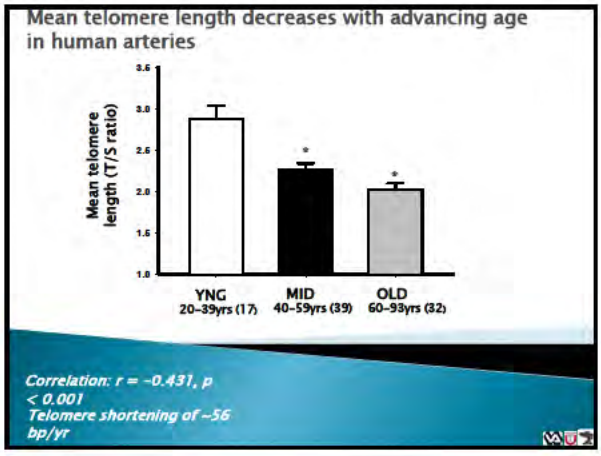


Telomere length determined by TRF to telomere length determined by qPCR correlation

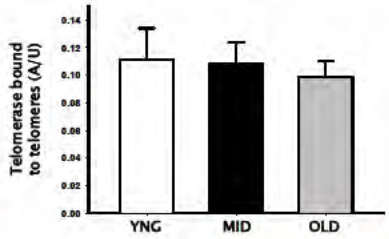
Chromatin Immunoprecipitation (ChIP)



Study results

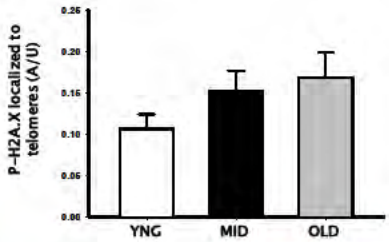


Aging does not alter telomerase activity in human arteries



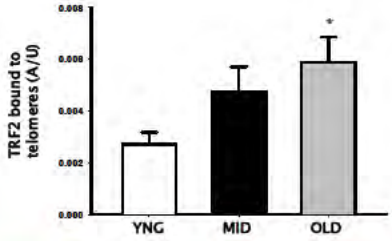
Correlation: r = −0.007, p = 0.477

p-H2A.X localized to telomeres (marker of telomere uncapping) is not altered with age human arteries



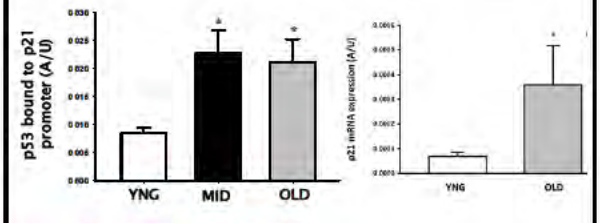
Correlation: r = 0.177, p = 0.052
YNG_OLD: p = 0.146

TRF2 bound to telomeres increases with advancing age



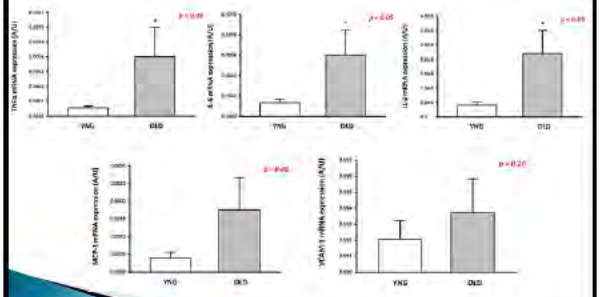
Correlation: r = 0.219, p < 0.05

Arterial aging is associated with augmented senescence signaling

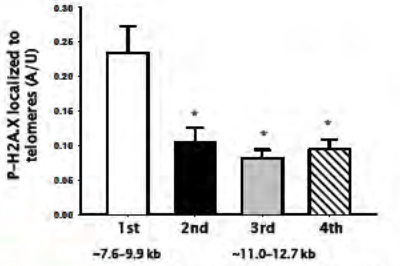


Correlation: r = 0.249, p < 0.05

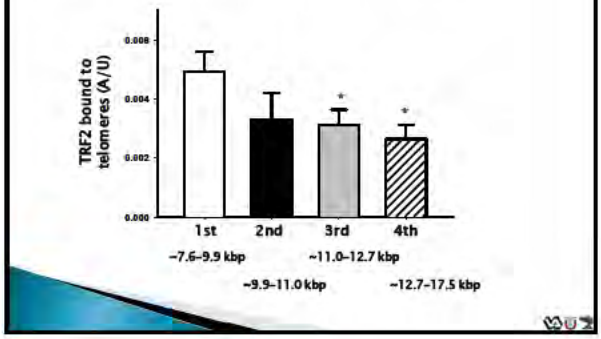
Age related changes arterial inflammation



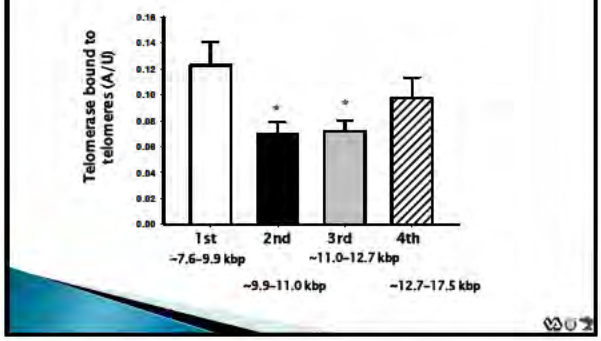
p-H2A.X localized to telomeres (marker of telomere uncapping) is related to shorter telomeres



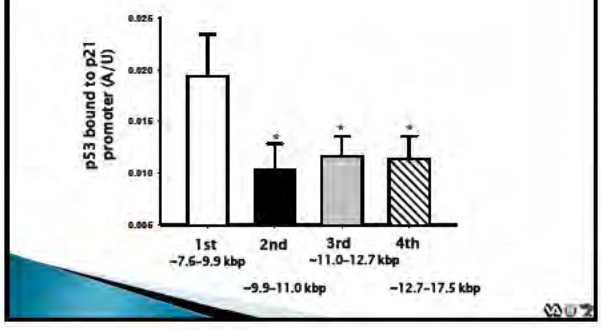
Higher TRF2 is related to shorter telomeres



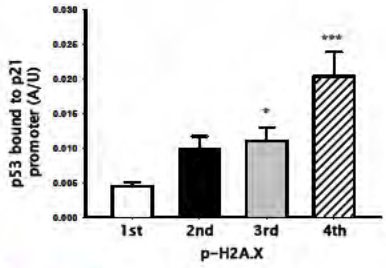
Telomerase activity across telomere length



Shorter telomeres are associated with augmented senescence signaling in human arteries



Uncapped telomeres are associated with augmented senescence signaling in human



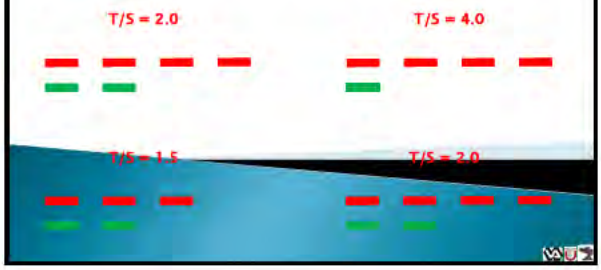
Conclusions

- Telomere length decreases with advancing age similar to rates in white blood cells
- Despite age related reductions in telomere leng h, telomerase activity is unchanged.
- Telomere uncapping does not occur with advancing age in human arteries
  - Telomere shortening with age may be insufficient to reach a critical leng h required for uncapping in human arteries
  - Greater TRF2 binding to telomeres with age may be a compensatory mechanism to prevent telomere uncapping wi h age-associated telomere shortening in human arteries
- Senescence signaling is elevated with advancing age in human arteries. This is *not completely explained* by telomere uncapping in human arteries
- Arterial Inflamma ion is associated with the altered telomere dynamics and senescence phenotype seen in old human arteries
- Consistent with cell culture data short telomeres are related to uncapping and senescent signaling in human arteries

Thank You!

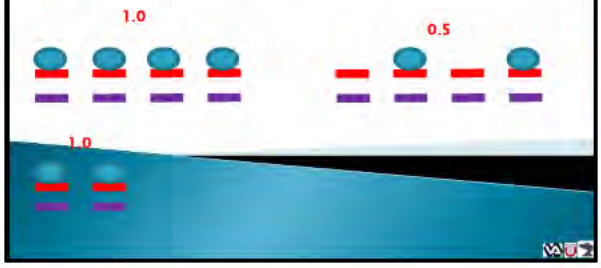
Telomere length normalization

T/S ratio: **Telomere signal**/**Albumin signal (SC)** from multiplex PCR reaction
*Albumin signal (SC) : copies of genome in sample, which corresponds to number of cells
**Thus, higher T/S ratio means more telomere per cell, or longer average telomere length



ChIP normalization

Ratio: **ChIP signal**/**Input signal (unbound fraction)** from separate PCR reactions
*Input signal: copies of ChIP target in sample, which corresponds to number of cells in ChIP
**Thus, higher ratio means more ChIP target bound by target protein per cell, rather than more material use in ChIP



Specific aims

- To document that age-related differences in EDD are associated with increased expression of the pro-inflammatory cytokines, TNF∝ and IL-6, and the senescence and apoptosis markers, p21, p16, BAX and activated Caspase-3, in human arterial tissue.
- To determine if age-related differences in arterial telomere structure, defined as differences in mean telomere length and telomeric localization of the double strand break marker; p-H2A.X, TRF2, and telomerase, are related to differences in arterial function (EDD) in human arteries with advancing age.

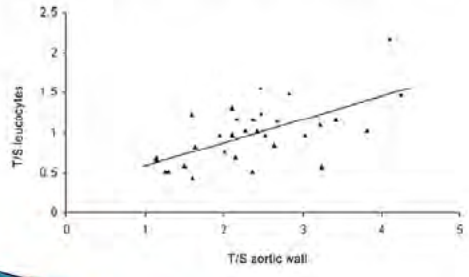
Additionally…

- Telomerase activity is not related to telomere length in human arteries
- Telomere uncapping is inversely related to telomere length in human arteries
- TRF2 binding to telomeres is inversely related to telomere length in human arteries
- Senescence signaling is inversely related to telomere length and directly related to telomere uncapping in human arteries

Innovation

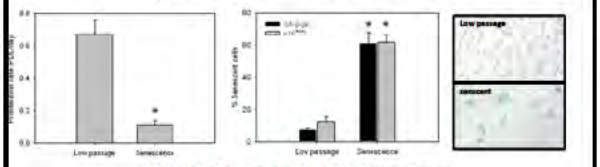
- Telomere dysfunction mediated cellular senescence has not been assessed with advancing age in human arteries.
- To date, technologies that demonstrate protein interactions with telomeric DNA have not been employed in vascular tissue.
- Develop biomarkers for vascular function that help clinicians better design and care for older individuals likely reducing their risk of developing CVD.
- Furthermore, the proposed studies will lead to future studies aimed at attenuating, preventing, or reversing "vascular aging".

Telomere length in leukocytes and telomere length in arterial tissue correlation



Wilson et al., 2008

Cellular Aging: Does it contribute to the vascular phenotype in older adults?



SA (beta) gal positive vascular cells in human atheroma

CA

Minamino, T. et al. Circ Res 2007;100:15 28

Conclusions thus far…

- Telomere shortening occurs with advancing age in human arteries
- telomerase activity with advancing age in human arteries
- No changes in telomere uncapping with advancing age in human arteries
- Greater TRF2 binding to telomeres with advancing age in human arteries
- Greater senescence signaling with advancing age in human arteries