Role of telomere dynamics in atherosclerosis and arterial aging

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No conflict of interests on this thematic
Active enzyme telomerase can replace the telomere DNA lost during each cell division thus preventing cell senescence.

Greider and Blackburn, 1985, 1987, 1989
TELOMERE

Telomere length

Number of cell divisions

Germinal cells

Stem cells

Somatic cells

Replicative senescence
THE CLINICAL SIGNIFICANCE OF
TELOMERE LENGTH

Is telomere length related
to the pace of aging
Accelerated arterial aging in subjects with shorter telomeres

- **High pulse pressure**: Jeancllos E, *Hypertension* 2000
- **Aortic Stiffness**: Benetos A, *Hypertension* 2001
- **Coronary atheroma**: Samani NJ, *Lancet* 2001
- **Endothelial Dysfunction**: Minamino T, *Circulation* 2002
- **Carotid plaques**: Benetos A, *Hypertension* 2004
- **Diabetes + Albuminuria**: Tentolouris N, *Diabetes Care* 2007
- **Heart Failure**: van der Harst P, *J Am Coll Cardiol* 2007
Shorter leucocyte telomeres length (LTL) in CHD patients
A systematic review and meta-analysis.

- 24 studies, 43,725 participants, 8400 patients with CVD (5566 with CHD and 2834 with cerebrovascular disease).
- Shortest versus longest third of LTL; RR for CHD: 1.54 (1.30 -1.83).
- Same when adjusted for conventional vascular RF: 1.42 (1.17 -1.73)

«...inverse association between LTL and risk of CHD independent of conventional vascular risk factors...»

Haycock PC et al. *BMJ.* 2014;349:g4227.
Telomere length regulation in the different periods of life

1. TL at birth: 8-12 Kb

2a. Childhood Attrition: 1.5-2 kb in the first 10 years

2b. Adult life attrition: 0.25 kb every 10 years

3. 60 y.o.: TL = 6.5 Kb

3 = 1 - (2a + 2b)
LTL and insulin resistance in the Danish Twin Registry

- Shorter LTL at baseline was associated with an aggravation in insulin resistance during the follow-up.
- The presence of insulin resistance at baseline was not associated with higher LTL attrition during the 12-year follow-up.

Simon Verhulst et al, Diabetologia 2016

LTL and carotid atherosclerosis in the French ERA study

- Shorter LTL was at baseline was associated with early development of carotid plaques
- The presence of carotid plaques at baseline was not associated with higher LTL attrition during the 9.5-year follow-up.

Simon Toupance et al, Hypertension 2017
Telomere length regulation in the different phases of life
How to explain the shorter telomeres in patients with cardio-metabolic diseases?

1. TL at birth: 8-12 Kb

2a. Childhood Attrition: 1.5-2 kb in the first 10 years

2b. Adult life attrition: 0.25 kb/ every 10 year

3. 60 y.o.: LTL= 6.5 Kb
In subjects with atherosclerotic CV disease the shorter LTL are related to early life attrition but not to shorter telomeres at birth.
Telomere length regulation in the different phases of life
How to explain the shorter telomeres in patients with cardio-metabolic diseases?

1. TL at birth:
   8-12 Kb

2a. Childhood Attrition:
   1.5-2.0 kb in the first 10 years

2b. Adult life attrition:
   0.25 kb/ every 10 years

3. 60 y.o.:
   LTL = 6.5 Kb
AORTIC VALVE STENOSIS AND TELOMERES
SIMILARITIES BETWEEN
ATHEROSCLEROSIS AND AORTIC VALVE STENOSIS

Aging
Risk factors
Lipid accumul.
Inflammatory cells infiltration
Oxidative stress
Cell death
Calcification

ATHEROSCLEROSIS

AORTIC VALVE STENOSIS
TELOMERE LENGTH IN AORTIC VALVES
EXPERIMENTAL DESIGN (IN VIVO AND IN VITRO STUDIES)

Patients undergoing aortic valve replacement surgery →

Aortic valve
Blood sample
Clinical data

Macrosopic dissection
Healthy Intermediate Calcified

Telomere restriction fragments (TRF) analysis

Statistic analysis

Transcriptomic analysis
Micro RNA analysis
Pr. Magnus Bäck
(Karolinska Institute)

DNA extraction from valve samples
Southern blot
Data analysis

Ilona Saraieva, Simon Toupance, Magnus Back, Athanase Benetos
TELOMERE LENGTH IN AORTIC VALVES CALCIFIED VS. NON-CALCIFIED SEGMENTS (DELTA 0.530 KB)

Non Calcified vs Calcified Aortic Valves (n=9)

p < 0.007

Ilona Saraieva, PhD thesis
THE TELOMERE LENGTH IN CALCIFIED SEGMENTS OF AORTIC VALVES IS SHORTER THAN IN THE HEALTHY SEGMENTS
SHORTER TELOMERE LENGTH IN CALCIFIED AORTIC VALVES

1. Do calcifications lead to shorter telomeres?
2. Do shorter telomeres increase the risk for calcifications?

Answering in *in vitro* studies
Question 1: Do calcifications lead to shorter telomeres?
Response 1: Measuring TL in VICs after induction of calcifications

Ilona Saraieva, PhD thesis
Question 2: Do shorter telomeres increase the risk of calcifications?

Response 2: Create cells with longer (senolytic) and shorter (H2O2) TL

- VICs +H2O2 → VICs
  - VICs +senolytic → VICs
  - VICs +H2O2 → VICs

- High Pi medium
  - VICs, shorter Telomeres
  - VICs, basic Telomeres
  - VICs, longer Telomeres

- Standard medium
  - VICs
  - VICs

Compare level of calcification, keep cells for possible mRNA and protein extraction.

Ilona Saraieva, PhD thesis
Aim of our studies:
Elucidate the mechanisms responsible for the relationship between short telomere and cardiovascular aging in order to propose preventive actions

Warfarin

Alizarin red staining

CTL
16uM
1uM
4uM

SAB-galactoside staining

Warfarin do not influence on hVICS calcification
Phosphate

Alizarin red staining

SAB-galactoside staining

It is possible to use different concentrations of Pi to obtain different levels of calcification in hVICS (1.5µM vs 2.8µM, higher concentration might have influence on viability).
There is no difference in amount of SAB-galactoside positive hVICs treated with different concentration of H2O2.

Next step: treat cells with H2O2 and measure telomeric length

Viability of cells treated with H2O2, p4

Viability of cells treated with H2O2, p5
There is no difference in amount of SAB-galactoside positive hVICs treated with different concentrations of dasatinib. Next step: treat cells with dasatinib (160μM) and measure telomeric length.

Viability of cells treated with dasatinib, p4

Viability of cells treated with dasatinib, p5
Telomerase activity in VICs cells

Telomerase activity in:
1. VICs, p4
2. VICs, p5
3. VICs, p5 (calcified cells)
4. VICs, p6
5. Hella cells (CTL+)
6. Lysis buffer (CTL-)
Telomerase Activation in Atherosclerosis and Induction of Telomerase Reverse Transcriptase Expression by Inflammatory Stimuli in Macrophages

Florence Gizard1,*, Elizabeth B. Heywood1,*, Hannes M. Findeisen1, Yue Zhao1, Karrie L. Jones1, Cèline Cudejko2,3,4,5, Ginell R. Post6, Bart Staels2,3,4,5, and Dennis Bruemmer1,†


**TERT is expressed in macrophages of human coronary artery atherosclerotic lesions.** Representative immunofluorescence images (objective magnification ×100) of an atherosclerotic lesion from a human coronary artery showing macrophages stained for CD68 (red, left upper panel) and TERT (green, upper right panel). Sections were counterstained with DAPI to visualize nuclei (blue, lower left panel) and merged to show the composite staining (lower right panel). Note the colocalization of CD68 and TERT immunoreactivity (lower right panel, yellow staining)

**Telomerase is activated in LDL-receptor-deficient mice fed an atherogenic diet.** Male LDL-receptor−/− mice were fed a standard laboratory diet or an atherogenic Western diet (n=8/group). After 12 weeks, telomerase activity was analyzed in aortic tissues using the telomeric repeat amplification protocol assay and quantified by ELISA (left panel). Characteristic 6-bp telomerase-specific laddering determined by Southern blotting from two representative mice per group is depicted on the right panel.
Telomerase activity in valves

Telomerase activity in:
1. BAV, H (2832)
2. BAV, I (2832)
3. BAV, C (2832)
4. BAV, C2 (2832)
5. BAV, C3 (2832)
6. BAV, I (2806)
7. BAV, C (2806)
8. Hella cells (CTL+)
9. Lysis buffer (CTL-)