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Age-related arterial telomere uncapping and senescence is greater in women compared with men

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ABSTRACT

Telomere uncapping increases with advancing age in human arteries and this telomere uncapping is associated with increased markers of senescence, independent of mean telomere length. However, whether there are sex specific differences in arterial telomere uncapping is unknown. We found that telomere uncapping (serine 139 phosphorylated histone γ-H2A.X in telomeres) in arteries was ~2.5 fold greater in post-menopausal women ($n = 17$, 63 ± 2 years) compared with pre-menopausal women ($n = 11$, 30 ± 2 years, $p = 0.02$), while there was only a trend towards greater telomere uncapping in older men ($n = 26$, 66 ± 2 years) compared with young men ($n = 11$, 31 ± 2, $p = 0.11$). Senescence markers, p53 bound to the *p21* gene promoter and *p21* gene expression, were 3–4 fold greater in post-menopausal compared with pre-menopausal women ($p = 0.01$–0.02), but only 1.5–2 fold greater in older compared with young men ($p = 0.02$–0.08). Blood glucose was related to telomere uncapping in women, while systolic blood pressure, pulse pressure and serum creatinine were related to telomere uncapping in men. Mean arterial telomere length decreased similarly in women and men with age ($p < 0.01$). Thus, the age-related increase in arterial telomere uncapping and senescence is greater in women than men, despite similar age-related reductions in mean telomere length in both sexes.

1. Introduction

In recent years, the relation between altered telomere function and cardiovascular disease (CVD) risk has grown to be appreciated. Although epidemiology and physiology studies typically focus on mean telomere length (Brouilette et al., 2003; Samani et al., 2001; Willeit et al., 2010), we recently demonstrated in arteries that other features of telomere biology (i.e., the uncapping of telomere ends) are better related to markers of cellular dysfunction (Morgan et al., 2013). Telomere ends form a loop structure, known as a cap, which prevents the chromosome ends from being recognized as double-stranded DNA breaks and initiating a DNA damage response (Griffith et al., 1999; Stansel et al., 2001; Takai et al., 2003). In human arteries, telomere uncapping from loss of the loop structure increases with advancing age and is associated with hypertension (Morgan et al., 2013, 2014). In arteries, greater telomere uncapping, independent of mean telomere length, is associated with increased markers of cellular senescence and inflammation (Morgan et al., 2013). However, whether there are sex specific differences in the age-related changes in telomere biology is unknown.

Increasing age is a major risk factor for CVD in both women and men, but for women, there is a greater incidence of CVD post-menopause compared to pre-menopause independent of traditional cardiovascular risk factors (Kannel et al., 1976). Previous studies of sex differences related to telomeres have typically focused on only telomere length in circulating immune cells, with most studies indicating that women have longer telomeres than men in leukocytes (Bekaert et al., 2007; Fitzpatrick et al., 2011; Gardner et al., 2014; Willeit et al., 2010). In addition, later onset of menopause and longer exposure to circulating estrogen is associated with greater leukocyte telomere length in women (Gray et al., 2014; Lin et al., 2011). Thus, not only is it unknown if telomere uncapping or mean telomere length are different in the arteries of women and men, but the role of menopause in women has yet to be studied.

Abbreviations: BMI, Body mass index; BUN, blood urea nitrogen; ChIP, Chromatin immunoprecipitation; CVD, cardiovascular disease; hTERT, telomerase; p21, cyclin-dependent kinase inhibitor 1A; p53, tumor suppressor p53; p-H2A.X, phosphorylation of histone γ-H2A.X at serine 139.

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The major consequence of telomere uncapping is the initiation of a DNA damage response and subsequent senescence signaling by the p53/p21 pathway (d'Adda di Fagagna et al., 2003; Takai et al., 2003). In human arteries, advancing age is associated with greater p53 bound to the *p21* gene promoter as well as greater *p21* gene expression (Morgan et al., 2013). Importantly, cellular senescence is associated with an aging phenotype (Baker et al., 2011; Herbig et al., 2006) and senescent cells in arteries have been associated with CVD (Matthews et al., 2006). However, it is currently unknown if markers of p53/p21 mediated senescence differ with advancing age in arteries from women and men.

Consequently, we sought to determine if telomere uncapping in resistance arteries, indicated by phosphorylation of histone γ-H2A.X at serine 139 (p-H2A.X) in telomeric chromatin, was different in women and men as well as between pre- and post-menopausal women. We also sought to determine if any elevations in telomere uncapping occurred concomitantly with greater activation of the p53/p21 senescence pathway. In addition, we examined if mean arterial telomere length and activity of telomerase differed between women and men with advancing age. Finally, we sought to determine if the clinical characteristics associated with telomere uncapping or shortening were different between women and men.

2. Materials and methods

2.1. Subjects

A total of 65 subjects undergoing a prophylactic melanoma-associated sentinel lymph node biopsy were enrolled in this study. Patients with a prior diagnosis of metastatic melanoma, prior chemotherapy treatment, and/or indication of melanoma metastasis (blood lactate dehydrogenase >618 U/L or positive sentinel lymph node biopsy) were excluded. To omit the peri-menopausal time period, subjects between the ages of 40 and 55 years were excluded. We enrolled 11 pre-menopausal women and 11 young men 20–39 years of age, and 17 post-menopausal women and 26 older men 56–85 years of age. The Institutional Review Boards of the University of Utah and the Salt Lake City Veteran's Affairs Medical Center approved all protocols, and written informed consent was obtained from all subjects prior to biopsy surgery.

2.2. Subject characteristics and arterial collection

Medical history and prescription medication use were obtained from medical records. Blood pressure, anthropometry and blood chemistry analysis were performed utilizing standard procedures. Arteries were collected during sentinel lymph node biopsy surgery performed at the Huntsman Cancer Hospital, University of Utah. Skeletal muscle feed arteries were excised from the inguinal (e.g., hip adductors or quadriceps femoris) or axillary (e.g. serratus anterior or latissimus dorsi) regions and were free of melanoma cells (Ives et al., 2013). Arteries were identified as skeletal muscle feed arteries by entry into the muscle bed, gross anatomy, coloration, and pulsatile bleed pattern (Ives et al., 2013). Arteries were cleaned of adipose and connective tissue, and washed to remove residual blood cells. The average size of each artery was approximately 2 mm in length, 0.5 mm in luminal diameter, and weighed 10–20 mg. Cleaned arteries were then snap frozen in liquid nitrogen and stored at −80 °C prior to performing the following assessments. All samples were assayed in triplicate, and replicate means were used for analysis.

2.3. Telomere uncapping

Chromatin immunoprecipitation (ChIP) was used to determine the amount of p-H2A.X (Santa Cruz Biotechnology, Inc.) localized to telomeres. ChIPs were performed as previously described (Morgan et al., 2013), and analyzed via qPCR for telomere content as described by Cawthon (Cawthon, 2009). Final values were expressed as the ratio of background corrected starting quantity (SQ) of telomeric DNA enriched by ChIP to telomeric DNA SQ in INPUT fraction. INPUTs represented 50% of telomeric DNA present in corresponding ChIP and were used to control for tissue concentration in samples with the calculation: (γ-H2 SQ − background SQ)/INPUT SQ = final value.

2.4. p53/p21-Induced senescence

ChIPs were performed to assess p53 bound to *p21* gene promoter (EMD Millipore Corporation) as previously described (Morgan et al., 2013), using a sequence-independent qPCR assay with FastStart SYBR Green Master Mix (Roche Diagnostics Corporation, Roche Applied Science). Additionally, *p21* mRNA expression was determined by qRT-PCR using the Quantitect Reverse Transcription kit (Qiagen, Inc.) and FastStart SYBR Green Master Mix (Roche Diagnostics Corporation, Roche Applied Science) according to the manufacturer's protocols. Final mRNA SQs were generated by standard curve and expressed as a ratio of target mRNA SQ to *18s* rRNA SQ (*18s* rRNA QuantiTect Primer Assay: Qiagen, Inc.). *18s* rRNA was used as a housekeeping gene transcript to control for tissue concentration in samples. *p21* mRNA primers: fwd-gacctgtcactgtcttgta, rev-cctcttggagaagatcagccg.

2.5. Mean telomere length

A sequence-independent multiplex qPCR technique using a SYBR Green master mix with 0.625 U AmpliTaq Gold 360 DNA polymerase (Life Technologies Corporation) was utilized to determine mean telomere length as described by Cawthon (Cawthon, 2009). Telomeric DNA (T) SQs and albumin SQs, used as single copy gene (S) to control tissue concentration in samples, were generated by standard curve and relative mean telomere length was expressed as the T/S ratio.

2.6. Active telomerase (hTERT)

ChIPs were performed to assess hTERT (Abcam) bound to telomeres as described above (Morgan et al., 2013, 2014).

2.7. Data analysis

Statistical analyses were performed with IBM SPSS (version 20, Armonk, NY). A 2 × 2 (age × sex) ANOVA was used to assess group differences and in cases of a significant F, post-hoc analyses were performed with a Bonferroni correction for pre-planned comparisons of age groups between each sex and sex groups within each age group. Pearson correlation analysis was used to assess bivariate relations of interest. In addition, partial correlations were used to assess the influence of subject characteristics on the relation between telomere length/uncapping and age. Variables that were not normally distributed (Shapiro–Wilk test) were log transformed. Values for telomere uncapping, senescence markers, telomere length and hTERT were normalized to the mean for the pre-menopausal women. Significance was set at $p < 0.05$. Values are presented as mean ± SEM.

3. Results

3.1. Subject characteristics

Pre-menopausal women and young men were not different in terms of any clinical characteristics except for serum creatinine and none of the subjects in these groups had a history of cardiovascular diseases or were taking cardiovascular-related medications. Likewise, post-menopausal women and older men were not different in terms of any clinical characteristics except for serum creatinine. Body mass index (BMI) and blood urea nitrogen (BUN) were greater with advancing