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Review

Acute coronary syndrome: Role of the telomere dynamic

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Telomeres, or historically named "terminal genes" are first discovered by Muller working on fruit fly in 1930s. Since then, the great progress was made in understanding the consequences of telomere erosion on the human health and disease states, as age related vascular diseases. The overlapping links between telomere dynamics in endothelial cells, endothelial progenitor cells and other cells laid in the vessel wall made telomere regulation as a hub node in keeping vascular health. Shortest telomeres paralleled with the advent of coronary artery diseases and its related risk factors, is well explainable by "Telomere hypothesis". But this needs further studies to determine the real state of short telomere length in atherosclerosis: marker or risk factor? As a marker, leukocyte telomere length was used in mirroring mean telomere length of the whole organism, but there are some debates about the best cell reflecting the exact telomere length. Impact of telomere biology on vascular system in the progression and development of atherosclerosis delights its targeting as a new therapeutic option for cardiovascular diseases. This needs in depth elucidation of the underlying mechanisms of telomere erosion in vascular beds.

Key words: Telomere length, endothelial cell dysfunction, atherosclerosis.

FULL TEXT

Telomeres, or historically named "terminal genes", are highly specialized structures at the end of chromosomes, which were first discovered by Muller working on the fruit fly in 1930s (Bailey and Goodwin, 2004). The role of these protective caps became more evident with the raised hypothesis of "problem of the terminal replication" by James (1972) and Olovnikov (1973) (Verdun and Karlseder, 2007) (Olovnikov, 2003). These repeated TTAGGG tracts make a D-loop-T-loop by folding 3 terminal overhang, which resembles a shelter for the susceptible ends (Rahman et al., 2008). This hanging end is structurally constructed by multiple adjacent quadruplex produced by intra-telomeric G base pairing (Oganesian, 2007). The presence of the 3 overhang is not helping without the cap. Uncapped 3 overhang acts as an invasive end for recombination, in a cell-cycle-specific period, as shown in yeasts (DuBois, 2002). Establishing a capped telomere, different proteins should participate. Shelterin is a complex built with six proteins: TRF1, TRF2, TIN2, Rap1, TPP1 and POT1 (de Lange, 2005). This complex makes the DNA repair system to ignore telomere as a double stranded DNA break point (Blackburn, 1991) (Greider, 1996). This magic structure needs a facilitator in keeping both length and function. $(TTAGGG)_n$ bricks are added to the telomeres through a ratcheting mechanism, repetitive dissociation from the newly synthesized telomeres, realigning and adding another six nucleotides at a time, via the action of telomerase (Artandi, 2006). Telomerase, a ribonucleo-protein composed of catalytic subunit (TERT), its own

Abbreviations: ATM, Ataxia telangiectasia mutated; **ADP,** adenosine diphosphate; **PARPS,** poly(ADP-ribosyl)polymerases; **vWF,** von Willebrand factor; **TERT,** Telomerase Reverse Transcriptase; **TEP,** telomerase associated protein; **EPCS,** endothelial progenitor cells; **EVA,** early vascular aging; **TMM,** telomere maintenance mechanisms; **SSDB,** single-stranded DNA breaks; **eNOS,** endothelial nitric oxide synthase; **RNP,** ribonucleoprotein; **HR,** homologues recombination; **NHEJR,** non-homologues end joining replication; **ALT,** alternative lengthening of telomere; **LTL,** Leukocyte telomere length; **STELA,** single telomere length analysis.

RNA template (TERC) and telomerase associated protein (TEP) which interacts dynamically with other components of telomere complex (Prathapam et al., 2005).

Unfortunately, telomeres are unstable structures. Any failure in these highly ordinate process, results in telomere deregulation: pace of aging and age related diseases; as atherosclerosis. Atherosclerosis, nation's number one killer, is mainly considered to be an age-related vascular disease (Burton et al., 2009). Recently paid attention "telomere hypothesis", running besides the previous concept of "response to injury", reminds atherosclerosis as a "telomere related disease" (Minamino and Komuro, 2006; Zhang et al., 2008). Telomeres, in their very ends, are cutting edges for the beginning of the disease chain. These critically short telomeres; which seen in the forced regions susceptible to atherosclerosis; promote chromosomal fusion implicated in the evolution of complex karyotypes, by the presence of Anaphase bridge, telomere associations and diccentric chromosomes (Wen et al., 2006). Telomere-driven cytogenetic evolutions can also evolve in TP-53 competent cells, which initiate the formation of cytogenetic aberrations (Wen et al., 2006).

Terminal ends of the cells, skilled in communication, tune the vascular behavior. Unwind vascular Olovnikov's clocks lead to cellular senescence and apoptosis, via activation of P16 (INK4a) signaling pathway: main gatekeeper of telomere-driven chromosomal abnormalities (Jacobs and de Lange, 2004) (Wen et al., 2006). Subsequently, vascular wall players, endothelial and smooth muscle cells, fall in replicative senescence: permanent exit from cell cycle, which is associated with disturbed cellular homeostasis (Hayashi et al., 2008). Senescent endothelial cell loses power to keep its function in its environment. It loses its attachment to its neighbors and this endothelial cell depletion makes a pro-coagulable state by exposing the thrombogenic sub-endothelial surface (Williamson, 2007). Detached endothelial cell makes a fenestra within the vessel wall, as a wound.

Risk factors for atherosclerosis accelerate telomere shortening in endothelial cells that are continuously exposed to stressors. But the impact of the ageing and local and systemic stressors on systemic telomere length is a virtue, which affects all players of athermanous plaque, besides endothelial cells, as immune and regenerative stem cells. Telomere and telomerase biology are some what different in various cells in the process of atherosclerosis.

In contrast to endothelial cells, telomerase activation and telomere stabilization occur in smooth muscle cells and T cell lymphocytes in the early stages of atherosclerosis (Minamino et al., 2001; Liu et al., 2005). Activated telomerase in smooth muscle cells, mainly attributed to the increased TERT phosphorylation induced by hypoxia and nuclear localization, afford them with increased life span and proliferative capacity (Minamino et al., 2001). This is in accordance with the "monoclonal origin of atherosclerosis" which is enhanced replication of monoclonal smooth muscle cells (Murry, 1997). This may be a defense mechanism in order to contract the wound in the vessel wall, but is not a permanent event. And finally, smooth muscle cells reach their critical telomere length which enters them to the senescent phase, due to the repeated cell divisions and atherogenic micro-environment. Enhanced senescence and apoptosis in the plaque's fibrous cap, smooth muscle cells and fibroblasts, make the lipid core unopposed as seen in the plaques vulnerable to rupture (Matthews et al., 2006). Further telomere shortening in endothelial cells in atherosclerotic plaques result in endothelial cell sloughing. The weakly protected plaque undergoes rupture with a little force as disrupted shear stress (Fukumoto et al., 2008). Telomere dysfunction in immune and inflammatory cells as monocytes/ macrophages impairs their functions (Damjanovic et al., 2007). These functionally impaired cells, as seen in lipid-laden macrophages, are unable to engulf lipid droplet thoroughly accumulated in the vessel wall and thus triggers lesional formation and progression (Schrijvers et al., 2007). Mentioned telomerase activation and subsequent enhanced replication of T cell lymphocytes seems to be due to the stimulatory effect of the exposed atherogenic antigens. But in the same direction with smooth muscle cells, in the late stages of atherosclerosis, increased apoptosis of T cell lymphocytes occurs. Overalls, at the late stages of atherosclerosis, the percentage of apoptotic cells is in its peak and telomerase activity in its nadir (Liu et al., 2005). These dynamic alterations in telomere length and cellular viability make a stable plaque vulnerable to rupture. In this setting, a brief viral or bacterial infection, mainly flu virus, Herpes virus, Cytomegalovirus, Epstein-Barr virus, Chlamydia Trachomatis, Chlamydia and Mycoplasma pneumonia worsen the subtle vascular disease by exacerbation of inflammatory load on the previously damaged endothelial cells (Muhlestein and Anderson, 2003). Therefore, the presence of a supportive "telomere cap" means a supportive "plaque cap", which determines the plaque fate.

Endothelial progenitor cells (EPCs), bone marrow-derived stem cells long favored as Rosetta stone in angiogenesis, are recruited to the intima in response to the signals derived from denuded intima layer and detached endothelial cells (Song, 2009). Senescent EPCs, impaired functionally and numerically, are less potentiated to reach the damaged vessel wall, which leads to non-healing intimal wound and progression of atherosclerotic plaques inward the lumen. But this is not the whole. In the middle stages of atherosclerosis, fibrofatty stage, fibrocytes, bone-marrow derived mescenchymal stem cells migrate to the adventitia after maturation via the interaction between chemokine (CC motif) receptor 7(CCR7) and its ligands in secondary lymphoid organs: Chemokine (C-C motif) ligand 19 and 21(Bellini, 2007) (Banas, 2002). CCR7-expressing fibrocytes migrated to

adventitia secret transforming growth factor- β and matrix metalloproteinase 9; two key elements in adventitia originated angiogenesis and lead to adventitia thickness (Metz CN. 2003) (Hartlapp, 2001). Fibrocytes differentiate into fibroblasts and myofibroblasts in media and trans-migrate toward intima and exert as protective cap for the plaque lipid core (Brigitte, 2007; Medbury, 2008). Fibrocyte senescence leads to adventitia shrinkage which indirectly results in further lumen narrowing due to the nature of negative remodeling (Deiner, 2007). Since fibrocytes actively participate in the formation of fibrous plaque, senescent fibrocytes lead to uncapped plaque which promotes inflammation and lumen narrowing: dynamic movement toward total vessel obstruction and untoward coronary event (Medbury, 2008).

By mentioned links, the relation between telomere shortening and recently paid attention subject of early vascular aging (EVA) syndrome are explainable. But the underlying mechanisms of accelerated telomere erosion proceeding endothelial cell dysfunction are more complex. The bottle neck of telomere loss is endothelial cell failure to keep telomere maintenance mechanisms (TMM). A brief review of the telomere structure and its requirements will shed lights to the paths lead to telomere dysfunction.

The average telomere length, like the average height, is heritable genetically, which highlights again the impact of genetic reservoir on the prevalence of cardiovascular diseases (Salpea, 2008). So, offsprings of parents with longer average telomere length and older fathers have longer terminal ends (Manestar-Blazic, 2004; Unryn, 200). Perhaps, it is longer telomere length in females which protect them against cardiovascular diseases (Terry, 2008). The association between short telomeres length and predisposition to atherosclerosis, as determined by carotid artery intimal medial thickness was demonstrated in the Framingham Offspring Study (FOS) (O'Donnell, 2008). As shown in Framingham Heart Study (FHS), shorter telomeres were seen with higher ratio of rennin/ angiotensin (Vasan, 2008). Critically length telomeres showed an inverse correlation with pulse pressure, biologic marker of vascular aging and predictor of increased mortality rate, in men (Benetos, 2001). This relation was inconsistent in females (Benetos, 2001). Effects of telomere length on the future cardiovascular risks have been determined in the West of Scotland Primary Prevention Study: a fate determining length (Brouilette, 2007).

Oxidative stress has direct effect on telomere length (Kurz et al., 2004). This effect has been determined through the formation of oxidatively modified Guanine bases, 8 -OxodG, in telomeric versus non-telomeric DNA (Kawanishi and Oikawa, 2004). The presence of these unpaired bases, interfering with replication fork, makes single-stranded DNA breaks (SSDB), but telomeric DNA in contrast to genomic is unable to repair the breaks (Dobbs et al., 2008). Decreased telomere length in the setting of excessive psychological stress, bipolar mood disorders and schizophrenia is attributed to the great burden of oxidative burst, mainly hydroxyl radicals (Fuster and Andres, 2006; Simon et al., 2006; Yu et al., 2008; Stephanson, 2003). Chronic oxidative stress is also an explanation for decreased telomere length in situations with increased risk of atherosclerosis as smoking, homosysteinemia, autoimmune disease, inflammatory bowel disease and chronic obstructive pulmonary disease (Morla, 2006; Richards et al., 2008; Gupta and Gollapudi, 2006; Risques et al., 2008). Alternatively, strategies to decrease oxidative stress (e.g. by over-expression of anti-oxidant enzymes or consumption of anti-oxidant agents as statins and N-acetyl systein) were associated with telomere rejuvenation (Mahmoudi et al., 2008; Voghel et al., 2008). Despite of major cause of decreased telomere length in atherogenic environment, the increased inflammation due to increased Leptin level and positive energy balance lead to telomere exhaustion in obess cases (Das et al., 2009; Lionetti et al., 2009). These factors, in addition to insulin resistance explain short telomeres in Diabetes and metabolic syndrome (Adaikalakoteswari et al., 2005).

Short telomeres may be partly due to the reduced telomerase activity, but interestingly the same pathologies affect both of them, as hypertension, oxidative stresses and increased C- reactive protein levels (Perez-Rivero et al., 2006; Satoh et al., 2008; Fujii et al., 2006). The impressive effect of atherogenic-hemodynamic on telomere length maintenance is recognizable by considering the shorter telomere length of the common iliac arteries with great burden of shear stress compared to internal thoracic artery (Chang and Harley, 1995). The cause of telomerase inactivation is not thoroughly understood in some settings. However, decreased telomerase activity is associated with senescent phenotype endothelial cells which means reduced NO production, impaired endothelial nitric oxide synthase (eNOS) activity and disturbed vascular integrity (Matsushita et al., 2001; Kurz, 2003). Mutually emerging evidences suggest that increased eNOS activity or NO bioavailability activate telomerase, with consequent transformation from pro-atherogenic to anti-atherogenic phenotype (Farsetti, 2009). Not only endothelial cell, but also EPCs are affected by impaired telomerase activity as shown by decreased population of functional EPCs with increased apoptosis rate among them (Fujii et al., 2006).

The overrate role of telomerase in telomere elongation caused overlooking its other probable roles. Recently, telomerase have been found to activate stem cells through mechanism which does not require its telomere-lengthening functions. Certainly, telomerase have more potentials than base-addition which are not yet understood. In this state, we just can expect that telomerase impairment plays great role, beside telomere attrition, in the development and progression of atherosclerosis.

Anyway, telomerase, driving factor in telomere maintenance, like other proteins, is regulated in different steps, as transcription, mRNA processing, translation, post-translational modifications, telomerase localization and access to telomeres. Down regulated telomerase expression in endothelial cells and plaque smooth muscle cells, forces them into crisis (Cao et al., 2002). Alternatively, telomerase expression alone rescued plaque smooth muscle cell senescence despite short telomeres, which means plaque rescue from rupture (Matthews et al., 2006). Telomerase expression is controlled via the positive and negative regulators, located in the TERT promoter. The combinatorial eNOS/ERalpha complex at the TERT estrogen response element site explains telomerase down regulation in endothelial cells with reduced nitric oxide (NO) environment (Zeng and Xu, 2008). The role of epistatic changes on the TERT promoters as DNA methylation is not yet evident in the process of endothelial cell dysfunction. Telomerase gene, by 15 exon-intron boundaries, possesses 9 isoforms, which the only functional one is full length variant and other deletion (α, β, α+β and γ) and insertion variants (I, II, III and IV) are totally frustrated by getting early terminate codons with no translation (Saeboe-Larssen et al., 2006). The role of alternative splicing in the impairment of rabbit telomerase activity was ruled out, but needs to be determined in human telomerase by further studies (Behjati et al., 2007). TERT- provided active-site residues for magnesium-catalyzed nucleotide addition are situated in the cleft of a structure that resembles a half-open right hand (Lue, 1999; Weilbaecher and Lundblad, 1999). Mutations in its invariant aspartate residues result in reduced telomerase activity (Weilbaecher and Lundblad, 1999). The rate limiting telomerase catalytic subunit, TERT, needs post translational phosphorylation for its proper action, mainly by PI 3 kinase/AKT, which is impaired due to oxidative stresses, decreased NO levels, impaired superoxide dismutase and so on (Ram et al., 2009; Zhou et al., 2009) This golden key pathway makes a hub node regulating telomerase activity, NO bioavailability and EPC differentiation, which seems a therapeutic target for atherosclerosis (Su et al., 2009; Tang et al., 2008). Some parts of pleitropic action of angiotensin converting enzymes are attributable to their "telomere protection" effects due to increased telomerase activity, EPC differentiation and NO production which is demonstrated to be through activation of this pathway; a powerful evidence for the interchanging cellular events leading to atherosclerosis (Xia et al., 2008; Dimmeler et al., 2001). Longer telomere length in females is also attributed to the estrogen induced TERT phosphorylation via activation of PI 3 kinase/AKT pathway (Doshida et al., 2006).

TERT participates with other components of telomerase subunits to make a functional enzyme. Full telomerase assembly is a highly complex process which needs processing of both RNA and protein components. Telomerase associated RNA exert functions beyond just being a template for telomerase core protein, as it is also involved in providing regions necessary for TERT binding and catalytic activity and some motifs important for stability and regulation (Collins, 2008). Recently, it has been demonstrated that TER can limit telomerase in primary cells and in cancer cell lines as well (Collins, 2008). Balanced amounts of TER and TERT may be needed for proper telomerase assembly and function. Possessing 5'-TMG cap and binding site for Sm proteins, remind the same processivity of TER with other RNAs, with small variation (Seto, 1999). This 451 - nucleotide RNA should be distinguished by RNA processing machine to ignore it from processing as mRNA, like addition of poly-Adenosine tail (Collins, 2008). Pseudouridine modifications by Box H/ACA, necessary for 3' end processing and TER accumulation, in addition with the presence of Sm proteins associated with telomerase holoenzyme, indicates a nucleolar stage of telomerase assembly (Romanova et al., 2009; Zappulla and Cech, 2009; Wong and Collins, 2006). The nucleolus involvement in telomerase maturation, cell-cycle and aging control makes plurifunctional nucleolus aging as a potential pace for aging (Lo et al., 2006). Full assembly of telomerase is a dynamic process, in which initial ribonucleoprotein (RNP) assembly of TER induces a TER conformational changes that promotes TERT binding (Collins, 2008). In human cells, this stepwise nucleocytoplasmic shuttling is orchestrated through exportin CRM1p and members of the 14-3-3 protein (Ferrezuelo et al., 2002; Gallardo et al., 2008) (Kimura et al., 2004). Shuttled TER participate in establishment of telomerase complex composed of different proteins with variety of accessory functions as heat shock protein 90 and its co-chaperons. Catalytically active telomerase exist in a HSP90- influenced equilibrium of assembly, disassembly and subunit degradation (Collins, 2008). The importance of highly co-ordinate process of telomerase assembly is evident by the consequent events of DKC1 mutation, seen in Dyskeratosis congenital (DC) (Lin et al., 2002). Impaired nucleolar TER assembly makes proliferative cells to experience lack of functional telomerase, despite of the presence of potential TERT (Mochizuki et al., 2004).

The assembled holoenzyme should leave cytoplasm for its proper action in nucleus. Intranuclear telomerase localization is a highly regulated process, which is dependent on cell cycle stage, transformation and DNA damage (Wong, 2002). Telomerase in and out of the nucleus proceeds through nuclear pores via importing/exporting complex and the small GTPase Ran (Haendeler et al., 2003). The GTP- concentration dependency across nuclear envelopes for directionality of transport is an interesting joint with localization and processivity. In the process of endothelial cell dysfunction, oxidative burden has been demonstrated to deplete human TERT by initiation of nuclear export (Haendeler et al., 2003). Interestingly, TERT import from cytoplasm is induced in T

cells and smooth muscle cells upon stimulation with growth factors and proliferative stimuli (Liu et al., 2001; Minamino and Kourembanas, 2001).

In organisms as *Saccharomyces cerevisiae,* telomerase repeat addition processivity is increased at critically short telomeres, which means that the elongation rate by telomerase depends on the danger signals from telomeres (Chang et al., 2007). The processivity rate of telomerase and the nucleotide addition is more in very short telomeres, but it is not analyzed in human with EVA syndrome. This alarm dependent strategy results in shift from non-extendible to extendible state. Like dNTP dependent fidelity of DNA polymerase, it has been shown that telomerase processivity is dependent to dGTP concentration (Hammond PW). In-depth evaluation of these mentioned regulatory networks in endothelial cells is needed for better understanding of telomerase dysfunction in promotion of endothelial cell dysfunction.

Imported telomerase makes a bipartite interaction with TER and single- stranded DNA by TERT-specific N- and C-terminal extensions from polymerase domains (Lue and Peng, 1998; Collins, 2008). Telomerase first binds to DNA substrate, aligning the DNA primer 3' end with the telomerase RNA template and holding a 5' region for the DNA primer in a separate anchor site (Lue and Peng, 1998). Binding of telomerase to DNA primer, subsequent reverse transcription of short RNA template, deoxynucleotide extension, primer translocation and dissociation will occur (Wyatt et al., 2007). Initiation of telomerase cycle needs binding of telomerase to the DNA primer, which is controlled negatively or positively, by proteins present at the chromosome terminus (Weilbaecher and Lundblad, 1999). Absence of these proteins prohibits telomere addition, due to the folded back 3' primer and buried within this nucleoprotein complex (Weilbaecher and Lundblad, 1999).

Telomeres have some alternative methods rather than telomerase for length maintance. Fruit fly, which we are indebted to them for major progresses made in telomere field, do not use telomerase for cap elongation (Villasante et al., 2007). In some organisms, telomere lengthening is based on the non-enzymatic telomere elongation mechanisms, as homologues recombination (HR) and non-homologues end joining replication (NHEJR) (Celli et al., 2006). This alternative lengthening of telomere (ALT) is most evident in tumors and lower organisms. This sister chromatid exchange, is not yet seen in endothelial cells in the process of atherosclerosis.

Telomeres are not simply added hexanucleotides. Telomeres processing initiated and progressed by Shelterin complex step-by-step. The 5′ end with the universal ATC-5′ sequence is generated under the precise action of POT1 (de Lange, 2005). Invasion of the generated 3′ overhang (100 - 400 nt long) to the adjacent D-loop (Duplex) with variable length remodel telomeres into duplex lariats, termed T-loops (telomeric) (de Lange, 2005; Griffith, 1999; Stansel, 2001). This telomere folding is mediated by TRF2, preferentially localized to the junction between the duplex repeats and the single-stranded overhang (Stansel, 2001). At this T-loop junction, TP53 is also co-localized, to facilitate the efficiency of TRF2-catalyzed t-loop formation (Zhang et al., 2005). Formation of T-loop needs TTAGGG-3' overhang of at least six nucleotides, hence 5' overhangs, blunt ends and non-telomeric termini are deficient in loop formation (Stansel, 2001). Even short telomeres are capable to construct protective capping loops in the excess amounts of TRF2 (Karlseder et al., 2004). But TRF2 over expression prohibits telomeric DNA (tDNA) to repair defects and consequent telomere shortening, without any effect on genomic DNA (Richter et al., 2007). So, the role of TRF2 in telomere maintance is two sides of a coin, with unknown underlying mechanisms. Any way, T-loops, should be untwined to "open state" and bound POT1 on 3' overhang should be un-loaded, in order to make telomeres accessible to telomeres and permit its action. TRF1, facilitator of loop formation, negatively regulate telomere length by tight binding to telomeric sequences (Hanaoka et al., 2005; Muramatsu et al., 2008). TRF1 and its 20 –amino acids internally deleted alternative spliced variant, Pin2, are involved in maintance of mitotic-spindle structure (Zhou et al., 2003). Over expression of Pin2/TRF1 forces cells toward apoptosis after induction of mitosis (Nakamura et al., 2002). Frustrated action of Pin2/TRF1 by phosphorylation via ataxia telangiectasia mutated (ATM) kinase is an explanation for accelerated telomere shortening and mitotic check point defects (Nakamura et al., 2002). The Pin2/TRF1 interaction is a delicate joint between mitotic-spindle maintance and telomere maintance mechanisms (Nakamura et al., 2002). Besides phosphorylation, Pin2/TRF1 function is restricted by ADP ribose polymerization via Tankyrase (TANKS1), member of poly-(ADP-ribose) polymerase (PARP) family (Zhou and Lu, 2001) (Rippmann et al., 2002; Seimiya and Smith, 2002). TANKS1 binds to the acidic domain of TRF1, through its ankyrin repeats and its failure to function, as shown in mice, results in anaphase arrest and makes telomeres inaccessible to telomerase: hidden telomeres (Seimiya and Smith, 2002; Chang et al., 2005). Down stream to the TANKS, F-box4 participates in degradation of Pin2/TRF1, in accordance with an Ubiquitin-Proteasome (Lee et al., 2006).

The role of shelterin dynamic in telomere shaping and function is evident by the studies on the amount of shelterin loading and telomere elongation. The reduced load of POT1 on short telomeres is associated with increased telomerase access (Zaug et al., 2005). Considering the capping function of shelterin on terminal overhangs arise questions about the enough amounts of shelterin for telomere function. It is not determined whether shelterin function on telomeres is length dependent or not.

In the other hand, telomere repair is not just flipping the switch. Repair of the damaged DNA bases is an NAD^+-

and ATP-dependent mechanism (Petermann et al., 2003). PARPs, involved in DNA-base excision repair, are activated by the induced DNA damages and released reactive oxygen species mainly from ischemic tissues (Bertram and Hass, 2008). In contrast to their conventional role in keeping genomic stability, hyperactive PARPs induce further damage and ROS production via promotion of energy crisis. ATP-depletion, one of the fundamental insults in the process of endothelial cell dysfunction, is promoted by activated PARPs to keep genomic stability (Mathews and Berk, 2008). Mutually, PARP activity aggravates inflammatory state of endothelial cells by participation in NF-kappaB transactivation (Benachour et al., 2007). It also can induce caspase-independent apoptosis and high- molecular weight DNA fragmentation by release of apoptosis-induced factor (van Wijk and Hageman, 2005). It is not clear that which percentage of energy is needed for making chromosomal stability, but it is apparent that ATP-depleted endothelial cells are more susceptible to further imbalance of energy stores. Perhaps, the process of DNA repair is incompletely performed or even blunted due to the inappropriate access to energy supplies. But these hypotheses await investigations.

The huge progresses made in understanding the basic concepts of atherosclerosis, seem to be partly due to the application of available models. As an age-related disease, Hutchinson-Gilford progeria syndrome (HGPS) and Wermer disease are suggested as the best candidates for the study of atherosclerosis (Ding and Shen, 2008). Premature aging and age-related diseases in these patients are associated with very short telomeres conflicted all cells sparing T lymphocytes (James, 2000). But *in vitro* studies encountered some problems due to the rapid entrance of endothelial cells to the senescence phase. Human Umbilical Vein Endothelial cells (HUVECs) are cells widely used for *in vitro* studies on atherosclerosis. In further studies need to evaluate telomerase activity, cells should be harvested in their subconfluent phase (Chang et al., 2005). It made some difficulty, especially when measures like NO production and vWF exocytosis needs to be evaluated in confluent endothelial monolayer. So, immortal endothelial cells via SV40 and TERT transfection made alternatives for researchers. TERT transfection into endothelial cells, in order to restore telomerase activity, provided them younger phenotypes which overcame the end-replication problem (Matsushita et al., 2001). These cells stably express surface markers of endothelial cells as CD31, CD34, vWF and eNOS and retain functional characteristic of normal cells as responsiveness to angiogenic growth stimuli, tube formation on matrigels and regulation of NO production and eNOS activation in response to extracellular stimuli (Shen et al., 2007). Studies on the telomere-attrition related endothelial cell dysfunction, with the application of the TERT-transected immortalized cells bypassed the senescent problem. But certainly the impact of interventions performed on the telomerase biology makes some differences with the normal events *in vivo*; an untoward bias in data interpretation.

Among animal models for studying atherosclerosis, pigs and especially knock out mice are used widely. Their great appreciable homology to human made them suitable candidates, but interestingly their telomere biology differs greatly. Both of these animals have telomeres longer than humans and in contrast to human, most of the mice cells have active telomerase (Houben et al., 2008). Extreme telomere length of mice will not shorten with aging and reach critical telomere length just in their 6^{th} generation (Cherif et al., 2003; Blasco et al., 1997). Rat telomeres are relatively long, but in contrast to mice shorten with aging (Cherif et al., 2003). Chickens have been shown to be intermediate: telomere biology like human and telomerase biology like mice (Venkatesan and Price, 1998). Among animals, dogs seem more applicable, both due to their similar telomere/telomerase biology and their availability (Venkatesan and Price, 1998). So, great attention should be paid in animal selection based on the study design and also gender- and tissue- specific variations in telomere length should also be considered. Author suggests the application of Ku86 knock out mice for studying the effects of telomere shortening in the process of atherosclerosis.

Negligible physiological changes in telomere length unrelated to cell division, makes telomere length a major indicator of cellular turn over (Rufer et al., 1999). But the effect of heritable chromosomal specific as well as genetic factors on telomere length arise questions about the superiority of measuring individual chromosomal versus mean telomere length in reflection of telomere kinetic. Among chromosomes, shortest and longest ones are attributed to 13p and female Xp, respectively. Telomeres shorter than median telomere length were observed in 17p while 19p has a telomere length far shorter than 17p (Mayer et al., 2006). Accelerated telomere shortening in inactivated X chromosome, is also interesting (Surralles et al., 1999). So, based on the observed differences in telomere length between individual chromosomes, measurement of mean telomere length seems more applicable. However, some doubts still remain about the application of the average telomere length, but we are not to measure shortest telomere length of cells. Trusting to mean telomere length, determination of the candidate cell for its measurement, is of great value due to the high intra- and inter- individual heterogeneity in intrinsic time table model of telomere biology (Baird, 2005). General beliefs are relied on the applicability of Leukocyte telomere length (LTL) in mirroring average telomere length of endothelial cells and their expansion potential. Off course developmental-stage dependent variations in telomere attrition rate within human leukocytes and the influential effect of chronic inflammation on LTL are not deniable (Frenck, 1998) (Rufer et al., 1999). The observed heterogeneity in

telomere length of these easily accessible cells makes ongoing challenge in finding the best candidate. Lymphocyte telomere length is longer than granulocyte' at birth, due to its stem cell, but telomere shortening is accelerated in lymphocytes rather than granulocytes, in later stages of life (Rufer et al., 1999). In addition, constant telomere attrition rate of lymphocytes in comparison with granulocytes, makes them better reflectors of systemic stimuli (Rufer et al., 1999). But in different diseases state, the candidate cell for telomere length measurement may be variable. It has been demonstrated that in type 2 Diabetes Mellitus monocyte telomere length reached its critical size while lymphocyte telomere length was within its normal length (Sampson et al., 2006). Hence, telomere length is a potential marker for development of cardiovascular diseases; it needs further studies in determination of the candidate cell in different settings with predisposition to atherosclerosis.

Questions about the cause and effect relationship between telomere length and risk of the cardiovascular diseases, made recent challenges about the efficacy of telomere length as predictive marker of coronary artery diseases. Off course, due to high inter-individual variation in telomere length its application as absolute risk marker for cardiovascular diseases needs more attentions. However, telomere length is a potential marker for patient risk stratification. This mitotic clock may alarm physicians for preventive interventions in high risk cases. Hence, the absolute benefits of Pravastatin have been demonstrated to be greatest in patients with shorter telomere length; telomere length may also be applicable as a prognostic marker and a guide for personalized medicine (Brouilette et al., 2007). To find its position as an absolute marker, telomere length follows and longitudinal studies of the same individuals are needed, but tedious western blot method for measurement of telomere length, made it laborious. Emergence of alternative methods as hybridization protection assay, Q-PCR, primed *in situ*, FISH, chromosome oriented-FISH, flow-FISH and single telomere length analysis (STELA) opened new horizons to the famous researches of Leonard Hayflic in 60th (Lin and Yan, 2005; Stindl, 2004). Inspite of these progresses, the challenge between telomere length and age related diseases will be ongoing, since the challenges about aging are yet to be tackled. Magnification of normal ageing process is needed for understanding its drastic effects. But really, our unknown data are more in this regard. As tight building syndrome, we do not know which is first in the process of aging: I) Telomere shortening or cytoplasm contraction and cell size enlargement. II) Are all the age related process occurs simultaneously or step by step, as first telomere shortening? III) Is there an interval between getting the critical telomere length and the consequently havoc events or soon after this threshold the events happens?

By increased prevalence of atherosclerosis and its complication despite the medical and interventional treatments, the emergence of new therapeutic strategies is needed. Inverse correlation of telomere length with plaque grade and numbers of atherosclerosis manifestations marks senescence prevention as a novel therapeutic target for atherosclerosis (Okuda et al., 2000; Wong et al., 2008). Providing a safe harbor for terminal ends in addition to unlimited supply of normal human vascular cells will find bright future application boards for the telomere related vascular diseases. It will be better to reinforce telomere capping functions or limit further telomere shortening. Strategies based on the over expression of TRF2 or potentiation of telomerase activity endow harnessing insights. To meet these needs, application of immortalizing enzyme, with a half life as long as a day, imparts great roles by affording cells with infinite life span and delay of cell-cycle exit. The advantages of permanent gain of telomerase function should be weighted with its potentials in induction of tumerogenic conversion in targeted cells. Recently discovered methods for "regulated telomerase expression" by Geron, broke the limits by making a safe margin for telomerase activity, hence no cellular transformation was seen after cellular telomerization by forced TERT expression. Another approach is application of telomere nanocircles, as artificial telomere lengthening (Lindstrom et al., 2002). Keeping telomere length is also achievable naturally by enough intakes of vitamin D and life style modifications, as decreasing psychological stress load and appropriate physical activity (Tuohimaa, 2009; Fuster and Andres, 2006; Kadi and Ponsot, 2009). Deep understanding of the underlying mechanisms of telomere deregulation and telomerase impairment in endothelial cells toward atherosclerosis, impart therapeutic options to manipulate them.

Conclusion

Telomere dynamics in the progression and development of atherosclerosis delights its targeting as a new therapeutic option for cardiovascular diseases. This needs in-depth elucidation of the underlying mechanisms of telomere erosion in vascular beds.

REFERENCES

Adaikalakoteswari A, Balasubramanyam M, Mohan V (2005). Telomere shortening occurs in Asian Indian Type 2 diabetic patients. Diabet. Med., 22(9):1151-1156.

Artandi SE (2006). Telomeres, telomerase and human disease. N. Engl. J. Med. Sep 21; 355(12): 1195-1197.

Bailey SM, Goodwin EH (2004). DNA and telomeres: beginnings and endings. Cytogenet. Genome Res., 104(1-4): 109-115.

Baird DM (2005). New developments in telomere length analysis. Exp. Gerontol, 40(5): 363-368.

Banas B, Wörnle M, Berger T, Nelson PJ, Cohen CD, Kretzler M, Pfirstinger J, Mack M, Lipp M, Gröne HJ, Schlöndorff D (2002). Roles of SLC/CCL21 and CCR7 in human kidney for mesangial proliferation, migration, apoptosis and tissue homeostasis. J. Immunol. May 1; 168(9): 4301-4307.

Behjati M, Salehi M, Moghim S, Dashti GR (2007). Evaluation of TERT mRNA alternative spliced variants in aortic endothelial cells of the

rabbit, in the fatty streaks stage of atherosclerosis. Acta Cardiol, 62(1): 7-12.
Bellini A, Mattoli S (2007). The role of the fibrocyte, a bone marrow-derived mesenchymal progenitor, in reactive and reparative fibroses. Lab. Invest. Sep; 87(9):858-70.
Benachour K, Hans CP, Suzuki Y, Naura A, Datta R, Belmadani S, Fallon K, Woods C, Boulares AH (2007). Poly(ADP-ribose) polymerase inhibition reduces atherosclerotic plaque size and promotes factors of plaque stability in apolipoprotein E-deficient mice: effects on macrophage recruitment, nuclear factor-kappaB nuclear translocation and foam cell death. Oumouna- Circulation. May 8; 115(18): 2442-50.
Benetos A, Okuda K, Lajemi M, Kimura M, Thomas F, Skurnick J, Labat C, Bean K, Aviv A (2001). Telomere length as an indicator of biological aging: the gender effect and relation with pulse pressure and pulse wave velocity. Hypertension, 37: 381-385.
Bertram C, Hass R (2008). Cellular responses to reactive oxygen species-induced DNA damage and aging. Biol Chem, 389(3): 211-220
Blackburn EH (1991). Structure and function of telomeres. Nature, 350(6319): 569-573.
Blasco MA, Lee HW, Hande MP (1997). Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell. 1997 Oct 3; 91(1): 25-34.
Brouilette SW, Moore JS, McMahon AD, Thompson JR, Ford I, Shepherd J, Packard CJ, Samani NJ; West of Scotland Coronary Prevention Study Group. (2007). Telomere length, risk of coronary heart disease and statin treatment in the West of Scotland Primary Prevention Study: a nested case-control study. Lancet. Jan 13; 369(9556): 107-114.
Burton DG, Giles PJ, Sheerin AN, Smith SK, Lawton JJ, Ostler EL, Rhys-Williams W, Kipling D, Faragher RG (2009). Microarray analysis of senescent vascular smooth muscle cells: A link to atherosclerosis and vascular calcification. Exp Gerontol. Jul 23.
Cao Y, Li H, Mu FT, Ebisui O, Funder JW, Liu JP (2002). Telomerase activation causes vascular smooth muscle cell proliferation in genetic hypertension. FASEB J, 16(1): 96-8
Celli GB, Denchi EL, de Lange T (2006). Ku70 stimulates fusion of dysfunctional telomeres yet protects chromosome ends from homologous recombination. Nat Cell Biol, 8(8): 885-890.
Chang E, Harley CB (1995). Telomere length and replicative aging in human vascular tissues. Proc Natl Acad Sci U S A. Nov 21; 92(24): 11190-4
Chang M, Arneric M, Lingner J (2007). Telomerase repeat addition processivity is increased at critically short telomeres in a Tel1-dependent manner in *Saccharomyces cerevisiae*. Genes Dev. Oct 1; 21(19): 2485-94
Chang MW, Grillari J, Mayrhofer C, Fortschegger K, Allmaier G, Marzban G, Katinger H, Voglauer R (2005). Comparison of early passage, senescent and hTERT immortalized endothelial cells. Exp Cell Res. Sep 10; 309(1): 121-36
Chang P, Coughlin M, Mitchison TJ (2005). Tankyrase-1 polymerization of poly(ADP-ribose) is required for spindle structure and function. Nat Cell Biol, 7(11): 1133-9.
Cherif H, Tarry JL, Ozanne SE, Hales CN (2003). Ageing and telomeres: a study into organ- and gender-specific telomere shortening, Nucleic Acids Res. 2003 Mar 1; 31(5): 1576-1583.
Collins K (2008). Physiological assembly and activity of human telomerase complexes. Mech. Ageing Dev., 129(1-2): 91-98.
Damjanovic AK, Yang Y, Glaser R, Kiecolt-Glaser JK, Nguyen H, Laskowski B, Zou Y, Beversdorf DQ, Weng NP (2007). Accelerated telomere erosion is associated with a declining immune function of caregivers of Alzheimer's disease patients. J. Immunol. Sep 15; 179(6): 4249-54.
Das B, Pawar N, Saini D, Seshadri M (2009). Genetic association study of selected candidate genes (ApoB, LPL, Leptin) and telomere length in obese and hypertensive individuals. BMC Med. Genet., 22(10): 1-99.
Deiner C, Loddenkemper C, Rauch U, Rosenthal P, Pauschinger M, Schwimmbeck PL, Schultheiss HP, Pels K (2007). Mechanisms of late lumen loss after antiproliferative percutaneous coronary intervention using beta-irradiation in a porcine model of restenosis. Cardiovasc Revasc Med, 8(2):94-98.
Dimmeler S, Aicher A, Vasa M, Mildner-Rihm C, Adler K, Tiemann M, Rütten H, Fichtlscherer S, Martin H, Zeiher AM (2001). HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. J. Clin. Invest., 108(3): 391-397.
Ding SL, Shen CY (2008). Model of human aging: recent findings on Werner's and Hutchinson-Gilford progeria syndromes. Clin. Interv. Aging. 3(3):431-444
Dobbs TA, Palmer P, Maniou Z, Lomax ME, O'Neill P (2008). Interplay of two major repair pathways in the processing of complex double-strand DNA breaks. DNA Repair (Amst). Aug 2; 7(8): 1372-1383.
de Lange T (2005). Shelterin: The protein complex that shapes and safeguards human telomeres. Genes Dev. Sep 15; 19(18): 2100-2110.
Doshida M, Ohmichi M, Tsutsumi S, Kawagoe J, Takahashi T, Du B, Mori-Abe A, Ohta T, Saitoh-Sekiguchi M, Takahashi K, Kurachi H (2006). Raloxifene increases proliferation and up-regulates telomerase activity in human umbilical vein endothelial cells. J. Biol. Chem. Aug 25; 281(34): 24270-8.
DuBois ML, Haimberger ZW, McIntosh MW, Gottschling DE (2002). A quantitative assay for telomere protection in Saccharomyces cerevisiae. Genetics,161(3): 995-1013.
Farsetti A, Grasselli A, Bacchetti S, Gaetano C, Capogrossi MC (2009). The telomerase tale in vascular aging: regulation by estrogens and nitric oxide signaling. J. Appl. Physiol., 106(1): 333-7.
Ferrezuelo F, Steiner B, Aldea M, Futcher B (2002). Biogenesis of yeast telomerase depends on the importin mtr10. Mol. Cell Biol., 22(17): 6046-6055.
Frenck RW Jr, Blackburn EH, Shannon KM (1998). The rate of telomere sequence loss in human leukocytes varies with age. Proc. Natl. Acad. Sci. U S A. May 12; 95(10): 5607-5610.
Fujii H, Li SH, Szmitko PE, Fedak PW, Verma S (2006). C-reactive protein alters antioxidant defenses and promotes apoptosis in endothelial progenitor cells. Arterioscler Thromb. Vasc. Biol., 26(11): 2476-2482.
Fukumoto Y, Hiro T, Fujii T, Hashimoto G, Fujimura T, Yamada J, Okamura T, Matsuzaki M (2008). Localized elevation of shear stress is related to coronary plaque rupture: a 3-dimensional intravascular ultrasound study with in-vivo color mapping of shear stress distribution. J Am Coll Cardiol. Feb 12; 51(6): 645-650.
Fuster JJ, Andres V (2006). Telomere biology and cardiovascular disease. Circ. Res. Nov. 24; 99(11): 1167-80.
Gallardo F, Olivier C, Dandjinou AT, Wellinger RJ, Chartrand P. EMBO J (2008). TLC1 RNA nucleo-cytoplasmic trafficking links telomerase biogenesis to its recruitment to telomeres. Mar 5; 27(5):748-757.
Gomperts BN, Strieter MR (2007). Fibrocytes in lung disease. J. Leukocyte Biol. 82: 449-456.
Greider CW (1996). Telomere length regulation. Annu Rev Biochem, 65: 337-365.
Griffith JD, Comeau L, Rosenfield S, Stansel RM, Bianchi A, Moss H, de Lange T (1999). Mammalian telomeres end in a large duplex loop. Cell. May 14; 97(4): 503-14.
Gupta S, Gollapudi S (2006). Molecular mechanisms of TNF-alpha-induced apoptosis in naïve and memory T cell subsets. Autoimmun Rev., 5(4): 264-8.
Haendeler J, Hoffmann J, Brandes RP, Zeiher AM, Dimmeler S (2003). Hydrogen peroxide triggers nuclear export of telomerase reverse transcriptase via Src kinase family-dependent phosphorylation of tyrosine 707. Mol. Cell Biol. Jul; 23(13): 4598-610.
Hammond PW, Cech TR (1997). dGTP-dependent processivity and possible template switching of euplotes telomerase. Nucleic Acids Res. Sep. 15; 25(18): 3698-704.
Hanaoka S, Nagadoi A, Nishimura Y (2005). Comparison between TRF2 and TRF1 of their telomeric DNA-bound structures and DNA-binding activities. Protein Sci., 14(1): 119-30.
Hartlapp I, Abe R, Saeed RW, Peng T, Voelter W, Bucala R, Metz CN (2001). Fibrocytes induce an angiogenic phenotype in cultured endothelial cells and promote angiogenesis *in vivo*. FASEB J., 15(12): 2215-24.
Hayashi T, Yano K, Matsui-Hirai H, Yokoo H, Hattori Y, Iguchi A (2008). Nitric oxide and endothelial cellular senescence. Pharmacol. Ther. Dec.; 120(3):333-9.

Houben JM, Moonen HJ, van Schooten FJ, Hageman GJ (2008). Telomere length assessment: biomarker of chronic oxidative stress? Free Radic Biol Med. Feb 1; 44(3): 235-46.
Jacobs JJ, de Lange T (2004). Significant role for p16INK4a in p53-independent telomere-directed senescence. Curr. Biol. Dec. 29; 14(24): 2302-8.
James SE, Faragher RG, Burke JF, Shall S, Mayne LV (2000). Werner's syndrome T lymphocytes display a normal *in vitro* life-span. Mech. Ageing Dev. Dec. 20; 121(1-3):139-49
Kadi F, Ponsot E (2009). The biology of satellite cells and telomeres in human skeletal muscle: Effects of aging and physical activity. Scand. J. Med. Sci. Sports. Sep. 17
Karlseder J, Hoke K, Mirzoeva OK, Bakkenist C, Kastan MB, Petrini JH, de Lange T (2004). The telomeric protein TRF2 binds the ATM kinase and can inhibit the ATM-dependent DNA damage response. PLoS Biol. Aug; 2(8):E240.
Kawanishi S, Oikawa S (2004). Mechanism of telomere shortening by oxidative stress. Ann N Y Acad. Sci., 1019: 278-284.
Kimura A, Ohmichi M, Kawagoe J, Kyo S, Mabuchi S, Takahashi T, Ohshima C, Arimoto-Ishida E, Nishio Y, Inoue M, Kurachi H, Tasaka K, Murata Y (2004). Induction of hTERT expression and phosphorylation by estrogen via Akt cascade in human ovarian cancer cell lines. Oncogene. Jun 3; 23(26): 4505-4515.
Kurz DJ, Hong Y, Trivier E, Huang HL, Decary S, Zang GH, Lüscher TF, Erusalimsky JD (2003). Fibroblast growth factor-2, but not vascular endothelial growth factor, upregulates telomerase activity in human endothelial cells. Arterioscler Thromb Vasc Biol. May 1; 23(5): 748-754.
Kurz DJ, Decary S, Hong Y, Trivier E, Akhmedov A, Erusalimsky JD (2004). Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. J. Cell Sci. May 1; 117(Pt 11):2417-2426.
Lee TH, Perrem K, Harper JW, Lu KP, Zhou XZ (2006). The F-box protein FBX4 targets PIN2/TRF1 for ubiquitin-mediated degradation and regulates telomere maintenance. J. Biol. Chem., Jan 13; 281(2): 759-768.
Lindstrom UM, Chandrasekaran RA, Orbai L, Helquist SA, Miller GP, Oroudjev E, Hansma HG, Kool ET (2002). Artificial human telomeres from DNA nanocircle templates. Proc. Natl. Acad. Sci. U S A. Dec. 10; 99(25): 15953-15958
Lin JH, Lee JY, Tsao CJ, Chao SC (2002). DKC1 gene mutation in a Taiwanese kindred with X-linked dyskeratosis congenita. Kaohsiung J. Med. Sci., 18(11):573-577
Lin KW, Yan J (2005). The telomere length dynamic and methods of its assessment. J. Cell Mol. Med., 9(4): 977-989
Lionetti L, Mollica MP, Lombardi A, Cavaliere G, Gifuni G, Barletta A (2009). From chronic overnutrition to insulin resistance: the role of fat-storing capacity and inflammation. Nutr. Metab. Cardiovasc. Dis., 19(2): 146-152.
Liu KR, J. Hodes, Weng NP. (2001). Telomerase activation in human T lymphocytes does not require increase in telomerase reverse transcriptase (hTERT) protein but is associated with hTERT phosphorylation and nuclear translocation. J. Immunol., 166: 4826-4830.
Liu SC, Wang SS, Wu MZ, Wu DC, Yu FJ, Chen WJ, Chiang FT, Yu MF (2005). Activation of telomerase and expression of human telomerase reverse transcriptase in coronary atherosclerosis. Cardiovasc. Pathol., 14(5): 232-240.
Lo SJ, Lee CC, Lai HJ (2006). The nucleolus: reviewing oldies to have new understandings. Cell Res. Jun; 16(6):530-538
Lue NF, Peng Y (1998). Negative regulation of yeast telomerase activity through an interaction with an upstream region of the DNA primer. Nucleic Acids Res. Mar 15; 26(6): 1487-1494.
Lue NF (1999). Sequence-specific and conformation-dependent binding of yeast telomerase RNA to single-stranded telomeric DNA. Nucleic Acids Res. Jun 15; 27 (12): 2560-2567.
Mahmoudi M, Gorenne I, Mercer J, Figg N, Littlewood T, Bennett M (2008). Statins use a novel Nijmegen breakage syndrome-1-dependent pathway to accelerate DNA repair in vascular smooth muscle cells. Circ. Res. Sep. 26; 103 (7): 717-725.
Manestar-Blazic T (2004). Hypothesis on transmission of longevity based on telomere length and state of integrity. Med. Hypotheses, 62(5): 770-772.
Matthews C, Gorenne I, Scott S, Figg N, Kirkpatrick P, Ritchie A, Goddard M, Bennett M (2006). Vascular smooth muscle cells undergo telomere-based senescence in human atherosclerosis: effects of telomerase and oxidative stress. Circ Res. Jul 21; 99(2): 156-164.
Mathews MT, Berk BC (2008). PARP-1 inhibition prevents oxidative and nitrosative stress-induced endothelial cell death via transactivation of the VEGF receptor 2. Arterioscler Thromb. Vasc. Biol., 28(4): 711-717.
Matsushita H, Chang E, Glassford AJ, Cooke JP, Chiu CP, Tsao PS (2001). eNOS activity is reduced in senescent human endothelial cells: Preservation by hTERT immortalization. Circ. Res. Oct. 26; 89(9): 793-798.
Mayer S, Brüderlein S, Perner S, Waibel I, Holdenried A, Ciloglu N, Hasel C, Mattfeldt T, Nielsen KV, Möller P (2006). Sex-specific telomere length profiles and age-dependent erosion dynamics of individual chromosome arms in humans. Cytogenet. Genome Res., 112(3-4): 194-201
Medbury HJ, Tarran SL, Guiffre AK, Williams MM, Lam TH, Vicaretti M, Fletcher JP (2008). Monocytes contribute to the atherosclerotic cap by transformation into fibrocytes. Int. Angiol. Apr.; 27(2): 114-123.
Metz CN (2003). Fibrocytes: a unique cell population implicated in wound healing. Cell Mol. Life Sci. Jul; 60(7): 1342-1350
Minamino T, Komuro I (2006). Antisenescence as a novel therapeutic strategy for vascular aging. Nippon Ronen Igakkai Zasshi. May; 43(3):347-350.
Minamino T, Mitsialis SA, Kourembanas S (2001). Hypoxia extends the life span of vascular smooth muscle cells through telomerase activation. Mol. Cell Biol. May; 21(10): 3336-3342
Minamino T, Kourembanas S (2001). Mechanisms of telomerase induction during vascular smooth muscle cell proliferation. Circ. Res. Aug 3; 89(3): 237-243
Mochizuki Y, He J, Kulkarni S, Bessler M, Mason PJ (2004). Mouse dyskerin mutations affect accumulation of telomerase RNA and small nucleolar RNA, telomerase activity and ribosomal RNA processing. Proc. Natl. Acad. Sci. U S A. Jul 20; 101(29): 10756-10761
Morla M, Busquets X, Pons J, Sauleda J, MacNee W, Agustí AG (2006). Telomere shortening in smokers with and without COPD. Eur Respir J. Mar; 27(3): 525-528
Muhlestein JB, Anderson JL (2003). Chronic infection and coronary artery disease. Cardiol Clin. Aug; 21(3): 333-362.
Muramatsu Y, Tahara H, Ono T, Tsuruo T, Seimiya H (2008). Telomere elongation by a mutant tankyrase 1 without TRF1 poly(ADP-ribosyl)ation. Exp. Cell Res. Mar 10; 314(5): 1115-1124.
Murry CE, Gipaya CT, Bartosek T, Benditt EP, Schwartz SM (1997). Monoclonality of smooth muscle cells in human atherosclerosis. Am. J. Pathol. Sep.; 151(3): 697-705.
Nakamura M, Zhou XZ, Kishi S, Lu KP (2002). Involvement of the telomeric protein Pin2/TRF1 in the regulation of the mitotic spindle. FEBS Lett. Mar. 13; 514(2-3): 193-198.
O'Donnell CJ, Demissie S, Kimura M, Levy D, Gardner JP, White C, D'Agostino RB, Wolf PA, Polak J, Cupples LA, Aviv A (2008). Leukocyte telomere length and carotid artery intimal medial thickness: The Framingham heart study. Arterioscler. Thromb. Vasc. Biol., 28(6): 1165-1171.
Olovnikov AM (2003). Redusome aging: commentaries. Adv Gerontol., 12: 28-45.
Oganesian L, Bryan TM (2007). Physiological relevance of telomeric G-quadruplex formation: a potential drug target. Bioessays. Feb.; 29(2): 155-165.
Okuda K, Khan MY, Skurnick J, Kimura M, Aviv H, Aviv A (2000). Telomere attrition of the human abdominal aorta: relationships with age and atherosclerosis. Atherosclerosis. Oct.; 152(2): 391-398.
Perez-Rivero G, Ruiz-Torres MP, Rivas-Elena JV, Jerkic M, Díez-Marques ML, Lopez-Novoa JM, Blasco MA, Rodríguez-Puyol D (2006). Mice deficient in telomerase activity develop hypertension because of an excess of endothelin production. Circulation. Jul 25; 114(4): 309-317.
Petermann E, Ziegler M, Oei SL (2003). ATP-dependent selection between single nucleotide and long patch base excision repair. DNA Repair (Amst). Oct. 7; 2(10):1101-1114.

Prathapam R, Witkin KL, O'Connor CM, Collins K (2005). A telomerase holoenzyme protein enhances telomerase RNA assembly with telomerase reverse transcriptase. Nat. Struct. Mol. Biol. Mar.; 12(3): 252-257.

Rahman R, Forsyth NR, Cui W (2008). Telomeric 3'-overhang length is associated with the size of telomeres. Exp. Gerontol. Apr.; 43(4): 258-265.

Richards JB, Valdes AM, Gardner JP, Kato BS, Siva A, Kimura M, Lu X, Brown MJ, Aviv A, Spector TD (2008). Homocysteine levels and leukocyte telomere length. Atherosclerosis. Oct; 200(2): 271-277

Risques RA, Lai LA, Brentnall TA, Li L, Feng Z, Gallaher J, Mandelson MT, Potter JD, Bronner MP, Rabinovitch PS (2008). Ulcerative colitis is a disease of accelerated colon aging: evidence from telomere attrition and DNA damage. Gastroenterology. Aug.; 135(2): 410-418.

Ram R, Uziel O, Eldan O, Fenig E, Beery E, Lichtenberg S, Nordenberg Y, Lahav M (2009). Ionizing radiation up-regulates telomerase activity in cancer cell lines by post-translational mechanism via ras/phosphatidylinositol 3-kinase/Akt pathway. Clin. Cancer Res. Feb. 1; 15(3): 914-923.

Richter T, Saretzki G, Nelson G, Melcher M, Olijslagers S, von Zglinicki T (2007). TRF2 overexpression diminishes repair of telomeric single-strand breaks and accelerates telomere shortening in human fibroblasts. Mech. Ageing Dev. Apr.; 128(4): 340-345.

Rippmann JF, Damm K, Schnapp A (2002). Functional characterization of the poly(ADP-ribose) polymerase activity of tankyrase 1, a potential regulator of telomere length. J. Mol. Biol. Oct. 18; 323(2): 217-224

Romanova L, Kellner S, Katoku-Kikyo N, Kikyo N (2009). Novel role of nucleostemin in the maintenance of nucleolar architecture and integrity of small nucleolar ribonucleoproteins and the telomerase complex. J. Biol. Chem. Sep. 25; 284(39): 26685-26694.

Rufer N, Brümmendorf TH, Kolvraa S, Bischoff C, Christensen K, Wadsworth L, Schulzer M, Lansdorp PM (1999). Telomere fluorescence measurements in granulocytes and T lymphocyte subsets point to a high turnover of hematopoietic stem cells and memory T cells in early childhood. J. Exp. Med. Jul 19; 190(2): 157-167.

Saeboe-Larssen S, Fossberg E, Gaudernack G (2006). Characterization of novel alternative splicing sites in human telomerase reverse transcriptase (hTERT): analysis of expression and mutual correlation in mRNA isoforms from normal and tumour tissues. BMC Mol. Biol., 29: 7-26.

Sampson MJ, Winterbone MS, Hughes JC, Dozio N, Hughes DA (2006). Monocyte telomere shortening and oxidative DNA damage in type 2 diabetes. Diabetes Care. Feb.; 29(2): 283-289.

Salpea KD, Nicaud V, Tiret L, Talmud PJ, Humphries SE; EARS II group (2008). The association of telomere length with paternal history of premature myocardial infarction in the European Atherosclerosis Research Study II. J. Mol. Med. Jul.; 86(7): 815-824.

Satoh M, Ishikawa Y, Takahashi Y, Itoh T, Minami Y, Nakamura M (2008). Association between oxidative DNA damage and telomere shortening in circulating endothelial progenitor cells obtained from metabolic syndrome patients with coronary artery disease. Atherosclerosis. Jun.; 198(2): 347-353.

Schrijvers DM, De Meyer GR, Herman AG, Martinet W (2007). Phagocytosis in atherosclerosis: Molecular mechanisms and implications for plaque progression and stability. Cardiovasc. Res. Feb. 1; 73(3): 470-480.

Seimiya H, Smith S (2002). The telomeric poly(ADP-ribose) polymerase, tankyrase 1, contains multiple binding sites for telomeric repeat binding factor 1 (TRF1) and a novel acceptor, 182-kDa tankyrase-binding protein (TAB182). J. Biol. Chem. Apr. 19; 277(16): 14116-14126.

Seto AG, Zaug AJ, Sobel SG, Wolin SL, Cech TR (1999). Saccharomyces cerevisiae telomerase is an Sm small nuclear ribonucleoprotein particle. Nature, Sep. 9; 401(6749):177-180

Shen JS, Meng XL, Schiffmann R, Brady RO, Kaneski CR (2007). Establishment and characterization of Fabry disease endothelial cells with an extended lifespan. Mol. Genet. Metab. Sep-Oct; 92(1-2): 137-144.

Simon NM, Smoller JW, McNamara KL, Maser RS, Zalta AK, Pollack MH, Nierenberg AA, Fava M, Wong KK (2006). Telomere shortening and mood disorders: preliminary support for a chronic stress model of accelerated aging. Biol. Psychiatry. Sep. 1; 60(5): 432-425.

Song MB, Yu XJ, Zhu GX, Chen JF, Zhao G, Huang L (2009). Transfection of HGF gene enhances endothelial progenitor cell (EPC) function and improves EPC transplant efficiency for balloon-induced arterial injury in hypercholesterolemic rats. Vascul. Pharmacol. Aug-Sep; 51(2-3): 205-213.

Stansel RM, de Lange T, Griffith JD (2001). T-loop assembly *in vitro* involves binding of TRF2 near the 3' telomeric overhang. EMBO J. Oct 1; 20(19): 5532-5540

Stephanson CJ, Stephanson AM, Flanagan GP (2003). Evaluation of hydroxyl radical-scavenging abilities of silica hydride, an antioxidant compound, by a Fe2+-EDTA-induced 2-hydroxyterephthalate fluorometric analysis. J. Med. Food. Fall; 6(3): 249-253.

Stindl R (2004). Tying it all together: telomeres, sexual size dimorphism and the gender gap in life expectancy. Med Hypotheses. 62(1): 151-4

Su KH, Tsai JY, Kou YR, Chiang AN, Hsiao SH, Wu YL, Hou HH, Pan CC, Shyue SK, Lee TS (2009). Valsartan regulates the interaction of angiotensin II type 1 receptor and endothelial nitric oxide synthase via Src/PI3K/Akt signalling. Cardiovasc. Res. Jun 1; 82(3): 468-475.

Surralles J, Hande MP, Marcos R, Lansdorp PM (1999). Accelerated telomere shortening in the human inactive X chromosome. Am. J. Hum. Genet. Dec; 65(6): 1617-22

Tang D, Lu J, Walterscheid JP, Chen HH, Engler DA, Sawamura T, Chang PY, Safi HJ, Yang CY, Chen CH (2008). Electronegative LDL circulating in smokers impairs endothelial progenitor cell differentiation by inhibiting Akt phosphorylation via LOX-1. J Lipid Res. Jan.; 49(1): 33-47.

Terry DF, Nolan VG, Andersen SL, Perls TT, Cawthon R (2008). Association of longer telomeres with better health in centenarians. J Gerontol A Biol Sci Med Sci. Aug; 63(8): 809-812.

Tuohimaa P (2009). Vitamin D and aging. J. Steroid Biochem. Mol. Biol. Mar.; 114(1-2): 78-84.

Unryn BM, Cook LS, Riabowol KT (2005). Paternal age is positively linked to telomere length of children. Aging Cell. Apr.; 4(2): 97-101

van Wijk SJ, Hageman GJ (2005). Poly (ADP-ribose) polymerase-1 mediated caspase-independent cell death after ischemia/reperfusion. Free Radic Biol Med. Jul 1; 39(1): 81-90

Vasan RS, Demissie S, Kimura M, Cupples LA, Rifai N, White C, Wang TJ, Gardner JP, Cao X, Benjamin EJ, Levy D, Aviv A (2008). Association of leukocyte telomere length with circulating biomarkers of the renin-angiotensin-aldosterone system: the Framingham Heart Study. Circulation, Mar 4; 117(9): 1138-1144.

Venkatesan RN, Price C (1998). Telomerase expression in chickens: constitutive activity in somatic tissues and down-regulation in culture. Proc. Natl. Acad. Sci. U S A. Dec. 8; 95(25): 14763-14768

Verdun RE, Karlseder J (2007). Replication and protection of telomeres. Nature, Jun 21; 447(7147): 924-931

Villasante A, Abad JP, Planelló R, Mendez-Lago M, Celniker SE, de Pablos B (2007). *Drosophila* telomeric retrotransposons derived from an ancestral element that was recruited to replace telomerase. Genome Res. Dec; 17(12): 1909-1918.

Voghel G, Thorin-Trescases N, Farhat N, Mamarbachi AM, Villeneuve L, Fortier A, Perrault LP, Carrier M, Thorin E (2008). Chronic treatment with N-acetyl-cystein delays cellular senescence in endothelial cells isolated from a subgroup of atherosclerotic patients. Mech. Ageing Dev. May;129(5): 261-270.

Weilbaecher RG, Lundblad V (1999). Assembly and regulation of telomerase. Curr. Opin. Chem. Biol. Oct; 3(5): 573-577.

Wen VW, Wu K, Baksh S, Hinshelwood RA, Lock RB, Clark SJ, Moore MA, Mackenzie KL (2006). Telomere-driven karyotypic complexity concurs with p16INK4a inactivation in TP53-competent immortal endothelial cells. Cancer Res. Nov. 15; 66(22): 10691-10700

Williamson MR, Shuttleworth A, Canfield AE, Black RA, Kielty CM (2007). The role of endothelial cell attachment to elastic fibre molecules in the enhancement of monolayer formation and retention and the inhibition of smooth muscle cell recruitment. Biomaterials. Dec; 28(35): 5307-5318.

Wong JM, Collins K (2006). Telomerase RNA level limits telomere maintenance in X-linked dyskeratosis congenita. Genes Dev. Oct 15; 20(20): 2848-2858.

Wong JM, Kusdra L, Collins K (2002). Subnuclear shuttling of human

telomerase induced by transformation and DNA damage. Nat Cell Biol. Sep; 4(9): 731-736.
Wong LS, de Boer RA, Samani NJ, van Veldhuisen DJ, van der Harst P (2008). Telomere biology in heart failure. Eur. J. Heart Fail. Nov.; 10(11): 1049-1056.
Wyatt HD, Lobb DA, Beattie TL (2007). Characterization of physical and functional anchor site interactions in human telomerase. Mol. Cell Biol. Apr; 27(8):3226-3240.
Xia L, Wang XX, Hu XS, Guo XG, Shang YP, Chen HJ, Zeng CL, Zhang FR, Chen JZ (2008). Resveratrol reduces endothelial progenitor cells senescence through augmentation of telomerase activity by Akt-dependent mechanisms. Br. J. Pharmacol. Oct; 155(3): 387-394.
Yu WY, Chang HW, Lin CH, Cho CL (2008). Short telomeres in patients with chronic schizophrenia who show a poor response to treatment. J Psychiatry Neurosci. May; 33(3): 244-247. Erratum in: J. Psychiatry Neurosci. 2008 Jul; 33(4): 343.
Zappulla DC, Cech TR (2006). RNA as a flexible scaffold for proteins: yeast telomerase and beyond. Cold Spring Harb. Symp. Quant Biol., 71: 217-224.
Zaug AJ, Podell ER, Cech TR (2005). Human POT1 disrupts telomeric G-quadruplexes allowing telomerase extension *in vitro*. Proc. Natl. Acad. Sci. U S A. Aug 2; 102(31):10864-10869.
Zeng L, Xu Q (2008). eNOS-ERalpha complex goes to telomerase. Circ. Res. Jul 3; 103(1):10-12
Zhang M, Zhou SH, Li XP, Shen XQ, Fang ZF (2008). A novel hypothesis of atherosclerosis: EPCs-mediated repair-to-injury. Med Hypotheses. 70(4):838-841.
Zhang Y, Cao EH, Qin JF (2005). Up-regulation of telomere-binding TRF1, TRF2 related to reactive oxygen species induced by As (2) O() in MGC-803 cells. Eur. J. Pharmacol. May 23; 516(1):1-9
Zhou MS, Schulman IH, Raij L (2009). Role of angiotensin II and oxidative stress in vascular insulin resistance linked to hypertension. Am. J. Physiol. Heart Circ. Physiol. Mar.; 296(3):H833- H839.
Zhou XZ, Perrem K, Lu KP (2003). Role of Pin2/TRF1 in telomere maintenance and cell cycle control. J. Cell Biochem. May 1; 89(1):19-37.
Zhou XZ, Lu KP (2001). The Pin2/TRF1-interacting protein PinX1 is a potent telomerase inhibitor. Cell. Nov. 2; 107(3):347-359.