NARRATIVE REVIEW

Cellular Senescence, Cardiovascular Risk, and CKD: A Review of Established and Hypothetical Interconnections

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Cellular senescence is associated with shortened or damaged telomeres and is characterized by permanent exit from the cell cycle, morphological changes, and altered function. It develops after repeated cell divisions and also can be induced prematurely by stress conditions. The senescent phenotype, depending on cell type and atherosclerosis phase, seems to be a proatherosclerotic one: it promotes endothelial dysfunction and appears to be implicated in plaque destabilization, as well as in endothelial progenitor cell alteration. Many traditional and nontraditional cardiovascular disease risk factors induce senescence in a variety of vascular cells. Several of these factors, such as diabetes, hypertension, oxidative stress, and inflammation, are clustered in patients with chronic kidney disease. In a limited number of recent studies, stress-induced premature cellular senescence in this biologically aged population also was described. The hypothesis that premature cellular senescence might be considered an additional atherosclerosis-inducing factor in patients with chronic kidney disease is proposed.

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INDEX WORDS: Telomeres; telomerase; renal failure; atherosclerosis; inflammation; oxidative stress; diabetes; hypertension; endothelial progenitor cell; p53.

Aging is a well-known risk factor for atherosclerotic cardiovascular disease (CVD).[1,2] Coronary heart disease (CHD), stroke, and peripheral vascular disease incidence increase with age.[1,2] Blood vessels undergo changes and their compliance decreases. The endothelium dysfunctions, its antithrombotic and vasodilatatory properties are reduced, and inflammatory activity increases.[3-6] However, it is unknown whether a common molecular mechanism exists behind these epidemiological and clinical observations.[7]

Patients with chronic kidney disease (CKD) experience increased CVD morbidity and mortality compared with the general population.[8] The clustering of many traditional (diabetes, hypertension, and so on) and nontraditional (oxidative stress, inflammation, and so on) CVD risk factors may explain this phenomenon in this population.[8,9] Furthermore, epidemiological data showed that biological age, at least regarding CVD morbidity and mortality, was often older than chronological age in these patients.[10-12] The question of whether biological aging correlates with the increased CVD mortality by means of a specific molecular mechanism therefore is highly relevant to studies of patients with CKD.

In the last few years, many experimental studies and some clinical data supported the hypothesis that the common process responsible for these phenomena is cellular senescence and telomere dysfunction.[7,13,14] In the first part of this review, the cellular senescence process and telomere regulation are introduced. In the second part, experimental and clinical data connecting atherosclerotic CVD to these molecular processes are discussed. Finally, the few studies that showed premature cellular senescence development in patients with CKD are covered, and although evidence is limited, a hypothesis proposing premature cellular senescence as a novel nontraditional CVD risk factor in this population is considered.

FEATURES AND MECHANISMS OF CELLULAR SENESCENCE

Phenotype and Pathways

Cellular senescence was first observed in vitro[15,16] when it was observed that normal cells in culture did not proliferate indefinitely, and after a period of rapid proliferation, their division

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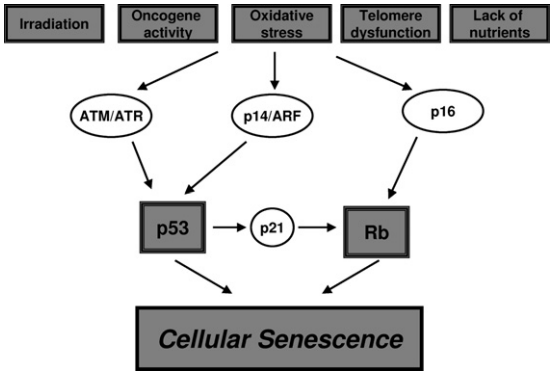

Figure 1. Initiating factors and signaling pathways in cellular senescence induction. A variety of intrinsic and extrinsic factors induce cellular senescence. These factors stimulate a number of cellular signaling pathways that result in the activation of p53, Rb protein, or both and induction of cellular senescence. p53 can activate senescence by activating Rb through p21 or independently of Rb. Rb is activated by either p21 or p16. Phosphorylation and activation of p53 is mediated by ataxia telangiectasia mutated (ATM)/ATM-related (ATR) involved in the DNA damage-response pathway or by alternative reading frame product of INK4a gene locus (ARF), a stress-dependent pathway.[22] (Adapted from Ben-Porath and Weinberg.[15])

rate slows and then ceases.[15,17] Cells remain viable, but they do not respond to mitogenic stimuli and their morphological characteristics and function change dramatically.[18-20] They lose their original shape, their volume increases, and they acquire a flattened cytoplasm ("fried egg" appearance).[14,15,21,22] These changes are accompanied by alterations in nuclear structure, gene expression, protein processing, and metabolism.[15,19,20] Furthermore, intercellular contact is lost and cells are tightly attached to the extracellular matrix,[23] changes that may alter tissue structure and function.[24-26] This described senescence model, which follows an extensive number of cell divisions, has been termed replicative senescence.[15] More recent data have shown that cells can enter senescence rapidly, independently of the number of cell divisions, in response to various physiological stresses (radiation, oxidative stress, lack of nutrients, DNA damage, and so on).[27-30] This type of senescence has been termed stress-induced premature senescence.[31]

Activation of senescence by different stress stimuli[15,22] is shown in Fig 1. Tumor suppressor proteins p53 and Rb, two transcription regulators, are crucial in the induction of senescence. It seems that p53 has a prominent role in mediating the response to DNA damage, oxidative stress, and telomere erosion or dysfunction. In humans, the p16→Rb pathway is activated in parallel by the same triggers, yet to a lesser extent than p53, and also by additional stimuli.[22,29,32,33] Linear (p53→p21→Rb; Fig 1) activation also was proposed.[22]

Although initially believed to be a cell-culture phenomenon, cellular senescence recently was observed in vivo as well.[17,34,35] The most common means of detecting cellular senescence is by colorimetric detection of β-galactosidase in cells under mildly acidic (pH 6.0) conditions, in contrast to the more strongly acidic (pH 4.0) conditions normally required to detect endogenous lysosomal β-galactosidase activity.[14,36] Other biomarkers include increased expression of p53, p21, and p16.[15,22,37-40]

Senescence is a fundamental cellular program that parallels that of programmed cellular death (apoptosis). Both molecular mechanisms restrict cellular proliferation. The reason a cell is driven to apoptosis versus senescence is not yet known.[41-45] The degree of stress[42] and cell-cycle phase[41] seem to be determining factors[14] (eg, higher doses of oxidative stress induce apoptosis, whereas lower and long-acting doses induce senescence). Moreover, apoptosis appears to occur more easily in senescent endothelial cells, yet seems to be blocked in other senescent cell types.[14] At the same time, factors involved in senescence signaling, such as p53, are also involved in apoptosis regulation through interaction with the BCL2 family of proteins.[32] In any case, cellular senescence as a biological mechanism, as well as the role that senescence has in the living organism, is, in contrast to apoptosis, not well understood.

Telomeres and Telomerase

Telomeres are protein-DNA complexes at the ends of eukaryotic chromosomes that protect chromosomes from fusion and degradation and prevent initiation of the DNA damage response (Fig 2).[46-53] Telomeres shorten after each cell division (Fig 2B and C).[15,54] When a critical number of cellular divisions is completed, eroded telomeres, interpreted by the cell as damaged DNA, signal the initiation of cellular senescence (Fig 2C).[15] This is the replicative senescence process, and in this case, the p53 pathway is mainly, if not solely, involved in triggering senes-

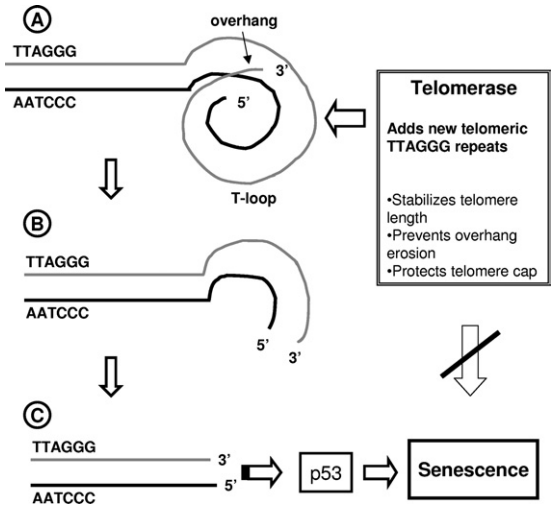

Figure 2. Telomere erosion, telomerase, and cellular senescence. (A) At the end of a telomere, a protective cap is formed. The T-loop configuration potentially offers protection and is formed by the invasion of the single-stranded overhang into an upstream double-stranded region of the telomere. A number of proteins (not shown) are also involved. (B) As the number of cellular divisions reaches a critical number, the protective telomere cup is eroded. (C) When erosion reaches a high level, telomere injury is sensed as DNA damage and cellular senescence is induced. As shown in the box to the right of panel A, telomerase not only adds new telomeric repeats, thus elongating telomeres, but also prevents overhang erosion and protects the telomere cap. In this way, telomerase activity delays replicative and stress-induced premature cellular senescence. (Adapted from Ben-Porath and Weinberg.[15])

cence.[22] However, telomeres are also involved in stress-induced premature senescence. It seems that this second pathway initiates not because of shortening, but because of changes in telomere structure (ie, alterations in the T loop and single-stranded overhang, as shown in Fig 2A and B) and function.[15,55,56] Thus, both telomere length and structural integrity are necessary for proper chromosome function and avoidance of DNA damage response and its consequent triggering of senescence.

Telomerase is a specialized reverse transcriptase that, in human cells, is composed of an RNA subunit (human telomerase RNA component [hTERC]) that is used as a template for the synthesis of telomeric repeats and a catalytic protein part (human telomerase reverse transcriptase [hTERT]). The enzyme not only produces telomeric repeats that elongate telomeres, but also prevents alterations in telomere structure, protecting the telomere cap (Fig 2).[46,57-59] Although it is believed that telomerase, with the exception of its overexpression in cancer cells, has an essential role only in reproductive cells and in cells with a rapid turnover, recent studies showed that its role in normal somatic cells may also be important.[57,60,61]

CELLULAR SENESCENCE AS AN EMERGING CARDIOVASCULAR RISK FACTOR

Vascular cellular senescence seems to be an in vivo phenomenon associated with atherosclerosis. Endothelial and vascular smooth muscle cells (VSMCs) in atherosclerotic plaques show morphological characteristics of senescence.[62,63] The senescent phenotype is evident in VSMCs found in carotid lesions of experimental animals, as well as in atherosclerotic lesions in human coronary arteries and aortic aneurysms.[64,65]

Cellular senescence seems to correlate with endothelial dysfunction and the entire inflammatory process of atherosclerosis. As shown in recent experimental and clinical studies, many well-known atherosclerotic CVD risk factors, both traditional and nontraditional, appear to induce cellular senescence. Cellular senescence biomarkers seem to predict atherosclerotic CVD events, whereas some antiatherosclerotic treatment modalities may act in part through the delay of cellular senescence.

Endothelial Dysfunction and Cellular Senescence

Aging transforms the phenotype of the vascular endothelial cell from antiatherosclerotic to proatherosclerotic.[14,66] Nitric oxide (NO) is a crucial factor for endothelial function.[67,68] Not only does NO regulate vascular tone and improve its antithrombotic and anti-inflammatory activity, but it also enhances endothelial cell survival by inhibiting apoptosis.[69,70] Aging downregulates endothelial NO synthase (eNOS) expression and activity and thus NO production.[71] Stable expression of hTERT, which increases telomerase activity and induces a younger phenotype in endothelial cells, restores eNOS activity, reestablishing properly functioning endothelium.[71] In addition, increasing NO bioavailability or eNOS activity activates telomerase and delays endothelial cell senescence.[72,73]

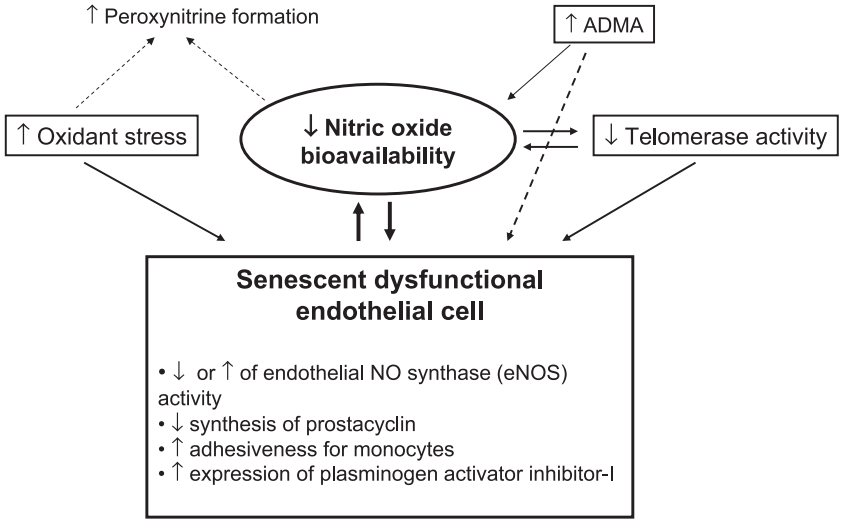

Figure 3. Relationship between endothelial cell senescence and nitric oxide (NO). Factors that induce endothelial cell senescence (oxidant stress,[74-76] asymmetrical dimethylarginine [ADMA],[77] reduced telomerase activity[71-73]) and senescent endothelium per se[71] decrease NO bioavailability, strongly contributing to endothelial dysfunction.

Mild chronic oxidative stress accelerates telomere erosion and the onset of senescence in normal endothelial cells.[74] Constitutive activation of Rac1, a protein that belongs to the Rho family of small guanosine triphosphatases and is a regulatory component of the plasma membrane reduced form of nicotinamide-adenine dinucleotide phosphate oxidase, enhances mitochondrial oxidative stress and induces premature senescence in endothelial cells.[75] Moreover, vascular aging in rat aortas appears to be initiated by enhanced superoxide production, followed by trapping of NO and subsequent peroxynitrite formation.[76] Interestingly, in this same study, eNOS expression and activity was increased, potentially as a compensatory mechanism.[76] Finally, asymmetrical dimethylarginine, an endogenous inhibitor of NOS, accelerates endothelial cell senescence, probably through increased oxygen radical formation and inhibition of NO production[77] (Fig 3).

Relationship Between the Inflammatory Atherosclerotic Process and Cellular Senescence

The main cell types involved in the inflammatory process of atherosclerosis, namely circulating monocytes (which adhere to the endothelium and transmigrate to the internal vascular wall, where they are transformed initially into macrophages and then, after ingestion of oxidized lipids, into foam cells), VSMCs (which migrate from the vascular media to the intima and participate in the formation of atherosclerotic plaques), and endothelial cells, are all influenced by the process of cellular senescence. It seems that whether the atherosclerotic process and its dramatic complications are induced by the senescence phenotype depends on the specific cell type involved, as well as on the phase of atherosclerotic plaque development (Fig 4, step 3).

Microarray analysis showed that different cell types that enter into senescence, including vascular endothelial cells, are locked in an activated state indicative of an inflammatory-type response.[78] Senescent lymphocytes also produce increased amounts of proinflammatory cytokines, such as tumor necrosis factor α, which may contribute to the inflammatory atherosclerotic process.[79,80] The initial event in atherosclerosis, monocyte adhesion to the endothelium, is induced when senescence is established in endothelial cells.[81] While overexpression of intercellular adhesion molecule 1 in senescent endothelial cells probably mediates enhanced adhesion,[81] exposure to tumor necrosis factor α (also overexpressed by senescent cells) further augments adhesion of monocytes to the endothelium[71] (Fig 4, steps 1 and 2).

Cell proliferation characterizes atherosclerosis development,[82] whereas resolution of inflammation is promoted by apoptosis of the accumulated inflammatory cells.[83,84] Reduced macrophage apoptosis, possibly in combination with the delay in the parallel process of cellular senescence, was associated in experimental studies with accel-

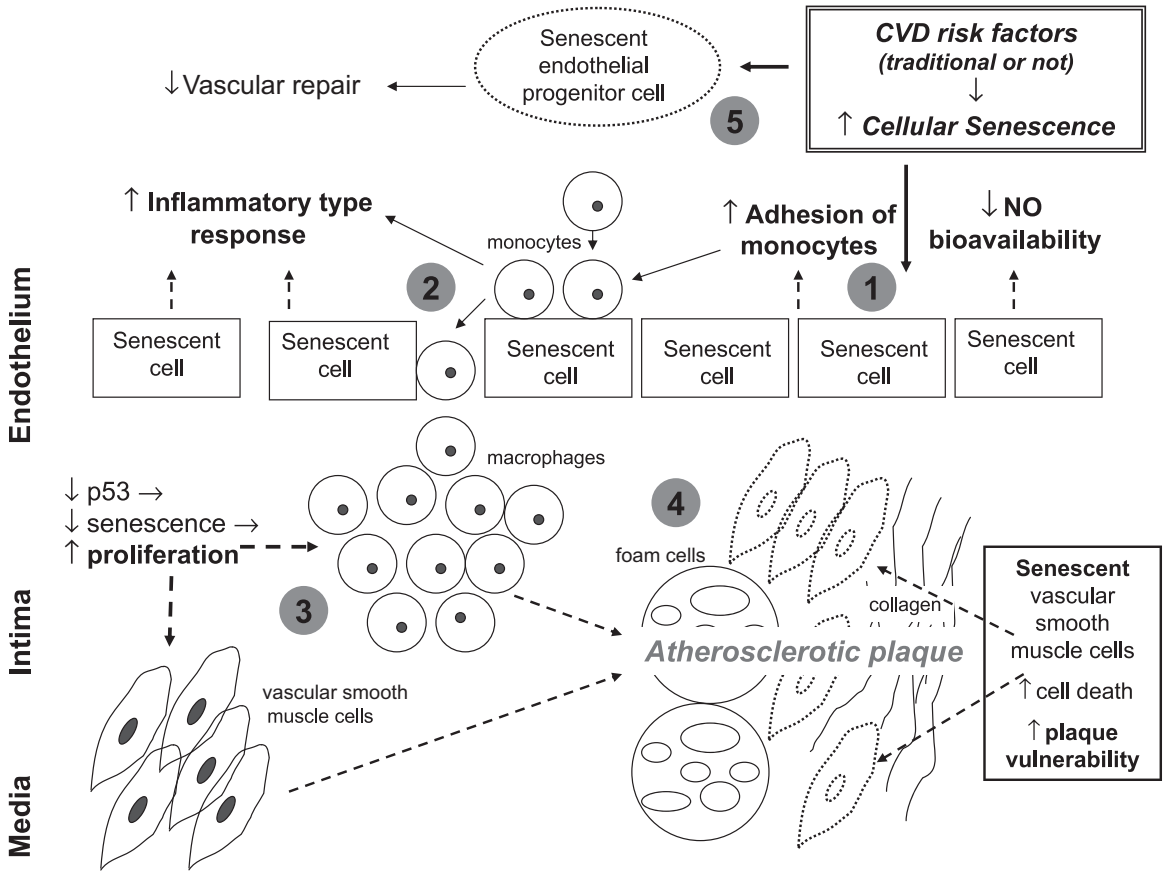

Figure 4. Cellular senescence, endothelial dysfunction, and the inflammatory process of atherosclerosis. 1. Inflammatory type response,[78] reduced nitric oxide bioavailability[71,76], and increased adhesiveness for circulating monocytes[71,81] characterize, among other properties, senescent endothelial cells. 2. This last property facilitates the initial event in the process of atherosclerosis: monocyte adhesion to endothelium and transmigration of these cells in the internal vascular wall (intima). 3. Proliferation of macrophages in intima and vascular smooth muscle cells (VSMCs) in media promotes atherosclerosis development; at this stage of the atherosclerotic process, inhibition and not induction of senescence in the specific cell types is what stimulates atherosclerosis progression.[86-90] 4. VSMCs in the fibrous cap of the atherosclerotic plaque, but not in media of the vessel wall, show characteristics of cellular senescence, increasing the probability of cell death and plaque destabilization.[91,92] 5. Oxidative stress and other traditional/nontraditional cardiovascular disease risk factors induce endothelial progenitor cell senescence, altering the vascular repair capacity of these cells.

erated atherosclerosis.[85] As shown in a number of in vivo studies using atherosclerosis-prone mice, a deficiency in p53, one of the main factors involved in senescence signaling (Fig 1), accelerates atherosclerosis development, possibly by increasing macrophage proliferation in atherosclerotic lesions.[86-88] Moreover, a recent publication showed that activation of peroxisome proliferator-activated receptor γ, the molecular target for insulin-sensitizing thiazolidinediones in patients with type 2 diabetes, suppresses telomerase activity in VSMCs, thus inhibiting their proliferation and preventing atherosclerosis development.[89] Finally, the same antiproliferative effect on lymphocytes and macrophages seems to be offered by short telomeres that protect apolipoprotein E–null mice from diet-induced atherosclerosis[90] (Fig 4, step 3).

VSMCs in fibrous caps of human atherosclerotic plaques, but not those found in the media of normal vessels, show characteristics of cellular senescence (β-galactosidase staining, expression of p16 and p21, telomere shortening, and reduced telomerase activity).[91] In vivo, plaque VSMCs show oxidative DNA damage; in vitro, oxidants induce premature cellular senescence.[91] In addition, higher expression of hTERT confers a younger phenotype in the same type of cells

Table 1. CVD Risk Factors Associated With Cellular Senescence Induction, Telomere Shortening, and Telomerase Activity Reduction

CVD risk factors that induce cellular senescence (in vitro)
Cigarette smoke extract[95]
High glucose exposure[73]
Treatment with angiotensin II[97]
Estrogen inhibition[73]
Traditional and novel CVD risk factors associated with telomere shortening in circulating blood cells (clinical studies)
Insulin resistance[101,108]
Impaired glucose tolerance[102]
Diabetes[102,103,104]
Body mass index[101]
Cigarette smoking[107]
Hypertension[108]
Pulse pressure[106]
Oxidative DNA damage[104]
↑ Catecholamines, cortisol[110]
Traditional and novel CVD risk factors associated with reduced telomerase activity in circulating blood cells (clinical studies)
Poor lipid profile, smoking, high systolic blood pressure, high fasting glucose level, abdominal adiposity[110]
Psychological stress[109]
↑ Autonomic reactivity to acute mental stress[110]

Abbreviation: CVD, cardiovascular disease.

despite telomere shortening.[91] Adenovirus-mediated transfer and overexpression of p53 in VSMCs in a murine atherosclerotic plaque model resulted in a marked decrease in cellular and extracellular content of the fibrous cap, subsequently transforming the plaque into a vulnerable one.[92] Death through apoptosis and possibly as a result of cellular senescence of VSMCs in the fibrous cap destabilizes the atherosclerotic plaque, increasing the probability of rupture.[93] Greater production of proinflammatory cytokines from senescent cells[80] may also contribute to plaque vulnerability[94] (Fig 4, step 4).

Atherosclerotic Cardiovascular Risk Factors and Cellular Senescence

Experimental Data

A number of in vitro and in vivo studies showed that such factors as cigarette smoking, high levels of glucose or advanced glycation end products, angiotensin II (AG-II), impairment of circadian rhythmicity, estrogen deficiency, hypertension, and excess endothelin production induce cellular senescence in many cell types and in experimental animals (Table 1).

Recently, it was shown that multiple exposures to cigarette smoke induced a classic senescence phenotype (β-galactosidase staining, flat and enlarged morphology, p16 overexpression).[95]

Exposure of human umbilical vein endothelial cells to high concentrations of glucose promoted cellular senescence and decreased telomerase activity.[73] When these cells were grown on glycated collagen, they expressed hallmarks of premature cellular senescence (staining with β-galactosidase and p53 and p14 overexpression)[96]; in addition, NO production decreased, whereas eNOS expression and nitrotyrosine-modified proteins increased.[96] In the same study, increased frequency of prematurely senescent cells also was observed in young Zucker diabetic rats compared with lean controls.[96]

Treatment of VSMCs with AG-II was observed to induce premature cellular senescence (overexpression of p53/p21) and increase the production of proinflammatory cytokines through nuclear factor-κB activation; both effects were neutralized by blocking the p21 pathway.[97] The same effects of AG-II were also observed in vivo in a mouse model of atherosclerosis.[97]

Circadian rhythms, including the rhythms of blood pressure, are regulated by a set of clock genes that generate circadian oscillation with a 24-hour cycle. Circadian expression of clock genes in senescent cells was significantly weaker than in young cells.[98] Introduction of telomerase completely prevents this reduction.[98] Estrogens induce hTERT expression and telomerase activity,[99] and estrogen treatment reduces the number of β-galactosidase–positive endothelial cells while also activating telomerase.[73] Finally, in a recent publication, a direct link between telomerase activity and hypertension was reported.[100] Mice lacking TERC ($TERC^{-/-}$) showed higher arterial pressure than wild-type mice as a result of an increase in plasma endothelin 1 levels, a consequence of endothelin-converting enzyme overexpression.[100]

Clinical Studies

Accelerated telomere shortening and, in some instances, reduced telomerase activity in peripheral-blood mononuclear cells (PBMCs) was associated with a number of established and putative CVD risk factors (Table 1).

Relative changes in telomere length correlated with the homeostasis model assessment of insulin resistance and changes in body mass index in a study of young adults with more than 10.1 to 12.8 years of follow-up.[101] In 2 recent studies, the presence of diabetes[102,103] and impaired glucose tolerance[102] correlated with telomere length in leukocyte DNA.[102] In another study, in a group of patients with type 2 diabetes, PBMC telomere erosion and oxidative DNA damage were significantly greater than in the control group.[104] Finally, in premenopausal women, insulin resistance and C-reactive protein (CRP) levels correlated inversely with leukocyte telomere length.[105]

Telomere length measured in white blood cells of 49 twin pairs from the Danish Twin Register was shown to be highly familial and inversely correlated with pulse pressure.[106] Obesity and cigarette smoking inversely correlated with telomere length in white blood cells in women,[107] whereas hypertension, oxidative stress, and increased insulin resistance were associated with shorter leukocyte telomere length in men in the Framingham Heart Study.[108]

In a carefully designed study, telomere length and telomerase activity in PBMCs were significantly lower in a group of 39 premenopausal women with high psychological stress (having a child who was chronically ill) than in 19 other mothers of healthy children who were under low psychological stress.[109] The same group of investigators found that low telomerase activity in leukocytes of 62 healthy women was associated with exaggerated autonomic reactivity to acute mental stress and increased nocturnal epinephrine levels.[110] In the same study, low telomerase activity in leukocytes was associated with smoking, poor lipid profile, high systolic blood pressure, high fasting glucose level, and greater abdominal adiposity, whereas telomere shortening correlated only with increased levels of stress hormones (catecholamines and cortisol).[110] The investigators proposed that low leukocyte telomerase activity constituted an early marker of CVD that is more sensitive than telomere shortening[110] (Table 1).

Senescence of Progenitor Cells and Cardiovascular Risk

Endothelial progenitor cells (EPCs) have an important role in endothelium integrity and damage repair.[111] Many CVD risk factors modulate progenitor cell levels and quality, consequently affecting the vascular repair capacity.[49] It seems that induction of senescence in these cells is an important mechanism mediating EPC dysfunction[14,111,112] (Fig 4, step 5). Ang-II accelerates EPC senescence through oxidative stress induction and reduction of telomerase activity.[113] The same effect results after exposure of EPCs to oxidized low-density lipoprotein.[114] Finally, CRP, an emerging CVD risk marker and potential risk factor, inhibits EPC survival and induces apoptosis through reduction of antioxidant defenses and telomerase inactivation.[115,116] These in vitro data also seem to be valid in vivo; EPC senescence is accelerated in both experimental hypertensive rats and patients with essential hypertension.[117]

Telomere Length and Prediction of Atherosclerotic Cardiovascular Events

In an increasing number of studies, telomere length measured in leukocyte or PBMC DNA correlated with incident or prevalent atherosclerotic CVD or other causes of morbidity and mortality. Although circulating blood cells are the most accessible tissue to measure such a biomarker of cellular senescence as telomere length, some drawbacks exist.[118] Telomere length in circulating blood cells is determined by a series of genetic,[49,119-121] epigenetic,[122,123] and environmental[101,107,109,119,124] factors that have to be taken into consideration when interpreting results of these studies.

Generally, it is commonly accepted that telomere length in circulating cells reflects the biological age of an individual. Furthermore, the same parameter could indicate some characteristics of these cells, such as production of higher amounts of inflammatory mediators[125] or the presence of chronically acting oxidative stress, which induce accelerated erosion of telomeres that might be directly implicated in the mechanism of atherosclerosis development and progression.[126-128]

Initially, Samani et al[129] showed that telomere length in leukocytes of 10 patients with angiographically detected severe CHD was significantly shorter than in 20 healthy controls, after adjustment for age and sex. Specifically, the investigators calculated that telomere size in patients with CHD was equivalent to that of healthy individuals who were 8.6 years older.[129] Two

years later, Cawthon et al[130] found that the mortality rate from heart disease was 3.18-fold greater in 143 healthy individuals older than 60 years with shorter telomeres in blood DNA.[130] In the same year, Brouilette et al[131] compared telomere length in leukocyte DNA of a group of 203 individuals with a premature myocardial infarction (<50 years) with that of 180 age- and sex-adjusted controls. They found that compared with subjects in the highest quartile length, individuals with shorter than average telomeres had a 2.8- to 3.2-fold greater risk of myocardial infarction.[131] Obana et al[132] found that hypercholesterolemic patients and/or those with diabetes with CHD had shorter PBMC telomeres than healthy controls. Similarly, Benetos et al[133] found that telomere length in DNA extracted from white blood cells was shorter in hypertensive men with carotid artery plaques than hypertensive men without plaques. Recently, Collerton et al[134] found that telomere length in PBMCs of very old patients (from the 85+ Newcastle study) was associated with left ventricular function. Conversely, in another recent publication, Bischoff et al[135] found no association between telomere length and survival in 812 subjects aged 73 to 101 years.

Two recent publications are probably the most important. In the first, Fitzpatrick et al[136] measured leukocyte telomere length in 419 randomly selected participants from the Cardiovascular Health Study and investigated associations with a number of CVD risk factors, as well as with incident CVD, after a follow-up of 7 years. Inverse associations were found between telomere length and diabetes, diastolic blood pressure, carotid intima-media thickness, and levels of glucose, insulin, and interleukin 6. In younger (≤73 years) individuals, each kilobase decrease in terminal restriction fragment length (a measure representative of telomere length), corresponded with a 3-fold increase in risk of myocardial infarction and stroke.[136] The investigators concluded that their findings supported the hypothesis that telomere attrition may be related to diseases of aging through mechanisms involving oxidative stress, inflammation, and progression of CVD.[136]

In the second study, which used a randomized case-control design, Brouilette et al[137] compared leukocyte telomere length at recruitment in 484 participants in the West of Scotland Primary Prevention Study who went on to develop CHD events with 1,058 matched controls who were free of events. Individuals in the middle and lowest tertiles of telomere length were more at risk of having a CHD event (odds ratios, 1.55 and 1.44, respectively) than individuals in the highest tertile.[137] It is worth noting that the risk of CHD associated with shorter telomeres was similar to the risk associated with many other traditional CVD risk factors; for example, odds ratios for body mass index, low-density lipoprotein cholesterol, and diabetes with hypertension were 1.08, 1.43, 1.51, and 1.65, respectively. In patients treated with pravastatin, the increased risk of CHD associated with shorter telomeres observed in the placebo group was significantly attenuated (odds ratio, 1.12 versus 1.93 in the middle tertile and 1.02 versus 1.94 in the lowest tertile of telomere length, respectively).[138] No difference in changes in values for low-density lipoprotein and high-density lipoprotein cholesterol, triglycerides, CRP, fibrinogen, and plasma viscosity were observed from recruitment to the end of year 1 with statin treatment in individuals with different telomere lengths. As the investigators concluded, leukocyte telomere length was associated with future CHD events in middle-aged high-risk men and may identify individuals who would benefit most from statin treatment.[137] As previously emphasized, the design and quality of this study were superior to all previous studies that investigated the association of telomere length and CVD risk.[139]

Treatment Options for Delay of Cellular Senescence: Cardiovascular Risk Reduction

Decreased CVD risk after statin treatment in West of Scotland Primary Prevention Study[137] may be caused by senescence prevention in EPCs through regulation of various cell-cycle proteins.[138] Moreover, statins seem to upregulate the expression of telomere repeat-binding factor, an important protein for telomere capping, thus preventing EPC senescence.[140] Finally, statins, such as atorvastatin, may delay senescence of endothelial cells by reducing overproduction of intracellular reactive oxygen species, consequently inhibiting nuclear export of TERT.[141] Similarly, *N*-acetylcysteine, a well-known anti-

oxidant, appears to have the same antisenescent effect in endothelial cells through reactive oxygen species reduction and inhibition of TERT nuclear export.[141]

Ebselen, a peroxynitrite scavenger, seems to prevent the increase in senescent endothelial cells observed in Zucker diabetic rats, a well-known experimental model of metabolic syndrome.[142] In addition, aspirin also was shown to prevent endothelial senescence, possibly by increasing NO bioavailability,[143] whereas L-arginine in asymmetrical dimethylarginine– or homocysteine-accelerated endothelial senescence seems to have the same effect through NO and heme-oxygenase-1 formation and induction.[144] Finally, raloxifene, a selective estrogen receptor modulator, seems to induce telomerase activity in umbilical vein endothelial cells through transcriptional and posttranscriptional regulation of hTERT.[145]

PREMATURE CELLULAR SENESCENCE IN PATIENTS WITH CKD

Evidence

Recently, a small number of publications focused on the investigation of telomere and telomerase biology in PBMCs of patients with CKD. Ramirez et al[146] examined PBMCs isolated from 15 hemodialysis (HD) patients and 15 age-matched controls. In a subpopulation of these cells, they found accelerated telomere shortening, increased p53 expression, and proinflammatory cytokine overproduction.[146] The percentage of cells with short telomeres correlated positively with CRP level. The investigators proposed that these senescent cells probably resulted from repeated activation and may have a pathophysiological role in the chronic inflammation described in this population.[146,147] Boxall et al[148] measured telomere length in PBMCs in 20 nondiabetic and 18 diabetic HD patients and 20 control subjects. They found no difference in mean telomere length between HD patients and controls, but they found an inverse correlation between telomere length and duration of HD in patients with diabetes.[148] Our group measured telomerase activity in PBMCs isolated from 42 HD patients and 39 control subjects and found that telomerase activity was detected in 43% of control subjects, but only 18% of HD subjects.[149] In individuals with detectable telomerase activity, the percentage of telomerase activity was significantly greater in controls. Although long-term and short-term HD patients had identical chronological ages, detectable telomerase activity was significantly lower in the former compared with the latter (13.3% $\pm$ 8.9% versus 75.0% $\pm$ 64.8%). We further investigated inflammation-oxidative stress-telomerase activity relationships in the same group of healthy controls and HD patients.[150] We found that in HD patients and control subjects, oxidized low-density lipoprotein and tumor necrosis factor α both inversely correlated with telomerase activity in PBMCs.[150] By separately examining HD patients, multivariate analysis showed that oxidized low-density lipoprotein and HD duration were the only significant predictors for percentage of telomerase activity in PBMCs.[150]

In summary, it seems that telomere-telomerase biological characteristics are altered in PBMCs of patients with CKD on HD therapy. Premature senescence appears to characterize this type of cell in this chronically sick population, and low-grade inflammation as well as oxidative stress may correlate with it.

The Case for Premature Cellular Senescence as a Nontraditional CVD Risk Factor in CKD

A number of traditional and nontraditional atherosclerotic CVD risk factors seem to be associated with premature cellular senescence induction, including telomere erosion and telomerase activity reduction (Table 1). Diabetes, hypertension, inflammation, and oxidative stress are the predominant causes and consequences of CKD. At the same time, they are also leading atherosclerotic risk factors in this population. The low-grade chronic inflammation frequently observed to be increased in these patients characterizes the senescence phenotype of many cell types and is also interrelated to atherosclerosis. Moreover, oxidative stress is a hallmark of CKD and is related to stress-induced premature cellular senescence and atherosclerosis. The limited published data for patients with CKD support the existence of premature cellular senescence, at least in PBMCs, as well as its correlation to inflammation and oxidative stress in this population. Furthermore, an increasing number of stud-

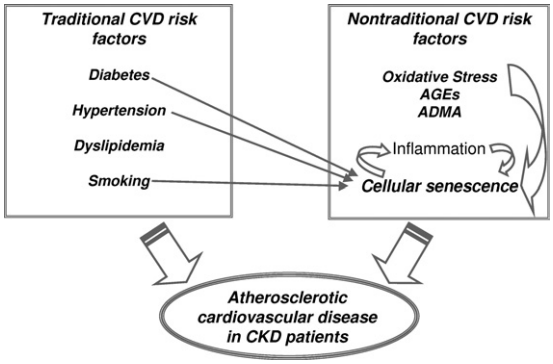

Figure 5. Cellular senescence as a nontraditional cardiovascular disease (CVD) risk factor in patients with chronic kidney disease (CKD). A number of traditional (diabetes, hypertension) and nontraditional (oxidative stress, advanced glycation end products [AGEs], asymmetrical dimethylarginine [ADMA], inflammation) CVD risk factors induce cellular senescence. Accumulation of senescence-inducing factors in patients with CKD may influence atherosclerosis development through premature cellular senescence. Thus, cellular senescence could be proposed as an emerging nontraditional CVD risk factor for this biologically aged population.

ies of the general population showed that telomere shortening in blood-circulating cells, as also described in patients with CKD, may be a new predictor of atherosclerotic CVD events.

In conclusion, after considering experimental data, extrapolating from clinical studies performed in the general population,[151] and examining the limited data published for renal patients, premature cellular senescence could be proposed as an emerging CVD risk factor for patients with CKD (Fig 5).

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