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Vascular endothelial senescence: from mechanisms to pathophysiology

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Erusalimsky JD. Vascular endothelial senescence: from mechanisms to pathophysiology. *J Appl Physiol* 106: 326–332, 2009; doi:10.1152/jappphysiol.91353.2008.—Most mitotically competent mammalian cell types can react to stress by undergoing a phenotypically distinctive and permanent form of growth arrest called “cellular senescence.” This response has been extensively characterized in cell culture and more recently it has been found to occur also in vivo in a number of tissues. In this review I will present the case for the occurrence of senescence in the vascular endothelium. I will also discuss the mechanisms and factors that modulate endothelial cell replicative capacity and the onset of senescence. Finally, I will examine the senescent phenotype and its possible consequences for the development and progression of vascular diseases.

endothelium; oxidative stress; telomere

DISEASES OF THE VASCULAR SYSTEM have long been considered to be age related in terms of their onset and progression (45). Ageing is associated with endothelial dysfunction (9, 87, 94), arterial stiffening and remodeling (49), impaired angiogenesis (72), defective vascular repair (94), and with an increasing prevalence of atherosclerosis (18, 81, 93). The reasons for these associations are still unclear, but it is plausible that organismal ageing and vascular disease may share common cellular mechanisms. One process that has been increasingly linked to both ageing and the development of vascular pathologies is cellular senescence (19, 27, 56).

Senescence is a stress and damage response phenomenon that locks up mitotically competent diploid cells in a permanent form growth arrest. Senescent cells undergo distinct changes in gene expression that may cause an impairment of cellular function. In endothelial cells these changes result in a phenotype that is pro-inflammatory, pro-atherosclerotic, and pro-thrombotic. Endothelial cell senescence can be induced by a number of factors implicated in vascular pathologies, particularly by sustained cell replication and oxidative stress. In this review I will examine the occurrence, mechanisms, and pathophysiological implications of this process as they emerge from cell culture and in vivo studies.

MECHANISMS OF SENESCENCE—AN OVERVIEW

Senescence was initially considered to reflect the finite capacity for division that normal diploid cells exhibit when propagated in culture (reviewed in Ref. 35), hence the term “replicative senescence.” At the molecular level senescence resulting from successive rounds of cell division has been linked to the progressive shortening and eventual dysfunction of telomeres, the physical ends of chromosomes (re-

viewed in Refs. 28, 77). In mammalian cells telomeres consist of a repeated DNA sequence (TTAGGG) that extends over a length of several thousand base pairs and associates to an array of specialized telomere binding proteins. Synthesis of telomeric DNA requires the presence of telomerase, a ribonucleoprotein complex that catalyses the addition of TTAGGG repeats to the 3'-end of the DNA chain, using as a template a complementary sequence within the RNA portion of the complex (50). The majority of human adult somatic cells either lack or have very low levels of telomerase (25). Under these conditions, and due to the inability of conventional DNA polymerases to replicate the end of the lagging strand, DNA synthesis during cell division results in a gradual loss of telomeric DNA (68). In addition, due to its high GGG content, telomeric DNA is particularly susceptible to oxidative damage and the generation of single strand breaks. Accordingly, the rate of telomere erosion is also greatly affected by the oxidative burden of the cell (91). Telomere erosion eventually compromises its functional integrity and leads to the induction of a DNA damage checkpoint response that halts the cell cycle permanently (15).

Senescence can also be triggered by telomere-independent events including non-telomeric DNA damage and persistent mitogenic stimulation (76). In cell culture these events induce an acute form of senescence, sometimes termed “stress-induced premature senescence” (82), which does not require extensive cell proliferation but which otherwise resembles that induced by damaged telomeres. Ultimately, the majority of senescence-inducing signals engage either or both the p53/p21 and p16/retinoblastoma protein tumor suppressor pathways, as the final effectors of the senescence program (reviewed in Ref. 8). The involvement of these mechanisms highlights the notion that senescence, although harmful in later life, might have evolved as barrier to cell transformation, promoting survival of the young to reach the stage of reproductive maturity (7).

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EVIDENCE THAT ENDOTHELIAL CELL SENEESCENCE OCCURS IN VIVO

While the occurrence of senescence in cell culture has been extensively documented, it is only recently that the importance of this phenomenon in vivo has begun to be appreciated. Senescent cells have been found in a number of mammalian tissues in association with ageing, age-related pathologies, hyperplastic lesions, and cellular stress (reviewed in Refs. 8, 19). In the case of the endothelium, firm evidence of its occurrence was obtained by several laboratories using senescence-associated β -galactosidase (SA- β -gal) as a histochemical marker. Using this method, senescent endothelial cells were found to accumulate after repeated balloon endothelial denudation of the rabbit carotid artery, an injury model that provokes endothelial and smooth muscle cell proliferation (24). Similarly, other laboratories demonstrated the presence of senescent endothelial cells overlying atherosclerotic plaques of human aorta and coronary arteries (57, 90) and in the aortae of diabetic rats (12). In addition, the occurrence of stress-induced endothelial cell senescence has recently been demonstrated in a murine model of oxidative stress (70).

The occurrence of endothelial cell senescence in vivo has also been inferred from examination of telomere length in the vasculature. A number of independent studies have shown that telomeres in the endothelium shorten with age and that this erosion is more pronounced in atherosclerosis-prone areas (2, 10, 67). Furthermore, a study examining the relationship between telomere length and coronary artery disease found that telomeres in endothelial cells derived from diseased portions of arteries were shorter than those from non-diseased regions (66).

FACTORS AFFECTING ENDOTHELIAL CELL SENEESCENCE

Studies on cultured endothelial cells have shown that the onset of senescence can be modulated by a plethora of factors affecting vascular function. These include mitogens (48), inflammatory molecules (5), angiotensin II (40), oxidants and antioxidants (26, 47), nitric oxide (89), high glucose (96), advanced glycation end-products (AGEs) (12), and mitochondria (74). Most of these factors influence senescence via two main processes: by altering the intracellular levels of cellular oxidative stress and/or by modulating telomerase activity (Fig. 1).

Oxidative Stress

Oxidative stress is a major stimulus for the induction of senescence (62). A substantial body of evidence indicates that in endothelial cells reactive oxygen species (ROS) generated from either intracellular or extracellular sources can induce or accelerate the development of senescence by acting at multiple subcellular levels (20). As indicated above, telomeres are particularly susceptible to oxidative damage. In addition, besides affecting telomeres directly, or indirectly via inhibition of telomerase (see below), ROS can induce senescence by telomere-independent mechanisms. The latter include direct damage to genomic DNA, mitochondrial damage, and activation of cytosolic stress response kinases or other redox-sensitive signaling proteins that have been implicated in senescence responses (Fig. 1).

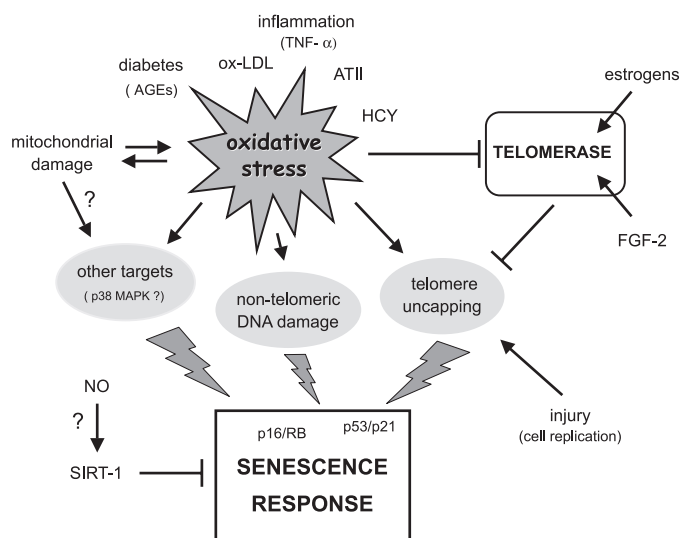


Fig. 1. Factors that induce endothelial cell senescence. ATII, angiotensin II; HCY, homocysteine; ox-LDL, oxidized LDL.

Oxidative stress and telomere damage. In endothelial cells, an association between oxidative stress, accelerated telomere shortening, and senescence has been suggested by studies in which the intracellular redox environment was manipulated by incubation with a vitamin C analog (26), by exposure to homocysteine (95), or by interference with the glutathione (GSH) redox cycle (47). In contrast, telomere dysfunction was not apparent when oxidative stress-induced senescence was caused by exposure to an AGE (12).

ROS as mediators of sustained mitogenic stimulation. Excessive mitogenic stimulation caused by forced expression of activated oncogenes is known to induce senescence (76). In endothelial cells this phenomenon has been observed when active forms of Akt (58), Ras (80), or Rac1 (16) were overexpressed. Promotion of senescence under these conditions is thought to result from a dysregulation of the cellular redox-balance leading to an increase in ROS production which in turn may stimulate p53 activity (reviewed in Ref. 20). The pathophysiological significance of these findings may lie in the fact that pro-atherogenic conditions, such as hyperinsulinaemia, chronic inflammation, and hypercholesterolaemia, are known to activate Akt- and Ras-mediated signaling in endothelial cells.

The role of mitochondria. ROS can damage mitochondrial DNA and other redox-sensitive components of this organelle, thus impairing mitochondrial function. Mitochondria by themselves generate ROS during normal respiration, and some studies suggest that when the normal function of the electron transport chain is affected ROS output may be augmented, thus increasing the oxidative burden of the cell (reviewed in Refs. 3, 52). Recently, the importance of mitochondria-derived ROS in the induction of endothelial cell senescence has been highlighted by a study examining the role of prohibitin-1 (PHB1) in this process (74). PHB1 is a constituent of the inner mitochondrial membrane thought to be important for the maintenance of mitochondrial functional integrity (65). It has now been demonstrated that knockdown of PHB1 in endothelial cells increases mitochondrial ROS generation, which causes cellular senescence (74). In this study the induction of senescence was

considered to be the consequence of a sustained ROS-dependent Akt activation. However, other ROS-induced signaling mechanisms or direct oxidative damage cannot be discounted at this stage. Aside from the precise identity of the downstream effectors of mitochondrial ROS, the significance of this finding may have implications beyond understanding the function of PHB1 in senescence. In this regard, the complex interplay between levels of NO, oxygen availability, and the redox environment may have an important role (21).

Modulation of Telomerase Activity

In endothelial cells levels of telomerase activity are substantially lower than those found in a typical cancer cell line or in cells from other renewable tissues (48). Nevertheless, expression in these cells could be physiologically relevant since its overall activity is growth regulated (39, 48) and inhibition by genetic means is associated with a reduction in the replicative capacity of the cells (22).

Studies on the regulation of this enzyme in endothelial cells have implicated endothelial cell mitogens (17, 48), nitric oxide (34, 89), inflammatory mediators (5), and oxidative stress (30, 47). Regulation has been reported to occur at multiple levels, including transcription (17, 48), post-translational Akt-mediated phosphorylation of the telomerase catalytic subunit (TERT) (5, 17), redox-controlled changes in intracellular localization (30), and oxidative modification of TERT (4).

The role of endothelial cell mitogens. A number of growth factors known to be important for endothelial homeostasis and angiogenesis have been investigated for their effects on telomerase activity and senescence (41, 48). Among these, FGF-2 was shown to enhance telomerase activity and to maintain cellular replicative life span, in association with an increase in TERT transcripts. In contrast, VEGF-A, at concentrations that had the same mitogenic effect as FGF-2 did, had no effect on TERT levels or activity and was not able to support long-term endothelial cell replication (48, 83). Another study, however, investigating the role of telomerase in angiogenesis, concluded that VEGF-A can activate telomerase via NO signaling (97). Other studies have shown that estrogens also increase telomerase activity in endothelial cells via transcriptional and post-translational mechanisms, the latter involving engagement of the PI3K-AKT pathway and subsequent phosphorylation of TERT (17, 41). Also in these cases the effects were accompanied by retardation of the onset of senescence.

The role of NO. It is generally assumed that NO counteracts endothelial cell senescence by stimulating telomerase activity and reducing telomere erosion (34, 89). However, experiments using a combination of pharmacological tools and silencing RNA technology suggest that this might not be the case (38). Indeed, manipulation of NO levels by either NO donors, NO synthase (NOS) inhibitors, or downregulation of endothelial NOS (eNOS) by RNA interference, had no effect on telomerase activity, cellular replicative capacity, or the accumulation of senescent cells (38). Similarly, eNOS inhibition or NO donors failed to affect telomerase activity or endothelial progenitor cell senescence in other studies (1, 5), in disagreement with initial claims from the same laboratory (89).

Recent findings examining the role of SIRT1 in the regulation of endothelial cell senescence (70) could shed some light on the above conflicting results. SIRT1 is a NAD⁺-dependent

protein deacetylase involved in the regulation of energy metabolism, stress responses, and cell survival (31). In endothelial cells, overexpression of SIRT1 prevents oxidative stress-induced premature senescence (69), most probably by promoting the deacetylation and consequent inactivation of p53 (51). Importantly, NO donors or a phosphodiesterase (PDE) III inhibitor known to increase eNOS activity (32) have been recently shown to upregulate SIRT1 expression and to inhibit the onset of senescence in cells subjected to oxidative stress, with the effects of the PDEIII inhibitor on senescence being abrogated by SIRT-1 downregulation. Notably, non-stressed cells exhibited high constitutive levels of SIRT-1 that were not affected by the putative increase in NO production (70). While the above study also showed an increase in telomerase activity upon inhibition of senescence, this effect may be attributed to the maintenance of the cells in a proliferative state (48) rather than to a direct effect of SIRT-1 or NO on telomerase. Taken together these findings suggest that NO may counteract senescence in the context of cellular stress, including inadvertent cell culture stress, through upregulation of SIRT1. It should be mentioned, however, that in cells with higher levels of telomerase such as immortalized cell lines, fibroblasts expressing telomerase ectopically and hematopoietic stem cells, SIRT-1 acts a negative regulator of growth and this behavior is associated with a decrease in telomerase activity (64). The notion that activation of SIRT-1 may have opposing effects in different cells and organisms is not new (43), thus suggesting that its role in counteracting endothelial cell senescence may be specific to this cell type.

The role of oxidative stress in the regulation of telomerase activity. Substances that induce oxidative stress and have pro-atherogenic properties such as TNF- α and oxidized LDL were reported to reduce telomerase activity in endothelial cells in association with the inhibition of the PI3K/AKT pathway (5). In addition, increased generation of ROS has been shown to promote the translocation of TERT from the nucleus to the cytoplasm (30), thus preventing the enzyme from accessing the telomere. Furthermore, evidence from other cell types (4, 33) suggests that oxidation of a TERT cysteine residue that is sensitive to the intracellular levels of GSH may account, at least in part, for the decrease in activity that occurs in endothelial cells upon inhibition GSH synthesis (47) or nitrosative stress (22). While it is not clear what is the relative contribution of each of these mechanisms to the inactivation of telomerase in vivo, it is likely that their importance may ultimately depend on the nature and duration of the stress.

THE ENDOTHELIAL SENESENT PHENOTYPE AND ITS PATHOPHYSIOLOGICAL CONSEQUENCES

Apart from the alterations related to the block in cell replication, senescent endothelial cells show characteristic changes in gene expression, morphology, and function (20). Some of these changes may be important in affecting the regenerative and angiogenic capacity of the endothelium, its reactivity and the progression of atherosclerosis, and its clinical sequelae (Fig. 2).

Changes Affecting Regeneration and Angiogenic Potential

As a direct consequence of the permanent growth arrest, endothelial cell senescence may impair the repair capacity of

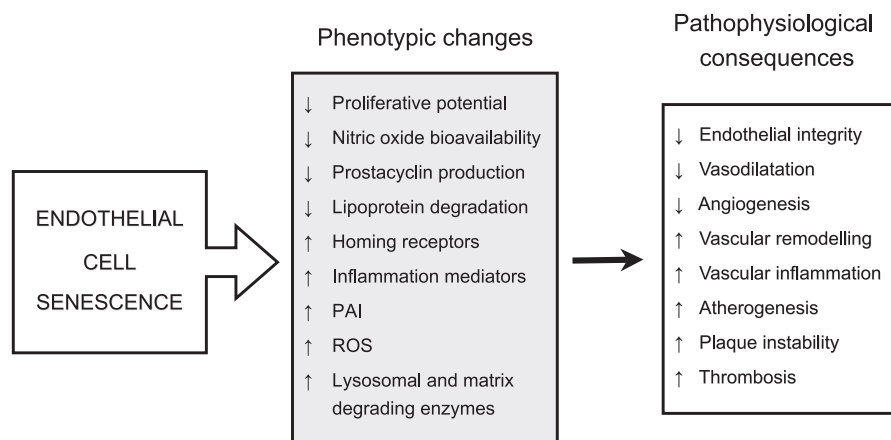


Fig. 2. The endothelial senescent phenotype and its pathophysiological consequences.

the endothelial lining (24). In this context the contribution of oxidative stress-induced senescence of circulating endothelial progenitor cells and in particular the involvement of angiotensin II in promoting this phenomenon, could also play an important role (36, 40, 42).

Endothelial senescence also results in changes in the expression levels of proteins associated with cellular architecture and cytoskeletal function (11, 44, 78, 90). These changes can affect the motility of the cells and these, in conjunction with the loss of replicative capacity may also reduce their angiogenic capacity. Indeed, a link between senescence and impaired angiogenesis has been established by several lines of evidence, including the demonstration that inhibition of telomerase reduces angiogenesis in tumor and therapeutic neovascularization models (23, 97), and that silencing of SIRT1 or PHB1 in endothelial cells, in addition to inducing senescence (69, 74), also abolishes their angiogenic properties (71, 74).

Pro-Atherogenic and Pro-Thrombotic Changes

Upon replicative senescence in culture, endothelial cells overexpress several proteins that typify the pro-inflammatory/pro-thrombotic phenotype of the endothelium in human atherosclerosis (61, 75, 88), including interleukin-1 α (54), the intercellular adhesion molecule ICAM-1 (53), and plasminogen activator inhibitor-1 (PAI-1) (14). ICAM-1 has been shown to increase during senescence induced by dysfunctional telomeres (57), whereas overexpression of PAI-1 has been demonstrated to occur both in replicative and stress-induced senescence (69, 95, 96).

A common feature of senescent endothelial cells is the presence of senescence-associated β -galactosidase (SA- β -gal) (86). This activity is a manifestation of an increase in lysosomal mass (46) and probably reflects the accumulation of autophagic vacuoles in the senescent cell containing non-degradable intracellular macromolecules and organelles. The increase in lysosomal mass has an uncertain underlying cause and while its pathophysiological significance in the arterial wall ought to be investigated, the possibility that the release of the vacuolar content could contribute to the inflammatory or pro-thrombotic phenotype, or even to the remodeling of the arterial wall, should be considered.

Senescent endothelial cells also undergo changes in the expression of proteins associated with the remodeling of the extracellular matrix (11, 29, 44, 78) and degrade less athero-

genic lipoproteins than their younger counterparts, suggesting that they have a reduced capacity to metabolize atherogenic lipids (90).

Changes Affecting Endothelium-Dependent Vasodilatation

Nitric oxide bioavailability is critical to normal endothelial function (60). Advanced age leads to impairment of endothelial NO production (84) and to increased inactivation of NO by superoxide (87), which contribute to age-related endothelial dysfunction (9, 98). A number of studies have investigated whether endothelial senescence may be involved in these phenomena. These studies have established that senescent endothelial cells have lower levels of eNOS activity and produce decreased levels of NO (37, 55, 73). This phenotypic change was also brought about after senescence was induced in aortic endothelial cells by introducing the dominant negative form of the telomere-binding protein TRF-2 (57). In contrast, endothelial cells bypassing senescence by ectopically expressing TERT displayed no decline in eNOS expression or NO production (55, 57). One of the most potent inducers of eNOS expression in endothelial cells is laminar shear stress. The effect of shear stress on eNOS expression was also markedly blunted in senescent endothelial cells, but again could be rescued by TERT overexpression. The same was true of the NO-mediated inhibition of monocyte adhesion to the endothelial monolayer (55). Levels of another important vasodilator, prostacyclin, were also found to be reduced in endothelial cultures undergoing senescence in vitro (63).

In contrast to endothelial cells that became senescent after successive rounds of replication, endothelial cells undergoing premature senescence due to AGE-induced oxidative stress actually increased eNOS expression, despite the fact that their ability to produce NO was reduced (12). Similarly, in the aortic wall of ageing rats in vivo, while NO production was impaired, eNOS was upregulated about sevenfold (87). This reduction in NO bioavailability was attributed to an increase in mitochondrial-derived ROS and concomitant production of peroxynitrite (87). Evidence of peroxynitrite formation and nitrosative stress, which could be prevented by anti-oxidant treatment, was also observed in association with senescence induced by AGEs in vitro (12) and by diabetes in vivo (6). While on the one hand these findings would be consistent with the occurrence of oxidative stress-induced senescence in the vasculature, it is important to point out that senescent endothelial cells produce

higher levels of ROS than their younger counterparts (85). Hence, oxidative stress is not only a stimulus for senescence but also an outcome of this process, which can also impinge on NO bioavailability.

Senescence and Apoptosis

The final fate of senescent cells may also have pathophysiological consequences because, given their altered phenotype, effective removal could be more beneficial for the maintenance of endothelial homeostasis. At present it is not clear whether in vivo senescent endothelial cells are removed by an active physiological mechanism, whether they accumulate indefinitely or simply succumb to the forces of flow, eventually shedding from the endothelial surface.

Studies examining the relationship between senescence and apoptosis in endothelial cells have yielded conflicting results. One study indicated that, unlike fibroblasts, the final fate of senescent endothelial cells is to undergo apoptosis (92). Other studies suggest that endothelial cell senescence by itself does not result in apoptosis, but rather it increases the sensitivity of these cells to apoptotic stimuli such as TNF- α and oxidized LDL (37, 80). In one case this effect has been attributed to the reduced levels of NO present in senescent cells (37) and this is consistent with the notion that physiological concentrations of NO promote cell survival (59).

Studies in HUVEC examining the effects of p53, p21, and p16 expression on the induction of apoptosis or senescence indicate that the p53/p21 pathway is involved in the induction of both phenomena, whereas the p16 pathway is only involved in the induction of senescence (13). In addition, these studies showed that the primary role of the p53/p21 pathway is in the control of apoptosis. Thus these findings suggest that in endothelial cells whether apoptosis occurs in association with senescence may also depend in part on the tendency of different types of stresses to engage one or both intracellular effector pathways.

SUMMARY AND FUTURE DIRECTIONS

The occurrence of endothelial cell senescence in the vasculature is gaining increasing recognition. Cell culture studies indicate that both cell turnover and oxidative stress may contribute to this phenomenon by inducing telomere shortening. Although the evidence for the occurrence of this process in the vessel wall in vivo is compelling, the existence of a causal relationship between telomere dysfunction and endothelial senescence awaits direct demonstration. In particular, while cell culture studies clearly show that endothelial cells express telomerase, that this activity is growth regulated and sensitive to the redox environment, and that telomerase promotes angiogenesis in models of disease, the relevance of these laboratory findings to human adult vascular homeostasis remains to be elucidated. In addition, oxidative stress may induce senescence by telomere-independent mechanisms and these may be more relevant in the context of vascular pathophysiology. Animal studies have confirmed that oxidative stress indeed induces senescence in vivo and that this may be particularly relevant in the context of type II diabetes (6). However, extending these findings to humans is currently hampered by the unavailability of non-invasive techniques to assess endothelial cell senescence in vivo. Development of new markers of

endothelial cell senescence amenable to clinical investigation will help to ascertain whether pharmacological interventions, such as statins and peroxynitrite scavengers that have demonstrable ability to reduce senescence in laboratory models of disease (6, 79), can also be effective in the human setting.

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