Premature senescence of endothelial cells: Methusaleh’s dilemma

Jun Chen and Michael S. Goligorsky
Departments of Medicine and Pharmacology, Renal Research Institute, New York Medical College, Valhalla, New York

Chen, Jun, and Michael S. Goligorsky. Premature senescence of endothelial cells: Methusaleh’s dilemma. Am J Physiol Heart Circ Physiol 290: H1729–H1739, 2006; doi:10.1152/ajpheart.01103.2005.—Senescence has been considered a programmed cellular response, parallel to apoptosis, that is turned on when a cell reaches Hayflick’s limit. Once cells enter the senescence program, they cease to proliferate and undergo a series of morphological and functional changes. Studies support a central role for Rb protein in controlling this process after it receives senescent signals from the p53 and p16 pathways. Cellular senescence is considered an essential contributor to the aging process and has been shown to be an important tumor suppression mechanism. In addition, emerging evidence suggests that senescence may also be involved in the pathogenesis of stem cell dysfunction and chronic human diseases. Under these circumstances cells undergo stress-induced premature senescence, which has several specific features. Focusing on endothelial cells, we discuss recent advances in our understanding of the stresses and their pathways that prompt the premature senescence response, evaluate their correlation with the apoptotic response, and examine their links to the development of chronic diseases and the impaired function of endothelial progenitor cells, with the emphasis on vasculopathy. Emerging novel therapeutic interventions based on recent experimental findings are also reviewed.

stress-induced premature senescence; endothelial progenitor cell; vasculopathy; metabolic syndrome; nephropathy

HISTORY OF SENESCENCE RESEARCH

Cessation of cell division after extended propagation in culture was first observed by Hayflick and Moorhead (61) in normal human fibroblasts in 1961, igniting research on cell senescence. This type of senescence, which usually requires weeks or months of culture, appeared to be related to the limited number of divisions that any particular cell type could undergo (thus determining its lifespan) and is termed replicative senescence. The bulk of evidence has linked senescence to the attrition of telomeres (60), which leads to chromosomal instability. In addition to telomere attrition, some stressors elicit a similar growth arrest within just a few days, referred to as stress-induced premature senescence (SIPS). The stressors that have been identified include activated oncogenes (17, 46, 117), DNA damage (50, 82, 96), oxidative stress (31, 119), suboptimal cell culture conditions (7, 8), and others (115, 143, 149). In either type of senescence, cells flatten and enlarge, acquiring a “fried egg” appearance. Initially thought to be a cell culture phenomenon, cell senescence has been more recently observed in vivo (30, 41, 44, 147). In addition to the above morphological changes, the recognized biomarkers of senescent cells include staining for β-galactosidase at pH of 6.0 [known as senescence-associated β-galactosidase (SA-β-gal) as opposed to the endogenous lysosomal enzyme detected at pH of 4.0 in normal cells]; decreased replicative capacity; increased expression of p53, p21, and/or p16 (vide infra) or other cyclin-dependent kinase inhibitors (e.g., p27 and p15); and accumulation of transcriptionally inactive heterochromatic structure [senescence-associated heterochromatic foci (SAHF)] (113). It is worth noting that many of the concepts related to senescence are developed from studies of human fibroblasts and from some immortalized cell lines; therefore, those concepts may not be fully relevant to primary endothelial cells.

REGULATION OF SENESCENCE

The above changes in cells undergoing replicative or SIPS have been mechanistically linked to the stabilization of p53 and hypophosphorylation of pRb. There is evidence supporting a linear p53-p21-pRb pathway (17, 32, 93) (Fig. 1). Overexpression of the tumor suppressor p53 results in transcriptional activation of many proteins, including p21, an inhibitor of the cyclin E/Cdk2 complex (87). Inhibition of this complex enables the maintenance of the hypophosphorylated (active) state of the Rb protein, which has been shown to interact with the transcription factor E2F family members and silence their transcriptional targets by invoking SAHF formation through the recruitment of heterochromatin proteins to E2F-responsive promoters (113). On the other hand, the activation of p16, an inhibitor of the cyclin D/Cdk4 and D/Cdk6 complex, provides an additional stimulatory pathway that maintains the Rb in its active state and hence leads to the induction of senescence (12, 84). Studies in mouse embryo fibroblasts have provided evidence that two other members of the Rb family, p107 and p130, may also be important factors for the senescent program because their inactivation was found to be necessary and sufficient to prevent senescence in Rb-mutated cells (39, 128). The contribution of these two proteins to the senescent programs in other cell types is currently under investigation.

Telomere shortening has been considered a hallmark of replicative senescence. It appears that the unprotected telomere structure can initiate DNA damage response and induce cell...
senescence by activating the ATM-p53-p21-pRb pathway (64), and that p16 has an important, but nonessential, role in this senescence process in the human fibroblast (20, 78). However, it may still possess a significant role in cells such as human prostate epithelial cells (79). Recent evidence suggests that telomere shortening may be an indispensable element for SIPS, which can usually be induced in days, and is unaltered by experimental telomere extension through exogenous over expression of human telomerase (hTERT) (53, 110). Oxidative stress and the oncogenic ras expression appear to activate the senescence program mainly by involving the p16-pRb pathway via its ability to bind and sequester MDM2 protein and inhibit the p16-dependent degradation of p53. The activation of p53 pathway may also initiate an apoptotic response. The balanced regulation of these two responses for apoptosis and senescence is currently under investigation.

It is worth noting that the signal cascades before the activation of p53 and p16 pathways remain poorly understood, and the signal pathway downstream of Rb/E2F that results in the various morphological and functional changes of senescence remains largely unexplored. There is also an ongoing debate as to the necessity and sufficiency of each of these tumor suppressor pathways in cell entry to senescence. In mouse embryo fibroblasts, these pathways may function independently, whereas both pathways may be involved in human fibroblasts (42, 76). It has been proposed that “distinct senescence programs can progress in parallel, resulting in mosaic cultures,” here some cells overexpress p21 or p16 or both (12).

Little is known about senescence and SIPS of endothelial cells. In aging vasculature, senescence has been found in association with the accumulation of mitochondria peroxynitrite in endothelial cells (147). Proatherogenic and proinflammatory factors like oxidized LDL, TNF-α, or hydrogen peroxide have been implicated in SIPS and shown to result in the inhibition of phosphoinositide 3-kinase/Akt and suppression of telomerase activity without discernible attrition of telomeres (16). Chronic exposure of human umbilical vein endothelial cells (HUVEC) to the noncytotoxic doses of tert-butyl-hydroperoxide or to an inhibitor of glutathione synthesis buthionine sulfoxamine resulted in the accelerated development of senescence after 30 versus 46 population doublings (88). This was accompanied by faster shortening of telomeres, their increased heterogeneity, and decreased telomerase activity, suggesting a key role for antioxidant cellular defense, specifically glutathione, in the maintenance of telomerase activity and telomere integrity. We found that culturing HUVEC on the surface of nonenzymatically glycated collagen I leads to increased proportion of SA-β-gal-positive endothelial cells in a much shorter period of time. Whereas changes of telomeres or in telomerase activity were undetectable compared with cells cultured on native collagen, oxidative stress was markedly increased (30).

Senescent endothelial cells are characterized by decreased production of nitric oxide (NO), changes in expression or phosphorylation of endothelial NO synthase (eNOS), decreased synthesis of prostacyclin, increased expression of plasminogen activator inhibitor-1, and enhanced adhesiveness for monocytes (37, 67, 97, 101, 111, 129). When human endothelial cells enter SIPS under the oxidative stress induced by glycated collagen (GC), we have found that despite an approximately twofold increase in the expression of immunodetectable eNOS, calcium ionophore-stimulated NO release was suppressed (<50% of control), and 3-nitrotyrosine (3NT)-modified proteins, the footprint of peroxynitrite production, accumulated. NO production and escape from senescence could be achieved by use of a NO donor (hydroxy-L-arginine), superoxide dismutase mimetic manganese (III) meso-tetrakis (4-benzoic acid) porphyrin, (MnTBAP), or peroxynitrite scavenger-antioxidant (ebselen), arguing in favor of oxidonitrosative stress as a proximal cause of SIPS in this model.

**RELATION BETWEEN SENESCENCE AND APOPTOSIS**

Many of the stressors described above can induce apoptosis as well as initiate the senescent program, and both of these responses have been shown to share some common elements in the stress-activated signal pathways. How the cell chooses between these two responses remains obscure. Several studies have attempted to elucidate the mechanism by which a specific outcome results from a certain type of stress. It has been demonstrated that cells in the S-phase commit to apoptosis when subjected to sublethal concentrations of H2O2; in contrast, cells in the G1 and G2/M phases commit to growth arrest (33). In addition, the degree of the stress seems to play an important role. Whereas a low dose of the oxidative stressor H2O2 can induce senescence in the diploid human fibroblast, apoptosis is the predominant outcome when cells are challenged with higher doses (14). This supports the hypothesis that senescence and apoptosis are two parallel outcomes that are activated after cells suffer irreparable damage. These two pathways may intersect at one or more as-yet-undefined

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**Fig. 1. Pathways inducing cell senescence and apoptosis.** A number of regulatory proteins transmit various stress signals and mediate cell entry into senescence through two cell cycle check-point pathways, p53/p21 and p16. Upregulation of p21 and p16 tumor suppressor can keep Rb in hypophosphorylated growth inhibition form, which prevents the binding of E2F to its targeted gene promoters. Studies have suggested that the DNA damage, initiated by unprotected telomere structure or other stressors, induce cell senescence mainly by activating the ATM-p53-p21-pRb pathway. The p16-pRb pathway may be activated through engagement of p38/MAPK cascade that is initiated by various stressors. Elevated ARF tumor suppressor gene products, p19ARF in mouse or p14ARF in human, can also input the stress signal to p53/p21 pathway via its ability to bind and sequester MDM2 protein and inhibit the MDM-dependent degradation of p53. The activation of p53 pathway may also initiate an apoptotic response. The balanced regulation of these two responses for apoptosis and senescence is currently under investigation.
point(s). Whereas cells are induced to apoptosis under stress, they will switch to senescence once their apoptosis pathway has been blocked either by exogenous overexpression of the bcl-2 gene (124) or by applying caspase inhibitors (122). On the other hand, it has been shown that aging enhances the sensitivity of endothelial cells toward apoptotic stimuli (67), and in some cases, the apoptosis program may spontaneously occur in senescent human and bovine endothelial cells (30, 144, 151, 161). In contrast to endothelial cells, human fibroblasts enter a stable growth arrest phenotype at the end of their lifespan and are resistant to various apoptotic stimuli (58).

Interestingly, instead of undergoing p53-dependent apoptosis under conditions of DNA damage induced by a variety of genotoxic stressors, senescent fibroblasts underwent necrosis instead, which may result from their failure to stabilize p53 (135). These results again exemplify the varied senescent programs seen in different cell types.

LYSOSOMAL-MITOCHONDRIAL INTERACTION IN SIPS: A HYPOTHESIS

Detection of senescent cells is based on the SA-β-gal assay, the molecular basis of which is still obscure (41). β-Gal is a lysosomal hydrolase cleaving β-linked terminal galactosyl residues from gangliosides, glycoproteins, and glycosaminoglycans. Its pH optimum is acidic (4–4.5), thus the appearance of the enzyme in the cytosol, as it occurs in cell senescence, could signify the loss of enzymatic activity. Why does the enzyme shift from the lysosomes to the cytosol? It has recently been demonstrated that p53 destabilizes and permeabilizes lysosomes (159), and this occurs before the effects of p53 induction on the mitochondrial permeability transition responsible for triggering apoptosis. Therefore, a plausible mechanism for the appearance of SA-β-gal cytoplasmic staining with the build-up of undegraded gangliosides is emerging. The role of p16, another marker of cell senescence, remains obscure. We hypothesize that as long as cellular gangliosides are below a certain threshold, the predominant effect of stress-induced p53 would be cell cycle arrest and senescence-like phenotype. Upon reaching a threshold level, gangliosides exert their effect on the mitochondrion leading, in tandem with the effect of p53, to the permeability transition, escape of cytochrome c, ATP, caspases and protease activating factor-1 (APAF-1), formation of apoptosomal complex (700 kDa), which cleaves downstream caspases and heralds cell’s commitment to apoptosis. Numbers along the pathways denote the sequence of events.

Fig. 2. Hypothetical involvement of dysfunctional lysosome in premature senescence. It has recently been demonstrated that p53 destabilizes and permeabilizes lysosomes, and this occurs before p53 effects on mitochondrial permeability transition and commitment to apoptosis. Appearance of β-Gal, a lysosomal hydrolase, in the cytosol, as it occurs in cell senescence, could signify the loss of its enzymatic activity and the subsequent build-up of undegraded gangliosides. We hypothesize that as long as cellular gangliosides are below a certain threshold, the predominant effect of stress-induced p53 would be cell cycle arrest and senescence-like phenotype. Upon reaching a threshold level, gangliosides exert their effect on the mitochondrion leading, in tandem with the effect of p53, to the permeability transition, escape of cytochrome c, ATP, caspases, and apoptotic protease-activating factor-1, formation of apoptosomal complex (700 kDa), which cleaves downstream caspases, and heralds the cell’s commitment to apoptosis (103). Thus, the accumulation of gangliosides, according to our hypothesis, serves as a switch of p53, and/or possibly p16, leading from cell senescence to apoptosis, as schematically depicted in Fig. 2. Though it is admittedly a simplified view of much more complex relations, it is a plausible roadmap toward understanding the pathogenesis of premature cell senescence and its potential link to apoptosis. In support of this scenario, the role of lipids in the maturation of phagolysosomes has recently been discovered (2). In our preliminary experiments, endothelial cells pretreated with an inhibitor of glucosylceramide-based glycosphingolipid synthesis, including ganglioside synthesis, d-threo-1-phenyl-2-decanoylamino-3-morpholinopropanol (10–100 nM) or its inactive analog, synthesized in Shayman’s laboratory (1, 92), were subjected to oxidative stress, and the proportion of apoptotic and senescent HUVEC was quantified 3 days later. Whereas inhibition of gangliosides synthesis did not appreciably affect cell senescence, it clearly prevented development of apoptosis (data not shown). These findings further support the proposed model where accumulation of gangliosides acts as a switch from senescence to apoptosis.

SENESCENCE AND DISEASES

In the field of cancer biology, suppression of senescence invariably predisposes animals to tumorigenesis, giving rise to the belief that cell senescence may have a protective role in vivo (3, 24, 100, 133, 137). Under certain irreparable stresses, normal cells will commit to a senescence program in which the senescent cells retain a relatively intact structure. One can speculate that this may help to maintain tissue structure in the event of sudden and diffuse cell death. However, senescence results in many deleterious structural changes, such as increased adhesion to extracellular matrix while losing cell-cell contacts and dramatic changes in chromatin structure and gene expression (113, 136, 138). In addition, senescence impairs normal cell function (25). In HIV-infected subjects, CD8+ T lymphocytes that displayed short telomeres could no longer proliferate and lost their cytotoxic efficiency to combat the virus (38, 43). Our in vitro studies indicate that prematurely senescent endothelial cells showed evidence of an impaired arginine-eNOS-NO functional system, changes similar to the aged endothelium of macrovasculature (147).

Evidence points to an important contribution of senescence to certain age-related diseases as has been suggested by
recent studies and the studies from other research groups. Accumulation of senescent myocytes with significant telomeric shortening has been detected in aged hearts with dilated cardiomyopathy (34). Cellular senescence also has a proposed role in the deterioration of renal graft function, which has been associated with telomere shortening and subsequent activation of p21 and p16 (81, 104). In human atherosclerotic lesions, overexpression of p53 and p21 has been found in nonproliferating endothelial and smooth muscle cells (71), and SA-β-gal-positive cells have been detected in human atherosclerotic plaques in the coronary artery and in injured rabbit carotid arteries (45, 107, 108). In Zucker diabetic fatty rats, we observed an increase in the number of SA-β-gal-positive cells in the aortic endothelium and at ostia of intercostal arteries accompanied by upregulated p53, p21, and p16 expression as well as elevated oxidative stress (18, 30). The development of diabetic microvasculopathy may also be related to the endothelial senescence as discussed in more detail later in this review.

Interestingly, it has been noticed that, even though mitotically inactive, senescent cells are far from being physiologically inert. Many genes in senescent cells display higher expression levels that do not merely correlate with cell cycle arrest (160). Senescent cells can secrete proteins, including degradative enzymes, inflammatory cytokines, and growth factors that may stimulate tissue aging and tumorigenesis and hence possess a more complex role in promoting chronic diseases (25, 26, 156). For instance, an immortal, but nontumorigenic, mouse mammary epithelial cell line will acquire tumorigenic capability and form malignant tumors when injected into mice with irradiation-induced senescent human fibroblasts (118). This was at least partly mediated by matrix metalloproteinase-3, an enzyme secreted by the senescent cells.

Recently, endothelial progenitor cells (EPCs) have been shown to have an important role in maintaining endothelium integrity and repairing its damage (145). Increasing evidence suggests that EPC may originate from several bone marrow cell populations. According to the initial description, EPCs are defined as cells expressing both hematopoietic stem cell markers, such as CD34 and CD133, and endothelial markers such as vascular endothelial growth factor receptor-2 and Tie-2 (4, 49, 59). Other reports suggest that myelo-monocytic cells can give rise to endothelial cells as well, especially the CD14+/CD34− myeloid cell population (131, 146). In addition, a subset of mesenchymal stem cells (termed multipotent adult progenitor cells) has the ability to differentiate into multiple cell types, including mature, functional endothelial cells (80, 123). They may represent a unique subset of EPCs. The above bone marrow-derived EPC can home to sites of ischemia and to damaged vessels. Infusion of EPCs was shown to augment capillary density and neovascularization of ischemic tissues, including infarcted myocardium (4, 85, 116), ischemic limbs (4, 5), or brain (162). Emerging evidence indicates that EPCs may play a critical role in the maintenance of intact vascular endothelium and in the repair of endothelial injury through the process of reendothelization as exemplified by atherosclerotic lesions (107, 121), direct mechanical injury to the endothelium (77), as well as inflammatory damage (158).

The balance between injury and repair is a life-long, carefully guarded process. The activation of local resident stem cells or the recruitment of circulating stem and progenitor cells may be a very important event during this process. A recent report indicated that early display of age-related endothelial dysfunction may be more strongly correlated to impaired EPC activity rather than decreased number when healthy young and old individuals were compared (63). Nonetheless, a study in the healthy individuals who bear cardiovascular risk factors has revealed a strong correlation between the number of circulation EPCs and the subjects’ combined Framingham risk factor score and the endothelial function (66). EPC level is suggested to be a surrogate biological marker for vascular function and cumulative cardiovascular risk.

Like any other cell type, EPCs are subjected to various stressors that could impair their function and proliferative capability. Capulating EPCs in healthy smokers exhibit impaired functional activities (66, 105). Hyperglycemia has been reported to reduce survival and impair function of EPCs (86). There is growing evidence that senescence may serve as an important mechanism mediating EPC dysfunction. Decreased numbers and an increased proportion of senescent EPC has been reported in patients with preeclampsia (141). Accelerated EPC senescence has also been noted in both experimental hypertensive rats and patients with essential hypertension (66, 75). Angiotensin II can induce EPC senescence through the induction of oxidative stress and influence telomerase activity (72). Oxidized low-density lipoprotein induces EPC senescence and leads to cellular dysfunction (74). EPCs from Type II diabetics exhibit impaired proliferation, adhesion, and engraftment in vascular structures (142). Except all of the pathological conditions mentioned above, EPC dysfunction has also been documented in Type I diabetes (95, 142), coronary artery disease (148, 155), atherosclerosis (107, 121), rheumatoid arthritis (54, 65), vasculitis with kidney involvement (69), and end-stage renal disease (27, 36).

**DIABETIC VASCULOPATHY AND SENESCENCE**

Even though the role of cell senescence in vascular biology remains underdeveloped, the adverse effects of endothelial SIPS appear to be obvious: the rapid development of macro- and microangiopathy as has been suggested by our recent studies and discussed briefly below.

Diabetic microvascular and macrovascular injury is central to the development of renal, retinal, neurological, and cardiovascular complications (22). In the early pathogenesis of diabetes, hyperglycemia perturbs several key vascular endothelial functions, leading to endothelial cell dysfunction, which is intimately linked to the impaired balance of the L-arginine-eNOS-NO system (52). These vascular alterations are thought to be of relevance to the onset and progression of diabetic complications (68, 114, 125).

Among the several proposed underlying mechanisms for hyperglycemia-induced vascular damage, evidence indicates a major role for increased advanced glycation end-products (AGEs) formation (29, 90, 132, 153). AGE precursors arise from the nonenzymatic reactions between glucose/glucose-derived dicarbonyls and cellular proteins, known collectively as Maillard reactions, which represent a series of reactions of rearrangement, dehydration, oxidation, and fragmentation of glucose or its adducts to protein (9). As a result of its chemical and structural alteration (62), AGEs cause excessive cytotoxic stress to the vascular system. In endothelial cells exposed to...
high glucose, intracellular AGE formation can occur within a week. In long-living tissue proteins, like collagen, AGE will accumulate over time at a rate that correlates with the protein half-life. During diabetes, AGEs are formed at an accelerating rate (157) resulting in very high tissue levels (23, 70).

We have examined the contribution of endothelial cell senescence to the pathogenesis of diabetic vasculopathy by using a glycated collagen I (GC)/endothelial cell culture system (30). We found that the senescent phenotype can be induced in early-passage HUVEC when cultured on a glycated matrix protein collagen I. An increased frequency of prematurely senescent cells, as judged by the presence of SA-β gal and overexpression of p53, p21, and p16, was similarly observed in the endothelium of aortas from 22-wk-old Zucker diabetic fat (ZDF) rats compared to Zucker lean (ZL) controls (Fig. 3, A and B).

Fig. 3. Vignettes illustrating the extent and mechanisms of vasculopathy in Zucker diabetic fatty rats and the results of treatment with ebselen (Ebs, E). A: en face view of SA-β-galactosidase expression by the aortic endothelium in 22-wk-old Zucker diabetic fatty rats and prevention of endothelial cell senescence with chronic ebselen treatment. B: thoracic aortic cross-sections and the expression of p53, p16, and p21 by endothelial cells. Representative images obtained from 22-wk-old ZL, ZDF, and ZDF + ebselen rat aortas. C: functional characteristics of prematurely senescent vasculature (a: diminished NO production; b: impaired vasorelaxation; c: maintenance of total pterin levels and reduction of tetrahydrobiopterin level). For NO production and aorta relaxation levels (Ca andCb): *P < 0.05 vs. ZL 22w, ZL 22w +E, and ZDF 22w + E; #P < 0.05 vs. ZDF 22w. For BH4 levels (Cc): *P < 0.05 vs. 8-wk-old ZL rats; **P < 0.01 vs. 22-wk-old ZL rats; ***P < 0.01 vs. 8-wk-old ZDF rats. For BH4 levels (Cc): *P < 0.05 vs. 22-wk-old ZL rats; #P < 0.05 vs. 22-wk-old ZDF rats. Compiled and modified from Brodsky et al. (18) and Chander et al. (28).
Separate experiments were performed to quantify the number of senescent endothelial cells in the aorta and correlate it with parameters characteristic of endothelial dysfunction, including the measurement of NO production, the vascular reactivity to acetylcholine, abundance of endothelial microparticles in the circulation, and the angiogenic competence of various vascular beds (18, 28, 48). Our results indicate that accumulation of senescent endothelial cells in the aorta of 16- to 22-wk-old ZDF rats is accompanied by reduced NO production and severely impaired acetylcholine-induced vasorelaxation (Fig. 3C, left and middle). The ability to form collateral blood vessels after femoral artery ligation is also noticeably reduced as assessed by laser Doppler flowmetry/imaging and histochemical detection of the capillary density in the affected striated muscle (18). Similar results were obtained by an ex vivo angiogenesis assay (19).

The development of microvasculopathy was also evaluated in the kidney of ZDF rats. At 22 wk of age, these animals developed proteinuria and a reduced creatinine clearance. Histological assessment showed that both the glomerulosclerosis and tubulointerstitial scarring index increased and focal segmental glomerulosclerosis was observed in 9.5 ± 1.8% glomeruli. Oil-Red-O staining revealed widespread lipid deposits in the circulation, and the angiogenic competence of various vascular beds has been tested with many promising results reported (11, 13, 109, 126, 130). Some investigators have been trying to preserve the endothelial progenitor cell population from early onset of senescence by using, for instance, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibition (statins) or estrogen (6, 73, 140). Detailed analysis of these strategies is beyond the scope of this review, and interested readers are referred to the excellent reviews mentioned above.

In the following, we focus our discussion on antioxidant therapy and summarize some of our in vitro and in vivo study results related to this strategy.

Whereas in short-lived species, antioxidant treatment has been successful in prolonging lifespan (83), this has not been the case in mammals. The lifespan was unchanged in middle-aged mice receiving lipoic acid or coenzyme Q10 antioxidant supplements but increased by 13% in mice receiving caloric restriction (91). Antioxidant therapy was reported to be ineffective in prolonging the lifespan in mammalian model systems, but there is evidence that antioxidant treatment protects against age-related dysfunction, including cognitive decline, as discussed in a recent review (51). On the other hand, targeted mutation of mouse p66Shc gene, which controls the production of ROS, induces resistance to stress and a 30% increase in lifespan. Improvement of vascular function in p66Shc ablated aging mice has been observed, and the p53/p21 stress response pathway is impaired in p66Shc−/− cells (106). The deletion of p66Shc was shown to reduce systemic and tissue oxidative stress, vascular cell apoptosis, and early atherogenesis in mice fed a high-fat diet (112). In this regard, it is intriguing to note that calorie restriction can also increase the resistance to oxidative stress (139), which is believed to contribute to the beneficial outcomes of calorie restriction.

Senescence has been classically viewed as a state of permanent growth arrest, and hence cells are rendered unable to re-enter the cell cycle. Though this concept is still widely accepted, recent studies have provided evidence indicating that under certain conditions senescence is reversible, at least in the early stages. Stable suppression of p53 expression in senescent mouse embryo fibroblasts through RNA interference leads to rapid cell cycle re-entry and to immortalization, indicating that both initiation and maintenance of senescence is p53 dependent (6). In a separate experiment, senescent human fibroblasts and mammary epithelial cells with low levels of p16 resumed robust growth after inactivation of p53, whereas those with high levels of p16 remained senescent. Exogenous expression of oncogenic ras can also induce limited growth in the same low p16 level senescent cell population (10), Caveolin-1 reduction, using an antisense and small interfering RNA strategy, can also induce senescent human fibroblasts to re-enter cell cycle upon epidermal growth factor stimulation (35).

We have tested the hypothesis that premature endothelial cell senescence is not only preventable but also reversible. Based on the finding of enhanced peroxynitrite formation in aging and stressed vasculature (30, 147), we performed a series of in vitro and in vivo experiments addressing the effect of a
bona fide peroxynitrite scavenger and antioxidant ebbselen. This seleno-organic compound scavenging peroxynitrite (99) was able to prevent and reverse senescence of early-passage HUVECs and preserve functional activity of eNOS in vitro by scavenging peroxynitrite (18, 30). The ability to prevent and reverse vascular dysfunction was tested in vivo by treating ZDF rats with ebsselen between the ages of 8 to 22 wk (treatment initiated when the hyperglycemia has just developed), 13 to 22 wk, or 16 to 22 wk (treatment initiated when vasculopathy was already present) (18, 28, 47). Our results indicate that ebsselen cannot only prevent macro- and microvasculopathy in Zucker diabetic fatty rats when administered from weeks 8 to 22 but partially reverses vasculopathy when administered from weeks 13 to 22 (i.e., starting shortly after the early onset of vasculopathy). This was accompanied by the prevention and reversal of endothelial cell senescence in the aorta. The above effects either diminish or disappear when administration of ebsselen starts at week 16 and lasts until 22 wk of age in Zucker diabetic fatty rats.

Macro- and microvascular complications of metabolic syndrome and diabetes are among leading causes of morbidity and mortality. Tight glucose control alone is insufficient to prevent macrovasculopathy, but there are indications that the soluble extracellular portion of the AGE receptor or AGE “breakers” are capable of preventing these complications (154). Notably, both agents are acting on the upstream, prereceptor mechanisms of endothelial dysfunction. Ebsselen therapy, on the other hand, targets downstream, postreceptor cellular consequences of endothelial oxidative stress. One of the possible attractive mechanisms of endothelial dysfunction. Ebsselen therapy, on the other hand, targets downstream, postreceptor cellular consequences of endothelial oxidative stress. One of the possible attractive features of selenorganic compounds is their combined peroxynitrite scavenging and antioxidant effect (99), thus, potentially not only preventing further oxidant stress, but also accelerating the clearance of preformed 3-NT-modified proteins. Indeed, the fact that ebsselen not only prevents, but also reverses, the preexisting vasculopathy makes it a promising therapeutic agent.

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REFERENCES

Invited Review

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