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Commentary

Ageing and atherosclerosis: Mechanisms and therapeutic options

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ABSTRACT

Atherosclerosis is the cause of most heart attacks and strokes, and is *par excellence*, a disease of ageing. Whilst disease prevalence and incidence increases with increasing decade of life, there is also evidence of accelerated cellular ageing in atherosclerosis. Such evidence includes impaired cell proliferation, early culture senescence and cell cycle markers of senescence *in vitro* and *in vivo*. Cell senescence is also characterised by loss of telomeres from the ends of chromosomes. Cellular ageing increases with disease severity, acting as a marker for disease, but also directly promotes atherosclerosis. Cellular ageing appears to be due to both abnormal proliferation of cells in an attempt to repair vessel damage, and a response to the damage itself. This review summarises the evidence of vascular cell senescence in atherosclerosis, the causes and consequences of accelerated cellular ageing in atherosclerosis, and identifies potential therapeutic options for both prevention and treatment.

1. Background

Coronary artery disease caused by atherosclerosis is one of the most frequent causes of death in the Western World. Atherosclerotic plaques consist of an accumulation of vascular smooth muscle cells (VSMCs) and blood-derived inflammatory cells, together with lipid and extracellular matrix proteins. In most cases, the plaque is associated with a dysfunctional endothelial cell lining, showing activation of adhesion molecules, and impaired vasorelaxation and anti-coagulant/anti-platelet properties. The most serious complications of atherosclerosis arise from the thrombotic occlusion of arteries consecutive to the erosion or rupture of an atherosclerotic plaque. These manifestations are directly associated with acute coronary syndromes, myocardial infarction and stroke.

The incidence of plaque rupture and thrombotic events is largely correlated with specific morphological and histological features described for unstable plaques [1]. In particular, vulnerable lesions have a thin fibrous cap overlying a large core of oxidized lipids and infiltrated inflammatory cells (Fig. 1A and B). In approximately 60% of cases, it is the fibrous cap of an atherosclerotic plaque that ruptures [2]. The structural components of atherosclerotic

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Abbreviations: 8-oxo-G, 8-oxo-guanine; A-T, ataxia telangiectasia; ATM, ataxia telangiectasia mutated (protein); ATR, ATM and Rad3-related (protein); DSBs, double-stranded (DNA) breaks; EC, endothelial cells; ECM, extracellular matrix; γ-H2AX, phosphorylated from of the histone protein H2AX; HMGCoA, 3-hydroxy-3-methylglutaryl coenzyme A; h-TERT, catalytic unit of the telomerase enzyme; ROS, reactive oxygen species; SIPS, stress-induced premature senescence; SAβG, senescence-associated β galactosidase; SSBs, single-stranded (DNA) breaks; VSMC, vascular smooth muscle cell.

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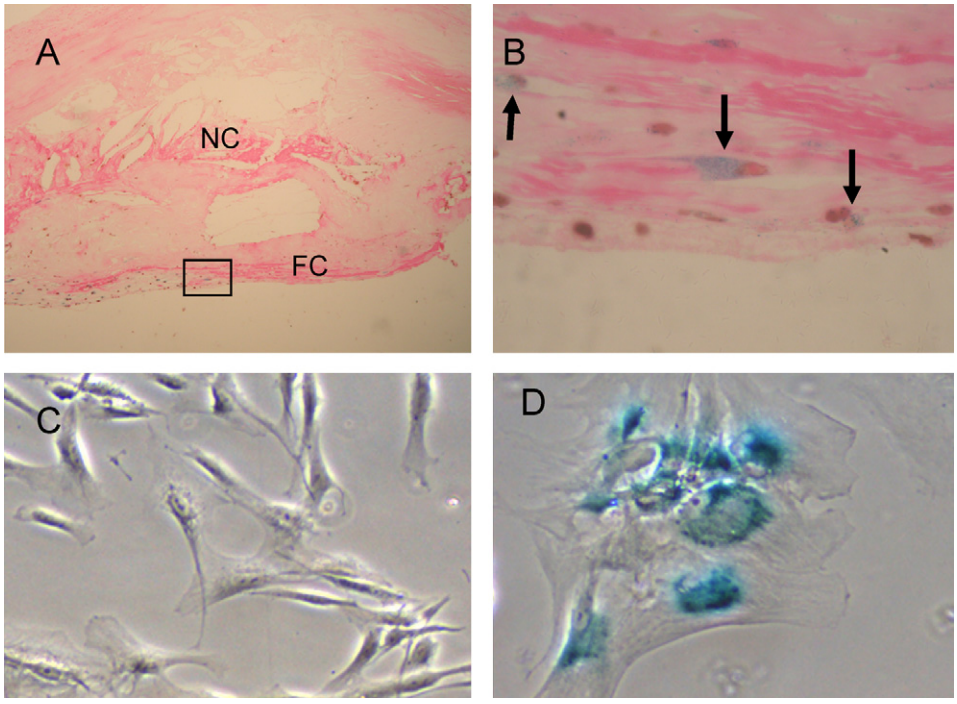

Fig. 1 – Evidence of senescence of human VSMCs. (A) Fast Red stain of a typical vulnerable plaque, demonstrating a thin fibrous cap (FC) overlying an extensive necrotic core (NC). (B) High power view of area outlined in (A) showing 8-oxo-G positive cell nuclei (brown) and SAβG staining (blue speckled cytoplasm) (arrows). In some cases, SAβG positive cells are also positive for 8-oxo-G, indicating evidence of DNA damage and senescence in the same cell. Most of the senescent VSMCs are located in the fibrous cap. (C) Phase contrast micrograph of normal human VSMCs in culture, also stained for SAβG. Cells are small and spindle-shaped, with no SAβG staining in early passages. (D) Phase contrast micrograph and SAβG staining of human plaque-derived VSMCs, showing larger, flattened cells, with high levels of SAβG staining (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

plaque caps consist primarily of VSMC-derived collagen, elastin, proteoglycans and extracellular matrix (ECM). In order to maintain stability, plaque caps require greater collagen and ECM content than the adjacent intima. As the cap ages, it thins, and becomes more inclined to rupture. Fibrous caps of unstable plaque contain less collagen and ECM components and fewer VSMCs than caps from stable and more intact plaques.

2. Evidence of cellular ageing and cell senescence in atherosclerosis

Cellular senescence can be defined as cell cycle arrest accompanying the exhaustion of replicative potential. Unlike quiescence, senescence is irreversible, and cells cannot initiate cell cycle transit in response to mitogens. Besides their characteristic morphology, senescent cells display undetectable rates of cell proliferation and characteristic patterns of gene expression, including markers such as senescence-associated β galactosidase (SAβG) activity [3] and cell cycle regulators. Importantly, cells undergoing replicative arrest have short telomeres, the structures comprising the ends of chromosomes. All of these features are present in cells in the atherosclerotic plaque.

2.1. *Cell proliferation*

Extensive characterisation of lesion development has shown that cell proliferation is low in early atherosclerosis (Fatty streak, Stary Type I lesion), peaks in the intermediate lesions (Stary II–IV), and declines in advanced fibroproliferative, complicated plaques (Stary type V lesions) [4,5]. Thus, contrary to popular belief, clinically important plaques show low levels of cell proliferation. Plaque VSMCs have been isolated from human endarterectomy specimens, subcultured *in vitro*, and their properties compared to those of VSMCs derived from the media of normal arteries [6–9]. Plaque VSMCs have an enlarged, flattened and stellar shape (Fig. 1C and D) with high amounts of cytoplasmic vacuoles and lysosomes. These are features of normal VSMCs undergoing replicative senescence in culture. A striking characteristic of plaque VSMCs *in vitro* is their restricted capability to proliferate [6–8,10,11]. Isolated plaque-derived VSMCs show lower rates of cell proliferation, and lower % of cells in S phase of the cell cycle [8,10]. In addition, plaque VSMCs undergo premature senescence. Normal VSMCs may be cultured for 10–20 passages under routine conditions, depending in part upon the age of the donor. In contrast, plaque VSMCs rarely remain dividing beyond 5–10 passages [8,10,11]. Whilst the lifespan of both plaque and normal VSMCs can be extended, in some cases to become immortalized [10,11], the extended lifespan of plaque

VSMCs is still less than normal VSMCs with the same genetic manipulation, and the rate of immortalisation of plaque VSMCs is lower than normal VSMCs. These studies reinforce the concept that plaque VSMCs are pre-senescent.

2.2. SAβG expression

Although senescent cells have very distinctive profiles of gene expression when compared to highly proliferating cells at early passage, finding specific markers that discriminate between senescent and non-proliferating quiescent or terminally differentiated cells has been more challenging. The activity of the senescence-associated β-galactosidase (SAβG) has been described as a marker of senescent cells *in vitro* and *in vivo*. β-galactosidase is a metabolic enzyme highly expressed in pre-senescent and senescent cells [12]. SAβG activity corresponds to β-galactosidase activity measured in pH conditions where only high levels of the enzyme are detectable, and proportionally correlate with lysosomal content [13]. Plaque VSMCs isolated from human endarterectomy samples not only possess a large and flattened shape, but also display high SAβG staining even at early stages of culture (Fig. 1D).

SAβG-positive cells are detected in both the endothelium and intimal VSMCs of advanced human atherosclerotic plaques, but not in the vessel media [14,15], suggesting that vessel wall senescence may be a feature of advanced plaques (Fig. 1C and D). SAβG staining is also found in VSMCs of the neointima of rabbit carotid arteries injured by repeated endothelial denudation [16]. However, within the plaque, many cells showing SAβG staining are macrophages due to their high lysosomal content, and double labelling is required to identify the origin of SAβG-positive cells.

2.3. Cell cycle regulators

VSMCs isolated from human plaques display significantly reduced percentage of cells in S-phase of the cell cycle, a higher percentage of cells in G_1, and a longer mean mitotic time compared with VSMCs isolated from normal vessels. Since cell cycle regulators controlling the G_1/S transition have a specific profile of expression in senescent cells, several studies have explored the pattern of expression of these specific genes in order to appreciate the proliferative status of VSMCs in pathological conditions.

The G_1/S transition is primarily regulated by the retinoblastoma protein pRB, product of the *rb* tumour suppressor gene. pRB exerts its negative regulation on the cell cycle through binding of E2F transcription factors, rendering them ineffective as transcription factors. As many E2F targets are genes required for S-phase entry, pRB regulates the G_1–S transition. The pRB/E2F complex is dissociated in early G_1 by phosphorylation of pRB by the cyclin-dependent kinases (CDKs) 2, 4 and 6. E2F becomes then available to activate the subset of genes required for S phase entry. While these kinases are constitutively expressed in cells, their activity depends on the expression of their respective activators, cyclin D_1, D_2 and D_3 for CDK4 and CDK6 and cyclin E for CDK2. As for fibroblasts, growth factors or serum-stimulated non-confluent VSMCs show increased pRB phosphorylation, increased expression of cyclin D and E and the induction of E2F-dependent S phase genes. Plaque VSMCs display an opposite pattern of pRB phosphorylation [17], which may explain their reduced E2F transcriptional activity.

The cell cycle is tightly regulated by multiple checkpoints, which allow the cell to verify the integrity of its DNA. If cell damage is detected, a series of cyclin-dependent kinase inhibitors, including $p16^{ink4}$, $p21^{cip1}$ or $p27^{KIP1}$ proteins, are expressed at high levels and directly or indirectly block the activity of the CDKs involved in G_1. Indeed, overexpression of p21 and p27 will force VSMCs into G_1 growth arrest [18]. When compared to control cells, plaque VSMCs have increased expression of p16 and p21, a reduction in pRB phosphorylation, and increased levels of E2F-1/pRB complex [17]. *In vivo*, $p27^{KIP1}$ expression is elevated in non-proliferating cells from normal and diseased vessels. In contrast, $p21^{CIP1}$ is elevated only in the atherosclerotic plaque [17,19] and co-localizes with elevated p53 [20], suggesting that the p21 transactivation may be dependent on p53 stabilization.

Confirmation of the role of these proteins in VSMC senescence comes from studies with single or double inactivation of p53, pRB or both. The lifespan of normal human VSMCs can be extended by inactivation of p53 or pRB alone, although inactivation of both p53 and pRB is more efficient and is required for full immortalisation [10,11]. In contrast, inactivation of p53 does not extend the lifespan of plaque VSMCs, and inactivation of pRB alone induces apoptosis in these cells [10]. Inactivation of both tumour suppressor genes is required for extension of lifespan of plaque VSMCs, and even then, immortalisation is rarely achieved [10,11]. Whilst this confirms that cell cycle arrest mediated via p53 and pRB are important for senescence of VSMCs, it implies that plaque VSMCs need inactivation of multiple checkpoints to bypass senescence.

2.4. Telomere length

Telomeres are DNA–protein complexes at the end of eukaryotic chromosomes that protect them from degradation, recombination or fusion. Telomeres consist of double-stranded repeats of the AATGGG sequence and are ended by a single stranded 3′ overhang folded in a structure referred as the T loop. Conventional polymerases need primers to elongate linear DNA, therefore cannot support the replication of the very end of telomeres. Telomeres are maintained by the enzyme telomerase, which contains an intrinsic RNA subunit (hTERC) and serves as a template for its catalytic subunit (hTERT). During division of somatic cells, low levels of telomerase do not allow full replication of telomere ends, resulting in progressive shortening with each division. As telomere shortening is an irreversible feature, the mean telomere length of a cell population can therefore be taken as an indicator of its replicative history.

There is also direct evidence of telomere loss in cells of the vessel wall in atherosclerosis. VSMC or endothelial cell telomere size is not uniform throughout the vasculature, and differences between the intima and media have been observed [21–23]. Vessels subject to high haemodynamic stress have significantly shorter telomeres in their intima when compared to arteries with low haemodynamic stress

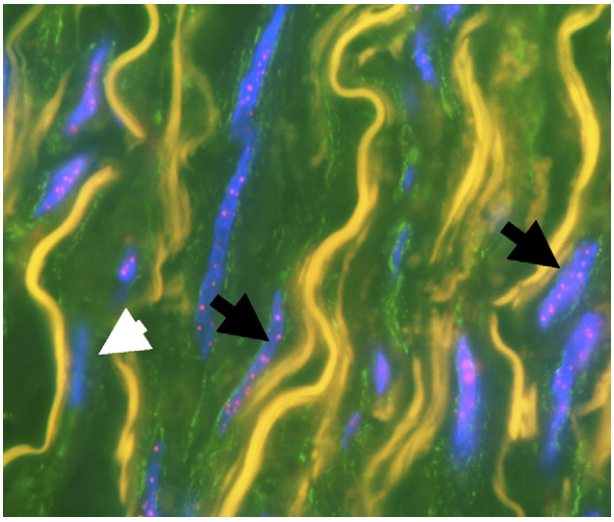

Fig. 2 – Telomere shortening in atherosclerosis. Combined in situ telomere fluorescence in situ hybridisation and fluorescence staining for VSMCs in a vessel wall. VSMC nuclei are shown as blue after staining with DAPI. Telomere signals are seen in the nuclei as pink dots. VSMCs are identified by fluorescein-conjugated antibodies to α-sm-actin (green). Examples of cells showing normal telomere signals are shown (black arrowheads) compared with a cell showing minimal signals (white arrowheads). Elastic lamina are identified by yellow autofluorescence (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

[22], suggesting that high cell turnover at sites of high stress cause short telomeres. In addition, telomere length in the media and intima of distal and proximal abdominal aorta has been shown to negatively correlate with predisposition to atherosclerosis [24]. The intima showed an age-dependent inverse correlation with age, with higher attrition rates in the distal than the proximal segments. The atherosclerosis grade inversely correlated with telomere length but the correlation was lost after adjustment for age [24]. More recently, in studies mapping telomere length in plaques we have found that plaque VSMCs show markedly shorter telomeres than VSMCs in the media, with critically short telomeres in VSMCs in the fibrous cap [25] (Fig. 2). Whilst these studies suggest that atherosclerosis is associated with telomere loss, they do not prove that telomere loss promotes atherosclerosis rather than just being a marker of increased cell turnover, nor do they identify the mechanism of telomere loss.

3. Mechanisms of cell senescence in atherosclerosis

Cell senescence may be triggered by two broadly different mechanisms. Replicative senescence may be induced by reduction of telomere length, changes in structure such as telomeric fusion or dicentrics, or loss of telomere-bound factors. Telomeres may trigger growth arrest via DNA damage responses at critical telomere lengths or structure. Cells subjected to sub-lethal stress due to DNA damage (UV and γ-irradiation, oxidative stress and treatment with histone deacetylase inhibitors) also undergo 'stress-induced premature senescence' – 'SIPS'. SIPS resembles replicative senescence, cells demonstrating a similar morphology and pattern of cell cycle regulators.

3.1. *Telomere shortening and senescence*

As indicated above, replicative senescence may be induced by reduction of telomere length, changes in structure such as telomeric fusion or dicentrics, or loss of telomere-bound factors. After repeated divisions, telomeres may reach a critical length or structure whereby irreversible growth arrest or senescence is triggered. In addition, there is substantial evidence of a cause and effect relationship between telomerase expression and manifestations of senescence. Ectopic expression of the catalytic subunit of telomerase (hTERT) can prevent replicative senescence in several cell types such as fibroblasts or epithelial cells, despite the fact that telomeres were not always protected. hTERT may also influence the interaction of telomeres with the nuclear matrix, increase chromosome stability, decrease telomere fusion, reduce spontaneous chromosome breaks and enhance DNA repair. These effects are independent of the effects of telomerase on telomere length. Telomere uncapping can also cause cell senescence independent of telomere length and telomerase activity.

Although ectopic expression of hTERT can dramatically extend the lifespan of normal human VSMCs [26], the evidence that hTERT expression or activity directly affects atherosclerosis is controversial. Human VSMCs and ECs express low levels of hTERT [27,28] and hTERT activity is readily detectable only when cells are proliferating [28]. Indeed, hTERT is a target of E2F-1 transcription factor, where mRNA expression of the enzyme increases when cells enter S phase. Phosphorylation of hTERT in vascular cells, along with its activation and nuclear translocation, offers an additional level of regulation of the enzyme activity at the post-translational level.

Whatever the mechanism of telomere damage, telomere dysfunction elicits a general DNA damage response and growth arrest via the activation of a protein kinase cascade including the kinases ataxia telangiectasia mutated (ATM) and ATM-related kinases (ATR). The DNA damage response involves the phosphorylation and recruitment of the kinases ATM, ATR and DNA protein kinase (DNA-PK) at the site of DNA damage. This results in the phosphorylation of histones such as H2AX, the association of DNA repair enzymes and cofactors (53BP1, MDC1/NFBD1 and NBS1), and activation of the transducer proteins ChK1 and ChK2. These subsequently result in activation of effector molecules such as the breast cancer susceptibility gene BRCA1, E2F transcription factors, p53 itself or the Cdc25A phosphatase (for review see [29] and Fig. 3). These proteins have multiple targets affecting processes such as transcription, DNA repair, apoptosis or cell cycle arrest. Thus, targeted damage of either telomeres or genomic DNA finally converge on to a common pathway characterised by the activation of at least one of the cell cycle

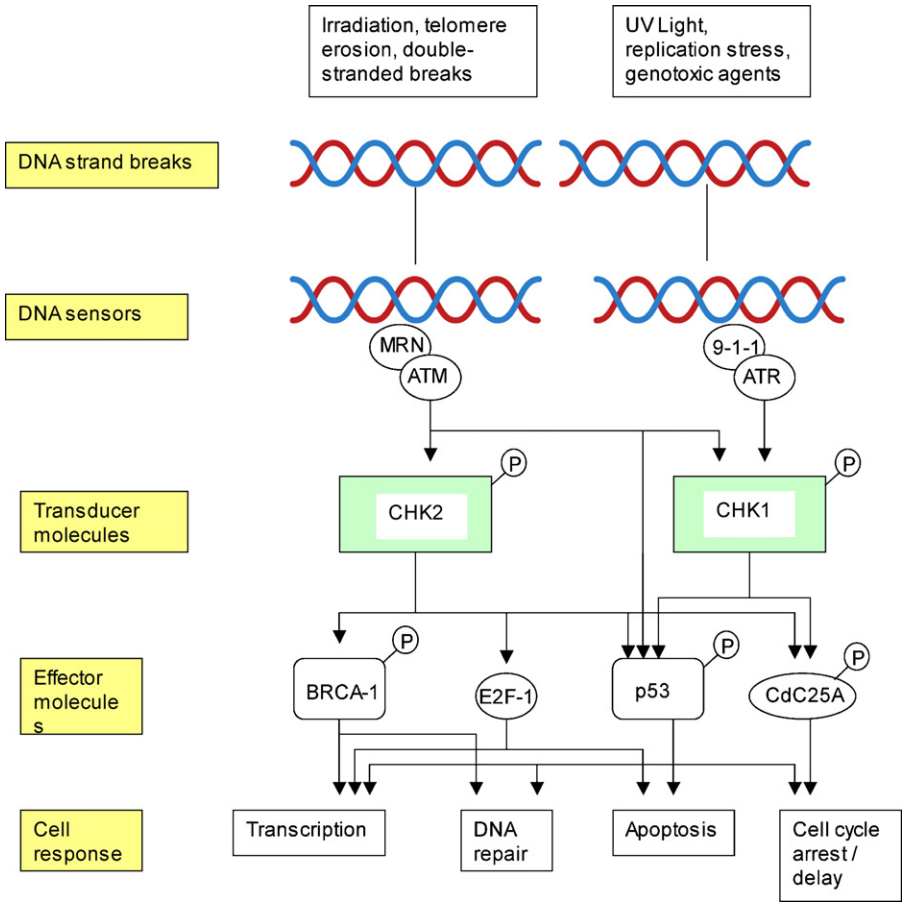

Fig. 3 – Schema of signalling pathways leading from DNA damage to the cell response. Abbreviations are as given in the text.

inhibitors $p21^{cip1}$ (via p53) or $p16^{ink4}$ (Fig. 4), responsible for inducing growth arrest and senescence (Fig. 4).

Senescence is also characterized by the presence of a greater number of immunochemically detected DNA damage foci. Each focus reveals the presence of the DNA repair complexes at the site of DNA damage. It is not certain, however, whether the DNA damage foci are associated with telomeric DNA. High resolution imaging of senescent fibroblasts shows that the foci-associated protein γ-H2A-X was found in telomeric DNA regions [30] and was co-immunoprecipitated with a sub-telomeric DNA region in a chromosome immunoprecipitation assay. In contrast, other studies found that γ-H2A-X does not colocalize with telomeric DNA or TRF2 proteins. Whatever the mechanism, these results support the hypothesis that telomere-mediated DNA damage response is involved in replicative senescence. Nuclear DNA damage foci are present during the cellular senescence, and for this reason the protein components of foci have been proposed as markers of senescence. It will be of particular interest to study these proteins in comparison to current markers of senescence and follow their behaviour in cardiovascular disease.

3.2. *Oxidative stress and senescence*

Cell division is not the only path to senescence. Irreversible growth arrest can also be triggered in response to a variety of stresses, and as such is termed 'stress-induced premature senescence' (SIPS). *In vitro*, SIPS can be elicited by over-expressing Ras and Raf oncogenes, radiation, or chemical agents producing any form of DNA damage and oxidant stress. Oxidative stress, in addition to excessive cell proliferation, is one of the most physiologically relevant triggers of cell senescence in pathological conditions. Intracellular generation of superoxide anions, hydrogen peroxide and hydroxyl radicals, can produce DNA strand breaks and various types of DNA bases modifications, including the highly mutagenic oxidation of guanine residues into 7,8 dihydro 8-oxo-guanine (8-oxoG).

Increased levels of ROS are found in atherosclerosis in all layers of the diseased arterial wall, and particularly in the plaque itself [31,32]. Whether generated by the cellular NAD(P)H oxidases, xanthine oxidase, myeloperoxidase or the mitochondrial oxidative metabolism, increased levels of ROS may account for the high levels of DNA lesions in atherosclerotic plaques. Higher levels of 8-oxoG DNA adducts are found in human carotid endarterectomy specimens compared with the normal media, associated with increased expression of DNA repair enzymes, such as DNA-PK and PARP-1 [33]. Evidence of fragmented DNA was also found in plaque macrophages and VSMCs [33]. A significant and reversible increase in DNA damage has also been observed in atherosclerotic plaques from cholesterol-fed rabbits [34], which

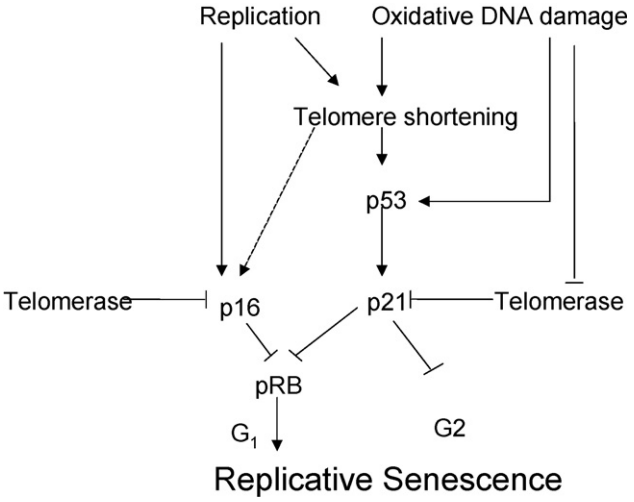

Fig. 4 – Schema of signalling pathways leading to replicative senescence. Plaque VSMC senescence occurs by a combination of replicative senescence and SIPS. Replication in VSMCs (which have low telomerase activity) induce telomere dysfunction and expression of p16. Reactive oxygen species induce DNA damage in both nuclear and mitochondrial DNA. DNA damage activates a damage response pathway involving activation of p53, with subsequent p21 transcription. p16 and p21 induce pRB hypophosphorylation and senescence. ROS also accelerate telomere loss during replication, in part by damage to telomeric DNA and reduction in telomerase activity.

regresses on withdrawal of high fat feeding. Altogether, these studies directly implicate oxidative DNA damage, DNA repair and cell senescence in atherosclerosis.

In vitro, oxidant stress induced by chronic hydrogen peroxide (H_2O_2) treatment, hyperoxic culture conditions or alterations of the cell's anti-oxidant properties can all accelerate senescence. Treatment of cultured fibroblasts with H_2O_2 can induce telomere single strand breaks that may promote telomere shortening and consequently induce premature cell cycle arrest by triggering pathways converging towards activation of the G_1-associated cell cycle inhibitors ($p21^{Cip1}$, $p16^{ink4}$). Increased telomere loss per division can also occur in individual cells due to a telomere-specific deficiency in base excision repair. This mechanism leads to preferential accumulation of ROS-induced single-stranded DNA breaks, preventing replication of distal segments of chromosomes when cells divide. Alternatively, repeated stress dramatically increases the proportion of cells undergoing growth arrest, suggesting that oxidative stress may exert selection pressure with replication of a subset of VSMCs *in vivo*. Oxidative stress can also induce premature senescence independent of telomere shortening. Pulsed treatments with low doses of H_2O_2 in fibroblasts can cause irreversible cell cycle arrest accompanied by an increase in SAβG staining without concomitant changes in telomere length.

Accumulation of oxidative damage to genomic DNA may contribute to SIPS, but also to replicative senescence since senescent cells *in vitro* have higher levels of 8-oxoG DNA base modifications. These results are consistent with the observation that senescent cells present more DNA damage foci, even in non-telomeric sequences. Therefore, the distinction between replicative and oxidative stress-induced senescence is not clearly delineated, and both pathways lead to SAβG overexpression, even though distinct cell cycle inhibitors may be involved.

4. Evidence for DNA damage in atherosclerosis

DNA damage is present both in the circulation of patients with atherosclerosis and the plaques themselves. DNA damage ranges from 'macro' damage, including deletions or additions of whole chromosomes or parts of chromosomes, to 'micro' damage, which includes DNA strand breaks, mutations of single bases, modified bases (including oxidation) or DNA adducts. For example, patients with coronary artery disease have a higher leukocyte micronucleus index (a marker of genetic instability) than healthy controls, which correlates with disease severity. There is also a significantly higher incidence and extent of a common mitochondrial DNA deletion (mtDNA4977). Premature atherosclerosis is a feature of defects in DNA repair pathways, such as Werner syndrome, a disease characterized by predisposition to cancer and early onset of symptoms related to normal ageing including osteoporosis, ocular cataracts, graying and loss of hair, diabetes mellitus, and atherosclerosis. Werner protein guards the genetic stability of cells, playing an integral role in base excision repair and at telomere ends.

This 'macro' DNA damage is also associated with biomarkers of carcinogenic exposure, such as DNA adducts or modifications to specific bases in atherosclerotic plaques. 'Bulky' aromatic DNA-adducts in VSMCs (most likely related to environmental exposure to genotoxic chemicals) are a predictor of atherosclerosis extent in humans even after adjustment for age, smoking, obesity, heart weight and genetic susceptibility markers. DNA strand breaks, oxidized pyrimidines and altered purines are also significantly higher in patients with coronary artery disease than controls, and human plaques show markers of oxidative damage, including DNA strand breaks, expression of 8-oxo-G (an oxidative modification of guanine residues in DNA (Fig. 1) and activation of DNA repair enzymes [33]. Strong nuclear and cytoplasmic immunoreactivity for 8-oxo-G is detected in plaque VSMCs, macrophages and endothelial cells, but not in VSMCs of adjacent normal media or normal arteries [33]. DNA damage is also a direct correlate of extent of atherosclerosis in experimental animals. For example, cholesterol feeding of rabbits induces oxidative damage in plaques, manifested by 8-oxo-G staining [34], DNA strand breaks, and apoptosis.

5. Causes of DNA damage

5.1. *Risk factors for atherosclerosis*

DNA can be damaged in numerous ways. Spontaneous damage due to replication errors, deamination, or depurina-

tion has to be repaired in addition to damage derived from oxidation and environmental chemicals. Many of the risk factors associated with atherogenesis, such as smoking and diabetes mellitus, may directly induce DNA damage. For example, smoking can cause oxidative DNA damage, inhibit DNA repair, and induce the production of advanced glycation end products, which themselves cause DNA mutation (reviewed in [35]). Similarly, advanced glycation end products have been implicated in the oxidation of low-density lipoprotein and elevated levels of 8-oxo-G. Although direct damage from specific risk factors or environmental agents may contribute to DNA damage in atherosclerosis, the most likely trigger of damage are reactive oxygen species.

5.2. *Reactive oxygen species*

It has been estimated that approximately 2×10^4 DNA damaging events occur in every cell/day [36]; a major portion of these occurs via reactive oxygen species (ROS). ROS include the superoxide anion ($^{\bullet}O_2^-$), hydrogen peroxide, hydroxyl radical, peroxynitrite and lipid peroxides, with their reactivity and half-life varying according to species. ROS are constantly produced within the cell, in particular via mitochondrial oxidative metabolism and pathological processes such as inflammation. In vascular cells, the primary source of ROS may be xanthine oxidase and NAD(P)H oxidases (*Noxs*). Other sources of ROS include cytochrome P450 isoenzymes, lipoxygenase, cyclooxygenase, hemoxygenase, and glucose oxidase. Myeloperoxidase is also produced by invading macrophages. ROS are also secondary messengers in specific signalling pathways, generated by specific plasma membrane oxidases in response to growth factors and cytokines.

DNA lesions caused by ROS include double- and single-stranded breaks (DSBs and SSBs), DNA–DNA and DNA–protein cross links and base modifications, including thymine glycol, 8-hydroxyguanine and 8-oxo-guanine. Certain types of damage can be traced back to specific insults. While superoxide and hydrogen peroxide are normally not reactive to DNA, both can be converted to the extremely reactive hydroxyl radical via the Fenton reaction. The hydroxyl radical can induce a vast array of damage to both nuclear and mitochondrial DNA. Hydroxyl radicals may induce base or nucleotide loss, adducts, and single and double strand breaks. In particular, hydroxyl radicals can interact with pyrimidine double bonds causing glycolytic damage (e.g. thymine glycol, uracil glycol) that can cause mis-pairing, transcriptional interrupts and if left un-repaired induce cell cycle arrest. Similarly, interaction of OH radicals with purines will generate formamidopyrimidines and other purine oxidative products that are usually recognised by the DNA repair mechanism. Thymidine oxidation, or the addition of methyl and alkyl groups, is frequently attributed to ROS of all types. Interestingly, 8-oxo-G, which frequently causes oxidative damage to deoxyguanines, is not recognised by the DNA repair system and is thus highly deleterious to cells. Un-repaired 8-oxoG will mis-pair with dA, leading to an increase in G to T transition mutations.ROS can induce both telomere-based senescence and SIPS. ROS induce DNA strand breaks, and base and nucleotide modifications, particularly in sequences with high guanosine content, such as telomeres. Indeed, telomeres are one of the DNA structures most sensitive to oxidative damage and ROS can accelerate telomere loss *in vitro*. Lipid products of oxidative stress such as 4-Hydroxynonenal (HNE) that are found in atherosclerotic plaques [37] inhibit telomerase expression and activity [38], and increased telomere loss/division can also occur in individual cells due to a telomere-specific deficiency in base excision repair, leading to preferential accumulation of ROS-induced single-stranded DNA breaks, preventing replication of distal telomeres when cells divide. Thus, it is possible that oxidative DNA damage to telomeres induces a DNA repair response that induces senescence. Not only do ROS induce senescence, senescent cells produce high levels of ROS, and contain higher levels of oxidatively damaged DNA [39]. *In vitro*, VSMCs from aged mice have decreased proliferation, yet generated higher levels of ROS in comparison with cells from younger mice, associated with decreased endogenous antioxidant activity, increased lipid peroxidation, and mitochondrial DNA damage [40].

6. Consequences of cellular senescence

6.1. *Vascular smooth muscle cells*

Although plaque rupture and erosion can induce vessel occlusion, a large proportion of ruptured plaques are clinically silent. For example, plaques demonstrate multiple sites of healed rupture, suggesting that the majority of disrupted plaques are repaired. Repair is due to a combination of VSMC invasion of thrombus, proliferation and matrix synthesis. The repair site is gradually remodelled over time, with alterations in matrix components, and resorption of thrombus. As plaque rupture and repair is also associated with plaque growth, the efficiency of the repair process critically determines whether the plaque undergoes subsequent rupture or causes lumen narrowing. VSMCs that have undergone senescence would be predicted to result in failure of efficient repair after plaque rupture. Although this prediction appears reasonable, there is no direct evidence for this, partly because of lack of a reliable animal model of plaque rupture.

6.2. *Endothelial cells*

Although cell turnover occurs in VSMCs as the plaque develops and after plaque rupture, endothelial cell replication is also increased at sites of plaque development. Cell replication occurs in response to cellular damage and cellular loss, in an attempt to maintain endothelial cell coverage. Exhaustion of replicative potential or senescence induced by DNA damage could therefore contribute to the findings that endothelial function decline with age. Thus, vasodilatation in conduit arteries such as the coronary arteries has been shown to decrease with age and this has been attributed to decreased generation and/or enhanced breakdown of endothelial vasodilators, as well as increased endothelial responsiveness to vasoconstrictors [41,42]. Consistent with this, endothelial NO production and endothelial NO synthase expression (eNOS) decline in endothelial senescent cells [43,44]. Vascular generation of superoxide anions (O_2^-) on the other hand, which are known to impair vascular relaxation by decreasing NO

bioavailability and by increasing peroxynitrite formation, increases [45]. Prostacyclin production is also attenuated whereas the production of two potent vasoconstrictors, thromboxane A2 and endothelin-1, is increased with both endothelial ageing and senescence.

There is extensive evidence that endothelial cell senescence contributes to atherogenesis (reviewed in [46–51]). Although many of the properties of senescent ECs identified above may contribute to this, senescent ECs also show enhanced interaction with monocytes and this appears to be a result of adhesion molecule and proinflammatory cytokine upregulation [46,52]. Senescent EC are also more sensitive to apoptosis and this may contribute to plaque erosion and progression [53].

6.3. *Blood cells*

Recent studies have identified an inverse relationship between peripheral leukocyte telomere length and either atherosclerosis or premature myocardial infarction [54–57]. Telomere length in leukocytes is also negatively associated with mortality due to cardiovascular disease [58]. In these studies, the average leukocyte telomere length in patients with severe coronary artery disease either before or after myocardial infarction was 300 bp shorter than those from control groups [54,55], corresponding to an equivalent difference of 11 years of age [55]. Despite this difference, the rate of decrease in telomere length per year was similar in both groups. Some studies have shown that telomere length is associated with cardiac events (mostly premature MI) in both small [55,59] and large cohorts of patients (for example the West of Scotland cohort [60]). However, other studies do not find such an association [61].

Several explanations have been proposed to support an association between blood cell telomere length and atherosclerosis. First, the telomere length of leukocytes, which is genetically determined at birth, may predispose to age-related diseases. In this model, senescent leukocytes contribute to atherosclerosis via an unknown mechanism. Second, inflammation associated with atherosclerosis accelerates leukocyte turnover, such that telomere loss is a marker of leukocyte senescence, but does not *per se* contribute to atherosclerosis. Inflammation would be both the inducer of telomere loss and predisposes to atherosclerosis, but leukocyte telomere loss *per se* does not promote atherosclerosis. In support of this, telomeres in T lymphocytes from patients with chronic inflammatory disease are significantly shorter than in the control group [62]. In addition, it has been proposed that age-related exhaustion of bone marrow vascular progenitor cells may increase the development of atherosclerosis.

6.4. *Senescence of multiple cell types*

Although there is considerable evidence of cell senescence in atherosclerosis, there are very few studies of accelerated senescence *in vivo* to demonstrate directly the role for cellular ageing. In most cases, whole body manipulations of the telomere structural proteins or telomerase have been used in mouse models of atherosclerosis to demonstrate any effect of cell senescence. Unfortunately, telomeres in mice have a very different structure than human telomeres, and these manipulations would be predicted to affect multiple cell types that contribute to the plaque. For example, ApoE null mice defective in hTERT show reduced atherosclerosis suggesting that decreased hTERT expression is protective in atherosclerosis [63]. In this study, hTERT was knocked-out in all cells that comprise the plaque, and the effects of telomerase deficiency were linked to impaired function of inflammatory cells. In contrast, mice in which various aspects of senescence are accelerated show increased atherosclerosis, suggesting that cellular senescence ultimately promotes atherogenesis [64]. It is therefore possible that manipulation of cellular senescence in VSMCs, ECs or inflammatory cells in mice can have variable effects on plaque development depending upon the stage of lesion formation.

In humans, VSMC senescence may exert profound effects on atherogenesis and stability of advanced plaques. Most patients with Hutchison Gilford Progeria Syndrome (HGPS), an accelerated ageing syndrome, die of atherosclerosis. VSMC depletion is a major feature in progeria, and normal ageing, and likely represents replicative senescence, telomere shortening, and decreased capacity for repair, as HGPS VSMCs are more susceptible to haemodynamic and ischaemic stress [65]. Replicative senescence and ongoing apoptosis in the fibrous cap would result in cap thinning, frequently seen in advanced human lesions, predisposing to plaque rupture. Senescent cells may also promote plaque instability by over-expressing proteins such as adhesion molecules [66], regulators of haemostasis [67], and matrix metalloproteinases [68].

Although senescence of vascular cells may impact directly on the development of atherosclerosis, senescence of non-vascular cells may indirectly promote atherosclerosis. For example, premature senescence of cells regulating lipid or glucose metabolism may induce a pro-atherogenic lipid profile or result in insulin resistance or diabetes. Along these lines, haploinsufficiency of the DNA damage sensor gene ATM results in multiple feature of the metabolic syndrome (hypertension, diabetes, insulin resistance, obesity) [69].

7. Prevention/treatment of DNA damage in atherosclerosis

The presence and biological consequences of DNA damage in atherosclerosis mean that both prevention and reversal of damage are therapeutic aims. *In vitro*, antioxidants can ameliorate ROS-induced DNA damage, although antioxidant trials in humans have been disappointing. In contrast, cholesterol lowering by diet is associated with a reduction in DNA damage and markers of DNA repair, at least in animal models. Drugs that have been proven to alter plaque progression and patient events have also been shown to alter vascular oxidative stress. In particular, HMGCoA reductase inhibitors ('Statins') reduce NAD(P)H oxidase activation [70,71] and superoxide production *in vitro*, in part by inhibiting the membrane translocation (and thus activity) of the small GTP-binding protein Rac-1 [72,73], a regulatory component of vascular NAD(P)H oxidase. Statins can also reduce superoxide production, and mRNA expression of specific *nox* subunits *in vivo*. This effect may underlie the observation that atorvas-

tatin reduces the degree of DNA damage of peripheral lymphocytes as well levels of oxidant stress in hyperlipidaemic patients.

Although drugs that reduce oxidative DNA damage may slow the development of premature vascular cell senescence, the more advanced the disease, the less tractable any therapeutic maneuvers are. For example, lipid lowering reverses activation of DNA damage earlier and to a greater extent than any reduction in markers of DNA damage [34]. This means that once senescent VSMCs and ECs are produced, such changes are irreversible. Only cellular replacement with more youthful cell types, either from circulating cells (ECs) or the adjacent healthy vessel wall (VSMCs), offers the chance of revitalising the vessel wall. Major advances in stem cell biology and knowledge about the mobilisation of progenitor cells will be required before this possibility can be achieved.

8. Conclusions

In summary, there is extensive evidence of free radical-induced DNA damage in atherosclerosis, and evidence of senescence of multiple cell types in the plaque. DNA damage, associated with repeated rounds of cell replication of ECs and VSMCs, results in cell senescence. Senescence will enhance atherogenesis and promote plaque rupture. Prevention and treatment of both DNA damage and cell senescence are major therapeutic targets in atherosclerosis.

REFERENCES

[1] Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. Arterioscler Thromb Vasc Biol 2000;20(5):1262–75.

[2] Loree HM, Tobias BJ, Gibson LJ, Kamm RD, Small DM, Lee RT. Mechanical properties of model atherosclerotic lesion lipid pools. Arterioscler Thromb 1994;14(2):230–4.

[3] Campisi J. The biology of replicative senescence. Eur J Cancer 1997;33(5):703–9.

[4] Gordon D, Reidy MA, Benditt EP, Schwartz SM. Cell proliferation in human coronary arteries. Proc Natl Acad Sci U S A 1990;87(12):4600–4.

[5] Lutgens E, de Muinck ED, Kitslaar PJ, Tordoir JH, Wellens HJ, Daemen MJ. Biphasic pattern of cell turnover characterizes the progression from fatty streaks to ruptured human atherosclerotic plaques. Cardiovasc Res 1999;41(2):473–9.

[6] Ross R, Wight TN, Strandness E, Thiele B. Human atherosclerosis. I. Cell constitution and characteristics of advanced lesions of the superficial femoral artery. Am J Pathol 1984;114(1):79–93.

[7] Mosse PR, Campbell GR, Wang ZL, Campbell JH. Smooth muscle phenotypic expression in human carotid arteries. I. Comparison of cells from diffuse intimal thickenings adjacent to atheromatous plaques with those of the media. Lab Invest 1985;53(5):556–62.

[8] Bennett M, Evan G, Schwartz S. Apoptosis of human vascular smooth muscle cells derived from normal vessels and coronary atherosclerotic plaques. J Clin Invest 1995;95:2266–74.

[9] Bauriedel G, Hutter R, Welsch U, Bach R, Sievert H, Luderitz B. Role of smooth muscle cell death in advanced coronary primary lesions: implications for plaque instability. Cardiovasc Res 1999;41(2):480–8.

[10] Bennett MR, Macdonald K, Chan SW, Boyle JJ, Weissberg PL. Cooperative interactions between RB and p53 regulate cell proliferation, cell senescence, and apoptosis in human vascular smooth muscle cells from atherosclerotic plaques. Circ Res 1998;82(6):704–12.

[11] Bonin L, Madden K, Shera K, Ihle J, Matthews C, Aziz S, et al. Generation and characterization of human smooth muscle cells lines derived from atherosclerotic plaque. Art Thromb Vasc Biol 1999;19:575–87.

[12] Dimri G, Lee X, Basile G, Acosta M, Scott G, Roskelley C, et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci 1995;92:9363–7.

[13] Kurz DJ, Decary S, Hong Y, Erusalimsky JD. Senescence-associated β-galactosidase reflects an increase in lysosomal mass during replicative ageing of human endothelial cells. J Cell Sci 2000;113(Pt 20/4): 3613–22.

[14] Vasile E, Tomita Y, Brown LF, Kocher O, Dvorak HF. Differential expression of thymosin beta-10 by early passage and senescent vascular endothelium is modulated by VPF/VEGF: evidence for senescent endothelial cells in vivo at sites of atherosclerosis. FASEB J 2001;15(2):458–66.

[15] Minamino T, Yoshida T, Tateno K, Miyauchi H, Zou Y, Toko H, et al. Ras induces vascular smooth muscle cell senescence and inflammation in human atherosclerosis. Circulation 2003;108(18):2264–9.

[16] Fenton M, Barker S, Kurz D, Erusalimsky J. Cellular senescence after single and repeated balloon catheter denudations of rabbit carotid arteries. Arterioscl Thromb Vasc Biol 2001;21:220–6.

[17] O'Sullivan M, Scott S, McCarthy N, Shapiro LM, Kirkpatrick PJ, Bennett MR. Differential cyclin E expression in human in stent stenosis vascular smooth muscle cells identifies targets for selective anti-restenotic therapy. Cardiovasc Res 2003;60:673–83.

[18] Tanner F, Boehm M, Akyürek L, San H, Yang Z-Y, Tashiro J, et al. Differential effects of the cyclin-dependent kinase inhibitors p27Kip1, p21Cip1 and p16Ink4 on vascular smooth muscle cell proliferation. Circulation 2000;101:2022–5.

[19] Tanner FC, Yang ZY, Duckers E, Gordon D, Nabel GJ, Nabel EG. Expression of cyclin-dependent kinase inhibitors in vascular disease. Circ Res 1998;82(3):396–403.

[20] Ihling C, Menzel G, Wellens E, Monting JS, Schaefer HE, Zeiher AM. Topographical association between the cyclin-dependent kinases inhibitor P21, p53 accumulation, and cellular proliferation in human atherosclerotic tissue. Art Thromb Vasc Biol 1997;17(10):2218–24.

[21] Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature 1990;345(6274):458–60.

[22] Chang E, Harley CB. Telomere length and replicative aging in human vascular tissues. Proc Natl Acad Sci U S A 1995;92(24):11190–4.

[23] Aviv H, Khan MY, Skurnick J, Okuda K, Kimura M, Gardner J, et al. Age dependent aneuploidy and telomere length of the human vascular endothelium. Atherosclerosis 2001;159(2):281–7.

[24] Okuda K, Khan MY, Skurnick J, Kimura M, Aviv H, Aviv A. Telomere attrition of the human abdominal aorta: relationships with age and atherosclerosis. Atherosclerosis 2000;152(2):391–8.

[25] Matthews C, Gorenne I, Scott S, Figg N, Kirkpatrick P, Ritchie A, et al. Vascular smooth muscle cells undergo telomere-based senescence in human atherosclerosis:

effects of telomerase and oxidative stress. Circ Res 2006;99(2):156–64.
[26] McKee JA, Banik SS, Boyer MJ, Hamad NM, Lawson JH, Niklason LE, et al. Human arteries engineered in vitro. EMBO Rep 2003;4(6):633–8.
[27] Minamino T, Kourembanas S. Mechanisms of telomerase induction during vascular smooth muscle proliferation. Circ Res 2001;89:237–43.
[28] Kurz D, Decary S, Hong Y, Trivier E, Akhmedov A, Erusalimsky J. Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. J Cell Sci 2004;117:2417–26.
[29] Mahmoudi M, Mercer J, Bennett M. DNA damage and repair in atherosclerosis. Cardiovasc Res 2006;71(2):259–68.
[30] Herbig U, Jobling W, Chen P, Chen D, Sedivy J. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21, but not p16. Mol Cell 2004;14:501–13.
[31] Warnholtz A, Nickenig G, Schulz E, Macharzina R, Brasen JH, Skatchkov M, et al. Increased NADH-oxidase-mediated superoxide production in the early stages of atherosclerosis: evidence for involvement of the renin-angiotensin system. Circulation 1999;99(15):2027–33.
[32] Hathaway CA, Heistad DD, Piegors DJ, Miller Jr FJ. Regression of atherosclerosis in monkeys reduces vascular superoxide levels. Circ Res 2002;90(3):277–83.
[33] Martinet W, Knaapen MW, De Meyer GR, Herman AG, Kockx MM. Elevated levels of oxidative DNA damage and DNA repair enzymes in human atherosclerotic plaques. Circulation 2002;106(8):927–32.
[34] Martinet W, Knaapen M, De Meyer G, Herman A, Kockx M. Oxidative DNA damage and repair in experimental atherosclerosis are reversed by dietary lipid lowering. Circ Res 2001;88:733–9.
[35] Basta G, Schmidt AM, De Caterina R. Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. Cardiovasc Res 2004;63(4):582–92.
[36] Ames B, Shigenaga K. Oxidative stress is a major contribution to aging. Ann NY Acad Sci 1992;663:85–96.
[37] Jurgens G, Chen Q, Esterbauer H, Mair S, Ledinski G, Dinges HP. Immunostaining of human autopsy aortas with antibodies to modified apolipoprotein B and apoprotein(a). Arterioscler Thromb 1993;13(11):1689–99.
[38] Pizzimenti S, Briatore F, Laurora S, Toaldo C, Maggio M, De Grandi M, et al. 4-Hydroxynonenal inhibits telomerase activity and hTERT expression in human leukemic cell lines. Free Radic Biol Med 2006;40(9):1578–91.
[39] Chen Q, Fischer A, Reagan JD, Yan LJ, Ames BN. Oxidative DNA damage and senescence of human diploid fibroblast cells. Proc Natl Acad Sci U S A 1995;92(10):4337–41.
[40] Moon S, Thompson L, Madamanchi N, Ballinger S, Papaconstantinou J, horaist C, et al. Aging, oxidative responses, and proliferative capacity in cultured mouse aortic smooth muscle cells. Am J Physiol Heart Circ Physiol 2001;280:H2779–88.
[41] Egashira K, Inou T, Hirooka Y, Kai H, Sugimachi M, Suzuki S, et al. Effects of age on endothelium-dependent vasodilation of resistance coronary artery by acetylcholine in humans. Circulation 1993;88(1):77–81.
[42] Blackwell KA, Sorenson JP, Richardson DM, Smith LA, Suda O, Nath K, et al. Mechanisms of aging-induced impairment of endothelium-dependent relaxation: role of tetrahydrobiopterin. Am J Physiol Heart Circ Physiol 2004;287(6):H2448–53.
[43] Sato I, Morita I, Kaji K, Ikeda M, Nagao M, Murota S. Reduction of nitric oxide producing activity associated with in vitro aging in cultured human umbilical vein endothelial cell. Biochem Biophys Res Commun 1993;195(2):1070–6.
[44] Matsushita H, Chang E, Glassford AJ, Cooke JP, Chiu CP, Tsao PS. eNOS activity is reduced in senescent human endothelial cells: Preservation by hTERT immortalization. Circ Res 2001;89(9):793–8.
[45] van der Loo B, Labugger R, Skepper JN, Bachschmid M, Kilo J, Powell JM, et al. Enhanced peroxynitrite formation is associated with vascular aging. J Exp Med 2000;192(12):1731–44.
[46] Minamino T, Miyauchi H, Yoshida T, Ishida Y, Yoshida H, Komuro I. Endothelial cell senescence in human atherosclerosis: role of telomere in endothelial dysfunction. Circulation 2002;105(13):1541–4.
[47] Freedman DA. Senescence and its bypass in the vascular endothelium. Front Biosci 2005;10:940–50.
[48] Erusalimsky JD, Kurz DJ. Cellular senescence in vivo: its relevance in ageing and cardiovascular disease. Exp Gerontol 2005;40(8/9):634–42.
[49] Minamino T, Miyauchi H, Yoshida T, Tateno K, Kunieda T, Komuro I. Vascular cell senescence and vascular aging. J Mol Cell Cardiol 2004;36(2):175–83.
[50] d'Alessio P. Aging and the endothelium. Exp Gerontol 2004;39(2):165–71.
[51] Brandes RP, Fleming I, Busse R. Endothelial aging. Cardiovasc Res 2005;66(2):286–94.
[52] Shelton DN, Chang E, Whittier PS, Choi D, Funk WD. Microarray analysis of replicative senescence. Curr Biol 1999;9(17):939–45.
[53] Zhang J, Patel JM, Block ER. Enhanced apoptosis in prolonged cultures of senescent porcine pulmonary artery endothelial cells. Mech Ageing Dev 2002;123(6): 613–25.
[54] Samani NJ, Boultby R, Butler R, Thompson JR, Goodall AH. Telomere shortening in atherosclerosis. Lancet 2001;358(9280):472–3.
[55] Brouilette S, Singh RK, Thompson JR, Goodall AH, Samani NJ. White cell telomere length and risk of premature myocardial infarction. Arterioscler Thromb Vasc Biol 2003;23(5):842–6.
[56] Nakashima H, Ozono R, Suyama C, Sueda T, Kambe M, Oshima T. Telomere attrition in white blood cell correlating with cardiovascular damage. Hypertens Res 2004;27(5):319–25.
[57] Benetos A, Gardner JP, Zureik M, Labat C, Xiaobin L, Adamopoulos C, et al. Short telomeres are associated with increased carotid atherosclerosis in hypertensive subjects. Hypertension 2004;43(2):182–5.
[58] Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years or older. Lancet 2003;361(9355):393–5.
[59] van der Harst P, van der Steege G, de Boer RA, Voors AA, Hall AS, Mulder MJ, et al. Telomere length of circulating leukocytes is decreased in patients with chronic heart failure. J Am Coll Cardiol 2007;49(13):1459–64.
[60] Brouilette SW, Moore JS, McMahon AD, Thompson JR, Ford I, Shepherd J, et al. Telomere length, risk of coronary heart disease, and statin treatment in the West of Scotland Primary Prevention Study: a nested case–control study. Lancet 2007;369(9556):107–14.
[61] Kurz DJ, Kloeckener-Gruissem B, Akhmedov A, Eberli FR, Buhler I, Berger W, et al. Degenerative aortic valve stenosis, but not coronary disease. is associated with shorter telomere length in the elderly. Arterioscler Thromb Vasc Biol 2006;26(6):1303.
[62] Wu K, Higashi N, Hansen ER, Lund M, Bang K, Thestrup-Pedersen K. Telomerase activity is increased and telomere length shortened in T cells from blood of patients with atopic dermatitis and psoriasis. J Immunol 2000;165(8):4742–7.

[63] Poch E, Carbonell P, Franco S, Diez-Juan A, Blasco MA, Andres V. Short telomeres protect from diet-induced atherosclerosis in apolipoprotein E-null mice. FASEB J 2004;18(2):418–20.
[64] Fenton M, Huang HL, Hong Y, Hawe E, Kurz DJ, Erusalimsky JD. Early atherogenesis in senescence-accelerated mice. Exp Gerontol 2004;39(1):115–22.
[65] Stehbens WE, Wakefield SJ, Gilbert-Barness E, Olson RE, Ackerman J. Histological and ultrastructural features of atherosclerosis in progeria. Cardiovasc Pathol 1999;8(1):29–39.
[66] Saito H, Papaconstantinou J. Age-associated differences in cardiovascular inflammatory gene induction during endotoxic stress. J Biol Chem 2001;276(31):29307–12.
[67] Yamamoto K, Takeshita K, Shimokawa T, Yi H, Isobe K, Loskutoff DJ, et al. Plasminogen activator inhibitor-1 is a major stress-regulated gene: implications for stress-induced thrombosis in aged individuals. Proc Natl Acad Sci U S A 2002;99(2):890–5.
[68] Zeng G, McCue HM, Mastrangelo L, Millis AJ. Endogenous TGF-beta activity is modified during cellular aging: effects on metalloproteinase and TIMP-1 expression. Exp Cell Res 1996;228(2):271–6.
[69] Schneider JG, Finck BN, Ren J, Standley KN, Takagi M, Maclean KH, et al. ATM-dependent suppression of stress signaling reduces vascular disease in metabolic syndrome. Cell Metab 2006;4(5):377–89.
[70] Wassmann S, Laufs U, Baumer AT, Muller K, Ahlbory K, Linz W, et al. HMG-CoA reductase inhibitors improve endothelial dysfunction in normocholesterolemic hypertension via reduced production of reactive oxygen species. Hypertension 2001;37(6):1450–7.
[71] Vecchione C, Brandes RP. Withdrawal of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors elicits oxidative stress and induces endothelial dysfunction in mice. Circ Res 2002;91(2):173–9.
[72] Wassmann S, Laufs U, Muller K, Konkol C, Ahlbory K, Baumer AT, et al. Cellular antioxidant effects of atorvastatin in vitro and in vivo. Arterioscler Thromb Vasc Biol 2002;22(2):300–5.
[73] Tsubouchi H, Inoguchi T, Sonta T, Sato N, Sekiguchi N, Kobayashi K, et al. Statin attenuates high glucose-induced and diabetes-induced oxidative stress in vitro and in vivo evaluated by electron spin resonance measurement. Free Radic Biol Med 2005;39(4):444–52.