Role of DNA Damage in Cardiovascular Disease

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Patients with some progeroid syndromes, such as Werner syndrome, exhibit atherosclerotic cardiovascular disease (CVD) at a young age as a manifestation of premature aging. Recent studies have revealed that most progeroid syndromes are caused by genetic defects in specific molecules involved in the DNA damage response, a cornerstone of genome stability. Ionizing radiation is one of the most potent genotoxic stimuli and causes various kinds of DNA damage. Further, there is increasing evidence that therapeutic radiation treatments can cause cardiovascular complications. Here, we describe the DNA damage and subsequent response, review recent advances in the understanding of the molecular basis of progeroid syndromes (especially those syndromes that involve CVD), review the pathological and epidemiological analysis of radiation-induced CVD, and discuss the possible role of DNA damage and the DNA damage response in the pathogenesis of atherosclerotic CVD. (Circ J 2014; 78: 42–50)

Key Words: Atherosclerosis; DNA repair; Genome instability; Progeria; Senescence

Progeroid syndromes are rare genetic disorders that recapitulate physiological aging at an accelerated rate. In 1995, Yosef Shiloh’s group identified ATM, the gene mutated in ataxia-telangiectasia. The following year, the gene responsible for Werner syndrome, WRN, which encodes a helicase, was identified by positional cloning. Since the time of those discoveries, the links between the DNA repair system and progeroid syndromes have been a major research focus in the field of molecular genetics, gerontology and oncology. Most of these syndromes are caused by mutations in genes encoding DNA repair proteins, and therefore, these syndromes involve genome instability. Growing evidence suggests that molecules that mediate the response to DNA damage and the subsequent cellular outcomes of this process can also contribute to the development of cardiovascular disease.

DNA Damage and DNA Damage Response

Genotoxic Stimuli and Types of DNA Damage

The human genome is under constant attack by endogenous and exogenous factors, and the protection and faithful repair of genomic DNA is critical for cell survival. DNA damage is caused by a wide variety of environmental agents (e.g., ionizing radiation (IR), ultraviolet (UV) radiation from sunlight, and numerous genotoxic chemicals) and by the byproducts of normal cellular metabolism (e.g., reactive oxygen species (ROS) and products of lipid peroxidation). IR and UV light can induce the formation of pyrimidine dimers, and IR can also induce oxidation of DNA bases, single-strand breaks (SSBs) and double-strand breaks (DSBs). Chemothapeutic agents can cause a variety of different DNA lesions, including alkylation of bases, covalent links between bases of the same DNA strand (intrastrand crosslinks) or of different DNA strands (interstrand crosslinks), SSBs and DSBs. Cigarette smoke contains genotoxic components and can cause a wide variety of DNA adducts and oxidative DNA damage. ROS, which are produced in the course of normal cellular metabolism, electron leaks in the electron transport chains, various enzymes, IR and UV, can induce base modifications, abasic sites, protein-DNA adducts, intra-/interstrand DNA crosslinks, SSBs and DSBs. Mis-incorporation and erroneous insertion and deletion of bases can also arise during DNA replication and recombination, as well as during the repair of some forms of DNA damage.

DNA Damage Response and Its Cellular Consequences

Cells have an evolutionally conserved pathway, termed the DNA-damage response (DDR), that senses, transduces the signal of, and repairs the damage with the goal of maintaining genomic integrity. This repair response can arrest the cell cycle in order to avoid propagating damaged DNA into daughter cells. Generally, DSBs are detected by the MRE11-RAD50-NBS1 (MRN) complex, which activates the primary kinase, ATM (ataxia-telangiectasia mutated), whereas SSBs are detected by RPA and the RAD9-RAD1-HUS1 (9-1-1) complex, which recruit another primary kinase ATR (ataxia-telangiectasia and Rad3-related). ATM/ATR phosphorylate mediators and downstream kinases, including H2AX, CHK1/2, p53, and...
BRCA1, to transduce DDR signaling.\textsuperscript{11}

Multiple repair pathways have evolved to counteract DNA damage and each is directed to a specific type of lesion. To date, 5 major DNA repair pathways have been identified: base excision repair (BER), nucleotide excision repair (NER), mismatch repair, homologous recombination (HR) and nonhomologous end joining (NHEJ).

If the DNA lesions are properly repaired, the cells resume normal proliferation after transient cell-cycle arrest. If the DNA damage is severe and/or remains un repaired, the cells undergo apoptosis or cellular senescence. DDR modulates many other cellular responses, including transcription and chromatin remodelling.

**Repair of Single-Strand DNA Damage**

BER is the primary DNA repair pathway that corrects single lesion or small alteration of bases, such as oxidation, alkyla tion, deamination and SSBs. The repair process occurs as follows: (1) excision of a damaged or inappropriate base, (2) inci sion at the resulting abasic site, (3) clean-up of termini to permit repair synthesis and/or nick ligation, (4) gap-filling, and (5) sealing of the final, remaining DNA nick.\textsuperscript{12,13}

NER is a more complex process involved in the removal of a lesion containing oligonucleotides and is a particularly important repair system for the removal of bulky DNA lesions.\textsuperscript{14} There are 2 types of pathway depending on the manner in which DNA damage is recognized: global genome repair (GGR) or transcription-coupled repair (TCR). GGR scans the genome constantly and repairs damage in inactive nontranscribed genes throughout the genome. By contrast, TCR is initiated when RNA polymerase stalls at a lesion during active transcription. In either system, after identification of a damaged site, the repair proteins are recruited to the site to confirm the presence of damage, excise the damaged DNA, and then fill in the gap.

**Repair of Double-Strand Breaks**

DSBs are life-threatening lesions that are repaired by a complex network of multiple DNA repair pathways. To date, at least 4 independent pathways are known to repair DSBs: HR, NHEJ, alternative-NHEJ, and single-strand annealing.\textsuperscript{15} Among these pathways, NHEJ and HR are evolutionarily conserved DNA repair pathways and maintain genomic stability in response to DSBs.\textsuperscript{16} The main differences in these pathways are the requirement for DNA homology (template) on the sister chroma tid in HR, and the accuracy of the repair. In mammalian cells, NHEJ is the predominant pathway and is utilized throughout the cell cycle.\textsuperscript{16} NHEJ repairs DNA damage by simply rejoining the ends of the DNA, and this process does not use a template to synthesize new DNA at the damaged sites (Figure 1). Thus, NHEJ is less accurate and may give rise to some deletions (ie, error-prone repair). Important proteins involved in NHEJ include the Ku70/80 heterodimer, DNA-dependent protein kinase, catalytic subunit (DNA-PKcs), and the DNA ligase IV/X-ray repair cross-complementing protein 4 (Xrcc4) complex.\textsuperscript{13}

HR is essential for preservation of genomic integrity. Multiple regulatory mechanisms have evolved to ensure that HR occurs at the correct timing and in the correct context. HR requires a sister chromatid for template purposes (Figure 2). A central player in HR is Rad51, which scans the genome for an intact copy of the broken DNA on the sister chromatid.\textsuperscript{17} During HR, the missing information on the broken strand is copied from the sister chromatid, so the damage is repaired without loss of genetic information. HR can only take place in dividing cells, because of the necessity of a sister chromatid. In most mammalian cells, the HR process is largely limited to the S-phase and to the repair of specific DNA lesions.\textsuperscript{16} By contrast, terminally differentiated cells or cells in the G0 or G1 phase rely on NHEJ rather than HR.

**Progeroid Syndromes/Genomic Instability Syndromes**

As mentioned before, most progeroid syndromes are caused by mutations in the genes encoding DNA repair proteins or by mutations in genes encoding protein components of the nuclear lamina. It is noteworthy that some syndromes predominantly manifest with senescence, others are associated with predisposition to cancer, and some have both phenotypes (summarized in the Table). Some syndromes are of particular interest to the study of CVD, because affected patients exhibit premature atherosclerosis, dyslipidemia and diabetes.

**Werner Syndrome**

Werner syndrome (WS) is a rare autosomal recessive disease that is highly prevalent in Japan.\textsuperscript{18} It is characterized by premature onset of the signs of aging, which includes graying and loss of hair, bilateral cataracts, scleroderma-like skin changes, short stature, thin limbs, atherosclerosis, diabetes, osteoporosis, and a high incidence of malignancies.\textsuperscript{18,19} Symptoms appear during the second or third decade of life, and the median age of death of affected patients is 46 years.\textsuperscript{20} The major causes of death in patients with WS are myocardial infarction.
Several lines of research have shown that cells derived from WS patients and WRN knock-down cells are hypersensitive to genotoxic reagents and display various types of chromosomal abnormalities. These observations support the notion that the WRN protein plays an important role in DNA repair. Indeed, WRN plays a pivotal role in DNA replication. Several studies suggest that WRN promotes recovery from replication fork stalling by preventing SSBs from being converted into DSBs, regressing the fork, allowing bypass formation, and resolving DNA structures at fragile sites. Further, WRN rap-

**Figure 2.** Repair of DNA double-strand break (DSB) by homologous recombination (HR). See the main text for details. DSB are recognized by the Mre11–Rad50–Nbs1 complex, which is recruited to the DSB site to generate a single-stranded DNA by resection. The single-stranded ends are bound by Rad51 which polymerizes and searches the homology, and then invades the homologous template. DNA polymerases use the intact copy to re-synthesize the deleted DNA sequences and DNA ligases join the synthesized fragments.
findings suggest that WRN plays an important role in telomere maintenance and that telomere attrition is a key mechanism in the pathogenesis of WS.

It is noteworthy that WS, Bloom’s syndrome, and Rothmund-Thomson syndrome exhibit some different clinical features but share overlapping phenotypes, despite the fact that the defective proteins in all of these syndromes belong to the RecQ-like DNA helicase family. For example, patients with Bloom’s syndrome do not show premature aging, whereas patients with WS and Rothmund-Thomson syndrome do. In addition, Bloom’s syndrome and Rothmund-Thomson syndrome are associated with sun-sensitivity, whereas this phenomenon is not common in patients with WS.

Telomeres are markedly shortened in WS cells, and the introduction of telomerase extends lifespan and reduces chromosomal aberration in these cells. Double-null mice for WRN and the telomerase RNA component (TERC), a component of the telomerase complex, recapitulate clinical features of WS patients, such as diabetes, cataracts, osteoporosis, hair graying, alopecia and premature death, whereas WRN deficiency alone does not lead to premature aging.

**Table. Progeroid Syndromes and Related Diseases**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Genetic defect</th>
<th>Affected cellular process</th>
<th>Clinical phenotype</th>
<th>Mean lifespan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ataxia-telangiectasia (AT*)</td>
<td>ATM</td>
<td>DSB repair (HR, NHEJ)</td>
<td>Cerebellar ataxia</td>
<td>20 years</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Telangiectasia</td>
<td></td>
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<td></td>
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<td></td>
<td>Immunodeficiency</td>
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<td></td>
<td></td>
<td></td>
<td>Cancer predisposition (Premature aging)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Insulin resistance)</td>
<td></td>
</tr>
<tr>
<td>Hutchinson-Gilford progeria syndrome (HGPS*)</td>
<td>Lamin A</td>
<td>Nuclear organization DSB repair Transcription</td>
<td>Premature aging Growth retardation Lipodystrophy Atherosclerosis No cancer predisposition</td>
<td>13 years</td>
</tr>
<tr>
<td>Werner syndrome (WS*)</td>
<td>WRN (RecQ helicase)</td>
<td>DSB repair (HR, NHEJ) DNA replication Telomere maintenance Transcription</td>
<td>Premature aging Short stature Thin limbs Atherosclerosis Diabetes Cancer predisposition</td>
<td>~50 years</td>
</tr>
<tr>
<td>Bloom syndrome</td>
<td>BLM (RecQ helicase)</td>
<td>HR DNA replication</td>
<td>Growth retardation UV sensitivity Immunodeficiency Cancer predisposition</td>
<td>27 years</td>
</tr>
<tr>
<td>Rothmund-Thomson syndrome</td>
<td>RecQL4 (RecQ helicase?)</td>
<td>Unknown</td>
<td>Premature aging Growth retardation Poikiloderma Cancer predisposition</td>
<td>Normal?</td>
</tr>
<tr>
<td>Cockayne syndrome</td>
<td>CSA, CSB</td>
<td>Transcription-coupled NER BER?</td>
<td>UV sensitivity Mental retardation Premature aging Kiposis No cancer predisposition</td>
<td>12 years</td>
</tr>
</tbody>
</table>

*AT, HGPS, and WS are discussed in detail in the main text.
BER, base excision repair; DSB, double-strand breaks; HR, homologous recombination; NER, nucleotide excision repair; NHEJ, non-homologous end joining; UV, ultraviolet.

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**Ataxia-Telangiectasia**

Ataxia-telangiectasia is an autosomal recessive disorder that is characterized by progressive cerebellar ataxia because of neuronal degeneration, telangiectasia (dilated blood vessels) in the conjunctivae and skin, immunodeficiency, predisposition to lymphoreticular malignancies, premature aging, growth retardation, and insulin resistance. Interestingly, heterozygotes
for the ataxia-telangiectasia mutated (ATM) allele have an increased risk of death from ischemic heart disease compared with noncarriers.\textsuperscript{35,36}

Ataxia-telangiectasia is caused by mutations in \textit{ATM}, which encodes the ATM protein. ATM is a serine/threonine kinase and has significant homology to phosphatidylinositol 3-kinase (PI3K). Upon DSB induction, ATM rapidly localizes to DNA damage sites, and the kinase activity increases. ATM phosphorylates itself, MRN, CHK2, DNA-PK, p53, 53BP1, and BRCA1, all of which are involved in DDR, including cell-cycle checkpoint, DNA repair, apoptosis, and senescence. Among these downstream targets of ATM, p53 modulates the transcription of many target genes, and plays a key role in DSB-induced cell senescence, apoptosis and cell-cycle arrest. Experiments with ATM, p53-double-null mice suggest that there are also ATM-independent, p53-dependent pathways for cell senescence and apoptosis.\textsuperscript{36,37} Cells from AT patients display a defective response to DSBs, increased chromosomal breakages, sensitivity to IR, and cell-cycle checkpoint defects. Thus, ATM likely functions as a trigger molecule for DDR and thereby promotes the maintenance of genomic stability and a reduction in the risk of cancer and other diseases.

\textbf{Hutchinson-Gilford Progeria Syndrome}

Hutchinson-Gilford progeria syndrome (HGPS) is a rare and severe premature aging syndrome. The incidence of this disorder is approximately 1 in 4,000,000 live births. HGPS is characterized by symptoms of advanced aging, including severe growth retardation, alopecia, lipodystrophy, scleroderma, decreased joint mobility, osteolysis, and progressive atherosclerosis. Death of affected children occurs at approximately 13 years of age, mostly from MI or stroke. Other features of aging, such as malignancies, diabetes, and neural degeneration, are not common.\textsuperscript{38,39}

In most cases, HGPS is caused by a single de novo point mutation in the LMNA gene, which encodes lamin A/C.\textsuperscript{40,41} Lamin A is a major component of the nuclear lamina and is thought to play a major role in maintaining the integrity of the nuclear envelope and in the regulation of transcription and replication. Lamin A is translated from LMNA as a precursor prelamin A. Prelamin A is modified several times, including farnesylation, methylation, and cleavage twice by the endopeptidase, ZMPSTE24. Mature lamin A does not contain the farnesylated Cys residue. The most common mutation found in HGPS is G608G (GGC>GGT) within exon 11. Although this single-base substitution is silent in terms of the coding sequence, it activates a cryptic splice site and causes the removal of 150 nucleotides. The resultant mutant prelamin A is internally deleted near the C-terminus, and is called progerin. Progerin lacks the second cleavage site, and as a result, cannot be correctly processed and remains farnesylated. Fibroblasts from HGPS patients show nuclear abnormalities, such as blebs of the nuclear envelope, thickening of the nuclear lamina, and clustering of nuclear pores.\textsuperscript{40-42} Mice lacking ZMPSTE24 also exhibit progeroid phenotypes and these nuclear abnormalities, whereas mice expressing nonfarnesylated prelamin A do not exhibit progeria.\textsuperscript{43,44} Suggesting that premature aging and nuclear abnormality in HGPS patients are, at least in part, resulting from accumulation of farnesylated prelamin. Of note, p53 target genes are markedly upregulated in Zmpste24-deficient mice, and organismal accelerated aging and cellular senescence phenotypes are partially recovered by p53 deficiency, which suggests activation of p53 plays pivotal role in the accelerated aging observed in HGPS.\textsuperscript{45}

Importantly, cells from HGPS patients and Zmpste24\textsuperscript{-/-} mice show increased DNA damage and defects in DDR.\textsuperscript{46} Foci of 53BP1 and the amount of γH2AX (phosphorylated histone H2AX) are increased at basal state in HGPS cells and tissue samples. In addition, ATM, ATR, and their downstream kinases, CHK2 and CHK1, are activated in HGPS cells, and these cells have elevated sensitivity to genotoxic reagents, indicating that abnormally processed prelamin A causes an increase in DNA damage, especially DSBs.\textsuperscript{47,48} Recruitment of DDR proteins to DNA damage sites is delayed and sustained after genotoxic stress. Thus, it is likely that HGPS cells possess some defects in the DNA repair machinery, especially for DSBs, whereas checkpoint signaling is preserved or even activated in these cells.\textsuperscript{47,49,50} The details regarding these defects in DNA repair have not been elucidated, and therefore, it is largely unknown how progerin causes abnormal DDR and the progeroid phenotype. Interestingly, a similar nuclear deformity, and increased progerin and DSBs are also observed in normally aged individuals, implicating progerin in physiological aging.\textsuperscript{51}

\textbf{Role of DNA Damage in Atherosclerosis}

\textbf{DNA Damage in Atherosclerotic Plaques}

Studies using human samples and animal models suggest that atherosclerotic plaques contain accumulated DNA damage and activated DNA damage response elements. Mahmoudi et al reported that human atherosclerotic plaques have more DSBs and activation of ATM when compared with normal tissue.\textsuperscript{52} Similarly, oxidative DNA damage is also increased with the marker for BER in human atherosclerotic plaques.\textsuperscript{53} These findings suggest that accumulation of DNA damage in atherosclerotic plaques is mediated, at least in part, by ROS. Increased apoptosis and senescence, which are possible consequences of the DNA damage response, have also been demonstrated in the cellular components of atherosclerotic lesions.\textsuperscript{35,45,55}

\textbf{Atherosclerosis in Progeroid Syndromes}

As mentioned earlier, the main causes of death of WS patients are MI and stroke. Considerable atherosclerotic plaque with calcium deposition is present in the coronary arteries and aorta of patients with WS.\textsuperscript{56,57} The precise mechanism by which WRN mutation results in accelerated atherosclerosis in the affected individuals remains unclear. Recently, Okabe et al reported that metabolic disorders, such as diabetes mellitus, hyperlipidemia, and hypertension, are closely related to atherosclerotic vascular disease in patients with WS.\textsuperscript{58} In addition, experiments using a mouse model of WS, in which the helicase domain of WRN is deleted, showed increases in visceral fat, and of fasting triglyceride and cholesterol levels followed by insulin resistance, all of which closely recapitulate the phenotype of human metabolic syndrome.\textsuperscript{59} Thus, metabolic abnormalities may be related to accelerated atherosclerosis in WS. Abnormalities in the immune system, extracellular matrix, and the coagulation system may also be involved in accelerated atherosclerosis and subsequent fatal vascular events in patients with WS.\textsuperscript{18,66} The direct effects of functional defects on vascular cells, such as vascular smooth muscle cells (VSMCs) and endothelial cells, have not been clearly demonstrated in patients with WS.

Human ATM heterozygotes have increased risk of death from ischemic heart disease.\textsuperscript{35} Studies using mouse ATM heterozygotes have also shown features of metabolic syndrome, including increased visceral fat, insulin resistance, and high blood pressure.\textsuperscript{46} ATM deficiency promotes atherosclerosis in apoE\textsuperscript{-/-} mice, which was attenuated by transplantation.
of the bone marrow of wild-type mice. Tissues from ATM-deficient mice showed increased mitochondrial DNA damage and ROS, and reduced oxidative phosphorylation, as well as increased nuclear DNA damage. Although the precise mechanisms are not fully elucidated, it appears that acceleration of atherosclerosis by ATM deficiency involves direct effects on vascular wall cells and disturbance of systemic metabolism resulting from both nuclear and mitochondrial DNA damage and increased ROS.46-48

Similar to WS, atherosclerotic CVD, such as MI and stroke, is the main cause of death in patients with HGPS. However, the histological features of vascular change vary among the published studies,49 probably because the diagnosis of HGPS in these few studies was not consistently based on genetic testing, and therefore, non-HGPS cases may have been included in the patient populations. Collectively, the vascular lesions of HGPS can be characterized as those of conventional atherosclerosis, except that VSMCs are lost in the media, and the lipid core and inflammation are not as profound. The thickened intima and thinned media in patients with HGPS is generally hypocellular, possibly because of degeneration of VSMCs and replacement by fibrosis.49-51 These features may be caused by the absence of other overt risk factors, such as diabetes or hyperlipidemia (in contrast to WS) and are reminiscent of vascular changes in the elderly. Recently, Olive et al described adventitial fibrosis in the arteries from 2 patients with genetically determined HGPS. The classic features of atherosclerosis, such as necrotic core, foam cells, and foci of chronic inflammation were observed in those samples. In addition, progerin was detected in the media, plaques, and adventitia of the coronary arteries (and preferentially in VSMCs and fibroblasts) in HGPS patients. A mouse model of HGPS exhibited severe loss of VSMCs and diminished response to a nitric oxide donor, sodium nitroprusside, and these findings mimic the vascular abnormalities seen in HGPS patients.52 The role of thrombosis in cardiovascular events in patients with HGPS is unclear. No obvious occlusion by thrombus has been documented, although healed plaque rupture was observed.54 In contrast, total or subtotal occlusion by unruptured plaque or fibrous intima has been documented.55 Further pathohistological studies are required to fully characterize the vascular lesions of HGPS.

Recently, 2 laboratories have generated induced pluripotent stem cells (iPSCs) from fibroblasts that were isolated from HGPS patients. A study by Zhang et al demonstrated that radiation doses above 0.5 Gy increase the risk of heart disease and stroke.56,57 The estimated increase in risk for heart disease is 14%/Gy, which is higher than that seen in those women who underwent radiotherapy for breast cancer. The Adult Health Study, which consists of biennial examinations of approximately 15% of the Life Span Study cohort members since 1958, has shown radiation dose-related increase in the prevalence of hypertension, elevated serum cholesterol, and aortic arch calcification.58 In addition, studies of atomic bomb survivors have revealed that radiation also causes persistent increases of inflammatory response markers, such as interleukin 6, CRP, and interferon γ, and alteration of T- and B-cell composition.59,60 Chronic inflammation, as well as increased blood pressure and serum cholesterol, may partially be responsible for the increased rate of cardiovascular events in atomic bomb survivors.

Experimental studies have provided insights into the mechanisms of accelerated atherosclerosis in response to radiation. Stewart et al showed that a single exposure to 14 Gy of radiation resulted in acceleration of atherosclerosis, as characterized by a high proportion of macrophages and granulocytes and by intraplaque hemorrhage, in apoE-/- mice.61 Fractionated radiation (2 Gy × 20) had similar effects,62 and studies suggest that radiation-induced atherosclerosis is characterized by acceleration of initial lesions and exacerbation of local inflammation.63 These clinical and experimental studies support the notion that IR has direct and harmful effects on the arterial wall. However, there is no firm evidence that definitively proves that DNA strand breaks and/or the subsequent DDR are involved in the acceleration of atherosclerosis, despite the fact that radiation doses in the context of radiation therapy or the atomic bomb are sufficient to induce DNA damage. IR produces ROS, and therefore, it can induce vascular pathology via oxidative stress. Further in vivo and in vitro studies are needed to elucidate the precise molecular mechanisms by which radiation accelerates atherosclerosis and increases the risk of coronary artery disease and stroke after radiotherapy.

**Other CVD Associated With DNA Damage**

HGPS patients display several other cardiovascular abnormalities. Thickening and calcification of the aortic and mitral valves are common, and studies have found that progerin accumulates within these altered structures. Other abnormalities include general hypertrophy of the left ventricle, interstitial fibrosis, and patchy MI. The fibrosis, which is prominent in the endocardium, may be caused both by local ischemia and altered expression of the components of extracellular matrix.58,39,62

Cardiac fibrosis and aortic stenosis are observed in a mouse model of WS. Interestingly, the level of hydrogen peroxide is increased in both the serum and the cardiac tissue of these mice, which is followed by accumulation of oxidative DNA damage in the heart.59 The incidence of aortic stenosis or cardiomyopathy is uncertain in WS patients. Besides accelerating atherosclerosis, IR can cause pericar-
Senescence, apoptosis and dysfunction over time. Additionally, the cells that senesce because of persistent DNA damage response secrete proinflammatory cytokines, chemokines, growth factors, and proteases, termed the senescence-associated secretory phenotype (SASP), which fuels chronic inflammation. These phenomena may accelerate atherosclerosis and/or increase plaque instability (Figure 3).

**Conclusions and Future Perspectives**

DNA damage and the consequences of the DNA damage response (e.g., cellular senescence and apoptosis) are present in atherosclerotic plaques. Studies of progeroid syndromes suggest that accumulated DNA damage causes persistent activation of the DNA damage response, telomere attrition, and genome instability, which, in turn, lead to progressive cellular senescence, apoptosis and dysfunction over time. Additionally, the cells that senesce because of persistent DNA damage response secrete proinflammatory cytokines, chemokines, growth factors, and proteases, termed the senescence-associated secretory phenotype (SASP), which fuels chronic inflammation. These phenomena may accelerate atherosclerosis and/or increase plaque vulnerability (Figure 3). It is likely that these cellular events target other tissues, including adipose tissue, as well as the vasculature. DNA damage/DNA damage response could be therapeutic targets for age-related CVD. DNA damage and progerin levels are increased in the normally aged vasculature, although the mechanisms by
which progerin increases DNA damage and/or alters DNA repair remain to be elucidated. Further characterization of the defective proteins in progeroid syndromes that contribute to the DNA damage response will provide insight into the molecular mechanisms underlying progression of atherosclerosis with aging.

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