Inflammation and Tissue Remodeling as Potential Therapeutic Targets

Review

DNA Damage and Senescence-Associated Inflammation in Cardiovascular Disease

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1. INTRODUCTION

Recent studies revealed that most of progeroid syndromes and some familial cancer syndromes are caused by mutations in genes encoding DNA repair proteins. Since the time of those discoveries, study of the links between the DNA repair system and human disease has been a major focus of research in the fields of molecular genetics, gerontology and oncology. “Cellular senescence,” a form of stable cell-cycle arrest, has been considered to be a protective mechanism against cancer. However, senescent cells induced by certain stresses secrete numerous proinflammatory cytokines, chemokines, and growth factors, a feature termed senescence-associated secretory phenotype (SASP). SASP is involved in the DNA repair. DNA damage activates a broad range of signaling pathway that leads to repair, cell cycle arrest, apoptosis and so on, which is called DNA damage response. Recent studies revealed that persistent DNA damage response triggers induction of cell senescence and senescence-associated secretory phenotype (SASP). Here, we review recent advances in the understanding of the molecular mechanisms by which SASP components are regulated, and discuss the possible roles of DNA damage and the DNA damage response, and SASP in the pathogenesis of cardiovascular disease.

Key words DNA repair; inflammation; progeria; senescence

2. DNA DAMAGE AND DNA DAMAGE RESPONSE

2.1. Genotoxic Stimuli and Types of DNA Damage

The genome is under constant attack by endogenous and exogenous genotoxic factors. Endogenous genotoxic factors include byproducts of normal cellular metabolism, such as reactive oxygen species (ROS), reactive nitrogen species, products of lipid peroxidation, and endogenous alkylating factors. DNA damage is also induced by a wide variety of exogenous agents, e.g., ionizing radiation (IR), UV radiation from sunlight, and genotoxic chemicals. In addition, DNA is damaged by spontaneous reactions, such as hydrolysis. IR and UV light can induce the formation of pyrimidine dimers, and IR can also induce the oxidation of DNA bases, single-strand breaks (SSBs) and double-strand breaks (DSBs). Chemotherapeutic agents can cause a variety of DNA lesions, including the 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 all induce base modifications, abasic sites, protein-DNA adducts, intra/inter-strand DNA crosslinks, SSBs and DSBs. Spontaneous hydrolysis causes abasic sites and deamination. In a mammalian cell, it is estimated that occurrence rate of spontaneous hydrolysis of DNA is as high as 104 per day. 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.

2.2. DNA Damage Response and Its Cellular Consequences

Cells have an evolutionally conserved pathway, termed the DNA-damage response (DDR), which senses, transduces the signal of, and repairs DNA damage for 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

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kinase, ATM (ataxia telangiectasia mutated), whereas SSBs are detected by replication protein A (RPA) and the RAD9-RAD1-HUS1 (9-1-1) complex. ATM is a serine/threonine kinase, and shares significant homology with 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 checkpoints, DNA repair, apoptosis, and senescence. Cells from AT patients display a defective response to DSBs, increased chromosomal breakage, 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.6)

There are multiple repair pathways to counteract DNA damage, such as base excision repair (BER), nucleotide excision repair (NER), mismatch repair, homologous recombination (HR) and non-homologous end joining (NHEJ). Each of these repair mechanisms is directed to a specific type of lesion (Fig. 1).

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 unrepaired, DDR induces cell death by apoptosis or permanent cell cycle arrest, i.e., cellular senescence. DDR modulates many other cellular responses, including transcription and chromatin remodeling.7)

2.3. Repair of Single-Strand DNA Damage

BER is the primary DNA repair pathway that corrects single lesions or the subtle alteration of bases, such as oxidation, alkylation, deamination and SSB. BER is involved in the removal of bulky DNA lesions. NER includes two sub-pathways, depending on the manner in which DNA damage is recognized: GG-NER or TC-NER. Double-strand breaks can be repaired by HR or NHEJ. (Color figure can be accessed in the online version.)

NER is a more complex process involved in the removal of a lesion containing oligonucleotides, and it is a particularly important repair system for the removal of helix-distorting (bulky) DNA lesions. These DNA lesions include cyclobutane pyrimidine dimers (CPDs) and pyrimidine-(6,4)-pyrimidone products (6-4PPs) induced by UV radiation, bulky chemical adducts induced by polycyclic aromatic hydrocarbons present in cigarette smoke, intranastral crosslinks induced by cisplatin, and several forms of oxidative damage such as 8,5'-cyclopurine-2'-deoxynucleotides.14) NER includes two sub-pathways, depending on the manner in which DNA damage is recognized: global genome NER (GG-NER) or transcription-coupled NER (TC-NER). GG-NER constantly scans the genome and repairs damage in inactive non-transcribed genes throughout the genome. In GG-NER, xeroderma pigmentosum, complementation group C (XPC) mainly detects damage that distorts the DNA-helix, such as 6-4PPs, whereas CPDs disturb the DNA-helix only mildly, and are poorer substrates for XPC. In repairing CPDs, UV-DDB/XPE supports XPC to recognize the damage. By contrast, TC-NER is initiated by stalled RNA polymerase II at a lesion site during active transcription, which is detected by Cockayne syndrome WD repeat protein A (CSA) and Cockayne syndrome protein B (CSB). After recognition of a damaged site, GG-NER and TC-NER use a common repair mechanism: repair proteins are recruited to the site to confirm the presence of damage, excise the damaged DNA, and then fill in the gap.

2.4. Repair of Double-Strand Breaks

DSBs are the most dangerous lesions, the repair of which is carried out by a complex network of multiple DNA repair pathways. DSBs can be repaired by NHEJ, alternative NHEJ, single-strand anneal-
In most mammalian cells, the HR process is largely limited to S-phase and to the repair of specific DNA lesions. By contrast, DSB repair in terminally differentiated cells, or cells in the Gₐ and Gₐ phase, relies on NHEJ rather than HR.

It is unclear whether alternative NHEJ is merely a backup for classical NHEJ or the components of alternative NHEJ have other functions in dsDNA processing. Future work will help to understand the exact role of alternative NHEJ and single-strand annealing.

3. PROGEROID SYNDROMES/GENOMIC INSTABILITY SYNDROMES

As mentioned before, most progeroid syndromes are caused by mutations in genes that encode DNA repair proteins. It is noteworthy that some syndromes predominantly manifest with senescence, others are associated with a predisposition to cancer, and some have both phenotypes (summarized in Table 1). Some syndromes, such as Hutchinson–Gilford progeria syndrome (HGPS), are of particular interest in the study of cardiovascular disease, as affected patients exhibit premature atherosclerosis. Clinical characteristics of and molecular mechanisms underlying these syndromes have been reviewed elsewhere.

4. SASP

In addition to growth arrest, senescent cells exhibit global changes in gene expression, including the secretion of inflammatory cytokines, chemokines, growth factors, extracellular matrices, matrix metalloproteases, and other proteases, collectively referred to as a senescence-associated secretory phenotype (SASP). SASP factors include interleukin (IL)-1α, IL-1β, IL-6, IL-8, CCL-2, GROα, GROβi, vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), matrix metalloproteinase (MMP)-1,-3 and -10, plasminogen activator, and PAI-1. Although SASP factors vary among cell types and according to varying stimuli, inflammatory cytokines are the most common molecules involved. Importantly, SASP is not just a consequence of senescence, but also induces senescence, as mentioned later.

Acosta et al. conducted an unbiased screening for small hairpin RNAs that extend the lifespan of primary human fibroblasts. They found that a chemokine receptor, CXCR2, was required for replicative, oncogene- and DNA damage-induced senescence. Additionally, the CXCR2 ligands, such as IL-8 and GRO-1, were also upregulated, and were found to mediate replicative and oncogene-induced senescence. The expression of these CXCR2 ligands was regulated by transcription factors nuclear factor-kappa B (NF-κB) and CCAAT/enhancer binding protein (C/EBP). To identify genes upregulated during oncogene-induced senescence, Kuilman et al. performed genome-wide expression microarray analysis of human diploid fibroblasts expressing BRAFV600E, and found that genes involved in cytokine and chemokine responses were specifically activated during OIS (oncogene-induced senescence). Among these genes, IL-6 was upregulated during OIS and downregulated during OIS bypass, irrespective of cell type, and independent of p16INK4A status. A transcription factor, C/EBPβ, was upregulated during OIS, and critically mediated the induction of IL-6, as well
as IL-8. Not only IL-6, but also its cognate receptor, IL-6R, was upregulated in OIS. Of note, the induction of C/EBPβ was also regulated by IL-6, suggesting that IL-6 and C/EBPβ may form a positive feedback loop and be causally involved in oncogene-induced amplification of inflammatory networks and senescence. A study by Rodier et al. showed that persistent DNA damage response induced by genotoxic stress, replication stress, or oncogenes, is essential for inflammatory cytokine secretion, such as IL-6 and IL-8.23) Especially, ATM, NBS1 and Chk2 are required for the secretion of inflammatory cytokines induced by radiation and replication stress. Interestingly, neither p53 nor pRB are required for IL-6 secretion induced by replication stress. These findings suggest that a persistent DNA damage response, but not cellular senescence per se, is crucial for SASP induction.

Subsequent studies have revealed that SASP factors secreted from senescent cells induce senescence in surrounding normal cells.24) Among numerous SASP factors, TGF-β family ligands, including BMP2 and inhibin A, play a major role in paracrine senescence arrest. Additionally, the study by Acosta et al. showed that cells expressing IL-1α showed oxidative DNA damage, the induction of p53 and p21[3P], and mimicked a SASP-like response in oncogene-induced senescent cells.25) Similarly, Campisi and colleagues showed that IL-1α, but not IL-1β, regulates IL-6 and IL-8 secretion in senescent cells induced by genotoxic stress.26) Thus, IL-1 signaling may be involved in both autocrine and paracrine senescence, whereas TGF-β signaling may play a role more specifically in paracrine senescence.

As mentioned above, NF-κB plays a critical role in transducing DNA damage signaling upon genotoxic stress in response to inflammation. Activation of NF-κB requires multiple steps: 1) NEMO (NF-κB essential modulator), a scaffold protein in the IKK complex, is sumoylated and then translocated into the nucleus, 2) NEMO is phosphorylated by ATM in the nucleus, and 3) monoubiquitination of NEMO translocates the NEMO–ATM complex from the nucleus to cytoplasm, and this NEMO–ATM complex activates IKK in cytoplasm.27) Zmpst24−/−, a model for Hutchinson–Gilford progeria syndrome, exhibit hyperactivation of NF-κB, which is dependent on ATM and NEMO, an increased expression of inflammatory cytokines, such as IL-6, CXCL1 and tumor necrosis factor α (TNF-α), and adhesion molecules, such as ICAM1. Additionally, genetic and pharmacological inhibition of NF-κB

| Disease | Genetic defect | Affected cellular process | Clinical phenotype | Mean lifespan |
|---------|----------------|--------------------------|--------------------|--------------|
| Ataxia telangiectasia (AT) | ATM | DSB repair | Cerebellar ataxia | 20 years |
| | | | Telangiectasia | |
| | | | Immunodeficiency | |
| | | | Cancer predisposition | |
| | | | Growth retardation (insulin resistance) | |
| Hutchinson–Gilford progeria syndrome (HGPS) | LMNA | Nuclear lamina function | Premature aging | 12–15 years |
| | | DSB repair | Growth retardation | |
| | | Transcription | Lipodystrophy | |
| | | | Atherosclerosis | |
| | | | Alopecia | |
| | | | Osteoporosis | |
| | WRN (RecQ helicase) | DSB repair (HR, NHEJ, BER, NER) | Premature aging | ca. 50 years |
| | | Telomere maintenance | Short stature | |
| | | Transcription | Thin limbs | |
| | | DNA replication | Skin ulcers | |
| | | | Atherosclerosis | |
| | | | Diabetes | |
| | | | Osteoporosis | |
| | | | Cancer predisposition | |
| | BLM (RecQ helicase) | HR DNA replication | Growth retardation | 27 years |
| | | | UV sensitivity | |
| | | | Immunodeficiency | |
| | | | Diabetes | |
| | | | Cancer predisposition | |
| | RECQL4 (RecQ helicase) | Telomere maintenance | Premature aging | |
| | | | Growth retardation | |
| | | | Poikiloderma | |
| | | | Cancer predisposition | |
| | CS4, CSB | Transcription-coupled NER | UV sensitivity | 12 years |
| | | | Mental retardation | |
| | | | Premature aging | |
| | | | Kiphosis | |
| | | | Growth retardation |

* AT, HGPS, and WS are discussed in detail in the main text.
prevents the development of progeroid features and extends longevity in these mice. These findings suggest that DDR- and NF-κB-dependent secretion of inflammatory cytokines is involved in physiological and pathological aging in vivo.29)

Kang et al. showed that GATA4, which is degraded by p62-mediated selective autophagy under normal conditions, is stabilized by ATM/ATR-mediated DNA damage signaling.27) Accumulated GATA4 induces IL-1α and TRAF3IP2 (tumor necrosis factor receptor-associated factor interacting protein 2), which activate NF-κB. A study by Laberge et al. demonstrated that rapamycin, which selectively inhibits the activity of mammalian targets of rapamycin complex 1 (mTORC1), suppresses the translation of IL-1α, resulting in transcriptional activity of NF-κB stimulated by ionized radiation.30) Thus, selective autophagy and translation are also involved in the regulation of SASP.

As mentioned above, SASP effects are diverse: protumorigenesis, senescence reinforcement, tissue repair, and so on. Thus, cellular senescence and SASP can either be beneficial or harmful depending on the pathophysiological context and surrounding microenvironment.

5. DNA DAMAGE AND SENESCENCE IN ATHERO-SCLEROSIS

A vast amount of evidence suggests that inflammation plays a pivotal role in the development of atherosclerosis and the incidence of acute coronary syndrome.31) Many coronary risk factors, including low density lipoprotein (LDL), smoking and aging, induce inflammation. Very recently, it has been shown that a monoclonal antibody targeting IL-1β significantly decreases the recurrence of cardiovascular events in patients with previous myocardial infarction and high C-reactive protein.32) Of course, further study is required: the CANTOS trial (Canakinumab Anti-inflammatory Thrombosis Outcomes Study) confirmed the causal role of inflammation in the development of atherosclerosis in a clinical setting. As mentioned thus far, DNA damage induces inflammation; increasing evidence suggests that DNA damage may provide the pathway that links coronary risk factors and inflammation.

Studies using human samples and animal models suggest that atherosclerotic plaques contain both accumulated DNA damage and activated DNA damage response elements. Mahmoudi et al. have reported that human atherosclerotic plaques have more DSBs and ATM activation compared with normal tissue.33) Similarly, increased oxidative DNA damage is noted by the marker for BER in human atherosclerotic plaques.34) We also observed that DSBs and oxidative DNA damage were present in human atherosclerotic plaques.35) These data suggest that the 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.36–38) Interestingly, senescent vascular smooth muscle cells (VSMCs) induced both by replication and DNA damage secrete high levels of SASP factors, such as IL-6, IL-8, MCP-1 in an IL-1α-dependent manner.39) The IL-1α-driven SASP promotes adjacent cells to a proatherosclerotic state. Additionally, senescent cells in human atherosclerotic plaques express IL-1α and colocalize with IL-6 and CD68. These findings are well consistent with those in a study by Acosta et al. and suggest the role of IL-1α-driven SASP and the resultant chronic inflammation in the development of human atherosclerosis.21)

A study by Roks and colleagues showed that ERCC1−/− and XPD−/− mice, in which NER is defective, reveals vascular senescence, elevated blood pressure, and dysfunctional vasodilatation at a young age. The dysfunctional vasodilatation is due to a decreased level of endothelial nitric oxide synthase and hyperactivity of phosphodiesterase in vascular smooth muscle cells (VSMCs). Additionally, Roks et al. found a strong association of single-nucleotide polymorphism in a putative promoter region of the DDB2/XPE gene with carotid-femoral pulse wave velocity, a marker for vascular stiffness in the AortaGen Consortium database.40) In another study, by Arditi and colleagues, deficiency in OGG1, a molecule responsible for the removal of 8-OH-dG, one of the most abundant oxidative base lesions, resulted in increases in plaque size and lipid content, oxidative mtDNA, and serum levels of IL-1β and IL-18 in LDL receptor knockout mice fed a Western diet. These phenomena are dependent on NLRP3 inflammasome.41) These findings suggest that an insufficiency or deficiency of NER and BER are involved in the development of atherosclerosis and cardiovascular disease.

6. CONCLUSION

DNA damage and 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. These may lead to progressive cellular senescence, apoptosis and dysfunction over time. It is still unclear whether DNA damage per se cause aging. Recently, a mouse model in which tissue-specific and temporally controlled DSBs can be induced has been developed.42) It will soon be determined whether induction of DSBs alone is sufficient to mimic pathological phenotype and functional decline in premature aging. It is evident that DDR activation plays a major role in inducing SASP in many, but not all, settings of stress-induced senescence. However, the signaling mechanism by which SASP is induced upon stress is highly dependent on the contexts, such as cell types, types of stress and types of tumors. Additionally, the roles of senescent cells and SASP components are also largely dependent on the microenvironments surrounding those cells/tissues and the pathophysiological contexts. Rigorous investigation is needed to elucidate the roles of SASP components and signaling molecules in pathological conditions, for instance, using mouse models.

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