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Ionizing Radiation-Induced Endothelial Cell Senescence and Cardiovascular Diseases

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Exposure to ionizing radiation induces not only apoptosis but also senescence. While the role of endothelial cell apoptosis in mediating radiation-induced acute tissue injury has been extensively studied, little is known about the role of endothelial cell senescence in the pathogenesis of radiation-induced late effects. Senescent endothelial cells exhibit decreased production of nitric oxide and expression of thrombomodulin, increased expression of adhesion molecules, elevated production of reactive oxygen species and inflammatory cytokines and an inability to proliferate and form capillary-like structures *in vitro*. These findings suggest that endothelial cell senescence can lead to endothelial dysfunction by dysregulation of vasodilation and hemostasis, induction of oxidative stress and inflammation and inhibition of angiogenesis, which can potentially contribute to radiation-induced late effects such as cardiovascular diseases (CVDs). In this article, we discuss the mechanisms by which radiation induces endothelial cell senescence, the roles of endothelial cell senescence in radiation-induced CVDs and potential strategies to prevent, mitigate and treat radiation-induced CVDs by targeting senescent endothelial cells. **© 2016 by Radiation Research Society**

INTRODUCTION

An increasing amount of clinical evidence has demonstrated that exposing the heart to ionizing radiation increases the risks of cardiovascular diseases (CVDs). Radiation-induced CVDs first became apparent in patients who received high doses of radiation to the heart during treatment of Hodgkin's disease (*1*) and breast cancer (*2, 3*). The manifestations of radiation-induced CVDs are usually observed more than a decade after radiotherapy, and include accelerated atherosclerosis, myocardial fibrosis, conduction abnormalities and injuries to cardiac valves (*4, 5*). In addition, long-term epidemiological studies conducted in Japanese atomic bomb survivors revealed that individuals who received an acute single dose of 1–2 Gy total-body irradiation (TBI) showed a significant increase in mortality from myocardial infarction >40 years postirradiation. The risk of CVD-related death in these survivors was increased by 17% per Gy after 0–4 Gy TBI (*6*). It has also been shown that the risk of CVD-related mortality increases with low-dose occupational exposures (e.g., radiologists, nuclear power plant workers and emergency workers from the Chernobyl accident) (*7–9*). Collectively, these findings suggest that the heart and vasculature might be more radiosensitive than was previously thought (*10*).

Currently, the only available clinical approach to reduce late cardiac complications of radiotherapy is to reduce cardiac exposure during the therapy, but this is not always possible. Although radiotherapy has been improved with treatment planning and radiation delivery, a significant subset of patients with thoracic cancers, including those of the lung, esophagus and proximal stomach, still receives considerable radiation doses to the heart (*11–13*). There are similar risks from the increasing radiological and nuclear threats that exist today. Therefore, a better understanding of the pathophysiology of radiation-induced CVDs is needed to develop more effective therapeutic strategies to prevent, mitigate and treat radiation-induced CVDs.

The heart is a multicellular organ. The adult human ventricles consist of about 33% cardiomyocytes, 24% endothelial cells and 43% mesenchymal cells (*14*). The cell turnover rate for cardiomyocytes is highest in early childhood and gradually declines to <1% per year in adulthood. In contrast, endothelial cell turnover in the heart is high throughout life (>15% per year), whereas mesenchymal cell exchange is relatively low in adulthood (<4% per year). Cardiomyocytes are terminally differentiated, quiescent and highly radioresistant. Endothelial cells are relatively resistant to radiation-induced apoptosis, but they readily undergo senescence or permanent cell-cycle arrest after exposure to a moderate or high radiation dose (0.5–10 Gy) (Fig. 1) (*15–30*). While the role of endothelial cell apoptosis in mediating radiation-induced acute tissue injury has been extensively studied (*29–37*), little is known about the role of endothelial cell senescence in the

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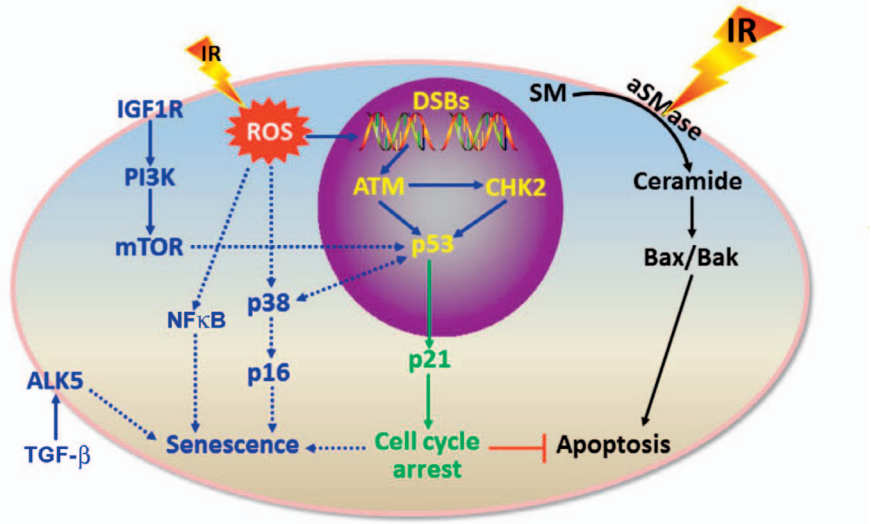

FIG. 1. Ionizing radiation induces endothelial cell apoptosis and senescence in a dose-dependent manner. Exposure of endothelial cells to a very high dose (>10 Gy) of ionizing radiation (IR) induces apoptosis via activation of the acidic sphingomyelinase (aSMase) that hydrolyzes sphingomyelin (SM) on the plasma membrane to generate ceramide and to induce Bax and Bak. However, exposure of endothelial cells to a moderate (>0.5 Gy) or high dose (<10 Gy) of radiation primarily induces senescence via multiple pathways, as shown. ALK5, TGF-β type 1 receptor kinase; ATM, ataxia-telangiectasia mutated protein kinase; CHK2, checkpoint kinase 2; DSBs, DNA double-strand breaks; IGF1R, insulin-like factor-1 receptor; mTOR, mechanistic target of rapamycin; NFκB, nuclear factor κB; p38, p38 mitogen-activated protein kinases; PI3K, phosphtidylinositol-3-kinase; ROS, reactive oxygen species; and TGF-β, tumor growth factor β.

pathogenesis of late effects caused by radiation (Fig. 2). Because endothelial cells are one of the major constituents of heart microvasculature and macrovasculature, induction of endothelial cell senescence may play an important role in radiation-induced CVDs. Therefore, in this article we focus our discussion on the mechanisms by which radiation induces endothelial cell senescence, effects of endothelial cell senescence on cardiovascular function and potential strategies for targeting senescent endothelial cells to prevent, mitigate and treat radiation-induced CVDs.

CELLULAR SENESCENCE: A DOUBLE-EDGED SWORD IN THE FIGHT AGAINST CANCER

Cells undergo senescence after extensive cell division or exposure to oncogenic or genotoxic stress such as radiation (*38–41*). Induction of cellular senescence is considered an important tumor-suppressive mechanism because it permanently arrests growth of genetically deranged cells with critically shortened telomeres or persistent DNA damage that may lead to genetic instability and cell transformation. More importantly, an increasing body of evidence indicates that induction of cellular senescence can stimulate the immune system to rapidly eliminate these genetically distorted cells (*42–44*). In addition, induction of senescence is an important function of radiation in cancer treatment, since it can potently induce senescence not only in cancer cells but also in endothelial cells (*45–47*). Induction of endothelial cell senescence inhibits angiogenesis, which may also contribute to the therapeutic effects of radiation on cancer.

However, if the rate of senescent cell production exceeds the immune system's capacity to clear them, or if the immune system is compromised and cannot efficiently remove senescent cells, senescent cells can accumulate in tissues (*39, 48*), which has been observed in animals and humans during aging and after radiotherapy. Under such circumstances, senescent cells can promote various age- and therapy-related pathologies, including CVDs, in a cell-autonomous and a non-cell-autonomous manner. For example, senescent cells produce increased levels of reactive oxygen species (ROS), which can induce oxidative damage to neighboring cells. In addition, senescent cells secrete a plethora of inflammatory mediators (e.g., cytokines and chemokines) and extracellular proteases, termed the senescence-associated secretory phenotype (SASP), which causes chronic inflammation and disruption of tissue structure and function (*38, 39, 49, 50*). Moreover, persistent accumulation of senescent cells in tissues can cause a decline in tissue stem and progenitor cells (*51–55*). This can occur intrinsically, if tissue stem and progenitor cells themselves become senescent, or extrinsically, if the cells making up the stem-cell niche undergo senescence and express SASP. Senescent tissue stem and progenitor cells exhibit significant defects in self-renewal, proliferation and differentiation, rendering them incapable of tissue maintenance, regeneration and repair. Therefore, induction of cellular senescence is a double-edged sword in the fight against cancer. Inhibiting the induction of senescence can be

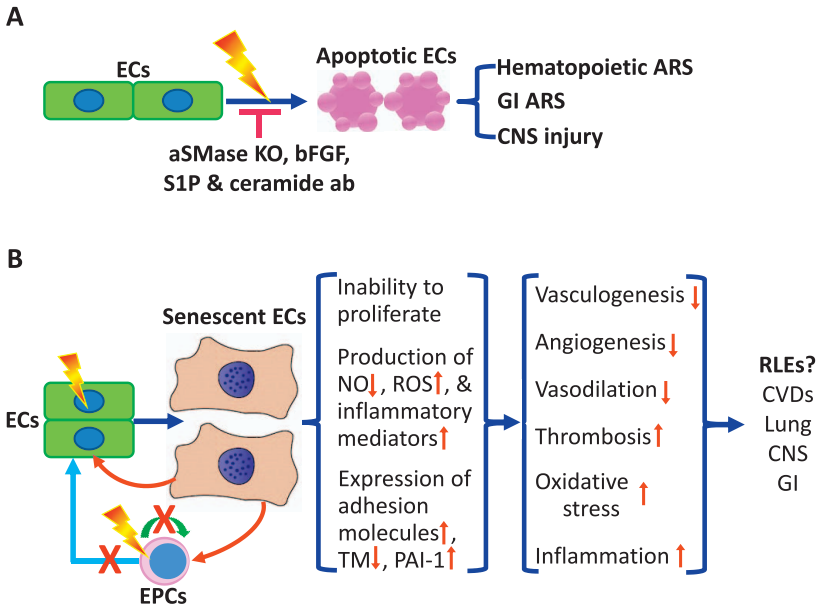

FIG. 2. Role of endothelial cell in radiation-induced acute tissue injuries and late effects. Panel A: Role of endothelial cell apoptosis in acute radiation syndrome (ARS) in the hematopoietic system, gastrointestinal (GI) system, and central nerve system (CNS). Acidic sphingomyelinase knockout (aSMase KO), basic fibroblast growth factor (bFGF), sphingosine-1-phosphate (S1P), and antibodies against ceramide (ceramide ab) can inhibit radiation-induced apoptosis in endothelial cells (ECs) and reduce radiation-induced acute injuries to various tissues. Panel B: Hypothetical roles of endothelial cell senescence in radiation-induced late effects (RLEs). EPCs, endothelial progenitor cells; NO, nitric oxide; ROS, reactive oxygen species; TM, thrombomodulin; PAI-1, plasminogen activator inhibitor-1; and CVDs, cardiovascular diseases.

detrimental by increasing tumorigenesis and decreasing tumor response to radiotherapy. In contrast, promoting clearance of senescent cells during aging and after irradiation can prevent their accumulation within tissues, and it may delay the onset and progression of age-related diseases and reduce late effects of radiation, such as CVDs. This hypothesis is supported by a recently reported finding that genetically selective elimination of $p16^{Ink4a}$ (p16)-positive senescent cells in normal *INK-ATTAC* transgenic mice via administration of the drug AP20187 significantly extended the animals' lifespans by delaying the onset and progression of age-related pathologies, including cardio-dysfunction (*56*). However, whether selective clearance of senescent cells can prevent and mitigate radiation-induced CVDs has not yet been investigated.

ENDOTHELIAL CELL SENESCENCE INDUCED BY IONIZING RADIATION

Endothelial cells from a variety of tissue origins and species, including human coronary artery, can undergo senescence after exposure to a moderate-to-high dose of radiation *in vitro* (*15–28*). In addition, emerging evidence indicates that cardiac endothelial cells senesce *in vivo* after local exposure to radiation (*57*). However, the underlying mechanisms by which radiation induces endothelial senescence have not been fully established. It has been suggested that diverse stimuli can induce cellular senescence in different cells via various upstream signal transduction cascades (including the p53-p21 pathway) that eventually converge on the p16-Rb pathway, whose activation inescapably prevents senescent cells from re-entering the cell cycle. The importance of the p53-p21 pathway is supported by the finding that activation of p53 and induction of p21 are transient events during the onset of senescence that subside when expression of p16 starts rising (*58–60*). Induction of senescence can be prevented by inactivation of p53 prior to upregulation of p16; however, once p16 is highly expressed, downregulation of p53 cannot reverse cell cycle arrest (*60, 61*). This indicates that activation of the p53-p21 pathway is an important role in initiation of senescence and that upregulation of p16 is required for maintenance of senescence.

However, endothelial cells are unique for the induction of senescence. Unlike other cells, it appears that the p53-p21 pathway is more important than the p16-Rb pathway for the induction of endothelial cell senescence, because knockdown of p53 expression, but not knockdown of p16 expression, inhibits endothelial cell senescence induced by a variety of stimuli (Fig. 1) (*16, 62–64*). The p53-p21 pathway may be activated in endothelial cells via induction of unrepairable DNA damage, persistent oxidative stress and expression of X-linked inhibitor of apoptosis-associated factor 1 and growth differentiation factor 15 (*16, 23, 62, 65–67*). Recently, it was reported that activation of the insulin/insulin-like growth factor 1 (IGF1)-phosphtidylinositol-3-kinase (PI3K)-Akt/mechanistic target of rapamycin (mTOR) pathway acts upstream of the p53-p21 pathway in mediating

endothelial cell senescence induced by radiation and high glucose (Fig. 1) (*21, 27, 28, 57, 68*). Radiation-induced senescence of endothelial cells was suppressed by specific inhibition of IGF1 receptor (IGF1R), PI3K or mTOR. The activation of the IGF1-PI3K-Akt/mTOR pathway may be attributable to downregulation of sirtuin 1 (SIRT1) (*22, 68, 69*). In addition, radiation-induced endothelial cell senescence also may involve: activation of p38, NFκB and TGF-β type 1 receptor ALK5; induction of endoplasmic reticulum stress; and downregulation of telomerase reverse transcriptase (*15, 22, 70–74*).

Radiation-induced senescent endothelial cells exhibit a variety of senescence-like phenotypes. These include changes in cell morphology, permanent cell-cycle arrest, increased staining for senescence-associated β-galactosidase (SA-β-gal) and elevated expression of p16 and p21. The cells are also defective in angiogenesis, having reduced ability to sprout, migrate and invade to form capillary-like structures in Matrigel® (*15, 70*). In addition, senescent endothelial cells produce increased levels of ROS, probably due in part to upregulation of NADPH oxidases, downregulation and/or upcoupling of endothelial nitric oxide synthase (eNOS) and induction of mitochondrial dysfunction (*75–78*). They acquire SASP by expressing increased levels of inflammatory cytokines and adhesion molecules (*15, 18, 25, 26, 57, 77, 79*). Radiation-induced senescent endothelial cells expressed decreased levels of thrombomodulin (*80, 81*) and increased levels of plasminogen activator inhibitor-1 (PAI-1) (*82, 83*). All these changes in senescent endothelial cells lead to endothelial dysfunction, which results in inhibition of angiogenesis, induction of oxidative stress and inflammation and dysregulation of vasodilation and hemostasis.

ROLE OF ENDOTHELIAL CELL SENESCENCE IN RADIATION-INDUCED CVDS

Although it has been extensively implicated in the pathogenesis of age-related CVDs (*82, 84–88*), the role of endothelial cell senescence in radiation-induced CVDs has yet to be determined (*89–91*). Radiation-induced CVDs may be in part attributable to a combination of effects on microvasculature and macrovasculature (*89–91*). Senescent endothelial cells are incapable of regenerating new cells to maintain the homeostasis of vasculatures and repair damaged blood vessels, which may contribute to the decreased density of cardiac capillaries and small coronary arterioles and to the accelerated atherosclerosis of large blood vessels, including rodent and human coronary arteries (*89, 90, 92–94*). Moreover, senescent endothelial cells can potentially impede the angiogenic activity of endothelial progenitor cells via SASP and increased production of ROS. Increased production of ROS, along with reduced production of NO due to downregulation of eNOS expression in senescent endothelial cells, can lead to a further decrease in NO bioavailability, which can impair endothelial-mediated vasodilation and cause hypertension, a major contributing factor of fatal age-related CVDs in humans (*75–78, 86, 95*). With increased levels of various inflammatory cytokines, adhesion molecules and PAI-1, and decreased levels of thrombomodulin, senescent endothelial cells are proinflammatory, prothrombotic and proatherogenic (*15, 18, 25, 26, 57, 77, 79–83*). Therefore, they are likely to play a very important role in radiation-induced atherosclerosis. Collectively, the combined deleterious effects of senescent endothelial cells on cardiac microvasculature and macrovasculature may lead to radiation-induced CVDs.

APPROACHES FOR TARGETING ENDOTHELIAL CELL SENESCENCE TO PREVENT, MITIGATE AND TREAT RADIATION-INDUCED CVDS

Because senescent endothelial cells likely play an important role in radiation-induced CVDs, targeting them with a therapeutic agent may be a novel strategy to prevent, mitigate and treat radiation-induced CVDs. While no such therapies are currently available, several strategies and candidate approaches are under investigation, as shown in Fig. 3. One strategy for targeting senescent endothelial cells is to prevent radiation from inducing senescence of endothelial cells. This could be achieved by inhibiting the IGF1-PI3K-Akt/mTOR pathway because its activation is important in mediating radiation-induced endothelial senescence. The pathway can be inhibited with a specific inhibitor of IGF1R, PI3k and mTOR, or with an activator of SIRT1 (*21, 22, 68, 69*). In addition, radiation-induced endothelial cell senescence could be inhibited by using antioxidants to scavenge ROS or by using a specific inhibitor to block activation of p38, NFκB and ALK5 (*15, 70*). However, these preventive strategies can be effective only if applied before or shortly after irradiation, when endothelial cells have not yet undergone senescence.

Alternatively, inhibition of SASP could be used to mitigate or treat radiation-induced CVDs because SASP likely mediates most of the deleterious effects of senescent endothelial cells on the cardiovascular system (*96*). The pathways involved in induction of cellular senescence substantially overlap those of SASP (e.g., p38, NFκB and mTOR). Specific inhibition of these pathways would not only block induction of senescence, as discussed above, but also suppress SASP (*97–101*). Targeting the Janus kinase (JAK) pathway, an upstream regulatory pathway of SASP, is another potential strategy for mitigating the effects of radiation-induced CVDs. It was recently reported that using RNAi or JAK inhibitors to target the JAK pathway suppressed development of SASP in human umbilical vein endothelial cells and human preadipocytes after exposure to radiation (*102*). Further, treating aged mice with a specific inhibitor of JAK-1 and JAK-2 (ruxolitinib) reduced systemic inflammation and frailty (*102*). It will be of great interest to determine whether inhibiting pathways such as

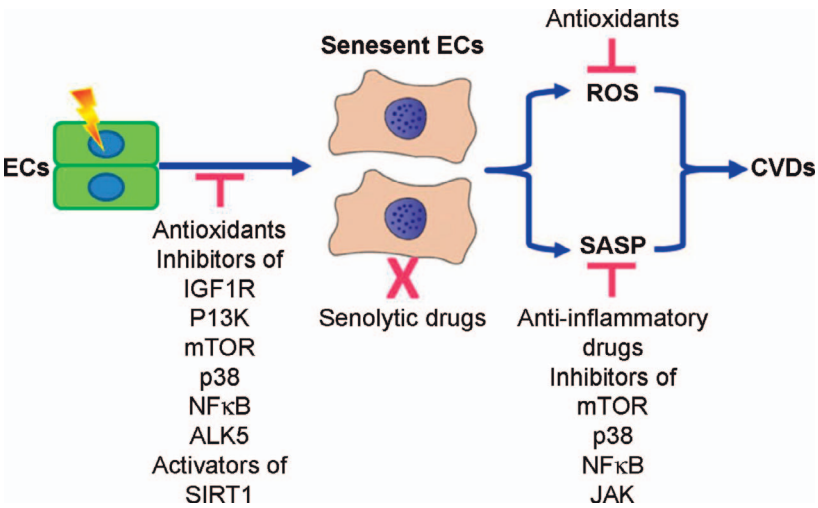

FIG. 3. Potential strategies to prevent, mitigate, and treat radiation-induced CVDs by targeting senescent endothelial cells. Antioxidants, inhibitors of insulin/insulin-like growth factor I receptor (IGF1R), phosphtidylinositol-3-kinase (PI3K), mechanistic target of rapamycin (mTOR), p38, NFκB and TGF-β type 1 receptor (ALK5), and the activators of sirtuin 1 (SIRT1) may be used to prevent radiation-induced CVDs by inhibiting the induction of endothelial cell (EC) senescence. Clearance of senescent cells with a senolytic drug that can selectively kill senescent cells including senescent endothelial cells has the potential to be developed as novel therapeutic strategy to mitigate and treat radiation-induced CVDs. Antioxidants and inhibitors of mTOR, p38, NFκB, and Janus kinase (JAK) may prevent, mitigate, and treat radiation-induced CVDs by scavenging senescent cell-produced reactive oxygen species (ROS) and inhibiting senescence-associated secretory phenotype (SASP), respectively.

JAK, which mediate radiation induction of SASP, can also prevent and mitigate radiation-induced CVDs. However, these approaches may require continuous treatment because the senescent endothelial cells remain in the heart and can express SASP again as soon as the pathway is no longer being inhibited.

The limitations associated with inhibition of senescence induction and SASP to prevent and mitigate radiation-induced CVDs could be overcome by clearing senescent endothelial cells with a small molecule that selectively kills senescent cells. This approach to mitigate and treat radiation-induced CVDs can be used after irradiation, when endothelial cells have already become senescent, and can be effective with an intermittent treatment. The seminal finding, discussed above, that AP20187 selectively eliminated senescent cells in transgenic mice, which prolonged their lifespans by delaying the onset of age-related pathologies, prompted efforts to identify senolytic drugs, small molecules that can selectively kill senescent cells without depending on a transgene (*103, 104*). Senolytic drugs have the potential for use not only as novel antiaging drugs but also as new medical countermeasures against radiation to mitigate and treat radiation-induced CVDs. However, finding a senolytic drug has been challenging because senescent cells are highly resistant to induction of apoptosis (*105*). We and others have used high-throughput screening of libraries, each containing thousands of compounds, to identify small molecules that selectively kill senescent cells, but only two nonspecific, cell-type-selective senolytic drugs have been discovered (*104*). We, therefore, took a targeted approach to identify senolytic drugs by individually titrating the cytotoxicity of hundreds of small molecules that participate in pathways predicted to be important for viability of senescent cells or maintenance of their phenotype. With this approach, ABT263, a Bcl-2/xL-specific inhibitor (*106*), was identified as the most potent broad-spectrum senolytic drug that selectively, potently and rapidly kills senescent cells in culture, regardless of cell type or species (*48*), including radiation-induced senescent human umbilical vein endothelial cells (preliminary data not shown). More importantly, oral administration of ABT263 to sublethally irradiated and normally aged mice effectively cleared senescent cells in several tissues, including senescent bone marrow hematopoietic stem cells and muscle stem cells, and it suppressed SASP in the lungs (*48*). We plan next to investigate in thoracic-irradiated mice whether ABT263 can effectively clear senescent endothelial cells and suppress SASP in the heart and whether ABT263 can mitigate and treat radiation-induced CVDs.

Using senolytic drugs such as ABT263 for mitigation and treatment of radiation-induced CVDs and other radiation-induced late effects (RLEs) has several advantages over conventional anti-inflammatory treatments. Senolytic drugs target the cells that may be fundamentally responsible for initiating and driving radiation-induced CVDs and other RLEs (Fig. 4) (*96, 107, 108*). Therefore, senolytic drugs should be more effective than anti-inflammatory therapeutics that target individual harmful

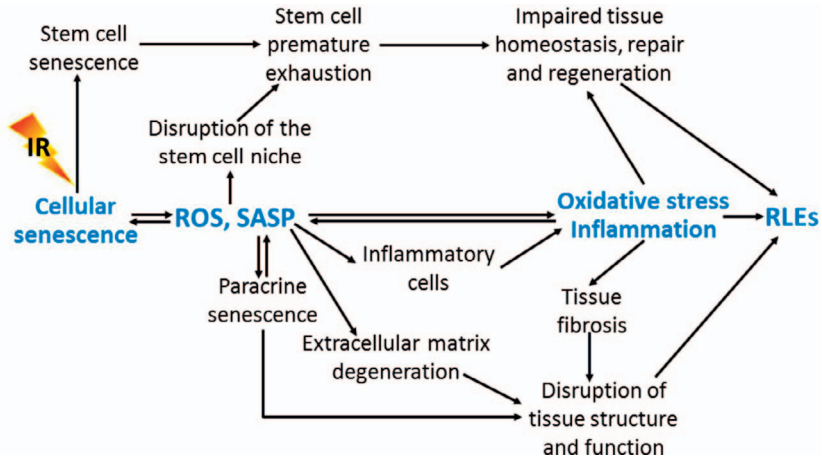

FIG. 4. Role of cellular senescence in radiation-induced late effects (RLEs). Senescent cells may mediate RLEs in part via increased production of reactive oxygen species (ROS) and expression of senescence-associated secretory phenotype (SASP).

inflammatory molecules (e.g., cytokine and chemokine) and less harmful, because, unlike anti-inflammatory agents, they do not interfere with the normal immune functions of the molecules. In addition, senolytic drugs may invigorate tissue stem and progenitor cells to improve tissue repair and regeneration because space occupied by senescent cells will be freed, allowing normal tissue stem and progenitor cells to repopulate and because SASP will be suppressed, improving the microenvironment for these cells. Therefore, senolytic drug treatment could be expected to have a much broader therapeutic effect than conventional anti-inflammatory treatments. Finally, senolytic drug treatment has the potential not only to mitigate radiation-induced CVDs and other RLEs, but also to prevent radiation-induced secondary malignancies and reduce cancer relapse and metastasis, because senescent cells are known to promote malignant transformation in neighboring cells and to stimulate proliferation and metastasis of resistant tumor cells, possibly in part through SASP (*79, 96, 107, 108*).

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