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Review

Radiation-induced Cell Death: a Multifaceted Sequel Shaping Radiation Effects

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Exposure of cells to ionizing radiation (IR) results in DNA damage, among which DNA double-strand breaks (DSBs) are the most deleterious to human health. Although DSBs are efficiently mended by DSB repair systems, intolerant radiation doses cause unreparable DSBs, which persistently activate DNA damage signaling pathway. As a result, cell death is executed. A number of cell death modes have been described, including apoptosis, premature senescence, necrosis, and autophagy. While the mode of cell death is dependent on the stimuli given to cells, apoptosis and premature senescence have been documented in several studies as the two representative modes of cell death in response to radiation exposure. Particularly, it is becoming more aware that premature senescence is the common cause of cell death in various non-hematopoietic tissues, including epithelial tissues, mesenchymal tissues, and endothelial and lymphatic cells. Further studies have demonstrated that secretory phenotype is common to senescent cells, and soluble factors secreted from senescent cells, such as cytokines, chemokines, growth factors, and matrix remodeling factors, are those deeply involved in the execution of both acute and late radiation health effects. Moreover, recent evidences have demonstrated a close connection between radiotherapy-induced senescence and the development of adverse effects. Current review overviews the modes of cell death caused by radiation exposure, and discusses the physiology of cellular senescence, molecular mechanisms of radiation-induced premature senescence, significance of premature senescence in tissue reaction and late effect. Moreover, recent approaches towards the amelioration of health effects by senolytic chemicals that enable elimination of senescent cells from tissues will be presented.

Key words: Ionizing radiation, cell death, senescence, tissue reaction, late effects

1. Introduction

Deposition of radiation energy to cells brings about various types of molecular changes, among which DSBs have been known to be the most critical type of DNA damage resulting in detrimental health effects. While DSBs are efficiently repaired by multiple DNA damage repair systems, such as non-homologous end-joining (NHEJ) and homologous recombination (HR)[1], if radiation dose is intolerable, some DSBs are left unrepaired while most other DSBs are mended by DSB repair systems, creating so-called unreparable DSBs. Such unreparable DSBs persist for a very long time[2-5], and they continuously activate ATM-dependent DNA damage checkpoint pathway, which gives rise to cell death. In

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Table 1. Major modalities of cell death

Mode	Morphological characterization	Key biochemical characterization
I. Macroscopic morphological classification		
Type I : apoptosis	cytoplasmic shrinkage, chromatin condensation, DNA fragmentation, membrane blebbing	
Type II : autophagy	cytoplasmic vacuolization	
Type III : necrosis	loss of membrane integrity, swelling of subcellular organelles	
II. Biochemical and molecular classification		
Apoptosis	See apoptosis, apoptotic body	caspases activation, CAD activation phosphatidylserine exposure
Necroptosis	cell swelling, membrane rupture moderate chromatin condensation	RIPK1, RIPK3, MLKL activation
Ferroptosis	mitochondria membrane rupture diminutive mitochondria with crista reduced	triggered by iron accumulation and lipid peroxidation, regulated by GPX4
Pyroptosis	rupture of plasma membrane, chromatin condensation, cell swelling, membrane bubbling	inflammatory caspase (caspase-1, -11) activation gasdermin D (GSDMD) activation
Autophagy-dependent cell death	autophagic vacuolization, membrane blebbing	LC3B-II accumulation, increased lysosomal activity
Anoikis	See apoptosis	loss of integrin-dependent anchorage
Entotic cell death (Entosis)	cell-in-cell formation	activation of adhesion proteins, LC3-mediated phagocytosis
Netotic cell death (NETosis)	chromatin fiber release, membrane nuclear membrane collapse	NETs formation
Lysosome-dependent cell death	Lysosome and plasma membrane rupture	lysosomal membrane permeabilization
MPT-driven necrosis	See necrosis	rely on cyclophilin D (CYPD)

CAD: caspase-activated DNase, RIPK: ubiquitinate receptor interacting serine/threonine kinase, MLKL: mixed lineage kinase domain like pseudokinase, GPX: glutathione peroxidase, NET: neutrophil extracellular traps, MPT: mitochondrial permeability transition.

addition to necrosis, which was widely recognized as a cell death modality, early observations defined that cells exposed to ionizing radiation were died predominantly by apoptosis[6]. It was demonstrated that radiation-induced apoptosis was regulated by p53, which is also a key player for regulating cell cycle progression to keep the integrity of the genome[7, 8]. Accordingly, a model, in which DSB-induced p53 activation transiently arrests cell cycle to make a time for repair, has been proposed. In the model, cells with low level of DSBs are assumed to complete DSB repair by inducing p53-dependent transient cell cycle arrest, while cells with unreparable amount of DSBs are forced to die by p53-dependent apoptosis. However, it now turns out to be clear that apoptosis induction is rather limited to a certain type of tissue, such as hematopoietic tissues, and most other epithelial tissues, such as epithelial tissues, mesenchymal tissues, as well as endothelial and lymphatic cells, show non-apoptotic cell death modalities in response to ionizing radiation[9,10]. Considering extensive advances in recent studies regarding cell death pathway, it is a right time to renew our knowledge on modes of cell death caused by radiation exposure. Therefore, this review overviews past and present studies with regard to cell death after radiation exposure, and emphasizes the significance of radiation-induced premature senescence in tissue reaction and late effects, together with possible ways to ameliorate radiation health effects by eliminating senescent cells from tissues.

2. Historical view

Historically, three types of cell death modality, which were classified by morphological changes, were proposed in mouse and rat prenatal tissues exposed to embryotoxic substances[11] (Table 1). Type I cell death or apoptosis is represented by cell shrinkage, plasma membrane blebbing, nuclear fragmentation, and DNA fragmentation, leading to the formation of small cytoplasmic vesicles, known as apoptotic bodies. Type II cell death or autophagy is manifested by visible cytoplasmic vacuolization, which contains subcellular organelles and cytoplasmic materials. Type III cell death or necrosis is characterized by the loss of membrane integrity and organelle swelling.

Subsequently, various types of cell death modalities have been documented, so that the Nomenclature Committee on Cell Death (NCCD) formulates criteria for the definition of cell death since 2005[12]. The NCCD has

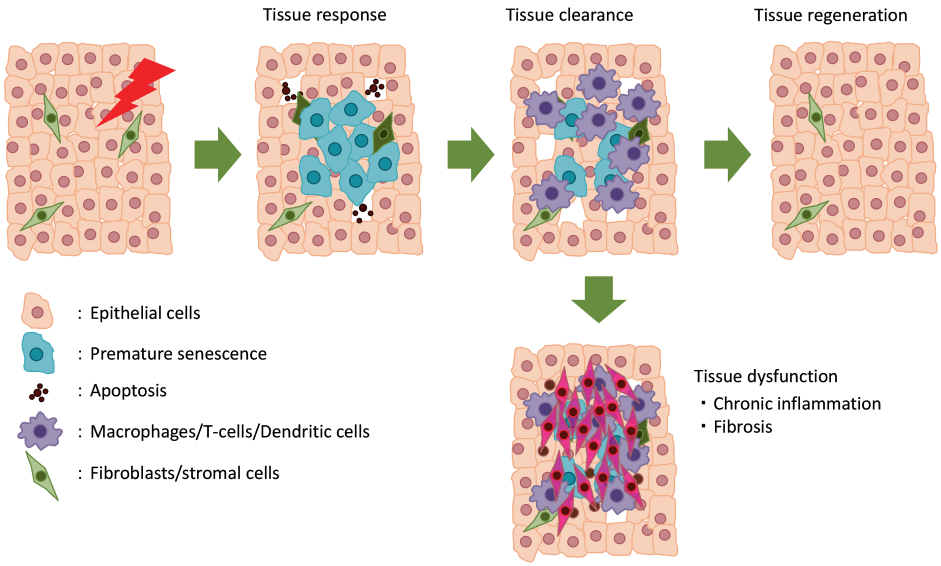

Fig. 1. Tissue response to IR. Radiation exposure to tissues brings about premature senescence as well as apoptosis of cells. In non-hematopoietic tissues, premature senescence is predominantly induced in various cells, such as epithelial cells and fibroblasts/stromal cells, which attracts immune cells to promote tissue regeneration. In case, where higher doses are delivered, cell death is more profound, resulting in manifestations of tissue dysfunction including chronic inflammation and fibrosis.

published five position papers so far, and the newest one was released in 2018[13]. Accordingly, cell death is divided into accidental cell death (ACD) and regulated cell death (RCD), in which the former is induced by unpredicted insults and the latter is more physiological and is biologically controlled. Cell death modalities associated with RCD include apoptosis, necroptosis, ferroptosis, pyroptosis, autophagy-dependent cell death, anoikis, entotic cell death (entosis), netotic cell death (NETosis), lysosome-dependent cell death, and mitochondrial permeability transition-driven necrosis[14] (Table 1). Additionally, cell death that stimulates adaptive immune response in the host is called immunogenic cell death[15]. Cellular senescence, as well as mitotic catastrophe, have not been considered to constitute forms of RCD by NCCD, however, these two play a pivotal role in the fate of cells in response to ionizing radiation, which is extensively discussed later.

3. Physiology of senescence in normal cells

Cellular senescence has been recognized as a cause of aging, as senescent cells accumulate in various tissues in aged humans and mice[16-18]. Cellular senescence was originally described in cultured normal human diploid cells, which ceased to grow after several rounds of cell divisions[19]. Telomere attrition, along with DNA damage accumulation and epigenetic reactivation of the *CDKN2A* locus, are the origin of endogenous factors that cause cellular senescence[20, 21]. Although cellular senescence is predominantly associated with aging, recent findings have illuminated its role in the embryonic development as well[22].

Cellular senescence is distinct from other non-proliferative states, such as quiescence at G0 phase and terminal differentiation. Irreversible and permanent cell cycle arrest is one representative feature of senescent cells, but they are still metabolically active. In culture, senescent cells are viable and stay for a quite long time[2-5], and several studies have proven that they secrete several soluble factors, which exert physiological response in tissue microenvironment, including inflammation[22-26]. Thus, senescence is primarily beneficial to damaged tissue because its facilitates tissue remodeling (Fig. 1). However, in elderly animals, accumulation of senescent cells in tissues gives rise to unfavorable decline in physiological function of them leading to age-related pathologies.

Since senescence is induced not only by shortened telomeres but also by endogenous DNA damage, it is expected to be a barrier against cancer development. At the same time, some studies have shown that senescence in bone marrow supports proliferation and survival of malignant cells. Thus, senescence is a multi-faced phenomenon that causes pleiotropic effects in a context-dependent manner, which is carefully considered below. Also, physiological senescence is stem from telomere attrition, while radiation-induced senescence does not involve telomere shortening, so that the definition, premature senescence, is used in this review.

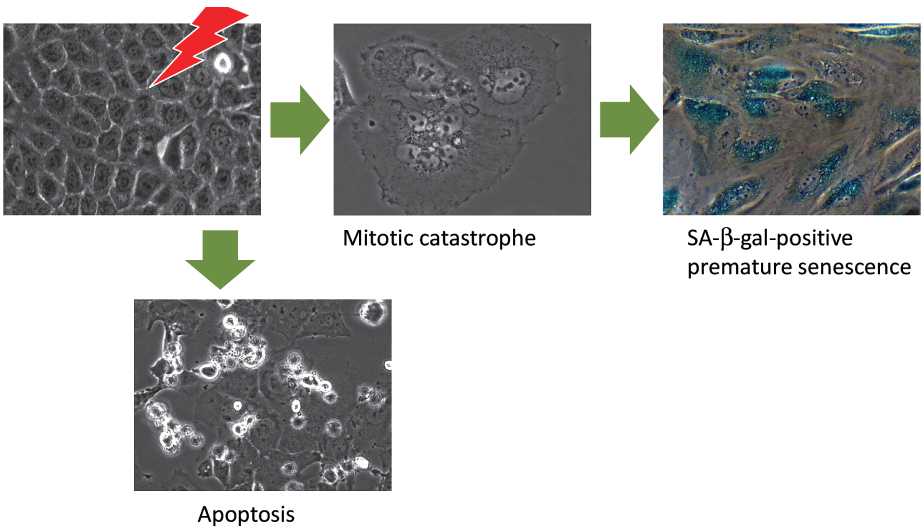

Fig. 2. Two representative cell death modes induced by IR. Exposure of epithelial cells to IR gives rise to various modes of cell death, among which premature senescence and apoptosis are the two major modes. In normal cells, immediate cell cycle arrest persists and senescence phenotypes become obvious, whereas, in cancer cells, it needs a few rounds of cell cycle before terminal growth arrest is executed. Because cancer cells are forced to divide even with multiple DSBs, mitotic catastrophe, in which multiple micronuclei, di-nuclei, and giant nuclei are emerged, is taken place. Only after the cell cycle is terminated, cancer cells exhibit common senescence phenotypes including SA-β-gal expression. Apoptosis induction is preferentially observed in hematopoietic cells, however, in some epithelial cells, like those in the crypts of small intestine, show apoptotic cell death.

4. Molecular bases for senescence induction in normal cells

Telomeres, which constitute the end structure of mammalian chromosomes, are the molecular clock that counts the number of cell divisions[27, 28]. In fact, DNA replication machinery is unable to synthesize DNA at its 5' terminal, which is known as end-replication problem, so that telomeres are eroded through successive cell divisions. Finally, critically shortened telomeres are presumed to unable to organize protective telomere structure, so-called T-loop. As a result, the end structures of telomeres, which are structurally equivalent to DSBs, are exposed and trigger DNA damage response[28]. Shortened telomeres are sensed by ATM, which results in phosphorylation and stabilization of p53[29]. Accumulated and activated p53 transactivates several downstream effectors that regulate cellular response to DSBs[30-35]. Among the factors responsible for DNA damage response, $p21^{WAF1/CIP1}$ protein is an essential one that controls cell cycle arrest. $p21^{WAF1/CIP1}$ is a nuclear protein that inhibits cyclin-dependent kinase (CDK) associated with RB phosphorylation, such as CDK2, CDK4 and CDK6, and thereby inhibiting cell cycle progression from G1 to S, which is generally called G1 arrest[36]. Because telomere attrition is not reversible, cell cycle arrest at G1/S border persists. Eventually, the level of $p21^{WAF1/CIP1}$ is decreased, but compensative activation of the $p16^{INK4A}$ locus continues irreversible cell cycle arrest[37]. Thus, senescent cells are not mere G1-arrested cells but are morphologically, physiologically and biochemically different from the quiescent cells and the terminally differentiated cells. In fact, morphology of senescent cells is totally different from that of proliferating cells, and they are really large and flattened (Fig. 2). They sometimes show bi-nuclei or giant nuclei, and exhibit a unique enzymatic activity, which is called senescence-associated β-galactosidase (SA-β-gal) activity[38](Fig. 2). Initially, SA-β-gal activity was thought to be a unique biochemical marker for senescence, however, it now becomes clear that augmented lysosomal activity is the nature of SA-β-gal activity. In fact, the swell of lysosomes are commonly observed in senescent cells.

In addition to ATM-p53 axis-dependent DNA damage response, it has been proposed that multiple pathways are involved in a full specification of senescence[39](Fig. 3). For example, cytoplasmic chromatin fragments (CCFs), which are brought about micronuclear chromatin fragmentation, and damage-associated molecular patterns (DAMPs) are involved in the secretory phenotype of senescence. A role of mTOR has also been discussed in deepening the senescence state[40]. All these pathways associated with cellular senescence were complied by the International Cell Senescence Association (ICSA), together with the recommendations on how to utilize responsible molecules as biomarkers[18].

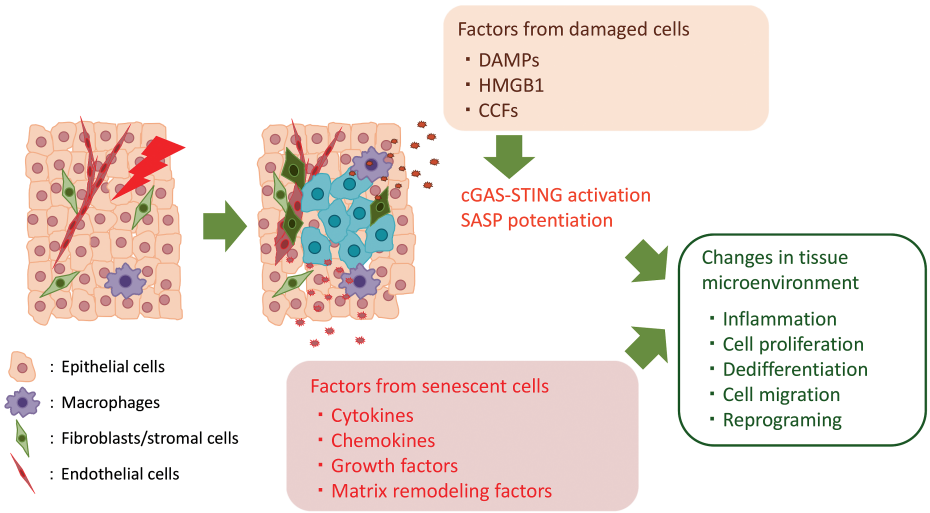

Fig. 3. Pathways involved in the manifestation of late effects/adverse effects of radiation exposure. Exposure of tissues to intolerable doses of IR results in massive cell death in various types of cells constituting tissue, which mediate release of several factors from damaged cells, including damage-associated molecular patterns (DAMPs), high mobility group box 1 (HMGB1), and creation of cytoplasmic chromatin fragments (CCFs). The latter is involved in the activation of the cyclic GMP-AMP (cGAMP) synthase (cGAS) - the stimulator of interferon genes (STING) pathway, which potentiates secretion of soluble factors from senescent cells. At the same time, a group of soluble factors are secreted from premature senescent cells, and they provoke changes in the local and systemic tissue microenvironment, which mediate late health effects caused by radiation exposure and adverse effects originated from radiation therapy.

5. Induction of premature senescence by IR in normal cells

Permanent cell cycle arrest was initially documented in normal human diploid cells exposed to ionizing radiation[41, 42]. Subsequently, several DNA damaging agents were confirmed to induce premature senescence[42]. Because most of the earlier studies used lymphocytes, apoptosis was believed to be the major cell death modality, which was the reason why studies towards radiation-induced cell death did not take senescence induction into consideration. However, accumulating evidences have sufficiently proven that premature senescence is more a common modality of cell death after radiation exposure in various cell types, including not only fibroblasts, but also epithelial cells, endothelial cells, myoblasts, and bone marrow- and adipose-derived stem cells[9, 10, 43-46]. Thus, premature senescence has now been integrated into the mode of cell death in response to radiation exposure, and it is indispensable for the comprehensive understanding of both tissue reaction and late effect caused by radiation exposure[47].

Historically, two types of cell death mode have been utilized in the field of radiation biology and radiation oncology, one of the definition is interphase cell death, and the other is mitotic cell death. While the former is mediated by apoptosis and necrosis, the latter process is undescribed until recently but is characterized by the induction of giant cells, bi-nucleated cells, or by multiple micronuclei. It is comparable with clonogenic cell death, whose definition is widely used in the radiation oncology researchers. It now appears to be clear that mitotic cell death is equivalent to premature senescence. In addition, interphase cell death in non-hematopoietic cells is executed primarily by premature senescence, so that it is the time to renew our view of cell death mode by introducing recent advances in radiation biology and oncology.

6. Mechanism of IR-induced premature senescence in normal cells

While physiological senescence is brought about the telomere attrition, radiation-induced premature senescence is coupled with persisted cell cycle arrest caused by unscheduled and persistent chromosomal breakages[4, 5, 37]. Upon irradiation, multiple DNA repair systems execute mending DSBs, however, if the amount of DSBs exceeds the threshold of DSB repair capacity, some fractions of DSBs are left unrepaired. Since induction of DSBs simultaneously activates ATM-dependent DNA damage signaling pathway, irrespective of the initial number DSBs, DNA damage signaling is persisted as long as unreparable DSBs are remained[2].

ATM is a product of the gene named ataxia-telangiectasia mutated, which is mutated in radiation sensitive disorder, Ataxia-telangiectasia (AT). ATM belongs to the superfamily of phosphatidylinositol 3-kinase

related kinases[48]. ATM is a serine-threonine kinase, whose activity is stimulated by reorganization of higher-order chromatin structure caused by DSBs[49]. Various targets have been discovered, which include histone H2AX, NBS1, BRCA1, p53, CHK2, etc. Once ATM is activated, these downstream effectors are phosphorylated and mediate/initiate DNA damage response[50-55]. Among them, p53 plays the central role in DNA damage response[30-35]. p53 is a well known tumor suppressor protein, whose gene is mutated or lost in various kinds of human cancers. p53 is a transcription regulator and activates or suppresses expression of a group of genes, whose products are involved in apoptosis induction as well as cell cycle arrest. p53 is also well-known as a guardian of the genome, so that it efficiently limits propagation of cells with unreparable DSBs after radiation exposure. Since DNA damage repair systems efficiently eliminate DSBs, the number of DSBs is decreased instantaneously after irradiation. Following the completion of DSBs repair, ATM is dephosphorylated, and DNA damage signaling is turned off. Conversely, if DSB repair is unable to complete, activation of ATM and p53 persists, and cell cycle arrest continues. This is how permanent cell cycle arrest is executed.

Unreparable DSBs induce not only G1 arrest but also S arrest and G2 arrest, the latter two are transient, so that every cell subsequently starts to proceed to the next cell cycle phases. In contrast to S and G2 arrest, G1 arrest is known to be permanent in normal human cells, so that cells are persistently arrested in G1 phase, leading to premature senescence. It is evidenced that mitotic cells at the time of exposure have micronuclei[3] in the next G1 phase, In addition, it was also reported that cells might proceed to the next G1 phase even without cell division, which results in 4N G1 cells[56]. Cell cycle termination is really a dynamic process. While many normal cells are able to terminal cell cycle immediately, there is obviously a case, particularly in cancer cells, in which a few cell cycling are required before cell cycle is finally terminated[2].

7. Induction of premature senescence by IR in cancer cells

Irrespective of the evidence that IR-induced premature senescence in normal cells requires the p53 function, which is often abrogated in cancer cells, IR has been reported to induce premature senescence in various types of cancer cells[46, 57-60]. In some cancer cells, residual p53 activity is still able to execute premature senescence within one or two rounds of cell cycle. In most other cancer cells, they need a few more cell cycles before senescent phenotypes become obvious. In such cancer cells, cell cycle is progressed even with multiple DSBs, so that they show mitotic catastrophe, which is manifested by multiple micronuclei, di-nuclei, or by giant cell formation (Fig. 2). Besides, as cell cycle is progressed, DNA is also replicated, resulting in the nuclei with polyploidy. Although the mechanism, by which cell cycle is terminated in cancer cells, has not been fully defined yet, premature senescence is a common phenomena, and IR-induced premature senescence is now integrated into so-called therapy-induced senescence (TIS) as a part[61-63].

Premature senescence is manifested by prolonged growth arrest, increased lysosomal activity, and SA-β-gal activity, which are common to premature senescence in normal cells, however, morphological change is not so significant as in normal cells (Fig. 2). While premature senescence is irreversible in normal cells, recent studies have shown that TIS is reversible[64-67], although the its frequency is quite low, such as 1 in 10^6 cells. Importantly, it has been demonstrated that cancer cells in TIS reveal up-regulation of stem cell markers, suggesting that TIS is somehow associated with reprogramming of cancer cells. It allows prolonged survival of cancer cells in a dormant state with stemness, and such cancer cells are assumed to contribute to recurrence of the diseases[68].

So far, most of the studies have been done *in vitro*, and its demonstration is limited to the studies using certain biological settings. Thus, the reversibility of TIS still needs to be proven in various culture systems, and whether escape from TIS *in vivo* will happen in reality has to be determined. Also, it is currently not known whether IR-induced premature senescence in cancer cells is reversible or not. Future studies will be necessary to define whether cancer cells are able to escape from IR-induced premature senescence, as it is indispensable for understanding of mechanisms underlying radio-resistance and accelerated repopulation.

8. Secreted phenotype of senescent cells

While senescent cells cease to divide, they are still metabolically active and exhibit up-regulation of several gene transcriptions, most of which encode secretory soluble factors. Interestingly, such secretory factors were commonly observed in various cell systems, and the phenomenon was named senescence-associated secretory phenotype (SASP)[22-25]. Discovery of paracrine function of senescent cells uncovers biological mechanisms underlying antagonistic pleiotropy of senescence[68, 69]. Many of the secretory factors are common over different cell types, and most of them are expected to affect surrounding tissue microenvironment. Until recently, several factors have been identified, which are categorized into cytokines, chemokines, other inflammatory factors, growth factors, matrix remodeling factors, ligands, and extracellular matrix[18-25, 70] (Fig. 3 and

Table 2. Soluble factors secreted from senescent cells

Factors	Functions	Cell systems secreted factors
IL-1a	Cytokine	Human endothelial/muscle, fibroblasts epithelial cell
IL-6	Cytokine	Human fibroblasts, keratinocyte, monocyte melanocyte, epithelial cell
IL-8	Cytokine	Human fibroblasts
MCP-1	Cytokine	Fibroblasts, liver stellate cell
CXCL1	Chemokine	Human fibroblasts
CCL2/5	Chemokine	Human fibroblasts
TGF-β1	Growth modulator	keratinocyte, epithelial cell
IGFBPs	Growth modulator	Fibroblasts, epithelial cell, endothelial cell
CSFs	Growth modulator	Fibroblasts
MMPs	Matrix remodeling factor	Fibroblasts
PAI-1	Matrix remodeling factor	Endothelial cell, fibroblasts

Table 2). For example, IL-1α and IL-6 are representative cytokines for inflammatory response. Ectopic IL-1α secretion is suggested to amplify senescence cycle, while IL-6 is involved in inflammatory response and reproduces immunosuppressive microenvironment. Growth factors, such as HGF and bFGF, stimulate proliferation of neighboring cells, and matrix remodeling factors facilitate reconstitution of tissue structures. Thus, the secretion of soluble factors by senescent cells could play pivotal roles in the cross-talk between senescent cells and surrounding tissue microenvironment, by which tissue homeostasis is maintained. Conversely, inability of the clearance of senescent cells or persistent senescence caused by higher doses of radiation exposure give rise to chronic inflammation and fibrosis, which are detrimental to physiological tissue functions (Fig. 1).

Several lines of evidences have shown that there is no obvious difference in SASP between normal cells and cancer cells, indicating that common mechanisms could be involved in the initiation and execution of SASP. For example, DAMPs, high mobility group box 1 (HMGB1), and CCFs are those released from damaged normal and cancer cells (Fig. 3), which are contributed to potentiation of SASP in both cells. The profiles of secreted factors are also very similar between normal and cancer cells exposed to IR. Thus, it is likely that IR-induced premature senescence, whether it is induced in normal cells or cancer cells, shares common features of SASP[71-73].

9. Crossroad where IR-induced premature senescence meets tissue reaction and late effect of radiation

Radiation-induced premature senescence is deeply associated with cell death in many cell types, indicating that it had better integrate premature senescence into the physiological processes leading to the manifestation of radiation effect. For example, while bone marrow death and intestinal death are predominantly caused by apoptosis, premature senescence could also be involved in the manifestation of acute radiation syndrome (ARS). In fact, several studies have demonstrated that senescence-associated secretory phenotype is involved in the manifestation of adverse effects following radiotherapy[46, 74-76].

Significance of premature senescence in tissue reaction is corroborated by the fact that many of the secretory factors are those mediating inflammatory response, which is obviously a symptom of ARS. Although further studies are needed, secretory factors that mediate tissue reaction could be the potential target to mitigate ARS.

In addition to ARS, several lines of results have suggested that premature senescence is also involved in the manifestation of late effects, in particular, cancer development. It is well established that ionizing radiation induces cancer, whereas the underlying mechanism has not been fully elucidated yet[47, 77]. Although ionizing radiation is well known to cause targeted effects, which are arisen in cells directly absorbed radiation energy, several studies have described that radiation exposure give rise to non-targeted effects[39, 78-83], which is the manifestation of the consequences of radiation exposure in cells that have never been exposed. For example, bystander effect is one of the non-targeted effects, which is mediated by secreted factors and/or through cell-to-cell communications[84]. Importantly, many of the secreted factors associated with bystander effect are apparently identical to those secreted from senescent cells. Considering that premature senescence is surely the major modality of cell death in many cell types, it is no doubt that radiation-induced premature senescence plays an indispensable role in executing non-targeted effects.

Since senescence-associated secretory factors have been proven as critical mediators to change tissue microenvironment and immunogenic microenvironment, they play a pivotal role in reinforcement of the state of senescence. Furthermore, secreted factors are involved in tissue reconstruction by stimulating cell growth, matrix remodeling, and angiogenesis. Particularly, attracting

immunogenic cells by senescent cell-derived cytokines and chemokines need to be discussed, since it is essential for tissue regeneration and is known to be involved in immunogenic cell death[85, 86]. While recruitment of immune cells gives rise to beneficial effects, such as potentiation of systemic immunity, which is know as abscopal effect[87-89], several literatures demonstrated that some secreted factors bring local chronic inflammation as well as immunosuppressive microenvironment[87-89], which play a role in propagation of the initiated cancerous cells. Thus, radiation-induced premature senescence plays pleiotropic roles, and it is likely that some late effects might be brought about secreted factors from senescent cells. This idea should be integrated into the mechanism of radiation-induced carcinogenesis. Since manifestation of secretory phenotype of senescence dislike a stochastic phenomenon, its dose-response should not be linear, and such possibility has to be discussed further in detail from the radiation protection point of view.

Of note, it has now been clear that several aging-related diseases are originated from senescence[20-22]. Since non-cancer health effects caused by radiation exposure resemble such pathological processes, radiation-induced premature senescence should also be discussed with respect to non-cancer effects.

10. Elimination of senescent cells and amelioration of adverse effects

Radiation-induced premature senescence could be involved in the execution of radiation health effect, so that removal of senescent cells is expected to mitigate short-term as well as long-term radiation effects. Since targeted elimination of senescent cells from tissues have already been proven to ameliorate age-related pathologies[20, 21, 90-92]. It is practical to consider immediate application of targeted elimination of premature senescent cells from radiation protection point of view. So far, the development of the unique transgenic *INK-ATTAC* mouse model, in which the drug-inducible apoptosis-activator gene was driven by the $p16^{INK4a}$ promoter, revealed that several types of age-related diseases were mitigated by eliminating the $p16^{INK4a}$-positive senescent cells from old mice. The same results were obtained by another transgenic 3MR mice, in which the herpes simplex virus-derived thymidine kinase gene was under the control of $p16^{INK4a}$ promoter. Accordingly, selective removal of senescent cells was examined in human cases[90-92].

In order to eliminate senescent human cells several senolytic approaches have been examined[90-92]. One representative approach is to use small chemical inhibitors targeting proteins involved in apoptosis. The most promising approach is to use inhibitors of pro-survival BCL family proteins[93-95]. For example, ABT-263, which is widely used senolytic drug, inhibits Bcl-2 families, including Bcl-2, Bcl-xL and Bcl-w[93, 94], and several previous studies have shown that ABT-263 effectively eliminate senescent cells both *in vitro* and *in vivo*[93-98]. Importantly, several clinical trials have been examined to improve the efficacy of radiotherapy by eliminating therapy-induced senescent cells[99-109].

Although the mechanism, by which senescent cells are eliminated by senolytic drugs, is not fully understood yet, our previous study demonstrated that ABT-263 alone had little or no effect on growing cancer cells. Since SA-β-gal-positive senescent cells were selectively eradicated by apoptosis[110], western blot analysis was performed, which demonstrated that expression of apoptosis-related proteins, such as Bcl-xL, Bcl-2, and Bcl-w were obviously up-regulated in radiation-induced premature senescent cells. Furthermore, the examination also revealed that the levels of Bax and Bad were commonly increased in senescent cells. Since apoptosis was not evident in senescent cancer cells without ABT-263, and ABT-263 alone was not effective to unirradiated cancer cells, it was suggested that augmented expressions of both apoptotic and anti-apoptotic factors were critical for efficient removal of premature senescent cells induced by radiation exposure.

Besides ABT-263, several bioactive compounds have been applied for senolysis. For example, the combination of the Src kinase inhibitor dasatinib and the flavonoid quecetin (so-called D+Q recipe), which was developed using a bioinformatical approach, has now been subjected to clinical trials[21, 92]. Several other clinical trials are underway or planned[92]. Although these clinical trials are towards the age-related diseases, the same approach should be applicable to mitigate the adverse health effects originated from radiation therapy, and to alleviate radiation health effects as well.

11. Conclusion

Exposure to ionizing radiation results in induction of DSBs, which are the most deleterious to human health. Although DSBs are efficiently mended by DSB repair systems, receiving intolerable doses of radiation gives rise to unreparable DSBs, resulting in persistent activation of ATM-p53 axis-dependent DNA damage signaling pathway in normal cells. Accordingly, cell death is brought about tissues/organs, and impermissible levels of cell death cause tissue reaction. Among cell death modalities observed after radiation exposure, premature senescence has now been recognized as a representative consequence of various non-hematopoietic tissues, including epithelial tissues, mesenchymal tissues, and endothelial and lymphatic cells. It has also been demonstrated that IR-induced premature senescence is a major cause of

death of cancer cells. Unexpected secretory phenotype associated with premature senescence has attracted enormous interests, as secreted soluble factors, such as cytokines, chemokines, growth factors, and matrix remodeling factors are indeed associated with the modification of tissue microenvironment, which is involved in the execution of radiation late effects as well as adverse effects after radiation therapy.

Although physiological significance of senescence induction and its relevance to radiation health risk have not been fully described yet, several studies have already shown its potential to promote novel strategies and techniques against radiation protection seems promising. Future studies are expected to deepen our knowledge of biological significance of IR-induced premature senescence in tissue reaction and late effects, and promote studies towards possible ways to ameliorate radiation health effects by eliminating senescent cells from exposed tissues. At the same time, deeper understandings of therapy-induced premature senescence should provide novel mechanistic insights into the effectiveness of radiation therapy, which should provide innovative strategies for cancer radiation therapy in the future.

Conflict of interest

The author declares that I have no conflict of interest.

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