Chapter X: Late Radiation Effects

Section e: Aging/Senescence

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Total body irradiation is known to induce multiple biomarkers in clinical signs of aging. Several studies of total body irradiated mice demonstrated classic signs of aging including: graying of hair, reduction of stem cell populations in the bone marrow, reductions in blood counts, and life shortening (8, 14-15). Partial body irradiation experiments demonstrated age-associated changes in these parameters, as well as, specific aging effects on targeted tissue volumes (8). For example, irradiation of one hind limb to high dose was demonstrated to induce loss of tissue flexibility (elasticity) and signs of age-associated osteoarthritis. Irradiation-induced delay in bone wound, or tissue/skin wound healing is usually associated with aging in experimental animal models was also reported (17-18).

There has been much interest in the mechanism by which irradiation induces tissue aging, and, particularly, how these changes relate to radiation "late effects". Other chapters in this web-based textbook address specific components of the irradiation late effects with respect to tissue and organ systems, and how these relate to clinical radiation therapy. It is the purpose of the present chapter to review current methodologies for studying two important components of irradiation-induced aging; namely, total body irradiation induced aging and irradiation-induced cellular senescence.

Total Body Irradiation-Induced Aging

The similarity of the mechanism of total body irradiation effects with those effects of aging has been elucidated from three areas of investigation: 1) study of nuclear reactor workers and irradiation exposed miners; 2) study of the sequellae of galactic cosmic irradiation and solar proton events providing continuous low dose rate exposure of astronauts during space travel; and 3) Atomic bomb or nuclear accident survivors. Nuclear reactor workers and miners have a history of prolonged chronic exposure to a low level of irradiation such that cumulative doses may be significant depending on the number of years of exposure, dose rate of exposure, and the quality of the radiation. Much information from Uranium miners has revealed an increase in the incidence of known radiation exposure events including: cataracts, and increased incidence of cancer including lung cancer.

A study of less than 100 astronauts after completing space missions of various durations has revealed a concern for an increase in cataracts, but also for concern that the doses sustained may approach those associated with neurodegeneration, and carcinogenesis. Less information is available from galactic cosmic irradiation owed to the relatively shorter observation period since the first space mission. Long-term clinical studies on these individuals are in progress, and will be evaluated. However, the confounding variables of prolonged weightlessness, psychosocial changes including changes in the microbiome that are associated with prolonged space travel, and dietary and metabolic changes associated with space missions will have to be taken into account in interpreting the data.

Another third set of data has come from the study of second and third generation survivors of the Hiroshima and Nagasaki atomic bomb events. Accumulation of genetic changes in the children and grandchildren of A. bomb survivors suggest the susceptibility of these individuals to both neurodegenerative and carcinogenic events associated with genetic change, changes in fertility,

neurocognitive changes, and potential for induction of mutations associated with inheritance of irradiation-induced changes.

Relatively, little information is available on irradiation-induced life shortening in the survivors of the Atomic bomb, due to multiple factors including: inconsistencies of data collection, relatively small numbers of individuals with known exposure levels, and multiple sources of combined injury, such as thermal burns, concussion, and infections. Each of these data sets provides some information with respect to the effects of ionizing irradiation on life shortening and aging of tissues and organ systems.

The most convincing evidence for irradiation-induced aging comes from experimental models. Studies with C. Elegans, Drosophila (fruit flies), Zebrafish, and rodent models indicates that ionizing irradiation induces life shortening. Studies with each of these model systems has also provided a test system for application of anti-aging drugs (14-15, 24). Studies with C. Elegans first documented beneficial effects of mitochondrial targeted antioxidants to prolong survival after irradiation, and, thus, ameliorate irradiation-induced life shortening (23). Other studies have documented significant life shortening in both male and female total body irradiated mice (male life shortening greater than that in female). There was also the therapeutic effect of increasing antioxidant stores prior to irradiation (14), and continuous administration of antioxidants in the diet with respect to decreasing life shortening effects of irradiation (15).

There remains much work to be done with respect to the effects of irradiation on accelerating the effects of aging. Several areas of research are important for new investigators to consider and include approaches to answering several important questions:

1. What is the mechanism of irradiation-induced depletion of antioxidant stores? Is this attributable to continuous oxidative stress in non-proliferating tissues, or because of tissue changes in the microenvironment? How does aging alter the ability of stromal cells to support hematopoietic or intestinal stem cells or stem cells in other organs?

2. Is there a change in DNA damage repair, as a result of epigenetic changes induced by irradiation? Are cells, tissues, and organs less likely to tolerate environmental stress following exposure to total body irradiation, in part, because their DNA repair capacity is compromised?

3. What are the epigenetic changes induced in total body irradiated animals, and how can these potentially be ameliorated by specific targeted agents?

4. What should be the molecular and cellular targets for designing new therapies for irradiation-induced life shortening? Should these therapies be the same targets as those being developed in therapy of non-irradiated individuals, who seek treatment for aging in general or specific age-related debilitating diseases such as Osteoarthritis, Alzheimer's Disease, Dementia, Atherosclerotic Cardiovascular Disease, post-trauma, thermal burn, and other hnon-irradiation-induced causes of tissue fibrosis?

5. What is the molecular mechanism of irradiation-accelerated aging, and is this process associated with broad multiple tissue events, or events localized to specific organs, tissues, and cells such as stem cells or cells of the microenvironment?

Irradiation-Induced Senescence

There has been great interest in the phenomenon of cellular senescence based upon discovery of the senescence associated secretory phenotype (SASP) (2). SASP includes: IL-1_a, $IL1_b$, IL-6, IL-8, CXCL1, bFGF, HGF, MAP-1, MMP-1, MMP-3, MMP-13, as well as, loss of laminβ1 and increased NFKβ signaling. Senescence is the condition by which cells permanently lose their ability to proliferate. Senescent cells are distinct from other cells in a tissue, which demonstrate quiescence (non-proliferating state, but capacity to proliferate at a later date). These two categories are a subject of intense interest, particularly, because some data suggests that the biomarkers used to detect senescent cells may reflect an incomplete separation of this phenotype from quiescence (4-7, 24). Cells that are termed to be senescent are those, which express certain biomarkers including: age-associated beta-galactosidase, increase in p21, increase in p16, telomere shortening, and observed increase in secretion of senescent cell associated secretory factors including inflammatory cytokines (2). Ionizing irradiation has been shown to induce increased numbers of senescent cells in tissue culture (13). Furthermore, total body irradiated mice demonstrate increase in accumulation of senescent cells in some organs such as spleen and bone marrow, but not in other organs such as brain (13).

A major problem in studying senescent cells has been the inability to sort them and separate them from tissues in which increased accumulation of such cells has been detected. Cell surface markers for senescent cells have not be reproducibly effective in separating senescent cells utilized (12). In contrast, quiescent cells have been sorted by a specific cell surface phenotype associated with stem cells in the bone marrow, and these cells have been shown to have decreased proliferative activity, and downregulated mitochondrial generation of ATP (It is well established that maintaining a large population of stem cells in quiescence is critical for long-term survival (9)).

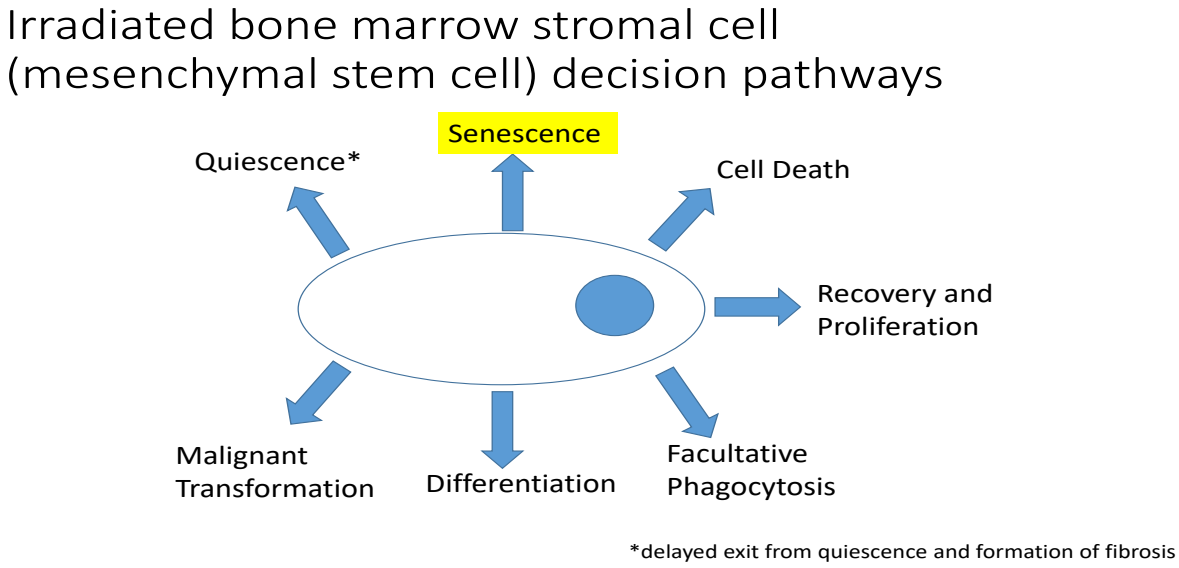
In animal models of increased oxidative stress, or genetic inability to repair DNA damage, a loss of stem cells has been associated with life shortening or failure production of enough differentiated cells to maintain homeostasis (10). Fanconi Anemia mice (Fancd2-/-) have a decreased number of hematopoietic stem cells, and this is associated with aging, and decrease in stem cell numbers occurs at a more rapid pace than that observed in wild type control littermate mice (9). Clinical or laboratory animal administration of small molecule drugs such as Metformin has been demonstrated to decrease the depletion of quiescent cells in the bone marrow and provide for longer generation of mature red blood cells, the differentiated progeny of bone marrow stem cells, and improved survival (9-10). How Metformin stabilizes marrow hematopoietic stem cells is not known. Whether this effect is directly on the stem cells themselves, or through "quieting" of the bone marrow microenvironment is not known. Fanconi Anemia mice are known to have an intrinsic hyperactive TGF-β signaling pathway (21). Since aging is associated with continuous production of TGF-β, which is a negative regulator of hematopoiesis by the cells of the bone marrow microenvironment this observation is logical, it is not clear whether a therapeutic effect of Metformin is directly on the bone marrow stem cells, or

by decreasing production of inhibitory regulators including TGF-β in cells of the microenvironment. The available data emphasize the difference between quiescence and senescence, the latter associated with permanent failure to proliferate or differentiate. Removing senescent cells with senolytic drugs such as desatinib and quercetin has been evaluated in an aging mouse model (24) and has shown some success in slowing the accumulation of age-associated events.

Cellular Pathways and Irradiation Response

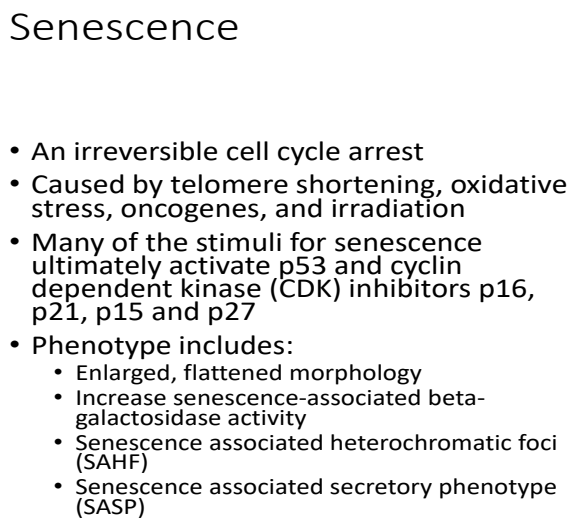
Ionizing irradiation is known to kill cells by inducing each of multiple cell death pathways. Direct induction of DNA strand breaks leads to signaling to the mitochondria producing apoptosis. Release of TGF-β and other inflammatory cytokines leads to induction of necroptosis in both irradiated and non-irradiated cells. Depletion of glutathione peroxidase for (mitochondrial glutathione peroxidase, which can reduce hydrogen peroxide to water) leads to ferroptosis. Other cell death pathways including: parthanatos (mitochondrial poly-Adp-Ribosyl-Prolimerase) PARP induce death, and pyroptosis (bacterial signaling or intracellular activation of Caspase-1) are some of the death pathways, which can result in elimination of cells from an irradiated tissue. Some cells in the irradiation field may repair and return to "normal" functioning. Other cells may proceed to the pathway of senescence (1), which is associated with a non-proliferative state, but by continued residence in tissues produce potentially damaging inflammatory cytokines called the senescence-associated secretory phenotype (SASP) (Fig. 2: Ref. Munoz-Espin, et al., Fig. 1, Nat Rev Mol Cell Biol, 15(7): 482, 2014 & He, et al., Fig. 1, Cell, 169(6): 1000-1011, 2017)). Irradiated cells may also differentiate to cell types with specific functions, and even become phagocytes, which can ingest and metabolize other cells in the tissue (Fig. 1).

Fig. 1:



Included in the possible decision pathways is the pathway towards malignant transformation including carcinogenesis and leukemogenesis. Whether cells sitting in a quiescent state, differentiated state, or even senescent state can proceed to the cancer phenotype is not known.

Fig. 2:



Accumulation of senescent cells has been both associated with an increase and a decrease in carcinogenesis within a tissue. The data supporting an anti-carcinogenic effect is that associated with production by the senescent cell cytokines that could dampen the proliferative capacity of premalignant cells to grow, invade or metastasize. The logic of this argument is that more cells going to senescence means less cells going to cancer (Figs. 3 – 4) (Fig. 3: Martinez-Samudio, et al., Cell, 170(5): 1044-1044, 2017) (Fig. 4: Che, et al., Trends Genet, S0168-9525(17): 30208-1, 2017).

Fig. 3:

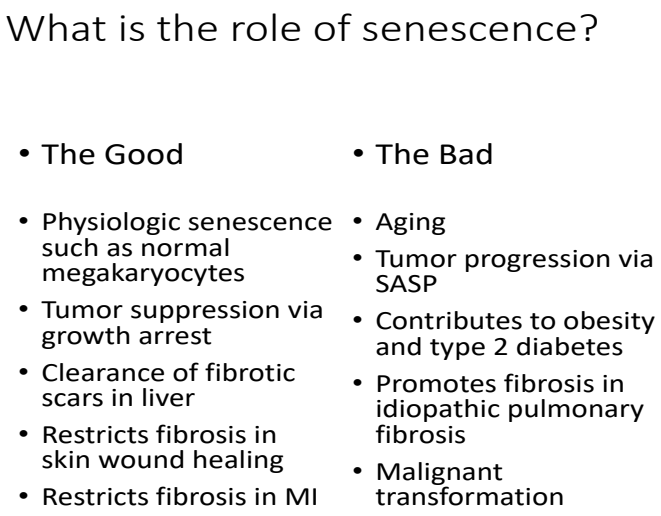


Fig. 4:

Senescent cells accumulate in response to aging and DNA damage signaling. Fanca-/- bone marrow stromal are radiosensitive compared to wild type:

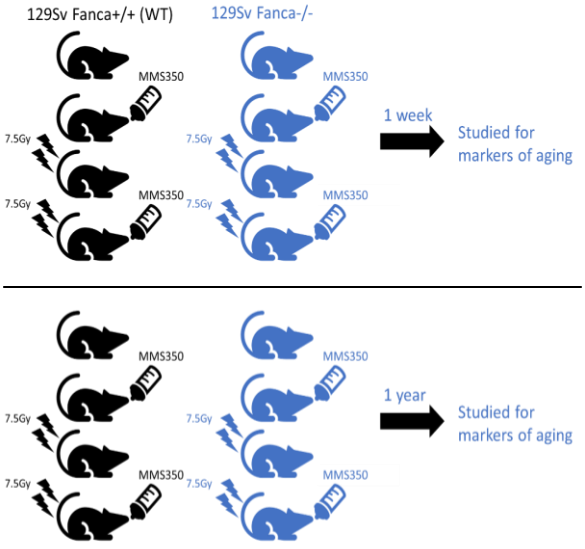
1. Fanca-/- bone marrow stromal cell lines show more radiation induced senescence *in vitro*
2. Fanca-/- mice show more radiation induced senescence and increase in other markers of aging *in vivo*
3. MMS350 ameliorates irradiation induced senescence and other markers of aging *in vitro* and *in vivo* (1)

Alternatively, a pro-carcinogenic effect of senescent cells is that associated with a chronic inflammatory state in tissues that can lead to transformation of premalignant cells to a fully malignant state. An example is the induction of acute myelogenous leukemia in TET-2 deficient mice that display a myeloproliferative, but non-cancer disorder that is converted to acute myelogenous leukemia by production of IL-6 that is initiated by an inflammation stimulated bacteria (22).

Anti-Aging Drugs, Anti-Senescence Drugs – Are These Radiation Mitigators?

Recent studies have demonstrated that chronic administration of the radiation mitigator, water-soluble-DMSO analog, MMS350, reduces senescence in total body irradiated Fanca-/-, as well as, control Fanca+/+ (129/Sv) mice (13). In these studies, sublethally irradiated mice were maintained for one year on continuous administration of MMS350 in the drinking water compared to those given regular drinking water (Fig. 5).

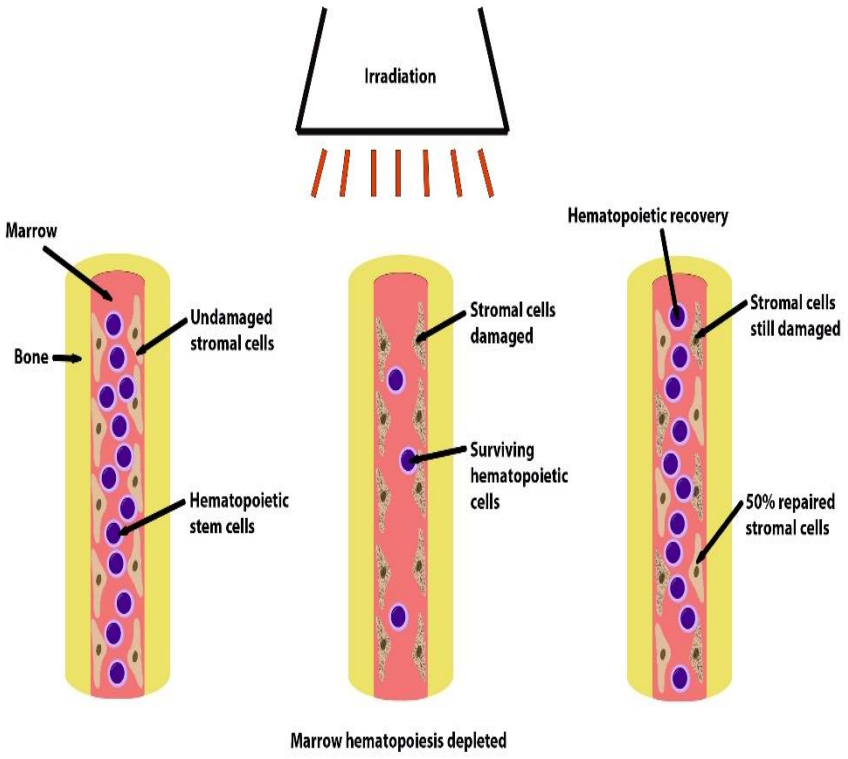
Fig. 5: Schematic of measuring MMS350 effects in TBI Fanca+/+ and Fanca-/- mice. Subgroups of mice were either sham irradiated or 7.5 Gy irradiated. All mice survived one year, since this was a sublethal TBI dose. One week prior to irradiation, mice were started on drinking water supplemented with no drug (control, regular water) or 400 µM MMS350. Mice remained on regular water or MMS350-supplemented water continuously after irradiation until time of sacrifice. Mice were sacrificed at one week or one year post-TBI to study markers of aging <u>in</u> <u>situ</u> and in explanted marrow in LTBMC. (Reprinted from reference #13 with permission of the publisher.)



Several parameters of aging included accumulation of senescent cells in a proliferative organ (spleen) or non-proliferative organ (brain) were different between organs, but did demonstrate age-related increase in the number of senescent cells in both organs (15). MMS350 administration decreased the number of cells rapidly proliferating in the spleen. Perhaps, more important, was the demonstration that bone marrow stromal cells, which inhibited from proliferation by total body irradiation showed restored proliferative capacity if MMS350 had been delivered in the water for a short one week interval, and continuously for one year (Fig. 6). The data demonstrate a repair capacity of slowly proliferating or non-proliferative cells in situ in total body irradiated animals, if given an antioxidant drug, which in this case, was also a radiation mitigator (Fig. 6).

Fig. 6: Comparison of in vivo with ex vivo cultured marrow stromal cell effects of an anti-senescence drug in total body irradiation.

Panel A:



Panel B:

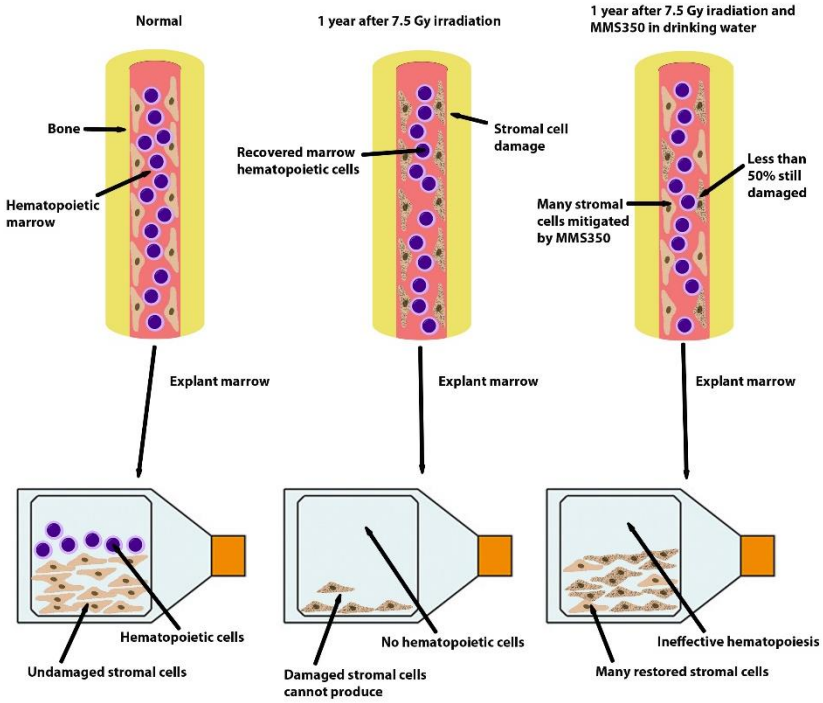


Fig. 6: Schematic representation of hypothesized mechanism of late irradiation effects at 1 year on the marrow microenvironment and amelioration by continuous MMS350 administration. A) Some marrow stromal cell damage repaired by 1 year after TBI to allow formation in situ to support hematopoiesis in vivo. B) Subtle marrow stromal cell damage is not repaired by 1 year and revealed by the stress of induction of proliferation in vitro in LTBMCs. Panels in Fig. 6A show the in vivo interaction of bone marrow stromal cells (mesenchymal stem cells) representing the hematopoietic microenvironment, with hematopoietic stem cells and their progeny, representing the hematopoietic stem cell compartment. After 7.5 Gy TBI, the majority of differentiated hematopoietic cells in vivo and some hematopoietic stem cells are absent (middle panel) compared to control unirradiated marrow (left panel), and associated with the acute reduction in peripheral blood counts (leukocytes, red blood cells, and platelets). While bone marrow stromal cell proliferation after explant at 1 week after TBI is reduced representing the acute effects of ionizing irradiation, this is not associated with impaired recovery in vivo (right hand panel) for support of hematopoietic cells that were in quiescence and resisted irradiation.

The panels in Fig. 6B demonstrate the effects of administration of MMS350 for 1 year after 7.5 Gy TBI when bone marrow is then explanted to culture. While there is recovery of hematopoiesis in vivo (top middle and right hand panels), there was impaired capacity of marrow stromal cells following explant to form a functioning hematopoietic microenvironment and support hematopoietic cells (lower middle and right hand panels). While MMS350 administration for one year after TBI (lower right hand panel) increased survival and proliferation of stromal cells, there was still incomplete recovery of support of

functioning hematopoiesis in vitro. (Reprinted from reference #13 with publisher permission.)

The drug MMS350 reduces another late effect of irradiation, radiation pulmonary fibrosis (16). The drug is an acute radiation mitigator improving survival when delivered intravenously 24 hrs. after total body irradiation (16). Continuous administration of MMS350 was associated with downregulation of RNA transcripts for multiple inflammatory cytokines and stress response genes (16).

Will an antiaging drug be an anti-senescence drug? Will an antiaging drug be a radiation mitigator? Will a radiation mitigator serve as both an antiaging and anti-senescence agent? These are all critical questions for determining a potential link between ionizing irradiation and aging.

Radiation mitigating drugs may not be anti-aging drugs. Controversy still persists as to whether those experimental animals (or patients), who experience significant radiation early effects, will be those at greater risk for radiation late effects. The answer to these questions must involve studies of both radiation protector (delivered before irradiation exposure) and radiation mitigator drugs (delivered after irradiation exposure). In the former case, by increasing antioxidant stores or increasing the availability of DNA repair enzymes, one may experimentally decrease the number of cells dying from subsequent acute radiation exposure and decrease acute radiation cytotoxicity (14, 16). In the case of a radiation mitigator drug, which is delivered 24 hrs or later after irradiation, DNA strand breaks have already been induced in cells, and communication from nucleus to mitochondria of pro-apoptotic molecules, and induction of inflammatory cytokines associated with necroptosis, lysosomal necrosis, ferroptosis, parthanatos, and pyroptosis may have been initiated and already occurred. Thus, a radiation mitigator would target events that shut down these death pathways that have already been initiated by irradiation. The same cell death pathways are operative and functioning during aging. So, an agent, which shuts down one or more radiation-induced death pathways could be an anti-aging drug. According to this logic, total body irradiation exposure induces continuous upregulation of these cell death pathways, and would accelerate aging, or induce early onset aging. A radiation mitigator drug might be an anti-aging drug.

The above discussion leads to two hypotheses: 1) The frequency of detection of senescent cells in human irradiated tissues, organs, and organ systems should increase with age and/or irradiation. 2) The signaling pathways common to aging and irradiation may be important targets for discovering anti-aging drugs. Irradiation exposure, accelerated aging, and irradiation-induced senescent cells appear to be related. The study of senescence is a fertile area for research in both radiobiology and aging and much more investigation is required.

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