

Full Length Article

Potential role of senescence in radiation-induced damage of the aged skeleton

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ARTICLE INFO

Keywords:

Radiation
Bone damage
Osteoporosis

ABSTRACT

Human aging-related changes are exacerbated in cases of disease and cancer, and conversely aging is a catalyst for the occurrence of disease and multimorbidity. For example, old age is the most significant risk factor for cancer and among people who suffer from cancer, > 60% are above the age of 65. Oxidative stress and DNA damage, leading to genomic instability and telomere dysfunction, are prevalent in aging and radiation-induced damage and are major cellular events that lead to senescence. Human exposures from nuclear fallout, cosmic radiation and clinical radiotherapy (RT) are some common sources of irradiation that affect bone tissue. RT has been used to treat malignant tumors for over a century, but the effects of radiation damage on tumor-adjacent normal tissue has largely been overlooked. There is an increase in the percent survivorship among patients post-RT, and it is in older survivors where the deleterious synergy between aging and radiation exposure conspires to promote tissue deterioration and dysfunction which then negatively impacts their quality of life. Thus, an aging skeleton is already pre-disposed to architectural deterioration, which is further worsened by radiation-induced bone damage. Effects of senescence and the senescence associated secretory phenotype (SASP) have been implicated in age-associated bone loss, but their roles in radiation-associated bone damage are still elusive. RT is used in treatment for a variety of cancers and in different anatomical locations, the sequelae of which include long-term morbidity and lifelong discomfort. Therefore, consideration of the growing evidence that implicates the role of senescence in radiation-induced bone damage argues in favor of exploiting current senotherapeutic approaches as a possible prevention or treatment.

1. Introduction

Cellular senescence, first described as a condition of irreversible cellular growth arrest obtained during serial passaging of cells was recognized by Hayflick and Moorhead in 1961 [1]. While replicative senescence, generally described by the limited capacity of cells to divide, is linked to shortening of telomeres [2–5], stress-induced cellular senescence can be generated by increases in reactive oxygen species (ROS), DNA damage, and cellular imbalances in proteostasis [6–13]. Furthermore, the oncogenes *ras* [14] and *raf* [15] have also been shown to cause cellular senescence. Senescence is a known mechanism which mitigates tumor proliferation [16–18] by maintaining early neoplastic cells in a viable albeit apoptosis-resistant arrest state. Accompanying their growth arrest, senescent cells produce an inflammatory secretome; the senescence associated secretory phenotype (SASP), which has also been linked with tumor proliferation and invasion [19–21]. Senescent

cells in normal organs cause tissue dysfunction and disease likely related to tissue-specific threshold effects [22–25].

Aging is the leading risk factor for the majority of chronic diseases and related comorbidities [26]. One such disease related to aging is osteoporosis, which causes bone loss and associated fragility fractures [27–31], resulting in substantial morbidity and mortality. One fundamental aspect of age-related bone loss is the uncoupling of bone-forming osteoblast function and bone-resorbing osteoclast activity [32]. A major cause for this uncoupling has been attributed to the increase in cellular senescence and the rise in inflammatory and other proteins of the SASP [33,34].

Ionizing radiation (IR) is a major inducer of free radicals, including ROS, leading to DNA damage. While oxidative stress and DNA damage-induced changes in bone (either due to aging or radiation) have been studied extensively, the role of cellular senescence in the pathophysiology of radiation-related osteoporosis is still only partially

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Received 5 November 2018; Received in revised form 4 December 2018; Accepted 8 December 2018

Available online 10 December 2018

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Table 1

The table highlights the significant changes seen in skeletal tissue during aging and upon exposure to radiation, either through clinical radiotherapy, cosmic radiation or nuclear fallout.

| Parameters of bone tissue changes | | Aging | Radiation |
|---|---------------------|------------------------------------|--|
| Pathological conditions | Osteopenia | ↑↑↑ [162] | ↑↑↑ [106,163] |
| | Osteoporosis | ↑↑↑ [162] | ↑↑↑ [116] |
| | Fracture healing | ↔ [164] | ↓↓↓ [165] |
| Trabecular bone volume | | ↓↓↓ [166,167] | ↓↓↓ [48,100,103,104,115] |
| Trabecular thickness | | ↓↓↓ [166] | ↑ [103,104] |
| Trabecular separation | | ↑↑↑ [166] | ↑↑ [48,100] |
| Trabecular number | | ↓↓↓ [166] | ↓↓ [103,104] |
| Osteoblast number | | ↓↓ [168] | ↓↓↓ [48,100,104] |
| Osteocyte | Number | ↓↓ [169–171] | ↓↓ [48] |
| | Canalicular network | ↓↓↓ [172] | ↓↓↓ [48] |
| Osteoblast function | BFR | ↓↓↓ [173] | ↓↓ [48] |
| Osteoclast number/osteoclast surface (per bone surface) | | ↓↓ [174] | ↓↓↓ With high dose focal RT [100,104,107] ↑↑ With low dose or whole body IR [87,175] ↑↑ [87] |
| Serum CTX/TRAP5b | | ↑↑ Women [176,177] Men [177] | ↑↑ [87] |
| Adipocyte number | | ↑↑ [178] | ↑↑↑ [48,103,109,179] |
| Bone marrow vasculature | | ↓↓ [180] | Vessel number ↓↓ [181] Necrosis ↑↑↑ [182–184] |
| | | – Angiogenesis ↓↓ [185,186] | – |

↑, ↑↑ or ↑↑↑: mild, moderate or high increase. ↓, ↓↓ or ↓↓↓: mild, moderate or high decrease. BFR: Bone formation rate, CTX: C-terminal telopeptide. TRAP: Tartrate-resistant acid phosphatase.

understood. However, due to extensive studies in rodents, we have a detailed understanding of changes inside the bone environment upon exposure to IR, many of which are similar to age-related osteoporosis (Table 1). Among the major changes seen in both IR- and age-related osteoporosis is the deregulation of mesenchymal stem cell function, increased bone marrow adiposity, reduction of osteoblasts on the bone surface and osteocyte apoptosis characterized by empty lacunae.

In this review we shall discuss the various sources of IR, their effects on bone and their capacity to cause cell senescence-related alterations in bone. A special focus will be given to clinical radiotherapy (RT) and the resulting changes in tumor-adjacent bone tissue.

2. Types of radiation exposure and effects on the skeleton

2.1. Clinical radiotherapy and bystander effects on the skeleton

From its discovery, the therapeutic efficacy for radiation to cure cancers of many histopathologies was identified and utilized, first in a crude manner using radium [35–38], and later with the implementation of X-rays, gamma-rays and more recently proton beam. For years the bystander effects, while recognized, were mostly overlooked in favor of the benefits for cancer treatment. Indeed, together with chemotherapy, RT has been very effective in killing cancer cells and reducing tumor size. With improved treatment regimens, RT in conjunction with chemotherapy is able to extend the life span of cancer survivors. With increased survivorship, the bystander effects of RT, including bone damage became a well-recognized late but unavoidable effect, resulting in a gamut of bone changes from osteopenia and osteoporosis to osteoradionecrosis (ORN) [39,40]. Radiation-associated insufficiency fractures also occur following the treatment of a variety of cancers including breast [41,42], anal, rectal and cervical cancers, where the radiated regions have a > 3-fold increase in fractures. Moreover,

fractures caused by radiation are painful, difficult to treat and are associated with very high rates of delayed union and nonunion [43,44], especially in older individuals [45,46]. In a retrospective study performed using Medicare claim records and a sample size of > 2500 women > 65 years of age who received conventional RT, approximately 9% of patients receiving RT had pelvic fractures [45]. To date, the mechanism of radiation-induced bone damage has not been fully elucidated [47–49]. We will attempt to summarize the scope of skeletal damage post-conventional and improved RT methodologies in various cancers.

Pelvic insufficiency fractures (PIFs) following RT were often sidelined as an age- or post-menopause-related condition. While age and post-menopausal osteoporosis predispose patients receiving RT for cancer treatment where the pelvis is in the field of radiation, PIFs have also been reported in pre-menopausal women receiving RT for cervical cancers [50]. Within the pelvis, fracture sites may include the sacrum, pubis, iliac crest and the acetabulum. Due to extensive follow-up studies all over the world, there is now convincing evidence that the PIFs reported post-RT are linked to the direct damage caused by IR [51–53]. The number of reported PIFs in gynecologic cancers range from 10 to 29% [51,54] and may even go up to 80% in certain cases, with age and menopause being the confounding factors. Even with the use of advanced RT like intensity modulated radiation therapy (IMRT), stereotactic body radiation therapy (SBRT) and image-guided radiotherapy there is still great concern for PIFs post-therapy.

RT is one of the most common treatment modalities to treat gynecologic cancers. Technological advancements have been made in the ways RT is locally administered to treat cervical and uterine malignancies. As mentioned above, until recently the problem of post-RT PIFs was underreported. As the survival rate of the uterine and cervical cancer patients have increased from a few years to > 5 years, the adverse effects of RT treatments became a stark reality [55] and one that pushed the field for improved techniques. Currently, most cervical cancer patients are treated with IMRT, which is a kind of external beam radiation therapy (EBRT) that allows enhanced conformality of the radiation dose to target when compared to the standard 3D plan. Often used together with cisplatin, a widely used chemotherapeutic agent [56], RT is usually used in locally advanced cervical cancers and sometimes combined with surgery for uterine cancers. Despite improved techniques, recent reports indicate a 30% chance of PIF with IMRT among patients > 50 years of age [57].

Radiation dose and the regimen often determine the extent of the bone damage. On average a dose of 45–50 Gy is delivered with external beam for the treatment of gynecological cancers [50]. The same study classified the various fractures based on their location, showing that 83% of fractures occur in the sacral area, 10% in sacrum and pubis, 3.5% in iliac crest and 3.5% in sacrum and acetabulum [50]. RT was given in conjunction with cisplatin and 5-fluorouracil, which may be additional variables contributing to bone anomalies post-RT. The majority of the patients who developed fractures were treated with primary RT which suggests that higher doses of RT were used to optimize tumor control.

Prostate cancer affects 200,000 men annually in the US and approximately 1.3 million globally, and 28% of the men diagnosed with prostate cancer were treated with pelvic EBRT. Pelvic conformal EBRT was linked with an increased risk of fractures of the hip by 76% [58]. The EBRT was often used in combination with androgen suppression therapy for intermediate and high risk prostate cancer patients and the therapy itself may further increase the risk of hip fractures. Sacral insufficiency fractures is a common consequence with increasing doses of SBRT used with hypo-fractionation, often with shorter latencies than the usual reported occurrence of sacral insufficiency fractures at 13 months post-SBRT [59].

Anal cancer, among all gastrointestinal cancers, is a rare disease with incidence rates of 1–2%, but there are at least 9000 cases per year in United States and 25–30,000 globally [60,61]. Among these 70% are

in women and 30% are in men. According to American Cancer Society statistics, annually there are about 1200 deaths on average in United States alone that can be directly attributed to anal cancer. The incidence rates may vary globally with variation in gender, race and lifestyle, but consistently it is a condition predominant in older individuals (> 60 years of age). Anal sphincter-preserving concurrent chemoradiation therapies are used in lieu of surgery for anal cancers. Several advanced RT methods have been generated for the treatment of anal cancers, including three-dimensional conformal RT, IMRT, and pencil-beam scanning proton therapy. These techniques use an image-guided approach to identify the location of the tumor which minimizes the safety margin around the target to reduce the risk of geometrical miss. These advanced RT methods are claimed to be sparing to the pelvis and bone marrow, but the outcomes from these treatments are still awaited.

Rib fractures are common with the use of RT for treatment of lung, breast and cancers that affect tissues in the thoracic region [62–65]. RT to the chest often leads to chest wall pain, and subsets of those patients who report chest wall pain have rib fractures as the etiology [64].

Lung cancer is the leading cause of death irrespective of gender, country and cancer type. SBRT is the standard of care for non-small cell lung cancer, when the cancer is considered inoperable. Ninety percent of patients suffering with lung cancer were found to be heavy users of tobacco [64]. The average rate of rib fracture varied in different studies ranging from 8% [66]–21% [64,67]. With the projected number of patients having lung cancer estimated to be about 250,000 per year in United States alone, approximately 50,000 of these patients may suffer rib fractures.

Breast cancer is another form of malignancy which requires the use of RT and is associated with rib fractures as a bystander effect. Conventional RT, SBRT and brachytherapy, all with the intention to minimize damage to healthy tissues near the cancer site, still pose a potential threat of spontaneous rib fractures [68–70]. Apart from fractures, breast pain are significant side effects of RT [71]. Brachytherapy was introduced for the treatment of breast cancer as advancement over EBRT and with minimal side effects, but a 5-year study revealed an increased fracture incident with brachytherapy as compared to EBRT [71] especially if the location of the tumor was close to the chest wall. Incidents of rib fractures among patients with breast cancer and treated with some form of RT range from 1 to 19% [72].

RT in combination with surgery and chemotherapy is a common treatment regimen for most head and neck cancers (HNC). With recent technological advancements, the side effects caused by RT may be reduced, but skeletal damage post-treatment is still a concern. HNCs are often transformed from the squamous cells of the soft mucosal lining of the mouth, nose and throat. HNCs can be characterized by the areas they affect such as oral cavity, oropharyngeal, nasal, paranasal sinus, nasopharyngeal, laryngeal and hypo-pharyngeal regions. Radiation could be the first line of treatment or used adjuvantly, depending on the location of the malignancy. More often RT is given as a course of treatment in locally advanced HNC, and post-RT morbidities may include xerostomia, mucositis, damaged collagen, blood vessel damage, bone loss, fractures and osteonecrosis.

ORN is a condition generally characterized by an overall degeneration of the bone tissue, loss of vasculature, hypo-cellularity and osteolysis. ORN mainly affects the mandible and has an early onset (ORN initiates < 2 years after RT) and a late onset (ORN initiates > 2 years post RT) [73].

A metastatic tumor in bone modifies the bone microenvironment causing osteolysis of existing bone and leading to further tumor formation in the local environment. Standard RT and SBRT in re-irradiation are the standard of care for spinal metastases, but are associated with a high risk of vertebral compression fractures [74]. There is evidence that patients who benefit from SBRT (i.e., suppression of spinal metastases) live for many more years, but during this period vertebral compression fractures were significantly high [75]. Solitary treatments

of SBRT tend to use a high dose of IR and are used in the cases of aggressive spinal metastases; however, this puts survivors at high risk of vertebral compression fractures.

2.2. Space exploration-cosmic radiation

Long term space exploration is one of the fascinating ideas undertaken by several space agencies across the world. However, the adversities expected in outer space beyond the low earth orbit limits this enthusiasm [76]. The different kinds of radiation that an astronaut is exposed to, may include particles constituting the Earth's magnetic field, particles trapped in the van Allen radiation belt, solar flares and high energy protons and ions of the solar system [77]. Neutron-based IR is another source of radiation which were reported both inside and outside of the international space station (ISS) [78,79], and the dose varies based on the location of the ISS. Microgravity and the overall long term exposure to cosmic IR contribute to the deterioration of healthy tissues including bone.

Radiation exposure during a stay at the ISS has been shown to cause DNA damage in human fibroblasts [80,81]. Moreover, given plans of future space exploration to other planets, long term exposure to cosmic radiation is a greater threat at the surface of Mars [82,83], since the “Red Planet” does not have an atmosphere. Thus, the extent of DNA damage followed by subsequent cellular, tissue and organ level compromise could be much more dramatic for a space mission to Mars [84]. Bone deterioration starts to become evident at 2 Gy, which may not be achieved during a six-month ISS stay (i.e., total radiation dose accumulates to only 80–160 milliSieverts (mSv) or about 0.08–0.16 Gy). However, the lack of an atmosphere leaves an astronaut in the direct cosmic radiation path, the dose equivalent of which is still unknown due to lack of evidence.

Using mouse models, several studies have mimicked the whole body radiation, also called total-body irradiation (TBI) that astronauts are exposed to in outer space. Based on these studies, reduction in bone volume is observed in the radiated group with whole-body low dose radiation or as a combination with microgravity [85,86]. Both with radiation alone and with microgravity, the bone loss was attributed to increases in bone resorption by osteoclasts [86,87]. Mechanisms of cosmic radiation damage that will affect long term space exploration are not well recapitulated in these mouse models and an array of unanticipated side effects may be generated by the accumulation of total radiation dose with time and prolonged stay without gravity.

2.3. Nuclear fallout

Nuclear fallout is a term used when radioactive particles are released in the environment, exposing the normal population to an array of radiation-related side effects. The first exposure of nuclear fallout was observed after the atomic bombing at Hiroshima and Nagasaki during the World War II. Immediate effects from the bombs were catastrophic, with any life form in the close vicinity of the bombs evaporating; any person within 3 km sustaining severe burns and the entire area being subjected to massive amounts of radioactivity. Apart from the 110,000 instant deaths, there were severe side effects observed for many years leading to a death-toll of 200,000 and attributable to the fallout following the bombing. While the capacity of these bombs then was massive, the cold war era saw bombs that exceeded the capacity by 1000–3000-fold.

Development and testing of nuclear weapons and damage to nuclear power plants (such as that occurring in Chernobyl and Fukushima) are major reasons for current day fallouts. Ample evidence is available to assess tissue damage following fallout, including that due to radioactivity as a pollutant through air, water and food. It is well accepted that apart from the naturally occurring forms of radioactive elements, the material from fallout has started to enter the food chain [88]. Bone particularly was shown to be affected indirectly by the radioactive

absorption of strontium (Sr90) [89–92]. Sr90 of all the 100 or so radioactive elements reported in fallouts is one of the most harmful to human cells. Radioactive Uranium (236)U and Pulonium (239)Pu originating from weapons testing were detected in European roe deer antlers and in human subjects, suggesting long-term incorporation of these elements in the skeleton [93,94]. In terms of time between the nuclear testing and the actual fallout, there was a range from several months to years following the initial date of testing [95].

With the rise of civil nuclear power plants in several countries, the chances of nuclear fallouts have increased exponentially, making it imperative to understand the radiation dosages and consequences of long-term exposures in healthy tissues, including bone.

3. Alterations in the skeleton as a response to radiation effects

3.1. Radiation induced DNA damage, apoptosis of bone cells and reduced bone formation

IR causes oxidative damage of cells and tissues by generating free radicals. These free radicals in turn affect lipids, proteins, DNA and other cellular constituents. Both low and high doses of IR have been shown to cause substantial oxidative damage, DNA damage, chromosome aberrations, genomic instability [96] and telomere modifications [97]. These molecular changes lead to cell death, mostly due to apoptosis and to a lesser extent by necrosis. Cells that survive the initial onslaught of these stress triggers have an altered phenotype. Accumulation of these altered cells with any IR-induced cellular changes may result in either malignancies or cellular senescence [98,99]. Double strand breaks (DSBs) are the most deleterious kind of DNA damage mostly identified by the phosphorylation of the histone variant, H2A and termed γ H2AX. DSBs start to accumulate within hours of IR exposure and generally this initial wave wanes away; however, in vivo DSBs have been reported even after several weeks. Accumulation of γ H2AX is found in irradiated bone marrow cells, osteoblasts and osteocytes in vitro [49] and in vivo [48,100].

IR sensitizes an array of bone cells to apoptosis, and while this is considered an early event, the free radicals generated by the initial dose causes a repetitive sequence of DNA damage and apoptosis. IR-induced DNA damage and apoptosis of osteoblasts and osteocytes in rodent models was observed 2 and 4 weeks post-radiation [48,49,100]. IR-induced osteoblast apoptosis in rodent models accounts [101–103] for 10–20% of the reduced bone formation rate (Table 1) as seen by double labeling experiments or by reduced serum osteocalcin [48,100,104]. Osteocytes on the other hand are more resilient to IR, but still

osteocytic canalicular shortening, DNA damage, apoptosis and empty lacunae (a sign of osteocyte cell death) (Fig. 1) are all observed post-RT [48,49,105].

3.2. Osteoclast function and bone resorption

Depending on the dose of IR and method, osteoclast activity has been shown to vary. While low dose whole-body IR (as seen with space radiation) increases osteoclast activity and bone resorption [87,103,106], high dose focal IR (as seen with RT) reduces the osteoclast number in a time dependent manner [48,100,104,107]. These studies indicate that the effect of IR-induced changes in osteoclast function have multiple variables. Adding to this variability are the differences in IR-induced osteoclast activities between trabecular and cortical bone. Best described by a study done using focal RT, it was shown that while the trabecular bone had reduced bone resorption and formation, the cortical bone was unaffected in terms of bone formation. Hence, uncoupling from physiological bone remodeling was seen with minimal osteoclast-based resorption, resulting in cortical thickening post RT [107]. Therapeutic intervention to block resorption post-IR may work under low-dose conditions, but since the osteoclast depletion post-RT takes place rather quickly, the use of any anti-resorptive agent at a later stage fails the purpose of preserving bone architecture.

3.3. Radiation and bone marrow adiposity

Bone marrow mesenchymal stem cells (MSCs) are progenitors that are considered multipotent in nature, with an ability to form fibroblasts, osteogenic cells, chondrocytes, myocytes and adipocytes. Bone marrow adiposity and bone formation are inversely proportional [108] with the balance between the two being maintained by the MSC pool. Several reports have confirmed that age, disease and IR shift MSC fate toward adipogenesis as opposed to osteogenesis, a characteristic seen with bone aging as well (Table 1, Fig. 1). Bone marrow is generally composed of “red marrow” which is comprised of hematopoietic cells, and “yellow marrow” which is comprised predominantly of fat cells. IR causes yellowing of the marrow, which is an indicator of increased adipocytes and is one of the clinical consequences of RT [109,110] that correlates well with loss of bone architecture easily detected by magnetic resonance imaging [111,112].

Both age-related osteoporosis and IR-related bone loss are thus characterized by a relative increase in marrow fat [113,114]. In rodents both low and high doses of radiation transform the bone environment such that the MSCs undergo lineage switching to preferentially form

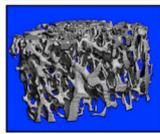
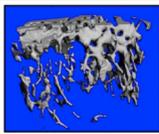
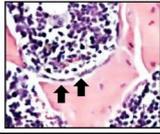
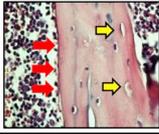
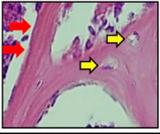
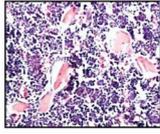
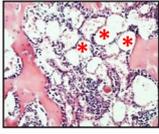
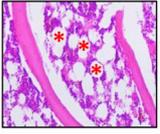
| Parameters | Non-irradiated (4m old) | Radiated (4m old) | Non-irradiated (24m old) |
|------------------------------|---|---|--|
| Trabecular bone architecture |  |  |  |
| Osteoblast and Osteocytes |  |  |  |
| Bone marrow adiposity |  |  |  |

Fig. 1. Comparison of radiation- and aging-induced bone damage.

Skeletal architecture deteriorates due to natural events such as aging or by external stressors such as radiation, as in clinical radiotherapy for the treatment of malignant or solid tumors. Some consequential events during radiation exposure or with aging that affect bone architecture include reduction in osteoblast-mediated bone formation (red arrows, loss of functional osteoblasts; black arrows: osteoblasts on bone surface of young or non-irradiated animals), reduction in functional osteocytes due to cell death (yellow arrows: empty lacunae post-osteocyte apoptosis) and increase in bone marrow adiposity (red asterisks). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

adipocytes [48,100,104,115,116]. While in vitro IR causes growth arrest in MSCs, in vivo they preferentially differentiate into adipocytes [48]. CCAAT/enhancer binding protein (C/EBP) and peroxisome proliferator-activated receptor (PPAR) families of transcription factors regulate adipocyte differentiation. Focal IR has been shown to increase PPAR- γ expression with direct correlation to adipocyte number and inverse correlation to bone volume [48]. Interestingly, PPAR- γ has been shown to cause cell senescence by inducing *p16^{INK4a}* expression [117]. Therefore, the increase in adiposity post-IR may be due to DNA damage directly in cells capable of undergoing adipogenesis or secondarily to cell senescence induced by DNA damage.

3.4. Radiation effects on hematopoietic cells and vascularity

Bone marrow hematopoietic stem cells (HSCs) and cells originating from them are sensitive to IR and exposure to IR can lead to severe bone marrow damage and leukemia. TBI is deleterious to bone marrow HSCs and blood vessels in an acute manner, where IR-induced damage of HSCs and swelling of blood vessels increase at 2 weeks post-TBI in animal models, but return to normal levels and morphology respectively after several weeks [100,118].

Endothelial cells undergo similar changes as HSCs, MSCs and mature bone cells. Damage to the smaller vessels occurs with acute IR [119] and with chronic IR to the larger vessels [120,121]. Bone marrow sinusoidal endothelial cells regain their original shape following a lethal dose of irradiation [122], suggesting that the IR-induced vascular damage is mostly an acute event. Species variability could be a factor, since in human subjects radiation-induced avascular necrosis is a known clinical entity, especially in the case of RT.

4. Contribution of cell senescence to radiation-induced bone damage

4.1. Cell senescence

While cellular senescence plays an important role in regulating uncontrolled growth of cells, the accumulation of senescent cells are likely responsible, at least in part, for a multitude of age- and disease-related co-morbidities including those associated with skeletal dysfunction. DNA damage and the response to DNA damage, as evident by the presence of DNA damage response checkpoint kinases ATM and CHK2, are considered important for the generation of senescence and the SASP [123]. Various aspects of cell senescence in age-related bone loss have been discussed in this issue by Farr et al.

4.2. Radiation-induced cell senescence

Radiation generates deleterious signals which can lead to cellular senescence. Radiation-induced oxidative stress, generation of free radicals, formation of DNA-DSBs and telomere dysfunction are all triggers for senescence. Telomerase deficient mice (*mTR*^{-/-}) with 40% shorter telomeres than wild type animals are exquisitely sensitive to gamma irradiation and telomere dysfunction is thought to be the major factor in causing this sensitivity [124]. Using SA- β -gal as a marker for senescence, several in vitro studies have shown that IR induces senescence in a time and dose dependent manner [125], which correlates with reduced cell proliferation as measured by Ki67, 5-Bromo-2'-deoxyuridine (BrdU) [126–128], or colony forming assays [48,129]. While both CDKN2A (p16) and CDKN1A (p21) have been shown to increase post-IR in cell cultures [130], CDKN1A seems to be the primary pathway to senescence post-IR in vivo [131,132]. Different tissues may preferentially express either p16 or p21 depending on the nature of IR-induced stress. A microarray study evaluating differentially expressed genes in patients who received TBI showed consistent expression of CDKN1A in white blood cells of all patients, but the study did not report CDKN2A in their dataset [133]. Among IR-induced genes in blood cells

following either TBI or an internal IR source, CDKN1A was reported by several groups irrespective of the source of IR [134], which included x-ray, ¹³⁷Cs γ -ray and ⁹⁰Sr.

4.3. Evidence of senescence in irradiated bone

The idea that senescence is responsible for osteoporosis is not a new one [135,136], but direct evidence was lacking until recently. Based on studies performed in physiologically and prematurely aged animals, it was shown that senescent myeloid cells, osteoblasts, osteoprogenitors, osteocytes, and MSCs exist and that a subset of bone cells express a SASP which may account for key aspects of the pathophysiology of osteoporosis [137–140].

Most of the studies showing senescence in MSCs and HSCs are in vitro studies, with the exception of the ones which report marrow HSC senescence in vivo using whole body irradiation [134,141–143]. DNA damage, an inducer of senescence, has been implicated as a cause of bone deterioration in whole limb radiation with low-dose IR and post-focal RT [48,100]; however, bone damage caused due to either model was not linked directly to cellular senescence as the major mechanism. Both conventional RT, which delivers high radiation doses to bone, as well as the newer, improved delivery versions that reduce side effects of RT, still cause osteoporosis.

4.4. Fracture healing and senescence following radiotherapy

Insufficiency fractures during aging and post-RT are quite evident; however the role of senescence in fracture healing in either of these conditions is largely unknown. Fractures that result as a consequence of age are slow to heal, as are those that occur post-RT [144,145]. While wound healing is promoted by senescence [146,147] and the SASP may reduce wound-related fibrosis [146,148], aging itself seems to delay wound healing [149]. The transient presence of senescent cells at the tissue repair site seems to be beneficial, while the chronic presence of senescent cells during aging likely has detrimental consequences. It is possible that RT generates senescent cells and the SASP in a chronic manner similar to aging, hence propagating the delay in fracture healing seen post-RT.

5. Targeting senescence as a therapeutic approach to prevent radiation induced bone damage

Accumulation of senescent cells and the SASP causes deleterious changes in tissue remodeling and leads to tissue dysfunction. A hallmark of age-related osteoporosis appears to be the chronic accumulation of senescent cells [137]. Thus clearance of senescent cells by senolytic drugs and reducing the SASP profile by senomorphic drugs may be viable approaches which can potentially improve bone integrity and function.

Evidence for the role of senescence and the SASP in causing age-related osteoporosis is based on the presence of senescent cells in osteoporotic bone [137], and the use of a senolytic cocktail (dasatinib, a tyrosine kinase inhibitor and quercetin, a plant based flavonol) or SASP inhibitor (Ruxolitinib, an inhibitor of the Janus kinase pathway) [150], that improved bone accrual in aged animals. Similar results were obtained by the genetic clearance of *p16^{INK4a}* cells, with the use of AP20187, a synthetic drug that initiates the dimerization of the membrane bound myristoylated FK506-binding-protein-caspase 8 fusion protein in the INK-ATTAC mouse model, where the transgene encodes for an inducible caspase 8 expressed solely in senescent cells [23].

ABT263 (Navitoclax, an inhibitor of the anti-apoptotic proteins BCL-2 and BCL-xL), another senolytic, was able to effectively deplete senescent bone marrow HSCs and senescent muscle stem cells in aged animals and also was able to mitigate TBI-induced pre-mature aging [143]. However, TBI is not mechanistically similar to RT in its effects on bone, and as discussed above, TBI increases bone resorption while RT

induces bone loss even though osteoclasts are substantially depleted. Based on its senolytic effects, ABT263 could still be a potential treatment for RT-induced osteoporosis and fracture non-unions. Similar inhibitors of BCL-2 and BCL-xL, Fisetin, A1331852 and A1155463 were also shown to work as senolytic drugs in vitro [151], but the in vivo efficacy of these compounds of treating RT-induced bone damage remains to be determined.

HSP90 inhibitors were identified as radio-sensitizers for cancer cells [152–155]. Recently, some of the same HSP90 inhibitors were shown to work as senolytic drugs [156,157]. 17-Dimethylamino-ethylamino-17-demethoxygeldanamycin (17-DMAG), a derivative of the HSP90 inhibitor geldanamycin, is a radio-sensitizer for cancer cells, but it also protects normal tissue from radiation damage [158]. Moreover, 17-DMAG has been shown to work as one of the rare senolytic compounds which can inhibit malignant tumors [159,160] but also promote vessel regeneration [161]. This makes 17-DMAG a potential compound for the treatment of fractures due to RT, since these fractures are known to have necrosis secondary to compromised vascularization.

The general approach of clearing senescent cells so as to protect against radiation-induced bone damage has not to date been evaluated in pre-clinical models. However, we now have potential candidate drugs that could ameliorate the skeletal deterioration caused by the effects of radiation on bone.

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