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Osteocyte Cellular Senescence

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Abstract

Purpose of review.—Senescent cells are now known to accumulate in multiple tissues with aging and through their inflammation (the senescence-associated secretory phenotype, SASP), contribute to aging and chronic diseases. Here we review the roles of senescent osteocytes in the context of bone loss.

Recent findings.—Numerous studies have established that senescent osteocytes accumulate in the bone microenvironment with aging in mice and in humans. Moreover, at least in mice, elimination of senescent cells results in attenuation of age-related bone loss. Osteocyte senescence also occurs in response to other cellular stressors, including radiotherapy, chemotherapy, and metabolic dysfunction where it appears to mediate skeletal deterioration.

Summary.—Osteocyte senescence is linked to bone loss associated with aging and other conditions. Senescent osteocytes are potential therapeutic targets to alleviate skeletal dysfunction. Additional studies better defining the underlying mechanisms as well as translating these exciting findings from mouse models to humans are needed.

Keywords

Osteocyte; Senescence; Aging; Radiotherapy; Chemotherapy; Type 2 Diabetes Mellitus

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Joshua Farr, Japneet Kaur, Madison Doolittle and Sundeep Khosla declare no conflict of interest.

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Introduction

Cellular senescence is a cell fate, like differentiation, proliferation, or apoptosis, that involves essentially irreversible proliferative arrest, tumor suppressor activation, altered chromatin organization, apoptosis resistance, and frequently is associated with increased protein synthesis [1]. The phenomenon was discovered in the early 1960s [2] by Leonard Hayflick who found that with prolonged serial culture, normal human embryonic fibroblasts eventually lost their capacity to divide, yet they remained viable. This growth arrest phase was coined "senescence" [2]. It is now well established that various types of stress converge to cause a cell to enter the cellular senescence program. Examples of senescence-inducing stressors include internal and external cell damaging insults such as DNA breaks, oncogenic stimuli, reactive metabolites and oxygen species, proteotoxic stress, and inflammation [1].

The pathways that drive a cell into senescence are largely centered on the cyclin-dependent kinase inhibitors, most notably $p16^{Ink4a}$ and $p21^{Cip1}$ (p53 is upstream of $p21^{Cip1}$), that in response to accumulating cell damage are upregulated as a protective mechanism to halt cell proliferation and prevent malignant transformation [1]. The resistance of senescent cells to apoptosis is acquired via activation of several senescent cell anti-apoptotic pathways (or SCAPs) [3]. Among the most common morphological features present with the proliferative arrest and apoptosis resistance of senescent cells, are the higher-order unfolding of satellite heterochromatin (termed senescence-associated distension of satellites [SADS] [4]) and the acquisition of DNA damage at sites of telomeres – *i.e.*, telomere-associated foci [5].

Despite their growth arrest, senescent cells are highly metabolically active and can acquire a distinctive pro-inflammatory secretome, termed the senescence-associated secretory phenotype (SASP) [6]. The SASP varies by senescent cell type and based on the senescence-inducing stressor, but is typically comprised of cytokines, chemokines, matrix-degrading metalloproteinases (MMPs), and growth factors that can transmit senescence to neighboring, previously healthy cells via a senescence-induced bystander effect [7]. Thus, by spreading local and systemic inflammation over prolonged periods, the SASP can drive stem cell dysfunction, aberrant remodeling, and tissue deterioration [1].

It is now evident that senescent cells accumulate *in vivo* with aging and at pathological sites of multiple chronic diseases [1]. Likewise, the biological relevance and consequences of senescent cells in aging, cancer, and various etiological conditions are becoming increasingly apparent [1]. This conceptual framework has predominantly stemmed from the discovery of genetic and pharmacological approaches to eliminate senescent cells *in vivo.* For example, the development of a novel transgene, termed *INK-ATTAC* [8] – *i.e.*, $p16^{Ink4a}$-linked apoptosis through targeted activation of caspase, has enabled the elimination of $p16^{Ink4a}$-positive senescent cells in mice. More recently, the identification of drugs, termed 'senolytics' [9], that target SCAPs to specifically kill senescent cells has led to a better understanding of the causal roles of senescent cells and has opened new opportunities to therapeutically target senescent cells to prevent or alleviate numerous comorbidities as a group, instead of one at a time [10], to thereby extend healthspan (*i.e.*, the period of life free of chronic disease). In several different models of aging and chronic disease, multiple groups

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have demonstrated that genetic or pharmacological elimination of senescent cells prevents or alleviates numerous comorbidities [8, 11–36].

There is also recent mounting interest in the roles of senescent cells in bone [37–39]. Indeed, this is a rapidly emerging area as, to date, multiple studies have investigated the contributions of senescent cells to bone loss in various settings (as summarized in Table 1). The purpose of this review is to summarize the existing knowledge of skeletal cellular senescence in mice and humans with aging as well as in preclinical models of cancer therapy and type 2 diabetes mellitus (T2DM), with a focus on senescent osteocytes, given the immense biological importance of the osteocyte in bone biology (reviewed elsewhere [40, 41] and in this *Issue of Current Osteoporosis Reports*).

Identification of Senescent Osteocytes in Bone with Aging

In studies in mice, our group identified senescent cells in bone and characterized their SASP *in vivo* [42]. From young (6-month-old) and old (24-month-old) female and male mice, we developed approaches using magnetic-activated cell sorting (MACS) to rapidly isolate enriched *in vivo* populations of various cell types from the bone microenvironment [42]. From mouse bone marrow, these populations included myeloid cells (CD14+), B cells (CD19+), and T cells (CD3+) as well as osteoblast progenitors (Lin−/Lepr+) [42]. In addition, we subjected mouse bones to serial liberase digestions to release cells, and then used MACS to first deplete for hematopoietic/endothelial markers (CD31/34/45/54−), followed by a positive selection for alkaline phosphatase (AP) to enrich for osteoblasts (AP+/CD31/34/45/54−) [42]. We then demonstrated that the remaining liberase-digested bone samples (termed "osteocyte-enriched bone samples") were highly enriched for several key osteocyte markers (*i.e.*, *Dmp1, Phex, Mepe, Sost, Fgf23*) [42], consistent with methods developed by the Bonewald laboratory [43]. These approaches allow for the rapid (within 2-3 hrs of sacrificing the animal) isolation of various major cell populations from the bone microenvironment, and subsequent analysis of *in vivo* gene expression signatures.

In each of the isolated cell populations, we then compared the levels of the senescence markers, $p16^{Ink4a}$ and $p21^{Cip1}$, between young and old mice. Our data established that $p16^{Ink4a}$ mRNA expression increased significantly (~5-10 fold) with aging in myeloid cells, B and T cells, osteoblast progenitors, osteoblasts, and osteocytes; results were similar in female and male mice [42]. By contrast, $p21^{Cip1}$ mRNA levels did not change with aging in the majority of the isolated populations. Interestingly, however, $p21^{Cip1}$ mRNA levels did increase significantly with aging in osteocytes from male mice [42]. Consistent with the higher expression of the senescence-related cyclin-dependent kinase inhibitors, $p16^{Ink4a}$ and $p21^{Cip1}$, we found that a subset of osteocytes in bone cortices of old mice displayed altered chromatin organization (*i.e.*, large-scale unraveling of peri-centromeric satellite heterochromatin or SADS), which is a consistent characteristic of senescent cells [4]. Using a threshold of 4 SADS per cell to define cellular senescence, we detected a significantly increased number of senescent osteocytes in bone cortices of old (~11% senescent) as compared to young (~2% senescent) mice [42]. To further confirm osteocyte senescence with aging, we isolated primary osteocytes from bones of young and old mice (using techniques developed by the Bonewald laboratory [44]) and measured DNA damage

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(53BP1) co-localized with telomeres (telomere-induced foci, TIF), another established biomarker of cellular senescence related to impaired DNA damage repair mechanisms [45]. With aging, our data demonstrated that the number of TIF+ osteocytes increased 6-fold, thus providing further evidence for an accumulation of senescent osteocytes in old bone [42]. The age-associated increase in the proportion of senescent osteocytes we observed *in vivo* was consistent with results from another group [46], which also found increased senescence markers (*e.g.*, *p16^{Ink4a}* mRNA expression and DNA damage) in osteocyte-enriched bone samples of old relative to young mice. In addition, Piemontese et al. [46] reported a potential connection in old mice between osteocyte senescence, increased production of RANKL, and endocortical resorption leading to thinning of the cortex and increased cortical porosity with aging. Taken together, these findings establish that at least a subset of osteocytes become senescent with old age in mice.

In our young (6-month) versus old (24-month) mouse study [42], we next aimed to examine the *in vivo* age-related changes in the SASP produced by various cells within the bone microenvironment by measuring the mRNA expression levels of a panel of 36 previously identified SASP factors [6]. Using Gene Set Enrichment Analysis (GSEA), we found relatively few SASP changes with aging in osteoblast progenitors, osteoblasts, B cells, and T cells [42]. By contrast, there was a profound age-associated SASP signature identified in both myeloid cells and in osteocytes as 23-26 of the 36 queried SASP factors significantly increased with aging in these cell populations [42]. Thus, although only a relatively small proportion of cells within bone marrow and bone itself become senescent with aging (*e.g.*, ~11% of osteocytes), it appears that these cell populations, particularly senescent myeloid cells and osteocytes, are likely capable of contributing to the development of a pro-inflammatory local bone microenvironment that contributes to skeletal dysfunction, at least in mice.

To establish whether our findings of increased cellular senescence in bone with aging in mice extended to humans, we isolated small needle bone biopsies from the posterior iliac crest of younger (27±3 years) versus older postmenopausal (78±5 years) women and examined senescence markers and SASP factors by rt-qPCR [42]. The bone biopsies contained bone marrow elements as well as both trabecular and cortical bone comprised predominantly of osteocytes. Despite their heterogeneity, consistent with our data in mice, bone biopsies from older women had significantly higher mRNA expression levels of the senescence markers, *p16^{INK4a}* and *P21^{Cip1}*, as well as several SASP factors (12 of the 36 queried) in comparison to biopsies from younger women [42]. These data thus suggest parallel findings between mice and humans, and prompted us to next ask the question of whether eliminating senescent cells prevents age-related bone loss. To address this question, we utilized both genetic and pharmacological approaches to eliminate senescent cells.

Eliminating Senescent Cells Using Genetic Versus Pharmacological Approaches

As noted earlier, global clearance of senescent cells can be achieved using *INK-ATTAC* [8] as this mouse model contains a “suicide” transgene driven by the p16^{Ink4a} promoter that

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permits the inducible elimination of senescent cells by activating an apoptosis cascade specifically in p16^{Ink4a}-positive cells resulting in their ablation in response to administration of a synthetic drug (AP20187). Several studies in models of aging and various chronic diseases have demonstrated that clearing senescent cells using *INK-ATTAC* prevents or delays multiple major chronic diseases of aging, including cardiovascular disease, frailty, hepatic steatosis, pulmonary fibrotic disease, metabolic dysfunction, neurodegenerative diseases, renal disease, osteoarthritis, and osteoporosis [8, 11–25]. Alternatively, systemic senescent cell clearance can be achieved pharmacologically by periodically administering senolytics that, as noted earlier, target SCAPs to specifically kill senescent cells [3]. For example, the combination of dasatinib (D; a tyrosine kinase inhibitor) plus quercetin (Q; a natural flavonoid present in many fruits and vegetables) (D+Q) was identified as among the first generation senolytics with established *in vivo* efficacy [26]. The combination D+Q was more senolytic than either alone and remained effective in eliminating senescent cells when delivered intermittently [26], thereby avoiding potential off-target, non-senolytic actions as these compounds have relatively short *in vivo* half-lives (<12 hrs). Consistent with the genetic *INK-ATTAC* approach, intermittent senolytic therapy has been shown to improve several aspects of healthspan in preclinical models of aging and chronic disease [26–36].

Effects of Senescent Cell Clearance on Age-Related Bone Loss

Based on the collective data demonstrating that senescent cells are present at the time and location of osteoporosis with aging in humans [42] as in mice [42, 46], we hypothesized that cellular senescence, including the age-associated accumulation of senescent osteocytes, has a causal role in mediating the pathogenesis of osteoporosis. In order to test this hypothesis, we utilized both genetic (*INK-ATTAC*) and pharmacological (senolytics D+Q) approaches to target senescent cells [20]. These interventional studies were initiated in mice at 20-months of age as our analysis of changes in *p16^{Ink4a}* throughout the murine lifespan indicated that expression of this senescence marker in osteocyte-enriched bone samples begins to increase significantly in both males and females around 18 months of age [20], which coincides with the timing of age-related bone loss in both sexes. Over the course of four months, old *INK-ATTAC* mice were treated twice weekly with AP20187, whereas old wild-type mice were treated once monthly with D+Q. Both approaches resulted in at least partial elimination of senescent osteocytes and improved bone mass, microarchitecture, and strength by suppressing bone resorption (reducing osteoclast numbers) and either maintaining (trabecular skeletal sites) or increasing (cortical skeletal sites) osteoblast numbers as well as indices of bone formation [20]. These data thus implicate cellular senescence at the nexus of skeletal aging. Furthermore, the absence of any effects of these interventions on the skeleton in young mice [20] points to their specificity in eliminating senescent cells that accumulate with aging, and the intermittent senolytic dosing strategy employed, along with the short *in vivo* half-lives of D+Q (<12 hrs), reduces the likelihood of potential off-target effects and undesired side effects on non-senescent cells. These findings provide “proof-of-concept” evidence in mice for selectively eliminating senescent cells, including senescent osteocytes, to potentially alleviate age-related bone loss. It is noteworthy, however, that clearance of senescent osteoclast progenitors was not enough to prevent bone loss in old mice [47], which further implicates senescent osteocytes as perhaps

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the key drivers of age-related bone loss. In addition to age-related osteoporosis, recent emerging data suggest that targeting cellular senescence in mice also has beneficial skeletal effects in the settings of radiotherapy- and chemotherapy-driven bone loss [35, 36], thus expanding the potential applications of this strategy to treat other conditions of skeletal dysfunction.

Radiotherapy-Induced Bone Loss

In addition to old age, other skeletal conditions have been identified in which senescent cells accumulate in bone and thus patients with such conditions may benefit from senolytic therapy. For example, mounting evidence has linked radiation therapy in cancer patients to chronic bone loss and cell senescence [48–50]. Radiation induces senescence by causing cell damage, including DNA breaks and chromatin disruption [49]. While radiation therapy is effective in slowing cancer, it consequently becomes detrimental to bone, as senescence and skeletal dysfunction ensue. Indeed, Chandra and colleagues [35] recently showed in young adult mice that senescent osteoblasts and osteocytes (characterized by increased senescence-associated β-galactosidase [SA-β-Gal] staining and TIFs) accumulate prematurely in the setting of focal radiation therapy (FRT)-induced bone loss. Consistent with another study [36], whole-bone mRNA analysis revealed that radiation caused increased expression of senescence ($p16^{Ink4a}$ and $p21^{Cip1}$) as well as several SASP genes [35]. Following FRT, mice treated with the senolytic cocktail, D+Q, had reduced TIF+ osteocytes, preserved bone formation, and attenuated bone loss as compared to vehicle-treated mice [35]. These results are consistent with previous findings in old mice [20] demonstrating that senolytic therapy eliminates senescent osteocytes and has beneficial effects on bone turnover.

Chemotherapy-Induced Bone Loss

Alongside radiation, chemotherapy is another strategy to induce growth arrest of cancer cells. However, activated cellular senescence is a common byproduct, as observed with numerous chemotherapeutic drugs, including bleomycin [51], cisplatin [52] and hydroxyurea [53], among others. Recently, Yao and colleagues [36] hypothesized that bone loss resulting from chemotherapy may be a consequence of increased skeletal senescence. Similar to radiation therapy, mouse long-bone samples enriched for osteocytes (flushed by centrifugation) from young mice treated with the chemotherapeutic, doxorubicin (DOXO), displayed upregulated $p16^{Ink4a}$ mRNA expression relative to osteocyte-enriched bones from vehicle-treated mice [36]. In addition, bone marrow cells depleted of hematopoietic/endothelial marker also exhibited robust elevated mRNA levels of $p16^{Ink4a}$ and $p21^{Cip1}$, as well as increased SASP (*e.g.*, *IL6*) and reduced levels of the apoptotic marker, *Hmgb1* [36]. Following DOXO-therapy, treatment of *INK-ATTAC* mice with AP20187 was sufficient to rescue chemotherapy-induced bone loss [36]. At the cellular level, DOXO-treated mice exhibited increased osteoclast number and reduced bone formation rates; these phenotypes were normalized after clearance of senescent cells with AP20187 [36]. These results that are thus consistent with findings in old *INK-ATTAC* mice treated with AP20187 [20]. Therefore, cellular senescence may represent a therapeutic target to preserve bone in patients with cancer following chemotherapy [36].

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Accelerated Osteocyte Senescence and Skeletal Fragility in Type 2 Diabetes Mellitus (T2DM)

Patients with T2DM are at higher risk for fractures despite normal or higher bone mineral density (BMD) [54, 55]; thus, fracture risk in these individuals may stem from poor bone quality [56]. We hypothesized that cellular senescence may represent a mechanistic link between skeletal fragility and T2DM [57] given that: i) bone cell senescence increases in old age [42, 46]; ii) eliminating senescent cells prevents skeletal fragility with aging [20]; and iii) emerging data showing that obesity prior to aging causes the premature accumulation of senescent cells at etiological sites including adipose tissue [24], liver [18], brain [22], and pancreatic β cells [25], contributing to metabolic dysfunction [58].

To examine whether T2DM causes accelerated osteocyte senescence and skeletal dysfunction [57], we recently leveraged a non-genetic wild-type mouse model that closely mimics human T2DM – *i.e.*, the high-fat diet (HFD)/streptozotocin (STZ) mouse of T2DM [59–61] that develops overt hyperglycemia, dysfunctional insulin secretion, insulin resistance, and β-cell deterioration. Additional advantages of this T2DM model of human metabolic dysfunction include both environmental (in obesity) and temporal (after skeletal maturity) control of T2DM onset [59–61]. After 3-4 months of metabolic dysfunction in young adulthood, we found that HFD/STZ (*i.e.*, T2DM) mice develop poor bone quality, similar to that which manifests in humans [62, 63], including significantly deteriorated trabecular and cortical bone microarchitecture, impaired bone material properties, and diminished biomechanical strength [57]. Mice with T2DM also displayed dysfunctional bone remodeling, including higher bone resorption and defective bone formation, based on bone histomorphometric analyses and circulating bone turnover markers [57]. Using mass spectrometry, we also found that T2DM mice had increased skeletal and circulating levels of the advanced glycation endproduct (AGE), N^{ε}-(1-carboxymethyl)-L-Lysine (CML), an established activator of the receptor for AGE (RAGE) pathway [64]. Finally, we demonstrated that T2DM led to the accelerated accumulation of senescent osteocytes, characterized by increased expression of the cyclin-dependent kinase inhibitors, $p16^{Ink4a}$ and $p21^{Cip1}$, in osteocyte-enriched bone samples, a greater proportion of SADS+ [4] osteocytes, and significantly more telomere-associated foci – TAF+ [5] osteocytes as compared to age-matched mice on low-fat control chow [57]. Interestingly, senescent osteocytes in mice with T2DM developed a unique pro-inflammatory SASP signature comprised predominantly of significantly upregulated levels of MMPs (*i.e.*, *Mmp3*, *Mmp9*, *Mmp12*, and *Mmp13*) and *Nfkb1* (NF-κB), a downstream target of the RAGE signaling pathway activated by CML [64]. Collectively, these observations point to the RAGE pathway and senescent cells as potential therapeutic targets to alleviate diabetic skeletal fragility [57]. However, whether there is a direct causal relationship between cellular senescence, RAGE signaling, and skeletal fragility in T2DM has not been established. This hypothesis needs to be tested.

Evidence for Cellular Senescence of Post-Mitotic Cells

In addition to senescent osteocytes, multiple other post-mitotic, terminally differentiated, tissue-resident cells develop senescent-like features, including SASP acquisition, and have been shown to mediate deleterious effects on neighboring cells in their respective tissues [65]. As early as the 1950's, lipofuscin – the "age pigment" – was reported to accumulate in

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long-lived post-mitotic cells such as neurons [66], cardiomyocytes [67], and osteocytes [68]. While most studies examining cellular senescence have focused on the growth arrest of proliferative-competent cells, further investigation of long-lived post-mitotic cells has led to exciting recent insights. For example, senescent neurons have been a focus in the context of major age-related cognitive diseases [21, 22, 34]. Interestingly, up to 80% of Purkinje and 40% of cortical, hippocampal, and peripheral post-mitotic neurons in the myenteric plexus of aged mice show markers of senescence [69]. Cardiomyocytes, another major post-mitotic cell type, have also been shown to take on a profound senescence phenotype with advancing age in both mice and humans [23]. Clearance of senescent cells in aged mice using both genetic and pharmacological approaches reduced cardiomyocyte senescence and alleviated cardiac hypertrophy and fibrosis [23]. Findings of senescence-like features in additional post-mitotic cells, including adipocytes [70] and hepatocytes [18], have led to interest in how these cells become senescent in the setting of little to no active replication to thereby induce DNA damage and telomere shortening. Although still unclear, it has been proposed that senescence of post-mitotic cells arises at least in part from mitochondrial dysfunction [23], therefore acting independent of cell division. Additional underling mechanisms are likely shared among post-mitotic senescent cells, including senescent osteocytes, a better understanding of which could provide novel insights into the pathophysiology of numerous conditions, including osteoporosis.

Senolytics in Human Clinical Trials

Senolytics are currently in clinical trials, and results from the first studies were recently reported. For example, the first-in-human pilot clinical trial of D+Q provided initial evidence that senolytic therapy may improve aspects of physical function as nine doses of D+Q over three weeks led to better walking distance and speed as well as ability to rise from a chair by five days after the final dose in 14 human subjects with idiopathic pulmonary fibrosis (IPF), a debilitating fibrotic disease associated with senescent cell accumulation in the lungs [71]. More recently, early interim findings from a phase 1, open-label, study of a 3-day oral course of D+Q in nine subjects with diabetic kidney disease showed that senolytics reduced the burden of senescent cells in adipose tissue by eleven days after the final dose [72]. As an extension to the aforementioned preliminary studies, placebo-controlled, human trials are currently underway as more information about safety, side effects, tolerability, and efficacy is needed. Furthermore, additional carefully monitored trials in human subjects with other conditions, including age-related bone loss, are initiating in the near future.

Conclusions

It is now clear that in mice and in humans, senescent cells, including senescent osteocytes, accumulate in the bone microenvironment with aging and in response to other cellular stressors (*e.g.*, radiotherapy, chemotherapy, T2DM; see Fig. 1). These senescent osteocytes contribute to the pro-inflammatory SASP in the bone microenvironment (and perhaps systemically), leading to an increase in bone resorption and decrease in bone formation. Whether the specific elimination of senescent osteocytes is sufficient to prevent age-related bone loss remains to be seen. Nevertheless, even a partial elimination of these senescent cells or inhibition of their SASP results, at least in mice, in preserved bone mass through

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favorable effects on bone metabolism. These pre-clinical studies have now set the stage for early-stage human trials, currently underway, examining whether reducing the burden of senescent cells in humans using senolytics can reduce bone resorption/increase bone formation and thereby prevent or ameliorate age-related bone loss, with the added advantage of potentially also delaying frailty [33] and reductions in muscle mass that may also contribute to fracture risk.

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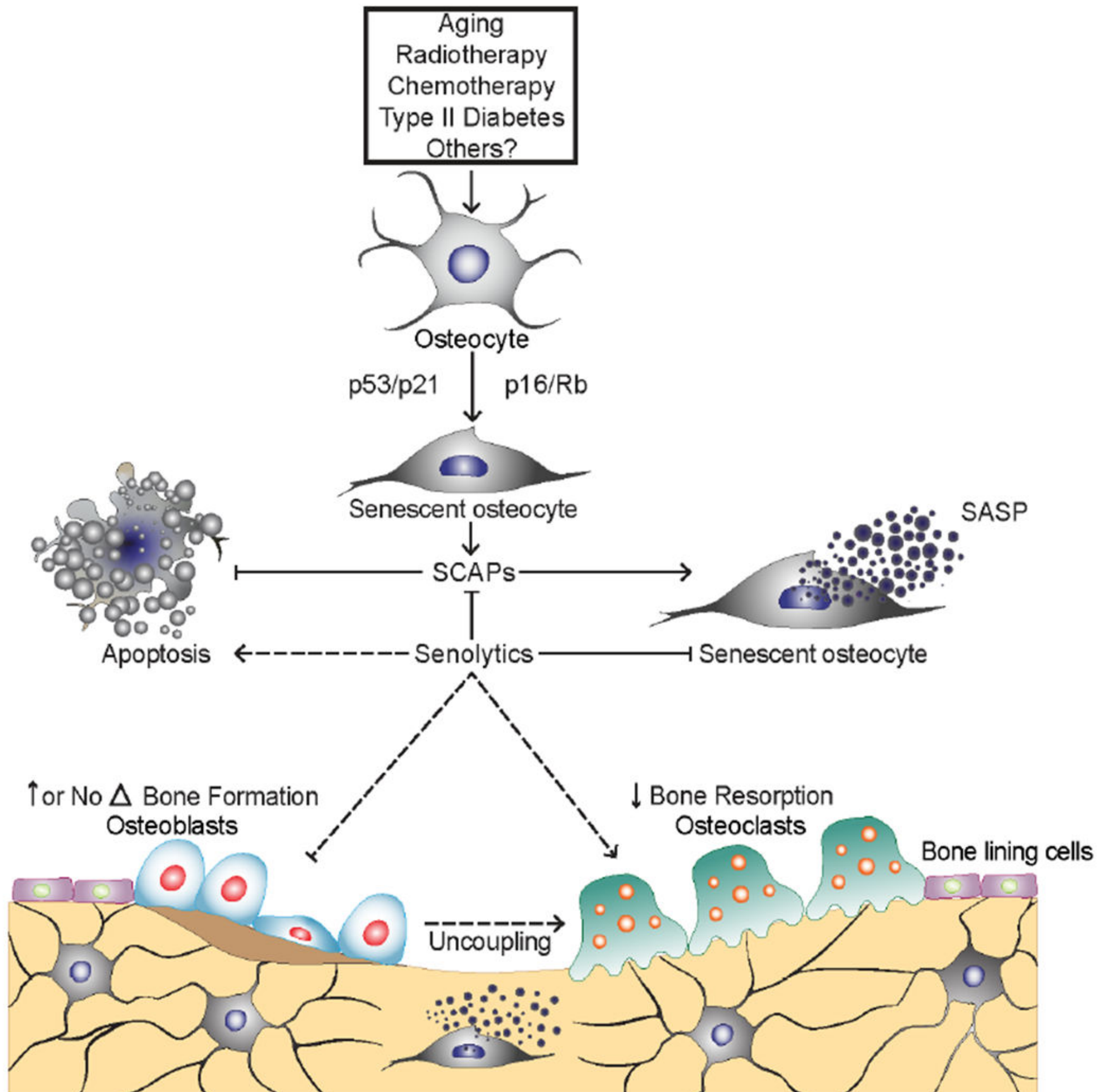

Fig. 1. Osteocyte cellular senescence in bone and effects of senolytics on bone metabolism.
Senescent cells, including senescent osteocytes, accumulate with aging, radiotherapy, chemotherapy, type 2 diabetes mellitus (T2DM) in bone, where their senescence-associated secretory phenotype (SASP) increases bone resorption by osteoclasts and reduces bone formation by osteoblasts. In old mice, senolytics reduce the burden of senescent cells, which leads to a reduction in bone resorption with either increased (cortical bone) or maintained (trabecular bone) bone formation, resulting in a beneficial 'uncoupling' between bone resorption and bone formation. Dashed lines indicate beneficial effects of senescent cells on

osteoclasts, osteoblasts, and the coupling between osteoclasts and osteoblasts. Adapted and reproduced with permission from [20], SpringerNature.

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Table 1:

Summary of senescence biomarkers in bone and effects of senolytic (genetic or pharmacological) approaches on senescence biomarkers in models of aging, type 2 diabetes mellitus (T2DM), and cancer therapy.

Reference	Animal or Human Characteristics	Senescence Biomarkers	Senolytic Approach	Senescence Biomarkers
Farr et al., 2016 [20]	C57BL/6 mice ♀♂ **Old** (24m) vs. young (6m)	↑*$p16^{Ink4a}$* (OP, OB, OCY, B, T, Myeloid) - ♀ & ♂ ↑*$p21^{Cip1}$* (OCY) - ♂ ↑*p53* (OCY, Myeloid) - ♀ ↑SASP, senescent OCY, TIF^{+} OCY	-	-
	Human bone biopsies♀ **Old** (72-87y) vs. young (23-30y)	↑*$p16^{Ink4a}$*, *$p21^{Cip1}$*, SASP	-	-
Farr et al., 2017 [42]	C57BL/6 mice ♀♂ **Old** (20m) vs. young (6-12m)	↑*$p16^{Ink4a}$* (OCY) ♀ & ♂	*INK-ATTAC*♀, 20m **AP20187** vs. VEH	↓*$p16^{Ink4a}$*, *EGFP*, ↓senescent OCY (SADS+)
			C57BL/6 ♂ 20m **D+Q** vs. VEH	↓*$p16^{Ink4a}$* ↓senescent OCY(SADS+)
Piemontese et al., 2017 [46]	C57BL/6 mice ♀♂ **Old** (20-21 m) vs. young (6-7m)	↑*$p16^{Ink4a}$* (OCY), DNA damage (γH2AX)	-	-
Chandra et al., 2020 [35]	C57BL/6 mice ♂ **R** vs. NR	↑*$p16^{Ink4a}$*, *$p21_{Cip1}$*, SASP ↑SA-β-Gal^{+} and TIF^{+} osteoblasts ↑TIF^{+} OCY	C57BL/6 ♂ 4m **D+Q** vs. VEH	↓*$p16^{Ink4a}$*, *$p21_{Cip1}$*,SASP ↓TIF^{+} OCY
Eckhardt et al., 2020 [56]	C57BL/6 mice ♂ 7m **HFD/STZ** vs. control	↑*$p16^{Ink4a}$*, *$p21_{Cip1}$*, SASP (OCY) ↑ senescent OCY (SADS+, TAF +)	-	-
Yao et al., 2020 [36]	FVB/NJ mice ♀, 6w **R** vs. NR	↑*$p16^{Ink4a}$*, SASP ↓ HMGB1	-	-
	FVB/NJ mice ↑, 6w **DOXO** vs. VEH	↑*$p16^{Ink4a}$*, SASP, SA-β-Gal^{+} ↓HMGB1	*INK-ATTAC*♀ 16w **AP20187** vs. VEH	↑$p16^{Ink4a}$, SASP ↓ HMGB1

Key: m, month; y, year; R, radiated; NR, non-radiated; HFD, high-fat diet; STZ, streptozotocin; DOXO, doxorubicin; OP, osteoprogenitor; OB, osteoblast; OCY, osteocytes; B, B-cells; T, T-cells; SASP, senescence-associated secretory phenotype; TIF, telomere dysfunction-induced foci; *INK-ATTAC*, $p16^{Ink4a}$-linked apoptosis through targeted activation of caspase; EGFP, enhance green fluorescent protein (encodes for the *INK-ATTAC* transgene); SA-β-Gal, senescence-associated β-galactosidase; D, dasatinib; Q, quercetin. The upward (↑) and downward (↓) arrows represent the results for the study group in **bold** versus the contralateral group.