COMMENTARY

SIMS

SENESCENT OSTEOCYTES

Senescent Osteocytes: Do They Cause Damage and Can They Be Targeted to Preserve the Skeleton?

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It has long been postulated that a primary reason for the prevalence of osteoporosis in aged populations is that cellular senescence reduces the ability of osteoblast progenitors to proliferate and differentiate into matrix-producing osteoblasts.(1) Recent work from Farr and colleagues(2) makes use of recently identified markers of senescence to suggest that multiple cell types within the bone microenvironment become senescent with age, including not only osteoblast progenitors, but also mature osteoblasts, B cells, T cells, and osteocytes. This leads the authors to suggest that, with aging, a subset of osteocytes become senescent and produce signals to neighboring myeloid lineage cells, which, in turn, stimulates the production and secretion of pro-inflammatory cytokines and chemokines, creating a toxic local microenvironment that may contribute to age-related bone loss. In addition, they suggest that targeted removal of senescent cells may provide

a therapeutic option for age-related osteoporosis.[2]

Replicative senescence as a concept was developed by Hayflick in the 1960s to explain why primary cultured cells lose their capacity to divide after a reproducible number of population doublings in vitro.[3] For many years, this was thought to be an artifact of cell culture, but the existence of senescence in vivo in both dividing and postmitotic cells is now widely accepted. Senescence is now thought not only to have anti-tumorigenic action, but also to contribute to tissue repair, embryonic development, and to age-related pathologies.[1] Accumulation of senescent cells, and a gradual increase in telomere dysfunction has been noted in almost all tissues and species studied to date.[1]

The suggestion that senescence may play a role in the pathogenesis of age-related osteoporosis stems from studies showing a relative decline in bone formation that occurs with age.[4] Roles for both telomere shortening and increased oxidative stress in the declining osteoblast function with age has been supported by studies in mouse models (for review, see Marie[5]). However, this age-related decline may not relate only to an intrinsic defect in the osteoblast lineage. Many cell types within the bone marrow microenvironment regulate osteoblast precursor differentiation,[6] and the signals they produce may also be altered with senescence. Osteocyte senescence may also alter osteoblast activity given that osteocytes regulate osteoblast differentiation by production of pro-osteoblastic cytokines and the Wnt inhibitor sclerostin.

Because aging is associated with a decline in bone formation[4] and a reduction in ability of osteoblast precursors to differentiate in vitro,[7,8] one logical outcome may be a reduced response of these cells to endogenous or exogenous factors that stimulate bone formation. However, this does not appear to be the case: bone formation is stimulated in postmenopausal women treated with parathyroid hormone (PTH),[9] and in both aged mice and in the SAMP/6 mouse model of age-associated bone loss, PTH response is robust.[10,11] Although Farr and colleagues[2] confirm increased senescence of osteoblast precursors with age, such senescence can be overcome by exogenous intermittent PTH treatment.

Multiple cellular pathways contribute to cellular senescence, including telomere shortening and DNA damage, increased oxidative stress, and oncogene activation.[1] Although there are multiple senescence inducers, they converge on two major effector

pathways: *p16*^{Ink4a} and *p19*^{Arf}, both encoded by *Cdkn2a/CDKN2A*).[1] Some 12 years ago *p16*^{Ink4a}, but not *p19*^{Arf}, mRNA levels were reported to be elevated in bone marrow from aged mice.[12] This was observed both in the hematopoietic lineage positive (Lin+) and lineage negative (Lin–) populations[12]; the latter would include mesenchymal osteoblast precursors. Farr and colleagues[2] provide evidence confirming that the same is true for multiple cell types within bone and bone marrow. They observed higher levels of *p16*^{Ink4a} mRNA in all cellular populations isolated from bones of old mice (24 months old) compared to adults (6 months old): osteoblasts, osteoblast progenitors, osteocytes, B cells, T cells, and myeloid cells. Such analysis depends heavily on the purity of cell populations isolated, and analysis of RNA from freshly isolated cells, particularly in the case of osteocytes, because primary osteocytes lose their phenotype when cultured.[13,14] It should be noted that although osteoblast and osteoblast progenitor populations were isolated with the use of previously published fluorescence activated cell-sorting (FACS) methods, the cells isolated as "osteocytes" in this work were not specifically purified. Instead, osteocytes were obtained from the remaining fragments of long bones and vertebrae after removal of those cells used to prepare osteoblasts. Confirmation of the presence of osteocytes was indicated by osteocyte marker mRNA, but the purity of these preparations remains questionable. Previous work has shown the value of purifying cells from bone digests using genetically altered mice with fluorescence targeted to the osteocyte with the DMP1 promoter,[15] and more recently it has been noted that even when these markers are used, additional purification by hemopoietic lineage depletion is required to eliminate contaminating osteoclasts.[14]

Although this data suggests a higher number of senescent cells in the bone microenvironment, elevated *p16*^{Ink4a} mRNA level alone is not sufficient evidence of senescence, because it is not upregulated only in this circumstance.[16] It is also not clear without further analysis whether the higher level of *p16*^{Ink4a} mRNA results from an increased rate of senescence or from accumulation of senescent cells. This is a particular concern for the assessment of osteocytes in rodent cortical bone which, unlike human cortical bone, does not remodel.[17] This, and the aforementioned problem of osteocyte purity, was at least partially addressed by the detection in osteocytes of senescence-associated distension of satellites (SADS) in tissue sections. In the cortical diaphysis of

24-month-old mice, 11% of osteocytes demonstrated SADS staining compared to approximately 2% in 6-month-old mice.[2] SADS indicates the unraveling of centromeres from their normal compact state postmitosis, and has been observed in multiple cell types as they enter senescence.[18] This confirmed the higher proportion of senescent osteocytes and suggests a greater rate of osteocyte entry into the senescent state in aged bone. Whether there are more senescent osteocytes in particular regions of the cortex (eg, areas that undergo less mechanical strain, osteocyte layers residing closer to the bone marrow microenvironment or closer to the periosteum), whether this is also observed in trabecular bone, and whether there are sex differences remain unknown.

Cellular senescence was also indicated by the detection of sites of unrepaired DNA damage, known as telomere dysfunction-induced foci (TIFs) in impure "osteocyte-enriched" preparations that were cultured for 7 days. However, as mentioned above, osteocytes, even highly purified ones, do not retain their phenotype when cultured.[13,14] It is hard to know whether this really indicates increased senescence in osteocytes, is a cell culture artifact, or indicates senescence in cells present in the impure preparation that have overgrown the culture.[19]

One outcome of cellular senescence is an increase in the expression of a range of chemokines, cytokines, and metalloproteinases. This acquisition of a proinflammatory phenotype has become known as the senescence-associated secretory phenotype (SASP), and is believed to be the major mechanism by which senescent cells alter their microenvironment.[20] Indeed, many of these factors have been noted to influence vascularization, bone formation, and to promote osteoclastogenesis and bone destruction (eg, G-CSF, VEGF-A, IL-6, MMP13, Cxcl1, and Nfkb1). Although myeloid cells showed a dramatic increase in SASP-associated proteins, only a mild increase in mRNA levels of some SASP-associated proteins was observed in the impure "osteocyte-enriched" preparations.[2] It remains an open question whether these increases accurately reflect changes in the osteocyte network, or result from the presence of contaminating cells with very dramatic changes in SASP-associated proteins. An elevation in $p16^{Ink4a}$ and SASP-associated mRNA levels in RNA extracted from human biopsies provides confirmation that such changes occur in human bone, although again the cell types most dramatically affected are not known. Is the elevation in SASP-associated protein in

myeloid cells the key contributor to age-related bone loss? Does the release of additional proteases in the bone microenvironment cleave membrane-bound receptors such as RANKL, leading to further bone destruction? How would changes in SASP-associated proteins lead to a reduction in osteoblast precursors with age? Which factors should be targeted to prevent age-related bone loss? As osteocytes undergo senescence, does their production of these agents alter the activity of osteoblasts and osteoclasts on the bone surface? Does their change in expression of these factors influence the surrounding bone matrix with age or cause perilacuno-canalicular resorption? These questions remain unresolved.

Regardless of the cellular source of SASP-associated chemokines, cytokines, and metalloproteinases, the suggestion that senescence within the bone microenvironment increases with age strengthens the evidence that cellular senescence could lead to age-related osteoporosis. Would removal of senescent cells provide a method to treat such bone loss? Could this approach prolong skeletal health? Better still, in the case of an already aging skeleton, could removal of senescent cells provide rejuvenation? A number of approaches have been tested in other organ systems to eliminate senescent cells with promising results. Experiments with genetically altered mice indicated that lifelong removal of senescent cells delayed onset of age-related pathologies of the eye, skeletal muscle, and adipose tissue, whereas deletion late in life slowed the progression of these pathologies.[21] No improvement in bone mass was detected, but this was only assessed at a cursory level.[21] A more recent study using a pharmacological approach by combining dasatinib and quercetin to reduce numbers of senescent cells resulted in a higher total bone mineral density in treated mice,[22] presumably by preserving skeletal structure. Whether this is achieved by retaining bone formation, or by inhibiting resorption is not known, nor is it clear whether the skeleton was maintained by removal of senescent cells from bone itself. Was skeletal structure maintained secondary to beneficial effects on other tissues known to regulate bone mass such as muscle or adipose? Is it possible to remove senescent osteocytes without increasing bone resorption and causing further bone loss? Alternatively, would the empty osteocyte lacunae fill with matrix, leading to bone tissue necrosis?

These results are promising and support the early hypothesis that age-related bone

loss may stem, at least in part, from cellular senescence of a range of cells within the bone microenvironment, including osteocytes. Whether the senescence of the osteocyte network is a major contributor to the bone loss and could be targeted to prevent age-related bone loss remains to be determined.

Disclosures

The author states that she has no conflicts of interest.

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