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Perspective Article

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Osteoblast progenitor fate and age-related bone loss

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Histologic studies indicate that the development of age-related bone loss is due to a decline in the number of osteoblasts present in the bone multicellular unit (BMU), compared to the demand for them[1]. The number of osteoblasts in the BMU is a function of the available supply of new osteoblasts from local progenitors and the life span of the mature cell. Based on these considerations, age-related bone loss must be due at least in part to a reduction in the production of osteoblasts from multipotential mesenchymal stem cells and/or increased osteoblast apoptosis. We have obtained evidence that aging has a deleterious effect on mesenchymal stem cell number via a reduction in self-renewal capacity; and that the production of osteoblasts from these progenitors during aging is reduced by the diversion of multipotential progenitors into the adipocytic lineage instead of the osteoblastic lineage.

Loss of early osteoblast progenitors with age

The cell populations responsible for continuous tissue regeneration belong to stem, transit amplifying, and mature compartments[2]. These cell types are distinguished by their ability to divide, differentiate, and serve as the mature, or "executive", cell of a particular tissue - matrix formation in the case of osteoblasts. Stem cells serve as the reservoir of executive cells of the tissue throughout life by virtue of their ability to divide and produce an identical stem cell (i.e. to self-renew) and/or a more differentiated cell. Transit amplifying cells are the earliest differentiated progeny of stem cells. Primitive members of this compartment can also undergo many rounds of self-renewal as they divide; however, this property is short-lived and eventually a more differentiated cell type is the more frequent outcome. Eventually, the non-dividing mature executive cell develops.

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The earliest osteoblast progenitor of adult bone marrow is designated as colony-forming unit-fibroblast (CFU-F) because of its ability to form colonies of fibroblastic cells when placed into culture. Many of these colonies contain osteoblast progenitors that differentiate to form a mineralized matrix *in vitro*, and will form bone following subcutaneous implantation into immunocompromised mice[3]. The cell that gives rise to these particular colonies is termed CFU-osteoblast (CFU-OB), which is considered a subpopulation of CFU-F. We recently showed that CFU-OB of adult murine bone marrow has high proliferative and finite self-renewal capacity[4]. Thus, they are early transit amplifying mesenchymal osteoblast progenitors, rather than quiescent stem cells. These properties make them well positioned for the minute-to-minute control of the supply of osteoblasts to the BMU. Indeed, the number of CFU-OB increases when bone remodeling and formation increases in sex steroid deficiency[5]; and decreases when bone remodeling and formation is decreased by glucocorticoid excess[6].

Osteoblast progenitors also decline in aging humans and rodents[7]. In the SAMP6 mouse model of early onset osteopenia with histologic features of age-related bone loss, CFU-OB number is reduced[8]. Preliminary studies with marrow from SAMP6 mice indicates that the decrease in CFU-OB is associated with a decrease in their self-renewal capacity (Jilka, unpublished). Interestingly, skin fibroblasts of SAMP6 mice also exhibit decreased proliferative capability *in vitro*[9]. The reduction of CFU-OB self-renewal during aging is consistent with findings of reduced self-renewal or proliferation of murine hematopoeitic progenitors as well, depending on genetic background[10].

The cause of age-related loss in stem cell self-renewal capacity may be due to telomere shortening, loss of sensitivity to, or production of, growth factors; or the cumulative effects of oxidative stress and DNA damage on cell function over the lifespan of the animal. Normally, when cells experience oxidative stress or DNA damage, the p53 protein activates cellular pathways that prevent replication or stimulate apoptosis. In this way damaged cells and cells that are potentially tumorigenic cannot develop further. Recent evidence

suggests, however, that the cost of this protective mechanism in regenerating tissues is eventual depletion of the pool of replicating stem cells[11]. Specifically, mice with so-called activating mutations in p53 exhibit increased p53-mediated tumor suppression, but they also had a reduction in lifespan that was associated with early degeneration of several organs, including the skeleton.

Diversion of mesenchymal progenitors into the adipocyte lineage

The differentiation pathway chosen by multipotential mesenchymal progenitors is another important determinant of the final number of osteoblasts that are ultimately derived from the progenitors. It has long been appreciated that with aging, marrow fat increases at the same time that osteoblast number is diminished. Thus, if bone marrow adipocytes form at the expense of osteoblasts during aging, bone loss may ensue[7].

Marrow of SAMP6 mice exhibit increased marrow fat; and they have decreased osteoblast progenitors but increased adipocyte progenitors[12]. Similar reciprocal changes were also found in 24-month C57BL/6 mice. It is well established that lineage specific transcription factors govern expression of a particular cell phenotype. In the case of osteoblasts, Runx2 and osterix play the dominant roles[13,14], whereas Peroxisome Proliferator-Activator Receptor-γ2 (PPARγ2) orchestrates the expression of the adipocyte phenotype[15]. Unlike Runx2 and osterix, PPARγ2 is a ligand-activated transcription factor. We recently reported that activation of PPARγ2 with rosiglitazone in the murine marrow-derived mesenchymal progenitor cells stimulated their differentiation to adipocytes and irreversibly blocked their ability to differentiate into osteoblasts[16]. The latter response appeared to be due to suppression of Runx2 and the synthesis of osteoblast-specific proteins.

Consistent with the *in vitro* findings, administration of rosiglitazone to mice caused bone loss[17]. Histomorphometric studies revealed a reduction in cancellous bone area that was associated with a decrease in bone formation rate and osteoblast number; and an increase in the number of marrow adipocytes. However, rosiglitazone had no effect on either the number or replicative capacity of CFU-OB. Thus, activation of PPARγ2 reduces cancellous osteoblast number by acting on the differentiation rather than the replication of uncommitted progenitors of osteoblasts and adipocytes. These findings support the possibility that the decline in osteoblast number, and the bone loss, that occurs with aging may be due in part to activation of PPARγ2, resulting in the development of adipocytes at the expense of osteoblasts.

The PPARγ2 ligand-binding site interacts with a wide variety of lipophilic compounds, including thiazolidinediones, prostaglandin J2, polyunsaturated fatty acids such as linoleic acid, and oxidation products of polyunsaturated fatty acids. Linoleic acid peroxidation occurs upon generation of oxygen free radicals, and hydroxyacids subsequently form under biologic conditions. Several lines of evidence suggest that such PPARγ2 ligands increase with advancing age. For example, levels of polyunsaturated fatty acids increase in various tissues during aging, and there is an age-related increase in oxidized low density lipoproteins that contain the PPARγ2 ligands 9-hydroxyoctadecadienoic acid (9-HODE) and 13-HODE[18-20].

We recently showed that the linoleic acid peroxidation products 9,10-epoxyoctadecenoic acid (9,10-EOA) and 9,10-dihydroxyoctadecenoic acid (9,10-DHOA) are PPARγ2 ligands; and that 9,10-DHOA is both pro-adipocytic and anti-osteoblastic[21]. Strikingly however, 9,10-EOA and the thiazolidine acetamide ligand GW0072 are anti-osteogenic without stimulating adipocyte differentiation. On the other hand, 9-HODE is pro-adipogenic without affecting osteoblast differentiation. These findings indicate that PPARγ2 stimulates multiple pathways that promote adipocyte differentiation, inhibit osteoblast differentiation, or both, depending on the nature of the activating ligand; and that fatty acid oxidation metabolites could be involved in age-related bone loss. Moreover, adipocytes themselves produce factors, possibly lipid-derived, that suppress osteoblast differentiation[22], setting up the possibility of a self-amplifying cascade in which increased adipogenesis leads to increased production of factors that both suppress osteoblastogenesis and stimulate additional adipogenesis.

Summary

Based on advances in the understanding of stem cell biology and the elucidation of transcription factors involved in lineage specification of mesenchymal progenitors, we have identified both intrinsic (reduction in self-renewal) and extrinsic (PPARγ2 ligands) mechanisms that may contribute to the age-related bone loss via a decline in osteoblast production from mesenchymal progenitors. Further studies are needed to determine the relative contribution of each mechanism, and to elucidate the role of genetic contribution to the prevention or potentiation of each mechanism.

References

1. Parfitt AM. Bone-forming cells in clinical conditions. In: Hall BK (ed) Bone Volume 1. The osteoblast and osteocyte. Telford Press and CRC Press, Boca Raton, FL; 1990:351-429.
2. Loeffler M, Potten CS. Stem cells and cellular pedigrees - a conceptual introduction. In: Potten CS (ed) Stem cells. Academic Press, San Diego, CA; 1997:1-27.
3. Kuznetsov SA, Krebsbach PH, Satomura K, Kerr J, Riminucci M, Benayahu D, Robey PG. Single-colony derived strains of human marrow stromal fibroblasts form bone after transplantation *in vivo*. J Bone Miner Res 1997; 12:1335-1347.
4. Di Gregorio GB, Yamamoto M, Ali AA, Abe E, Roberson P, Manolagas SC, Jilka RL. Attenuation of the self-renewal of transit-amplifying osteoblast progenitors in the murine bone marrow by 17 beta-estradiol. J Clin Invest 2001; 107:803-812.

5. Jilka RL, Takahashi K, Munshi M, Williams DC, Roberson PK, Manolagas SC. Loss of estrogen upregulates osteoblastogenesis in the murine bone marrow: evidence for autonomy from factors released during bone resorption. J Clin Invest 1998; 101:1942-1950.
6. Weinstein RS, Jilka RL, Parfitt AM, Manolagas SC. Inhibition of osteoblastogenesis and promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids: potential mechanisms of their deleterious effects on bone. J Clin Invest 1998; 102:274-282.
7. Robey PG, Bianco P. Cellular mechanisms of age-related bone loss. In: Rosen C, Glowacki J, Bilezikian JP (eds) The aging skeleton. Academic Press, San Diego, CA; 1999:145-157.
8. Jilka RL, Weinstein RS, Takahashi K, Parfitt AM, Manolagas SC. Linkage of decreased bone mass with impaired osteoblastogenesis in a murine model of accelerated senescence. J Clin Invest 1996; 97:1732-1740.
9. Lecka-Czernik B, Moerman EJ, Reis RJ, Lipschitz DA. Cellular and molecular biomarkers indicate precocious *in vitro* senescence in fibroblasts from SAMP6 mice - evidence supporting a murine model of premature senescence and osteopenia. J Gerontol [A] 1997; 52A:B331-B336.
10. De Haan G, Van Zant G. Genetic analysis of hemopoietic cell cycling in mice suggests its involvement in organismal life span. FASEB J 1999; 13:707-713.
11. Tyner SD, Venkatachalam S, Choi J, Jones S, Ghebranious N, Igelmann H, Lu X, Soron G, Cooper B, Brayton C, Hee Park S, Thompson T, Karsenty G, Bradley A, Donehower LA. p53 mutant mice that display early ageing-associated phenotypes. Nature 2002; 415:45-53.
12. Kajkenova O, Lecka-Czernik B, Gubrij I, Hauser SP, Takahashi K, Parfitt AM, Jilka RL, Manolagas SC, Lipschitz DA. Increased adipogenesis and myelopoiesis in the bone marrow of SAMP6, a murine model of defective osteoblastogenesis and low turnover osteopenia. J Bone Miner Res 1997; 12:1772-1779.
13. Ducy P, Zhang R, Goeffroy V, Ridall AL, Karsenty G. Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. Cell 1997; 89:747-754.
14. Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR, De Crombrugghe B. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell 2002; 108:17-29.
15. Rosen ED, Spiegelman BM. PPARgamma: a nuclear regulator of metabolism, differentiation, and cell growth. J Biol Chem 2001; 276:37731-37734.
16. Lecka-Czernik B, Gubrij I, Moerman EJ, Kajkenova O, Lipschitz DA, Manolagas SC, Jilka RL. Inhibition of Osf2/Cbfa1 expression and terminal osteoblast differentiation by PPARgamma2. J Cell Biochem 1999; 74:357-371.
17. Jilka RL, Lecka-Czernik B, Ali AA, O'Brien CE, Weinstein RS, Manolagas SC. Activation of PPARγ2 by Rosiglitazone Causes bone loss associated with increased marrow adiposity and decreased osteoblast number in mice. J Bone Miner Res 2001; 16:S319 (abstract).
18. Bolton-Smith C, Woodward M, Tavendale R. Evidence for age-related differences in the fatty acid composition of human adipose tissue, independent of diet. Eur J Clin Nutr 1997; 51:619-624.
19. Jira W, Spiteller G, Schramm A. Increase in hydroxy fatty acids in human low density lipoproteins with age. Chem Phys Lipids 1996; 84:165-173.
20. Nourooz-Zadeh J, Pereira P. Age-related accumulation of free polyunsaturated fatty acids in human retina. Ophthalmic Res 1999; 31:273-279.
21. Lecka-Czernik B, Moerman EJ, Grant DF, Lehmann JM, Manolagas SC, Jilka RL. Divergent effects of selective peroxisome proliferator-activated receptor-{gamma}2 ligands on adipocyte versus osteoblast differentiation. Endocrinology 2002; 143:2376-2384.
22. Maurin AC, Chavassieux PM, Frappart L, Delmas PD, Serre CM, Meunier PJ. Influence of mature adipocytes on osteoblast proliferation in human primary cocultures. Bone 2000; 26:485-489.