

Review

Plasticity and regulation of human bone marrow stromal osteoprogenitor cells: potential implication in the treatment of age-related bone loss

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Summary. Human bone marrow stroma contains pluripotent mesenchymal progenitor cells that can give rise to many mesenchymal lineages, including chondroblasts, adipocytes or osteoblasts. The differentiation of these cells towards a specific lineage is dependent on hormonal and local factors activating specific transcription factors. Attempts have been recently made to identify osteoprogenitor cells in the human bone marrow and to identify the molecular mechanisms responsible for lineage-specific differentiation of human bone marrow stromal cells. Using a clonal pluripotent human bone marrow stromal cell line with tri-potential characteristics, we have provided evidence for a controlled reciprocal regulation of osteoblast/chondroblast and osteoblast/adipocyte differentiation of human bone marrow stromal cells. We have also shown that administration of TGF β that regulates the expression of specific osteoblast and adipocyte transcription factors can promote osteoblast differentiation and inhibit adipocyte conversion of rat marrow stromal cells *in vivo*. This indicates that the reciprocal relationship between osteoblastogenesis and adipogenesis can be manipulated *in vivo* in order to improve bone formation. Future studies will have to identify key signals for lineage-specific differentiation of human marrow stromal cells. This may result in the development of therapeutic strategies to promote the differentiation of these cells towards the osteoblast lineage and to inhibit excessive bone marrow adipogenesis associated with aging.

Key words: Bone marrow, Human, Osteoblasts, Adipocytes, Plasticity, Aging

Introduction

Aging is associated with bone loss resulting from decreased connection of bone trabeculae, leading to decreased bone resistance and increased risk of fractures (osteoporosis). Osteoporosis is a multifactorial disease with genetic, nutritional and hormonal components (Eisman, 1999; Marie, 2001). The non genetic causes of bone loss occurring with aging are multiple (Riggs et al., 2002). They include decreased sex hormone production, particularly in women at menopause, decreased calcium and protein uptake, decreased vitamin D metabolism, increased secretion of parathormone (PTH) and decreased physical activity. The causes of bone loss with aging are multiple and result in an imbalance between bone resorption and bone formation. The mechanisms of the increased bone resorption occurring with aging are now better understood (Manolagas, 2000). In contrast, the causes of the reduction of bone formation with age remain relatively unknown. Slowing of bone formation occurring with aging is believed to result in part from a reduction in the number of osteoprogenitor cells (Jilka et al., 1996; D'Ippolito et al., 1999; Nishida et al., 1999) whereas the osteoblastic differentiation potential does not seem to vary with age and osteoporosis in the human bone marrow (Justesen et al., 2002). With aging, in parallel to the slow reduction in bone-forming cell number, the number of adipocytes increases in the bone marrow, suggesting an inverse connection between osteoblastogenesis and adipogenesis (Gimble et al., 1996). Studies of human bone marrow cells led investigators to suggest that alterations in bone formation and adipogenesis during aging may result from reduced stromal cell differentiation into osteoblasts and preferred differentiation into adipocytes. Recent attempts have been made therefore to identify osteoprogenitor cells in the human bone marrow and to identify the molecular mechanisms responsible for their differentiation. In this short review article, we will review the knowledge on the plasticity and molecular

mechanisms of regulation of osteoblast progenitors in the human bone marrow in relation with bone aging.

Plasticity of human bone marrow stromal cells

The bone marrow stroma is a complex system composed of mesenchymal cells (MCSs) which contribute to regeneration of different tissues (Bianco and Robey, 2000). Almost three decades ago, the presence of osteogenic cells was identified in the bone marrow (Friedenstein et al., 1976). Since that time, several attempts have been made to isolate MSCs from the bone marrow stroma. Initially, heterogeneous populations of human bone marrow stromal cells were obtained (Haynesworth et al., 1992; Rickard et al., 1996; Fromigué et al., 1997). Thereafter, immortalised human bone marrow stromal cells have been developed and characterized (Hicok et al., 1998; Houghton et al., 1998; Fromigué et al., 2001). Recently, human bone stromal cell progenitors have been successfully isolated (Oyajobi et al., 1999; Stewart et al., 1999; Walsh et al., 2000) using the Stro-1+ antibody recognising a cell membrane protein (Gronthos and Simmons, 1995). More recently, mesodermal progenitor cells that can differentiate into other lineages have been isolated from the human bone marrow stroma (Colter et al., 2001; Qi et al., 2003). Some rare postnatal cells, called Multipotent Adult Progenitor Cells (MAPCs) were found to give rise to chondroblasts and osteoblasts as well as other cell types such as endothelial cells, neurons or hepatocytes, among other cell types (Jiang et al., 2002), suggesting that some MSCs may have the capacity to differentiate into cells of different embryonic origin. However, at the present time, stem cells with continuous autoreplication and multipotential behaviour have not yet been identified in the human bone marrow.

The plasticity of MSCs has been well documented. MSCs can give rise to skeletal cells (osteoblasts, chondrocytes) and non-skeletal cells, including adipocytes, vascular smooth muscle cells, skeletal and cardiac muscle cells and hematopoietic-supportive stromal cells (Owen, 1988; Pereira et al., 1995; Prockop, 1997; Dennis et al., 1999) (Fig. 1). As mentioned above, it has been known for several years that bone marrow stromal cells are able to differentiate into osteoblasts or to form bone when implanted ectopically (Friedenstein et al., 1987; Haynesworth et al., 1992; Pereira et al., 1995; Kuznetsov et al., 1997; Prockop, 1997). MSCs can differentiate into osteogenic cells under stimulation with a combination of dexamethasone, ascorbic and phosphate (Ashton et al., 1985). MSCs can differentiate into adipocytes or osteoblasts in rats, mice and humans (Bennett et al., 1991; Beresford et al., 1992; Thompson et al., 1998; Hicok et al., 1998; Nuttall et al., 1998) under stimulation of a variety of cocktails. Recent data also indicate that human MSCs can differentiate into chondrocytes under stimulation with Transforming Growth Factor β (TGF β) when cultured in aggregates (Pittenger et al., 1999; Ahdjoudj et al., 2001; Sekiya et

al., 2002). Differentiation towards one lineage is dependent on local, hormonal and mechanical factors (Owen, 1988; Prockop, 1997; Thompson et al., 1998). The finding that clonal MSC can differentiate into adipocytes, osteoblasts or chondrocytes in different species (Dennis et al., 1999; Negishi et al., 2000) including humans (Pittenger et al., 1999; Muraglia et al., 2000; Ahdjoudj et al., 2001), indicates that a single MSC may have multipotential competence. Indeed, we found that clonal F/STRO-1⁺ A-positive human marrow stromal cells express mRNA markers or protein of the osteoblast lineage [Cbfa1/Runx2, osteocalcin (OC), alkaline phosphatase (ALP), type 1 collagen], of the chondrocyte lineage (aggrecan, types 2, 9 and 10 collagen), and of the adipocyte lineage (PPAR γ 2, C/EBP α , aP2, G3PDH, lipoprotein lipase, leptin) under basal conditions (Ahdjoudj et al., 2001) suggesting a common origin for the three lineages (Fig. 1). Others found that bone marrow adipocytes can become osteogenic in vitro whereas osteoblast precursor cells can differentiate into adipocytes (Bennett et al., 1991; Diascro et al., 1998), suggesting that plasticity can occur at least when cells are not fully mature (Nuttall et al., 1998; Park et al., 1999; Pittenger et al., 1999). However, separation between the osteoblastic and adipocyte lineages occurs relatively late during human bone marrow MSC differentiation. We found that clonal MSCs maintain the expression of osteoblast phenotypic markers while starting to express adipocyte genes (Ahdjoudj et al., 2001). Whether plasticity of human MSCs still occurs at all stages of maturation of each cell lineage remains to be determined.

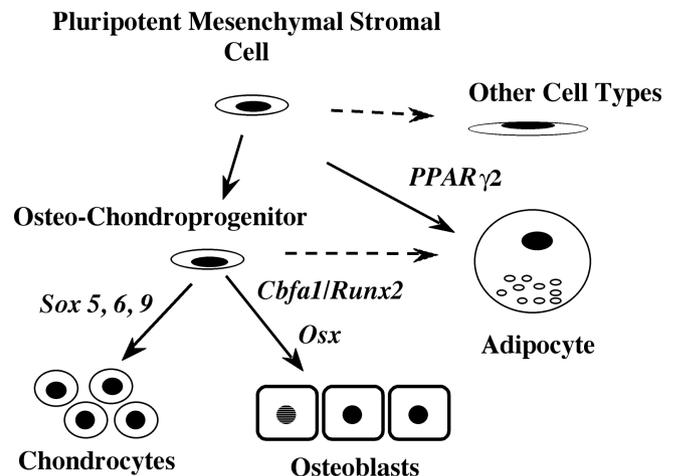


Fig. 1. Plasticity of human bone marrow stromal cells. Mesenchymal stromal cells can give rise to skeletal cells (osteoblasts, chondrocytes) and non-skeletal cell types. The differentiation towards chondroblasts, osteoblasts and adipocytes is under the control of the indicated specific transcription factors.

Expression of genes during MSC differentiation into osteoblasts and adipocytes

The osteogenic differentiation of human bone marrow stromal cells is characterized by the sequential expression of genes that are governed in part by *Cbfa1/Runx2* (Fig. 2), the main transcription factor required for osteoblast differentiation (Ducy et al., 1997, 1999). *Cbfa1* controls the expression of osteoblast genes by binding to the OSE2 sequence in the promoter of several genes (Ducy et al., 1997). Moreover, *Cbfa1/Runx2* is regulated post-transcriptionally (Xiao et al., 1997). It is interesting that in human MSCs the activity but not the expression of *Cbfa1/Runx2* increases with osteoblast differentiation (Shui et al., 2003). However, it is likely that, in addition to *Cbfa1/Runx2*, other transcription factors may play a role in MSC differentiation into chondroblasts, osteoblasts and other lineages (Qi et al., 2003) (Fig. 2).

Adipocyte differentiation is dependent on the expression of C/EBP and PPAR γ families of transcription factors which regulate the expression of adipocyte differentiation genes (Rosen and Spiegelman, 2000). PPAR γ 2 expression is the main transcription factor required for adipocyte differentiation *in vitro* and *in vivo* (Hu et al., 1995; Kubota et al., 1999; Rosen et al., 1999). Pharmacological inhibition of PPAR γ 2 or genetic alteration of PPAR γ 2 *in vitro* inhibits adipocyte differentiation, further emphasizing the importance of this factor in adipogenesis (Oberfield et al., 1999; Gurnell et al., 2000; Wright et al., 2000). Several studies have shown that the differentiation of MSCs into the adipocyte lineage requires PPAR γ 2 expression. In murine marrow stromal cells, overexpression of PPAR γ 2 represses *Cbfa1/Runx2* and osteoblast gene expression (Hicok et al., 1998; Lecka-Czernik et al., 1999). Furthermore, PPAR γ agonists inhibit osteoblast differentiation of bone marrow stromal cells (Lecka-Czernik et al., 1999; Parhami et al., 1999; Tintut et al.,

1999). For example, we have found that linoleic acid inhibits *Cbfa1/Runx2* expression and at the same time it promotes PPAR γ 2 expression in clonal human bone marrow stromal F/Stro-1⁺A cells (Ahdjoudj et al., 2001). *In vivo*, heterozygotic mice for PPAR γ show increased osteoblast number and bone volume (Akune et al., 2001). This further indicates that PPAR γ 2, a positive promoter of adipogenesis, negatively regulates osteoblast differentiation *in vitro* and *in vivo* (Fig. 3).

Mechanisms of regulation of MSC differentiation

The osteogenic process in long bones is controlled by numerous hormones and growth factors. These factors act by increasing cell proliferation, differentiation and apoptosis. MSC proliferation is in part promoted by growth hormone and PTH (Kassem et al., 1994; Gevers et al., 2002), among other hormonal factors. Local factors such as Insulin-like growth factor (IGF)-I (Thomas et al., 1999a,b), TGF β (Machwate et al., 1995; Fromigué et al., 1997) and Fibroblast growth factor (FG2) (Zhang et al., 2002) stimulate bone marrow stromal cell growth. These factors may thus contribute to expand the population of mesodermal osteoprogenitors during skeletal development, growth and postnatal bone remodeling.

The differentiation of MSCs towards osteoblasts or adipocytes is regulated by several molecules, including hormones and local growth factors (Fig. 2). These agents may act in part by increasing the expression or activity of *Cbfa1/Runx2* and downstream genes. Glucocorticoids were among the first agents identified to promote osteogenic differentiation of MSCs (Rickard et al., 1997). We and others found that dexamethasone increases the expression of ALP, an early osteoblast

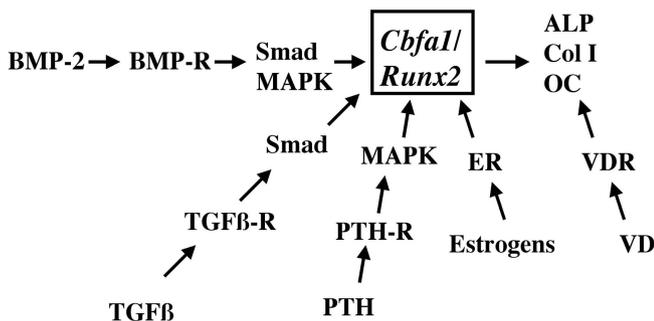


Fig. 2. Osteoblast differentiation of MSCs is dependent on the expression and activity of the transcription factor *Cbfa1/Runx2* under the control of hormonal (PTH, estrogens, 1,25-dihydroxyvitamin D (VD)), and local factors (BMP, TGF β) that act on their receptors to activate signaling pathways that regulate the differentiation of human marrow stromal cells.

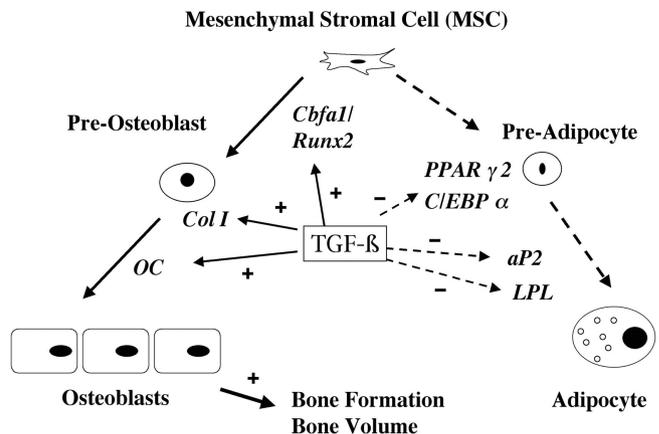


Fig. 3. Control of the osteoblast/adipocyte conversion by TGF β in vivo. In osteopenic (hypokinetic) rats, treatment with TGF β upregulates osteoblast differentiation and downregulates adipogenic differentiation genes in bone marrow stromal cells, resulting in concomitant improvement of osteogenesis and reduction of adipogenesis (from Ahdjoudj et al., 2002).

gene, in human bone marrow stromal cells and that it promotes the differentiation of MSCs into mature osteoblasts *in vitro* (Fromigué et al., 1997; Rickard et al., 1997). In clonal human bone marrow stromal F/Stro-1⁺ cells, we found that dexamethasone increases Cbfa1/Runx2, ALP and osteocalcin expression (Ahdjoudj et al., 2001). In addition to glucocorticoids, 1,25-dihydroxyvitamin D promotes ALP expression in human MSCs (Fromigué et al., 1997). Estrogens (Dang et al., 2002; Okazaki et al., 2002) and PTH signalling (Chan et al., 2001) also promote the differentiation of MSCs into osteoblasts by promoting Cfa1/Runx2 expression. Bone Morphogenetic Protein-2 (BMP-2) also promotes osteoblast differentiation in human bone marrow stromal cells (Fromigué et al., 1998; Gori et al., 1999). Recent data indicate that BMP and retinoic acid signalling cooperate to repress adipogenesis and promote osteoblast differentiation (Skillington et al., 2002). Moreover, sonic hedgehog (shh) (Spinella-Jaegle et al., 2001) and leptin promotes osteoblast differentiation of MSCs (Thomas et al., 1999b) (Fig. 2). Interestingly, all these molecules also act on MSCs to inhibit adipocyte differentiation (Gimble et al., 1995; Gori et al., 1999; Thomas et al., 1999a,b; Chan et al., 2001; Spinella-Jaegle et al., 2001; Dang et al., 2002) which further supports the hypothesis that there is a reciprocal control of MSC differentiation into osteoblasts or adipocytes (Gimble et al., 1996). In support of this concept is the evidence that differentiation towards one pathway restricts expression of other lineage-specific genes. Indeed, we found that exposure of clonal F/STRO-1⁺ human bone marrow stromal cells to linoleic acid increased expression of adipocytic genes and cytoplasmic triglyceride accumulation, and suppressed dexamethasone-induced stimulation of osteoblast marker genes. This provides evidence for a controlled reciprocal regulation of osteoblast/adipocyte differentiation of human bone marrow stromal cells (Ahdjoudj et al., 2001).

The signaling pathways that control the adipocyte/osteoblast differentiation processes in MSCs have begun to be understood. BMP and TGFβ are known to control Cbfa1/Runx2 expression through Smad proteins and other signaling pathways including MAP kinases (Yamaguchi et al., 2000). Molecules that activate MAPK such as TGFβ and PTH inhibit adipocyte differentiation (Chin et al., 1999; Chan et al., 2001). Cytokines that activate MAPK also inhibit adipogenic differentiation *in vitro* and *in vivo* (Beresford et al., 1992; Hauner and Hochberg, 2002). Activation of MAPK may result in PPARγ2 phosphorylation in its N-terminal domain (Adams et al., 1997; Hu et al., 1995), which results in inhibition of its transcriptional activity. Whether this mechanism also occurs in human MSCs, however, remains to be determined. It is likely that signals transduced by BMP, TGFβ, PTH and other local factors act in concert to regulate Cbfa1/Runx2 and PPARγ2 expression, resulting in differentiation toward

the osteoblastic or adipocyte lineage (Fig. 2).

MSCs: A clue for correcting the alterations of bone formation and adipogenesis with aging?

In concert with the decreased osteogenic potential of bone marrow cells with age, adipocytes accumulate in the bone marrow stroma. In neonates, adipocytes are barely present in the bone marrow stroma but the number and size of stromal adipocytes increase with aging and more than 90% of the bone marrow cavity is occupied by adipocytes in the aged bone (Rozman et al., 1989; Charbord et al., 1996; Kirkland et al., 2002; Verma et al., 2002). Interestingly, adipocyte accumulation in the human bone marrow stroma correlates with trabecular bone loss with aging (Meunier et al., 1971; Burkhardt et al., 1987). Such an increase in bone marrow stroma adipocytes is also observed in experimental conditions. For example, estrogen deficiency causing trabecular bone loss in ovariectomised rats is associated with increased adipogenesis in the bone marrow stroma (Martin and Zissimos, 1991). Mice with premature aging (SAMP 6 strain) also show decreased bone formation and increased number of adipocytes in the bone marrow stroma (Jilka et al., 1996). In hypokinetic rats, bone loss resulting from a decreased osteoblast number (Machwate et al., 1993) is associated with increased adipocyte number and size in the bone marrow cavity (Ahdjoudj et al., 2002). This inverse correlation between the two differentiation processes suggests that the alteration of osteoblasto-genesis and adipogenesis occurring in osteopenic conditions including aging results from an abnormality of the differentiation of the common precursor cell (Gimble et al., 1996).

This opens the interesting possibility that promoting MSC differentiation into osteoblasts may reduce adipocyte differentiation in the bone marrow. In support of this hypothesis is our recent finding that osteoblast/adipocyte differentiation of MSCs can be influenced *in vivo*. We used a rat model in which skeletal unloading decreases Cbfa1/Runx2, osteocalcin and type I collagen mRNA levels, and in parallel increases the expression of PPARγ2 and adipocyte differentiation genes lipoprotein lipase (LPL) and αP2 in the bone marrow stroma. We showed that administration of TGFβ2 not only corrected the abnormal expression of Cb1/Runx2, osteocalcin and type I collagen mRNAs, but also decreased PPARγ2 and normalized αP2 and LPL expression and reduced adipocyte number and volume in the bone marrow stroma in unloaded rats. Moreover, the increased osteoblast differentiation and reduced adipocyte differentiation induced by TGFβ2 resulted in improved osteogenesis and prevention of bone loss in this model (Ahdjoudj et al., 2002) (Fig. 3). This provides evidence that the reciprocal relationship between osteoblasto-genesis and adipogenesis can be manipulated *in vivo* in order to improve bone formation. It remains to be shown

that such manipulation may also lead to improved bone formation and bone mass in aged animals.

Conclusion

Recent progress has been made in the identification of factors that govern, regulate and promote MSC differentiation towards osteoblasts or adipocytes. However, the mechanisms of differentiation of MSCs towards other lineages remain largely unknown. The use of human clonal pluripotent human stromal cell lines may help in deciphering the molecular mechanisms of lineage-specific differentiation in humans. It will be important in the future to identify the signaling and molecular pathways that are important for lineage-specific differentiation of human MSCs. This may result in the development of therapeutic strategies to promote the differentiation of human bone marrow stromal cells towards the osteoblast lineage and to inhibit the excessive bone marrow adipogenesis associated with bone aging.

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