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In Osteoporosis, differentiation of mesenchymal stem cells (MSCs) improves bone marrow adipogenesis

Ana María Pino[1], Clifford J. Rosen[2] and J. Pablo Rodríguez[1,*]

[1] Laboratorio de Biología Celular y Molecular, INTA, Universidad de Chile,
[2] Maine Medical Center Research Institute, Scarborough, Maine, USA.

ABSTRACT

The formation, maintenance, and repair of bone tissue involve close interlinks between two stem cell types housed in the bone marrow: the hematologic stem cell originating osteoclasts and mesenchymal stromal cells (MSCs) generating osteoblasts. In this review, we consider malfunctioning of MSCs as essential for osteoporosis. In osteoporosis, increased bone fragility and susceptibility to fractures result from increased osteoclastogenesis and insufficient osteoblastogenesis.
MSCs are the common precursors for both osteoblasts and adipocytes, among other cell types. MSCs´ commitment towards either the osteoblast or adipocyte lineages depends on suitable regulatory factors activating lineage-specific transcriptional regulators. In osteoporosis, the reciprocal balance between the two differentiation pathways is altered, facilitating adipose accretion in bone marrow at the expense of osteoblast formation; suggesting that under this condition MSCs activity and their microenvironment may be disturbed. We summarize research on the properties of MSCs isolated from the bone marrow of control and osteoporotic post-menopausal women. Our observations indicate that intrinsic properties of MSCs are disturbed in osteoporosis. Moreover, we found that the regulatory conditions in the bone marrow fluid of control and osteoporotic patients are significantly different. These conclusions should be relevant for the use of MSCs in therapeutic applications.

Key words: MSCs, osteoporosis, adipogenesis, bone marrow microenvironment

BACKGROUND

The formation, maintenance, and repair of bone tissue depend on fine-tuned interlinks in the activities of cells derived from two stem cell types housed in the bone marrow interstice. A hematologic stem cell originates osteoclasts, whereas osteoblasts derive from mesenchymal stem cells (MSCs). Bone tissue is engaged in an unceasing process of remodelling through the turnover and replacement of the matrix: while osteoblasts deposit new bone matrix, osteoclasts degrade the old one.

Bone marrow provides an environment for maintaining bone homeostasis. The functional relationship among the different cells found in bone marrow generates a distinctive microenvironment via locally produced soluble factors, the extracellular matrix components, and systemic factors (Raisz, 2005; Sambrook and Cooper, 2006), allowing for autocrine, paracrine and endocrine activities. If only the main cellular components of the marrow stroma are considered, the activity of adipocytes, macrophages, fibroblasts, hematopoietic, endothelial and mesenchymal stem cells and their progeny bring about a complex range of signals.

Osteoporosis is a bone disease characterized by both decreased bone quality and mineral density. In postmenopausal osteoporosis, increased bone fragility and susceptibility to fractures result from increased osteoclastogenesis, inadequate osteoblastogenesis and altered bone microarchitecture.

The pathogenesis of the disease is hitherto unknown, hence the interest in basic and clinical research on the mechanisms involved (Raisz, 2005; Sambrook and Cooper, 2006). Cell studies on the origin of postmenopausal osteoporosis initially focused on osteoclastic activity and bone resorption processes; then on osteoblastogenesis, and more recently on the differentiation potential of mesenchymal stem cells (MSCs) (Shoback, 2007). Moreover, distinctive environmental bone marrow conditions appear to provide support for the development and maintenance of unbalanced bone formation and resorption (Nuttall and Gimble, 2004; Tontonoz et al., 1994). In this review, we consider the participation of the differentiation potential of MSCs, the activity of bone marrow adipocytes and the generation of a distinctive bone marrow microenvironment.

MESENCHYMAL STEM CELLS (MSCs)

Bone marrow contains stem-like cells that are precursors of nonhematopoietic tissues. These cells were initially referred to as plastic-adherent cells or colony forming-unit fibroblasts and subsequently as either mesenchymal stem cells or marrow stromal cells (MSCs) (Minguell et al., 2001; Lindnera et al., 2010; Kolf et al., 2007). There is much interest in these cells because of their ability to serve as a feeder layer for the growth of hematopoietic stem cells, their multipotentiality for differentiation, and their possible use for both cell and gene therapy (Minguell et al., 2001; Kolf et al., 2007). Friedenstein et al. (1970) initially isolated MSCs by their adherence to tissue culture surfaces, and essentially the same protocol has been used by other investigators. The isolated cells were shown to be multipotential in their ability to differentiate in culture or after implantation *in vivo,* giving rise to osteoblasts, chondrocytes, adipocytes, and/or myocytes.

Corresponding authors: J. Pablo Rodríguez. Laboratorio de Biología Celular. INTA, Universidad de Chile. El Líbano 5524, Macul. Casilla 138-11, Santiago, Chile. jprodrig@inta.uchile.cl
Phone: 56-2-9781452. Fax number: 56-2-2214030

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MSCs populations in the bone marrow or those that are isolated and maintained in culture are not homogenous, but rather consist of a mixture of uncommitted, partially committed and committed progenitors exhibiting divergent stemness (Baksh et al., 2004). These heterogeneous precursor cells are morphologically similar to the multipotent mesenchymal stem cells, but differ in their gene transcription range (Baksh et al., 2004). It has been proposed that in such populations, cell proliferation, differentiation and maturation are in principle independent; stem cells divide without maturation, while cells close to functional competence may mature, but do not divide (Song et al., 2006).

Several molecular markers identify committed progenitors and the end-stage phenotypes, but at present there are no reliable cell markers to identify the uncommitted mesenchymal stem cells. Given the difficulty to identify a single marker to evaluate the population of stem cells, various combinations of these markers may be used (Seo et al., 2004; Lin et al., 2008; Xu et al., 2009). Therefore, MSCs are mainly defined in terms of their functional capabilities: self-renewal, multipotential differentiation and transdifferentiation (Baksh et al., 2004).

Hypothetically, the fate of MSCs appears to be determined during very early stages of cell differentiation ("commitment"). During this mostly unknown period, both intrinsic (genetic) and environmental (local and/or systemic) conditions interplay to outline the cell's fate towards one of the possible lineages. Based on microarray assays comparing gene expression at the stem state and throughout differentiation, it has been proposed that MSCs multilineage differentiation involves a selective mode of gene expression (Baksh et al., 2004; Song et al., 2006). It appears that "stemness" is characterized by promiscuous gene expression, where pluripotential differentiation results from the maintenance of thousands of genes at their intermediate expression levels. Upon commitment to one fate, only the few genes that are needed for differentiation towards the target tissue are selected for continuous expression, while the rest are downregulated (Zipori, 2005; Zipori, 2006).

The gene expression profile of undifferentiated human MSCs (h-MSCs) show high expression of several genes (Song et al., 2006; Tremain et al., 2001), but the contribution of such genes in preserving h-MSC properties, such as self-renewal and multilineage differentiation potential, or in regulating essential signalling pathways is largely unknown (Song et al., 2006). Several factors like age (Zhou et al., 2008), culture condition (Kultere et al., 2007), microenvironment (Kuhn and Tuan, 2010), mechanical strain (McBride et al., 2008) and some pathologies (Seebach et al., 2007; Hofer et al., 2010) appear to affect MSCs´ intrinsic activity.

MSCs´ commitment towards either the osteoblast or adipocyte lineage is determined by a combination of regulatory factors in the cells´ microenvironment. The adequate combination leads to the activation of lineage-specific transcriptional regulators, including Runx2, Dlx5, and osterix for osteoblasts, and PPARγ2 and a family of CAAT enhancer binding proteins for adipocytes (Murunganandan et al., 2009). Although the appropriate collection of regulatory factors required for suitable differentiation of MSCs is largely unknown, the TGF/BMPs, Wnt and IGF-I signals are briefly considered.

Several components of the BMP family are secreted in the MSCs´ microenvironment (Lou et al., 1999, Gori et al., 1999; Gimble et al., 1995); BMP-2/4/6/7 have been identified as mediators for MSCs differentiation into osteoblasts or adipocytes (Muruganadan et al., 2009). The intracellular effects of BMPs are mediated by an interaction with cell surface BMP receptors (BMPRs type I and type II) (Gimble et al., 1995). It seems that differentiation into adipocytes or osteoblasts is highly dependent on the type of receptor I expressed by the cells, so that adipogenic differentiation requires signaling through BMPR IA, while osteogenic differentiation is dependent on BMPR IB activation (Gimble et al., 1995). The active receptors trigger the activation of Smad proteins, which induce specific genes. Under osteogenic differentiation, BMP action promotes osterix formation through Runx2-dependent and Runx2-independent pathways, thereby triggering osteogenic differentiation (Gori et al., 1999; Shapiro, 1999).

In addition to the role of BMPs in bone formation, BMPs also positively mediate the adipogenic differentiation pathways (Haiyan et al., 2009). It has been demonstrated that there is a binding site for Smad proteins in the promoter region of PPARγ2 (Lecka-Czernik et al., 1999), and over-expression of Smad2 protein suppresses the expression of Runx2 (Li et al., 1998). These observations suggest that adequate content of osteoblasts and adipocytes in the bone marrow is dependent on balanced signaling through this pathway. Moreover, considering the distinct role assigned to BMPRIA and BMPRIB, the temporal gain or loss of a subtype of BMP receptors by MSCs could be critical for commitment and subsequent differentiation (Gimble et al., 1995144).

Wnt signaling in MSCs is also decisive for the reciprocal relationship among the osteo/adipogenic pathways. Activation of the Wnt/β-catenin pathway directs MSCs differentiation towards osteoblasts instead of adipocytes (Bennett et al., 2005; Ross et al., 2000; Moldes et al., 2003). Animal studies have shown that activation of the Wnt signaling pathway increases bone mass, preventing both hormone-dependent and age-induced bone loss (Bennett et al., 2005). Furthermore, Wnt activation may control cell commitment towards osteoblasts by blocking adipogenesis through the inhibition of the expression of both C/EBP and PPARγ adipogenic transcription factors, as demonstrated in vivo in humans (Qiu et al., 2007), in transgenic mice expressing Wnt 10b (Bennett et al., 2005) and *in vitro* (Rawadi et al., 2003). MSCs´ self-renewing and maintenance of the undifferentiated state appear to be dependent on appropriate canonical Wnt signaling, promoting increased proliferation and decreased apoptosis (Boland et al., 2004; Cho et al., 2006). The overexpression of LRP5, an essential co-receptor specifically involved in canonical Wnt signaling, has been reported to increase proliferation of MSCs (Krishnan et al., 2006). In addition, disruption *in vivo* or *in vitro* of β–catenin signaling promoted spontaneous conversion of various cell types into adipocytes (Bennett et al., 2002). Moreover, the importance of this pathway for bone mineral density has been highlighted by the observation that genetic variations at either the LRP5 or Wnt10b gene locus are associated with osteoporosis (Brixen et al., 2007; Usui et al., 2007).

Also, insulin-like growth factor-I (IGF-I) signalling is clearly an important factor in skeletal development. The IGF regulatory system consists of IGFs (IGF-I and IGF-II), Type I and Type II IGF receptors, and regulatory proteins including IGF-binding proteins (IGFBP-1-6) and the acid-labile subunit (ALS) (Rosen et al., 1994). The ligands in this system (i.e. IGFs) are potent mitogens, and in some circumstances differentiation factors, that are bound in the circulation and interstitial fluid

as binary (to IGFBPs) or ternary complexes (IGF-ALS-IGFBP-3 or -5) with little free IGF-I or -II. IGF bio-availability is regulated by the interaction of these molecules at the receptor level; hence changes in any component of the system will have profound effects on the biologic activity of the ligand. The IGFBPs have a particularly important role in regulating IGF-I access to its receptor, since their binding affinity exceeds that of the IGF receptors. The IGF system is unique because the IGFBPs are regulated in a cell-specific manner at the pericellular microenvironment, such that small changes in their concentrations could strongly influence the mitogenic activity of IGF-I (Jones and Clemmons, 1995; Hwa and Rosenfeld, 1999; Firth and Baxter, 2002). IGFs are expressed virtually by all tissues, and circulate in high concentrations. Although nearly 80% of the circulating IGF-I comes from hepatic sources, both bone and fat synthesize IGF-I and these tissues contribute to the total circulating pool. Locally produced IGF-I predominates over circulating IGF-I in maintaining skeletal integrity (Rosen et al., 1994; Kawai and Rosen, 2010), and both ALS and IGFBP-3 participate in regulating bone function. However, the possible autocrine/paracrine roles of IGF-I and IGFBPs in marrow (Liu et al., 1993; Peng et al., 2003) or in osteoblast (Zhao et al., 2000; Zhang et al., 2002; Wang et al., 2007) are practically unknown.

RELATIONSHIP BETWEEN THE OSTEO- / ADIPOGENESIS PROCESSES - THE FAT THEORY FOR OSTEOPOROSIS

Since in the bone marrow MSCs are the common precursor cells for osteoblast and adipocytes, adequate osteoblast formation requires diminished adipogenesis. As pointed out above, MSCs commitment and differentiation into a specific phenotype depends on hormonal and local factors (paracrine/autocrine) regulating the expression and/or activity of master differentiation genes (Nuttall and Gimble, 2004; Muruganadan et al., 2009) (Figure 1). A reciprocal relationship has been postulated to exist between the two differentiation pathways whose alteration would facilitate adipose accretion in the bone marrow, at the expense of osteoblast formation, thus decreasing bone mass (Reviewed in Rosen et, al 2009; Rodríguez et al.. 2008; Rosen and Bouxtein, 2006). Such unbalanced conditions prevail in the bone marrow of osteoporosis patients, upsetting MSC activity and the microenvironment (Nuttall and Gimble, 2004; Moerman et al., 2004; Rosen and Bouxtein, 2006). This proposition is known as the fat theory for osteoporosis. Moreover, this alteration of osteo-/adipogenic processes is also observed in other conditions characterized by bone loss, such as aging, immobilization, microgravity, ovariectomy, diabetes, and

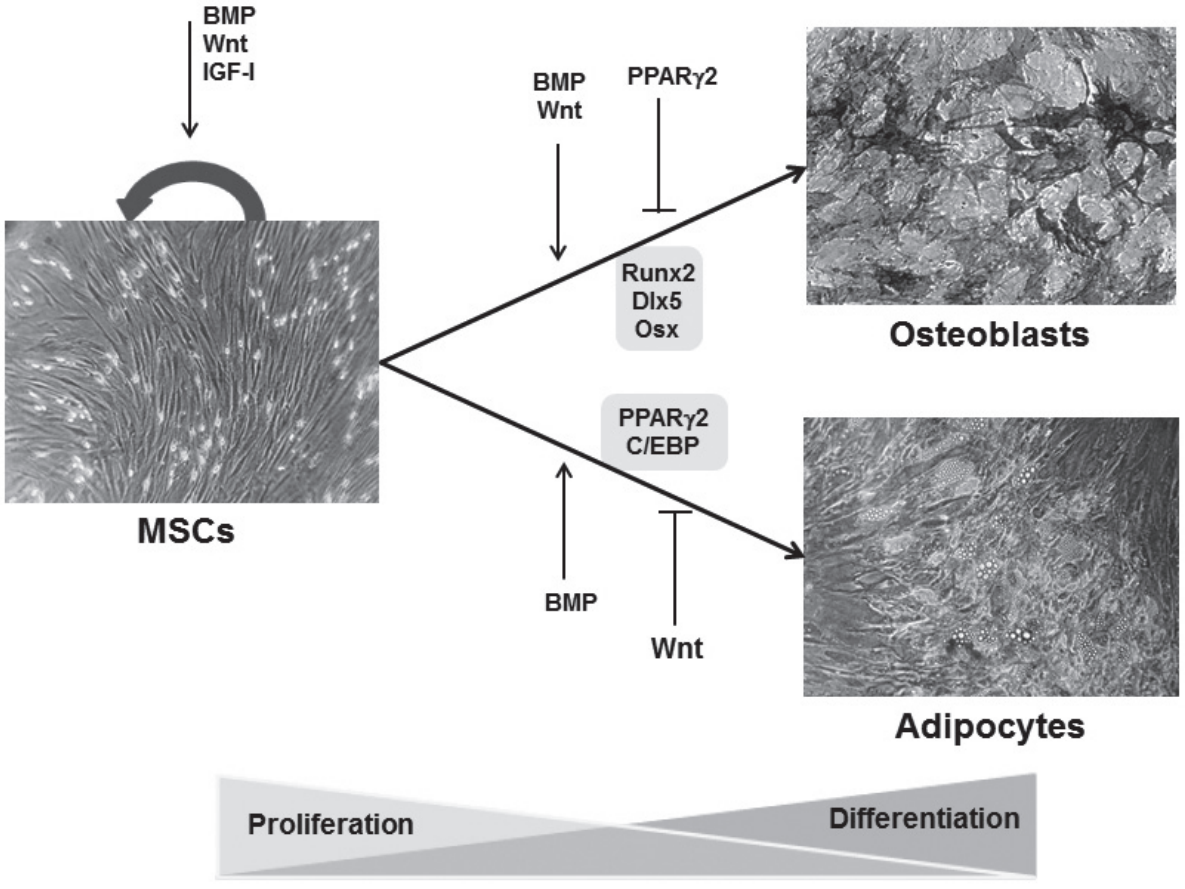

Figure 1: Schematic representation of mesenchymal stem cells (MSCs) differentiating into osteoblasts or adipocytes. Cell differentiation depends on specific hormonal and local factors regulating the expression and/or activity of master differentiation genes (enclosed in grey box). Abbreviations: MSCs: Mesenchymal stem cells, BMP: Bone Morphogenetic Protein, Wnt: IGF-1: insulin-like growth factor-1, Runx2: Runt-related transcription factor 2, Dlx5: Distal-Less Homeobox 5, Osx: Osterix, PPARγ2: Peroxisome proliferator-activated receptor gamma 2, C/EBP: CCAAT/enhancer-binding protein

glucocorticoid or tiazolidindione treatments, highlighting the harmful consequence of marrow adipogenesis in osteogenic disorders (Wronski et al., 1986; Moerman et al., 2004; Zayzafon et al., 2004; Forsen et al., 1999).

Cell studies comparing the differentiation potential of MSCs derived from osteoporotic patients (o-MSCs) with that of control MSCs (c-MSCs) have shown unbalanced osteogenic/adipogenic processes, including increased adipose cell formation, counterbalanced by reduced production of osteogenic cells (Nuttall and Gimble, 2004; Rodríguez et al., 2008; Rosen and Bouxtein, 2006). Further research on MSC differentiation has shown that activation of PPARγ2, a master transcription factor of adipogenic differentiation, positively regulates adipocyte differentiation while acting as a dominant negative regulator of osteogenic differentiation (Lecka-Czernik et al., 1999; Jeon et al., 2003; Khan and Abu-Amer, 2003). In contrast, an increase in bone mass density was observed in a PPARγ deficient mice model; even the heterozygous deficient animals showed high bone mass and increased osteoblastogenesis (Cock et al., 2004). On the other hand, Runx2 expression by MSCs inhibits their differentiation into adipocytes, as may be concluded from experiments in Runx2-/- calvarial cells, which spontaneously differentiate into adipocytes (Kobayashi et al., 2000).

In vivo observations further support the fat theory. Early studies observed that osteoporosis was strongly associated with bone marrow adipogenesis. Iliac crest biopsies showed that bone marrow from osteoporotic patients had a considerable accumulation of adipocytes in relation to that of healthy elderly women (Moerman et al., 2004; Meunier et al., 1971). More recently, increased bone marrow adiposity measured by in vivo proton magnetic resonance (^{1}H-MRS) has been associated with decreased bone mineral density in patients with low bone density (Griffith et al., 2005; Yeung et al., 2005; Blake et al., 2008).

In newborn mammals there is no marrow fat; however the number of adipocytes increases with age such that in humans over 30 years of age, most of the femoral cavity is occupied by adipose tissue (Moore and Dawson, 1990). The function of marrow fat is largely unknown; in humans it was first considered to be 'filler' for the void left by trabecular bone during aging or after radiation. Later, these cells have been proposed to have a role as an energy source, or as modulators of adjacent tissue by the production of paracrine, and autocrine factors (reviewed in Rosen et al., 2009). In fact, adipokines, steroids, and cytokines (Lee et al., 2002; Pino et al., 2010; Rosen et al., 2009;) can exert profound effects on neighboring marrow cells, sustaining or suppressing hematopoietic and osteogenic processes (Omatsu et al., 2010; Krings et al., 2012; Rosen et al., 2009; Rodríguez et al., 2008). Thus, the function of bone marrow adipose tissue may be similar to that of extra medullary fat. As such, it has been well established that unbalanced production of signaling products from subcutaneous or visceral fat modulates several human conditions including obesity, lipodystrophy, atherogenesis, diabetes and inflammation. Recent studies in mice, suggest a complex fat phenotype in the bone marrow, presenting mixed brown and white adipose properties (Lecka-Czernik, 2012). Further work is needed to find out whether differences in the quality or quantity of marrow fat, take part in deregulated bone remodelling in some bone diseases.

STUDIES ON THE ACTIVITY OF OSTEOPOROTIC MSCs

Because of their ability to self-renew, human MSCs can be expanded and differentiated in vitro, offering many perspectives for tissue engineering and regenerative medicine approaches. However, there is scarce information on whether specific diseases affect the properties of MSCs, because of the difficult accessibility to human bone marrow in health and disease (Cipriani et al., 2011; Corey et al., 2007).

Our research has focused on the properties of MSCs isolated from bone marrow of control and osteoporotic post-menopausal women. We grouped our observations on functional characteristics of o-MSCs and c- MSCs in three categories, which are summarized in Table I, as follows:

a) General activities: h-MSCs isolated from osteoporotic and control donors have similar CFU-F, but different proliferation rates. O-MSCs showed significantly diminished proliferation rate and decreased mitogenic response to IGF-I. The pERK/ERK ratio is increased in o-MSCs, compared with control c-MSCs. In other cell types, activation of the MEK/ERK signalling pathway enhances the activity of adipogenic transcription factors (Prusty et al., 2002). We also observed decreased TGF-β production by o-MSCs, as well as decreased capacity to generate and maintain a type I collagen-rich extracellular matrix, both conditions supporting cell differentiation into the adipocyte phenotype. Then, considering that the lineage fate of MSCs is dependent on early activation by specific BMPs, PPARγ and Wnt signaling (Ross et al., 2000; Rawadi et al., 2003; Westendorf et al., 2004; Baron and Rawadi, 2007), we compared the expression level of some genes related to these pathways in c- and o- MSCs. Results obtained by RT-PCR showed that in c- and o-MSCs the expression level of mRNA for β-catenin, Dkk-1, and BMPRIB was similar; while the level of mRNA for Wnt 3a was undetectable in both types of samples. The expression level of mRNA for GSK-3β, LRP6 and Osx was lower in o-MSCs than in c-MSCs, while the mRNA level for Ror2, Wnt 5a, BMPRIA showed doubtful. To further quantify the expression level of GSK-3β, LRP6, Osx, Ror2, Wnt 5a, BMPRIA real time RT-PCR was performed. As shown in Table I, statistically significant decreased mRNA levels for GSK-3β, LRP6 and Osx (0.64, 0.26 and 0.18 fold, respectively) were observed in o-MSCs, as compared to c-MSCs. In addition, mRNA levels for Ror2, Wnt 5a, and BMPRIA were similar in both types of cell samples.

   These data suggest impaired regulation by the BMPs and Wnt pathways in o-MSCs, representing some intrinsic deviation from control cells that might underlie the impaired self-renewal, and adipogenic/osteogenic differentiation potential observed in o-MSCs. mRNA levels for Ror2, Wnt 5a, and BMPRIA were similar in both types of cell samples.

b) Differentiation potential of cells: under osteogenic differentiation conditions, cells derived from osteoporotic donors had diminished alkaline phosphatase activity and less calcium deposition, compared with cells from control donors, in agreement with their reduced ability to form mature bone cells. On the other hand, the increased

adipogenic potential of o-MSCs was tested by incubating cells in adipogenic medium; under this condition o-MSCs showed favoured adipogenesis compared with c-MSCs. In conjunction, these observations sustain the notion that in the bone marrow of osteoporotic women, fat overload occurs at the expense of osteogenesis (Meunier et al., 1971).

c) Adipocyte characteristics: Adipocytes derived from both MSCs types were similar in cell size and granularity (unpublished observations); however, the fluorescence index in adipocytes originated from c-MSCs was significantly higher than those from o-MSCs (Table I), suggesting that c- and o-adipocytes differ in the quality of their lipid content. As far as we know, this is the first observation on qualitative differences in the lipid content among c- and o-adipocytes, matching some observations in the quality of lipids in the bone marrow fluid (Li et al., 2012).

STUDIES ON THE ACTIVITY OF BONE MARROW FLUID OF POST-MENOPAUSAL WOMEN

Distinctive environmental bone marrow conditions appear to support the development and maintenance of the balance between bone resorption and bone formation. Knowledge is

TABLE I

Functional characteristics of osteoporotic- and control- MSCs

	Condition	Incubation Time (days)	c-MSCs	o-MSCs	Reference
General Activities:					
Total Colonies Number (CFU-F)	Basal	14	12.7±5.6	14.1±2.6	Unpublished observations
Proliferation rate	Basal		High	Low	Rodríguez et al. 1999
IGF-1 mitogenic response (0 – 50 ng/ml)	Basal	4	Yes	No	Rodríguez et al. 1999
p-ERK/ERK	Basal	3	0.55±0.05	1.3±0.25	Rodríguez et al. 2004
TGF-β Synthesis (units/10^6 cells)	Osteogenic	14	16	7	Rodríguez et al. 2000
Collagen Type I Synthesis (μg/10^6 cells)	Basal	1	10.2±1.9	5.1±2.7	Rodríguez et al. 2000
GSK-3β mRNA level (relative to c-MSCs)	Basal	-	1.06±0.21	0.56±0.05*	Unpublished observations
LRP6 mRNA level (relative to c-MSCs)	Basal	-	1.00±0.30	0.197±0.05*	Unpublished observations
Osx mRNA level (relative to c-MSCs)	Basal	-	1.023±0.48	0.098±0.04*	Unpublished observations
Differentiation potential:					
Alkaline Phosphatase Activity (μmol PNP/min/10^6 cells)	Osteogenic	12	19.4±1.16	7.8±0.28	Rodríguez et al. 1999
Calcium Deposition (μg/plate)	Osteogenic	16	34±0.5	14.5±1.1	Hess et al, 2005
Adipocytes (%)	Adipogenic	14	11.5±3.3	22.3±6.5	Hess et al, 2005
Adipocytes characteristics:					
Granularity	Adipogenic		326±147	493±152	Unpublished observations
Size	Adipogenic		87.5±23.8	95.2±3.7	Unpublished observations
Fluorescence Index	Adipogenic	14	3.64±0.43	2.13±0.15*	Unpublished observations

Basal: Non differentiation condition; OS: Osteogenic differentiation condition; AD: Adipogenic differentiation condition; * $p<0.05$.
GSK-3β: Glycogen Synthase Kinase-3
LRP6: low-density lipoprotein-related receptor protein-6
TGF-β: Transforming growth factor beta
Osx: Osterix
IGF-1: Insulin-like growth factor 1
pERK: Phospho-Extra-cellular regulated kinase
ERK: Extra-cellular regulated kinase

scarce about the intramedullar concentration of compounds with recognized regulatory effects on bone formation or resorption and is limited to some pathologic conditions or estimated from measurements in plasma (Wiig et al., 2004; Iversen and Wiig, 2005; Lee et al., 2002; Khosla et al., 1994). Measurement of soluble molecules found in human bone marrow has been particularly difficult, not only because of tissue seclusion, but also because of the complicated anatomy and blood perfusion of bone. Since it may be expected that concentrations measured in the bone marrow fluid (BMF) more reliably reflect the physiologically relevant levels in the interstitial compartment surrounding the bone cells than values found in blood, we isolated the extracellular bone marrow fluid by directly spinning bone marrow samples for 20 min at 900xg. Considering the complex organization in such a regulatory milieu, we opted for evaluating some molecules recognized as markers of adipocyte, proinflammatory or osteoclastic/osteoblastic activity (Pino et al., 2010).

The concentrations of cytokines or receptors measured in the bone marrow extracellular fluid from control and osteoporotic human donors are indicated in Table II. In addition, the concentrations of IGF-I and its IGFBPs were analyzed, as well as the C-terminal telopeptide cross-links of type I collagen (CTX). Results summarized in Table II indicate significantly different concentrations of regulatory molecules in the extracellular fluid of control versus osteoporotic women; this last group was characterized by higher content of proinflammatory and adipogenic cytokines. Also, osteoporotic samples showed decreased leptin bioavailability, suggesting that insufficient leptin action may characterize the osteoporotic bone marrow (Pino et al., 2010). In addition, bioavailability of IGF-I appears diminished in o-BMF, as shown by the increased IGFBP3/IGF-I ratio.

Taken together our results and those of other researchers identify significant differences between functional properties of control and osteoporotic MSCs, displayed *in vitro*, in cells under basal or differentiating conditions. Moreover, it can be concluded that such divergence prevails also *in vivo*, because the bone marrow fluid of osteoporotic patients characterizes by unfavourable content of several regulatory molecules. Therefore, the properties of both MSCs and bone marrow microenvironment are significantly impaired in osteoporotic patients, negatively affecting bone formation.

CONCLUSIONS

In the pathogenesis of osteoporosis, impairment of both MSCs functionality and microenvironment add to the known detrimental effect of increased osteoclast activity, resulting in decreased bone formation.

O-MSCs are characterized by intrinsic functional alteration leading to poor osteogenic capability and increased adipogenesis. Osteoporotic bone marrow microenvironment differs from the control microenvironment by increased concentration of pro-adipogenic and pro-inflammatory regulatory factors.

The content and/or quality of adipocytes in the bone marrow appear critical to delineate impairing of MSCs; in this

TABLE II

Regulatory activity in bone marrow fluid of post-menopausal women

Regulating Factor concentration	Control BMF	Osteoporotic BMF	Reference
Interleukin-6 (pg/mL)	4.8±2.5	6.2±2.5*	Pino et al. 2010
Soluble interleukin-6 receptor (ng/mL)	33.7±13.1	47.0±13.7*	Pino et al. 2010
TNF-α (pg/mL)	72.3±55.0	148.9±82.0*	Pino et al. 2010
Adiponectin (μg/mL)	9.5±2.4	5.7±2.7*	Pino et al. 2010
Soluble RANKL (pmol/L)	0.27±0.16	0.14±0.05*	Pino et al. 2010
Osteoprotegerin (pmol/L)	2.9±0.9	4.4±1.8*	Pino et al. 2010
Leptin (ng/mL)	14.5±11.3	7.0±4.4*	Pino et al. 2010
Soluble leptin receptor (ng/mL)	44.6±14.7	48.9±17.8	Pino et al. 2010
Leptin bioavailability	0.33±0.22	0.15±0.16*	Pino et al. 2010
IGF-1 (ng/ml)	76,1±25,4	48,2±18,5*	Xian et al. 2012
IGFBP-3 (ng/ml)	24,52±5,98	27,88±8,52	Unpublished observations
IGF-1/IGFBP-3	3.1	1.72	Unpublished observations

BMF= Bone marrow fluid. *$p<0.05$.
TNF-α: Tumor necrosis factor alpha
RANKL: receptor activator of Nuclear Factor κ Beta ligand
IGFBP: Insulin-like growth factor binding protein

sense osteoporosis could be homologated to other age-related diseases such as obesity, atherogenesis and diabetes, which are characterized by extramedullar unbalanced adipocyte formation and signaling.

Currently it is not known how damaged o-MSCs emerge, further work is needed to ascertain the role of the microenvironment, and genetic and epigenetic factors, as proposed for other stem cells-related pathologies.

The conclusion that intrinsic properties of MSCs are altered in osteoporosis should be relevant for the therapeutic use of MSCs, which represent an interesting promise for regenerative medicine for several severe human diseases.

The possibility of reversing o-MSCs impairment opens new perspectives for osteoporosis therapy.

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REFERENCES

ASTUDILLO P, RÍOS S, PASTENES L, PINO AM, RODRÍGUEZ JP (2008) Increased adipogenesis of osteoporotic human-msenchymal stem cells (MSCs) characterizes by impaired leptin action. J Cell Biochem 103: 1054-1065

BAKSH D, SONG L, TUAN RS (2004) Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy. J Cell Mol Med 8: 301-316.

BARON R, RAWADI G (2007) Minireview: Targeting the Wnt/β-catenin pathway to regulate bone formation in the adult skeleton. Endocrinology 148:2635–2643.

BENNETT CN, LONGO KA, WRIGHT WS, SUVA L J, LANE TF, HANKENSON KD, MACDOUGALD OA (2005). Regulation of osteoblastogenesis and bone mass by Wnt10b. Proc Natl Acad Sci USA 102: 3324-3329.

BENNET CN, ROSS SE, LONGO KA, BAJNOK L, HEMATI N, JOHNSON KW, HARRISON SD, MACDOUGALD OA (2002) Regulation of Wnt signaling during adipogenesis. J Biol Chem 277:30998-31004.

BIANCO P, ROBEY PG (1999) Diseases of bone and stromal cell lineage. J Bone Miner Res 14: 336-41.

BLAKE GM, GRIFFITH JF, YEUNG DK, LEUNG PC, FOGELMAN I (2008) Effect of increasing vertebral marrow fat content on BMD measurement, T-Score status and fracture risk prediction by DXA. Bone 44: 495-501

BOLAND GM, PERKINS G, HALL DJ, TUAN RS (2004) Wnt 3a promotes proliferation and suppresses osteogenic differentiation of adult human mesenchymal stem cells. J Cell Biochem 93:1210-1230.

BRIXEN K, BECKERS S, PEETERS A, PITERS E, BALEMANS W, NIELSEN TL, WRAAE K, BATHUM L, BRASEN C, HAGEN C, ANDERSEN M, VAN HUL W, ABRAHAMSEN B (2007) Polymorphisms in the low-density lipoprotein receptor-related protein 5 (LRP5) genes are associated with peak bone mass in non-sedentary Men: results from the Odense Androgen Study. Calcif Tissue Int 81:421-429.

CHO HH, KIM YJ, KIM SJ, KIM JH, BAE YC, BA B, JUNG JS (2006) Endogenous Wnt signaling promotes proliferation and suppresses osteogenic differentiation in human adipose derived stromal cells. Tissue Eng 12:111-121.

CIPRIANI P, MARRELLI A, LIAKOULI V, DI BENEDETTO P, GIACOMELLI R (2011) Cellular players in angiogenesis during the course of systemic sclerosis. Autoimmunity Rev 10:641-646.

COCK TA, BACK J, ELEFTERIOU F, KARSENTY G, KASTNER P, CHAN S, AUWERX J (2004) Enhanced bone formation in lipodystrophic PPAR$\gamma^{hyp/hyp}$ mice relocates haematopoiesis to the spleen. EMBO reports 5: 1007–1012.

COREY SJ, MINDEN MD, BARBER DL, KANTARJIAN H, WANG JCY, SCHIMMER AD (2007) Myelodysplastic syndromes: the complexity of stem-cell diseases. Nat Rev Cancer 7: 118-129.

FIRTH SM, BAXTER RC (2002) Cellular actions of the insulin-like growth factor binding proteins. Endocr Rev 23:824–854.

FORSEN L, MEYER HE, MIDTHJELL K, EDNA TH (1999) Diabetes mellitus and the incidence of hip fracture: results from the Nord-Trondelag Health Survey. Diabetologia 42: 920-925.

FRIEDENSTEIN AJ, CHAILAKHJAN RK, LALYKINA KS (1970). The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet 3:393-403

GIMBLE JM, MORGAN C, KELLY K, WU X, DANDAPANI V, WANG CS, ROSEN V (1995) Bone morphogenetic proteins inhibit adipocyte differentiation by bone marrow stromal cells. J Cell Biochem 58: 393-402.

GORI F, THOMAS T, HICOK KC, SPELSBERG TC, RIGGS BL (1999) Differentiation of human marrow stromal precursor cells: bone morphogenetic protein-2 increases Osf2/Cbfa1, enhances osteoblast commitment, and inhibits late adipocyte maturation. J Bone Miner Res 14: 1522-1535.

GRIFFITH JF, YEUNG DK, ANTONIO GE, LEE FK, HONG AW, WONG SY, LAU EM, LEUNG PC (2005) Vertebral bone mineral density, marrow perfusion, and fat content in healthy men and men with osteoporosis: dynamic contrast-enhanced MR imaging and MR spectroscopy. Radiology 236: 945-951

HAIYAN H, TAN-JING S, LI X, HU L, HE Q, LIU M, LANE MD, TANG QQ (2009) BMP signaling pathway is required for commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage. Proc Natl Acad Sci USA 106:12670-12675.

HESS R, PINO AM, RÍOS S, FERNÁNDEZ M, RODRÍGUEZ JP (2005) High affinity leptin receptors are present in human mesenchymal stem cells (MSCs) derived from control and osteoporotic donors. J Cell Biochem 94:50-57.

HOFER EL, LABOVSKY V, LA RUSSA V, VALLONE VF, HONEGGER AE, BELLOC CG, WEN HC, BORDENAVE RH, BULLORSKY EO, FELDMAN L, CHASSEING NA (2010) Mesenchymal stromal cells, colony-forming unit fibroblasts, from bone marrow of untreated advanced breast and lung cancer patients suppress fibroblast colony formation from healthy marrow. Stem Cells Dev 19: 359-370.

HWA V, OH Y, ROSENFELD RG (1999) The insulin-like growth factor-binding protein (IGFBP) superfamily. Endocr Rev 20:761–787

IVERSEN PO, WIIG H (2005) tumor necrosis factor a and adiponectin in bone marrow interstitial fluid from patients with acute myeloid leukemia inhibit normal hematopoiesis. Clin Cancer Res 11:6793–6799.

JEON MJ, KIM JA, KWON SH, KIM SW, PARK KS, PARK SW, KIM SY, SHIN CS (2003) Activation of peroxisome proliferator-activated receptor-γ inhibits the Runx2-mediated transcription of osteocalcin in osteoblasts. J Biol Chem 278: 23270-23277.

JONES JI, CLEMMONS DR (1995) Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 16:3–34.

KAWAI M, ROSEN CJ (2010) The IGF-I regulatory system and its impact on skeletal and energy homeostasis. J Cell Biochem 111:14–19.

KHAN E, ABU-AMER Y (2003) Activation of peroxisome proliferator-activated receptor-γ inhibits differentiation of preosteoblasts. J Lab Clin Med 142: 29-34.

KHOSLA S, PETERSON JM, EGAN K, JONES JD, RIGGS BL (1994) Circulating cytokine levels in osteoporotic and normal women. J Clin Endocrinol Metab 79:707–611.

KOBAYASHI H, GAO Y, UETA C, YAMAGUCHI A, KOMORI T (2000) Multiline-age differentiation of Cbfa1-deficient calvarial cells in vitro. Biochem Biophys Res Commun 273: 630-636.

KOLF CM, CHO E, TUAN RS (2007) Biology of adult mesenchymal stem cells: regulation of niche, self-renewal and differentiation Arthritis Research & Therapy 9:204 -213.

KRINGS A, RAHMAN S, HUANG S, LU Y, CZERNIK PJ, LECKA-CZERNIK B (2012) Bone marrow fat has browne adipose tissue characteristics, which are attenuated with aging and diabetes. Bone 50: 546-552.

KRISHNAN V, BRYANT HU, MACDOUGALD OA (2006) Regulation of bone mass by Wnt signaling. J Clin Invest 116: 1202-1209.

KUHN NZ, TUAN RS (2010) Regulation of stemness and stem cell niche of mesenchymal stem cells: implications in tumorigenesis and metastasis. J Cell Physiol 222: 268-277.

KULTERE B, FRIEDL G, JANDROSITZ A, SÁNCHEZ-CABO F, PROKESCH A, PAAR C, SCHEIDELER M, WINDHAGER R, PREISEGGER KH, TRAJANOSKI Z (2007) Gene expression profiling of human mesenchymal stem cells derived from bone marrow during expansion and osteoblast differentiation. BMC Genomics 8: 70-84.

LECKA-CZERNK B (2012) Marrow fat metabolism is linked to the systemic energy metabolism. Bone 50: 534-539.

LECKA-CZERNIK B, GRINNELL SJ, MOERMAN EJ, CAO X, MANOLAGAS SC, OBRIEN CA (1999) Identification of a Smad binding element in the PPARγ2 promoter: A potential site of cross-talk between osteoblastogenesis and adipogenesis signaling pathways. J Bone Miner Res 14: Suppl1, S1056.

LECKA-CZERNIK B, GUBRIJ I, MOERMAN EA, KAJKENOVA O, LIPSCHITZ DA, MANOLAGAS SC, JILKA RL (1999) Inhibition of Osf2/Cbaf1 expression and terminal osteoblast differentiation by PPAR-gamma2. J Cell Biochem 74: 357-371.

LEE WY, KANG MI, OH ES, HAN JH, CHA BY, LEE KW, SON HY, KANG SK, KIM CC. (2002) The role of cytokines in the changes in bone turnover following bone marrow transplantation. Osteoporos Int 13:62–68.

LI J, TSUJI K, KOMORI T, MIYAZONO K, WRANA JL, ITO Y, NIFUJI A, NODA M (1998) Smad2 overexpression enhances smad4 gene expression and suppresses Cbfa1 gene expression in osteoblastic osteosarcoma ROS17/2.8 cells and primary rat calvaria cells. J Biol Chem 273: 31009-31015.

LI X, SHET K, RODRIGUEZ JP, PINO AM, KURHANEWICZ J, SCHWARTZ A, ROSEN CJ (2012) Unsaturation Level Decreased in Bone Marrow Lipids of Postmenopausal Women with Low Bone Density Using High Resolution HRMAS NMR. ASBMR 2012 Annual Meeting American Society of Bone and Mineral Research. October 12-15, 2012, Minneapolis, Minnesota, USA.

LIN NH, MENICANIN D, MROZIK K, GRONTHOS S, BARTOLD PM (2008) Putative stem cells in regenerating human periodontium. J Periodont Res 43: 514–523.

LINDNERA U, KRAMERA J, ROHWEDEL J, SCHLENKE P (2010) Mesenchymal stem or stromal cells: toward a better understanding of their biology? Transfus Med Hemother 37:75–83

LIU JP, BAKER J, PERKINS AS, ROBERTSON EJ, EFSTRATIADIS A (1993) Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). Cell 75:59–72.

LOU J, XU F, MERKEL K, MANSKE P (1999) Gene therapy: adenovirus-mediated human bone morphogenetic protein-2 gene transfer induces mesenchymal progenitor cell proliferation and differentiation in vitro and bone formation in vivo. J Orthop Res 17: 43-50.

MCBRIDE SH, FALLS T, KNOTHE TATE ML (2008) Modulation of stem cell shape and fate B: mechanical modulation of cell shape and gene expression. Tissue Eng Part A 14: 1573-1580.

MEUNIER P, AARON J, EDOUARD C, VIGNON G (1971) Osteoporosis and the replacement of cell populations of the marrow by adipose tissue. A quantitative study of 84 iliac bone biopsies. Clin Orthop Relat Res 80:147-154.

MINGUELL JJ, ERICES A, CONGET P (2001) Mesenchymal stem cells. Exp Biol Med 226:507-520.

MOERMAN EJ, TENG K, LIPSCHITZ DA, LECKA-CZERNIK B (2004) Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: the role of PPAR-γ 2 transcription factor and TGF-β/BMP signaling pathways. Aging Cell 3:379–389.

MOLDES M, ZUO Y, MORRISON RF, SILVA D, PARK BH, LIU J, FARMER SR (2003) Peroxisome-proliferator-activated receptor gamma suppresses Wnt/beta-catenin signalling during adipogenesis. Biochem J 376: 607-613.

MOORE SG, DAWSON KL (1990) Red and yellow marrow in the femur: Age-related changes in appearence at MR imaging. Radiology 175: 219-223.

MURUGANADAN S, ROMAN AA, SINAL CJ (2009) Adipocyte differentiation of bone marrow-derived mesenchymal stem cells: cross talk with the osteoblastogenic program. Cell. Mol. Life. Sci 66:236-253.

NUTTALL M, GIMBLE JM (2004) Controlling the balance between osteoblastogenesis and adipogenesis and the consequent therapeutic implications. Curr Opin Pharmacol 4:290-294.

OMATSU Y, SUGIYAMA T, KOHARA H, KONDOH G, FUJII N, KOHNO K, NAGASAWA T (2010) The essential functions of adipo-osteogenic progenitors as the hematopoietic stem and progenitor cell niche. Immunity 33: 387-399.

PENG XD, XU PZ, CHEN ML, HAHN-WINDGASSEN A, SKEEN J, JACOBS J, SUNDARARAJAN D, CHEN WS, CRAWFORD SE, COLEMAN KG, HAY N (2003) Dwarfism, impaired skin development, skeletal muscle atrophy, delayed bone development, and impeded adipogenesis in mice lacking Akt1 and Akt2. Genes Dev 17:1352–1365.

PINO AM, RÍOS S, ASTUDILLO P, FERNÁNDEZ M, FIGUEROA P, SEITZ G, RODRÍGUEZ JP (2010) Concentration of adipogenic and pro inflammatory cytokines in the bone marrow supernatant fluid of osteoporotic women. J Bone Min Res 25, 492-498

PRUSTY D, PARK B-H, DAVIS KE, FARMER SR (2002) Activation of MEK/ERK signaling promotes adipogenesis by enhancing peroxisome proliferators-activated receptor γ (PPARγ) and C/EBPα gene expression during the differentiation of 3T3-L1 preadipocytes. J Biol Chem 277: 46226-32.

QIU W, ANDRESEN TE, BOLLERSLEV J, MANDRUP S, ABDALLAH BM, KASSEM M (2007) Patients with high bone mass phenotype exhibit enhanced osteoblast differentiation and inhibition of adipogenesis of human mesenchymal stem cells. J Bone Min Res 22: 1720-1731.

RAISZ LG (2005) Pathogenesis of osteoporosis. Concepts, conflicts and prospects. J Clin Invest 115:3318-3325.

RAWADI G, VAYSSIERE B, DUNN F, BARON R, ROMÁN-ROMÁN S (2003) BMP-2 controls alkaline phosphatase expression and osteoblast mineralization by a Wnt autocrine loop. J. Bone Miner. Res. 18, 1842–1853.

RODRÍGUEZ JP, ASTUDILLO P, RÍOS S, PINO AM (2008) Involvement of adipogenic potential of human bone marrow mesenchymal stem cells (MSCs) in osteoporosis. Curr Stem Cell Res and Ther 3, 208-218.

RODRÍGUEZ JP, RÍOS S, FERNÁNDEZ M, SANTIBÁÑEZ JF (2004) Differential activation of ERK1,2 MAP kinase signaling pathway in mesenchymal stem cell from control and osteoporotic postmenopausal women. J Cell Biochem 92: 745-754

RODRÍGUEZ JP, MONTECINOS L, RÍOS S, REYES P, MARTÍNEZ J (2000) Mesenchymal stem cells from osteoporotic patients produce a type I collagen-deficient extracellular matrix favoring the adipogenic differentiation. J Cell Biochem 79: 557-565.

RODRÍGUEZ JP, GARAT S, GAJARDO H, PINO AM, SEITZ G (1999) Abnormal osteogenesis in osteoporotic patients is reflected by altered Mesenchymal Stem Cells dynamics. J Cell Biochem 75: 414-423.

ROSEN CJ, ACKERT-BICKNELL C, RODRÍGUEZ JP, PINO AM (2009) Marrow fat and the bone micro-environment: developmental, functional and pathological implications. Crit Rev Eukaryot Gene Expr 19: 109-124.

ROSEN CJ, BOUXSEIN ML (2006) Mechanisms of disease: is osteoporosis the obesity of bone? Nature Clinical Practice Rheumatology 2: 35-43.

ROSEN CJ, DONAHUE LR, HUNTER SJ (1994) Insulin-like growth factors and bone: The osteoporosis connection. Proc Soc Exp Biol Med 206:83–102.

ROSS SE, HEMATI N, LONGO KA, BENNETT CN, LUCAS PC, ERICKSON RL, MACDOUGALD OA (2000) Inhibition of adipogenesis by Wnt signaling. Science 289, 950– 953.

SAMBROOK P, COOPER C (2006) Osteoporosis. Lancet 367:2010-2018.

SEEBACH C, HENRICH D, TEWKSBURY R, WILHELM K, MARZI I (2007) Number and proliferative capacity of human mesenchymal stem cells are modulated positively in multiple trauma patients and negatively in atrophic nonunions. Calcif Tissue Int 80: 294-300.

SEO B, MIURA M, GRONTHOS S, BARTOLD PM, BATOULI S, BRAHIM J, YOUNG M, ROBEY PG, WANG CY, SHI S. (2004) Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 364: 149–155.

SHAPIRO IM (1999) Discovery: Osf2/Cbfa1, a master gene of bone formation. Clin Orthop Res 2(1): 42-46

SHOBACK D (2007) Update in osteoporosis and metabolic disorders. J Clin Endocrinol Metab 92:747-753.

SONG L, WEBB NE, SONG Y, TUAN RS (2006) Identification and functional analysis of candidate genes regulating mesenchymal stem cells self-renewal and multipotency. Stem Cells 24: 1707-1718.

TONTONOZ P, HU E, GRAVES RA, BUDAVARI AI, SPIEGELMAN BM (1994) mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. Genes Dev 8: 1224-1234.

TREMAIN N, KORKKO J, IBBERSON D, KOPEN GC, DIGIROLAMO C, PHINNEY DG (2001) Micro SAGE analysis of 2,353 expressed genes in a single cell-derived colony of undifferentiated human mesenchymal stem cells reveals mRNAs of multiple cell lineages. Stem Cells 19: 408-418.

USUI T, URANO T, SHIRAKI M, OUCHI Y, INOUEET S (2007) Association of a single nucleotide polymorphism in *Wnt10b* gene with bone mineral density. Geriatr Gerontol Int 7: 48-53

WANG YM, NISHIDA S, BOUDIGNON BM, BURGHARDT A, ELALIEH HZ, HAMILTON MM, MAJUMDAR S, HALLORAN BP, CLEMENS TL, BIKLE DD (2007) IGF-I receptor is required for the anabolic actions of parathyroid hormone on bone. J Bone Miner Res 22:1329–1337

WESTENDORF JJ, KAHLER RA, SCHROEDER TM (2004) Wnt signaling in osteoblasts and bone diseases. Gene 341: 19–39.

WIIG H, BERGGREEN E, BORGE BA, IVERSEN PO (2004) Demonstration of altered signaling responses in bone marrow extracellular fluid during increased hematopoiesis in rats using a centrifugation method. Am J Physiol Heart Circ Physiol 286:H2028–H2034.

WRONSKI TJ, WALSH CC, IGNASZEWSKI LA (1986) Histologic evidence for osteopenia and increased bone turnover in ovariectomized rats. Bone 7: 119-123.

XIAN L, WU X, PANG L, LOU M, ROSEN CJ, QIU T, CRANE J, FRASSICA F, ZHANG L, RODRIGUEZ JP, JIA X, YAKAR S, XUAN S, EFSTRATIADIS A, WAN M, CAO X (2012) Matrix IGF-1 Regulates Bone Mass by Activation of mTOR in MSCs: Implications for the Aging Skeleton. Nat Med 18, 1095-1101.

XU J, WANG W, KAPILA Y, LOTZ J, KAPILA S (2009) Multiple differentiation capacity of STRO-1+/CD146+ PDL mesenchymal progenitor cells. Stem Cells Dev. 18: 487–496.

YEUNG DK, GRIFFITH JF, ANTONIO GE, LEE FK, WOO J, LEUNG PC (2005) Osteoporosis is associated with increased marrow fat content and decreased marrow fat unsaturation: a proton MR spectroscopy study. J Magn. Reson Imag 22: 279-285

ZAYZAFON M, GATHINGS WE, MCDONALD JM (2004) Modeled microgravity inhibit osteogenic differentiation of human mesenchymal stem cells and increases adipogenesis. Endocrinology 145: 2421-2432.

ZHANG M, XUAN S, BOUXSEIN ML, VON STECHOW D, AKENO N, FAUGERE MC, MALLUCHE H, ZHAO G, ROSEN CJ, EFSTRATIADIS A, CLEMENS TL (2002) Osteoblast specific knockout of the insulin-like growth factor (IGF) receptor gene reveals an essential role of IGF signalling in bone matrix mineralization. J Biol Chem 277:44005-44012.

ZHAO G, MONIER-FAUGERE MC, LANGUB MC, GENG Z, NAKAYAMA T, PIKE JW, CHERNAUSEK SD, ROSEN CJ, DONAHUE LR, MALLUCHE HH, FAGIN JA, CLEMENS TL (2000) Targeted overexpression of insulin-like growth factor I to osteoblasts of transgenic mice: Increased trabecular bone volume without increased osteoblast proliferation. Endocrinology 141:2674–2682.

ZHOU S, GREENBERGER JS, EPPERLY MW, GOFF JP, ADLER C, LEBOFF MS, GLOWACKI J (2008) Age-related intrinsic changes in human bone-marrow-derived mesenchymal stem cells and their differentiation to osteoblasts. Aging Cell 7: 335-343.

ZIPORI D (2005) The stem state: Plasticity is essential, whereas self-renewal and hierarchy are optional. Stem Cells 23: 719-726.

ZIPORI D (2006) The stem state: mesenchymal plasticity as a paradigm. Curr Stem Cell Res Ther 1: 95-102.