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

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Abstract

The formation, maintenance, and repair of bone tissue involve close interlink 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' activities 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 out 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.

Keywords

MSCs; osteoporosis; adipogenesis; bone marrow microenvironment

Background.

The formation, maintenance, and repair of bone tissue depend on fine-tuned interlink 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

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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 an 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; Lindner 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.

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, MSCs' fate appears to be selected 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 would be 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-MSCs' 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 (Kulter 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, which should be present in the cell microenvironment. The adequate combination lead to the activation of lineage-specific transcriptional regulators, including Runx2, Dlx5, and osterix for osteoblasts, and PPAR γ 2 and a family of CAAT enhancer binding proteins for adipocytes (Muruganandan 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 (Muruganandan 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 PPAR γ 2 (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 showed that activation of the Wnt signaling pathway increased 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 PPAR γ 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 require 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 would prevail in the bone marrow of osteoporosis patients, upsetting MSCs' activities and their 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 the osteo-/adipogenic processes are also observed in other conditions characterized by bone loss such as aging, immobilization, microgravity, ovariectomy, diabetes, and 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), showed 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 MSCs differentiation has shown that activation of PPAR γ 2, 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 PPAR γ 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 to 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 (¹H-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 adipocyte number increases with age such that in humans older than 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 at first considered to be a '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 adipocyte phenotype. Then, considering that lineage fate of MSCs is dependent on early activation by specific BMPs, PPAR γ 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-BMSCs the expression level of mRNA for β -catenin, Dkk-1, and BMPRIIB was similar; while the level of mRNA for Wnt 3a was undetectable in both types of samples. The expression level of mRNA for GSK-3 β , LRP6 and Osx was lower in o-BMSCs than in c-BMSCs, while the mRNA level for Ror2, Wnt 5a, BMPRIA showed doubtful. To further quantify the expression level of GSK-3 β , LRP6, Osx, Ror2, Wnt 5a, BMPRIA real time RT-PCR was performed. As shown in Table I, statistically significant decreased mRNA levels for GSK-3 β , LRP6 and Osx (0.64, 0.26 and 0.18 fold, respectively) were observed in o-BMSCs, as compared with c-BMSCs. 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 of forming 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. The knowledge of

the intramedullary concentration of compounds with recognized regulatory effects on bone formation or resorption is scarce, 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 IGF-BPs 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 IGF-BP3/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 characterize by intrinsic functional alteration leading to poor osteogenic capability and increased adipogenesis. Osteoporotic bone marrow microenvironment differentiates from the control one 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 sense osteoporosis could be homologated to other age-related

diseases such as obesity, atherogenesis and diabetes, which are characterized by extramedullary unbalanced adipocyte formation and signaling.

Currently it is not known how damaged o-MSCs surge, further work is needed to ascertain the role of microenvironment, genetic and epigenetic and 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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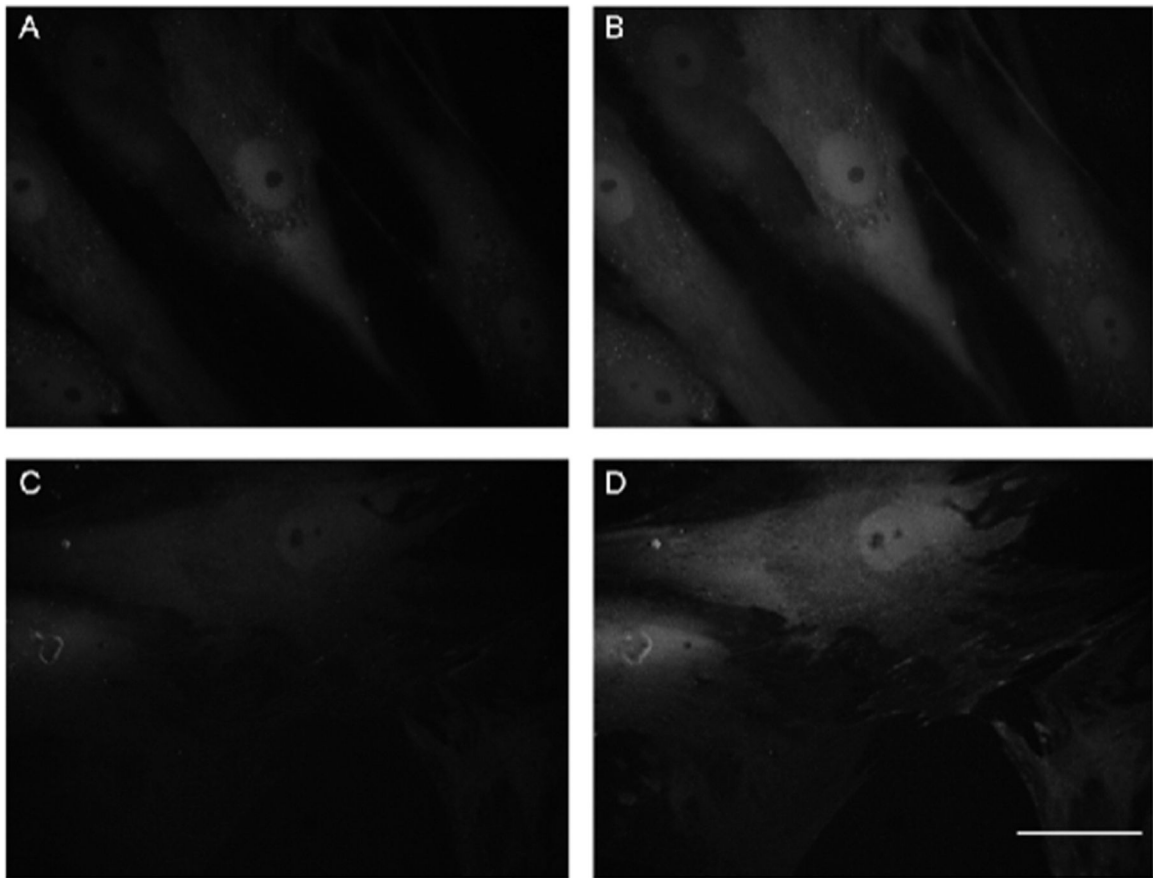


Figure 1.

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, PPAR γ 2: Peroxisome proliferator-activated receptor gamma 2, C/EBP: CCAAT/enhancer-binding protein

Table I.

Functional characteristics of osteoporotic- and control- MSCs.

	Condition	Incubation Time (days)	c-MSCs	o-MSCs	Reference
General Metabolic:					
Total Colonies Number (CFU-F)	Basal	14	12.7±5.6	14.1±2.6	Personal communication
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*	Personal communication
LRP6 mRNA level (relative to c-MSCS)	Basal	-	1.00±0.30	0.197±0.05*	Personal communication
Osx mRNA level (relative to c-MSCS)	Basal	-	1.023±0.48	0.098±0.04*	Personal communication
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			326±147	493±152	Personal communication
Size			87.5±23.8	95.2±3.7	Personal communication
Fluorescence Index	Adipogenic	14	3.64±0.43	2.13±0.15*	Personal communication

Basal: Non differentiation condition; OS: Osteogenic differentiation condition; AD: Adipogenic differentiation condition;

* p<0.05.

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 (μ g/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 (in press)
IGFBP-3 (ng/ml)	24,52±5,98	27,88±8,52	Personal communication
IGF-1/IGFBP-3	3.1	1.72	Personal communication

BMF= Bone marrow fluid.

^{*}
p<0.05.