**REVIEW ARTICLE**

# Adipogenesis and osteoblastogenesis: trans-differentiation in the pathophysiology of bone disorders

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**Abstract**

Mesechymal stem cells as pluripotent cells are involved in the differentiation of adipocytes under regulation of genes and transcription factors. The plasticity observed between adipocytes and osteoblasts differentiation is the basis of trans-differentiation, observed in both experimental and clinical level. This review analyzes not only the adipose tissue as an endocrine organ but also the underlying mechanism of trans-differentiation between adipocytes and osteoblasts. Fat and bone tissue interaction is altered by activation or silencing of genes, signaling molecules and transcription factors. Disorders of this interaction include ectopic ossification syndromes and other bone disorders like osteoporosis and multiple myeloma. Further research will reveal the instinct mechanisms of this imbalance in the pathophysiology of many metabolic disorders such as diabetes mellitus, atherogenesis e.t.c. Hippokratia 2011; 15 (1): 18-21

**Key words:** mesenchymal stem cells, adipogenesis, osteoblastogenesis, osteoporosis, ectopic ossification syndromes, multiple myeloma, review

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Mesenchymal stem cells (MSCs) are pluripotent, potentially differentiated cells with high mitotic index. The differentiation mechanisms of their precursor forms (progenitors) and their development into mature cells (osteoblasts, adipocytes, etc) are not fully understood. Probably silencing or over-expression of genes in specific stages of differentiation of MSCs is fatal for each cell line. Similarly, the activation of specific signalling pathways involved in differentiation of MSCs seems to play an important role in the process of maturation of cells[1].

Osteoporosis, defined by the International Osteoporosis Foundation (IOF), is a multifactorial disease characterized by systemic bone loss and deterioration of bone microarchitecture. Clinical studies have shown that bone loss in osteoporotic patients and in people with age-dependent bone loss, is associated with increased adipose tissue in the bone marrow[2,3]. Experimental models of mammals after ovariectomy, immobility or treatment with glucocorticoids revealed the deposition of fat cells into the bone marrow[4-9]. Notice that fat cells in bone marrow have common origin with MSCs, potentially differentiated in osteoblasts. It is speculated that the presence of dynamic equilibrium between adipogenesis and extensive formation of bone is the "melting point" in the prevention or therapeutics of diseases characterized by disturbances of the balance (Table 1).

## Adipogenesis and hormones of fat tissue: the "new" endocrine organ

Adipogenesis is the process by which undifferentiated precursor cells differentiate into mature adipocytes. At the stages of embryogenesis, but also in the evolu-

**Table 1:** Therapeutic targets of balancing osteoblasts and adipocytes on molecular level.

| Family | Molecular target |
|---|---|
| Nuclear hormone receptors | Androgen receptor, estrogen receptor, glucocorticoid receptor, vitamin $D_3$ receptor, PPAR, 12/15 lipoxygenase |
| Protein hormone/cytokine transmembrane receptor | Adiponectin, BMP, RANKL, notch/jagged, noggin, interleukin-6, interleukin-11, leptin |
| Cytoskeleton proteins | RhoA |
| G-protein-coupled receptors | PTH, GNAS1, IGF-I |
| Wnt signaling pathway | LRP5, β-catenin |

tion of the human organism, the deposition of fat tissue in the bone marrow is a passive process of storing fat cells within the marrow cavities that are not required to haematopoiesis. The complex process of differentiation of adipocytes is characterized by changes in cellular morphology, hormone sensitivity, gene expression and secretory capacity. In particular, adipocytes secret local factors, such as adepsin, leptin, adiponectin, TNF-α (Tumor Necrosis Factor-α) and angiotensinogen. These factors are characterized by significant pleiotropic effect on the local microenvironment of bone marrow cells, including osteoblasts[10-12].

Further studies indicate the trans-differentiation process (diversion during differentiation of a cell line to another of common origin) between the cells of the bone marrow, in the differentiation and maturation mechanisms of adipocytes[13,14]. One of the main characteristics of osteoblasts and adipocytes is plasticity which is observed in both experimental and clinical level. It seems that the excretion of some or sometimes all of the adipokines, are fatal for the adipocyte which can be converted into osteoblast. Today the adipose tissue is not considered to be a storage tissue but is characterized as an endocrine organ with an important role in the secretion adipokines (leptin, adiponectin) and hormones (estrogen, vitamin $D_3$, androsterone, cortisone), involved in the pathophysiology of different disorders entities, such as diabetes, obesity, inflammation and atherogenesis. In particular, leptin seems to be an indicator of the energy state of human organism. Lack of leptin, leads to obesity with significant involvement in the mechanisms regulating bone metabolism (trans-differentiation). In particular, leptin controls the RANKL/OPG axis (Receptor Activator for Nuclear Factor k B Ligand / Osteoprotegerin) by inhibiting the expression of RANKL and inducing the ORG in pre-osteoblasts and mononuclear cells in circulation (Figure 1). The diversion of an adipocyte into osteoblast is considered to be a multifactorial process regulated by all these factors[10,11,15-19].

Physiologically, adipogenesis is strictly controlled by hormones, cytokines, nutrients, alterations in the expression and/or activity of transcription factors that directly affect the different mechanisms of adipocyte

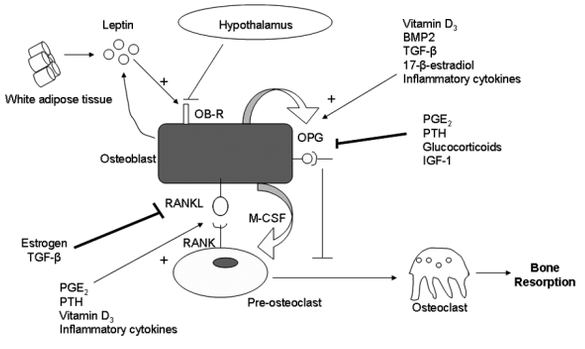


**Figure 1:** The interaction of white adipose tissue and other factors in differentiation of osteoblast.

differentiation. Transcription factors such as CEBP-α,-β,-δ (CCAT/enhancer binding protein-α,-β,-δ), PPARγ (Peroxisome proliferator-activated receptor-γ) and SREBP1 (sterol-regylatory element-binding transcription factor 1) are the "lead players" of adipocyte differentiation. Taking account that C/EBPδ is expressed in early differentiation of pre-adipocyte while in the final stages of maturation C/EBPα is expressed[11,12]. Alongside FgF10 (Fibroblast growth factor-10) acts synergistically in the final stages of maturation of fat cells, according to studies in knockout mice ($Fgf10^{-/-}$)[20].

Particularly PPAR proteins are investigated and their implication in controlling the balance between osteoblastogenesis and adipogenesis. The PPARs' are transcription factors that appear in three different isoforms: PPAR-α, PPAR-γ and PPAR-β/δ. The transcription factor PPARγ ligands include the long chains and oxidized derivatives of fatty acids and thiazolidinedione compounds. This factor is expressed in two isoforms, RPARγ1 and PPARγ2 due to alternative splicing or promoters of primary genes. Synthetic agonists of PPARγ such as derivatives thiazolidinedione are used as insulin-sensitive substances in the treatment of diabetes mellitus type II. However, adipogenesis is an unwanted effect of these compounds for the treatment of metabolic diseases in general. Studies have shown that the activation of PPARγ receptor, promotes adipogenesis of stromal cells in bone marrow and inhibits osteoblastogenesis[10,11,21]. Especially PPARγ2 induces the differentiation process of adipose tissue associated with bone metabolism. It seems that the ligands of PPARγ not only activate this nuclear receptor but also suppress the Cbfa-1 (Core binding factor a1)[11,14,21,22]. Furthermore studies focus on lipoxygenase inhibitors which reduce the concentration of oxidized fatty acids, by inhibiting the ligands of PPARα and/or PPARγ, thereby promoting adipogenesis and suppressing bone regeneration. Current research must be focus on the genes that encode nuclear receptors, in order to clarify the mechanisms underlying adipogenesis[11,14].

**Notch/Delta signalling pathway and RhoA gene in adipogenesis**

The signalling pathway of Notch/Delta factors and their receptors associated with the EGF (Epidermal Growth Factor) are under current research. Overexpression of notch *in vitro* inhibits osteogenesis, but promotes adipogenesis in cell models, through downregulation of the Wnt receptor function and the switch of *β*-catenin (necessary factors for the process of adipogenesis). The Wnt peptides and their receptors, interact with LRPs (Low-density Receptor-related proteins)[10,11,23,24]. Thus mutations of LRP5 are associated with loss of bone mass. Substances, such as lithium, alter Wnt signalling pathway by inhibiting adipogenesis. Undifferentiated stromal cells and pre-adipocytes involved in Wnt / LRP signalling pathway, express Pref-1 (Preadipocyte factor-1), a factor which inhibits adipogenesis, causing skeletal abnormali-

ties[11,25]. In addition dlk protein, a member of EGF family of proteins, contributes to the inhibition of adipogenesis hematopoiesis, glandular and neuroendocrine cell differentiation through intervention of Notch1 signalling pathway, with induced bone regeneration[11].

In human MSCs culture, the insertion of the RhoA gene has been shown to induce the bone cell differentiation with stretched form. The shape of the cell, the tendency of cytoskeleton and the particular gene expression, significantly influence the differentiation of MSCs into bone cells. Exercise and particularly mechanical stress seem to strengthen the bone[11]. Therefore mechanical stress and collagenous matrix somehow triggers the MSCs in the bone marrow in the process of differentiation into bone cells[11,26].

**Hereditary disorders associated with ectopic ossification and other bone disorders**

POH (Progressive Osseous Heteroplasia) is a rare disease characterized by ectopic ossification, starting in childhood in adipocytes of hypodermic layer. In the years this process is changing at the lower connective tissue. Genetic studies in patients with POH demonstrated important mutations of *GNAS1* gene (GNAS1 - Guanine Nucleotide-Binding α-polypeptide 1), that are characterized by reduced levels of the α-functional subunit of G-proteins[27].

Similar mutations in the *GNAS1*, are observed in patients with AHO (Albright's hereditary osteodystrophy). AHO was first described in 1942 and is characterized by morphological and endocrine abnormalities. Shortness of the metatarsal bones and round facies are the major malformations described in patients with AHO. In addition these patients have a significant resistance to parathyroid hormone with hypocalcemia, hyperphosphatemia. There may also have resistance to other hormones (ovarian, thyroid, neuro-pituitary gland). Surprisingly the inheritance of the mutant gene from the father leads to POH, while from the mother to AHO (location in the 20$^{th}$ chromosome, 20q12-q13.2), as a result of genomic imprinting. Studies in knockout mice demonstrated the different expression of GNAS1 in tissue level. Although we know that this gene is expressed in various tissues, what it is not known yet is the exact mechanism by which the mutation is transmitted to GNAS1. However the mutation leads to ectopic ossification of adipocytes' origin that transforms fat cells into osteoblasts[27-29].

A disease that its entity has some common phenotypic features with POH is FOP (fibrodysplasia ossificans progressive). FOP is an autosomal dominant disease that is characterized by skeletal abnormalities and progressive development of bone segments in tendons and muscles. It is observed that fibroblasts convert into osteocytes by endochondral ossification. Studies in Drosophila melanogaster revealed mutations in the genetic locus ddp, with a similar phenotype of FOP. Ddp genetic locus encodes a protein that belongs to TGF-β superfamily of proteins (transforming growth factor-β), called BMP2 (Bone morphogenetic protein-2). However, in human peripheral monocytes of patients with FOP, there was an overexpression of BMP4, mRNA a morphogenetic protein responsible for any endochondral heterotopic ossification[27,30]. This morphogenetic protein seems to be a key factor for involving not only bone regeneration process but also the transition between fat and bone cell.

A great example of this imbalance is Multiple Myeloma (MM). MM cells suppress osteoblast formation and differentiation and thereby inhibit bone formation. Under physiological conditions the osteogenic differentiation of mesenchymal cells is tightly regulated either by system hormones, such as parathyroid hormone (PTH), estrogens and glucocorticoids or by local growth factors, including the BMP family, interleukins, TGF-β, insulin growth factor (IGF) and fibroblast growth factor 2. Moreover these factors activate specific intracellular signal pathways that modify the expression and activity of several transcription factors in mesenchymal and osteoprogenitor cells, which result in osteoblastic differentiation. Further studies also are necessary to clarify a possible connection between adipogenesis, osteoblastogenesis and the pathophysiology of MM[31-36].

Several mechanisms are potentially involved in MM - induced inhibition of osteoblast formation and differentiation. MM cells inhibit osteoblastogenesis by blocking Runx2 activity in mesenchymal and osteoprogenitor cells through direct cell-to-cell contact with the involvement of very late antigen a (VLA-4) / vascular cell adhesion molecule 1 (VCAM-1). Soluble factors as interleukin (IL-7) may contribute to the suppression of Runx 2 activity by MM cells. Direct production of the Wnt inhibitor Dickkopf-1 (Dkk-1), secreted frizzled related protein (sFRP)-3 and rarely sFRP-2 by MM cells as well as the overproduction of hepatocyte growth factor could inhibit osteoblast formation. Finally, IL-3 overproduction in the MM microenviroment is involved in the inhibition of osteoblast formation and differentiation[36].

**Conclusion**

Understanding the process of differentiation of adipocytes and trans-differentiation into osteoblasts is crucial in order to identify genes and other factors that may contribute to the pathophysiology of hereditary ectopic ossificans and other bone or joint disorders[37]. It is known that disturbances of the balance between osteoblastogenesis and adipogenesis lead to metabolic diseases such as obesity, diabetes e.t.c. Therefore therapeutic interventions must focus on manipulating the "thin line" between osteoblastogenesis and adipogenesis.

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