OSTEOBLASTS AND BONE FORMATION

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Abstract

Bone is constantly being remodelled in a dynamic process where osteoblasts are responsible for bone formation and osteoclasts for its resorption.

Osteoblasts are specialized mesenchymal cells that undergo a process of maturation where genes like core-binding factor $\alpha 1$ (Cbfa1) and osterix (Osx) play a very important role. Moreover, it was found recently that the Wnt/β-catenin pathway plays a part on osteoblast differentiation and proliferation. In fact, mutations on some of the proteins involved in this pathway, like the low-density lipoprotein receptor related protein 5/6 (LRP5/6), lead to bone diseases.

Osteoblasts have also a role in the regulation of bone resorption through receptor activator of nuclear factor-κB (RANK) ligand (RANKL), that links to its receptor, RANK, on the surface of pre-osteoclast cells, inducing their differentiation and fusion. On the other hand, osteoblasts secrete a soluble decoy receptor (osteoprotegerin, OPG) that blocks RANK/RANKL interaction by binding to RANKL and, thus, prevents osteoclast differentiation and activation. Therefore, the balance between RANKL and OPG determines the formation and activity of osteoclasts.

Another factor that influences bone mass is leptin, a hormone produced by adipocytes that have a dual effect. It can act through the central nervous system and diminish osteoblasts activity, or can have an osteogenic effect by binding directly to its receptors on the surface of osteoblast cells.

Keywords: Osteoblasts; Bone Formation; Wnt/β- -catenin Signalling; Leptin; Cbfa1.

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Resumo

O osso está em constante remodelação num processo dinâmico, em que os osteoblastos são responsáveis pela sua formação e os osteoclastos pela sua reabsorção.

Os osteoblastos derivam de células mesenquimatosas que sofreram um processo de maturação, no qual genes como o *core-binding factor $\alpha 1$* (Cbfa1) e o*sterix* (Osx) assumem um papel de grande importância. Foi descoberto recentemente que a via de sinalização Wnt/β-catenina tem um papel relevante na diferenciação e proliferação do osteoblasto. Na realidade, mutações em determinadas proteínas envolvidas nesta via, como o co-receptor *low-density lipoprotein receptor related protein 5/6* (LRP5/6), são causa de doenças ósseas.

Os osteoblastos desempenham também um papel importante na regulação da reabsorção óssea através do *receptor activator of nuclear factor-κB* (RANK) *ligand* (RANKL) que interage com o seu receptor RANK, expresso na superfície de pré-osteoclastos, induzindo a diferenciação e fusão destas células. Por outro lado, os osteoblastos secretam um receptor solúvel, a osteoprotegerina (OPG), que bloqueia a interacção RANK/RANKL através da sua ligação ao RANKL, prevenindo assim a diferenciação e activação do osteoclasto. Desta forma, o balanço entre RANKL e OPG determina a formação e actividade do osteoclasto.

Outro factor que influencia a massa óssea é a leptina, uma hormona produzida pelos adipócitos, que tem um efeito duplo. Pode actuar através do sistema nervoso central e diminuir a actividade do osteoblasto, ou pode exercer um efeito osteogénico através da interacção com os receptores presentes na superfície do osteoblasto.

Palavras-chave: Osteoblastos; Formação do Osso; Sinalização Wnt/β-catenina; Leptina; Cbfa1.

Introduction

Bone must be stiff, slightly flexible and light in order to make loading possible and facilitate movement. In particular, bone has a specific characteristic that distinguishes it from other materials: this tissue can respond according to the location and extent of the damage, remove it and replace it with new bone.[1]

Bone is composed by cells and an extracellular matrix which becomes mineralized by the deposition of calcium hydroxyapatite, giving the bone rigidity and strength. Bone has three distinct cell types: the osteoblasts, or bone-forming cells, the osteoclasts, or bone-resorbing cells, whose functions are intimately linked,[2] and the osteocytes, which are osteoblasts entrapped within lacunae. In order to balance bone formation and resorption in healthy individuals, osteoblasts secrete factors that regulate the differentiation of osteoclasts and osteocytes secrete factors regulating the activity of both osteoblasts[3] and osteoclasts.[1] Bone is constantly being resorbed by osteoclasts and then replaced by osteoblasts in a process called bone remodelling.[2] Resorption is much faster than formation: an area of bone can be resorbed in 2-3 weeks but it takes at least three months to rebuild it.[4] Bone resorption is probably the first event that occurs in response to a mechanical stress signal.[5] In fact, resorption and formation are tightly coupled, so that after resorption a formation phase occurs and, normally, the amount of bone resorbed will be formed in the succeeding phase (Figure 1). This coordination arises from the linkage between osteoblasts and osteoclasts, which is mediated by the release of growth factors from bone during resorption.[6]

On the other hand, osteoblasts, have, in addition to their classic role as bone forming cells, the ability to regulate bone resorption through the expression of receptor activator of nuclear factor-κB (RANK) ligand (RANKL), which links to its receptor, RANK, on the surface of pre-osteoclast cells, inducing their differentiation and causing bone resorption. On the contrary, the soluble decoy receptor (osteoprotegerin, OPG), also produced by osteoblasts, is able to block RANK/RANKL interaction by binding to RANKL and, thus, prevent osteoclast differentiation and activation, reducing bone resorption.[6]

In this article, we will review osteoblast biology and the processes that influence bone formation and resorption through this cell.

Osteoblasts

Osteoblasts have a very important role in creating and maintaining skeletal architecture; these cells are responsible for the deposition of bone matrix and for osteoclasts regulation. Osteoblasts are mononuclear, not terminally differentiated, specialized cells.[7] When they are active, a large Golgi apparatus and an abundant rough endoplasmic reticulum is visible. In addition, osteoblasts form tight junctions with adjacent osteoblasts and have regions of the plasma membrane specialized in vesicular trafficking and secretion.[8] As they differentiate they acquire the ability to secrete bone matrix.[6] Ultimately, some osteoblasts become trapped in their own bone matrix giving rise to osteocytes which, gradually, stop secreting osteoid.[8] Osteocytes are the most abundant cells in bone; these cells communicate with each other and with the surrounding medium through extensions of their plasma membrane.[9,10] Therefore, osteocytes are thought to act as mechanosensors, instructing osteoclasts where and when to resorb bone and osteoblasts where and when to form it.[1,10]
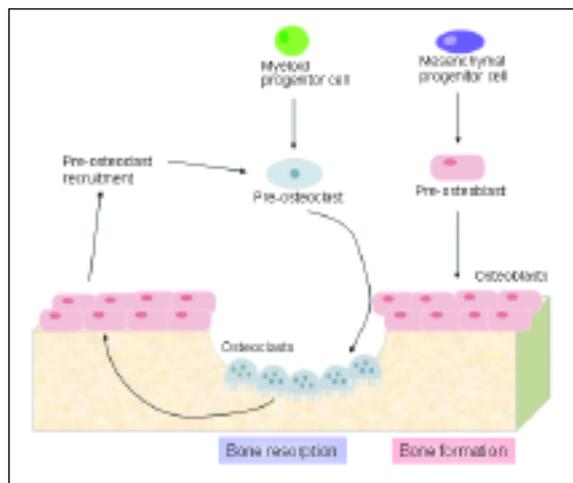

Figure 1. Bone is constantly being resorbed by osteoclasts and formed by osteoblasts in a coupled process, mediated by the release of growth factors from bone during resorption and by molecules produced by osteoblasts, which regulate the differentiation of osteoclasts. (Osteoblasts and osteoclasts symbols were adapted from Rheugulation Database, knowledge base on rheumatoid arthritis, www.rheugulationdb.com)

Wnt pathway on osteoblastogenesis

Wnts are secreted glycoproteins that are found in all animal species.[11] The human genome encodes 19 W*nt* genes that bind to cell-surface receptors and mediate a cascade of events that regulate a variety of cellular activities, including cell fate, determination, proliferation, migration, polarity and gene expression. Wnts activate three distinct intracellular signalling cascades: the Wnt/β-catenin pathway, the Wnt/Ca^{2+} pathway and the Wnt/planar polarity pathway. The Wnt/β-catenin pathway is frequently referred to as the canonical pathway and it promotes cell fate determination, proliferation and survival through the increase of β-catenin levels and alteration of gene expression by the transcription factor Lymphoid enhancer factor/T cell factor (Lef/Tcf).[12] Activation of this signalling pathway occurs with binding of Wnt to Frizzled (Fz), a transmembrane receptor, and low-density lipoprotein receptor related protein 5/6 (LRP5/6) co-receptors[11,13] (Figure 2). In the absence of a Wnt ligand, the cytosolic level of β-catenin is kept low by its phosphorylation and degradation, thereby suppressing the expression of Wnt-responsive genes.[12] Inappropriate activation of Wnt signalling contributes to cancer and reduced Wnt signalling has been associated with osteoporosis.[11,14]

The first indication that Wnt signalling has an important role in bone formation came from studies in humans where mutations that inactivate the Wnt co-receptor LRP5 were shown to cause osteoporosis. Moreover, gain-of-function mutations in this receptor increased Wnt signalling and resulted in a high bone mass phenotype, both in humans and mice,[15] due to an elevated number of active osteoblasts which seem to be protected from apoptosis. In fact, the mutation G171V on LRP5 is a missense mutation[14] that decreases the interaction between LRP5 and the Wnt antagonist Dickkopf1 (Dkk1), thereby diminishing its inhibitory effect on endogenous Wnt signalling.[3] In mice, null mutations in LRP5 resulted in bone loss after birth, due to decrease in bone formation and osteoblast proliferation.[4] Therefore, loss-of-function mutations in LRP5 lead to low bone density whereas gain-of-function mutations cause high bone mass.[14] It was also found that a series of genes involved in the Wnt signalling pathway are induced during fracture repair in rodents, giving strength to the relation between Wnt signalling and LRP5 on bone.[4]
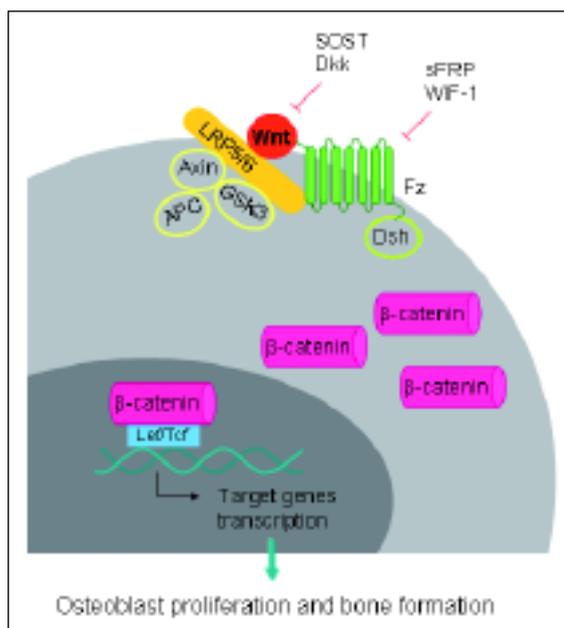

Figure 2. Canonical Wnt signalling has multiple roles in osteoblastogenesis, is essential for osteoblast lineage differentiation early in the development and, postnatally, is involved in osteoblast proliferation, survival and lifespan. Binding of Wnt to Frizzled and LRP5/6 receptors induce a signalling cascade that allows the accumulation of β-catenin in the citosol. β-catenin then enters the nucleus where it promotes the transcription of target genes. APC – Adenomatous Polyposis Coli; Dkk – Dickkopf; Dsh – Dishvelled protein; Fz – Frizzled receptor; GSK3 – Glycogen Synthase Kinase-3β; LRP5/6 – Low-density Lipoprotein Receptor Related Protein 5/6; sFRP – secreted frizzled-related protein; WIF-1 – Wnt inhibitory factor; Lef/Tcf – lymphoid enhancer factor/T cell factor. (Adapted from Krishnan et al.)[13]

LRP5 is expressed at low levels in various tissues, shows little temporal changes, and constitutes a key factor in bone regulation. This effect is linked to the role of LRP5 on the Wnt signalling pathway. LRP5 interacts with proteins from the Wnt family to form a complex with Fz, leading to the activation of the canonical Wnt signalling pathway.[4] In fact, β-catenin activity is essential for the differentiation of mature osteoblasts and, consequently, for bone formation. However, lack of this molecule does not change the differentiation of osteoprogenitor cells into the early osteoblastic precursors but, instead, it blocks the expression of Osterix (Osx) and, as a consequence, these cells acquire a chondrogenic phenotype.[3] The effect of LRP5 on bone mass is mediated by the β-catenin Wnt signalling pathway and, *in vitro*, it results in

the expression of an early osteoblast marker, alkaline phosphatase (ALP). Although this signalling pathway is involved in the regulation of osteoblastogenesis and bone formation, a specific Wnt protein that triggers its activation has still not been identified.[15] In fact, several Wnt genes, such as Wnt1, Wnt4, Wnt5α, Wnt9α/14 and Wnt7b are expressed in either osteoblast precursors or adjacent tissues during development, and Wnt3α and Wnt10b are expressed in bone marrow,[13] but only Wnt10b mutants express a postnatal decrease in bone mass.[3] The expression of Wnt10b in mesenchymal progenitor cells, *in vitro*, induces the expression of the transcription factors core binding factor α1 (Cbfa1), Distal-less homeobox 5 (Dlx5) and Osx, stimulating osteoblastogenesis. These observations substantiate the fact that Wnt signalling influences the differentiation of the precursor cells by increasing the expression of key osteoblastogenic transcription factors[15] (Figure 3). On the other hand, Wnt10b inhibits the transcription factors CCAAT/enhancer-binding protein α (C/EBPα) and peroxisome proliferator-activated receptor γ (PPARγ), blocking adipogenesis.[15] Thus, Wnts have also a role on mesenchymal precursor cells lineage commitment and adipogenesis is most likely
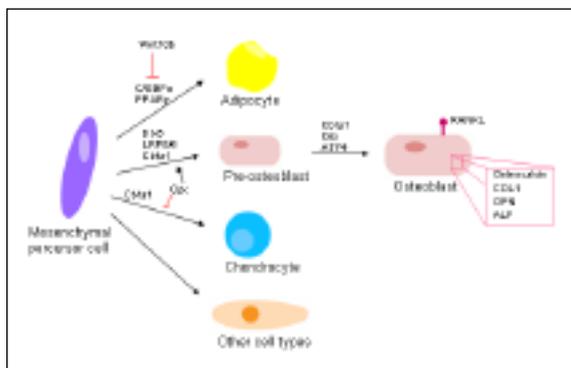

Figure 3. Mesenchymal precursor cells give rise to several cell types, among which are osteoblasts. ALP – alkaline phosphatase; ATF4 – activating transcription factor 4; cbfa1 – core binding factor α1; COL1 – collagen type Iα1; C/EBPα - CCAAT/ /enhancer-binding protein α; Dlx5 – distal-less homeobox 5; LRP5/6 – low – density lipoprotein receptor related protein 5/6; Osx – osterix; OPN – osteopontin; PPARγ – peroxisome proliferators-activated receptor γ; RANKL – receptor activator of nuclear factor-κB ligand. (Osteoblasts and osteoclasts symbols were adapted from Rheugulation Database, knowledge base on rheumatoid arthritis, www.rheugulationdb.com)

the default pathway for the cells that do not receive proper inductive signals to become osteoblasts, chondrocytes, myocytes or other mesodermal cells.[12] As a matter of fact, a relationship between osteoblast and adipocyte differentiation might exist, where an increase in osteoblast differentiation is associated with a decrease in adipocyte differentiation.

Wnt signalling is tightly regulated by secreted antagonists. An example of this regulation is the interaction of Wnt with the receptor Fz which is inhibited by members of the secreted frizzled-related protein family (sFRP) and Wnt inhibitory factor (WIF-1).[12] On the other hand, LRP5/6 activity is antagonized by sclerostin (encoded by the *SOST* gene) and members of the Dkk family.[13] Canonical Wnts upregulate Dkk2 expression which, in turn, controls some of the processes required for terminal osteoblast differentiation, mostly by removing the cells from the cell cycle. In fact, Dkk2 is important in late stages of osteogenic differentiation, particularly for the formation of mineralized matrix.[16] LRP5 and LRP6 are receptors for the Wnts and for other types of molecules such as sclerostin, which is produced by osteocytes, inhibiting the proliferation and maturation of osteoblasts and promoting their apoptosis. Mutations that reduce *SOST* function, in humans, cause abnormal growth of bone tissue, giving rise to a disease named sclerosteosis. Also, the function of mature osteoblasts, including the ability to synthesize extracelular matrix proteins requires LRP5 as well as the signalling protein activating-transcription factor 4 (ATF4).[5]

Wnt/β-catenin pathway acts by attenuating osteoclastogenesis[13] through transcriptional regulation of OPG, since the expression of this molecule was found to be upregulated by canonical Wnt signalling in an *in vitro* screen for Wnt-regulated genes. OPG is also a direct target of the β-catenin-Tcf complex in osteoblasts, and Tcf1 is probably a transcription factor required for OPG regulation.[3]

Genes expressed in osteoblasts

Osteoblasts derive from mesenchymal precursor cells[8,17] which also originate chondrocytes, myoblasts, adipocytes and tendon cells, depending on the transcription factors that regulate the pathway.[4,5,18] Three of these transcription factors are Cbfa1, Osx and ATF4, which have been identified as controllers of the osteoblastic lineage.[5,18] In the absence of Cbfa1 and Osx, no osteoblasts are formed. Also, bone morphogenic proteins (BMPs),

members of a family of secreted growth factors, provide important and specific signals that are essential for full osteoblastogenic differentiation.[5] In cell culture, osteoblasts resemble fibroblasts; the only morphological trait osteoblast specific is located outside the cell in the form of a mineralized extracellular matrix. Moreover, all the genes expressed in fibroblasts are also expressed in osteoblasts. In fact, these cells have only two specific transcripts, one encoding Cbfa1 and other encoding osteocalcin, an inhibitor of osteoclast function that is only expressed when these cells are completely differentiated.[2]

Cbfa1, also known as Runx2, Osf2 and AML3,[4] has all the properties of a differentiation factor for the osteoblast lineage. During embryonic development, Cbfa1 is expressed just before osteoblast differentiation and only in mesenchymal cells committed to become either chondrocytes or osteoblasts. Subsequently, the expression of this transcription factor becomes limited to osteoblasts[2] and is required for the expression of osteoblast-specific proteins, such as osteocalcin.[8] Cbfa1-null mice lack osteoblasts. However, they are able to develop cartilage with late chondrocyte maturation.[2,4] Also, through regulation of osteocalcin expression, Cbfa1 controls bone formation by differentiated osteoblasts. Binding sites for Cbfa1 are also present in the regulatory sequences of most genes that are required for the synthesis of extracellular matrix.[2] Osx is another factor involved on osteoblast differentiation and acts downstream of Cbfa1 to induce differentiation of osteoblasts. Mice that are deficient in Osx develop a normal-shape skeleton composed only by cartilage, without osteoblasts or mineralized matrix. However, their cartilage is normal, containing fully differentiated chondrocytes, which points to a specific role of Osx in osteoblast differentiation.[4]

During differentiation, osteoblasts express a characteristic pattern of genes that distinguish them from other cell types. Collagen type Iα1 (COL1) is expressed from the beginning of osteoblast differentiation and is the main structural component of bone matrix. Osteopontin (OPN), a non-collagenous matrix protein, and ALP are important in stabilizing the matrix. Osteocalcin is another non-collagenous protein that is almost exclusively expressed in bone and is up-regulated in the late differentiation stage. This stage coincides with the onset of mineralization suggesting that osteocalcin may play a part in the regulation of matrix mineralization.[19]

RANKL and OPG

RANKL is a member of the Tumour Necrosis Factor (TNF) superfamily of cytokines. Also known as TNFSF11, TRANCE, OPGL, ODF and CD252,[20] RANKL was initially identified as a cytokine produced by T cells and needed for their interaction with dendritic cells[21] and later it was found that this protein mediates the differentiation of T and B lymphocytes.[22] RANKL is produced as a membrane-bound protein in osteoblasts, bone marrow stromal cells, activated T lymphocytes[23] and smooth muscle cells[24] and cleaved into a soluble form by a metalloprotease.[23] The cell-bound form is the most common and is expressed by many cell types. On the other hand, the expression of the secreted form is restricted to activated T cells[25, 26] and mast cells.[27] Structurally, RANKL has an organization very similar to that of other TNF family members, with a short intracellular domain and a long extracellular domain, where the first exons encode for the intracellular domain, and the extracellular domain is encoded by the middle and last exons. In fact, through analysis of the phylogeny of the TNF family, it was found that RANKL is closely related to CD40L and that these molecules appear to derive from an ancestor gene that diverged from other TNF family members.[20]

TNF family members mediate several biological processes and RANKL is specially important in bone, in the immune system[22] and in mammary epithelium.[28] In bone, the expression of RANKL on osteoblast cells allow the maturation, differentiation and activation of osteoclasts by binding to its receptor, RANK (Figure 4), on osteoclast precursors, in the presence of macrophage-colony stimulating factor (M-CSF).[29,30] Deletion of the RANKL gene in mice resulted in severe osteopetrosis and a complete lack of osteoclasts, as a result of an inability of osteoblasts to support osteoclastogenesis.[20] Analysis of RANKL promoter revealed the presence of binding sites for two potent stimulators of this protein, vitamin D and glucocorticoids, as well as the binding site for Cbfa1.[26,30]

OPG, also known as TNFRS11B, OCIF and TR1, was first identified in 1997 as being a protein that exhibits a protective effect on bone.[31] Although, it is expressed ubiquitously and abundantly in many tissues and cell types.[26] OPG is a member of the
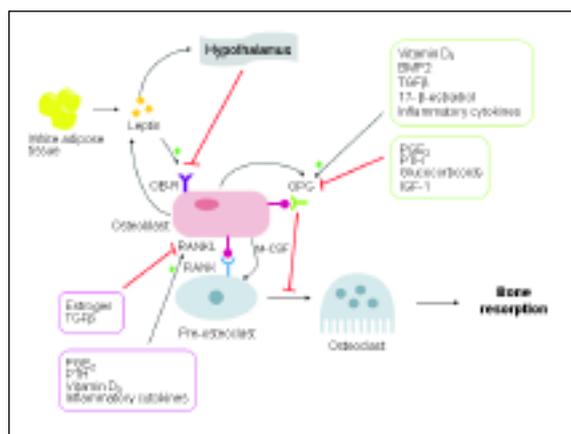
Figure 4. Osteoblasts secrete two proteins, RANKL and OPG, which are responsible for the communication between osteoclasts and their precursors. These two molecules have antagonistic effects on bone mass; while RANKL induce bone resorption, OPG blocks it. Leptin can act through the hypothalamus blocking osteoblast activity, or it can act directly on osteoblast receptors enhancing osteoblast function. IGF-1 – insulin-like growth factor 1; M-CSF – macrophage-colony stimulating factor; OB-R – leptin receptor; OPG – osteoprotegerin; PGE₂ – prostaglandin E₂; PTH – parathyroid hormone; RANK – receptor activator of nuclear factor-κB; RANKL - RANK ligand; TGFβ – transforming growth factor β. (Osteoblasts and osteoclasts symbols were adapted from Rheugulation Database, knowledge base on rheumatoid arthritis, www.rheugulationdb.com)

TNF receptor superfamily, which consists of transmembranar proteins that evoke signal transduction, mediating several biological responses, such as cytotoxicity, apoptosis and cell survival, proliferation and differentiation.[31,32] OPG was identified only as a soluble protein,[31,33] closely related to CD40,[29] which is able to bind to its respective ligand, thereby preventing the activation of cellular targets. In fact, the main function of OPG is to antagonize RANKL effects, by interrupting the signalling between osteoblasts and osteoclast progenitors[28,34] (Figure 4). Indeed, over expression of OPG in mice resulted in osteopetrosis, due to inhibition of osteoclast maturation, whereas OPG-deficient mice exhibit osteoporosis.[25]

The human OPG gene is located on chromosome 8, in an area closely linked to other proteins involved in bone-forming activity, which raised the possibility that this region may enclose a cluster of genes involved in the regulation of bone development and metabolism.[31] The promoter region of the OPG gene has also several polymorphisms that could result in altered binding of transcription factors, possibly affecting gene transcription,[35] and result in changes in bone mineral density which, ultimately, might lead to bone metabolic diseases.[34]

RANKL and OPG are both produced by osteoblasts and have an important role in regulating osteoclasts.[5,6] It has been proposed that several factors can regulate the RANKL/OPG ratio and, thus, regulate osteoclastogenesis. Among these factors are vitamin D_3,[31] IL-1α, IL-1β, TNFα, TNFβ, bone morphogenic protein (BMP) 2, transforming growth factor β (TGFβ) and 17β-estradiol that increase OPG levels, whereas prostaglandin E_2 (PGE₂), parathyroid hormone (PTH),[29] glucocorticoids and insulin-like growth factor-1 (IGF-1) decrease them.[36] There is also evidence pointing to a change of RANKL/OPG ratio in response to gene transcription activity due to polymorphisms enclosed in their promoter regions.[35] On the other hand, PTH, PGE₂, inflammatory cytokines and vitamin D_3 stimulate RANKL, whereas the expression of this molecule is attenuated by estrogen and TGFβ.[37]

In an *in vitro* study, RANKL mRNA levels were found to be higher in undifferentiated cells and decrease 5-fold during osteoblasts differentiation, whereas OPG mRNA levels were much lower in undifferentiated cells and increased 7-fold during differentiation. Moreover, these findings are in agreement with the fact that only undifferentiated cells can support osteoclastogenesis, while partially or completely differentiated cells cannot.[6] Accordingly, the amount of RANKL and OPG expressed by osteoblasts depends on their stage of differentiation: pre-osteoblast cells express high levels of RANKL and relatively low levels of OPG, thus stimulating osteoclast differentiation and function. On the other hand, more mature osteoblasts express higher levels of OPG, in comparison to RANKL levels, inhibiting osteoclast differentiation and function.[5] Hence, a high RANKL/OPG ratio in bone microenvironment is the main molecular mechanism that determines osteoclastogenesis.[6,23] RANKL and OPG mRNA levels were shown to correlate with altered resorption in response to physiological stimuli, such as calcium concentration and hormonal treatment.[19]

The role of Leptin

Leptin was first identified as an hormone secreted

by the white adipose tissue.[23] It acts by binding to a hypothalamus receptor and regulates body weight through appetite suppression and increased energy expending.[4] Leptin is known to act in the immune system, reproduction, development, hematopoiesis, angiogenesis and in the skeletal tissue.[38]

Two opposing mechanisms have been suggested to explain the effect of leptin on bone metabolism. On one hand, leptin can act locally to promote the development of osteoprogenitor cells and stimulate osteoblasts to form new bone and, on the other hand, leptin can act through the central nervous system decreasing osteoblast activity[23] (Figure 4). Mice genetically deficient in leptin, or leptin receptor, have an obese phenotype, with a high bone mass.[2,38] However, when leptin is injected intracerebroventricularly, there is a decrease in bone-forming activity and a return to the bone mass seen in a wild-type mice, suggesting that leptin acts through a central pathway after binding to its receptor in the hypothalamic nuclei.[38,39] In addition, increased serum levels of leptin decrease bone mass suggesting that serum levels of this hormone might control bone mass through a neuronal pathway.[40] It is still unclear which are the mechanisms relating leptin, central nervous system and bone. However, it is known that in the hypothalamic nuclei leptin receptors are co expressed in neurons expressing the appetite-suppressing neuropeptides proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) and in neurons expressing the appetite-inducing neuropeptide Y (NPY) and agouti-related peptide (AgRP). Interestingly, intracerebroventricular injection of leptin results in reduced expression of NPY and AgRP and increased expression of POMC. Thus, this provides preliminary evidence that the melanocortin pathway might be involved in leptin signalling, at least for its appetite regulator role.[41]

When leptin acts directly on osteoblast and chondrocyte surface receptors it can have an osteogenic effect.[38,39] There are six isoforms of the leptin receptor (OB-R), created by different splicing variants of the gene *ob*, but only four of them (OB--Ra, OB-Rb, OB-Rc and OB-Rf) are expressed on osteoblasts. From these, just OB-Ra and OB-Rb are able to mediate leptin-induced signalling transduction into the cell.[42] It was also found that primary human osteoblasts transcribe, translate and secrete leptin and that leptin expression fluctuates during its differentiation. In fact, in mesenchymal cells, leptin expression is present and in proliferating osteoblasts is suppressed, while it reappears in late stage osteoblasts. Accordingly, it can be argued that leptin increases bone formation by enhancing human osteoblast proliferation, collagen synthesis and mineralization.[43] Moreover, leptin serves as an osteoblastic antiapoptotic agent by reducing the mRNA levels of Bax-α/Bcl-2, which facilitate the transition of mature osteoblasts to osteocytes.[39,43]

Due to the fact that RANKL and OPG are involved in the interaction between osteoblasts and osteoclasts, the influence of leptin upon these molecules was investigated. In an *in vitro* study, it was found that leptin decreases RANKL mRNA expression but had no effect on OPG mRNA levels.[23] This observation raised the hypothesis that leptin can affect bone remodelling through the RANKL/OPG pathway. This effect may depend on leptin serum levels which, in humans, are influenced by food intake, periods of growth and reproduction.[23].

Conclusions

Cbfa1 and Osx genes are critical in osteoblast differentiation. In addition, the Wnt/β-catenin pathway plays a role not only on osteoblast differentiation but also on its proliferation. In fact, mutations in some of the proteins involved in this pathway, like LRP5/6 lead to bone diseases. Osteoblasts are also influenced by leptin in a dual way: inhibitory, through the central nervous system, or stimulatory through the direct binding on surface receptors. On the other hand, osteoblasts are responsible for the RANKL/OPG balance which is the major determinant of osteoclast differentiation.

Although most of the current therapeutic options for osteoporosis antagonize bone resorption, the increasing knowledge on osteoblast regulation is creating new hypothetical targets that can transform the future of osteoporosis management.

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