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Bone cells and the mechanisms of bone remodelling

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1. ABSTRACT

Bone is a peculiar connective tissue which functionally interacts with many other organs and tissues, including bone marrow, lymphoid tissue, kidney, adipose tissue, endocrine pancreas, brain and gonads. Bone functions are accomplished by three principal cell types: the osteoblasts, cells of mesenchymal origin having osteogenic functions, the osteoclasts, giant multinucleated cells arising from the monocyte-macrophage line and devoted to resorb bone, and the osteocytes, the latter arising from mature osteoblasts that, once deposited the bone matrix, remain trapped in it, becoming quiescent cells. Osteocytes are known for their role as mechanosensors, however, old and new evidence showed their active contribution to mineral homeostasis. Moreover, the cross-talk between bone cells is crucial, since a correct bone homeostasis relies on a right coupling between osteoblast and osteoclast functions. Any deregulation of this coupling is responsible for bone disease condition, which reflects on other organs with which bone interacts.

2. INTRODUCTION

2.1. The bone tissue

Bone is a peculiar connective tissue, made up of cells and of an extracellular matrix which, at variance with all the other tissues, is mineralized. Therefore, it is composed of an organic component, mainly formed by fibres of collagen (type I, 90% of the total protein), and of an inorganic component, responsible of the 65% of tissue weight and constituted by mineral salts, in which the most abundant is the hydroxyapatite (Ca10 (PO4)6 (OH)2). The composition and organization of the bone matrix gives this tissue special mechanical properties such as stiffness, rigidity, tensile strength, and an extraordinary lightness.

Three principal cell types are present in the bone: the osteoblasts, cells of mesenchymal origin having osteogenic functions, the osteoclasts, giant multinucleated cells arising from the monocyte-macrophage line and devoted to resorb bone, and the osteocytes. The latter

account for over 90% of total bone cells and arise from mature osteoblasts that, once deposited the bone matrix, remain trapped in it, becoming quiescent cells. However, despite this apparent quiescence, osteocytes are known for their role as mechanosensors, a pivotal function in the regulation of bone remodelling. Moreover, emerging studies suggest that osteocytes contribute to mineralization and systemic phosphate regulation (1), while old and new evidence showed the ability of osteocytes to remove and replace the bone matrix, thus actively contributing to mineral homeostasis (2-4).

Many important functions are accomplished by the bone tissue: (i) mechanical, supporting the whole body and allowing locomotion; (ii) protective, shielding many vital organs and the bone marrow; (iii) metabolic, regulating the homeostasis of calcium and phosphate; (iv) endocrine, contributing to the global energy balance (5,6) and male fertility (7).

2.2. Bone as a central tissue: cross-talk with other organs

More and more evidence over the last years pointed out the close linking between bone and other organs, with a reciprocal regulation that may be disrupted under pathological conditions. Herein, we will briefly report the well described interactions of bone with the immune system, the kidney and the adipose tissue, and will comment on the recently recognized relationships of bone with glucose homeostasis and male fertility.

2.2.1. Bone and immune system

The interplay between bone and immune system is one of the most widely held topics, which consolidated a new interdisciplinary field: the osteoimmunology. This is not surprising, since bone and immune system share several molecular pathways crucial for their homeostasis, an example for all being the Receptor Activator of NFkappaB Ligand (RANKL)/RANK pathway (8-10). Considerable support to the understanding of the bone-immune system cross-talk came from the studies by the Takayanagi's group on the autoimmune disease rheumatoid arthritis. Indeed, they identified a subset of T lymphocytes, named osteoclastogenic TH cells, able to induce osteoclast differentiation and improve their function, directly or by producing high quantities of InterLeukin (IL)-17 (10).

Another link between bone and immune system is suggested by the fact that immune cells and osteoclasts share the same origin: the hematopoietic stem cells present in the bone marrow. Consistently, it has been shown that osteoclasts can promote mobilization of hematopoietic progenitors by degrading endosteal components (11).

Finally, recent evidence has clearly showed that the differentiation of the hematopoietic stem cell progeny is regulated by a subtype of osteoblasts, named Spindle-shaped N-cadherin+ Osteoblasts (SNOs), located to the endosteum, which form the hematopoietic stem cell (HSC) endosteal niche (12). To this regard, a key evidence came from the Calvi's group, who demonstrated that transgenic mice with a constitutively active ParaThyroid Hormone (PTH) receptor under the control of alpha1collagene promoter showed an increased number of osteoblasts paralleled by an increase of HSC (12). Moreover, PTH systemic intermittent treatment of mice expanded HSC (12). This finding was corroborated by the evidence that in bone marrow recipient mice the engraftment of the most primitive HSC occurred near the endosteum (13).

2.2.2. Bone and kidney

Since bone and kidney are both responsible for the regulation of calcium and phosphate balance, a reciprocal influence is obvious, and secondary involvement of bone in patients with renal disease and *vice versa* are very frequent clinical events (14). Typically, chronic renal failure induces hyperphosphatemia and hypocalcemia, with a consequent stimulation of PTH secretion and acidosis. All these alterations concur to develop a specific bone disease called renal osteodystrophy. Moreover, the pathological picture of bone in renal failure has a broad spectrum, ranging from high turnover to low turnover bone lesions, which could be also associated with defective mineralization, leading to a significant increase of bone fractures (15).

As far as the involvement of kidney in patients with primary bone disease is concerned, it is known that bone cells produce molecules able to directly regulate kidney function, such as the Fibroblast Growth Factor 23 (FGF23), a protein mainly secreted by osteoblasts and osteocytes (16). In the kidney, FGF23 favours phosphate excretion and suppresses 1,25 (OH)2 vitamin D3 synthesis (14). To accomplish this function, FGF23 requires a cofactor, called klotho, that is primarily expressed at the level of renal tubules. Indeed, the close relationship between FGF23 and klotho was strongly suggested by the fact that FGF23 knock out and klotho mutant mice showed a very similar phenotype (17). In the presence of klotho, FGF23 binds its receptor expressed by kidney tubular cells, thus inhibiting the synthesis of the sodium-phosphate co-transporter, with a consequent reduction of renal phosphate reabsorption and of serum phosphate concentration (18). The relevance of this FGF23-dependent axis was shown in some diseases characterised by elevated levels of FGF23, such as some inherited forms of osteomalacia and in fibrous dysplasia (19,20).

2.2.3. Bone and adipose tissue

Several studies have demonstrated a positive relationship between bone density and fat mass (21,22). Several pathways are shared by the two tissues (22) and among the bone-seeking molecules produced by adipocytes, leptin is the adipokine that has been most studied. Indeed, it was shown that this hormone regulates bone mass by increasing osteoblast proliferation and differentiation and nodule formation (23), as well as by inhibiting osteoclast differentiation (24).

Indeed, leptin action on bone is more complex, since it is able to indirectly influence bone mass through the central nervous system. In particular, it was observed that leptin administration into the third ventricle of the brain led to bone loss due to inhibition of bone formation and increase of bone resorption (25,26) and these effects

seem to be mediated by beta-adrenergic receptors present on osteoblasts (27). The same studies also revealed that serotonin could mediate leptin's functions in this context, as demonstrated by the ability of leptin to regulate serotonin turnover and transport in brain. Moreover, mice lacking the leptin receptor in serotonergic neurons developed a high bone mass phenotype, together with increased appetite, decreased energy expenditure and obesity, with a phenotype closely resembling the ob/ob mice, which are a natural mutant strain deficient in leptin (27,28).

2.2.4. Serotonin-mediated control of bone mass

Serotonin is a bioamine produced by brainsteam neurons and enterochromaffin cells of the duodenum, the latter form accounting for the 95% of the total amount. Recent evidence pointed out a role for the gut-derived serotonin in the LDL-receptor related protein 5 (LRP5)-dependent control of bone mass (29). As it will be described in more detail, loss- and gain-of-function mutations in the LRP5 gene affect bone formation causing osteoporosis and high bone mass, respectively, and this has been well explained by the fact that LRP5 is a co-receptor for Wingless-int (Wnt), which in turn binds to Frizzled and this complex triggers a beta-catenin dependent signal that promotes osteoblast differentiation (30). On the other hand, it has been demonstrated that gut-derived serotonin inhibited bone formation by hampering osteoblast proliferation through a cAMP Response Element-Binding (CREB)-dependent mechanism that reduced cyclin D1 expression (29). The link between wnt pathway and gut-derived serotonin raised by the evidence that LRP5 inhibited the expression of tryptophan hydroxylase (Thp1), the rate-limiting enzyme for serotonin biosynthesis (29). Therefore, in contrast with previous papers, this work pointed out the ability of LRP5 to favour bone formation by inhibiting serotonin synthesis in enterochromaffin cells, thus attributing to this hormone a new functional meaning.

As far as brain-derived serotonin is concerned, it has been observed that this neurotransmitter is a positive regulator of bone mass, as demonstrated by experiments employing mice deficient for the tph2 gene, which codes for this specific serotonin (31). In these mice it was demonstrated that brain serotonin inhibited the synthesis of epinephrine with a consequent decrease of the sympathetic tone. This decline is relayed in osteoblasts by reduced signalling mediated by the beta 2 adrenergic receptor, which was found to reduce osteoblast proliferation and increase osteoclast resorption (31).

2.2.5. Bone and glucose metabolism

Also this field is just emerged. Recent studies pointed to osteocalcin as a new bone hormone, regulating pancreatic insulin production. Osteocalcin is a 5 kDa non-collagenous protein produced by mature osteoblasts and osteocytes (32,33). The first evidence of the involvement of osteocalcin in glucose metabolism came from the Karsenty's group, who demonstrated that osteocalcin null mice had high blood glucose, along with a low pancreatic beta cell mass and insulin content (5). Osteocalcin can be found in two forms, carboxylated on 3 glutammate residues or uncarboxylated. This latter type seems to be important for insulin secretion by beta-cells of the pancreatic islets and for peripheral tissue sensitivity to insulin (5). Accordingly, Ferron *et al.* (34) demonstrated that chronic infusion of uncarboxylated osteocalcin increased serum insulin and reduced blood glucose. It was also shown that acidification induces the decarboxylation of osteocalcin, pointing to osteoclasts as key cells involved in the release of the active form of this hormone. Moreover, osteoblasts express insulin receptors, and insulin binding controls osteoblast development and osteocalcin expression by suppressing the Runx2 inhibitor Twist2 (35) as well as increases collagen synthesis, ALkaline Phosphatase (ALP) expression and glucose uptake (6,35), and decreases osteoprotegerin expression (6), thus enhancing osteoclast formation.

2.2.6. Bone and male infertility

The cross-talk between bone and reproductive apparatus is well known, since estrogens and androgens represent the principal hormones preserving bone mass through direct/indirect mechanisms stimulating osteogenic functions and inhibiting osteoclast resorption ability (36). What has been recently discovered is the ability of bone to regulate male gonad function and fertility. Indeed, the Karsenty's group showed another unexpected hormonal role of uncarboxylated osteocalcin produced by osteoblasts, demonstrating, by cell-specific loss- and gain-of-function animal models, that this protein is able to stimulate the production of testosterone by Leydig cells, while it has no effect on the female gonad (7). Furthermore, they identified a G-coupled receptor for osteocalcin that is expressed and transduces its signal in Leydig cells, leading to a CREB-dependent activation of the transcription of genes coding for enzymes required for testosterone synthesis.

3. CELLS OF THE BONE TISSUE

3.1. Osteoblasts

Osteoblasts are cells sharing a common pluripotent mesenchymal stem cell (MSC) with other cells of connective tissues, including adipocytes, chondrocytes, fibroblasts and myoblasts (37). Although representing only 4-6% of total resident cells, osteoblasts attend the crucial function of building the bone.

3.1.1. Osteoblastogenesis

Osteoblast differentiation from MSCs follows a specific programme of gene expression (37). The first step of osteoblastogenesis is the commitment of MSCs towards an osteo/chondroprogenitor, which is under the control of the Wnt and of the Bone Morphogenetic Proteins (BMPs) pathways (Figure 1A). Indeed, it has been demonstrated that Wnt10b not only promotes this commitment but at the same time prevents differentiation of preadipocytes by suppressing the adipogenic transcription factors CCAAT Enhancer Binding Protein alpha (C/EBP alpha) and Peroxisome Proliferator-Activated Receptor gamma (PPARgamma) (38).

Wnt signalling is generally involved in many cell functions (39), however its crucial role in bone metabolism emerged after the discovery that loss- and gain-of-function

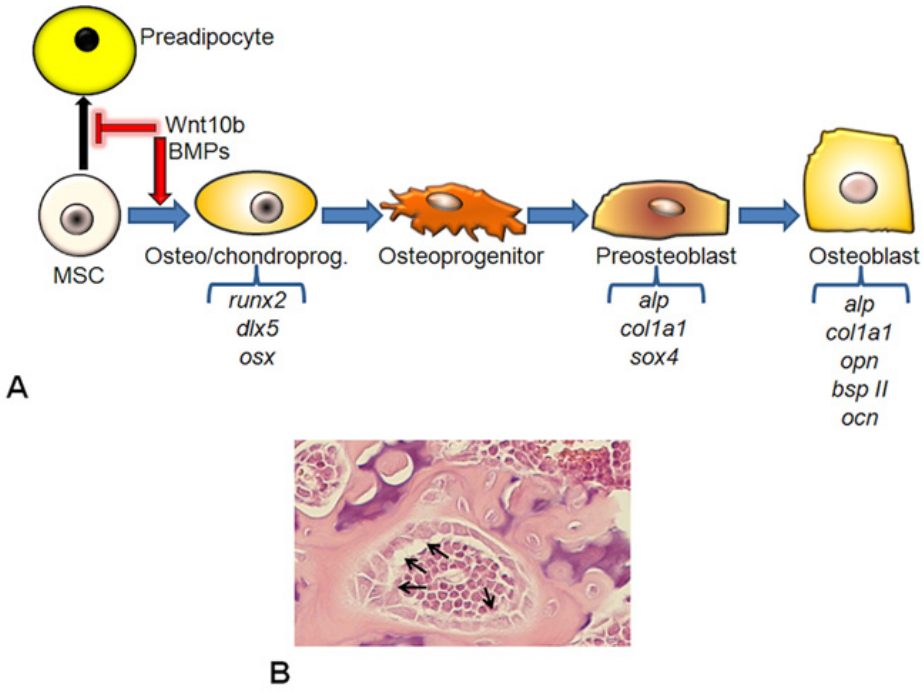

Figure 1. The osteoblast. (A) Schematic diagram of the osteoblastogenesis: osteoblasts arise from a pluripotent mesenchymal stem cell (MSC) which, under proper stimuli, is committed toward an osteo/chondro-progenitor (osteo/chondroprog.), followed by an osteoprogenitor, a preosteoblast and an active, mature osteoblast. Specific markers are indicated for each differentiation stage. (B) Bone tissue section stained with hematoxylin/eosin showing cuboid mature osteoblasts (arrows). Original magnification 20X .

mutations in the Wnt co-receptor LRP5 in humans led to the osteoporosis-pseudoglioma syndrome (40) and to the high bone mass syndrome (41,42), respectively. When Wnt binds to Frizzled and to LRP5/6 receptor, this complex triggers a signal that blocks Glycogen Synthase Kinase 3 beta (GSK3beta) activity, with a consequent prevention of beta-catenin phopshorylation. In the hypophosphorylated form, beta-catenin is more stable, so that it accumulates in the cytoplasm and, upon reaching a specific concentration, translocates to the nucleus where it regulates transcription of Wnt target genes. In contrast, in the absence of Wnt, GSK3beta phosphorylates beta-catenin, thus promoting its ubiquitination which prevents Wnt signalling (30).

BMPs, the other factors involved in the early step of osteoblastogenesis, belong to the Transforming Growth Factor (TGF) beta superfamily. As for the Wnt pathway, mutations in the BMP genes in animals and humans allowed to highlight the role of these proteins in bone metabolism (43).

The osteo/chondroprogenitor committed cell is characterised by the expression of the osteoblast-specific transcription factors Runt-related transcription factor 2 (Runx2), Distal-less homeobox 5 (Dlx5), and Osterix (Osx), the latter downstream of Runx2 (Figure 1A).

Dlx5 is expressed in most developing skeletal elements and its expression is also induced during bone fracture healing. Osteogenic functions of dlx5 are under the control of BMPs through a protein kinase A dependent mechanism (44).

Runx2 is a master gene of osteoblast differentiation, as also demonstrated by the fact that Runx2 null mice lack osteoblast differentiation which results in the absence of bone, while chondrocytes of cartilage templates fail to undergo hypertrophy (45). In humans, runx2 is haploinsufficient and causes CleidoCranial Dysplasia (CCD), an autosomal dominant disease with abnormalities in bones formed by intramembranous ossification (46). Several factors, such as BMPs, TGF-beta, PTH and FGFs promote runx2 activation.

Osx expression in MSCs progenitors is stimulated by BMPs and Insulin like Growth Factor (IGF)-1 (47). Even if Osx is a downstream target of runx2, its BMP-2-induced activation follows both runx2-dependent (48) and -independent (49) pathways. Another gene recently found to be involved in osteoblast differentiation is Sox4, a transcription factor that stimulates osteoblast proliferation, differentiation and function by acting upstream of osx and independent of runx2 (50).

Activation of Wnt, together with Runx2 expression, further promotes osteoblast commitment at the expense of chondrocyte differentiation. Following the lineage commitment, osteoprogenitors undergo a proliferative stage, hence they acquire ALP expression, that is one of the earliest markers of osteoblast differentiation.

As pre-osteoblasts cease to proliferate, they undergo morphological changes, lacking a spindle-shaped conformation and becoming large cuboidal differentiated osteoblasts, which are highly enriched in ALP and are able to secrete bone matrix proteins such as type I collagen, osteopontin (OPN) and Bone SialoProtein (BSP) II. Finally, upon osteoblast maturation and activation, they express genes involved in bone matrix mineralization as well as osteocalcin (ocn), a marker of post-proliferative mature osteoblasts with hormonal activity (5-7) (Figure 1A).

3.1.2. Osteoblast morphology

When observed by light microscopy, active osteoblasts are usually found lining the bone matrix they are producing (Figure 1B). Osteoblasts appear as cuboid-shaped cells with a round nucleus located at the base of the cell and with a strong basophilic cytoplasm with PAS-positive granules containing the precursors of bone matrix glycoproteins. These cells also present cytoplasmic processes that extend into the osteoid matrix and are in contact with the osteocyte processes. The plasma membrane of osteoblasts is particularly enriched in ALP, which is the characteristic osteoblast marker (51).

Active osteoblasts observed by transmission electron microscopy show the typical features of cells involved in an intense protein synthesis: a well developed rough endoplasmic reticulum, with dilated cisternae, a prominent Golgi complex, several free ribosomes, and an euchromatic nucleus with a voluminous nucleolus.

3.1.3. Osteoblast functions

Osteoblasts are the bone cells devoted to deposit the uncalcified bone matrix, named osteoid, and to subsequently provide for its mineralization. The former function is accomplished by secretion of type I collagen, non collagen proteins, such as OPN, BSP II, osteonectin and osteocalcin, and proteoglycans, preferentially biglycan and decorin.

Once deposited, organic bone matrix is mineralized following two principal steps. During the first step, there is the formation of hydroxyapatite crystals within the so called matrix vesicles, which are extracellular membrane-layered vesicles that form from the membrane surface of chondrocytes, osteoblasts and odontoblasts (52). This process is not completely known, however it has been demonstrated to involve calcium-binding proteins, including calbindin D9k, BSPII, calcium-binding phospholipids such as phosphatidylserine, and calcium channel-forming annexins, which allow the accumulation of calcium within the matrix vesicles (52 and reviewed in this book by Wuthier), while two main proteins provide phosphate intake: type III Na/Pi co-transporter (53) and the PHOSPHO1 phosphatase (54). Hydroxyapatite crystals form within matrix vesicles once the accumulation of calcium and phosphate overcomes the point of solubility, thus giving rise to Ca3 (PO4)2 (tricalcium phosphate), eventually leading to hydroxyapatite after hydroxylation.

During the second step, mineral crystals are elongated into the extracellular space, filling the gap between collagen fibrils. The crucial event is the formation of the first stable crystal, called critical nucleus, which is formed when clusters of ions come together with the right orientation and with a sufficient energy. The critical nucleus is then more and more increased in size by the addition of ion clusters, leading to the secondary nucleation (52).

Elongation of hydroxyapatite requires also a correct ratio of Pi to inorganic pyrophosphate (PPi), the latter inhibiting the formation of hydroxyapatite (55). PPi is formed by NPP1 (Nucleotide Pyrophosphatase/Phosphodiesterase Isozyme) from nucleotide triphosphates. PPi is also provided by *ANKH* (human homolog of the mouse progressive ankylosis, *ank*, gene), which is located in the membranes of hypertrophic chondrocytes and osteoblasts and transports PPi from the cell to the extracellular space (56). In contrast, tissue-non-specific ALP hydrolyses PPi to generate two molecules of Pi, so promoting matrix mineralization (57). Patients with hypophosphatasia caused by a deficiency of ALP, also show abnormal bone mineralization (58).

Another important function accomplished by osteoblasts is the regulation of osteoclast differentiation. Indeed, two principal cytokines stimulating osteoclast differentiation are produced by osteoblasts: Macrophage-Colony Stimulating Factor (M-CSF) and RANKL (59), whose mechanisms of action will be described in more detail in the next paragraphs.

3.2. Osteocytes

3.2.1. Osteocyte formation

At the end of the bone formation phase osteoblasts may undergo different fates: they can be subjected to apoptosis, become inactive osteoblasts or bone lining cells (Figure 2) or are trapped in the bone matrix as osteocytes, non-proliferative terminally differentiated cells regularly dispersed throughout the mineralized matrix (Figures 2,3A). Although osteocytes represent the most abundant bone cells, the precise mechanisms of osteocyte formation and the specific functions attended by them are still only in part known. Franz-Odendaal *et al.* (60) proposed eight recognizable transitional stages from the osteoblast to the osteocyte. As already described, starting from a proliferating preosteoblast, this cell differentiates towards a preosteoblast and then a mature, active osteoblast. The latter, if designated to become an osteocyte, slows down matrix production and is more and more embedded in it. At this point we have an osteoblastic osteocyte or type I preosteocyte, which progresses towards an osteoid osteocyte (type II preosteocyte), a type III preosteocyte, a young osteocyte and finally an old osteocyte (Figure 3A). This evolution is characterised by a dramatic change of osteoblast shape, from a polygonal morphology to a flat cell with several dendrites extending in a polarized manner towards the mineralizing front, which is followed by dendrites extending towards the vascular space or bone surface (61).

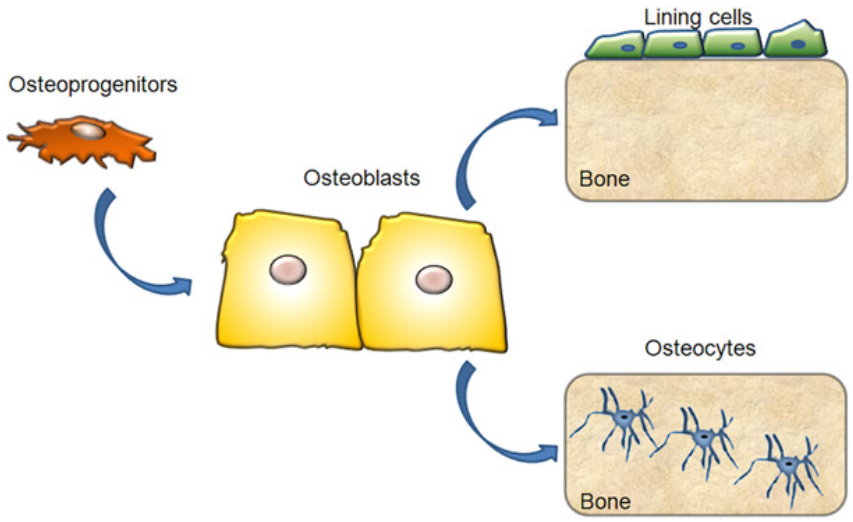

Figure 2. The fate of the osteoblast. Once deposited the bone matrix and accomplished its mineralization, a mature osteoblast could acquire a resting phenotype becoming a lining cell or differentiate towards an osteocyte.

Recent findings also indicated that osteocyte dendritic formation requires the cleavage of collagen and other matrix proteins (62,63), since null mice for the Matrix Type 1-MetalloProteinase (MT1-MMP), which is involved in collagen and other matrix proteins degradation, show osteocytes with a reduction of the number and the length of dendritic processes.

Like the bone matrix components, the final fate of the osteocytes is to be digested during osteoclast bone resorption (61).

3.2.2. Osteocyte morphology and markers

When observed at the microscopic level, osteocytes appear as spider-shaped cells encased in a lacuna (Figure 3B), evenly dispersed throughout the mineralized matrix and connected to each other, to cells lining the bone surface and to the bone marrow through their dendritic processes located in tiny canals called canaliculi (4,64,65). These allow osteocytes to be linked each other metabolically and electrically via gap junctions, through which they exchange ions and other small molecules. It has recently been demonstrated that dendrites are not static but rather subjected to repeated extension and retraction (61). Moreover, young osteocytes also show motile properties of their cell body within the lacuna (61).

Until a few years ago, the markers described for osteocytes were limited to low or no ALP, high casein kinase, high osteocalcin expression, and high CD44. More recently, new specific osteocyte markers have been identified, among which sclerostin, which reaches the highest expression in the old, deeply embedded osteocytes (66). Sclerostin is the product of the SOST gene, whose mutation causes high bone mass in human, since sclerostin serves as a negative regulator of the Wnt/beta-catenin pathway by blocking LRP5 (67). Other osteocyte markers are related to phosphate homeostasis, including Phosphate-regulating neutral endopeptidase on chromosome X (Phex) (68), Matrix Extracellular Phosphoglycoprotein/Osteocyte/osteoblast Factor 45 (MEPE/OF45) (69), FGF23 (16) and Dentin Matrix Protein 1 (DMPl) (1) (Figure 3A).

3.2.3. Osteocyte functions

Over the years, several studies have disproved the classical vision of the osteocyte as an inactive cell, and besides its traditional role of mechanosensor, several other functions have been identified.

As mechanosensors, osteocytes perceive variations of mechanical strain and respond by translating this kind of stimuli into biochemical signals that affect bone resorption and/or formation. Indeed, it has been demonstrated that following mechanical loading, there is an increase in osteocyte content of glucose 6-phosphate dehydrogenase (70), along with an increase of c-fos, TGF-beta and IGF-1 mRNAs (71). Moreover, it has been shown that shear stress inhibits osteocyte apoptosis induced by serum starvation, and that substrate stretching prevents dexamethasone-induced apoptosis (72).

Another important function of osteocytes is the regulation of phosphate homeostasis, which is accomplished through the DMP1 and FGF23 pathways by regulating renal phosphate reabsorption (1,73). Moreover, they play an active role in mineralization as also suggested by osteocyte expression of DMP1 and MEPE, which seem to be positive and negative regulators of mineral deposition, respectively. Current data support the hypothesis that these molecules may be transducers of strain and participate in the mineral homeostasis of the perilacunar space (74).

Finally, early studies indicate the ability of osteocytes to regulate calcium homeostasis by removing and subsequently replacing the mineralized matrix (3,4,75). First evidence for a pro-osteolytic action of osteocytes

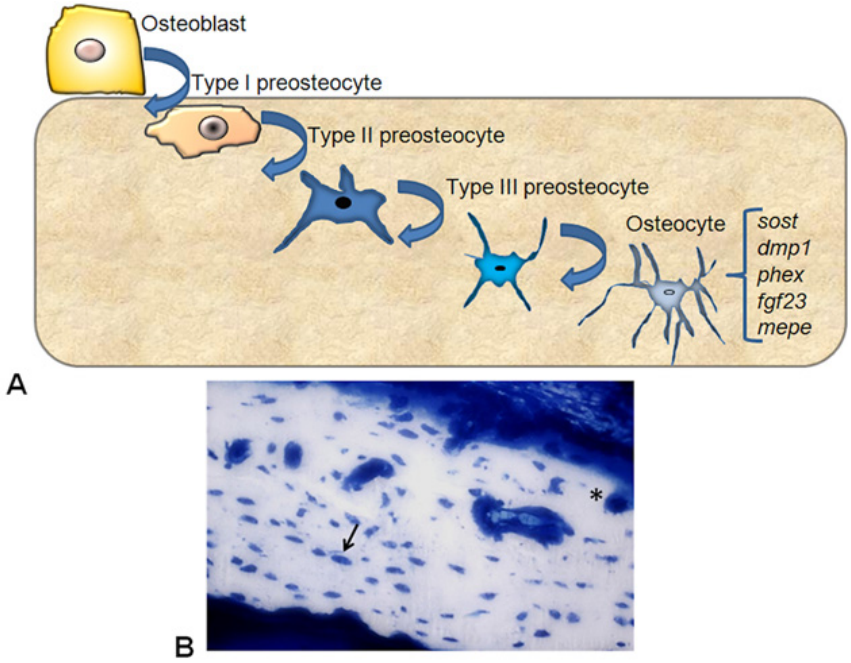

Figure 3. The osteocyte. (A) Schematic cartoon showing osteocyte differentiation: once deposited the bone matrix, the osteoblast could be entrapped in it, and undergo a differentiation process towards a type I, II and III preosteocyte and finally to an osteocyte, which expresses specific markers such as sost, dmp1, phex, fgf23 and mepe. (B) Bone histological section stained with methylene blue/azure II showing several osteocytes encased in lacunae (arrow) and newly formed preosteocytes (asterisk). Original magnification 40X.

came from *in vivo* experiments with immobilized rats that presented with periosteocytic osteolysis (76). Indeed, periosteocyte osteolysis was observed in bone biopsies from patients affected by hyperparathyroidism (77), renal osteodystrophy (78), Paget's disease of bone (2) and bone metastases (79).

As far as the role of osteocytes in matrix formation is concerned, little evidence is present. Using a laying hen model subjected to a low calcium diet followed by a calcium replenishment period, it was observed the presence of collagen fibrils apparently just synthesized in the osteolytic lacunae of compact bone, which were also surrounded by toluidine blue-positive materials, together with a fluorescent mineralization label by tetracycline treatment (3,75). Some years later, Tazawa *et al.* (80) demonstrated that in bone sections from rats subjected to chronic treatment with PTH, osteocyte lacunae contained a hematoxylin positive matrix with features resembling an immature bone matrix. Finally, data obtained using atomic force microscopy and scanning acoustic microscopy indicate that the perilacunar matrix surrounding the osteocyte is distinct from the rest of the bone matrix, since it has a lower elastic modulus or is less mineralized than the surrounding matrix (64).

3.3. Osteoclasts

3.3.1. Osteoclastogenesis

The pathway of osteoclast differentiation is now well established (59). First evidence showing that circulating blood contains cells able to differentiate into osteoclasts were performed in 1981 by Marks and Walker (81). They connected normal and osteopetrotic mice in parabiosis experiments and showed the hematogenous origin of this cell. Subsequently, Burger and coworkers (82) demonstrated, by *in vitro* experiments with bone marrow-derived cells, that osteoclasts originate by differentiation of precursors belonging to the CFU-M (Colony Forming Unit-Macrophage) lineage (Figure 4A). The molecule essential for the initial commitment of osteoclast differentiation is the transcription factor PU.1, belonging to the family of Ets transcription factors, which recognizes a purine-rich sequence 5'-GGAA-3'. The essential role of PU.1 in osteoclastogenesis was revealed for the first time in 1997 (83), by a report showing that its expression increased as marrow macrophages differentiate into osteoclasts. Moreover, the tight association between PU.1 and osteoclastogenesis was confirmed by the analysis of PU.1 deficient mice phenotype, characterised by the absence of osteoclasts and macrophages (83). PU.1 drives the expression of the *c-fms* gene, encoding for the tyrosine kinase receptor of M-CSF as well as of the receptor RANK, that upon the binding with its ligand, RANKL, induces the fusion of osteoclast precursors (84).

The binding of RANKL to its receptor activates the fusion and differentiation of osteoclast precursors into mature osteoclasts. TRAF6 (TNF Receptor Activated Factor 6) is recruited and activates MAP kinases and IκB (10), leading to the nuclear translocation of transcription factors such as c-fos, c-jun, ATF2 (Activating Transcription Factor 2) and Nuclear Factor-kappaB (NF-

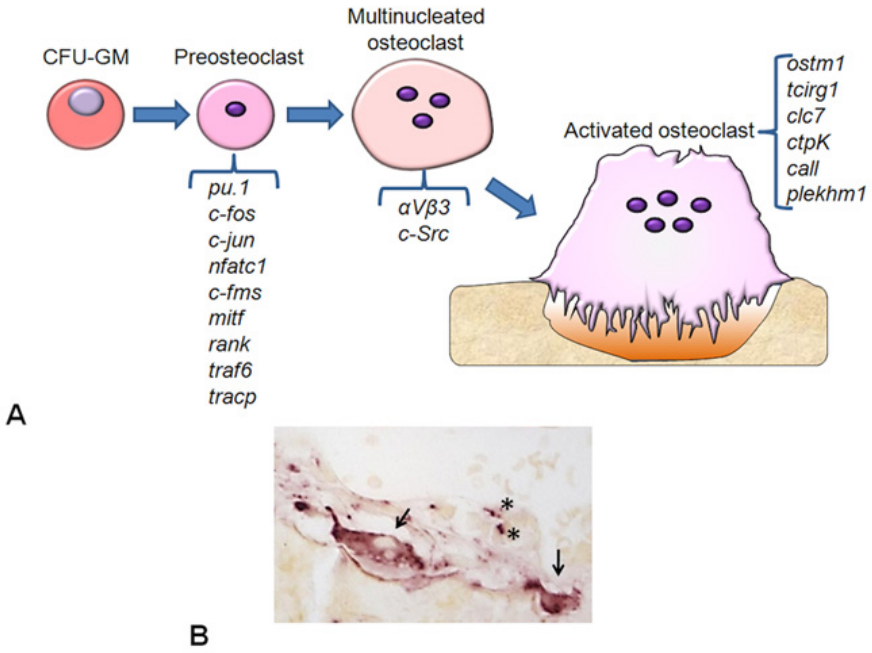

Figure 4. (A) Schematic representation of the osteoclast differentiation process: starting from a CFU-GM (Colony Forming Unit-Granulocyte Monocyte), under proper stimuli there is the commitment towards preosteoclasts, which fuse to give rise to multinucleated osteoclasts. The osteoclasts next polarize, and adhere to the bone matrix thus becoming active. (B) Bone tissue section showing osteoclasts (arrows) and preosteoclasts (asterisks) purple-stained for the specific marker TRAcP. Original magnification 40X.

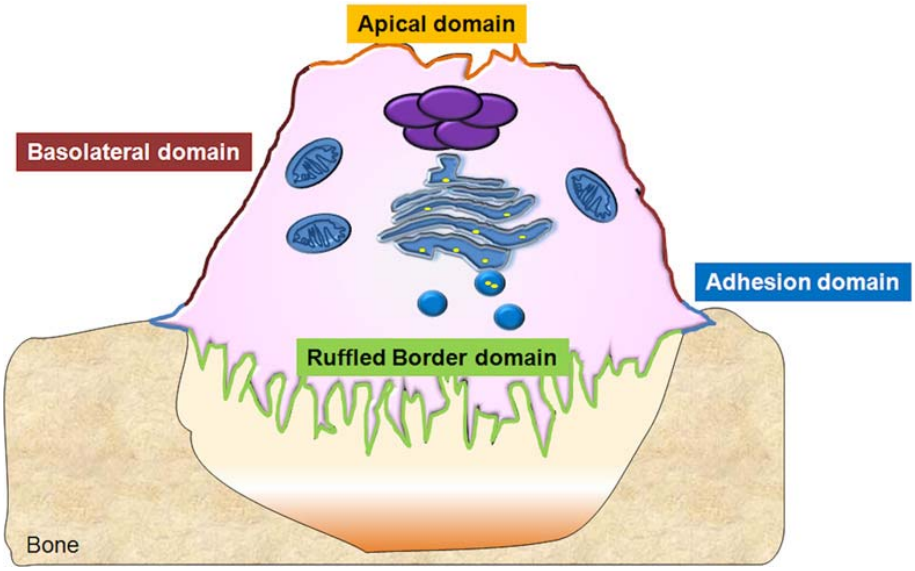

Figure 5. Schematic diagram showing a polarized osteoclast, which presents with the indicated plasma membrane domains.

kappaB), that drive the expression of osteoclast specific genes.

Another transcription factor important for the osteoclast differentiation is the basic-loop-helix-leucine zipper protein MITF (MIcrophthalmia-associated Transcription Factor). Osteoclasts express two different isoforms of Mitf, Mitf-A and Mitf-E (85). While Mitf-A is expressed in macrophages and osteoclasts at similar levels, Mitf-E expression increases during osteoclast differentiation. MITF interacts with PU.1 to increase the transcription of osteoclast specific genes, including ctsk and acp5, encoding cathepsin k and Tartrate Resistant Acid Phosphatase (TRAcP), respectively. Moreover, Hu and coworkers (86) showed that MITF and PU.1, following stimulation with M-CSF, interact with zing finger protein

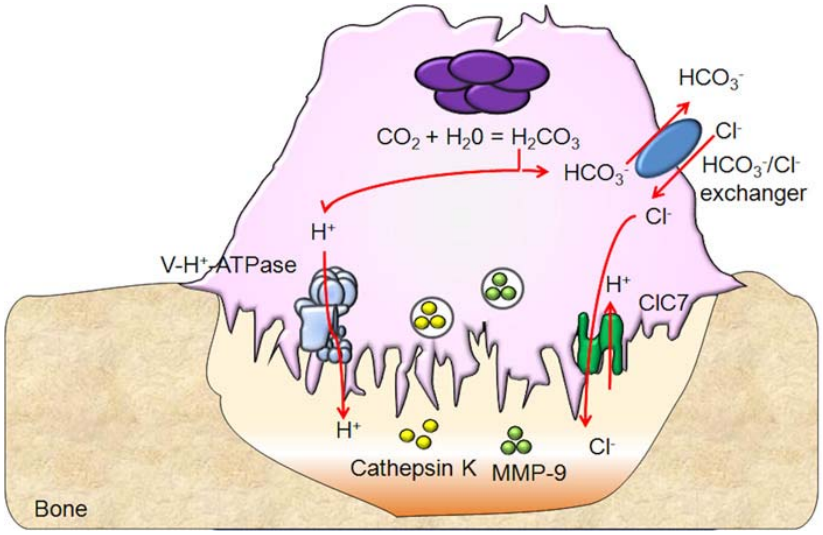

Figure 6. Representation of the molecular mechanisms involved in osteoblast bone resorption.

EOS, a member of the Ikaros family, to reduce their expression. This transcriptional inhibition is removed when osteoclast progenitors are treated with RANKL, causing a robust induction of ctsk and acp5.

NFATc1 (Nuclear Factor of Activated T-cells, cytoplasmic 1) is another transcriptional factor forming a complex with PU.1 and MITF to regulate the expression of osteoclast-specific genes. Particularly, it is activated by NFATc2 and NF-kappaB, and is subjected to an auto-amplification loop in a process requiring the activation of the calcium/calmodulin-dependent protein calcineurin (10).

3.3.2. Osteoclast morphology

The osteoclast is a peculiar cell whose function is to destroy the tissue to which it belongs (59). In fact, in histological sections, osteoclasts appear attached to the bone matrix, in small depressions, named "Howship's lacunae", which are the result of their bone resorbing activity (Figure 4B). They are polykarya containing 4 to 50 nuclei, derived from the fusion of mononuclear cells belonging to the monocyte-macrophage lineage (87). The fusion increases the size of the cell up to reach a diameter of 20-100 microm, enabling it to resorb a large area of tissue. In fact, while macrophages degrade targets in lysosomes by an internalization process, the osteoclasts are able to degrade the bone matrix extracellularly, creating an "extracellular lysosomal compartment" in front of the bone matrix to be resorbed (59,87). Under the light microscope, the several nuclei appear different in shape: some are round and other irregular. A recent study (88) demonstrated, by RNA FISH assays, that these nuclei differ for their transcriptional activity, confuting two previous reports (89,90) showing that all nuclei in osteoclasts have equal transcriptional activity.

Ultrastructural studies showed that osteoclasts contain many mitochondria, due to the high levels of energy expenditure required during bone resorption. Moreover, several lysosomes containing acid cathepsin K and TRAcP, are observed, along with endosomes, rough and smooth endoplasmic reticulum, and multiple Golgi complexes, typically one per each nucleus.

3.3.3. Osteoclast function

As described above, osteoclasts are the cells responsible for the degradation of the bone matrix. To resorb bone, they polarize, acidify the resorbing lacuna and secrete proteolytic enzymes.

Polarization is a crucial step for osteoclast activity (91). In fact, it is possible to identify 4 different domains of the plasma membrane (Figure 5). The area facing the bone matrix to be resorbed is organized in a specialized domain, named "ruffled border", characterised by deep and irregular foldings of the membrane (92). A peripheral domain is described to represent the "adhesion area" by which osteoclasts attach to the bone matrix, segregating the resorbing lacuna from extracellular fluids. The remaining membrane represents the basolateral domain and contains many proteins important for ion transport and response to signals. Moreover, an apical domain, opposite to the ruffled border, is identified and its function is correlated with the transcytosis of products from the resorbing lacuna to extracellular fluids (91,93,94).

The polarized osteoclasts resorb bone through two different processes: the acidification of the resorbing lacuna to dissolve the inorganic matrix, and the secretion of proteolytic enzymes to digest the organic components of the bone tissue.

To acidify the bone resorbing lacuna (pH~4), osteoclasts need a proton source (Figure 6). The Carbonic Anhydrase II (CAII) hydrates the carbonic anhydride (CO2) to form carbonic acid (H2CO3), that spontaneously dissociates in bicarbonates (HCO3-) and protons (H+). The latter are actively transferred to the resorbing lacuna by a proton pump located in the ruffled border domain, the vacuolar-type H+-ATPase. The bicarbonate ion is

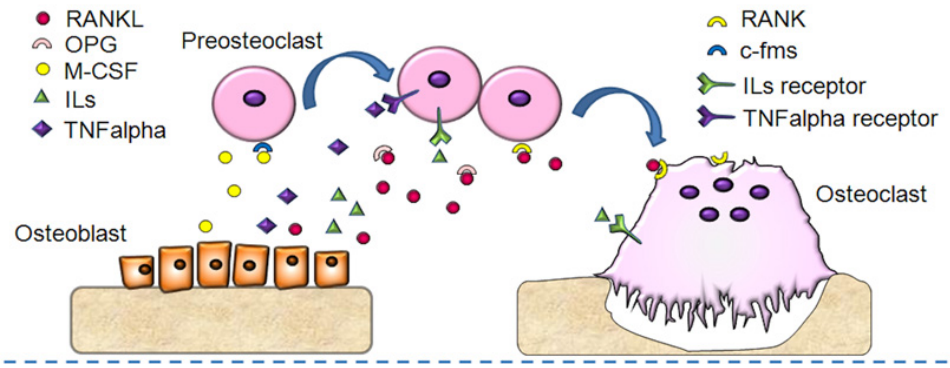

Figure 7. Schematic representation of the pathways regulating osteoclast differentiation.

exchanged with chloride (Cl-) by a HCO3-/Cl- exchanger located in the basolateral domain. The Cl- ion is translocated to the lacuna by a Cl-/H+ antiport, balancing the charge of ions across the membrane. All these processes are essential to liberate calcium and phosphate and to uncover the organic matrix, ready now to be digested by proteolytic enzymes. Osteoclasts then secrete into the resorbing lacuna acidic hydrolases, including cathepsin K, released by the fusion of lysosomes with the ruffled border, and MMPs, such as the MMP-9.

4. OSTEOBLAST-OSTEOCLAST CROSS-TALK

Osteoblasts and osteoclasts talk each other to guarantee a correct bone homeostasis, which in turn relies on a right coupling between their functions. Although it is well known that osteoblasts are able to regulate osteoclast formation and resorbing function by secreting a plethora of factors, only recently evidence is accumulating about the ability of osteoclasts to influence osteoblast behaviour.

4.1. How osteoblasts regulate osteoclasts.

The hypothesis that osteoblasts could regulate osteoclast differentiation goes back more than 30 years ago (95) and evidence of this theory accumulated more and more over time. One of the first indication that correct osteoclast differentiation is dependent on osteoblasts came from the op/op animal model of osteopetrosis, in which osteoclasts do not form due to the lack of M-CSF produced by osteoblasts and stromal cells (96). This factor, as already described, is expressed by osteoblasts as a surface molecule which binds to its receptor, c-fms, located on osteoclast precursors, thus stimulating their proliferation and subsequent differentiation (Figure 7).

Although the crucial role of the RANKL/RANK pathway in osteoclast formation and function is clearly known, the knowledge on RANK and RANKL was born in an immunological context. They are molecules expressed by T cells and dendritic cells, respectively, and their physical interaction increases the ability of dendritic cells to stimulate naïve T cell proliferation and dendritic cell survival (10). At the same time, a molecule expressed by osteoblasts, named Osteoclast Differentiation Factor (ODF) (8), was identified to increase osteoclast formation. Osteoprotegerin (OPG) was instead recognized as an osteoblast secreted factor belonging to the TNF receptor family that, in contrast to RANK, inhibited osteoclast development and bone resorption acting as a decoy receptor. A molecule able to interact with OPG, that was named OPG-Ligand (OPGL) (97), was also identified and found to be identical to RANKL and ODF.

In the bone context, osteoblasts represent the main source of RANKL, which is in large part produced as a membrane surface molecule, therefore the RANKL/RANK signalling relies on the cell-cell contact between the osteoblasts and the osteoclast precursors (Figure 7). Surface RANKL can be released as soluble RANKL after shedding by metalloproteinases, such as MMP-14, A Disintegrin And Metalloproteinase (ADAM)-10 (98) and MT1-MMP (99).

In contrast, OPG plays an osteoprotective role, since it has the same extracellular structure of RANK but lacks the transmembrane and cytosolic domains so that it binds to RANKL preventing its interaction with RANK, with a consequent inhibition of osteoclastogenesis (Figure 7).

Osteoblasts also secrete several cytokines that stimulate osteoclast formation, such as IL-1beta, IL-6, PTH related Peptide (PTHrP) and Tumour Necrosis Factor (TNF)-alpha (Figure 7).

4.2. How osteoclasts regulate osteoblasts

During the physiological process of bone remodelling, it is important that bone resorption is followed by the bone formation phase, to maintain unaltered the bone mass. It is now well established that, during bone resorption, growth factors, including TGF-beta, IGF-I and BMPs are released from the bone matrix and stimulate osteoblast activity (100) (Figure 8A). However, several studies demonstrated that osteoclasts are able to stimulate osteoblast activity also in a bone resorption-independent manner. Histomorphometric analysis performed on iliac crest biopsies of osteopetrotic patients seem to confirm this osteoclast/osteoblast cross-talk (101). In fact, in the form of osteopetrosis characterised by an increased number of non-functional osteoclasts, so called “osteoclast-rich” form, an increased number of osteoblasts was also found, while a

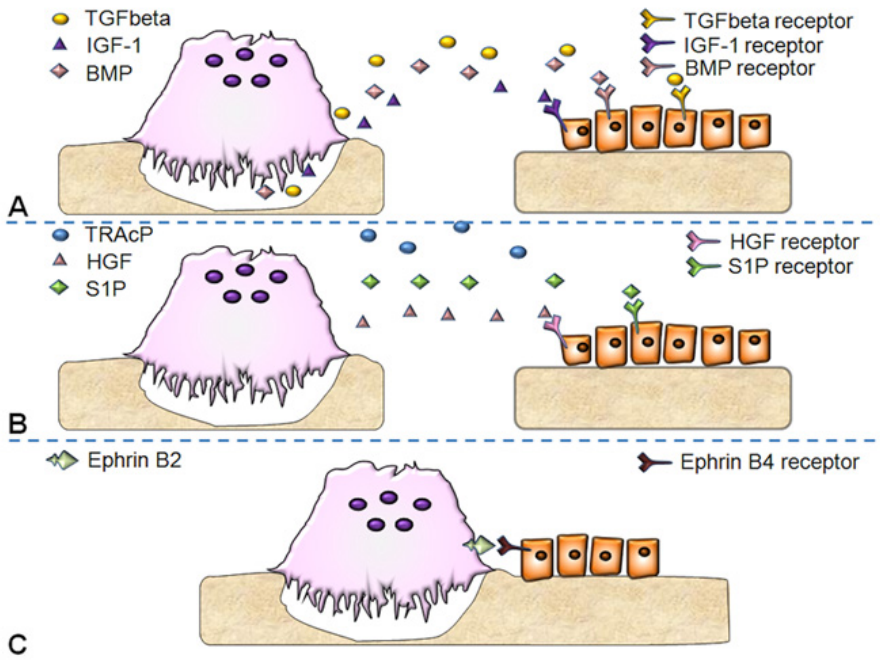

Figure 8. Pathways involved in osteoclast-induced regulation of osteoblast homeostasis according to bone resorption (A) dependent or (B) independent mechanisms. (C) Schematic representation of the EphB4/Ephrin B2 bi-directional signalling.

reduction of osteoblasts surface/bone surface has been observed in patients affected by "osteoclast-poor" osteopetrosis.

Many efforts have been made to identify the molecules responsible for osteoclast-osteoblast cross-talk. Indeed, by treating mouse osteoblast precursor-like cells, MC3T3-E1, with conditioned media from mouse osteoclast-like cell line, RAW264.7 treated with RANKL, Kubota and co-workers identified the so called OsteoBlastogenesis Inhibitory Factor (OBIF), subsequently classified as Platelet Derived Growth Factor (PDGF) BB (102). It was also shown that PDGFBB promoted osteoblast proliferation but at the same time inhibited osteoblast differentiation.

Another molecule involved in osteoclast-osteoblast coupling could be the Hepatocyte Growth Factor (HGF), whose receptors are expressed by both osteoblasts and osteoclasts. Interestingly, it has been demonstrated that osteoclasts secrete HGF, thus leading to a paracrine/autocrine regulation (103) (Figure 8B)

The Sphingosine 1 Phosphate (S1P) is another mediator of osteoclast-osteoblast cross-talk, whose production and secretion is increased in bone-marrow macrophage cultures by RANKL treatment. It has been demonstrated a dual role of this factor, which triggers a negative loop in osteoclasts in response to RANKL, and stimulates migration and survival of osteoblasts (104) (Figure 8B).

TRAcP belongs to Purple Acid Phosphatases (PAPs) family, a group of enzymes that catalyze the hydrolysis of phosphorylated substrates. It could be another important protein implicated in the osteoclast-osteoblast coupling, as it was shown to stimulate osteoblast activity (101). Moreover, the relevance of TRAcP in osteoblast-osteoclast cross-talk was also demonstrated by the analysis of transgenic mice overexpressing TRAcP, that present with a reduction of bone volume but increased bone formation (105). The mechanism by which TRAcP is able to stimulate osteoblast activity is not well defined. Several studies demonstrated that trypsin treatment as well as pH =7.0 convert TRAcP activity into an ATPase (106), a step that could be relevant since it is known that extracellular nucleotides like ADP and ATP stimulate DNA synthesis and proliferation in MC3T3-E1 mouse osteoblast-like cells (107).

Finally, two very recent studies from Briggs (108) and Lausch (109), revealed that loss of TRAcP activity due to missense mutations in the ACP5 gene is responsible for the immune-osseous disease SPondyloENChondroDysplasia (SPENCD), whose patients are characterised by a wide range of autoimmune manifestations in association with intracranial calcification and skeletal dysplasia. This clinical picture is also characterised by a dramatic up-regulation of INterFeron (INF) alpha and type I INF-stimulated genes. The study pointed to the role of TRAcP in osteopontin dephosphorylation, which, consistently, in these patients is more phosphorylated. This finding further strengthens the close relationship between bone and immune system.

4.3. Osteoblast-osteoclast bidirectional signalling

Eph receptors include a wide family of tyrosine kinases characterised by the fact that the interaction with

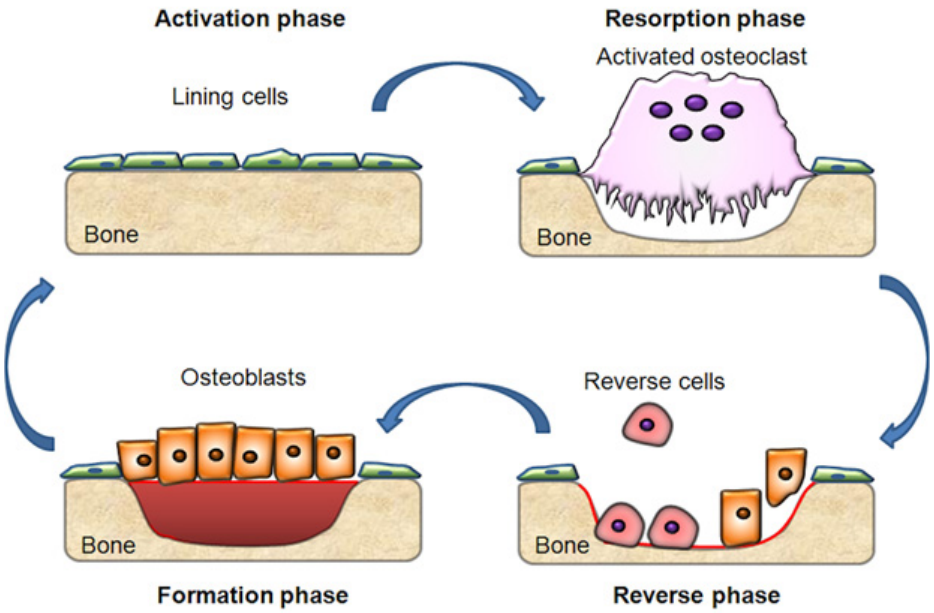

Figure 9. Schematic representation of the phases of the bone remodelling process.

their specific eph ligands results in a bidirectional signalling (110). Besides the many functions already ascribed to this pathway, such as regulation of cell migration, proliferation and cancer development, recent evidence has underlined a peculiar role in bone homeostasis.

Eph receptors include two subfamilies, Eph A and B, which interact with ephrin ligand A and B, respectively (111). The structure of Eph A and B receptors is characterised by an extracellular region which binds the ephrin ligand, and an intracellular region containing a juxta-membrane domain, a tyrosine kinase domain, a Sterile ALPHA Motif (SAM) and a PDZ binding domain. Ephrin B ligands are transmembrane proteins, while ephrin A ligands are attached to the extracellular cell membrane by a glycosyl-phosphatidyl-inositol binding.

Bidirectional signalling triggered by ephB/ephrin B1 has been investigated in the bone context first by Compagni and colleagues (112), who found that ephrin B1 knockout mice were lethal at birth and showed several skeletal abnormalities. Indeed, missense mutations or deletions of the gene coding for ephrin B1 have been associated with the human X-linked CranioFrontoNasal Syndrome (CFNS) (113).

Recent studies demonstrated that ephrin B2 expression increased during osteoclast differentiation, while osteoblasts express both ephrin B2 and its receptor (Figure 8C). Moreover, by interacting with ephB4 receptor located on osteoblasts, ephrin B2 triggered a forward signalling that, in turn, favoured osteoblast formation, while the reverse signalling, triggered by ephrinB2, inhibited bone resorption (114). These results were confirmed by the evidence that the conditional mice overexpressing ephB4 in osteoblasts showed an increase of bone mass and of bone formation rates, together with a decrease of osteoclast number. Therefore, the concurrence of ephB4/ephrinB2 forward and reverse signalling leads to a switch from resorption to formation.

5. BONE REMODELLING

5.1. Physiology of bone remodelling

Bone is the unique tissue physiologically undergoing continuous destruction and rebuilding. This peculiar property is known as bone remodelling, a dynamic process that is mandatory for the accomplishment of the following functions: i) removal of primary and infantile bone, and deposition of the mechanically competent secondary bone, ii) old bone renewing, iii) removal and substitution of ischemic or microfractured bone and iv) regulation of calcium homeostasis. Bone remodelling relies on the correct balance between bone resorption by osteoclasts and bone deposition by osteoblasts, whose functions must be tightly coupled not only quantitatively, but also in time and space (115).

Bone remodelling takes place in the so called Bone Multicellular Units (BMUs) throughout the skeleton and is accomplished according to the four following phases (Figure 9).

Activation phase. Different inputs, such as microfractures, alteration of mechanical loading sensed by the osteocytes and some factors released in the bone microenvironment, including IGF-I, TNF-alpha, PTH and IL-6, can trigger this phase, all of them converging on stromal and/or lining cells, the latter being quiescent osteoblasts, which in turn secrete factors able to recruit osteoclast precursors. To this regard, lining cells increase their own surface expression of RANKL, which interacts with its receptor RANK expressed by preosteoclasts, thus

allowing preosteoclast fusion and differentiation toward multinucleated osteoclasts. Another factor involved in this phase is the Monocyte Chemoattractant Protein-1 (MCP-1) a cytokine secreted by stromal or bone lining cells in response to PTH or inflammatory cytokines, such as TNF-alpha and IL-1beta, which recruits osteoclast precursors to the site of bone resorption (116).

Resorption phase. This phase takes 2-3 weeks in humans. Once differentiated, osteoclasts polarize, adhere to the bone surface and begin to dissolve bone. As already described, this function requires the acidification of the bone matrix to dissolve the inorganic component and subsequently the release of lysosomal enzymes, such as cathepins K, and of MMP-9, both in charge for the degradation of the organic component of bone (59).

Reverse phase. This phase lasts 9 days in humans, during which osteoclast activity is arrested, likely by promoting osteoclast apoptosis. Moreover, during this phase, the so-called reverse cells appear, whose role has not yet been completely clarified. Indeed, it is known that they are macrophage-like cells with a likely function of removal of debris produced during matrix degradation. Another hypothesized function for these cells is the release of factors that inhibit osteoclast and/or stimulate osteoblasts (115).

Formation phase. This phase takes 4-6 months in humans. Bone matrix resorption leads to the release of several growth factors herein stored, including BMPs, FGFs, TGFbeta, and IGF-II, which are likely responsible for the recruitment of the osteoblasts to the reabsorbed area. Indeed, as already told in the previous paragraph, osteoblast recruitment and differentiation also rely on osteoclast secretion of molecules, such as S1P, PDGF BB, HGF and Myb-Induced Myeloid protein-1 (mim-1). Once recruited, osteoblasts produce the new bone matrix, initially not calcified (osteoid), then promoting its mineralization, thus completing the bone remodelling process. Osteoblasts that remain encased in the new bone matrix eventually differentiate into osteocytes, other osteoblasts become quiescent and form flattened lining cells on the bone surface until a new remodelling cycle is started, while the remaining osteoblasts die by apoptosis (115).

5.2. Deregulation of bone remodelling and related diseases

The correct balance between bone deposition and resorption is crucial for the proper maintenance of the bone mass and the loss of this coupling is the starting point for skeletal pathologies, such as osteoporosis. This is a systemic, skeletal disorder characterised by low bone mass with a high susceptibility to fractures. Primary osteoporosis is a disease of elderly people, usually divided into postmenopausal (type I) and age-related (type II), essentially caused by the fact that the production of bone by means of osteoblasts cannot compensate for bone resorption by osteoclasts (117). Estrogens withdrawal and, to a lesser extent, androgen decline are the principal determinant of primary osteoporosis, since sex hormones act on osteoblasts favouring their survival and, at the same time, inducing osteoclast apoptosis through activation of the FASL/FAS pathway (118). Moreover, estrogens suppress the production of osteoblast-derived pro-osteoclastogenic cytokines, such as IL-1, IL-6, TNF-alpha, and RANKL (119), and stimulate the secretion of OPG (120).

Osteoporosis may also be secondary to a number of chronic diseases with an onset at any age (121). Indeed, many chronic diseases have a local or a systemic inflammatory basis, which has deleterious effects on bone mass (122). Another cause inducing severe osteopenia, eventually leading to osteoporosis, is mechanical unloading, due to muscle failure, disuse, paralysis or absence of gravity in astronauts (123).

Most drugs currently available for the treatment or prevention of osteoporosis, such as estrogens and selective estrogen receptor modulators, bisphosphonates and agents blocking the RANKL/RANK pathway (denosumab), are antiresorptive. However, it is desirable to identify novel agents that could have an anabolic function in order to improve and restore bone mass. Currently, only strontium ranelate, that besides inhibiting osteoclasts enhances bone formation, and intermittent administration of PTH are known for their osteoblast anabolic effect, therefore this field of research is clearly open to new discoveries and more effective treatments.

Bone remodelling is altered also in cancer-induced bone diseases as it occurs in multiple myeloma and bone metastases. Both are characterised by high morbidity caused by bone pain, hypercalcaemia, pathologic fractures, spinal cord and nerve root compression. According to their clinical and histological features, secondary bone cancers can be classified as osteolytic, osteosclerotic (or osteoblastic) and mixed. For instance, bone metastases of breast cancer and multiple myeloma are predominantly osteolytic, while the nature of bone metastases in prostate cancer is preferentially osteoblastic (124). However, a substantial body of evidence showed that osteoclast activation is required not only in osteolytic but also in osteosclerotic metastases, and that the bone resorption phase is a pre-requisite for the subsequent deposition of bone, so that both arise from a deregulation of the bone remodelling process (125).

Destruction of bone in osteolytic metastases is mediated by the osteoclasts rather than by the cancer cells themselves (124), since it has been observed that, once colonized the bone, tumours produce factors that directly or through the osteoblasts induce the formation of osteoclasts. Among them, there is the PTHrP, which in turn elicits RANKL expression by bone marrow stromal cells. Breast cancer cells also produce M-CSF, PGE2 (Prostaglandin E2) and several pro-inflammatory cytokines, such as IL-1, IL-6, TNF-alpha, GM-CSF and IL-8, which stimulate osteoclasts formation and enhance the bone-resorption activity (126). The increased destruction of bone matrix due to an exacerbated osteoclast activity is not compensated by an adequate bone formation and, as worse, leads to the release of tumour-seeking factors therein stored, such as TGF-beta,

IGFs, FGFs, PDGF and BMPs, that further stimulate cancer expansion. These events result in the so called "vicious circle" which replaces the "virtuous circle" of normal bone remodelling, progressively increasing bone destruction and tumour burden (124).

6. SUMMARY AND PERSPECTIVE

Bone is a specialized connective tissue with the peculiarity of a mineralized extracellular matrix, which gives this tissue the mechanical properties necessary to attend the locomotion and organ protection functions and, at the same time, confers to bone an extraordinary lightness. Despite its rigid structure, bone is a very dynamic organ, the only one undergoing destruction and rebuild several times during life. Bone remodelling is crucial to acquire a correct bone mass and for calcium homeostasis and it is strictly dependent on the correct functional coupling between two principal bone cells: the osteoclasts, devoted to resorb bone and the osteoblasts, with osteogenic functions. Indeed, these two cell types talk and regulate each other by releasing paracrine factors and this cross-talk is crucial for the maintaining of their coupling.

A correct balance between these two functions is mandatory for a correct bone mass, while a deregulation of this balance leads to pathological conditions that not only involve the bone, but have also an impact on other organs. This is explained by the increasing evidence clearly demonstrating the centrality of the bone tissue, which actively interacts with other organs, contributing to their homeostasis and regulation. The interplay between bone and immune system is one but not the only example, as very recently endocrine functions have been identified for the bone, which regulates kidney function, energy metabolism and male gonad functions.

It is clear though that the vision of the bone as a static tissue has been profoundly changed over the years, so that when we look at this organ we cannot ignore its influence on other tissues, especially in a pathologic contest. Therefore, a deeper understanding of the relationships between bone and other organs is likely to help identify new strategies for the management of previously unrecognized pathological conditions due to the disruption of this physiological cross-talk.

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Abbreviations: ALP: alkaline phosphatase, ATF2: activating transcription factor 2, BMPs: bone morphogenetic proteins, BSP II: bone sialoprotein II, CA II: carbonic anhydrase II, CFU-M: colony forming unit-macrophage, Dlx5: distal-less homeobox 5, DMP1: dentin matrix protein I, FGF23: fibroblast growth factor 23, GSK3beta: glycogen synthase kinase 3 beta, HGF: hepatocyte growth factor, IGF-1: insulin-like growth factor-1, LRP5: LDL-receptor related protein 5, MCP-1: monocyte chemoattractant protein-1, M-CSF: macrophage-colony stimulating factor, MITF: microphtalmia-associated transcription factor, MMP: Metalloproteinase, MT1-MMP: membrane type 1-MMP, NFATc1: nuclear factor of activated T-cells, cytoplasmic 1, OPG: osteoprotegerin, OPGL: OPG ligand, Osx: osterix, PDGF: platelet-derived growth factor, PTH: parathyroid hormone, RANK: receptor activator of NFkappaB, RANKL: Rank ligand, Runx2: runt-related transcription factor 2, S1P: sphingosine 1 phosphatase, TGF-beta: transforming growth factor beta, TNF-alpha: tumour necrosis factor alpha, TRAcP: tartrate resistant acid phopshatase, TRAF6: TNF receptor activated factor 6.

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