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REVIEW

The molecular mechanism behind bone remodelling: a review

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Abstract Bone is a connective tissue and guarantees protection and support of organ function. Contrary to the common view, bone is a dynamic tissue that constantly undergoes turnover in order to maintain stability and integrity. In this process called bone turnover or bone remodelling, two effector cell types are involved. Osteoclasts, specialised for bone resorption, and osteoblasts, responsible for bone formation, are key players in bone turnover. In the past decade, a lot of information about signal pathways, osteoblast–osteoclast communication and osteoclast activation concerning bone remodelling has arisen. In this publication, we aim to review molecular and biochemical insights with respect to the bone remodelling process. The bone remodelling process is of fundamental importance for craniofacial growth, orthodontic tooth movement and regenerative dentistry.

Keywords Bone remodelling · Osteoblasts · Osteoclasts · Runx2 · Regenerative dentistry

General function and structure of bone

Bone is a hallmark of all vertebrates and absolutely essential in terms of organ protection and support, brain and lung function, locomotion, support of haematopoiesis in the bone marrow, storage of minerals (i.e. calcium, phosphate) and providing attachment to muscles. Bone tissue is largely composed of type I collagen amounting to about 90% of total bone protein. The remaining organic component consists of non-structural proteins like growth factors, blood protein, osteonectin and osteocalcin. The inorganic phase of bone is mainly composed of mineral hydroxyappatite ($Ca_{10}(PO_4)_6(OH)_2$). Due to its material consistency, bone has a relatively flexible character as well as compressive strength. The mature bone is composed of two different types of tissue: The hard outer layer of bone, the so-called cortical bone, is responsible for the stability of the skeleton. This tissue appears smooth, white and solid. The cortical bone contains osteons (Haversian systems), which are composed of a central canal (Haversian canal) surrounded by lamellae of bone matrix. Within the lamellae, there are osteocytes embedded in tiny spaces (lacunae). The Haversian canal encompasses blood vessels and nerve cells throughout the bone and communicates with osteocytes in lacunae through canaliculi. Moreover, the corticalis has an outer membrane, the periosteum. The periosteum consists of an outer fibrous layer and an inner one that has osteogenic potential and enables the bone to enlarge [31]. The inside of bone is assembled by a trabecular network (spongiosa) and harbours bone marrow or embryonic connective tissue. The spongiosa ensures elasticity and stability of the skeleton and accounts for the main part (about 70%) of bone metabolism [50].

Bone remodelling

In order to maintain stability and integrity of bone, it is constantly undergoing remodelling, with about 10% of bone material being renewed each year [40]. Bone remodelling is a complex process that involves bone resorption performed by osteoclasts, followed by bone formation carried out by osteoblasts. In this process, these cells closely collaborate in

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basic multicellular units (BMU) [23, 25, 50]. The bone remodelling cycle involves different sequential steps, namely resorption, reversal and formation (Fig. 1) [23, 25]. The osteoclast stimulation occurs in BMU, which are small cortical and trabecular bone covering areas, where the remodelling process takes place. In these sites, osteoclastogenesis precedes with the expression of receptor activator of nuclear factor κ B ligand (RANKL) by bone lining cells, which in turn binds to the RANK that exists as a surface receptor on the membrane of pre-osteoclasts. This binding promotes an intricate and distinct signalling cascade for osteoclast activation and commitment [70]. The expression of RANKL is up-regulated in the presence of interleukin-1 (IL-1), tumour necrosis factor α (TNF-α) or vitamin D, whereas transforming growth factor ß (TGFß) or estrogens have an opposite effect. It is recognised that the expression of RANKL is up-regulated in malignant tumour cells, where it is involved in bone destruction. In the resorption stage, chemokines or chemotactic cytokines (MCP-1 or monocyte chemoattractant protein-1) are secreted by stromal cells or bone lining cells to attract and stimulate recruitment of osteoclast precursors for bone resorption. Furthermore, MCP-1 is produced in response to the presence of parathyroid hormone or inflammatory cytokines such as TNF-α and Interleukin-1ß [22, 41, 46]. Regarding bone turnover, osteocytes are considered to influence bone remodelling. Osteocytes, in bone embedded

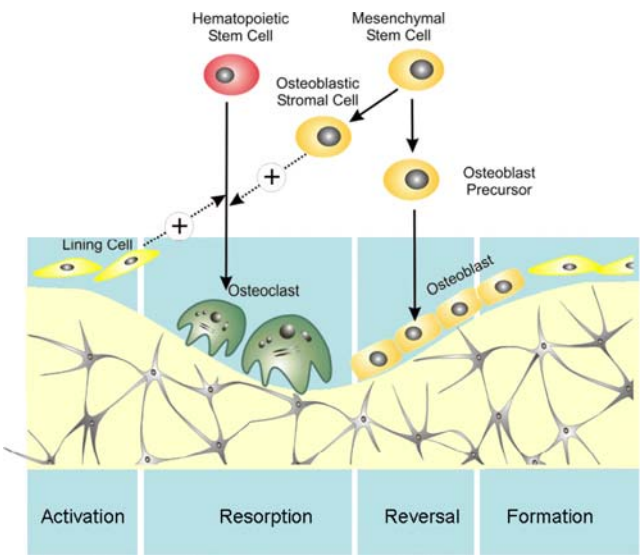

Fig. 1 Overview of bone remodelling. Resting phase—the bone surface is coated by resting flattened lining stromal cells. Resorption phase—after triggering the remodelling process, osteoclasts degrade old bone matrix. Reversal phase and bone formation—after bone removal, osteoclasts undergo apoptosis and osteoblasts become activated and lay down new bone material in the trench. Resting phase—with the replacement of old bone by new one, the osteoblasts form resting flattened lining cells on the surface of bone

spider-shaped cells, have been proposed to sense microcracks and microfractures, which trigger subsequent osteoclast differentiation and bone resorption [6, 46]. Osteocytes are connected with other cells on the bone surface or to each other by cytoplasmic processes, which traverse the bone. It is thought that osteocytes can regulate the expression of RANKL in osteoblasts, thus, influencing osteoclastogenesis [7]. It was demonstrated that radiolabelled parathyroid hormone localises to osteocytes, where PTH suppresses the expression of sclerostin, which is exclusively synthesised by osteocytes. Sclerostin exerts an antagonising action on the wnt signalling pathway by binding to the LRP5/6 receptor, which results in an inhibition of osteoblastogenesis and in an increased osteoblast apoptosis. Thus, osteocytes exert a negative regulation of osteoblast number and formation of bone by secreting sclerostin [19]. A special receptor for PTH, parathyroid hormone receptor 1 (PTHR1), is expressed in osteocytes and in osteoblasts [16]. O'Brien et al. [51] demonstrated that the activation of PTHR1 in transgenic mice decreases the expression of sclerostin, which results in a higher bone mass. However, a directed activation of the osteocyte specific PTHR1 was unexpectedly accompanied by an increase of osteoclast number and accelerated bone resorption, which was also manifested by an elevated level of RANKL and M-CSF. Nevertheless, the PTHR1 activation in osteocytes led to a higher rate of bone remodelling with a positive balance resulting in more bone [51]. In the further course of resorption, the osteoclast progenitors are differentiated to fully active osteoclasts that dig trenches with a depth of 40–60 μm in the bone [23]. The possibility has been discussed that during bone resorption liberated matrix, proteins from the bone may involved in osteoblast commitment, such as bone morphogenetic proteins (BMPs) and insuline-like growth factors -II, which in turn would reverse the process from bone resorption to bone formation. However, this assumption is questionable because of the matrix protein degrading effect of cathepsin-K and other proteases, which are secreted by the osteoclasts. In addition, some evidences were found that the coupling of liberated growth factors from the bone is dispensable for osteoblast commitment [46]. The resorption phase is followed by the reversal phase comprising the differentiation of osteoblast precursors and the discontinuation of bone resorption with osteoclast apoptosis. Osteoblasts become probably activated by secretion products from osteoclasts [46]. The osteoblast activating effect of osteoclast secretion products like sphingosine 1-phosphate (S1P), myb-induced myeloid protein-1 (mim-1), a B polypeptide chain platelet-derived growth factor homodimer (PDGF-BB) and a hepatocyte growth factor (HGF) has been demonstrated [46]. Secreted S1P binds to an osteoblast S1P receptor, which results to an up-regulation of RANKL expression and in an enhanced

osteoblast survival [46]. PDGF-BB supports a better proliferation of osteoblast pre-cursors, whereas the differentiation of these pre-cursors is suppressed. Secreted HGF from osteoclasts stimulates DNA synthesis and proliferation in osteoblasts as well in osteoclasts by binding to HGF receptor that is found in both cell types [46]. After osteoblast activation, osteoblasts lay down new bone material (i.e. collagen type I, osteocalcin, osteopontin, etc.) until the resorbed bone is entirely replaced by a new one [25]. Osteoblasts that become encased in the new bone matrix during bone formation are transformed to osteocytes, the most abundant cellular component of the bone [17]. The osteoblasts become quiescent at the end of bone remodelling, and they form flattened lining cell on the bone surface until a new remodelling cycle is triggered. The osteocyte product sclerostin may involve in this process by antagonising the canonical wnt signalling pathway, which leads to a reduced osteoblast activity. Osteopetrotic diseases like Van Buchem disease and sclerosteosis are caused by a loss of function mutation of the *sost* gene product sclerostin. Therefore, sclerostin is regarded as a key inhibitor that regulates the normal extent of bone formation and consequently protects against the deleterious effects of uncontrolled bone formation [55].

Osteoblasts: regulation and bone formation

Osteoblasts are effector cells for bone formation with the widely known ability to form bone tissue by secretion of alkaline phosphatase, type I collagen, proteoglycan, bone sialoprotein and osteopontin. Osteoblasts do not function individually but are found in clusters along the bone surface where they produce bone matrix by deposition of collagen 1. Subsequently, the collagen matrix becomes mineralised, presumably through the activity of alkaline phosphatase. Although the precise function of alkaline phosphatase is less clear, it has been assumed to be involved in the bone mineralisation process [71]. Besides their role in bone formation, osteoblasts are also involved in osteoclast differentiation as they produce RANKL or OPG to modulate osteoclast activity [62]. Osteoblast cells originate from pluripotent mesenchymal stem cells of the bone marrow [31]. A number of transcription factors and biochemical factors tightly regulate osteoblast recruitment, function and maturation. Osteoblast differentiation is promoted by the secretion of lipid-modified glycoproteins of the wingless (wnt) family, BMP-2 and several transcription factors.

In order to give a general survey, we aim to describe important signalling pathways that induce osteoblastogenesis and to define essential transcription factors such as Runx2 and Osterix.

Canonical wnt pathway

Wnt proteins are involved in signalling pathways during development and later stages of life. Regarding bone formation, wnt proteins activate a specific type of the intracellular signalling cascade called the canonical wnt signalling or wnt/ß-catenin pathway (Fig. 2) [54]. Binding of a secreted wnt protein through a low-density lipoprotein receptor-related protein 5 and 6 (LRP5/6) co-receptor leads to the disruption of a protein complex, consisting of adenomatous polyposis coli (APC), axin and glycogen synthase kinase 3 (GSK3). Consequently, the phosphorylation of ß-catenin by GSK3 is inhibited, thus, causing hypophosphorylation of ß-catenin. In this way, stabilised ß-catenin is accumulated in the cytoplasm and translocated to the nucleus where it interacts with T cell factor (TCF)/lymphoid enhancer binding factor transcription factors to support gene expression of wnt-responsive genes [5, 37]. In the absence of wnt, APC and axin act as a scaffold protein complex to support the phosphorylation activity and binding of GSK3 to the substrate ß-catenin, whereas phosphorylated ß-catenin is marked for degradation by the ubiquitin-dependent proteasome [37]. In fact, suppression of GSK3 activity by an artificial inhibitor (e.g. CHIR99021) caused an increase of bone mass [4]. In another case, an inactivating mutation of the wnt-specific co-receptor LRP5/6 caused osteoporosis [21]. Furthermore, results from quantitative PCR analysis pointed out that elevated wnt10b concentrations in a bone-marrow-derived cell culture was proportional to an increase of osteoblastogenic transcription factors (e.g. Osterix, Runx2) [4]. Thus, it has become clear that the wnt protein-induced signal cascade stim-

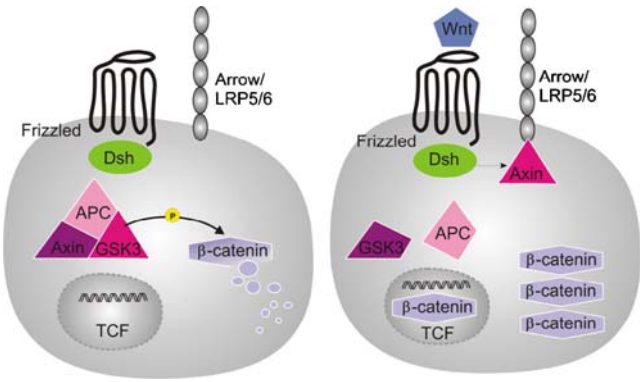

Fig. 2 Overview over the canonical wnt pathway. In the absence of wnt, the scaffold protein complex promotes the hyperphosphorylation of ß-catenin by GSK, whereby ß-catenin becomes degraded by the ubiquitin-dependent proteasome. The binding of wnt to the frizzled receptor results in activation of dishevelled and to an interruption of the scaffold proteins, axin and APC. This results in a hypophosphorylation of ß-catenin, which allows translocation of ß-catenin into the nucleus where it becomes associated with the TCF-transcription factor and participates in the initiation of transcription of target genes

ulates osteoblastogenesis. A number of secreted antagonist proteins regulate the canonical wnt pathway: For example, the antagonists of wnt protein are the secreted frizzled-related proteins, wnt inhibitory factor-1 that binds to wnt protein and prevents wnt activity [73] and members of the dickkopf family [45]. Dickkopf binds to LRP5/6 to make up a complex with the transmembrane Kremens protein (kringle-containing protein marking the eye and the nose) that trigger the removal of LRP5/6 from the cell surface [37, 45]. Thus, the number of wnt-specific receptors is reduced. Sclerostin (a SOST gene product), another antagonist of wnt protein and negative regulator of bone formation, binds to the LRP5/6 receptor and prevents binding of wnt protein [37, 68].

Bone morphogenetic proteins

BMP belongs to the large super family of TGFß. BMPs were extensively researched and were demonstrated to play an important role during embryonic development [30] as well as in cartilage and bone formation [8]. For instance, some BMPs were found in bone [67]. In vitro studies involving cell cultures have shown that BMPs are able to convert the differentiation pathway of myoblastic cell lines into that of osteoblast lineage. Furthermore, it was demonstrated that the expression of osteoblast specific proteins, such as alkaline phosphatase osteocalcin and the osteogenic transcription factor Runx2, is induced by BMPs [31, 32]. BMPs induce osteoblast differentiation through a Smad dependent signal pathway (Fig. 3): The secreted BMP dimer binds cooperatively to types I and II serine/threonine kinase receptor. Upon binding of BMPs to type II receptor, type I receptor kinase is phosphorylated by the activated type II receptor kinase. Receptor-regulated Smad (R-Smad; Smad 1, 2, 3, 5 and 8) proteins interact transiently with type I receptor kinase and become phosphorylated at SSXS motif at their C-termini [36]. Consequently, these phosphorylated R-Smad proteins associate with the common partner co-Smad protein (Smad 4) to form a heteromeric complex that is subsequently translocated into the nucleus. There, they interact with transcription factors, for example with Runx2, to participate in gene transcription [53]. Additionally, it has been reported that an inhibitory class of Smad proteins, i. e. Smad 6 and 7, interacts with phosphorylated type kinase I or with co-Smad and, thus, prevents the activation of R-Smad or reduces the scale of R-Smad/co-Smad complex formation [2].

Transcription factors that regulate osteoblast differentiation

Runx2/cfba1, a member of the Runt-related family of transcription factors, is an important transcription factor

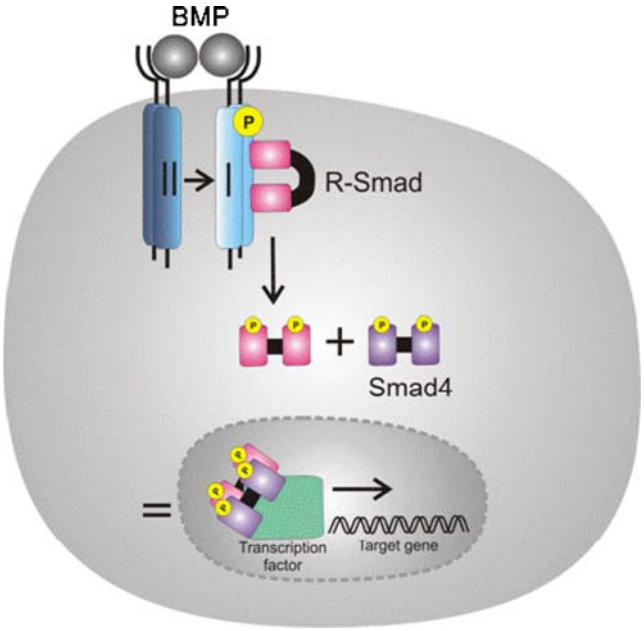

Fig. 3 Overview of the TGFß superfamily signalling pathway. Upon BMP activation, type II receptors transphosphorylate type I receptors. Type I receptor phosphorylates R-Smad proteins, which in turn associate with Smad-4 (co-Smad) in a complex. This complex moves across the nuclear membrane into the nucleus, where the Smad–protein complex induces specific gene expression

for osteoblast differentiation. As mentioned, signal pathways like wnt/ß-catenine and the TGF-ß/BMP pathway can modulate the transcriptional factor to induce an osteogenic phenotype [10, 18, 27]. It has been shown that null mutants of mice that do not express Runx2 fail to form any bone tissue and that osteoblast maturation is blocked [34]. Moreover, Ducy [13] demonstrated that Runx2 can activate osteoblast-specific genes in non-osteoblastic cells and that the expression rates of *osteocalcin*, *osteopontin*, *alkaline phosphatase* and *type I collagen* genes were variably expressed when cells were transfected with different isoforms of Runx2 [24]. Thus, it is obvious that transcription factor Runx2 plays a significant role for osteoblastogenesis. Osterix, a zinc finger-containing transcription factor, is similar to Runx2, an essential transcription factor for osteoblast differentiation. Studies made by Nakashima et al. [48] have demonstrated that *Osterix*-null mutants of mice show a similar phenotype like *Runx2*-null mutants in terms of the lack of bone formation owing to the absence of osteoblast differentiation. It was noted that in osteogenic cells of Osterix-null mutant, the transcription level of *Runx2* was in the same scale with those of wild-type osteoblasts [48]. However, no Osterix transcripts were detected in Runx2-null mice [49]. Therefore, it is assumed that Osterix must be acting downstream of Runx2 in the differentiation pathway of osteoblasts.

Osteoclasts: regulation and bone resorption

Osteoclasts are multi-nucleated cells specialised in bone resorption. The first step is involved in recruitment and dissemination of osteoclast progenitors, stemming from haematopoietic monocyte-macrophage lineage [38, 57]. It was noted that osteoclast activity and formation is dependent from the presence of two different cytokines, macrophage colony-stimulating factors (M-CSF) and RANKL and calciotropic hormones (Fig. 4) [31, 64]. M-CSF binds directly to its receptor, c-fms, on the surface of the early osteoclast progenitor and, thereby, provides signals required for proliferation [64]. Furthermore, M-CSF enhances osteoclast activity owing to prevent osteoclast apoptosis [20, 57]. Indeed, Yoshida [74] demonstrated the important role of M-CSF in rodents, where mutations in the coding region of M-CSF caused a severe osteopetrotic phenotype due to the functionally inactivated M-CSF. However, administration of M-CSF to the rodents with inactivated M-CFS led to the restoration of impaired bone resorption [15, 28, 33]. As mentioned, the second important osteoclast key activator is RANKL. RANKL is a member of the TNF gene family, which is predominantly expressed on the surface of osteoblast stromal cells. Systemic and local factors like parathyroid hormone, IL-1 or prostaglandins

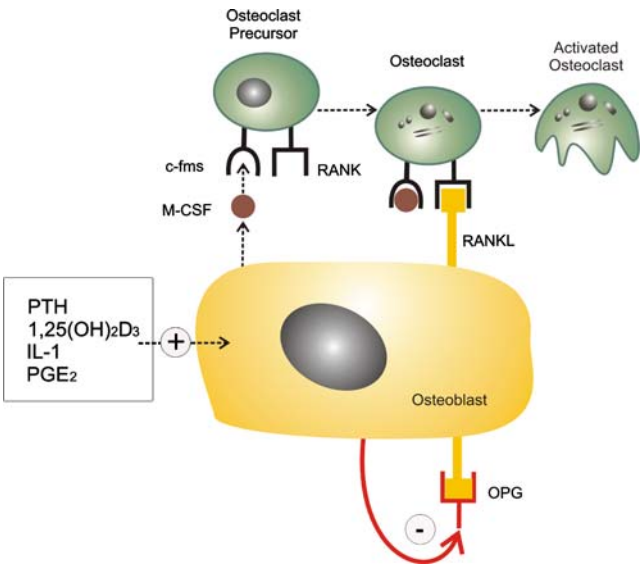

Fig. 4 Overview of cell–cell interaction between osteoblast and osteoclast. M-CSF acts on osteoclast precursors to control their proliferation and differentiation. The osteoblasts produce a protein called receptor activator of nuclear factor kappa B ligand; this can bind to the receptor RANK that is localised on the osteoclast precursor and stimulate it to differentiate to fully activated osteoclast. OPG that is secreted by osteoblasts competes with RANK for RANKL. Parathyroid hormone (*PTH*), interleukin-1 (*IL-1*), prostaglandin (*PGE2*) and *1,25 (OH)$_2$ vitamin D$_3$* are stimulators of bone resorption in that they stimulate the expression of RANKL in osteoblast cells

can induce osteoclast formation by increasing expression of RANKL on the surface of immature osteoblasts and marrow stromal cells [10, 31, 35, 39, 57, 61]. The osteoclast differentiation by RANKL and its RANK is accomplished by cell–cell interaction between osteoblasts and osteoclasts. RANKL that is membrane bound on the osteoblast surface thereby binds to RANK, which is placed on the surface of the osteoclast [56, 65]. For instance, it has been observed for decades that in vitro differentiation of monocytes to osteoclasts occurs only with co-cultured osteoblasts [63]. Moreover, studies by Odgren [52] and Dougall [12] pointed out the crucial role of RANK and RANKL concerning osteoclast activation. Thus, cell-to-cell signalling by RANKL and RANK is necessary for osteoclast differentiation. Another cytokine that is expressed by osteoblasts, osteoprotegrin (OPG) acts as decoy receptor for RANKL and, thereby, inhibits osteoclast differentiation [60, 72]. Thus, it was assumed that the proportion between RANKL and OPG could regulate bone resorption and bone strength [35]. Under inflammatory conditions, such as rheumatoid arthritis or myeloma, the proportion between RANKL to OPG is increased, thus, favouring increased osteoclastogenesis [57]. Three calciotropic hormones acting in different ways control osteoclast activity [56]: Vitamin D promotes the differentiation of osteoclasts from monocyte macrophage stem cell precursors in vitro. In vivo, enhanced osteoclastic bone resorption was observed when vitamin D was applied at high doses [26, 66]. This effect is caused by the stimulation of RANKL production by osteoblasts. Parathyroid hormone is a peptide secreted by cells of the parathyroid gland [56]. It is secreted in response to changes in blood calcium and affects bone formation and resorption. It acts antagonistically to calcitonin by increasing the concentration of calcium in blood plasma. Parathyroid hormone binds to osteoblasts and induces the production of surface proteins (M-CSF and RANKL) that stimulate the maturation and action of osteoclasts [56]. Calcitonin is synthesised primarily by the parafollicular C-cells of the thyroid in response to the calcium concentration within the C-cells. Several studies concluded that calcitonin inhibits bone resorption by acting directly on osteoclasts through its receptor [9, 56].

The initial step of bone resorption is migration of osteoclasts to the bone area to be remodelled, where they become adherent to the bone. The attachment of osteoclasts is accomplished by formation of podosomes containing filamentous actin and $\alpha_v\beta_3$ integrin, which interact with bone matrix proteins, such as osteopontin and vitronectin [14, 47, 66]. The intimate contact between the bone matrix and the osteoclasts is reflected by the polarisation of the latter, with the plasma membrane forming three distinct areas: first, the basolateral membrane which is not in

contact with bone material; second, the sealing zone that is closely attached to the bone; and third, the ruffled border which is ring-shaped and surrounded by the sealing zone. The ruffled border faces the bone matrix and is in direct contact with the matrix. The sealing zone forms a diffusion barrier to ensure a defined proton and protease concentration secreted by the ruffled border in the evolving resorption lacunae underneath the osteoclast cell [69]. Active osteoclasts form a resorptive front side, attaching to the bone matrix. Osteoclasts are capable of dissolving bone material, such as mineral hydroxyapatite, by secretion of hydrochloric acid. A V-type ATPase, which is present in the ruffled membrane of osteoclasts, translocates protons into the resorption lacuna and, thus, acidifies the environment (pH~4.5). Moreover, a carbonic anhydrase II is present in osteoclasts to supply the need of protons. To ensure electro neutrality, a chloride channel (CLC7) simultaneously transports the counter ion chloride [69]. Because of demineralisation of bone material, the organic phase of bone becomes more accessible. It is assumed that lyosomal cathepsin K and, to a lesser extent, matrix metalloproteinases are responsible for the degradation of organic bone matrix. Present data show that cathepsin K plays a major role in degradation of type-1 collagen under acidic conditions [58]. In contrast, matrix metallo proteinase-9 (gelatinase B or MMP9), a second abundant protease, from osteoclastic origin barely degrade the organic matrix. Rather, MMP-9 is considered to be involved in the initiation of bone degradation by the removal of the collagenous layer from the bone surface and cleaning the resorption pits from remaining collagen after cathepsin K digestion [11, 42]. During bone resorption, a high concentration of calcium up to 40 mM was observed in the microenvironment of the resorption site [59] that inhibits osteoclast activity [43]. The rise of calcium results in an alteration of the calcium level in the osteoclasts is followed by actin reorganisation and podosomal disassembly, and, consequently, leads to apoptosis [43, 44]. Even TGF-ß that blocks bone resorption has been reported to promote osteoclast apoptosis, which is reflected in nuclear and cytoplasmic condensation and fragmentation of DNA [25]. Besides calcium and TGF-ß, other substances like estrogens, some kinds of bisphophanats and taxomifen were able to induce osteoclast apoptosis [1, 3, 29].

Conclusion

Bone is a connective tissue designed to withstand mechanical forces. It undergoes remodelling in certain time intervals or in response to microdamages. Bone turnover is a complex process influenced by the mutual actions of osteoblasts and osteoclasts, which effect bone renewal. A variety of factors such as hormones and cytokines is involved in the differentiation and regulation of osteoblast and osteoclast cells. Disturbances in signal pathways, bone responsive transcription factors that affect bone remodelling can cause severe bone diseases (e.g. osteopetrosis, osteoporosis). The understanding of the molecular and biochemical mechanisms that enable the bone to maintain stability and integrity is essential to devising therapeutic strategies in all fields of regenerative medicine and dentistry.

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Conflict of interest The authors declare that they have no conflict of interest.

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