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Importance of osteocyte-mediated regulation of bone remodelling in inflammatory bone disease

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Summary

Although the impact of osteoblast-osteoclast crosstalk in bone remodelling has been intensively studied, the importance of osteocytes, descendants of osteoblasts, in this process has for a long time been neglected. During their embedding phase, osteocytes undergo considerable phenotypic transformation, from a cuboidal, highly metabolically active osteoblast secreting extracellular matrix to a small, stellate, quiescent osteocyte with numerous long dendrites. Osteocytes are encysted in cavities (lacunae) and their dendritic extensions are located in tunnels (canaliculi) forming a remarkable, highly branched, lacunar-canalicular signalling network that spans the entire bone matrix. Osteocytes and their dendrites can communicate directly with each other and through the release of effector proteins such as sclerostin and nuclear factor κB ligand (RANKL), influence osteoblast and osteoclast formation. This allows osteocytes embedded within the bone matrix to communicate and coordinate activity of cells on the bone surface to adapt to mechanical needs and hormonal changes.

Besides their importance in sustaining physiological bone homeostasis, accumulating evidence suggests that dysregulated osteocyte function and alterations in the osteocyte lacunar-canalicular network structure are characteristics of skeletal diseases.

This review highlights some aspects of osteocyte communication with osteoclasts and mesenchymal stromal cells, the importance of blood vessel-osteocyte interaction and describes central functions of these cells in rheumatoid arthritis, osteoarthritis, osteomyelitis and osteoporosis. Within the last decade new technologies and tools have facilitated the study of osteocyte biology and the search for therapeutic targets to address bone fragility in the near future.

Keywords: *osteocytes, inflammation, bone remodelling, musculoskeletal disorders*

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The function of osteocytes in the bone remodelling process

Bone tissue is simultaneously resorbed at millions of skeletal sites throughout the body and is replaced shortly afterwards. This continuous bone remodelling process is required to replace old bone tissue and to repair bone micro-cracks. In adults it has been estimated that 10% of bone is replaced every year and that this is crucial for maintenance of a healthy skeleton [1, 2]. Bone remodelling throughout life is strictly controlled through a complex network that controls the crosstalk between osteoblast and osteoclast cells. During bone renewal, small areas of bone are resorbed by osteoclasts, which in turn leads to osteoblast recruitment that fill these regions with new bone tissue. This coupling of osteoclast and osteoblast activity is termed the "basic multicellular unit" or BMU. Bone formation and bone resorption are coupled in the BMU through factors that are entrapped within the bone matrix and released upon its degradation by osteoclasts. The BMUs in trabecular and cortical bone show differences in their structure and how they remove and replace bone. In trabecular bone the BMUs are located on the bone surface and are covered by a thin canopy layer of elongated mesenchymal cells covering the whole bone remodelling area and separating it from the bone marrow. The BMUs of cortical bone are located inside the bone within cylinders that have a pointed "cutting zone" formed by osteoclasts [3]. Osteoblast precursors migrate along the abluminal surface of blood vessels [4] to form new bone along the cement line. Cement lines, visible by their reduced mineralisation [5], follow the scalloped surface created by osteoclasts and represents the interface between old and new bone.

Until recently, osteoclasts were described as initiating bone remodelling; however the trigger for this initiation was not known [3]. Evidence has accumulated in the last few years that osteocytes are indeed fact key initiators and drivers of bone remodelling.

Osteocytes are descendants of matrix-producing osteoblasts, encysted in cavities (lacunae) and are located deep inside the bone matrix. The exact life span of osteocytes is unknown; however, it is estimated that they can survive for decades if undisturbed in locations with a slow bone turnover rate [6]. Osteocytes are known to play a crucial role in sensing mechanical loading and regulation of calcium and phosphate homeostasis.

For decades it has been well known that osteocytes can remove and remodel their surrounding bone matrix struc-

tures. Osteocytes are able to demineralise their perilacunar/canalicular matrix, a mechanism defined as "osteocytic osteolysis". It occurs during various physiological and pathophysiological conditions such as immobilisation, hyperparathyroidism and lactation, and has an important function in calcium homeostasis.

Qing et al. demonstrated that lactating mice have larger osteocyte lacunae than virgin controls to mobilise bone mineral. Seven days post-lactation new bone is formed around the lacunae and their size returns to virgin levels. Osteocytes seem to use similar molecular mechanisms to osteoclasts to remove mineralised bone matrix. They up-regulate osteoclast markers such as tartrate-resistant acid phosphatase (TRAP) and cathepsin K in response to elevated parathyroid hormone-related peptide (PTHrP) [7]. Additionally, they create an acidic environment in their lacunar-canalicular space through carbonic anhydrase 2 and the proton pumping vacuolar ATPases. However, they lack a sealed resorption pit and therefore it is not clear which mechanisms protect them from the acidic milieu [8, 9]. Further studies are needed to understand the precise mechanisms of osteocytic osteolysis and its differences from osteoclast-mediated bone resorption.

Recent studies have revealed that osteocytes control bone remodelling by communication with cells located in their surrounding environment. Each cell exhibits 40–100 dendritic extensions [10], which, by extending through the bone matrix in channels called canaliculi, form connections to neighbouring osteocytes, blood vessels and cells on the bone surface. Murine canaliculi are between 50 and 100 nm in diameter [11] and dendrites are connected to each other via gap junctions [12], building an active and responsive network inside the bone that links osteocytes to each other and to cells at the periosteal and endosteal surfaces as well as endothelial cells of the capillaries.

Crosstalk of osteocytes with osteoclasts and osteoblasts

Whereas osteoblasts (and consequently osteocytes) originate from the mesenchymal lineage, osteoclasts are of haematopoietic origin and are derived from the monocyte/macrophage lineage. During osteoclast differentiation, the monocyte/macrophage-derived osteoclast precursors fuse with each other forming large multi-nucleated cells that are unique in their ability to degrade the bone matrix. Two osteoblast-derived factors, macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor κB ligand (RANKL), are essential for osteoclastogenesis, and the number of osteoclasts is severely reduced in mice in which the activity of either one of these factors is impaired [13–17]. RANKL signals via its receptor RANK, present on osteoclast precursors and mature osteoclasts. Loss-of-function mutations in either *TNFSF11* or *TNFRSF11A*, the genes encoding RANKL and RANK, respectively, result in osteoclast-poor osteopetrosis characterised by increased bone mass due to defective osteoclast differentiation [18–21]. RANKL and M-CSF are also expressed by osteocytes supporting the generation of functional osteoclasts at sites of bone remodelling [22]. Interestingly, mice deficient in M-CSF display osteocyte defects as well as osteopetrosis [23]. This could be owing either to direct effects of M-CSF on the differentiation and/or survival of osteocytes, or to the absence of osteoclast- and/or macrophage-dependent signals. Nakashima et al. [24] demonstrated that purified osteocytes have a greater capacity to support osteoclastogenesis by release of RANKL than osteoblasts or bone marrow stromal cells. In agreement with this, it has been demonstrated that RANKL secreted by osteocytes is essential for bone remodelling whereas RANKL produced by osteoblasts is redundant [25]. The action of RANKL can be blocked by osteoprotegerin (OPG), a soluble decoy receptor that binds to RANKL, preventing it from binding to RANK [26], and the RANKL/OPG ratio is a key determinant of the degree to which osteoclast-mediated bone resorption occurs at a given site [27–29]. OPG expression by osteocytes was found to be highly increased upon ovariectomy, possibly as a protective or reparatory mechanism in response to increased bone loss [30].

In order to exert their function, osteoclasts adhere to the bone surface via an actin ring (or sealing zone) that surrounds a ruffled border, enabling them to create a local acidic resorptive microenvironment [21, 31]. Bone is a rich source of growth factors, which are embedded in the bone matrix and released upon osteoclast-mediated bone resorption [32]. One of these growth factors, which is highly present in the bone matrix and released and activated as a result of osteoclast activity is transforming growth factor-β (TGF-β) [33–35]. TGF-β is a pleiotropic cytokine controlling a wide variety of cellular responses during bone remodelling affecting, amongst others, the differentiation of both osteoclasts and osteoblasts. Importantly, TGF-β couples bone resorption and formation, since the active TGF-β1 released during osteoclast-mediated bone resorption induces migration of bone mesenchymal stem cells to the resorptive sites where they undergo osteoblastic differentiation in response to the signals provided by the local microenvironment generated by the osteoclasts [36]. Whereas osteoclasts recruit osteoblasts to sites of bone remodelling, the osteoclasts and their progenitors are thought to be directed to these locations in an osteocyte-dependent manner. Osteocytes undergo apoptosis as a result of microdamage or lack of mechanical stimulation due to disuse or weightlessness, which promotes recruitment and/or differentiation of osteoclast precursors to the sites where osteocyte apoptosis had occurred [37–39]. Increased expression of the osteoclast-promoting cytokines RANKL and vascular endothelial growth factor (VEGF) and decreased expression of OPG in osteocytes was found to coincide with up-regulation of caspase-3 and to be blocked by inhibiting osteocyte apoptosis [40–42]. The RANKL released from the apoptotic osteocytes subsequently controls osteoclast localisation and differentiation and bone resorption, followed by recruitment of osteoblast precursors [41, 42]. It is noteworthy that osteocyte-specific deletion of connexin 43, which mediates the communication between osteocytes and osteoblasts, is required for their survival, leading to an enrichment of osteoclasts in areas where osteocytes have undergone apoptosis and at the same time reduced their OPG expression levels [43]. Together, this evidence demonstrates that bone remodelling and the recruitment of osteoclast and osteoblast precursors is targeted to sites where osteocyte apoptosis has occurred by localised shifting of the RANKL/OPG ratio.

Osteocytes also regulate osteoblast mineralisation by targeting the Wnt signalling pathway through the release of bone anabolic Wnt proteins and their inhibitors the Dickkopf related protein 1 (DKK1) and sclerostin. Overexpression of Wnt1 in mice results in bone formation by increasing osteoblast number and activity [44]. Wnt inhibitor sclerostin and DKK1 are known to block Wnt proteins by binding LRP 5/6 receptors leading to increased bone volume and density [45–47].

Crosstalk of osteocytes and mesenchymal stromal cells

Mesenchymal stromal cells (MSCs) are multipotent cells but do not fulfil all stem cell criteria [48]. MSCs can be isolated out of many tissues and the characteristics of these cells can differ; however, there are three minimum criteria to be fulfilled: they must be plastic-adherent under standard culture conditions; they must occupy the potential to differentiate into adipocytes, osteoblasts and chondroblasts *in vitro*; and they must express the surface markers CD105, CD73 and CD90 and lack haematopoietic markers including CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR [49].

At present, little is known about the influence of osteocytes on MSCs. Conditioned media from the osteocytic cell line MLO-Y4 increases the osteogenic differentiation potential of MSC compared with supernatant of the osteoblastic cell line MC3T3-E1 [50]. Moreover, co-cultures of osteocytes with MSCs leads to greater calcium deposition than osteoblasts cultured with MSCs [51], indicating an important role for MSC-osteocyte interactions on driving bone mineralisation. Despite the lack of knowledge concerning the soluble signals affecting osteogenic differentiation, osteocytes seem to have an active stimulatory impact on bone formation rates.

Osteocyte signalling is not only involved in osteogenic, but also in adipogenic differentiation of MSCs. Fairfield and colleagues found that sclerostin, an inhibitor of bone formation mainly released by osteocytes, induces adipogenesis in bone marrow-derived MSCs [52]. Knockout of the *Sost* gene (encoding sclerostin) or the pharmacological inhibition of sclerostin decreased bone marrow adipose tissue formation, demonstrating that the bone marrow niche is regulated by osteocytes in the extracellular matrix through sclerostin release. To our knowledge, there are currently no reports on the influence of osteocytes on chondrogenic differentiation of MSCs.

Osteocyte-blood vessel interactions and their impact on bone remodelling

The importance of blood vessels in endochondral bone formation in the embryo has long been understood, with vascularisation of the bone primordium always preceding ossification. More recently the precise role of the blood vessel endothelial cells in guiding osteoblasts to sites of bone formation [4, 53] and regulating their maturation has been identified [54, 55]. In some instances it appears that the blood capillaries themselves can undergo mineralisation [56] and thus guide trabecular patterning.

In the context of trabecular bone modelling, the interaction of osteocytes with bone endothelial cells has not yet been investigated in detail; however, studies of the small bones of the ear has demonstrated that osteocyte lacunae align in parallel to bone capillaries, reminiscent of the patterning observed in the cortical bone osteons of larger mammals [53]. Indeed, analysis by scanning electron microscopy of human bone [57] and of optically cleared mouse long bones has demonstrated direct interaction of osteocyte canaliculi with endothelial cells and with the capillary-associated osteoclasts that are responsible for cutting the micro-canals that house these capillaries [58]. Given that osteocytes are the main producers of RANKL [24], it seems likely that osteocyte signalling guides the formation of transcortical capillaries by osteoclasts. Osteocytes also secrete prostaglandin E2 which has regulatory effects on angiogenesis and vascular permeability [59, 60], and therefore may also control bone oxygenation and metabolism.

In an initial study to assess the effect of inflammation on transcortical capillaries, Grüneboom et al. [58] showed that chronic arthritis in mice increased transcortical vessel number within 62 days of arthritis onset, demonstrating that changes in the bone vascular system can be highly dynamic. This increase in vessel number was successfully blocked with bisphosphonate treatment, indicating the requirement of osteoclasts for new bone vessel formation. An increase in cortical canals has also been observed in patients with rheumatoid arthritis, in some cases very early in disease [61]. It remains to be seen whether the increase in bone capillaries is a driver of disease and damage in arthritis or secondary to the metabolic requirements of the inflamed tissue, however this represents an exciting and novel opportunity for future work.

The techniques now exist to visualise the bone in much higher detail that was previously possible. Advances in micro-computed tomography (microCT), including synchrotron-mediated microCT, are now able to give resolution below 1 μm [62, 63], allowing visualisation of cortical vessels. A particularly important development in this field is optical clearing of tissue which, for the first time has allowed 3D visualisation of immuno-labelled whole bones [64].

Communication between osteocytes in bone diseases

Although osteocytes are located deep inside the mineralised bone matrix, they do not exist isolated without connection to their environment. Osteocytes build a dense lacunar-canalicular network that affects bone remodelling by aiding communication between one cell and another. This network is also highly dynamic, osteocytes can extend and retract their dendrites [65] adapting to environmental changes to maintain bone homeostasis.

Patients with musculoskeletal disorders such as osteoporosis, rheumatoid arthritis, osteoarthritis and osteomyelitis suffer from imbalance of bone remodelling. It still remains largely unknown how the architecture of the osteocyte network is involved in this process and how this might influence bone loss and repair, especially under disease conditions. The importance of the osteocyte network during degenerative bone disorders will be discussed in the following sections.

Rheumatoid arthritis

Rheumatoid arthritis is a systemic autoimmune disease characterised by joint inflammation, pain, fatigue, and destruction of articular cartilage and bone. In particular, localised osteoclast-mediated bone resorption at the interface between inflammatory synovial tissue and subchondral bone is responsible for erosions seen in rheumatoid arthritis patients. The impact of osteocytes and their network on inflammatory bone loss due to the communication with adjacent osteoclasts in rheumatoid arthritis is not yet understood.

Recently, *in vitro* studies have demonstrated that stimulation of osteocytes with serum from patients with rheumatoid arthritis, containing elevated levels of inflammatory cytokines, increases the RANKL/OPG ratio, which leads subsequently to enhanced osteoclastogenesis and bone destruction [66]. Moreover, osteoblastogenesis is reduced as a result of enhanced expression of DKK1 and sclerostin by osteocytes, both negative regulators of bone regeneration. Pathak and co-authors found that osteocytes produce pro-inflammatory cytokines such as tumour necrosis-α (TNFα), interleukin (IL)-6 and IL-1β, key factors in rheumatoid arthritis pathogenesis, which are up-regulated by rheumatoid arthritis -serum treatment *in vitro*. Therefore osteocytes might provide a new therapeutic target to treat inflammatory bone loss in rheumatoid arthritis [67].

Osteoarthritis

Osteoarthritis is a chronic, degenerative joint disease featuring articular cartilage degradation, osteophyte formation, muscle weakness and inflammation that has a number of reasons including mechanical joint instability, obesity, genetic pre-disposition and ageing. Osteoarthritis has been long considered as a primary disorder of articular cartilage; however, the impact of bone in the pathology of osteoarthritis is gaining increasing interest. It is commonly accepted that subchondral bone sclerosis is a hallmark of osteoarthritis [68, 69]. As a result of abnormal bone remodelling, subchondral bone is thickened and hyper-mineralised in osteoarthritis and these changes have been detected prior to any evidence of cartilage damage [70]. Moreover, phenotypic changes of osteocytes might be involved in the increase of bone volume and calcification: Studies from Jaiprakash et al. and Tate et al. demonstrated that the osteocyte bodies in subchondral bone from patients with osteoarthritis are rounder with fewer, more disorganised dendrites and decreased connectivity compared to healthy controls [71, 72]. They also identified reduced expression of sclerostin and increased expression of dentin matrix protein 1 (DMP1), a regulator of bone mineralisation, in osteoarthritis osteocytes that correlated with increased subchondral bone volume.

The importance of the osteocyte network for osteoarthritis development has recently been shown in a mechanically-induced post-traumatic osteoarthritis mouse model. The authors revealed that an intact osteocyte network in the subchondral bone contributes to the development of osteoarthritis, suggesting that targeting may ameliorate severity of the disease [73].

Although the role of osteocytes and their network in osteoarthritis remains to be elucidated, these current studies suggest a critical regulatory function of osteocytes in bone homeostasis and mineral metabolism during osteoarthritis development.

Osteomyelitis

Osteomyelitis is characterised by inflammation of the bones and the surrounding tissues due to bacterial infections leading to bone deformations and destruction. This infection is very painful and not easy to treat, especially when it becomes chronic. Unfortunately, the rate of clinical failures in treatment is high, and the consequence is often loss of function and/or amputation [74, 75].

One of the main pathogens is *Staphylococcus aureus*, which persists in the bone by invading osteoblasts and osteoclasts, which provides protection from the immune system [76–78]. The internalisation of S. *aureus* by osteoblasts leads to decreased osteoblast activity characterised by reduced proliferation, increased apoptosis, decreased expression of osteogenic markers such as collagen type I, osteopontin, osteocalcin, and decreased alkaline phosphatase (ALP) activity [79–81]. Additionally, infected osteoblasts display an imbalanced RANKL/OPG ratio, shifting the bone remodelling process towards bone destruction. Osteoblasts infected by *S. aureus* show increased expression of RANKL and prostaglandin E_2 (PGE_2) [79, 81, 82]. PGE_2 is a hormone-like molecule, which induces RANKL expression in an autocrine and paracrine manner via activation of the EP4 receptor on osteoblasts [83].

Osteoclasts and their precursors can also be infected by *S. aureus*. Infected precursors release pro-inflammatory cytokines that trigger osteoclastogenesis of uninfected cells, whereas the infection of mature osteoclasts lead to increased resorption activity and subsequent bone loss [78]. Of note, De Mesy Bentley et al. [84] demonstrated that in a murine mouse model of osteomyelitis *S. aureus* invades into the osteocyte lacunae, proliferates and migrates throughout the canaliculi network. Recently, they identified invasion of *S. aureus* in the osteocytic-canalicular system of an amputated limb from a patient suffering from diabetic foot ulcer and *S. aureus* chronic osteomyelitis [85]. These new findings reveal that microorganisms not only infect osteoclasts and osteoblasts, but are also able to migrate through the dense osteocyte network, providing a new mechanism to spread and sustain the infection.

Osteoporosis

Patients with osteoporosis display an imbalance between bone resorption and bone formation leading to bone loss and a higher fracture risk associated with an increase in morbidity and mortality. Osteoporosis is most commonly associated with ageing, affecting mostly postmenopausal women. It is assumed that the bone loss seen in these patients is linked to osteocyte death triggered by oestrogen deficiency [86]. Tatsumi et al. analysed osteocyte function in an osteocytes deletion model. This mouse model is based on a diphtheria toxin receptor-mediated cell knockout (TRECK) system under an osteocyte specific promoter. After a single injection of diphtheria toxin approximately 80% of osteocytes (but no osteoblasts) are deleted. This triggers $TRAP^+$ osteoclast invasion into the cortical bone and recruitment of ALP^+ osteoblasts to the intracortical cavities created by osteoclasts. Additionally, long-term effects such as decreased trabecular and cortical bone mass

and microarchitecture deterioration occur, demonstrating a role for osteocytes in bone loss in osteoporosis [87].

Since the ability to sense and respond to mechanical load depends on many factors, such as shape of the cell and the cytoskeleton, it is likely that the mechanical stimulation of osteocytes in osteoporosis is affected [88]. Interestingly, osteocyte-ablated mice exhibited resistance to bone loss induced by limb-unloading [87]. Moreover, an ulnar-loading model demonstrated that enhanced loading reduces sclerostin release by osteocytes and promotes bone formation. On the contrary, unloading of the hind limbs lead to an upregulation of *sost* transcript [89] and subsequently inhibits bone formation. These results clearly demonstrate that osteocytes need mechanical load to sense and adapt to external stimuli.

The mechanical load on bone is amplified in the interstitial fluid flow, which is the movement of fluid throughout the lacunar-canaliculi system [90]. The resulting fluid shear stress in osteocytes controls the expression of signalling proteins that influences the differentiation or activity of osteoblasts and osteoclasts [89, 91, 92]. Ciani and colleagues showed enhanced fluid movement in the cancellous bone across the lacunar-canaliculi system in ovariectomised rats after mechanical stimulation [93]. In this model of postmenopausal osteoporosis, it could also be shown that oestrogen deficiency resulted in increased canaliculi size and lacunar-canalicular porosity [94]. This demonstrates that altered interstitial fluid flow in oestrogen-deficient rats is associated with an alteration of transmission signals. All together, these studies propose osteocytes as important mechanosensors regulating bone remodelling by releasing signal proteins to maintain bone homeostasis.

Therapies for bone repair

Therapeutics to treat bone loss have until recently focused on targeting osteoclasts. More recently drugs that promote osteoblast function have also been developed and the potential for targeting osteocyte activity is an exciting new avenue for therapy. Antiresorptive agents such as bisphosphonates are the most common medication to target osteoclast-mediated bone resorption in patients suffering from osteoporosis [95]. One major property of bisphosphonates is their ability to bind strongly to the bone mineral hydroxyapatite. Osteoclasts, during their resorption process, take up and internalise bisphosphonates from the bone surface. Bisphosphonates act by driving apoptosis of osteoclasts or through the inhibition of farnesyl pyrophosphate (FPP) synthase, preventing post-translational prenylation of GTPases that are required for the ruffled border formation of osteoclasts. As a consequence osteoclast resorption is inhibited [96, 97]. Another antiresorptive drug is denosumab, a monoclonal antibody against RANKL. However, treatment with bisphosphonates or RANKL inhibitors is able to prevent excessive bone resorption, and do not affect bone formation. One possible reason for that is that osteoclasts release crucial factors from the mineralised matrix such as active transforming growth factor-β (TGFβ) or insulin-like growth factor-1 (IGF-1) that are needed to enhance migration of mesenchymal stromal cell to the site of resorption to promote bone formation [36, 98]. Thus, new drugs that are able to inhibit bone resorption while increasing bone formation are needed.

Most recently, the potential for therapeutic-targeting of osteocytes has been realised with anabolic drugs such as anti-sclerostin antibodies for osteoporosis treatment coming onto the market. Sclerostin, mainly expressed by osteocytes, exerts its inhibitory actions on bone formation by negatively regulating the canonical Wnt signalling pathway, and therefore its inhibition is a very promising strategy to promote bone formation. Neutralising antibodies to sclerostin have been developed to treat patients with osteoporosis. Interestingly, these antibodies display dual effects by increasing bone formation as well as decreasing bone resorption. Phase III studies of the monoclonal anti-sclerostin antibody romosozumab (AMG785) have demonstrated positive results. In 2016, the FRAME study (Fracture study in postmenopausal women with osteoporosis) was published, in which 7180 postmenopausal women received a monthly dose of romosozumab or a placebo for one year, followed by denosumab treatment every 6 month for one year. After 12 and 24 months, the risk for new vertebral fractures decreased in the romosozumab group [99]. The FRAME study was followed by the ARCH study (Active Controlled Fracture Study in Postmenopausal women with osteoporosis at high risk), published in 2017. In this study, 4093 patients received monthly subcutaneous injections of romosozumab or weekly oral administration of alendronate, followed by alendronate only for another 12 months. The risk for new vertebral fractures was 48% lower in the romosozumab-to-alendronate group than in the alendronate-to-alendronate group; however, for the first time, an imbalance in cardiovascular adverse events in the romosozumab group compared with the alendronate only group was observed [100]. An explanation for this trend could be that sclerostin is constitutively expressed in the aorta [101] and is upregulated in foci of vascular and valvular calcification, but the function of sclerostin in cardiovascular tissue remains unknown [102, 103]. Unfortunately, because this study was not designed as a cardiovascular risk study it lacked a placebo-only control. Thus, further work is required to understand the cardiovascular risks of targeting sclerostin in these patients. The last phase III study, the BRIDGE study (placebo-controlled study evaluating the efficacy and safety of romosozumab in treating men with osteoporosis), tested romosozumab in men and was published in 2018. Two hundred and forty-five men were treated with romosozumab or placebo once a month for 1 year. The outcome of the study was significantly higher bone mineral density of the lumbar spine in the romosozumab group compared with placebo control. Again, there was an imbalance in adverse cardiovascular events in the romosozumab group [104]. In 2019, the US Food and Drugs Administration approved the drug romosozumab (trade name Evenity) for patients suffering from postmenopausal osteoporosis with high fracture risk. Excluded are women with a cardiovascular event one year before initiation of the treatment.

Conclusion

Despite their importance in controlling bone homeostasis, the precise function of osteocytes and the molecular mechanisms underpinning how osteocytes influence bone remodelling, especially under disease conditions, are far from clear. Several studies have revealed that osteocytes

release signalling molecules that can either promote or impair osteoblastogenesis and osteoclastogenesis, and also seem to be important regulators for new blood vessel formation. It is likely that not only osteocytes themselves but also the pattern of the osteocyte lacunar-canalicular network plays a key role in maintaining healthy bone homeostasis. Advancing our understanding of osteocyte biology should prove useful in developing novel therapies to preserve osteocyte dendricity and viability thereby protecting against bone loss and maintaining bone health.

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