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REVIEW

Osteocyte: the unrecognized side of bone tissue

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Abstract
Introduction Osteocytes represent 95% of all bone cells. These cells are old osteoblasts that occupy the lacunar space and are surrounded by the bone matrix. They possess cytoplasmic dendrites that form a canalicular network for communication between osteocytes and the bone surface. They express some biomarkers (osteopontin, β3 integrin, CD44, dentin matrix protein 1, sclerostin, phosphate-regulating gene with homologies to endopeptidases on the X chromosome, matrix extracellular phosphoglycoprotein, or E11/gp38) and have a mechano-sensing role that is dependent upon the frequency, intensity, and duration of strain.
Discussion The mechanical information transmitted into the cytoplasm also triggers a biological cascade, starting with NO and PGE_2 and followed by Wnt/β catenin signaling. This information is transmitted to the bone surface through the canalicular network, particularly to the lining cells, and is able to trigger bone remodeling by directing the osteoblast activity and the osteoclastic resorption. Furthermore, the osteocyte death seems to play also an important role. The outcome of micro-cracks in the vicinity of osteocytes may interrupt the canalicular network and trigger cell apoptosis in the immediate surrounding environment. This apoptosis appears to transmit a message to the bone surface and activate remodeling. The osteocyte network also plays a recognized endocrine role, particularly concerning phosphate regulation and vitamin D metabolism. Both the suppression of estrogen following menopause and chronic use of systemic glucocorticoids induce osteocyte apoptosis. On the other hand, physical activity has a positive impact in the reduction of apoptosis. In addition, some osteocyte molecular elements like sclerostin, connexin 43, E11/gp38, and DKK1 are emerging as promising targets for the treatment of various osteo-articular pathologies.

Keywords Apoptosis · Micro-cracks · Osteocyte · Osteoporosis · Remodeling

Introduction

Osteocytes constitute the main cellular component of mammalian bones. Osteocytes represent more than 95% of all the bone cells (20,000 to 80,000 cells/mm^3 of bone tissue); there is approximately 20-fold more osteocytes than osteoblasts in a bone [1–3]. Osteocyte density has been evaluated at 31,900 and 93,200 cells/mm^3 in bovine and murine bone, respectively [4].

In human bone, Frost estimated that the mean half life of osteocytes is 25 years [5]. Meanwhile, bone remodeling induces a bone tissue turnover of 4% to 10% per year [6], so the expected duration of the life of an osteocyte is not easy to determine and is not likely to be this long. The life duration of osteocytes is higher than that of osteoblasts, which is an estimated 3 months in human bone [6], and 10–20 days in mice-woven bone [7].

The role of osteocytes remained unknown for a long time and was probably underestimated. Over the last 15 years, many publications have aimed to highlight the role of this cell. Osteocytes are distinctive and isolated cells that are embedded within the bone matrix, whereas osteoclasts, osteoblasts, and lining cells are present at the bone surface. The specific location of these cells within the bone matrix may explain

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some astonishing metaphors in the literature, such as "choreography from the tomb" [8] and "buried alive" [3]. In another title, they are named "martyrs for the integrity of bone strength" due to the physiological role played by their apoptosis on bone remodeling regulation [9].

Morphology

Osteocytes have a dendritic morphology, while their cell body has fusiform shape in long bones or sometimes rounded in flat bones (Figs. 1 and 2) [10]. These cells are localized in an osteocytic lacuna and have been buried in the matrix [8]. They present some cytoplasmic "extensions": the dendrites that extend into channels in the matrix called "the canaliculi". Osteocytes communicate with each other and with the cells at the surface of the bone tissue via these dendrites [11]. The lacuno-canalicular system which represents only 1% of interstitial fluid volume constitutes a molecular exchange surface area which has been estimated at 400-fold higher than the Havers and Volkmann system and 133-fold higher than the trabecular bone system (1,200 m^2 versus 3 and 9 m^2, respectively for rat male [12]).

Some studies have shown that according to the type of bone formed and the activity of the osteoblasts involved, the newly formed osteocytes can adopt a variable size and morphology in comparison with older osteocytes already embedded in the matrix [13]. Moreover, the morphology of embedded osteocytes is dependent on the bone type. Indeed, osteocytes found in trabecular bone are more rounded than osteocytes from cortical bone [14], with the latter adopting an elongated morphology [14]. In humans, osteocytes measure approximately 10 μm across the short axis and 20 μm along the long axis in long bones [4, 15].

The dimensions of the murine osteocyte lacunae are around 5 by 20 μm [16] with a gap present between the cell and the lacunar wall. Likewise, the cytoplasmic processes are approximately half the diameter of the canaliculi, with murine canaliculi ranging between 50 and 100 nm in diameter [16]. Osteocytes display polarity in terms of the distribution of their cell processes with the majority coming from the cell membrane facing the bone surface [11].

Origin and biomarkers

Osteoblasts are involved in bone matrix mineralization. At the end of the bone-forming phase, osteoblasts have one of three different fates: (1) they are embedded in the bone as osteocytes, (2) they are transformed into inactive surface osteoblasts called bone-lining cells, or (3) they undergo programmed cell death (apoptosis). An osteocyte is an old osteoblast buried in the matrix that it has itself produced; some of the pre-osteoblastic and osteoblastic characteristics remain detectable in these cells (osteopontin, integrin β3). During the process of bone formation, some osteoblasts are left behind in the upwardly advancing newly formed osteoid material and become entombed in the matrix as an "osteoid osteocyte". During the process of "burial", the future osteocyte maintains contact with the advancing osteoblasts at the surface by extending cellular processes [17], while the surrounding osteoid matrix becomes mineralized [17]. Aubin has suggested that only 10–20% of osteoblasts differentiate into osteocytes [17]. Molecular

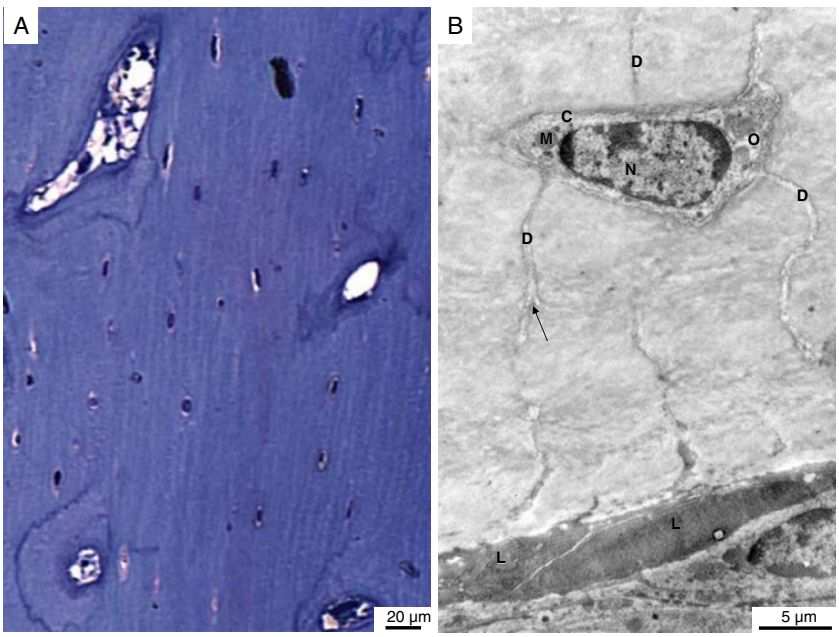

Fig. 1 Bone tissue and osteocyte morphology. **a** Histological section of Wistar rat tibia after toluidine blue staining showing the fusiform shape of osteocytes. **b** Transmission electron microscopy of osteocyte in cortical bone tissue. *O* osteocyte, *L* lining cell, *M* mitochondria, *N* nucleus, *C* cytoplasm, *D* dendrites, *arrow* dendrite process dichotomy

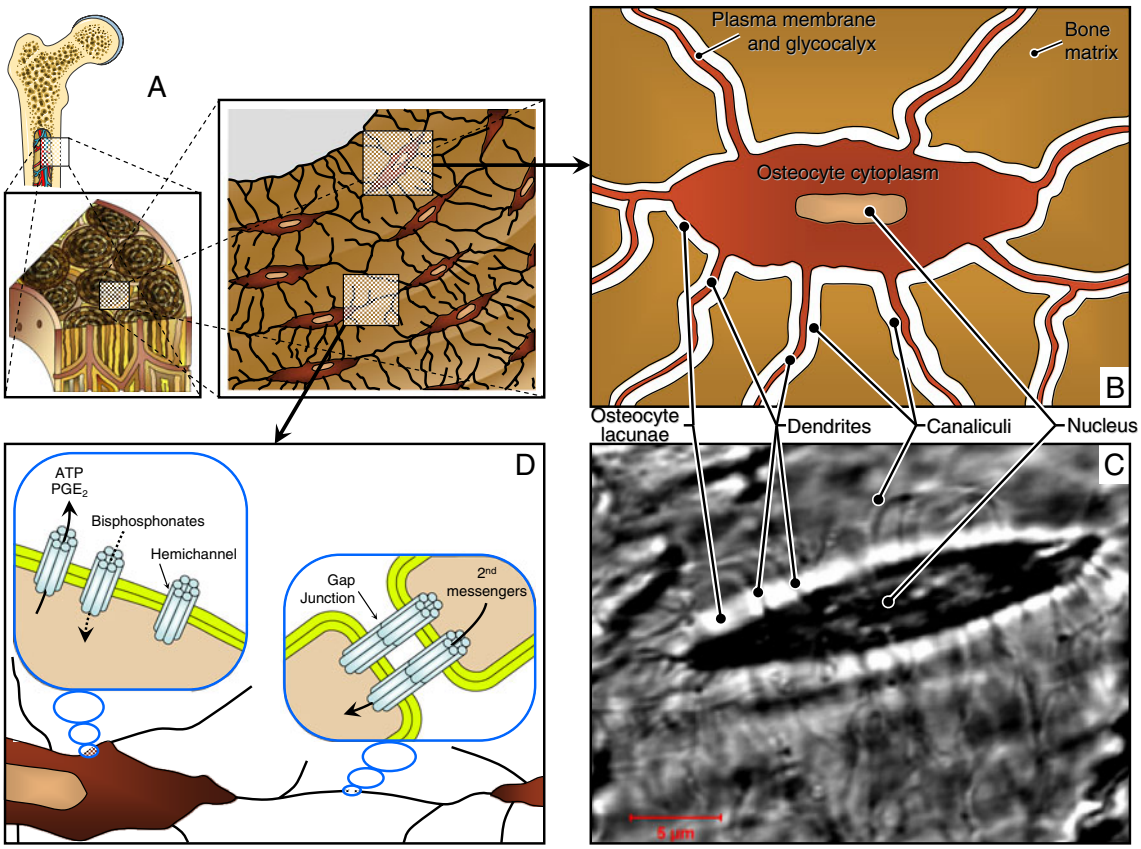

Fig. 2 Bone tissue anatomy, tissue and cellular architecture. **a** Multi-scale bone tissue anatomy: organ, osteon, osteocyte, and cytoplasmic processes. **b** Schematic representation of an osteocyte. Osteocytes are embedded into a mineralized bone matrix. **c** Confocal microscopy imaging of an osteocyte from Wistar rat tibia. **d** Osteocyte communication. Connexin-composed connexons form hemi-channels that control the passage of the E2 prostaglandin or ATP between the osteocyte cytoplasm and the extracellular compartment. These hemi-channels are also implied in the mechanism of action of bisphosphonates. Two separate hemi-channels from two adjacent osteocytes can contact each other to form a gap junction and can thereby exchange information. Gap junctions are thus involved in the communication between two osteocytes

mechanisms regulating these processes are not fully understood and may depend on the location of bone formation, on the species, on the age and/or gender, and on the mode of ossification [3].

Cells at this early stage of osteoblast to osteocyte differentiation have been variously named as “large osteocytes”, “young osteocytes”, “osteoid osteocytes”, or “pre-osteocytes” [18]. These cells are larger than mature osteocytes and have numerous ribosomes, a well-developed endoplasmic reticulum and a wide Golgi complex, both involved in the synthesis of proteins and mucopolysaccharides [3]. The study of the different markers (membrane, nuclear, and cytoplasmic markers) allows for the profiling of osteocytes to determine the stage of cell maturation (young osteocyte versus mature osteocyte) [3]. Osteocyte differentiation is accompanied by the progressive reduction of several bone markers (alkaline phosphatase, bone sialoprotein, osteocalcin, collagen type I, Runx2), the preservation of some markers (osteopontin, β3 integrin, E11/gp38 antigen), and the appearance of new markers (CD44, dentin matrix protein 1 (DMP1), matrix extracellular phosphoglycoprotein (MEPE)) [3] (Table 1). Once the osteoid mineralizes, osteocyte ultrastructure undergoes further changes including a reduction in the endoplasmic reticulum and Golgi apparatus corresponding to a decrease in protein synthesis and secretion [19]. At this stage, many of the previously expressed bone markers are down regulated in the osteocyte (including alkaline phosphatase, bone sialoprotein, osteocalcin, collagen type I, Runx2) (Table 1) [19].

Currently, the molecular control of osteocytogenesis is largely unknown. Most of the in vitro osteocyte characteristics have been based on the use of the murine osteocyte-like cell line MLO-Y4 [20]. These cells, isolated from the long bones of transgenic mice, were characterized by long dendritic processes, osteopontin and connexin 43 expression, low levels of collagen type I, and alkaline phosphatase expression [20]. Osteocytogenesis is accompanied by increased expression of the genes for MEPE and DMP1, which have been associated with the osteocyte phenotype. In rodents, the restriction of mechanical stimulation (exercise) results in

Table 1 Molecular markers involved in osteocyte differentiation

Biomarkers	Osteoblast	Young osteocyte	Mature osteocyte
Alkaline phosphatase	++	-	-
Bone sialoprotein	++	+/-	+/-
Osteocalcin	++	+/-	+/-
Collagen type I	++	+/-	+/-
Runx2	++	-	-
Osteopontin	++	++	++
β3 integrin	++	++	++
E11/gp38 antigen	+/-	++	-
CD44	+/-	++	++
Sclerostin	-	+/-	++
DMP1	-	++	+/-
MEPE	-	++	++

DMP1 Dentin matrix acidic phosphoprotein 1, *MEPE* matrix extracellular phosphoglycoprotein; ++ present, +/- variable expression, - absent or present at levels below detection

increased levels of the hypoxia-related hypoxia-induced transcription factor-1 alpha protein in osteocytes [21].

A recent study has determined a role for matrix metalloproteinase-2 in the regulation of osteocyte production and the generation of an appropriate canalicular system [22]. DMP1 is an extracellular matrix protein member of the SIBLING family [23]. A recent work has emphasized the relative osteocyte specificity of this molecule and implicated it in osteocyte function and signaling [24]. Toyosawa et al. noted an osteocyte specificity of DMP1 in rat bone located on cell processes and in the peri-cellular matrix [24]. It is possible that DMP1 is a target molecule for CBFA-1 and is absent in CBFA-1 knockout animals. DMP1 knockout is associated with a hypo-mineralized phenotype linked with elevated fibroblast growth factor 23 (FGF23) and defective osteocyte lacuno-canalicular network formation [25].

In 2000, two studies described a factor synthesized by osteoblasts and osteocytes, this factor being named either osteoblast/osteocyte factor-45 (OF45) or MEPE [26, 27]. The protein OF45/MEPE is another member of the SIBLING family that is known to influence mineralization directly in a BMP2 stimulated in vitro model of osteogenesis and to inhibit phosphate uptake by renal cell lines [28]. Targeted disruption of OF45/MEPE results in increased bone mass and a degree of resistance to age-related trabecular bone loss [29].

Mechano-reception and mechano-transduction

Long time regarded as simply a cell at the end of its lifetime, embedded in a mineral matrix, the osteocyte is now seen as the cell at the center of and the initiator of the bone remodeling process [30]. Indeed, osteocytes form an interconnected network of cells having the capacity to detect mechanical pressures and loads. Although it is widely accepted that the osteocyte is the cell responsible for sensing mechanical strain, there is debate within the field as to whether the osteocyte cell body or dendrites are primarily responsible for mechano-sensation [31, 32]. Nevertheless, this mechanical variation stimulates osteocytes to emit specific signals to cells present at the bone surface in response to this mechanical stimulus [33] (Fig. 3).

Mechanical strains and weight bearing loading forces play a major role in the triggering of the bone remodeling process all along the adult life [34]. Indeed, the bone tissue is able to adapt continuously to these mechanical loads by adding bone matrix to improve resistance to increased loads or by resorbing bone in response to a decrease in use. The parameters locally influencing this balance between formation and bone resorption are now well characterized in vivo and include frequency, intensity, and duration of the mechanical stimulus [35, 36]. Thus, bone mass is influenced by the applied tension peak [35], whereas the bone formation rate is modulated by the stimulus frequency [36]. The existence of load and discharge cycles leads to a

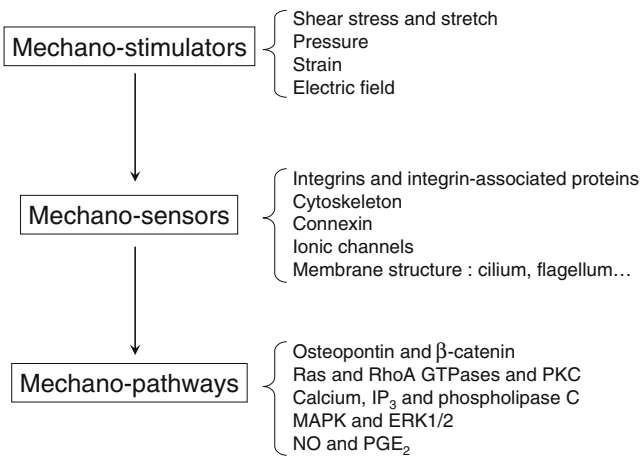

Fig. 3 Mechano-transduction. The osteocyte mechano-transduction involves a stimulus (mechano-stimulator), a receptor apparatus (mechano-sensor), and a consequent signaling cascade (mechano-pathway)

greater increase in the bone formation than when this same stimulus is applied only once [35, 36]. Lastly, bone quality and strength are improved if the mechanical stimulus is applied by short increments rather than over long periods [37]. The effects of these mechanical variations are well known and characterized macroscopically, and the most recent studies now seek to determine how mechanical modifications are detected at the cellular level and how this mechanical transduction is carried out.

Theoretical models and experimentations suggest that the lacuno-canalicular interstitial fluid flow varies with the extra-vascular pressure and with variations of mechanical loads applied to bone tissue and osteocytes [38]. Thus, mechanical forces applied to the bone induce interstitial fluid movements along canaliculi and osteocyte lacunae, and consequently, cause shear stress at the cellular level and deformations of the osteocyte plasma membrane [39, 40]. Theoretical models showed that shear stress applied to the osteocyte membrane during a physiological load was about 8–30 dyn/cm^2 [38]. It would be a major breakthrough in this mechano-reception field if one were able to measure interstitial fluid flows and pressures along the lacuno-canalicular system. It has also been suggested that this mechanical information could be directly detected by ciliar or flagellar structures of the cellular membrane [41, 42]. These cilia systems are already known to be critical to mechano-reception in other organs, for example, in the inner ear. Moreover, as osteocytes seem sensitive to many other factors like hypoxia [43], it is highly possible that the osteocyte mechano-reception is carried out via several modalities [33].

Whatever the mechanism of this mechano-reception, osteocytes are able to respond to mechanical stimulation by modulating the expression and the secretion of many molecules [44], including insulin-like growth factors (IGF-I and IGF-II) [45–47], osteocalcin [45, 48], sclerostin [49, 50], *c-fos* [45, 46], the synthesis prostaglandin enzymes G/H [45, 51], prostanoids [52, 53], and nitric oxide (NO) [54–56]. Osteocyte mechano-reception may stimulate the Wnt/Lrp pathway as a negative regulator of sclerostin secretion [49], whereas the sclerostin itself is a negative regulator of the bone formation. Following the perception of the mechanical message and conversion into a chemical message, the osteocyte can propagate its message by two nonexclusive methods: firstly, by diffusion of produced molecules (for example, the paracrine effects of NO secretion), and secondly, by a method of local transmission through gap junctions. These junctions form a connection between the cytoplasms of two adjacent cells. Gap junctions are formed by molecules of the connexin family and *Cx43* is the main connexin found in bone [20, 57]. These communicating junctions allow for the passage of molecules with a molecular weight of less than 1 kDa, such as prostaglandin PGE_2 [53, 58]. Furthermore, Cx43-constituted hemi-channels were reported to be essential transducers of the anti-apoptotic effects of bisphosphonates on osteocytes [59]. For this reason, these communicating junctions are currently perceived as being critical to the osteocyte mechano-transmission.

Apoptosis, micro-cracks and bone remodeling

Osteocytes are cells that not only play a physiological role during their lifetime, but also achieve functions through their apoptosis (Fig. 4). Micro-cracks have a deleterious effect on the bone tissue if they are in excess, with possibilities of micro-fissures, micro-fractures, and fractures due to bone deficiency. However, the micro-cracks and the breaking of the lacunar network seem to play an important physiological role [60]. It has been observed that pro-apoptotic molecules are elevated in osteocytes found in the vicinity of the micro-crack, whereas anti-apoptotic molecules are expressed 1–2 mm from the micro-crack [60], suggesting that the apoptotic area may be restricted to the neighborhood of the micro-crack.

The message transmitted by osteocyte apoptosis travels through the canalicular network to the surface of the bone tissue and is sent on to the progenitor cells. The real nature of this message is not known. It may consist in fluid movement, biochemical signals, or electrical stimuli [33, 51]. This message leads to the initiation signals for remodeling, thereby, stimulating the bone resorption/formation cycle. It should be noted that this phenomenon could directly stimulate resorption without passing through the usual message pathways that involve the lining cells. This direct initiation could be linked to the molecular expression of (RANKL) Receptor Activator for Nuclear Factor κ B Ligand, also known as TNF-related activation-induced cytokine (TRANCE), osteoprotegerin ligand (OPGL), and ODF (osteoclast differentiation factor) by osteocytes and especially by the cytoplasmic dendritic processes in the canaliculi [61]. Furthermore, it has been demonstrated that osteocytes may be involved in a process of direct stimulation of osteoblasts. This modeling phenomenon does not require a preceding local resorption. This type of bone formation could exist near fractures or during growth [61, 62].

Until recently, no mechanism has been demonstrated which explains the death of osteocytes either in old age or in association with osteoporosis and osteoarthritis [60]. More recently, Noble reported that the sudden suppression of ovarian function in young women with endometriosis taking (GnRH) Gonadotropin-releasing hormone, also known as Luteinizing-hormone-releasing hormone (LHRH) and luliberin analogs is associated with high prevalence rates of detectable deoxyribonucleic acid breaks in osteocytes [15]. Animal experiments show that both modulation of mechan-

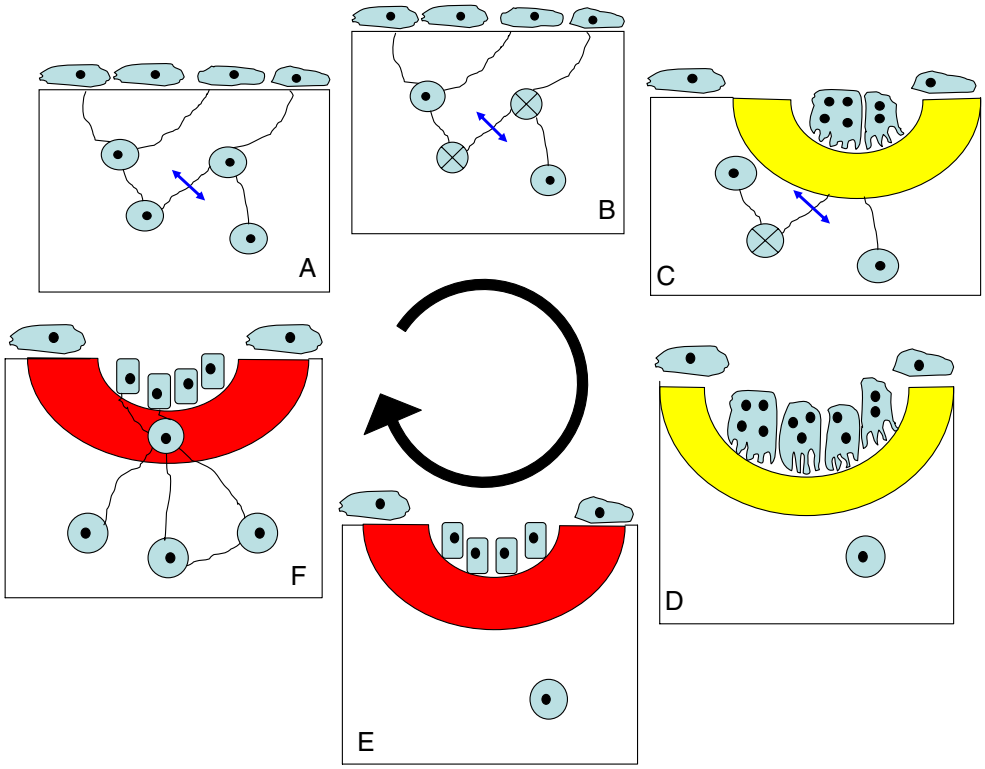

Fig. 4 Osteocyte death—the sequence of events. **a** A micro-crack (⟷) severes the canaliculi of several osteocyte dendritritic processes. **b** The micro-crack induces osteocyte apoptotic death (⊗) and a biochemical signal is transmitted to the lining cells at the surface of the bone tissue. **c** Lining cells (▭) and osteocytes (⊙) both release local factors that attract cells in the circulation and cells in marrow into the remodeling compartment where osteoclastogenesis occurs. **d** Osteoclasts ( ) resorb the matrix and the micro-crack (lacunae resorption:). **e** Reversal phase formation of the cement line, osteoblasts deposit newly formed osteoid matrix: . **f** Newly reformed canaliculi and an osteocyte network

ical loading and ovariectomy induce an increase in apoptosis of osteocytes and lead to micro-damages [63–66].

It is most likely that osteocyte death through apoptosis occurs at very high and at very low strain levels. The simplest explanation of the high rate of apoptosis of osteocytes seen after plastic deformation is that the micro-cracking of the matrix leads to mechanical damage of the osteocyte cell processes [60]. Noble et al. hypothesized that the death of osteocytes by apoptosis serves as a homing signal for osteoclasts and thus acts to encourage local bone resorption [67]. Alternatively, living osteocytes might act to inhibit osteoclastic resorption by expression of osteoprotegerin or other osteoclast inhibitors [29]. Osteocytes are also capable of generating the protein osteopontin, which contains a (RGD) is a standard amino acid abbreviation, meaning Arginine (Arg or R) – Glycine (Gly or G) – Aspartic Acid (Asp or D) integrin recognition site and appears able to induce osteoclast or osteoblast attachment to the bone matrix depending on the circumstances [68]. Osteocytes interact with the extracellular matrix through integrins [69, 70] and/or through dendritic processes in the canalicular wall [71]. Fluid movement through the canaliculi resulting from mechanical loading may induce a deformation of the extracellular matrix, generate tension in the tethering elements, or directly disturb the cell membrane [71, 72]. These changes may be transduced via integrin clustering into resultant survival signaling [73]. Moreover, dying osteocytes serve as initiators of targeted bone remodeling in response to physiologic or excessive strain to prevent the accumulation of micro-damages [6].

Osteocytes, by their apoptosis, are cells whose sacrifice acts to protect the integrity of bone strength [9]. Micro-cracks, apoptosis of the osteocytes, and the classical remodeling all lead to the resorption of the fragile matrix zone beyond the micro-crack. It is thought that the zones made up of old, highly mineralized bone are the most subject to micro-cracks. One theory of bone turnover is a finalist perspective, whereby, the remodeling leads to the disappearance of the micro-cracks and surrounding old mineralized bone and replaces this imperfect or “old” bone with newly formed bone that has properties that are more favorable.

A detectable space is present between the mineralized bone surface and the osteocyte surface. This space contains non-mineralized extracellular matrix enriched in non-collagenous proteins and proteoglycans. This matrix facilitates the

formation of bone fluids and the regulation of osteocyte activity through soluble factors [74]. Numerous studies have suggested that osteocytes orchestrate bone remodeling, regulating osteoblast and osteoclast activities [44, 73]. While the exact process, which leads to the initiation of bone remodeling at a specific site is currently unknown, many data suggest that osteocytes play a crucial role [44].

In 1968, Baud showed that some osteocyte lacunae exhibit irregular borders and considered this shape as reminiscent of osteocyte osteolysis activity [18]. The hypothesis of osteocyte osteolysis recently got some support from studies in rodents [75, 76]. Few studies have attempted to assess whether or not osteocytes may also have matrix deposition and mineralization ability [76–78]. The parathormone could have an influence on osteocyte osteolysis and matrix synthesis [76].

Phosphate and vitamin D metabolism

Phosphate is very important at the cellular level, where it is a component of many metabolic cycles (for example, during the synthesis of adenosine triphosphate (ATP)) and in various essential cellular functions, such as muscular conduction or coagulation [79]. At the tissue level, phosphate ions are necessary for the bone matrix mineralization [80]. However, as extreme variations in the levels of circulating phosphate (hypophosphatemia and hyperphosphatemia) have harmful effects [79, 81], several pathways exist to control phosphatemia. These control loops make it possible to avoid large variations in phosphate production and/or degradation while allowing bone mineralization to continue.

Historically, the first discovery of these loops controlling phosphate involves the parathyroid hormone (PTH) and vitamin D axes [82, 83]. This loop also controls calcemia and phosphatemia. It is well known that hypocalcemia leads to the production of PTH, which in turn acts on osteoblasts to stimulate the release of calcium and phosphate from the skeletal reservoir [83] most likely via the upregulation of RANKL, the osteoblastic signal for osteoclastogenesis. It is unknown whether PTH acts directly on osteoclasts to increase the resorption of the mineral matrix [61] as PTH receptors have never been identified on osteoclasts. PTH also causes a reduction in the urinary calcium excretion from the distal tubule, an inhibition of the phosphate urinary reabsorption from the proximal tubule, and a stimulation of the production of di-hydroxylated vitamin D, the active form of vitamin D [82]. The renal production of active vitamin D, as a di-hydroxylated metabolite, leads to an increase in calcium and phosphate absorption by the small intestine enterocytes [82]. Finally, this PTH/activated vitamin D axis is able to counterbalance hypocalcemia while modulating phosphatemia [84].

More recently, a second pathway for the regulation of phosphate has been identified. FGF23, Klotho, phosphate-regulating gene with homologies to endopeptidases on the X chromosome (PHEX), and DMP1 have all been implicated in this second pathway [85]. Fibroblast growth factor 23 (FGF23) is a low molecular weight, fibroblastic growth factor of 32 kDa [86, 87]. It is produced and secreted mainly by osteocytes, but also by bone marrow venous pericytes, ventrolateral thalamic nodes, and lymphatic elements [88, 89]. The osteocyte basal expression of FGF23 is low and increases when the DMP1/PHEX pathway is inhibited [90]. FGF23 is produced in response to an increase in the levels of di-hydroxylated vitamin D [89], and it transmits a signal on binding to a dimeric trans-membrane receptor. Indeed, this receptor is composed by both an isotype of the FGF receptor (type-1c, -3c or -4) and by an essential cofactor which is represented by the Klotho glycoprotein [91, 92]. The gene coding for this Klotho protein was identified as one of the genes that controls aging as its loss or mutation leads to an accelerated senescence with the early appearance of ectopic calcifications, muscular and cutaneous atrophies, osteoporosis, arteriosclerosis, and pulmonary emphysema [89, 91, 92]. Moreover, the disruption of the Klotho-FGF23 axis may be a consequence of the expression of progerin that is involved in the development of progeria (Hutchinson–Gilford syndrome) [93]. Constitutively, the Klotho protein is expressed only in some organs (including the parathyroid gland, kidneys, testicles, ovaries, brain, pituitary gland, and choroids plexuses) [90, 94]. However, the normal biological action of the binding of FGF23 on its Klotho receptor is observed primarily at the parathyroid gland and kidneys. Osteocytes produce FGF23 in response to high rates of di-hydroxylated vitamin D and this production causes an inhibition of the renal reabsorption of phosphate from the distal tubule and a reduction in the di-hydroxylated vitamin D production [92, 95]. In addition, the binding of osteocyte FGF23 on parathyroid glandular cells will induce a suppression of PTH secretion leading to increased calciuria and phosphaturia [96]. In summary, FGF23 produced by osteocytes in response to increased rates of di-hydroxylated vitamin D will act on kidneys and parathyroid gland via Klotho and lead to a reduction of phosphatemia [97].

An excess of FGF23 leads to a hypophosphatemia and can lead to an osteomalacia. FGF23 is secreted in excess by some mesenchymal tumors [98, 99]. Constitutively, FGF23 is mildly expressed by osteocytes and its synthesis is tightly controlled by the combined and inhibiting action of the DMP1 and PHEX proteins [90, 100]. The dentin matrix protein 1 (DMP1) protein may be capable of interacting with the PHEX protein [85]. Phosphate-regulating gene with homologies to endopeptidases on the X chromosome (PHEX) is expressed by osteocytes and osteoblasts and is an endopeptidase of the plasma membrane's external surface

[23, 101]. Biologically, both the native DMP1 protein and the PHEX enzyme cause an inhibition of FGF23 expression by acting negatively on the promoter sequences of this growth factor [25]. Therefore, increases in FGF23, in response to an increased di-hydroxylated vitamin D, could be inhibited by DMP1 or PHEX [85]. However, the intricate mechanism of this regulation currently remains unclear.

Effects of osteoporosis and physical activity on osteocytes

Osteoporosis is the consequence of a disturbance in the existing balance between osteoblastic bone formation and osteoclastic bone resorption [102]. This disease occurs mainly in post-menopausal women, and it is characterized by an increase in bone resorption without enough compensating formation of new bone [103, 104]. Osteoporosis is also defined by a loss of bone strength and a reduction of mass with a deterioration of bone quality, leading to an increased fracture risk [105].

It is presently unknown exactly how the suppression of estrogen can induce osteocyte apoptosis. If estrogen suppression has a direct effect on osteocytes, the two most probable possibilities are (1) the anti-oxidant effect of estrogen is critical for osteocytes that are vulnerable to anoxia or at least to hypoxia [43]; or (2) estrogens act directly via their osteocyte receptor [63]. Osteoporosis induced by chronic glucocorticoid treatment is often complicated by an area of local bone necrosis that is associated with the apoptosis of fracture-boarding osteocytes [106]. In this situation, the osteocyte apoptosis constitutes a cumulative and irrevocable effect that stops the lacuno-canalicular network and prevents the repair of micro-cracks, directly inducing bone fragility, and probably playing a major role in corticosteroid-induced osteoporosis and surrounding of fractures.

Recent work highlights the positive impact of physical activity on bone quality in post-menopausal women [107, 108]. However, the exact response of the osteocyte to physical activity or to the absence of mechanical loading is still largely unknown. Aguirre showed that mechanical stimulation of mouse osteocytes in culture attenuated apoptosis [109]. On the other hand, the reduction of mechanical loading in a tail-suspension murine model mimicking weightlessness increased the prevalence of osteocyte apoptosis, which was followed by osteoclast recruitment and associated bone resorption [109]. The same group also showed that mechanical stimulation of bone tissue activates extracellular regulated kinases pathways through integrin recruitment [73]. This serine–threonine kinase activated a kinase-induced decline in osteocyte apoptosis [73]. The global role of the osteocyte in mechano-sensing is generally accepted and the modeling processes usually include the triggering role of the osteocyte [4]. However, the molecular pathways involved in the mechano-sensing phenomenon remains debated [10, 44].

Therapeutic opening

The osteocyte is now regarded as being both at the center of bone remodeling and as the initiator of the bone remodeling processes. The biology and functions of this cell are becoming the subject of more and more studies and are leading to a better understanding of the role and actions of osteocytes. In addition, molecular elements controlling osteocyte functions have now been identified and many have become possible therapeutic targets for osteo-articular pathology treatment. Among the most recent and most promising targets, the sclerostin protein that is encoded by the SOST gene, the connexin 43 which forms gap junctions, the E11/gp38 glycoprotein coded by the human E11 gene or murine gp38 gene, and the Wnt/β-catenin pathway controlled by the Dkk1 protein, have all been identified [110]. Sclerostin globally plays a negative role on bone formation [49], and this molecule is preferentially produced by the osteocytes and pre-osteocytes [3, 50].

The osteocyte SOST gene encodes the sclerostin protein [49, 111]. Its expression is controlled by the levels of PTH, and an increase in PTH secretion causes a reduction of SOST expression [112, 113]. However, this SOST regulation is also varied according to the bone localization. Indeed, an increase in PTH leads to the reduction of SOST only in bone epiphysis and diaphysis, while SOST expression rates remain unchanged in the metaphysis [62, 112]. It should be noted, however, that as sclerostin is expressed in mature osteocytes, particularly those close to cartilage zones that are not resorbed, it is very likely that sclerostin expression plays a role in the local targeting of bone remodeling [114–116]. For this reason, both the SOST gene and its sclerostin protein represent very promising therapeutic targets for the regulation of targeted bone remodeling [117, 118].

Connexin 43, which acts to form gap junctions between osteoblasts and osteocytes, is a current hot topic and is the subject of many studies. Osteocytes are able to contact adjacent cells by forming a connection using half-channels called “connexons” (Fig. 2). When two cells form a whole channel by the attachment of their separate connexons, a gap junction is formed and the cells are able to exchange information in the form of ions or small molecules [119]. Osteoblast and osteocyte connexons are formed by the juxtaposition of six connexin 43 proteins [120]. These connexin 43-composed connexons have the capacity to control the passage of E2 prostaglandin or ATP produced in response to mechanic stimulations [57]. Moreover, con-

nexin 43 is deemed to be involved in the mechanism of action of bisphosphonates in osteoblasts and osteocytes [121–123]. Finally, it has recently been reported that connexin 43 could be regarded as a key molecule in inter-osteocyte communication [124, 125]. Connexin 43 may be involved in the regulation and the amplification of the osteocyte responses to mechanical stimulation and also in cell survival and apoptosis or in the differentiation from osteoblasts into osteocytes [126]. Thus, connexin 43 could be a potential therapeutic target.

The podoplanin protein (otherwise known as GP38, T1 alpha, or E11/gp38 protein) is coded by the E11 human or the gp38 murine gene and is selectively expressed by osteocytes in response to mechanical stimulation [127, 128]. This protein is also necessary for osteocyte dendrite elongation in response to shear stress [129]. However, as dendrite formation is an active process and not a passive mechanism, it is likely that the E11/gp38 protein is critical not only during dendritic formation, but also for osteocyte viability and function [129]. Indeed, its neutralization by antibodies led to the rapid degradation of dendritic podocytes, whereas its over-expression caused the formation of tubule-like membrane expansions [130, 131]. This molecule, therefore, seems to play an essential role in the osteocyte mechanical load perception capacities and may become a therapeutic target of interest.

The Wnt/β-catenin pathway is currently regarded as an important regulator of bone mass and bone cellular functions [44]. Indeed, this pathway is implicated in the differentiation and proliferation of osteoblasts as well as the synthesis of bone by osteoblasts, and it has been shown to play a role in the transmission of osteocyte signals that are generated by mechanical loads to the bone surface cells [132–135]. This pathway is particularly well controlled by several molecular inhibitors including the SOST gene and the Dkk1 gene and protein [136]. Indeed, mutations in SOST or Dkk1 in humans and in mice lead to an increase in bone mass [110, 117, 137–139]. Clinical trials aimed at the treatment of osteoporosis have shown that the Wnt/β-catenin pathway also induces anabolic effects within the clinical treatment setting [117, 140]. Thus, this Wnt/β-catenin pathway and/or its regulators (such as SOST or Dkk1) are also interesting therapeutic targets for new treatments of bone pathologies.

Summary and perspective

In conclusion, the osteocyte is no longer regarded as only an old osteoblast or as an inactive cell embedded in the bone matrix. Therefore, the functions of the viable osteocyte are now viewed as being equally or possibly more important than the functions of the dead or dying osteocyte. In fact, the osteocyte is currently considered as the mechano-sensing cell of the bone tissue. The osteocyte is thus perceived as being at the center of bone remodeling by coordinating both osteoblast activity and osteoclast resorption, but also as the initiator of the bone remodeling processes by locally sensing bone matrix deformation (i.e., cracked zones or overused areas). In that way, significant differences in osteocyte tridimensional morphologies and lacunae have been recently reported in human cortical bone from different pathologies with various bone mineral density (osteoarthritis, osteopenia, and osteopetrosis) [141], assuming that osteocytes may acquire phenotypical and adaptative differences according to various external mechanical strains. Furthermore, the osteocyte apoptosis has been recently reported as being insufficient for repair of micro-damage without physiological loading stimulation [142]. While manipulation of osteocyte responsiveness to mechanical loading offers potential for therapies aimed at preventing bone loss, the exact relationship between osteocyte morphology, bone architecture, mechano-responsiveness, and apoptosis is complex and needs further study.

Next to its mechano-sensibility, the osteocyte has also been shown to play a major role in phosphate homeostasis through PHEX, DMP1, and FGF23. Recent studies have furthermore shown that its sclerostin expression plays a critical role in the bone mass regulation and may be important in bone anabolic responses to PTH. At last, a recent study has demonstrated that the osteocyte marker DMP1 and SOST are regulated by muscle-related genes such as myogenin and Mef2 [143], supporting the idea that muscle-related gene network may play a role in the osteocyte cytoskeleton contractility and movements.

In summary, in the last few years, the perception of the osteocyte has changed from being viewed as an old inactive osteoblast to a highly active mechano-sensing and secretory cell that plays major roles in regulating osteoblast and osteoclast activity. Molecular pathways that control osteocyte functions might therefore become potential therapeutic targets for the treatment of bone diseases.

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