Endocrine function of osteocytes

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Summary
Osteocytes represent the most abundant cell type of the skeletal system. They have access to a large cellular surface area within the lacunocanalicular network. This network additionally provides connection to the vascular system, a prerequisite for secretion of endocrine regulators into the circulation. The best established endocrine function of the osteocyte network is the regulation of phosphate homeostasis by secretion of Fgf23, a hormone inhibiting renal phosphate reabsorption. Recently, several additional osteocyte-derived factors have been suggested to influence phosphate homeostasis, either directly or in an Fgf23-dependent manner. Moreover, osteocytes are also the major producers of Wnt signaling modulators, such as Sclerostin or Dkk1. Since these molecules primarily act as inhibitors of bone formation, there might be an additional influence of osteocyte-derived molecules on glucose handling and energy metabolism. In fact, osteocalcin, a long-known bone matrix protein and biomarker of bone formation, is now considered to act as a hormone controlling insulin production by pancreatic β-cells and insulin sensitivity of target organs. Since the endocrine functions of osteocytes are only beginning to be uncovered, it appears likely that additional osteocyte-derived molecules with systemic influences on whole body homeostasis might be identified in the future.

Osteocytes represent the most abundant bone-associated cell type with an overall number recently being estimated to 42 billion in an adult skeleton (1). However, not only their number is impressive, but also their exceptional longevity, with an average lifetime being estimated to 25 years or even longer (2). While the existence of osteocytes has already been acknowledged in historical anatomical descriptions, they were often considered to be quiescent and their physiological relevance remained a mystery for a long time. Studies regarding the functions of osteocytes are still complicated by the fact, that they are embedded within the mineralized bone matrix and therefore not easily accessible for analysis. Moreover, although some investigators were able to establish ex vivo cell cultures with osteocyte characteristics, it is questionable if these tissue culture systems are truly comparable to the complexities of the osteocyte network in vivo (3). More specifically, the location of osteocytes within a three-dimensional fully mineralized matrix is not only important to obtain the osteocyte-specific morphological features, but most likely it is also required for their physiological function(s).

The functions of osteocytes

In fact, when osteocytes, presumably derived from cells of the osteoblast lineage, are embedded into newly deposited bone, they change their morphology and form highly branched dendritic processes. The thereby generated lacuno-canalicular network allows direct interconnection of osteocytes via their processes, but also connection to the bone surface. The physiological functions that have been attributed to osteocytes throughout the last decades primarily include detection of physical damage such as microfractures, but also mechanosensing abilities following shear stress within the lacuno-canalicular network (4, 5).

Therefore, osteocytes have been proposed to have an important regulatory function in

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the context of bone remodeling and were suggested to produce numerous signaling molecules that act locally on other bone-associated cells such as osteoclasts, osteoblasts and lining cells. The identification of such specific molecules was quite difficult for a long time, mostly explained by the limitations of ex vivo systems, as discussed above. During the last two decades however, triggered by the availability of novel methods in molecular genetics, this problem was essentially solved, since it became possible to study the function of specific genes in vivo (using mouse models) and/or to identify disease-causing gene mutations in humans with inherited skeletal disorders, some of them affecting osteocytes. Based on results obtained through the use of these methods it became increasingly evident that osteocytes, in addition to modulating bone metabolism, also have the ability to influence other remote organs.

Osteocytes as endocrine cells

That osteocytes can have an endocrine function was not too surprising and is supported by several arguments. In fact, although the average diameter of canaliculi is rather narrow, studies with dyes of different molecular sizes have shown that this network is permissible for molecules with a size of up to 70 kD, thereby allowing transport of signaling molecules and/or hormones (6). Moreover, since the osteocyte network is connected with the bone surface, it was always conceivable to speculate that osteocyte-derived molecules can enter the circulatory system. While this is also true for some highly relevant factors regulating bone remodeling, such as Sclerostin and possibly Rankl, a true endocrine function of an osteocyte-derived molecule is best established for Fgf23 (7, 8). More specifically, based on hallmark evidence discussed below, Fgf23 is now considered to represent the most relevant hormone controlling phosphate homeostasis, and its primary origin is the osteocyte network.

In contrast to osteocalcin, whose function as a bone-derived hormone has been demonstrated primarily in mice, there is no doubt that Fgf23 also controls phosphate homeostasis in humans, as there are several hyperphosphatemic and hypophosphatemic disorders being caused by mutations of FGF23 itself or of genes involved in controlling Fgf23 expression, modification or signaling (9–12).

Fgf23, an osteocyte-derived hormone regulating phosphate homeostasis

Phosphate is an extraordinarily important ion for the function of cells and organisms. Imbalances in systemic phosphate levels, such as hyper- or hypophosphatemia, are highly detrimental, especially since they cause ectopic calcification or severe osteomalacia, respectively. Therefore several mechanisms have evolved to facilitate the strict regulation of intra- and extracellular phosphate levels. Since bone is a major reservoir of phosphate, it is not too surprising that it is primarily involved in the regulation of phosphate homeostasis. A key component of this regulatory system is the osteocyte-derived hormone Fgf23, which controls systemic phosphate levels together with the classical regulators of mineral homeostasis, i.e. 1,25-dihydroxyvitamin-D$_3$ (1,25(OH)$_2$D$_3$) and parathyroid hormone (PTH) (13). While PTH is mostly produced by the parathyroid gland, 1,25(OH)$_2$D$_3$ is synthesized in a multi-step process with the last activating step mediated by the kidney (14, 15). Compared to PTH and 1,25(OH)$_2$D$_3$, both being known for decades, the discovery of Fgf23 as a key regulator of mineral homeostasis was uncovered much later, i.e. 15 years ago (10). More specifically, it was reported that activating mutations of FGF23, causing resistance to proteolytic degradation, are found in individuals with autosomal dominant hypophosphatemic rickets (ADHR), a disorder characterized by hyperphosphaturia causing low serum phosphate levels and severe osteomalacia (10). Nearly at the same time, Fgf23 was identified by a differential cDNA screening approach as a molecule, whose increased expression in the respective tumour cells is causing oncogenic osteomalacia, an acquired disorder sharing many similarities to ADHR (12). These ground-breaking discoveries immediately offered a molecular explanation for the pathogenesis of another related disorder, i.e. X-linked hypophosphatemic rickets (XLHR), which was known to be caused by inactivating mutations of an osteocyte-specific endopeptidase termed Phex (Phosphate-regulating gene with homologies to endopeptidases on the X chromosome) (9). More specifically, while it was previously known that the phenotype of mice carrying a spontaneous deletion of the Phex gene (Hyp mice) was caused by a circulating factor promoting renal phosphate loss, this factor was then identified as Fgf23 (16–18).

The mechanism of Fgf23 action

These initial events leading to the identification of Fgf23 have triggered a large number of studies aiming at understanding the role of this novel hormone at a cellular and molecular level. It was shown that Fgf23 is predominantly expressed by osteocytes (11, 19), and since its predicted molecular weight is 32 kD, it can be transported through the lacuno-canaliculck network into the vascular system. The action of Fgf23 is mediated by a specific receptor complex consisting of the FGFR1 isoform IIIc and the co-receptor Klotho, whose mutual inactivation in humans causes a hyperphosphatemic disorder due to Fgf23-resistance (20). Although Fgf23 target organs also include parathyroid and pituitary glands, as well as the choroid plexus, the most important site of action is the proxi-
mal tubule of the kidney (Fig. 1). Here Fgf23 acts by decreasing the expression of at least three genes with high relevance in mineral homeostasis, i.e. Slc34a1, Slc34a3 and Cyp27b1 (21, 22). While the first two genes encode sodium/phosphate co-transporters facilitating renal phosphate reabsorption, the latter gene encodes 25(OH)D-1alpha-hydroxylase, the enzyme generating 1,25(OH)2D. As a consequence there are two relevant actions of Fgf23 mediated by the kidney. First, Fgf23 promotes renal phosphate loss by reducing the expression of the relevant sodium/phosphate co-transporters, thereby causing hypophosphatemia in states of Fgf23 excess (21). Second, Fgf23 inhibits the synthesis of 1,25(OH)2D3, a function that is probably best underscored by the high circulating 1,25(OH)2D3 levels found in Fgf23-deficient mice or patients with inactivating Fgf23 mutations (exhibiting hypophosphatemic familial tumoral calcinosis) (22, 23). Therefore, and underscored by additional effects on PTH production, Fgf23 is now considered to represent a key component of a complex endocrine system controlling mineral homeostasis, in mice and humans (7, 8). Moreover, the development of Fgf23 antagonists or neutralizing antibodies is a promising approach to treat hypophosphatemic disorders (24, 25).

The complexities of Fgf23 regulation in osteocytes

With respect to the osteocyte-origin of Fgf23 however there are still some open questions, especially regarding the regulatory systems controlling its production. In fact, since the Phex gene encodes a transmembrane protein with an extracellular endopeptidase domain, it was initially speculated that Fgf23 is a substrate for Phex, and that the lack of Fgf23 cleavage would explain the high circulating levels of Fgf23 in Hyp mice or XLHR patients (26). Apparently however, this hypothesis could not be confirmed, and instead it was found that Fgf23 is transcriptionally activated in cortical bone and primary osteoblasts from Hyp mice (27–29). Similar observations were made in Dmp1-deficient mice, which also display hypophosphatemic rickets, as it is the case in humans, where DMP1 mutations were found to cause autosomal recessive hypophosphatemic rickets (ARHR) (11). In other words, there are three human disorders with similar phenotype but different inheritance (ADHR, XLHR and ARHR), and all of them are primarily caused by excess circulating Fgf23 (9–11).

Moreover, since the three genes mutated in these disorders (i.e. FGF23, PHEX and DMP1, respectively) are all predominantly expressed by osteocytes, it is obvious that this cell type has a unique function in the control of phosphate homeostasis, and that identifying the underlying protein interactions is highly relevant to understand phosphate handling disorders (Table 1). Therefore, since some knowledge regarding these mechanisms has accumulated in the last decade, it is quite important to discuss the respective findings and the current hypotheses to fully explain the osteocyte’s role in mineral homeostasis.

### Regulation of Fgf23 by Phex

One of the keys to understand these complexities is surely the identification of physiologically relevant endogenous substrates of the putative endopeptidase Phex, which was the first player known to have a profound role in the osteocyte-mediated regulation of phosphate homeostasis. More specifically, immediately after the discovery of inactivating PHEX mutations in XLHR patients and a partial Phex gene deletion in Hyp mice (9, 30), it was speculated that this transmembrane protein, through its extracellular endopeptidase do-

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**Table 1** Hypophosphatemic Disorders caused by excess circulating Fgf23. XLHR, Xlinked hypophosphatemic rickets; ADHR, autosomal dominant hypophosphatemic rickets; ARHR, autosomal recessive hypophosphatemic rickets; OOM, oncogenic osteomalacia

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Mutated gene</th>
<th>Identification</th>
<th>Fgf23 status</th>
</tr>
</thead>
<tbody>
<tr>
<td>XLHR</td>
<td>PHEX</td>
<td>1995 (9)</td>
<td>Increased transcription</td>
</tr>
<tr>
<td>ADHR</td>
<td>FGF23</td>
<td>2000 (10)</td>
<td>Increased protein stability</td>
</tr>
<tr>
<td>ARHR</td>
<td>DMP1</td>
<td>2006 (11)</td>
<td>Increased transcription</td>
</tr>
<tr>
<td>OOM</td>
<td>–</td>
<td>2001 (12)</td>
<td>Ectopic expression</td>
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main, acts by cleaving a specific substrate, whose accumulation in XLHR patients or Hyp mice would cause the two major pathologies, i.e. hypophosphatemia and osteomalacia. After it was essentially ruled out that Fgf23 is cleaved and thereby inactivated by Phex, which would have been a likely possibility (27, 28), more recent studies have identified a specific peptide motif, termed ASARM (acidic serine aspartate-rich MEPE-associated motif), as a substrate for Phex (31–33). More specifically, the ASARM motif occurs in several members of the SIBLING (small integrin-binding ligand, N-linked glycoprotein) family of proteins, such as Dmp1, Mepe, Bsp and Osteopontin, all of them proposed to be regulators of extracellular matrix mineralization (34). For example, since elevated amounts of Osteopontin were observed in the bone matrix of Hyp mice, and since Osteopontin can act as an inhibitor of mineralization, its accumulation in the PHEX-deficient bone matrix might explain, at least in part, the observed osteomalacia in XLHR or Hyp mice (35). Apparently however, the transcriptional activation of Fgfs23, the most relevant consequence of Phex inactivation is caused by a more complex mechanism, which is just beginning to be unraveled (Fig. 2) (8).

Since Phex is able to interact with Mepe and cleave within the ASARM-peptide, it counteracts the effects on Fgf23 expression (37). This putative pathway might also represent a novel target for the treatment of mineralization defects, since the administration of SPR4, a synthetic Phex-related peptide sequestering ASARM-peptides, has been shown to ameliorate the skeletal phenotype of Hyp mice (38). In contrast, the serum levels of Fgfs23 were not normalized by the treatment, thereby confirming previous studies suggesting the existence of a hypophosphatemia-independent bone mineralization defect of Hyp mice, which further complicates the system (29, 38–40). An additional potential regulator of Fgfs23 levels is the gene ENPP1 coding for the ectonucleotide pyrophosphate/phosphodiesterase 1. Inactivating mutations of ENPP1 were identified in some patients suffering from ARHR (41). In addition to the increased phosphate wasting an elevated plasma level of Fgfs23 was observed in several of these patients. While the known function of Enpp1 is the production of mineralization inhibiting pyrophosphate molecules (42), the pathomechanism linking this function to the regulation of Fgfs23 still needs to be elucidated.

**Fig. 2**

Hypothesized transcriptional regulation of Fgfs23 by Phex. A: Membrane bound Phex interacts with membrane bound α1β3-integrin and soluble Dmp1. This trimeric complex leads to an intracellular signal that inhibits the transcription of Fgfs23. B: The formation of the Phex-Dmp1-α1β3-integrin complex can be inhibited by ASARM-motif containing peptides, that compete with Dmp1 for interaction with Phex. Thus the lack of the active complex leads to an uninhibited and therefore increased rate of Fgfs23 transcription.

The role of ASARM-peptides in the modulation of Phex activity

In fact, it was shown that Phex, together with Dmp1 and α1β3-integrin, forms a trimeric receptor complex located on the osteocyte plasma membrane, and that this complex leads to a signal suppressing the transcription of Fgfs23 (32, 36). It was further shown that short ASARM-containing peptides can act as competitive inhibitors of the Phex-Dmp1-α1β3-integrin complex, thereby inducing Fgfs23 expression (32, 37, 38). Of note, short peptides containing the ASARM-motif can also be generated under physiological conditions, for instance by the proteolytic cleavage of Mepe (Matrix extracellular phosphoglycoprotein) (34).

**Fgf23-independent functions of Mepe and Sfrp4**

Another level of complexity is again related to Mepe, since this protein seems to exert an own endocrine function. More specifically, it was found that there is a correlation between serum Mepe and phosphate levels (43), and that Mepe (or its ASARM-containing peptide), by inhibiting of renal phosphate reabsorption and intestinal phosphate absorption, causes phosphate wasting in an Fgfs23-independent manner (44). Finally, osteocytes were reported to produce another molecule with the potential to influence phosphate homeostasis, i.e. Sfrp4 (Secreted frizzled-related protein). More specifically, sFRP4, encoding a soluble protein binding to a family of secreted Wnt-signaling inhibitors, was found over-expressed in tumours causing oncogenic osteomalacia, and sFRP4 injection into rats promoted phosphaturia (45). Since sFRP4 is however expressed in several tissues it cannot be regarded as an osteocyte-specific endocrine regulator, and its molecular actions as a phosphate regulating hormone are not fully clarified yet. In conclusion, although there are quite a lot of remaining questions about the mechanisms explaining the similarities between ADHR, XLHR, ARHR and oncogenic osteomalacia, it is undoubted that Fgfs23 is an osteocyte de-
Indirect influence of osteocytes on energy metabolism

Besides the above-discussed osteocyte-specific genes involved in the regulation of phosphate homeostasis, osteocytes are also key players in the coordination of bone remodeling. More specifically, recent evidence suggested that osteocytes (together with hypertrophic chondrocytes) are the most relevant source of Rankl, the major cytokine promoting osteoclastogenesis and bone resorption in mice and humans (46, 47). Since Rankl expression however is also detectable in osteoprogenitor cells and in activated T cells, the physiological relevance of these findings, which are based on Cre-LoxP-technology in mouse models, is not fully established so far. In contrast, another molecule specifically regulating bone remodeling was found expressed in a truly osteocyte-specific manner and acts as an inhibitor of bone formation (48). This molecule was identified by human genetics in individuals with high bone mass disorders, i.e. van Buchem disease or sclerosteosis, and termed Sclerostin (49, 50). The mutations within the Sclerostin-encoding gene (termed SOST) are either causing decreased transcription (van Buchem disease) or protein inactivation (sclerosteosis), and Sost-deficient mice were found to display high bone mass due to excessive bone formation, similar to the patients with SOST mutations (51).

Sclerostin, a modulator of Wnt signaling

After the initial discovery of Sclerostin as a key regulator of bone formation in mice and humans, several investigators have provided strong evidence for a role of this protein as an antagonist of Lrp5-mediated Wnt signaling (52–55). More specifically it was found that Sclerostin is specifically secreted by osteocytes and that its actions on osteoblasts to decrease their activity (48). Although Sclerostin has weak homologies to a family of BMP antagonists it was shown early on that it binds to the transmembrane protein Lrp5, representing a coreceptor for ligands of the Wnt family (52, 53). Particularly interesting was the finding that the binding site of Sclerostin was destroyed by mutations of the Lrp5 extracellular domain, since these mutations were previously identified to cause excessive bone formation in individuals with inherited high bone mass (54, 56). Only recently, it was fully confirmed that the Sclerostin-Lrp5 interaction is indeed relevant in vivo, since the anti-osteoblastic effect of transgenic over-expression of Sclerostin in mice was only observed on an Lrp5-intact genetic background and fully abolished by two different Lrp5 mutations causing high bone mass (57).

Given the potential relevance of this interaction it was reasonable to utilize Sclerostin as a molecular target to treat bone loss disorders in an osteoblastic manner.

Conclusion

Considering the large surface area available for osteocytes to secrete bioactive molecules, it is not too surprising that this cell type can exert an endocrine function to affect other target organs. Although the concept that the skeleton acts as a source of physiologically relevant hormones is quite new with endocrine functions of specific cell types and molecules just beginning to be uncovered, there is one excellent example for an osteocyte-derived hormone, which has been truly established. More specifically, based on hallmark genetic and experimental evidence in mice and humans, Fgf23 is now known to represent to most important endocrine regulator of phosphate homeostasis, with a (patho)physiological relevance comparable to PTH. Although the precise molecular mechanisms controlling Fgf23 production by osteocytes are quite complex and still unresolved, it is quite logical that one of the hormones involved in mineral metabolism is actually bone-derived. In the same line of thought, since osteoblasts largely depend on glucose as an energy supply (67), it is reasonable to speculate that ucOsteocalcin is part of a feed-forward loop to control glucose homeostasis.

Therefore it is at least possible that additional hormones derived from skeletal cell types or from osteocytes in particular will be identified in the future, that for instance regulate muscle mass or function. Regardless of these speculative issues however, it is already obvious at present, that the osteocyte is a highly relevant cell type, not only for bone remodeling, but also for the whole organism.

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Importantly, this approach appears to be successful, as a monoclonal antibody neutralizing Sclerostin has been found to cause the highest increase of bone mineral density in osteoporotic individuals, when compared to alendronate or teriparatide (58). In terms of endocrine functions it can be stated that Sclerostin, in contrast to Fgf23, most likely does not act as a hormone itself. However, since it regulates bone formation and thereby osteocalcin production, there might be an indirect influence on glucose metabolism, as suggested by several studies in mouse models.

The role of Osteocalcin in energy metabolism

Osteocalcin is long known as a bone-specific matrix protein and a serum biomarker of bone formation. It is one example of a protein (together with blood coagulation factors and Matrix-Gla-protein), which is posttranslationally modified in a vitamin K-dependent manner to generate γ-carboxyglutamate (Gla) residues (59). While the 3 Gla residues within the murine osteocalcin protein increase its affinity to calcium and the mineralized bone matrix, there is recent evidence that the osteocalc-mediated process of decarboxylation at position Gla13, which generates Glu13, is an important step in activating an endocrine function of osteocalcin, which in its undercarboxylated form (hereafter termed ucOsteocalcin) influences energy metabolism (60, 61). More specifically, it was shown, through mouse genetics and in vitro experiments that ucOsteocalcin acts directly on beta-cells in the pancreatic islets where it stimulates insulin secretion (60). This endocrine function is mediated by interaction of ucOsteocalcin with the G-protein coupled receptor GPRC6A expressed by beta-cells (62). In addition, ucOsteocalcin has been shown to stimulate insulin sensitivity of target organs (60).

Consistently, Osteocalcin-deficient mice were found to display hyperglycemia, insulin resistance and adiposity (60). An opposing phenotype was observed in mice lacking OST-PTP (Osteotesticular protein tyrosine phosphatase), an enzyme facilitating insulin receptor dephosphorylation in osteoblasts, thereby activating expression of Osteoprotegerin, an antagonist of Rankl, and causing reduced osteocalcin decarboxylation by osteoclasts (60, 61).

Relevance of putative Osteocalcin functions

Whether these findings obtained in mouse models are relevant for human physiology, however, is controversially discussed, in contrast to Fgf23, where the transferability between mice and human studies is undoubted (63). In fact, although it was reported that Osteocalcin serum concentrations in humans correlated with glycemia and adiposity and that they are decreased in patients with type II diabetes mellitus, it has to be noted that not all studies addressing this question found such a clear correlation (64). The same applies for other putative functions of ucOsteocalcin that were identified in Leydig cells or the control of anxiety or learning behavior (65, 66). In any case, since Osteocalcin is not expressed in an osteocyte-specific manner, and since its activation appears to require osteoclast-mediated decarboxylation, there is, at most, an indirect influence of osteocytes on glucose metabolism.

The key role however of Sclerostin as a negative regulator of bone formation is undoubted, and there is no reason to rule out the existence of additional endocrine regulators primarily released by osteocytes.

Conflict of interest

The authors declare that there are no conflicts of interest.

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