HubLE | *MY MENTOR & I* Perspective

Bone and kidney crosstalk

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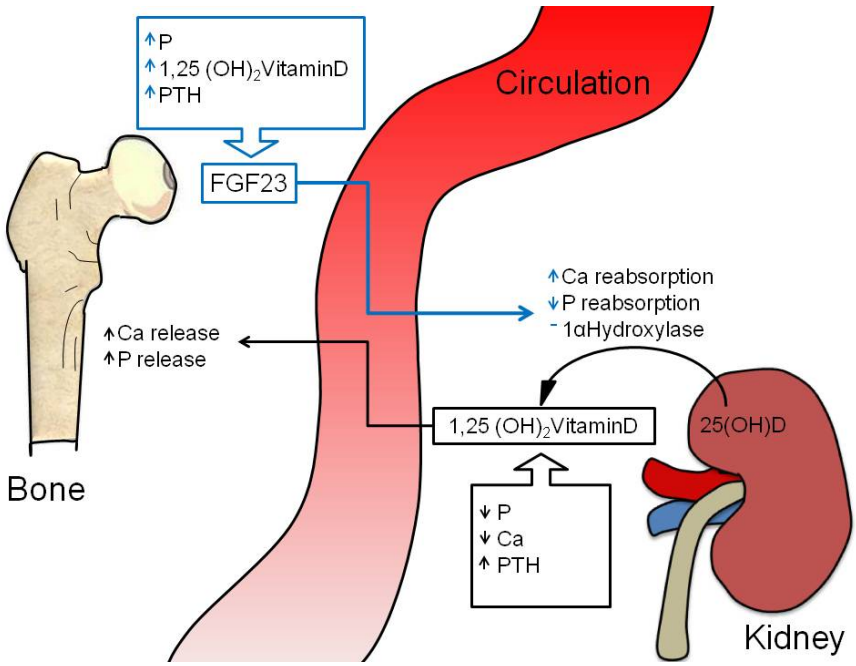
GRAPHICAL ABSTRACT



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Giulia Battafarano is a PhD student at the DAHFMO-Unit of Histology and Medical Embryology at the University of Rome 'Sapienza'. Her research aims to understand the skeletal alterations in patients affected by kidney diseases. In particular, she is studying the primary bone defects in cystinosis, analysing the effects of cystinosin deficiency on bone cells. She hopes that her study will lead to resolving the growth impairment and osteopenia observed in these patients.

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ABSTRACT

The regulation of mineral metabolism results from the interplay of four endocrine organs forming the parathyroid-intestine-bone-kidney axis. In this context, the skeleton stores minerals (calcium and phosphate) within its matrix and orchestrates the regulation of their levels, interacting with the kidney. Here, we give an overview of the main pathways involved in the crosstalk between these two organs. Moreover, we describe the endocrine network characterised by bone- and kidney-derived hormones, and further signals, i.e. parathyroid hormone, that target them.

Introduction

The skeleton is the largest organ in mammals and, besides providing mechanical support, the frame for locomotive muscle attachment and protection of internal organs, it plays other functions, such as energy, mineral metabolism, fertility and appetite regulation.[1,2]

Minerals, mostly calcium, phosphate and bicarbonate, are structural constituents of bones, and the skeleton works as their storage, releasing them when needed.[3]

Mineral balance is maintained by co-ordination of many endocrine signals between the kidney, bone and other organs, such as parathyroid glands and intestine.

Mineral metabolism: calcium and phosphate

Calcium

Calcium is involved in several biological functions, such as cell signalling, neural transmission, muscle function, blood coagulation, membrane and cytoskeletal regulation, secretion and biomineralisation.[4]

Calcium levels are finely regulated in intracellular and extracellular compartments. Total body calcium content is approximately 1kg, and 99% resides in the skeleton, whereas the extraskeletal component accounts for only 1%.[5]

Even with great variability, of 1g calcium introduced by diet, half is absorbed by the gastrointestinal tract, mainly by the duodenum, in a vitamin D-dependent manner.[6] About 10g calcium per day is filtered by glomeruli in the kidneys, and up to 99% is reabsorbed by two mechanisms: paracellular passive reabsorption and a transcellular active process mediated by CaSR (the calcium-sensing receptor) and TRPV5 (transient receptor vallinoid 5).[7] The reabsorption occurs mainly in the proximal tubules, where it is largely driven by diffusion through the paracellular shunt.[8,9] About 10% of filtered load is actively reabsorbed by the distal tubule.[10] Only 50-250mg calcium is excreted in urine during a day.[5] Hormonal regulation of tubular calcium reabsorption and urinary excretion contribute to the maintenance of calcaemic physiological range.

The most relevant hormones that control calcium homeostasis are parathyroid hormone (PTH) and vitamin D.[11]

Phosphate

The skeleton is also the reservoir of phosphate. Indeed, most of the phosphorus builds, with calcium, skeletal hydroxyapatite, whereas the extraskeletal phosphate accounts for ~15%, constituting phosphoproteins, phospholipids and nucleic acids.[12] The phosphate balance is maintained by fibroblast growth factor 23 (FGF23), PTH and vitamin D.[13,14] The phosphate introduced via the diet is absorbed in the small intestine, with both calcium-dependent and -independent mechanisms. The main process is through passive absorption that depends on the amount of phosphorus in the gut.[15] However, there is also an active sodium-dependent mechanism, mediated by NaPi2b (sodium-dependent phosphate transport protein 2B), and stimulated by $1,25(OH)_2$ vitamin D.[16,17] As for calcium, the kidney contributes to the regulation of phosphate levels in the blood by its reabsorption in convoluted and in proximal tubules.[11] Phosphate reabsorption by the proximal tubular cells involves uptake across the brush

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border membrane mediated by NaPi2a and NaPi2c, which collectively account for the reabsorption of ~80% of filtered phosphate.[17]

In this context, FGF23 is the main emerging phosphatonin that protects cells and tissues from high levels of phosphorus.[18] Indeed, increased oral phosphate intake results in increased FGF23 secretion and systemic circulation, while in dietary phosphate restriction, lower serum levels of FGF23 are observed.[16] To regulate phosphate homeostasis, FGF23 establishes a complex interaction with PTH and vitamin D by which FGF23 suppresses PTH secretion and $1,25(OH)_2$ vitamin D synthesis.

PTH: endocrine signal outside bone and kidney

The parathyroid gland evolved to operate as calciostat, responding to alteration of serum calcium levels with PTH secretion.[10] PTH is secreted as a protein of 84 amino acids. It is synthesised as a propeptide containing a presequence of 25 amino acids and a prosequence of 6 amino acids.[19] Presequence and prosequence are then cleaved off in the endoplasmic reticulum and the full length PTH of 84 amino acids is stored in vesicles.

PTH is released in response to a reduced concentration of extracellular calcium and it acts to restore calcium levels.[19,20] Parathyroid glands sense extracellular calcium concentration through the CaSR on their cell membrane. The interaction of calcium with the extracellular domain of the CaSR results in stimulation of phospholipase C-β (PLCβ) activity via $G\alpha_{11}$. PLCβ catalyses the formation of inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). The accumulation of IP3 induces the release of calcium into the cytosol from intracellular stores, whereas DAG activates the mitogen-activated protein kinase (MAPK) cascade. In contrast to other cells that release their product in response to increased calcium levels, these events lead to reduced PTH secretion from the parathyroid chief cells.[19-21]

PTH has a very short circulating half life and it is primarily metabolised in the liver into amino- and carboxy-terminal fragments, leading to several forms of PTH in the circulation. However, the amino terminus (known as 1-34 PTH) is the only one that can bind to the PTH receptor carrying out the biological effect on target organs.[22] The carboxy terminus is cleared by filtration in glomeruli and it accumulates during renal failure.[23]

The tissue response to PTH is mediated by PTH receptor type 1 (PTHR1) belonging to G-protein-coupled receptor family 2.[22,24] When PTH binds its receptor, several downstream pathways are activated in a tissue-specific manner, involving protein kinase A and protein kinase C (PKC) activation, cyclic AMP signalling and MAPK pathways.[19,22]

Biological effect of PTH

PTH acts directly on the kidney, inducing $1,25(OH)_2$ vitamin D production and calcium reabsorption, mainly in the distal convoluted tubule.[8] In addition to serum calcium, PTH regulates phosphate levels, inhibiting phosphate reabsorption by proximal tubules and causing hypophosphataemia. Indeed, PTH reduces NaPi2a and NaPi2c expression in the apical membrane of proximal tubular cells.[25-27] Even if a direct effect on intestinal calcium and phosphate absorption is not clear, PTH promotes their absorption through vitamin D released from kidney.[11]

PTH-mediated effects on bone seem to be dose-dependent. Indeed, chronic elevation of PTH, as in hyperparathyroidism, induces osteoclast resorption, whereas intermittent administration of 1-34 PTH has anabolic functions, stimulating osteoblast activity.[26,28] Furthermore, since PTHR1 localises in osteoblasts, osteocytes and stromal cells, but not in bone marrow haematopoietic cells or osteoclasts, the stimulation of bone resorption is probably indirect and mediated by osteoblast production of osteoclastogenic factors.[29]

Vitamin D: kidney-derived hormone that acts on bone

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Vitamin D is a fat-soluble secosteroid that acts as a hormone, interacting with a specific cytosolic receptor. It is involved in the regulation of nearly 3% of the human genome.[30] Vitamin D was first identified for its central role in calcium and phosphate metabolism. Indeed, it regulates mineral homeostasis and skeletal health, modulating intestinal absorption of calcium and phosphate, renal calcium reabsorption and co-ordinating with parathyroid functions. Regarding the direct effects on bone cells, van Driel and van Leeuwen described the complex interaction between vitamin D and osteoblasts.[31] Indeed, osteoblasts express vitamin D receptor (VDR). However, the effects of vitamin D on osteoblasts remain unclear because both osteoblast-specific deletion and overexpression of VDR lead to increased bone mass.[32-34]

Moreover, vitamin D promotes bone resorption, stimulating osteoclast differentiation and activity. Even if osteoclasts and their precursors express VDR,[35,36] vitamin D regulates osteoclast formation and function via osteoblast production of osteoclastogenic macrophage colony-stimulating factor and RANKL (receptor activator of nuclear factor kappa-B ligand).[37] Interestingly, osteoclast precursors from VDR-knockout mice can be induced by vitamin D to differentiate in the presence of wild type osteoblasts.[38] Moreover, further studies revealed non-classical actions of vitamin D, since its deficiency has been correlated with other conditions including autoimmune, cardiovascular, renal and neurodegenerative diseases, depression and cancer.[39]

The biologically active form of vitamin D is $1,25(OH)_2$ vitamin D (also known as calcitriol), obtained by metabolic conversion of its precursors: vitamin D_2 and vitamin D_3 (often called ergocalciferol and cholecalciferol respectively). The major source of vitamin D3 in humans is via the skin, which synthesises it from 7-dehydrocholesterol through sunlight exposure. Vitamin D2 and D3 are also introduced through the diet from vegetable- and animal-derived food sources respectively.[40] These precursors are then transported to the liver, where they are hydroxylated by vitamin D 25-hydroxylase, encoded by the *CYP2R1* gene, to generate 25-hydroxyvitamin D (25(OH) vitamin D). This is the most abundant circulating form of vitamin D.[41,42]

Because of their lipophilic nature, all the circulating metabolites of vitamin D are carried bound to vitamin D-binding protein (DBP). 25(OH) vitamin D-DBP complex is filtered through the glomerulus and internalised by proximal tubular cells via megalin-mediated endocytosis. Herein, 25(OH) vitamin D is released from DBP and it is either converted by 25-hydroxyvitamin D 1α-hydroxylase to 1,25-dihydroxyvitamin D ($1,25(OH)_2$ vitamin D) or it can be recycled into the circulation.[43] Normal serum levels of 25(OH) vitamin D are also maintained, ensuring extrarenal calcitriol synthesis.[44] Indeed, 1α-hydroxylase is also expressed in other tissues, such as bone.[45]

Serum phosphorus, calcium, FGF23 and other factors can affect the renal production of $1,25(OH)_2$ vitamin D. The pleiotropic actions of $1,25(OH)_2$ vitamin D are mediated by its binding to the VDR.[44] VDR is a nuclear receptor belonging to subfamily 1, and its binding to vitamin D ligand leads to genomic and non-genomic responses in target cells. To mediate genomic effects, the VDR-ligand complex forms a heterodimer with the retinoid X receptor (RXR) in the cytoplasm. This complex then translocates to the nucleus, where it binds to vitamin D-responsive elements in the promoter of VDR-responsive genes. Here, VDR-RXR recruits basal transcriptional factors, co-activators and co-repressors to induce/repress the transcription of target genes by RNA polymerase II.[46]

In addition, the VDR also contains an alternative $1,25(OH)_2$ vitamin D-binding A pocket that, after the binding to its ligand, can induce rapid non-genomic responses at the membrane level, independently from the VDR-RXR signalling. Furthermore, some form of vitamin D can also act on the retinoic acid-related orphan receptors.[47,48]

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$1,25(OH)_2$ vitamin D renal synthesis and catabolism are tightly co-ordinated with the calcium/PTH axis and the phosphate/FGF23 axis.

Calcium/vitamin D/PTH axis

In hypocalcaemia, PTH secretion is rapidly enhanced to restore calcium balance. High levels of serum PTH induce both calcium release from bone, activating its resorption, and 1α-hydroxylase expression and activity in the kidney. In turn, calcitriol synthesis is stimulated and intestinal calcium absorption is induced in the small intestine by enhancing expression of transient receptor potential cation channel (TRPV6) and calcium-binding protein. Moreover, $1,25(OH)_2$ vitamin D enhances calcium reabsorption by the kidney.[42] Upon calcium level normalisation, calcium and calcitriols inhibit PTH secretion and renal 1α-hydroxylase by negative feedback.[44]

FGF23: bone-derived hormone that acts on kidney

FGF23 is an endocrine hormone produced in the bone by osteocytes and osteoblasts. It targets several organs, mainly through FGF receptor 1c (FGFR1c)/α-klotho.

FGF23 is the most recently discovered member of the FGF superfamily; it was identified by three different groups around 2000. Yamashita and colleagues identified FGF23 preferentially expressed in the ventrolateral thalamic nucleus,[49] whereas the ADHR Consortium associated FGF23 with autosomal dominant hypophosphataemic rickets (ADHR).[50] At the same time, Shimada *et al.* discovered the effects of FGF23 on tumour-induced osteomalacia.[51]

The FGF superfamily constitutes 22 FGF members, classified in seven subfamilies.[52] Fifteen are paracrine-acting, four have an intracellular activity and do not interact with FGF receptors, and three have endocrine functions. The endocrine hormones are FGF19, FGF21 and FGF23. In contrast to other FGF members, their structure lacks a heparin-binding domain, allowing them to enter the circulation and execute their endocrine function.[53] Eighteen FGF ligands bind to FGF tyrosine kinase receptors (FGFRs), characterised by three extracellular immunoglobulin-like domains and a long cytoplasmic C tail. The FGF-FGFR interaction leads to the activation of multiple downstream effectors, such as PLCγ-PKC, JAK-STAT, PI3K-Akt-mTor and Grb2-Ras-Raf-MAPK.[54]

There are many germline and somatic mutations of FGFRs. While the somatic alterations are particularly frequent in human cancer, the germline mutations cause musculoskeletal phenotypes. Activating mutations of FGFR1 cause craniofacial dysmorphy, synostosis of skull and humerus, and dwarfism, whereas inactivating genetic alterations lead to syndactyly. Activating mutations of FGFR2 induce craniosynostosis, syndactyly and digitopalmar fusion. Achondroplasia and hypochondroplasia are associated with gain-of-function mutations of FGFR3. Genetic lesions of FGFR4 and FGFR5 and their effects on the human skeleton are not known.[55]

For the endocrine function of FGFs, a co-receptor is required, and this is provided by the klotho family.[56] Endocrine FGFs and klotho gene families are a complex system that co-evolved with the endoskeleton.[55]

The klotho family features three members: α, β and γ. They share a similar structure but α-klotho is the only one found in the circulation.[57] Indeed, membrane-bound α-klotho can be cleaved by ADAM10 and ADAM17 metalloproteinases, resulting in the secretion of a soluble molecule.[58]

α-Klotho is an obligate co-receptor for FGF23, and it was identified serendipitously when a transgenic experiment accidentally disrupted the α-klotho promoter.[59]

α-Klotho coupling with FGFR1 confers high affinity to circulating FGF23 and specificity to its target organs.[55] Indeed, even if FGF23 receptors are largely expressed, the FGF23 downstream signalling has been detected only in those tissues characterised by high

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expression of α-klotho, including the kidney, parathyroid glands and brain.[16,60]

FGF23 directly inhibits renal phosphate reabsorption by suppressing NaPi2a activity in proximal tubular cells, leading to phosphaturia. Moreover, it inhibits 1α-hydroxylase and PTH release from parathyroid glands. Thus, FGF23 indirectly reduces intestinal absorption of dietary phosphorus, by the reduction of circulating $1,25(OH)_2$ vitamin D.[16,61] Furthermore, it has been recently shown that FGF23 also regulates calcium handling through TRPV5 in renal distal tubules.[62]

Physiologically, FGF23 is regulated by stimulating and inhibiting factors. Saito *et al*. demonstrated a positive correlation between $1,25(OH)_2$ vitamin D, phosphate, PTH and FGF23 levels *in vivo*,[61] whereas negative regulation of FGF23 from PHEX (phosphate-regulating gene with homologies to endopeptidases on the X chromosome) and DMP1 (dentin matrix acid phosphoprotein 1) in bone is also known.[63,64]

Quarles proposed a model to explain DMP1 and PHEX regulation of FGF23 in osteocytes.[65] DMP1 is subjected to cleavage by metalloproteinases to create an N and a C terminus. The C terminus interacts with PHEX via the ASARM (acidic, serine- and aspartic acid-rich motif) and in turn, FGF23 promoter is inhibited. Moreover, both PHEX and DMP1 regulate the mineralisation of extracellular matrix, thus co-ordinating bone phosphate accretion with renal phosphate conservation.[65]

Mutations of FGF23 and α-klotho can cause low FGF23 levels with hyperphosphataemia due to renal phosphate retention and high levels of $1,25(OH)_2$ vitamin D, leading to familial tumoural calcinosis. On the other hand, gene mutations of PHEX and DMP1 result in increased FGF23, renal phosphate wasting, low levels of $1,25(OH)_2$ vitamin D and hypophosphatemia.[55]

Phosphate/vitamin D/FGF23 axis

FGF23 function in the regulation of phosphate homeostasis is co-ordinated with vitamin D and PTH. Indeed, calcitriol increases phosphate serum levels directly by inducing intestinal absorption and indirectly by stimulating its tubular reabsorption through the suppression of PTH.[16] Even if calcitriol enhances NaPi2b protein expression in the gut, it has been demonstrated that its upregulation also occurs in vitamin D-null mice. This result confirms that other factors, such as FGF23, also affect intestinal phosphate transporters.[66]

Both high phosphate serum levels and circulating calcitriol induce FGF23 synthesis in bone.

The interaction between vitamin D, PTH and FGF23 in phosphate homeostasis is not completely understood. Indeed, it remains to be clarified if PTH directly affects FGF23 production.[16]

Chronic kidney disease

Mineral homeostasis is preserved by an orchestra of endocrine hormones, as described above. Just as in physiological conditions, kidney and bone also influence each other in pathological conditions.

Chronic kidney disease (CKD) is an international public health problem affecting 5-10% of the world's population.[67] Chronicity is conventionally defined as renal impairment lasting for at least 3 months; to assess staging and severity of the disease, estimated or measured glomerular filtration rate is used.[68] For adults, the condition is considered to be CKD when the glomerular filtration rate is $\leq 60 ml/min/1.73m^2$, because herein alteration of calcium, phosphorous, PTH and vitamin D is detectable. For children, the glomerular filtration rate at which CKD is determined is higher ($<89 ml/min/1.73m^2$).[69] Changes in mineral parameters play an essential role in CKD pathophysiology. Indeed, in CKD, kidney function declines, leading to a progressive deterioration of mineral homeostasis, with increased FGF23, phosphate and PTH plasma levels, and a reduction of klotho and $1,25(OH)_2$ vitamin D.[55]

Specifically, the decrease in vitamin D has been related to the high levels of FGF23 produced by osteocytes to compensate for phosphate

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retention.[30] FGF23-mediated inhibition of 1α-hydroxylase and concurrent 24α-hydroxylase stimulation lead to calcitriol degradation and reduction. Moreover, the decrease in glomerular filtration rate and reduced megalin expression by proximal tubular epithelial cells impair 25(OH) vitamin D uptake.[44] Thus, the decrease in kidney function with progressive decline in serum calcitriol levels leads to hypocalcemia, secondary hyperparathyroidism and its complications, such as secondary osteoporosis.[30]

Since the increase in FGF23 precedes other changes in mineral metabolism in CKD, and it has been proposed as an early biomarker of acute kidney injury, it has been suggested that FGF23 could be involved in the transition from acute kidney injury to CKD. Moreover, it has been shown that FGF23 maintains a linear relationship with glomerular filtration rate in the transition from mild to moderate CKD whereas, in advanced stages, their relationship becomes exponential.[70] In parallel, klotho can be considered a parameter as sensitive as FGF23. Indeed, the rise of FGF23 levels in CKD is associated with simultaneous klotho reduction.[71,72] Furthermore, FGF23 exacerbates klotho deficiency via its suppression, thus leading to a vicious cycle of FGF23 excess and klotho deficiency that may lead to CKD-related complications such as cardiovascular disease.[70]

Kuro-o and Moe describe CKD progression as a process of adaptative response of the FGF23/α-klotho endocrine axis to the progressive loss of functional nephrons. While the number of nephrons reduces, it will be necessary for the remaining nephrons to compensate, in order to maintain phosphate balance.[Insert REF?] The increase in FGF23 production represents a compensatory response; this can induce further nephron loss due to the increased phosphate excretion per nephron, thus injuring the kidney. Considering the theoretical threshold of 0.5µg/day of phosphate excretion per nephron in healthy individuals, the loss of 50% of nephrons in CKD is sufficient to attain kidney damage. This can induce an amplifying loop in which a reduced number of nephrons induces FGF23 production that, in turn, enhances phosphotoxicity and further nephron loss.[55]

Chronic kidney disease-mineral and bone disorder

Since mineral metabolism is essential in bone tissue homeostasis, its alterations in CKD lead to bone abnormalities in patients. In the past decade, it has been suggested that the extra skeletal calcification that occurs in CKD may result from altered mineral and bone metabolism of CKD. Indeed, abnormal mineral metabolism, bone and extraskeletal calcification are closely related, and all together contribute to the morbidity and mortality of patients with CKD.[73] Since the traditional definition of renal osteodystrophy did not accurately encompass the broader clinical syndrome, the acronym CKD-MBD was coined in 2006. In contrast to renal osteodystrophy, which considered only the alterations of bone morphology displayed by CKD patients, CKD-MBD is defined as 'a systemic disorder of mineral and bone metabolism due to CKD manifested by either one or a combination of the following: abnormalities of calcium, phosphorus, PTH or vitamin D metabolism; abnormalities in bone turnover, mineralisation, volume, linear growth or strength; vascular or other soft-tissue calcification'.[73]

The alterations of bone morphology that occur in CKD-MBD can be assessed by histomorphometry and are classified according to turnover, mineralisation and volume (the TMV system).[69] This scheme provides a clinically relevant description of underlying bone pathology and helps to define pathophysiology and therapeutic approaches. The TMV classification system precisely describes the pathologic abnormalities in CKD patients.

- Osteomalacia is characterised by low bone turnover with abnormal mineralisation. Depending on severity and duration, the bone volume may be low to normal.

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- Adynamic bone features a low bone turnover with normal mineralisation and bone volume from low to normal.
- Mild hyperparathyroid-related bone disease (mild HPT) represents abnormality with medium turnover and a bone volume depending on the duration of the disease process.
- Osteitis fibrosa, also known as advanced HPT, is characterised by abnormalities along a continuum with mild HPT, with high bone turnover.
- Mixed uraemic osteodystrophy is described as high turnover, normal bone volume and abnormal mineralisation.[69]

CKD-MBD represents a complex disorder characterised by alterations of interconnected organs that influence each other. The complexity of this system makes it difficult to understand the causes and effects of these dysfunctions.

One molecular pathway emerging as crucial in CKD-MBD pathogenesis is the Wnt pathway. Several studies reported increased circulating levels of the Wnt inhibitor sclerostin in individuals with impaired renal function compared with those with normal kidney function.[74,75] Moreover, sclerostin values progressively rise across the stages of CKD and are associated with cardiovascular events in a non-dialysed CKD population.[76] Although the causes of this increase are debated, they are probably due to increased sclerostin production.

So far, bone has been considered the major source of sclerostin. Sabbagh and colleagues reported that the repression of Wnt/β-catenin signalling within osteocytes and the increased bone expression of sclerostin occur early in a mouse model of CKD.[77]

However, a new concept of Wnt pathway activation as a developmental programme reactivated during renal injury is emerging.[78] Early stage CKD is characterised by nephrogenesis programme reactivation in order to repair the kidney. The Wnt pathway co-ordinates tubular epithelial proliferation and polarity during nephrogenesis. When canonical Wnt signalling is activated, Wnt inhibitor expression increases in order to regulate the pathway in a negative loop. In contrast to Wnt glycoproteins that, once secreted, have autocrine/paracrine effects, during renal injury, the Wnt inhibitors also act as systemic factors entering the circulation and targeting extrarenal tissues.[78]

Circulating sclerostin has been also associated with vascular disease and mortality in CKD-MBD.[74,76] All together, these studies implicate sclerostin in the cross talk between kidney, bone and vasculature and its involvement in renal-bone-vasculature disease pathogenesis. Thus, a deeper understanding of the Wnt pathway may open new avenues for CKD-MBD prevention and treatment.[74]

Conclusion

In conclusion, bone and kidney functions are closely related. They co-ordinate each other to maintain mineral homeostasis through the release of endocrine factors. Likewise, their connection results in pathologic conditions such as CKD-MBD. Moreover, bone and kidney share common molecular pathway alterations that need to be better investigated in order to improve our understanding of CKD-MBD pathogenesis and the translational implications.

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