New Insights into the Molecular Basis of Kidney Governing Bone Theory

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ABSTRACT

Kidney governing bone theory plays an important role in treating bone metabolic disease such as osteoporosis, and many tonifying kidney prescriptions/herbs are widely used in Traditional Chinese Medicine (TCM). However, the exact biological basis of kidney governing bone theory in the context of new advances in biology is still not fully established. In this paper, the content of kidney governing bone theory in biology has been fully demonstrated from different aspects. We first propose that bone and kidney mutually affect each other in pathology and physiology, particularly through homeostasis of calcium, phosphorus and fibroblast growth factor-23 (FGF-23). Next, we identify that tonifying kidney prescriptions/herbs exert bone protective effects, thus treating osteoporosis by regulating bone formation and bone resorption. Furthermore, the exact molecular mechanisms of tonifying kidney prescriptions, herbs and their effective components in treating osteoporosis have been systematically reviewed. Finally, we come into the conclusion that kidney regulating bone mineral homeostasis, bone protective effects of tonifying kidney herbs and regulatory effects on bone homeostasis are all the manifestations of kidney governing bone theory. Therefore, the new insights into kidney governing bone theory in biology will promote the development of clinical practices, and drugs discovery in treating osteoporosis.

Key words: Osteoporosis, Kidney governing bone theory, Tonifying kidney prescriptions/herbs, Bone homeostasis, Effective components, Osteoclast, Osteoblast

Kidney governing bone theory in TCM proposes that kidney plays a crucial role in controlling bone functions. The book "Essentials of Chinese Medicine" states...
discussed to demonstrate the contents of kidney governing bone theory. Finally, we came into the conclusion that the contents of kidney governing bone theory are manifested as at least three aspects: kidney and bone mutually regulate each other in maintaining mineral homeostasis; tonifying kidney exerts bone protective effects; and tonifying kidney prescriptions/herbs/effective components regulate the differentiation and activity of bone cells (Osteoblasts and osteoclasts) thus to maintain bone homeostasis.

KIDNEY AND BONE MUTUALLY REGULATE EACH OTHER IN MAINTAINING MINERAL HOMEOSTASIS

1. Kidney controls calcium, and phosphorus homeostasis in bone

Kidney governing bone theory emphasizes that kidney plays important roles in regulating normal functions of bone. The regulatory effects of kidney on bone may be attributed to the kidney’s regulatory effects on mineral homeostasis. As a consequence, mineral homeostasis plays an important role in maintaining normal functions of bone.[15]

Bone is composed of crystal minerals, predominantly calcium and phosphorus. Skeleton system is the reservoir for calcium, and similar roles for phosphorus.[15]. Nearly 99% calcium and 84% phosphorus in humans are deposited as crystals in bone. Interestingly, homeostasis of calcium and phosphorus in bone is mainly determined by the reabsorption of kidney. Therefore, abnormalities of kidney’s reabsorption always lead to deficiency of calcium and phosphorus. Defects in calcium (Ca\(^{2+}\)) homeostasis are major causes of morbidity and mortality in elderly chronic kidney disease (CKD) patients.[16]. Studies have shown that chronic hypocalcaemia or net negative Ca\(^{2+}\) balance is associated with osteopenia and osteoporosis, resulting in fractures, morbidity and mortality.[17].

Moreover, kidney reabsorbs most of calcium and phosphorus in human, and guarantees homeostasis of minerals in bone.[18]. The average daily diets of adults contain roughly 1000 mg of Ca\(^{2+}\). In normal conditions, bone Ca\(^{2+}\) absorption and bone Ca\(^{2+}\) reabsorption are well-balanced. Absorbed dietary Ca\(^{2+}\) is excreted by kidney, and Ca\(^{2+}\) is freely filtered through the glomeruli in the kidney.[15]. 50% to 60% of filtered Ca\(^{2+}\) is reabsorbed in the proximal tubule via paracellular pathway coupled to sodium reabsorption[19,20]. For phosphorus homeostasis, common diets contain 0.8-1.5g of phosphorus daily, and 70 to 90% of dietary phosphorus reabsorbed by kidney, a process enhanced by active vitamin D, is predominantly distributed in bone.[15].

2. Bone FGF-23 regulates kidney function

Kidney governing bone theory indicates that bone also exert regulatory effects on kidney functions, although little is known about it. In the past several years, intensive investigations have shown that FGF-23 functions as an important regulator in controlling kidney’s function.[21]. FGF-23, a key regulator of phosphorus and vitamin D metabolism, its regulators were mainly secreted by bone (Osteocytes and osteoblasts)[21]. It has revealed that circulating FGF-23 rises dramatically in patients with decline of renal function, and functions as a key initiating role in development of abnormal mineral metabolism in patients with CKD.[22]. Studies have shown that disordering of FGF-23 and bone metabolism occurred in the early course of CKD.[23]. Furthermore, FGF-23 may function as a key initiating factor in disordered miners and bone metabolism, particularly in patients with CKD.[24]. In clinic, dysregulation of FGF-23 and its regulators result in osteomalacia, implicating the role of osteocytes in regulation of skeletal natalization. Alterations in skeletal mineralization are also associated with CKD patients, who usually have dramatically increased expressions of FGF-23 and its regulators.[24]. Traditionally, these abnormalities have been ascribed to changes in parathyroid hormone (PTH) and vitamin D axis, which leads to subsequent alterations in calcium and phosphorus metabolism.[25], and abnormalities of kidney’s function.

3. Manifestations of kidney governing bone theory between bone and kidney

Therefore, we propose that kidney governing bone theory in calcium and phosphorus metabolism is manifested as the following two points (Figure 1): 1) Kidney reabsorbs calcium and phosphorus homeostasis thus to guarantee bone functions; 2) Bone releases regulators, such as FGF-23, to maintain normal functions of kidney. Therefore, we came into the conclusion that bone and kidney mutually affect the functions of each other by regulating the metabolism of calcium, phosphorus and FGF-23, which partially demonstrate the biological bases of kidney governing bone theory.

BONE PROTECTIVE EFFECTS OF TONIFYING KIDNEY PRESCRIPTIONS/HERBS IN TREATING OSTEOPOROSIS

According to kidney governing bone theory, many tonifying kidney prescriptions/herbs are widely used in treating osteoporosis. In this part, based on clinical practice in TCM, bone protective effects of prescriptions/herbs were fully investigated.

1. Clinical investigations

Nearly thirty years ago, bone-protective effect of tonifying kidney prescriptions were first investigated in the management of osteoporosis by the instruction of kidney governing bone theory[2]. Tonifying kidney prescriptions were used to evaluate the therapeutic efficacy on osteoporosis by measuring kidney-deficiency symptoms and bone mineral density (BMD) from then on.[26]. Studies have established that tonifying kidney prescriptions not only improve clinical symptoms but also delay the developmental process of osteoporosis[26]. Therapeutic effects of kidney tonifying principles and Bushen Jiangu (BSJG) capsule on postmenopausal osteoporosis compared with
calcium-treated group in double-blind trials. The result shows that 3 months after treatment with BSJG capsule, symptoms of 92.85% patients were improved. 6 months after treatment, BMD of lumbar vertebra of 68.8% patients were significantly higher than that before treatment[27]. This finding indicates that BSGJ capsule could prevent and improve postmenopausal osteoporosis. For the therapeutic mechanisms, it is probable that BSGJ capsule improves bone formation and inhibits bone resorption[27]. A 5-year multicenter follow-up study[28] has shown that kidney-tonifying herbal Fufangs with phytoestrogenic epimedium prevent bone loss in postmenopausal osteoporosis. 194 postmenopausal women were recruited in this study, and all subjects were given oral administration of herbal Fufangs or placebo. At the end of 5 years, BMD in treatment group were increased dramatically from baseline compared to control group. Furthermore, fracture incidence is 2.4 fold lower in comparison with control group. This study identifies that herbal Fufang not only exerted beneficial effects on prevention of postmenopausal bone loss, but also showed its potential for reduction in fragility fracture incidence. Investigations[29] were also performed to evaluate therapeutic effect and safety of mixture for Nourishing Kidney and Strengthening Bone (NKSB). Among 160 cases of osteoporosis, 96 patients were treated with mixture for NKSB, 32 patients in control group were given placebo capsule, and 32 patients in blank group were given no drug. After one year treatment, BMD, clinical symptoms, and bone gla protein were significantly improved in comparing with controls. This finding indicates that NKSB can enhance BMD, promote osteogenesis and inhibit bone resorption, hence, treating osteoporosis.

2. Animal investigations
Numerous studies have indicated that tonifying kidney prescriptions/herbs administration displays a bone protective role and rescues bone loss in osteoporosis mice. Kidney-tonifying herbal Fufangs (Bushen Zhuanggu Granules, BZG) are able to prevent osteoporosis in ovariectomy (OVX)-rats[30]. Mechanistic studies indicate that BZG exert bone protective effects by enhancing BMD, bone architecture and strength. Tonifying kidney herbs, epimedi, fructus ligustri lucidi and fructus psoraleae (ELP), are one herbal prescription to treat osteoporosis in TCM. Recently investigations[31] have shown that ELP treatment prevent both osteoporosis and mast cell accumulation in unload-induced rats. Meanwhile, ELP significantly inhibits osteoclastic bone resorption by inhibiting histamine and tumor necrosis factor-α release. Zibushenjing Fang (ZBSF)[32] is composed by many tonifying kidney herbs, and study has shown that ZBSF could increase femur length in mice. Furthermore, studies have shown that tonifying kidney herb lavage[33] treatment increases all physiological indexes and biomechanical of rat femoral bone. This study indicates that tonifying kidney could effectively prevent bone loss and exert enhancement on bone biomechanical properties. Molecular studies have shown that tonifying kidney herbs treated rats-serum contains increased levels of TGF-β1 and hepcidin, which may be the mechanism underlying the promotion of osteogenic differentiation in bone MSCs[34].

For herbs investigations, studies have shown[35] that dried roots of rehmannia glutinosa (RG), a tonifying kidney herbal medicine with a long history of safe use in the treatment of osteoporosis, can significantly reverse BMD decrease in femurs and lumbars in OVX-induced rats, which indicates that RG is able to rescue OVX-induced bone loss without influencing hormones such as estrogen. Du-Zhong (DZ) is a tonifying kidney herbal medicine in treating bone fractures[36]. Studies have shown that daily oral administration of DZ is able to significantly prevent OVX-induced decrease in biomechanical quality of femur. The mechanical changes were associated with the prevention of BMD decrease, and the improvement in microarchitecture. Furthermore, 16 weeks of DZ treatment improve bone biomechanical quality through modifications of BMD, and trabecular microarchitecture. These results indicate that DZ might be a potential alternative medicine for treatment of postmenopausal osteoporosis.
3. Bone protective effects and kidney governing bone theory

Animal and clinical investigations have shown that tonifying kidney prescriptions/herbs displayed bone protective effects (Figure 2). Therefore, we propose that the bone protective effect of tonifying kidney prescriptions/herbs is one of the biological manifestations of kidney governing bone theory.

Bone homeostasis is composed of bone formation (Osteoblastogenesis) and bone resorption (Osteoclastogenesis). However, the precise regulatory effects of tonifying kidney prescriptions/herbs on osteoblastogenesis and osteoclastogenesis are still not understood. Therefore, next question is to fully identify the molecular mechanism of how tonifying kidney prescriptions/herbs exert their effects on maintaining bone homeostasis.

MOLECULAR MECHANISM OF KIDNEY GOVERNING BONE THEORY

As we all know, prescription/herbs are composed by numerous effective components/active principles, and it is very difficult to perform the molecular mechanism study by using whole prescription/herbs. Therefore, specific effective components from tonifying kidney prescriptions/herbs are important instruments to understand the molecular mechanisms of how tonifying kidney prescription/herbs exerting effects on osteoporosis. These effective components always obtain exact structure form, high concentrations, and are easy to purchase from the market. Therefore, next aim is to identify the molecular mechanism of kidney governing bone theory in bone remodeling by selecting typical effective components from tonifying kidney prescriptions/herbs (Figure 2, 3).

Currently, an increasing number of effective components/active principles from tonifying kidney prescription/herbs in TCM have been identified to exert an anabolic effect on osteoporotic mice model, as such compounds as epimedium-derived flavonoids (EF)[3], icariin[4], osthole[5] and psorale[6]. They have been established to exert bone protective effect on bushen (Tonifying kidney) based on the TCM theory. However, the exact molecular mechanism of these effective components on osteoblastogenesis and osteoclastogenesis are not systematically investigated.

1. Stimulation of osteoblast differentiation/bone formation

EFs have been reported to prevent bone loss in OVX-induced rats and late postmenopausal women[3]. Studies established that EF stimulates osteoblast formation in bone MSCs from OVX-induced osteoporosis rats. Mechanistic studies have shown that EFs significantly increase osteogenesis in bone MSCs by increasing of alkaline phosphates (ALP) activity, and mRNA expression of Runx2. This finding indicates that EFs exerts anabolic effects on osteoporotic bone by concomitantly promoting osteogenic differentiation. Icariin has been[4] found exerting stimulatory effects on bone formation in osteoprotegerin(OPG) KO mice, and the underlying mechanism are due to significantly unregulate the expression of BMP-2, BMP-4, RunX2, OC, Wnt1, and Wnt3a in OPG KO mice. Furthermore, icariin also significantly increased the expression of Axin2, DKK1, TCF1, and LEF1, which are the direct target genes of β-catenin signaling. This study demonstrates that icariin significantly reverses the phenotypes of OPG-deficient mice through the activation of Wnt/β-catenin-BMP signaling. Osthole stimulates differentiation of osteoblasts, and thus to stimulate bone formation and prevent bone loss in OVX-induced osteoporotic mice. Molecular studies identified that osthole enhances the expression of Wnt family (Wnt1, Wnt-3a, Wnt4) in MSCs. Further investigations indicate that osthole stimulates osteoblastogenesis by activating Wnt/β-catenin-BMP signaling[5]. Psoralen[6] promotes osteoblastogenesis from primary mouse calvarias osteoblasts in a dose-dependent manner by up-regulating the expression of osteoblast marker genes including type I collagen, osteocalcin and bone sialoprotein and ALP activity. Further studies demonstrated that psoralen up-regulate the expression of Bmp2 and Bmp4 genes, increase the protein level of phospho-Smad1/5/8, and activate BMP reporter activity, as well as the expression of osterix. These results

Figure 2. Tonifying kidney prescriptions/herbs/effective components exert bone protective effect in bone remodeling:1) Clinical and animal studies have fully identified that tonifying kidney prescriptions display bone protective effects in treating osteoporosis;2) Clinical and animal investigations have shown that tonifying kidney herbs prevents bone loss in osteoporotic patients and animals;3) Molecular studies have established that effective components regulating bone homeostasis by enhancing bone formation, and suppressing bone resorption.
suggest that psoralen stimulates bone formation through the activation of BMP signaling thus to promote osteoblast differentiation, and psoralen may be a potential anabolic agent to treat patients with osteoporosis. Oleanolic acid (OA) is one of active ingredients from Chinese medicinal herb Nuzhenzi (Fructus Ligustri Lucidi). OA and its derivatives possess several promising pharmacological activities, such as hepatoprotective effects, and anti-inflammatory, antioxidant, or anticancer activities[7]. Our previously work have revealed that OA exerts bone protective effects in OVX-rats by stimulating osteoblastogenesis[8]. Molecular study has shown that OA stimulates osteoblastogenesis by activating notch signaling.

2. Inhibitory effects on osteoclast differentiation
Recent studies indicate that effective components from tonifying kidney prescriptions/herbs exert regulatory effects on osteoclastogenesis[37,38], which indicate that bone resorption is also controlled by tonifying kidney prescription/herbs. These findings also demonstrate that the mechanisms of how these botanicals exert their effects on bone remodeling are far from our previously knowledge.

Series of studies have revealed that glycosides or derivative of OA prevent bone loss in O VX mice and inhibit osteoclastogenesis in vitro. Recently, studies have shown that OA exerts inhibitory effects on osteoclastogenesis and osteoclast activity[39]. This study has shown that OA dose-dependently inhibits receptor activator for nuclear factor-κB ligand (RANKL)-mediated osteoclastogenesis and formation of functional osteoclasts in bone marrow macrophages (BMMs). Moreover, OA significantly increases BMD in OVX mice partly by inhibiting osteoclast activity. Mechanistically, study has shown that OA inhibits osteoclastogenesis not by affecting on RANKL-induced activation of NF-κB, JNK, and p38 and ERK pathways in BMMs, but by suppressing RANKL-induced expression of NFATc1 and c-Fos in BMMs, which are two key transcriptional regulators of osteoclastogenesis. Moreover, OA significantly suppresses the expression of RANKL-induced osteoclast genes encoding MMP9, Ctsk, TRAP and Car2 in BMMs. Interestingly, the inhibition of OA on osteoclastogenesis and the expression of RANKL-induced NFATc1 and osteoclast genes are not affected even if BMMs were pretreated by RANKL[38]. Osthole prevents bone loss in 5/6 nephrectomy-induced osteoporotic mice by depressing the expression of CTSK and TRAP. Mechanistic study has shown that osthole rescues bone loss in 5/6 nephrectomy-induced osteoporotic mice by depressing the expression of NFATc1 and c-Fos[39,40]. Therefore, more effective components from tonifying kidney prescriptions/herbs will be performed for further study, and new molecular mechanism will be identified in the future.

CURRENT UNDERSTANDING OF KIDNEY GOVERNING BONE THEORY IN BIOLOGY
In this paper, we endeavor to propose biological schematic review for kidney governing bone theory in TCM. Here we
have shown that there are at least three manifestations of kidney governing bone theory in biology: 1) Kidney and bone mutually affect each through metabolism of calcium, phosphors and FGF-23 (Figure 1); 2) Tonifying prescriptions/herbs exert bone protective effects on bone remodeling thus to treating osteoporosis (Figure 2); 3) Mechanism bases of kidney governing bone theory including the stimulation of osteoblastogenesis and inhibitory of osteoclastogenesis (Figure 3).

EXPERT OPINIONS ON FUTURE STUDY OF KIDNEY GOVERNING BONE THEORY

Currently, there are two main categories of medications that are marketed in treating osteoporosis. One is targeting osteoblasts by stimulating bone formation, such as recombinant human parathyroid hormone (rhPTH) (1-34) [41]. The other is targeting osteoclasts by inhibiting bone resorption, and there are several major antiresorptive drugs (agents capable of inhibiting osteoclast formation and/or function) on the market in treating osteoporosis [42–44]: estrogen, selective estrogen receptor modulators (SERMs), bisphosphonates, calcitonin, and denosumab. However, all marketed drugs either lack satisfactory efficacy or have potential to cause serious side effects in management of osteoporosis. Moreover, as the population continues to age, osteoporosis is likely to become an even more prevalent and serious health and societal problem. Thus, more efficacious and safer antiresorptive drugs in treating osteoporosis are urgently needed in pursuit of better effect.

Tonifying kidney prescriptions/herbs have been shown to exert bone protective effects in different animal models and clinical trials. Molecular studies have shown that tonifying kidney prescriptions/herbs simultaneously inhibit differentiation of osteoclasts and stimulate osteoblastogenesis, which indicate both bone formation and bone resorption are regulated by tonifying kidney herbs. As safer effective components with fewer side effects are necessary in treating osteoporosis, tonifying kidney herbs may serve as drugs discovery in light of tonifying kidney governing bone theory. More importantly, tonifying kidney prescriptions/herbs are independently regarded as enriching Yin or Yang categories in TCM, such as prescriptions of Warming kidney Yin and Nourishing kidney Yang. Our previous work has shown that osteoblasts and osteoclast were independently considered as Yin and Yang in bone remodeling compartments [45]. Therefore, it is very interesting to identify the molecular mechanisms of the enriching Yin and Yang prescriptions/herbs in treating osteoporosis, and establish their effects on osteoclast (Yang) and osteoblast (Yin). Currently, tonifying kidney prescriptions/herbs have been shown to simultaneously regulate bone formation and bone resorption. However, the similarity and difference between Yin-Yang prescriptions/herbs in controlling bone formation and bone resorption are still not fully identified. Moreover, the effects of tonifying kidney herbs that exerted on bone miner metabolism and FGF-23 are still not fully investigated till now.

Therefore, ongoing work should focus on: 1) Are tonifying kidney herbs exerting their effects on miner metabolism? Such as homeostasis calcium and phosphorus and the expression of FGF-23; 2) Molecular studies on tonifying kidney prescriptions/herbs should be performed to find more effective and safe drugs to regulate bone formation and bone resorption; 3) Further investigations are needed to identify the similarity and difference among tonifying kidney Yang and nourishing kidney Yin in the managements of osteoporosis.

DISCLOSURE:

All authors state that they have no conflicts of interest.

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