

From Osteoblast to Osteoclast: New Insights of Yin-Yang Theory in Bone Remodeling

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ABSTRACT

Yin-Yang theory plays critical role in traditional Chinese medicine (TCM) science. However, there are many competing interpretations of Yin-Yang theory in the context with the development in biology, and no consensus has been established. Here we first propose that osteoblast should be regarded as Yin, while osteoclast should be considered as Yang in bone remodeling compartment (BRC). Our conceptions are consistent with the following key findings: 1)osteoblast and osteoclast both derive from the embryonic ectoderm; 2)osteoblast and osteoclast work collaboratively in BRC to maintain bone homeostasis; 3)the activities of osteoblast and osteoclast are coupled to remain dynamic balance; 4)osteoblast and osteoclast exert their function in ceaseless successions. Moreover, we argue that Yin-Yang relationships exist between osteoblast and osteoclast: osteoblast secretes the receptor activator for nuclear factor- κ B ligand (RANKL) and the monocyte/macrophage colony-stimulating factor (M-CSF) and osteoprotegerin (OPG) to positively or negatively regulate osteoclast differentiation and function. Meanwhile, osteoclast and bone resorption in turn release cytokines, such as transforming growth factor- β (TGF- β), insulin-like growth factor-1 (IGF-1) from bone matrix, to regulate osteoblast differentiation and function. Next, the unlimited division of Yin-Yang can be applied to divide sublevel of Yin-Yang inside osteoblast or osteoclast. Finally, Yin-Yang relationship of osteoblast and osteoclast is relative. Therefore, we come into the conclusion that the relationships between osteoblast and osteoclast as established in contemporary biology reflect the classic Yin-Yang in bone remodeling. The new Yin-Yang concepts of osteoblast and osteoclast may strengthen basic theory and clinical practice in TCM.

Key words: Yin-Yang theory, Bone remodeling, Osteoblast, Osteoclast, Bone homeostasis

Bone dynamic tissue that undergoes continual adaptation during vertebrate life to attain and preserve skeletal size, shape, and structural integrity and to regulate mineral homeostasis which named bone remodeling^[1,2]. Bone remodeling is responsible for growth and mechanically induced adaptation of bone and normal functions. Normally, bone remodeling is a tightly regulated process securing repair of micro damage and replacement of old bone with new bone through sequential osteoclastic resorption and ectoblastic formation. Bone resorption (mediated by osteoclast) and bone formation (mediated by osteoblast) work competitively but cooperatively in balanced manners to guarantee no alterations and maintain bone homeostasis^[3] after each remodeling cycles. The ceaseless successive movements between osteoblast and osteoblast in bone remodeling represent the properties of Yin-Yang.

Yin-Yang theory represents one of the core foundations of traditional Chinese medicine (TCM). Zhuangzi, an ancient Chinese philosopher, said "Material force moves and flows in all directions and in all manners. Its two elements (yin and yang) interact and unite to establish He (harmony), so it gives rise to the concrete. Thus the multiplicity of things is produced."^[4]. Interestingly, we found that osteoblast and

osteoclast operate collaboratively but competitively in bone remodeling compartment (BRC) to maintain bone homeostasis like Yin-Yang. During bone remodeling circle, osteoclast positively and firstly activate bone remodeling cycles by removing old bone^[2]. Meanwhile, osteoblast are negatively and secondary recruited to build new bone after the activation of osteoclast^[2]. Therefore, osteoblast is considered as Yin within BRC, and osteoclast is regarded as Yang within BRC.

Molecular study also established that osteoclast and osteoblast are coupled to maintain bone homeostasis in bone remodeling. Importantly, bone remodeling that operated by osteoblast and osteoclast is coordinated by receptor activator of nuclear factor kappa-B ligand (RANKL). RANKL plays as switch of the bone formation and bone resorption^[2, 5]. On one side, RANKL^[6] stimulates the differentiation of osteoblast thus to enhance bone formation by binding to osteoprotegerin (OPG) on osteoblast. On the other side, RANKL stimulates osteoclastogenesis and enhances bone resorption by binding to receptor activator of nuclear factor kappa-B (RANK). Therefore, we propose that RANKL/OPG/RANK system can positively or negatively work together by regulating

osteoclast and osteoblast in bone homeostasis. Based on the molecular bases, we argue that the roles of osteoblast and osteoclast in bone remodeling can be regarded as Yin and Yang.

THE YIN-YANG PROPERTIES OF OSTEOBLAST AND OSTEOCLAST IN BONE REMODELING

In normal bone remodeling, osteoclast positively and firstly activates bone remodeling cycles by removing old bone^[2]. Meanwhile, osteoblast is negatively and secondary recruited to build new bone after the activation of osteoclast^[2]. Here we propose that osteoblast (bone formation) should be considered Yin, while osteoclast (bone resorption) should be regarded as Yang (Figure 1A). This conception is consistent with the following key findings in biology^[7, 8]: 1) osteoblast and osteoclast both derive from the embryonic ectoderm, indicating that these two cells essentially have the same developmental origins like the Yin-Yang (Figure 2A); 2) osteoblast and osteoclast operate collaboratively within BRC to maintain bone homeostasis; 3) the activities of osteoblast and osteoclast are kept in balance manners to maintain bone homeostasis (Figure 2C); 4) osteoblast and osteoclast exert their function in a circular and periodic manner in bone homeostasis. These processes are similar to the Yin-Yang ceaseless movement according to Yin-Yang theory.

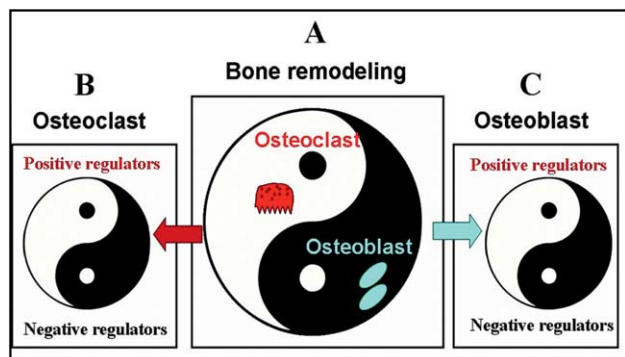


Figure 1. The Yin-Yang properties of osteoclast and osteoblast in bone remodeling, and the unlimited division of Yin-Yang properties inside osteoclastogenesis and osteoblastogenesis

A: In normal bone remodeling compartments (BRC), osteoclast positively and firstly activate bone remodeling cycles by removing old bone. Therefore, osteoclast is regarded as Yang within BRC. Meanwhile, osteoblast is negatively and secondary recruited to build new bone and increase bone density after the activation of osteoclast. Therefore, osteoblast is considered as Yin within BRC.

B: According to unlimited division properties of Yin-Yang, we propose that osteoclast (Yang) can be further divided into sublevels of Yin-Yang parts inside itself. Positive regulators that stimulate osteoclastogenesis are considered as Yang, and negative regulators which suppress osteoclastogenesis are regarded as sublevel Yin.

C: Meanwhile, positive regulators that stimulate osteoblastogenesis are considered as Yang while the negative regulators are regarded as sublevel Yin.

YIN-YANG RELATIONSHIPS BETWEEN OSTEOCLAST AND OSTEOBLAST IN BONE REMODELING

We argue that Yin-Yang relationships exist between osteoblast and osteoclast in bone remodeling (Figure 2C): 1) Osteoblast and osteoclast can mutually stimulate the differentiation of the other; 2) Osteoblast inhibits the differentiation of osteoclast and vice versa; 3) Osteoblast and osteoclast can mutually enhance the activity of the other; 4) Osteoblast restrains the activity of functional osteoclast and vice versa. In the following paragraph, the relationships between osteoblast and osteoclast will be systematically assessed in light of Yin-Yang theory by selecting typical regulators in bone remodeling.

1. OSTEOBLAST AND OSTEOCLAST MUTUALLY STIMULATE THE DIFFERENTIATION OF THE OTHER IN BONE REMODELING

1.1 Osteoblast stimulate the differentiation of osteoclast

Normally, osteoblast secretes RANKL and M-CSF to stimulate the differentiation of osteoclast (Figure 2B). Numerous studies

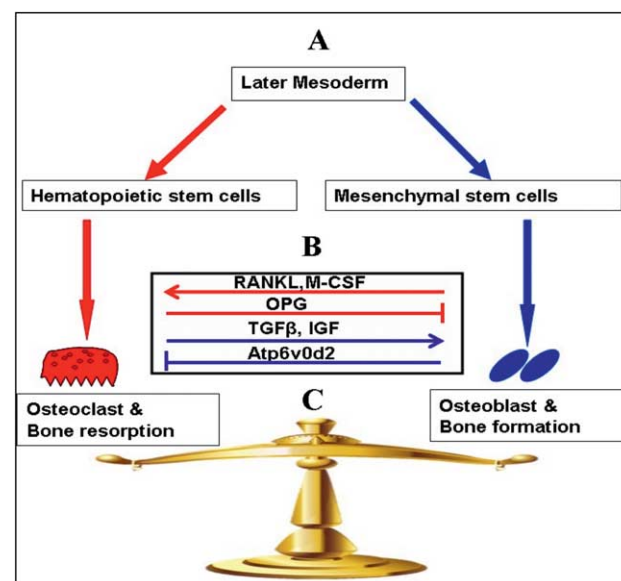


Figure 2. Some physiological and molecular bases of Yin-Yang properties for osteoclast (Bone resorption) and osteoblast (Bone formation) in bone homeostasis

A: Hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), originate from later mesoderm, separately differentiate into osteoclast (Yang) and osteoblast (Yin). These physiological relationships like Yin-Yang which are originate from Yuan in TCM theory.

B: Both the formations and functions of osteoblast and osteoclast are mutually regulated by the other through different factors. For these regulators, RANKL, M-CSF, TGFβ, IGF are positive regulators, and OPG, Atp6v0d2 are negative regulators between osteoclast (Yang) and osteoblast (Yin).

C: Just like the Yin-Yang balance, the activation of osteoclast (bone resorption, Yang) and osteoblast (bone formation, Yin) are coupled in bone remodeling to maintain bone homeostasis and function.

indicated that HSCs differentiate into osteoclast by the stimulations of RANKL and M-CSF^[9, 10]. In addition, non canonical Wnt5a, one transcriptional factor that secreted by osteoblast, also promotes the development of osteoclast by activating RANK signaling^[11]. Moreover, studies shown that osteoblast express the receptor of tumor necrosis factor α , β (TNF- α , β)^[12] and interleukin-1(IL-1)^[13]. As we all know, TNF- α , β and IL-1 play positive roles in regulating the differentiations of oteoclast by activating RANK signaling (Figure 2B). Therefore, Osteoblast stimulates the differentiation of osteoclast by activating or releasing positive regulators, which like the Yin stimulates Yang according to Yin-Yang theory.

1.2 Osteoclast stimulates the differentiation of osteoblast

Interestingly, osteoclast also plays positive roles in regulating the differentiation of osteoblast in bone remodeling. During normal bone remodeling, the activity of osteoclast (Bone resorption) remove old bone by dissolving bone mineral and bone matrix. Transforming growth factor beta (TGF- β), insulin-like growth factors (IGF), and bone morphogenetic protein (BMPs) are released after bone resorption^[9,14]. Molecular studies indicated that the above cytokines exert stimulatory effects in osteoblastogenesis^[14]. In addition, recently study established that osteoclast also stimulate osteoblast differentiation by releasing hepatocyte growth factors (HGFs)^[15]. Therefore, we come into the conclusion that osteoclast stimulate the differentiation of osteoblast in bone remodeling. These relationships between osteoclastogenesis and osteoblastogenesis like the Yang stimulate Yin in Yin-Yang theory.

2. OSTEOBLAST INHIBITS THE DIFFERENTIATION OF OSTEOCLAST AND VISE VISA

2.1 Osteoblast inhibits the differentiation of osteoclast

According to Yin-Yang properties, Yin and Yang can also inhibit the differentiation of the other. Therefore, we identify that osteoblast also inhibit the differentiation of osteoclast besides the stimulatory effects. In addition to the stimulatory effects of osteoblast that exerted on osteoclastogenesis by producing RANKL and M-CSF^[9,10], osteoblast simultaneously expresses osteoprotegerin (OPG)^[16, 17] to competly combine to RANKL. The binding of OPG with RANKL leads to the inactivation of RANK signaling thus to inhibiting the differentiation of osteoclast. Therefore, the expression of OPG on osteoblast significantly suppresses the differentiation of osteoclast. Osteoblast (Yin) negatively regulates the differentiation of osteoclast (Yang).

2.2 Osteoclast inhibits the differentiation of osteoblast

Previously studies indicated that osteoclasts are fully controlled by osteoblast.

However, recently studies shown that osteoclast also exert inhibitory effects on osteoblastogenesis. Studies shown that osteoclast express v-ATPase V0 subunit d2 (Atp6v0d2) near the ruffle border^[18] in mature osteoclast. Molecular bases indicate that Atp6v0d2 not only necessary for the activity of osteoclast by supplying the protons and energy, but also inhibit the differentiation of pre-osteoblasts into mature osteoblasts^[18]. According to Yin-Yang theory, the inhibitory effects of osteoclast that exerted on the differentiation of osteoblast are considered as Yang inhibits Yin in bone remodeling.

2.3 Osteoblast restrains the activity of functional osteoclast and vise visa

2.3.1 Osteoblast restrains the activity of functional osteoclast

According to Yin-Yang theory, Yin-Yang can restrain the activity of the other within certain limitation in order to keep the Yin-Yang balance. Interestingly, molecular studies indicated that osteoblast express OPG on its membrane to restrain the activity of functional osteoclast and bone resorption^[16]. This finding indicates that OPG is the target to prevent bone loss, and numerous studies were performed by targeting OPG in order to find new drugs with better effects in treating osteoporosis^[17]. For example, Denosumab, a fully human monoclonal antibody for RANKL, inhibits the activity of mature osteoclast. Denosumab was recently approved for the treatment of postmenopausal osteoporosis in women at a high or increased risk of fracture by the FDA in the United States and by the European Medicines Agency in Europe in 2010^[19]. Therefore, the restricted effects of osteoblast that exerts on osteoclast can be regarded as Yin restrains Yang in bone remodeling.

2.3.2 Osteoclast restrains the activity of functional osteoblast

Meanwhile, osteoclast also restrains the activity of functional osteoblast in bone resorption. Molecular studies shown that osteoclast secretes sclerostin^[20] in bone remodeling to restrain the activity of osteoblast and inhibits bone formation. Studies indicate that sclerostin exert inhibitory effect on ostoblast activity by binding BMP5 and BMP6 thus to suppressing the expression of alkaline phosphates (ALP) in osteoblast^[21]. In addition to sclerostin, the expression of semaphorin3A in osteoclast also significantly restrains the activity of in pre-osteoblast (bone formation)^[22]. According to Yin-Yang theory, the negative effect that osteoclast played in regulating the activity of functional osteoblast are regarded as Yang restrains Yin according to Yin-Yang theory.

2.4 Osteoblast and osteoclast can mutual enhance the activity of the other

2.4.1 Osteoblast enhance the activity of osteoclast

The Yin-Yang theory holds that idea that Yin and Yang can enhance the activity of the other, and the molecular mechanism should be identified to investigate it. Molecular studies found that RANKL and M-CSF not only play important roles in promoting osteoclast formation, but also

enhance the activity of mature osteoclast and bone resorption^[9, 10]. Lots of studies identified that RANKL and M-CSF activate series of downstream signaling pathways to start bone resorption, including matrix metalloproteinase 9 (MMP9), cathepsin (CTSK), tartrate resistance acid phosphatase (TRAP) and so on^[9, 10]. Therefore, the existence of RANKL and M-CSF are the conditions for normal activity of osteoclast. Lacking of RANKL and M-CSF lead to impaired osteoclast activity and depressed bone resorption in mature osteoclast^[23]. Hereby, the existences of osteoblast (Yin) are necessary to promote/enhance the activity of osteoclast (Yang).

2.4.2 Osteoclast enhance the activity of osteoblast

During normal bone resorption, the activities of osteoclast release TGF- β , IGF, and BMPs^[9, 14] to enhance the activity of mature osteoblast. Molecular studies indicate that TGF- β , IGF, and BMPs not only stimulate osteoblastogenesis, but also largely promote the activity of osteoblast and bone formation^[9, 14]. In addition to the above regulators, studies established that functional osteoclast also express CTSK to positively enhance the activity of osteoblast and bone formation^[16] to maintain bone homeostasis. Therefore, molecular studies indicate that the existence of osteoclast and bone resorption is the pre-conditions of osteoblast formation and bone formation. According to Yin-Yang theory, the relationships of mature osteoclast that enhancing the activity osteoblast in bone remodeling are considered as Yang enhances Yin.

THE UNLIMITED DIVISION OF YIN-YANG IN BONE REMODELING

The Yin-Yang theory holds that Yin-Yang exist in different levels, and Yin-Yang can be divided into sublevel of Yin-Yang^[4]. According to this idea, if we argue that osteoblast and osteoclast are regarded as Yin-Yang in bone remodeling, the sublevel of Yin-Yang should be identified in bone remodeling.

As we expected, many studies have found that there are positive regulators or negative regulators inside osteoblast differentiation or osteoclast differentiation^[22-24]. Interestingly, we find that the positive regulators, including signaling pathways, cytokines, proteins, genes, stimulate the differentiation of osteoblast and osteoclast. Therefore, the positive regulators are regarded as Yang (Figure 2B and 2C). However, negative regulators, including signaling pathways, cytokines, and proteins, genes (Figure 2B and 2C), exert inhibitory effects on formation of osteoblast and osteoclast. Therefore, the positive regulators are considered as Yang.

Therefore, we identified that relationships in Yin-Yang theory are also consistent to the differentiation and function inside osteoblast and osteoclast. Both positive and negative regulators have been implicated in controlling the differentiation and function of osteoblast and osteoclast^[22-24]. These total different regulators exist inside osteoblast or osteoclast, and the regulators are regarded as the sublevel of Yin and Yang in bone remodeling.

THE RELATIVITY OF YIN-YANG IN BONE REMODELING

The Yin-Yang theory holds that the roles of Yin and Yang is relative^[4]. Therefore, the Yin-Yang relationship of osteoblast and osteoclast is relative, and the roles of Yin or Yang properties are not permanent according to different systems in bone remodeling^[25]. For example, osteoblast is considered as Yin in contrast to osteoclast^[26, 27], while the osteoblast is regarded as Yang in contrast to adipocyte or osteocyte^[28, 29].

In addition to Yin-Yang relationship of osteoblasts and osteoclasts, there is another typical Yin-Yang in bone remodeling: osteoblast and adipocyte. Both two different from MSCs, but play varied effects in bone remodeling^[28, 29]. Osteoblast increase bone density by stimulating bone formation while adipocyte decrease bone marrow by changing MSCs into fats^[30]. This two cells play paradox roles in bone remodeling although they were differentiated from same stem cells. According to Yin-Yang theory, osteoblast and adipocyte are separately regarded as Yin and Yang in bone remodeling.

EXPERT OPINION OF THE FUTURE STUDY ON YIN-YANG THEORY

Taken together, we conclude that the relationships between osteoblast and osteoclast as established in contemporary biology reflect the classic Yin-Yang in TCM. The new insights of Yin-Yang in bone remodeling will promote our understanding of Yin-Yang contents in biology, and many clinical practices will be benefits a lot from it. What is more, many botanicals and their prescription are widely used in clinical practice in TCM basing on Yin-Yang theory. For example, the prescriptions of Warming kidney Yang and Nourishing kidney Yin, both two are effective regiments to treat osteoporosis in light of Yin-Yang theory in Longhua hospital in Shanghai.

However, the molecular mechanisms underlying these prescriptions in treating osteoporosis are still not fully investigated. Lots of effective components or principles from herbs in these two prescriptions exert stimulatory effects on osteoblast formation and enhance bone formation thus to prevent bone loss in osteoporosis mice model^[31-39]. But recently, several studies also show that these effective components also inhibit osteoclast formation and function thus to prevent bone loss^[40-42]. Interestingly, according to the Yin-Yang theory, all these herbs can be regarded as Yin or Yang in TCM theory.

Therefore, it is necessary to identify the molecular bases on how bone remodeling is regulated by these herbs or their effective components. Importantly, whether these effective components from Yin or Yang exert their effect by targeting osteoclast and osteoblast are still not identified. Our ongoing work will systematically investigate the effects of these Yin-Yang herbs or their effective components that exert on osteoblast and osteoclast separately. We believe that the identification of molecular mechanism underlying bone remodeling particular osteoblast and osteoclast may promote

the development of drugs discovery, basic TCM theory and management of bone metabolic disease in clinic.

DISCLOSURE:

All authors state that they have no conflicts of interest.

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