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Review

Regulation of transcriptional network system during bone and cartilage development

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ABSTRACT

Background: Bone and cartilage are essential skeletal tissues, which not only function as structural basis of locomotive organs, but also regulate calcium homeostasis, phosphate metabolism, hematopoiesis, and glucose turnover. Several hormones and cytokines in cooperation with their downstream transcription factors regulate bone and cartilage development; therefore, it is important to understand the precise mechanisms of this regulation.

Highlight: Genetic studies in human and mouse have provided a wealth of information regarding the transcription factors implicated in bone and cartilage development. Moreover, innovative molecular cloning techniques identified several new transcription factors that play indispensable roles in controlling the development of bone and cartilage. The mechanisms controlling the expression of these transcription factors have been meticulously elucidated, so that the transcriptional network system, which seemed so complex and mysterious not so long ago, has become considerably clearer in recent years.

Conclusion: Recent advances in our knowledge about transcriptional network systems contributed to understanding the molecular underpinnings of regulation and pathological disease mechanisms in bone and cartilage.

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1. Introduction

Development of bone and cartilage, both of which are major components of skeletal tissues, is precisely and cooperatively regulated by several transcription factors. Bone resorption by osteoclasts and bone formation by osteoblasts underlie bone remodeling, which is critical for bone development and metabolism. On the other hand, cartilage is formed by a sequential differentiation of chondrocytes from undifferentiated mesenchymal cells. Recent advances in molecular biology and genetics, including studies by our laboratory, have considerably improved our understanding of the molecular mechanisms of bone and cartilage development, particularly with regards to the involvement of several transcription factors in this process. In this review, we would like to introduce the concept of the transcriptional

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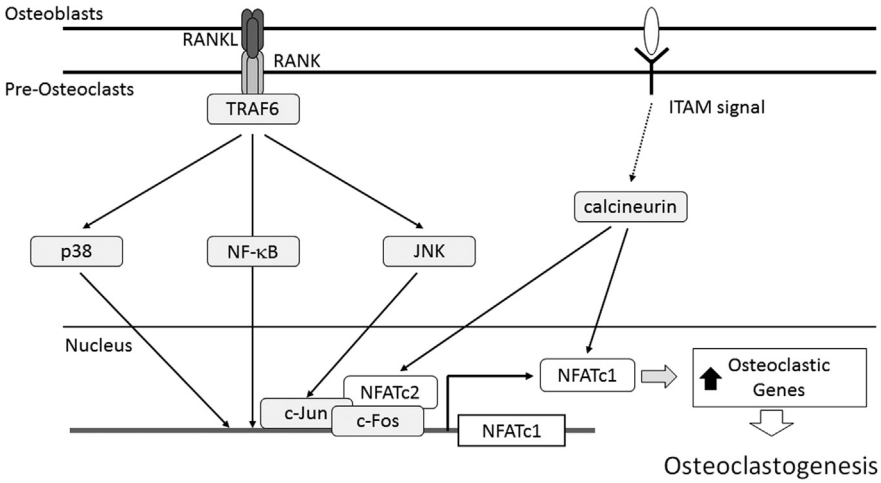

Fig. 1. Regulation of RANKL signaling during osteoclast differentiation. RANKL stimulates NF-κB, JNK/c-Jun, and p38 pathways through RANK and TRAF6, and subsequently induces NFATc1 in co-operation with ITAM and calcineurin signaling. NFATc1 stimulates osteoclast formation by controlling the expression of osteoclastic genes.

network system that regulates bone and cartilage development, and focus on the role of transcription factors and regulation of their expression.

2. Regulation of osteoclast formation by RANKL signaling and NFAT1c

Osteoclasts, which are the only type of cells able to resorb bone tissues, are differentiated from hematopoietic stem cells via the monocytes/macrophage lineage [1]. Two major molecules, M-CSF and RANKL, are essential for osteoclast development [1]. In addition, RANKL is required for bone resorbing function and survival of osteoclasts [1,2]. Because RANKL belongs to the TNF superfamily, RANKL mediates activation of NF-κB, JNK/c-Jun, and p38 pathways through RANK and TRAF6 [2,3]. Mouse genetic studies clearly demonstrated that RANKL, RANK, TRAF6, NF- κB, c-Fos, and c-Jun are indispensable for osteoclast differentiation (Fig. 1) [4–9]. The p38 pathway also seems to be necessary for the osteoclastogenesis, because a p38 inhibitor markedly suppressed osteoclast formation *in vitro* (Fig. 1) [10]. Collectively, these signaling pathways and their downstream transcription factors cooperatively regulate osteoclast formation.

It has been hypothesized that osteoclast formation may be under a control of an osteoclast-specific transcription factor. Indeed, four independent studies revealed that NFATc1 (NFAT2) is the osteoclast-specific transcription factor, which is critical for the osteoclastogenesis (Fig. 1). Microarray studies showed that NFATc1 is a direct target gene of RANKL [11,12]. NFATc1 was also identified as a transcriptional partner of the c-Jun/c-Fos complex during the osteoclastogenesis [9]. In addition, NFATc1 is a transcriptional target of c-Fos [13]. Most strikingly, NFATc1 is sufficient to induce osteoclast formation in the absence of RANKL [12]. Essential role of NFATc1 *in vivo* has been shown in mouse models [14,15]. NFATc1 regulates several osteoclast marker genes including *TRAP*, *cathepsin K*, *DC-STAMP*, and *NFATc1* itself (Fig. 1) [12,14,16]. Although auto-amplification of NFATc1 would be a very important mechanism for osteoclastogenesis [14], NFATc2 (NFAT1) has been shown to initiate the induction of NFATc1 in osteoclast precursor cells [9,14]. NFATc1 expression is also controlled by c-Jun, c-Fos, NF- κB, and p38 [9,17]. NFAT signals seem to be involved in the RANKL-mediated bone resorbing activity, presumably by activating c-Src [15].

It is known that transcription factors of the NFAT family are regulated by the phosphatase calcineurin, and that NFATc1 is dephosphorylated and activated by calcineurin during the osteoclastogenesis [18]. In addition, an essential role of signaling mediated by the immunoreceptor tyrosine-based activation motif (ITAM) for the activation of NFATc1 and osteoclastogenesis has been demonstrated (Fig. 1) [19]. Although cyclosporin A and FK506 are effective inhibitors of calcineurin, both of them have complex effects on bone metabolism [20]. Since VIVIT peptide and INCA compounds suppress NFAT activity by selectively inhibiting the interaction between NFAT and calcineurin [21,22], these and similar compounds may be used for treatment of bone destruction by osteoclasts without affecting the phosphatase activity of calcineurin.

3. Regulation of the osteoblastogenesis by BMP2 signaling and transcription factors

Bone morphogenetic protein 2 (BMP2), which is a member of the TGF-β superfamily, has a very powerful osteogenic and osteoblastogenic activity *in vitro* and *in vivo* [23,24]. SMAD signaling, mediated by SMAD1, SMAD5, and SMAD4, plays a central role in BMP2 signaling and osteoblast differentiation [23,25,26]. The complex of SMAD1/5 and SMAD4 relays the BMP2 signal to the nucleus as a transcriptional regulator (Fig. 2) [26,27].

BMP2 has been shown to manifest its osteogenic activity via upregulation of several transcription factors including RUNX2 and Osterix [28–30]. RUNX2 has been identified as an essential transcription factor for bone formation and osteoblast differentiation [31,32]. Mutations in the *RUNX2* gene cause cleidocranial dysplasia, a genetic skeletal disorder [33]. In addition, RUNX2 regulates the expression of osteoblastic genes such as osteocalcin, bone sialoprotein (BSP), and osteopontin [31,34,35]. Interestingly, SMAD signaling pathway controls both the expression and function of RUNX2 during the BMP2-regulated osteoblast differentiation (Fig. 2) [36–38]. Moreover, impaired interaction of RUNX2 with SMAD molecules is involved in the pathogenesis of cleidocranial dysplasia [39]. Cbfβ has been identified as an essential transcriptional partner of RUNX2 during bone formation (Fig. 2) [40]. C/EBPβ, TAZ, and pRb also interact with RUNX2 and upregulate its osteogenic activity (Fig. 2) [41–44]. Recently, TAZ has been demonstrated to function as a transcriptional mediator of Wnt signaling [45]. It is, therefore, likely that TAZ links BMP2 signals to the Wnt pathway, which is important for bone formation [46].

Osterix (*Sp7*), a BMP2 target gene during osteoblast differentiation, plays a critical role in bone formation and osteoblastogenesis [47].