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REVIEW ARTICLE

Regulation of osteoblast differentiation mediated by BMP, Notch, and CCN3/NOV

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Summary Osteoblasts originate from common progenitors, which are capable of differentiating into other mesenchymal cell lineages such as chondrocytes, myoblasts and adipocytes. Various hormones and cytokines regulate osteoblast differentiation of mesenchymal progenitors to osteoblasts. Among these, bone morphogenetic proteins (BMPs) are the most potent inducers and stimulators of osteoblast differentiation: BMPs not only stimulate osteoprogenitors to differentiate into mature osteoblasts but also induce non-osteogenic cells to differentiate into osteoblast lineage cells. BMPs are important local factors that regulate Runx2, which is an essential transcription factor for osteoblast differentiation. The Notch signaling pathway is involved in a variety of cellular function, including cell proliferation, differentiation and apoptosis. Notch signaling has a dual effect on osteoblast differentiation. In terms of stimulation, functional Notch signaling is essential not only for BMP-2-induced osteoblast differentiation but also for BMP signaling itself. CCN3/NOV, a member of the CCN family of proteins, exerts inhibitory effects on BMP-2-induced osteoblast differentiation via its involvement in the BMP and Notch signaling pathways. Thus, osteoblast differentiation is critically regulated by the intimate interaction of various signaling molecules including BMP, Notch and CCN3/NOV.

Contents

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1. Introduction

Skeletal tissue is composed of various types of mesenchymal cells such as osteoblasts, chondrocytes, myocytes, and adipocytes. These cells originate from common pluripotent progenitors called mesenchymal stem cells [1]. During the differentiation of these cell lineages, various hormones and cytokines critically regulate osteoblast differentiation. Among these, bone morphogenetic proteins (BMPs) are strong inducers of osteoblast differentiation and bone formation [2—4]. BMPs were originally identified as proteins that induced ectopic bone formation when implanted into muscular tissue [5]. Several lines of evidence have demonstrated that BMP-2 induces or promotes the expression of runt-related gene 2 [Runx2, alternatively called core-binding factor alpha 1 (Cbfa1)] [6—8] and osterix [9,10], which are essential transcription factors for osteoblast differentiation and bone formation [11—13], as well as osteoblast differentiation markers such as alkaline phosphatase (ALP), type I collagen and osteocalcin in various cells. Thus, BMPs are key molecules in the regulation of osteoblast differentiation.

The Notch signaling pathway is highly conserved among species and plays a fundamental role in a wide variety of processes during embryonic development and in the adult [14]. The *Notch* gene encodes a single transmembrane receptor member. The ligands for Notch including Delta1, Delta3, Delta4, Jagged1, and Jagged2 are also transmembrane proteins, and Notch signaling mediates cell—cell communication by regulating the choice of binary cell fate and the maintenance of stem cell populations [15]. In addition, it has been revealed that cross-talk between Notch and BMP signaling tightly regulates the cell fate in various types of cell lineages [16—19]. Although several studies using osteoblastic cell lines have demonstrated that Notch signaling is involved in the regulatory mechanism underlining osteoblast differentiation, these remain controversial [19—23].

The CCN family of proteins comprise the following six members; CCN1/cysteine-rich 61(CYR61), CCN2/connective tissue growth factor (CTGF), CCN3/nephroblastoma overexpressed gene (NOV), CCN4/Wnt-induced secreted protein 1 (WISP1), CCN5/WISP2, and CCN6/WISP3. These proteins have been demonstrated to possess a growth factor-like activity and to regulate cell growth and tissue formation. It has been reported that CCN proteins regulate the differentiation of skeletal mesenchymal cells such as muscle cells [24,25], chondrocytes [26,27] and osteoblasts [28—31]. Importantly, CTGF/CCN2 binds to BMP-4 and antagonizes its action [32]. We have also demonstrated that CCN3/NOV associates with Notch1, and that it inhibits myogenic differentiation [24]. Since these reports highlight the importance of CCN proteins in osteoblast differentiation, we investigated the role of CCN3/NOV in osteoblast differentiation [31]. Our study indicates that CCN3/NOV regulates osteoblast differentiation by interacting with BMP and Notch signalings.

Thus, osteoblast differentiation is regulated by multiple factors that interacting with BMPs. In this review, we will describe the roles of BMP, Notch and CCN3/NOV in osteoblast differentiation.

2. The role of BMPs in osteoblast differentiation

2.1. BMP signaling pathway

BMPs bind to two types of serine—threonine receptor, termed BMP type I receptor (BMPR-I) and type II receptors [33]. Both types of receptor are necessary for the transduction of BMP signals. Four BMPR-I receptors [(TRS (ALK1), ACVR1 (ALK2), BMPR-IA, and BMPR-IB)] have previously been cloned. Three type II receptors (BMPR-II, ActR-IIA, and ActR-IIB) are involved in the BMP signaling pathway. BMPR-I binds BMPs directly in the absence of type II receptors. This is in contrast to the transforming growth factor-beta (TGF-β) type I receptor that does not bind ligands in the absence of the TGF-β type II receptor [34,35]. BMPs preferentially bind at the N-terminal extracellular domain of BMPR-I, which is connected by a single transmembrane region to the C-terminal cytoplasmic kinase domain. BMPR-I has a cytoplasmic juxtamembrane region that is rich in glycine and serine residues (GS domain). Ligand-binding phosphorylates serines and threonines in the GS domain thereby activating BMPR-II; this event transmits downstream BMP signals through BMP-specific Smads and p38 MAPK to regulate BMP-responding genes.

A number of signal-transducing molecules of the TGF-β superfamily, termed Smads, have previously been identified (Fig. 1) [33,36,37]. To date, eight mammalian Smad pro-