Journal of Oral Biosciences 57 (2015) 179–184

Contents lists available at ScienceDirect

Journal of Oral Biosciences

journal homepage: www.elsevier.com/locate/job

Review

Inhibition of bone morphogenetic protein-induced osteoblast differentiation

Shoichiro Kokabu [a,b,*], Shizu Tsuchiya-Hirata [c], Hidefumi Fukushima [d], Goro Sugiyama [a], Jonathan W Lowery [e], Takenobu Katagiri [f], Eijiro Jimi [a,g]

[a] Division of Molecular Signaling and Biochemistry, Department of Health Promotion, Kyushu Dental University, 2-6-1 Manazuru, Kokurakita-ku, Kitakyushu, Fukuoka 803-8580, Japan
[b] Department of Oral and Maxillofacial Surgery, Faculty of Medicine, Saitama Medical University, 38 Morohongo, Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan
[c] Division of Endodontics and Restorative Dentistry, Department of Oral Function, Kyushu Dental University, 2-6-1 Manazuru, Kokurakita-ku, Kitakyushu, Fukuoka 803-8580, Japan
[d] Department of Physiological Science and Molecular Biology, Fukuoka Dental College, 2-15-1 Tamura, Sawara-ku, Fukuoka 814-0193, Japan
[e] Department of Biomedical Science, College of Osteopathic Medicine, Marian University, 3200 Cold Spring Road, Indianapolis, IN, USA
[f] Division of Pathophysiology, Research Center for Genomic Medicine, Saitama Medical University, 1397-1 Yamane, Hidaka-shi, Saitama 350-1241, Japan
[g] Center for Oral Biological Research, Kyushu Dental University, 2-6-1 Manazuru, Kokurakita-ku, Kitakyushu, Fukuoka 803-8580, Japan

ARTICLE INFO

Article history:
Received 13 April 2015
Received in revised form
12 May 2015
Accepted 13 May 2015
Available online 7 July 2015

Keywords:
Bone regenerative medicine
Osteoporosis
Adipogenesis
Transcriptional cofactor
Inflammation

ABSTRACT

Background: Bone morphogenetic proteins (BMPs) induce ectopic bone formation *in vivo* and osteoblast differentiation of various cells *in vitro*. Therefore, BMPs are thought to be useful in bone regeneration medicine and for treating bone-related diseases. However, clinical application of BMPs is not widespread.
Highlight: BMP signal transduction and BMP-induced osteoblast differentiation are negatively regulated at several steps. BMP-3 acts as an antagonist to activin receptor type 2B and suppresses osteoblast differentiation of bone marrow stromal cells (BMSCs). Targeted disruption of *Bmp-3* in mice increases trabecular bone formation and bone mass. A selective inhibitor of classical NF-κB pathway enhances BMP-2-induced ectopic bone formation *in vivo*. NF-κB inhibits BMP-induced osteoblast differentiation by directly targeting SMAD proteins. p65, the main subunit of NF-κB, interacts with SMAD4 and interferes with the DNA binding of SMAD complex, thus suppressing BMP-induced osteoblast differentiation. Transducin-like enhancer of split 3 (TLE3), a member of Groucho/TLE family, represses the transactivation of RUNX2, one of the master regulators of osteoblast differentiation, thus suppressing BMP-induced osteoblast differentiation of BMSCs.
Conclusion: In addition to BMP-3, NF-κB, and TLE3, numerous inhibitors suppress BMP-induced osteoblast differentiation. Therefore, a precise understanding of mechanisms underlying the inhibition of osteoblast differentiation may help develop novel methods for treating bone-related diseases or for the tissue engineering of the bone.

Contents

* Corresponding author at: Division of Molecular Signaling and Biochemistry, Department of Health Promotion, Kyushu Dental University, 2-6-1 Manazuru, Kokurakita-ku, Kitakyushu, Fukuoka 803-8580, Japan. Tel.: +81 93 285 3048.
E-mail address: r14kokabu@fa.kyu-dent.ac.jp (S. Kokabu).

http://dx.doi.org/10.1016/j.job.2015.05.005
1349-0079/

1. Introduction

Bone morphogenetic proteins (BMPs) induce ectopic bone formation when implanted into the muscle tissue and stimulate osteoblast differentiation of various cell types [1,2]. BMPs are the most thoroughly studied bone regeneration molecules and have received United States Food and Drug Administration (FDA) approval for application in bone regeneration in humans. However, clinical use of BMPs is limited by difficulties associated with their optimal delivery, incomplete identification of target cells involved in bone regeneration, and lack of understanding of systems that modulate BMP signaling or osteoblast differentiation.

BMP signaling is transduced by 2 types of transmembrane serine/threonine kinase receptors, namely, type I and II receptors [3,4]. To date, 3 type I BMP receptors have been identified. Of these, ALK3 (BMPRIA) only binds to BMPs, ALK6 (BMPRIB) binds to BMPs and anti-Mullerian hormone, and ALK2 (ACVR1) binds to BMPs and activins. Among type II BMP receptors, BMPRII recognizes only BMPs while activin receptor type 2A and 2B (ACVR2A and ACVR2B, respectively) recognize both BMPs and activins [5]. A BMP-bound type II receptor phosphorylates a type I receptor, which in turn phosphorylates downstream substrates such as receptor-regulated SMADs (R-SMADs), including SMAD1, SMAD5, and SMAD8 (SMAD9) and mitogen-activated protein kinases such as ERK, JNK, and p38 [2]. SMAD signaling plays a central role among the downstream effectors of BMP receptors and plays an important role in osteoblast differentiation [6]. R-SMADs are phosphorylated by BMP receptors at 2 serine residues in Ser-X-Ser (SXS) motif at the C terminus [7–9]. Phosphorylated R-SMADs form heteromeric complexes with SMAD4 and directly activate the transcription of BMP-responsive genes such as *ID1*, *ID2*, and *ID3* (Fig. 1) [10–13]. Both Osterix and RUNX2 are essential factors for osteoblast differentiation, and their expression is activated by BMP–SMAD signaling [14,15].

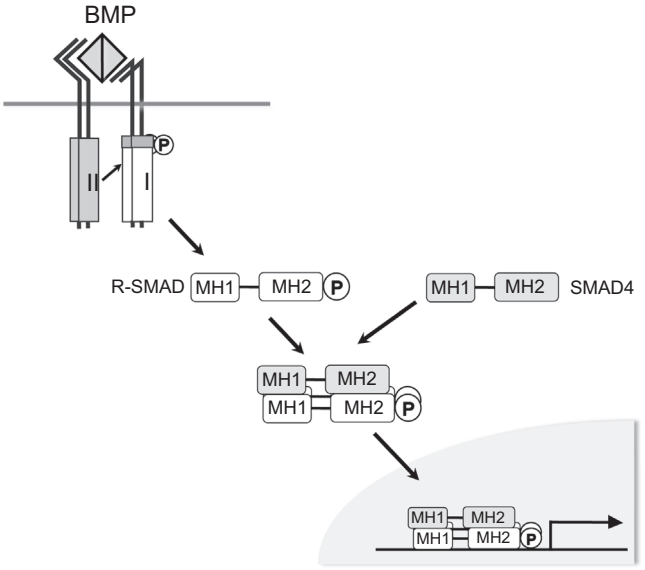

Fig. 1. BMP–SMAD signal transduction. BMPs bind to BMP type I and II receptors expressed on the surface of target cells. The type II receptors activate the type I receptor by phosphorylation. The activated type I receptor kinase phosphorylates 2 serine residues at the C-termini of SMAD1, SMAD5, and SMAD8 (SMAD1/5/8). The phosphorylated SMAD1/5/8 form heteromeric complexes with SMAD4 and translocate to the nucleus to regulate the transcription of target genes.

BMP signaling and BMP-induced osteoblast differentiation are negatively regulated at each step by various factors. In this review, we will focus on BMP signaling and its inhibitory mechanisms during osteoblast differentiation. In addition, we will discuss strategies for treating bone-related diseases such as osteoporosis as well as for the tissue engineering of the bone by using BMPs.

2. Extracellular factors

Several secreted, extracellular proteins bind to BMPs and prevent their binding to specific receptors. Noggin, chordin, follistatin, gremlin, and proteins belonging to DAN family acts as BMP antagonists in various animal species [3,16]. Expression of some of these antagonists such as noggin and gremlin is upregulated by BMPs, suggesting that these antagonists are a part of a negative feedback loop [17,18].

BMP-3 is the most abundant BMP in the bone matrix and is mainly secreted by osteoblasts and osteocytes [19,20]. When BMP-3 was originally purified and cloned in 1988, it was thought to have osteoinductive activity similar to BMP-2 and BMP-4 [21]. However, adult mice lacking BMP-3 have increased bone mass while mice having increased levels of skeletal BMP-3 show delayed endochondral ossification with spontaneous rib fractures [19,22]. Experiments on *in vitro* cultures of primary bone marrow stromal cells (BMSCs) have shown that overexpression of BMP-3 suppresses osteoblast differentiation while loss of BMP-3 increases the colony-forming units of fibroblasts and osteoblasts. These results indicate that BMP-3 acts as an antagonist to osteoinductive BMPs. This ability of BMP-3 to affect osteoblast differentiation is due to its interaction with ACVR2B because knockdown of endogenous *Acvr2b* in BMSCs reduces the suppressive effect of BMP-3 on osteoblast differentiation (Fig. 2) [20]. These findings are consistent with the receptor usage of BMP-3. BMP-3 cannot bind to BMPRII or ACVR2A because of structural constraints [23,24] and only interacts with ACVR2B among BMP type II receptors. Biochemical studies indicate that BMP-3 antagonizes osteoinductive BMPs by acting as a decoy ligand. Moreover, BMP-3 cannot act as a signaling molecule after binding to ACVR2B [23]. Thus, the name “BMP-3” is historical rather than based on function.

3. Intracellular factors

SMAD6 and SMAD7 inhibit the kinase activity of type I receptors by directly interacting with them in the cytoplasm [25,26]. SMAD ubiquitination regulatory factor 1 (SMURF1), a member of HECT family of E3 ubiquitin ligases, interacts with SMAD1 and SMAD5 through PPAY motif in their linker regions, thus triggering their ubiquitination and degradation [27]. SMURF2, which is also a HECT family protein, preferentially targets SMAD1 and induces its ubiquitination and proteasome-mediated degradation [28].

Different phosphatases, such as small C-terminal domain phosphatase 1 (SCP1) and protein phosphatase magnesium-dependent 1A (PPM1A), suppress BMP activity by dephosphorylating the C-terminal SXS motif of SMADs [29–31]. To determine