HOSTED BY
ELSEVIER
CrossMark
Available online at www.sciencedirect.com
ScienceDirect
Food Science and Human Wellness 3 (2014) 127–135
Food Science and Human Wellness
www.elsevier.com/locate/fshw

Bone morphogenetic proteins: Relationship between molecular structure and their osteogenic activity

Jian Yang, Pujie Shi, Maolin Tu, Yun Wang, Meng Liu, Fengjiao Fan, Ming Du *

School of Food Science and Engineering, Harbin Institute of Technology, Harbin 150090, PR China

Received 5 December 2014; accepted 26 December 2014

Abstract

Bone morphogenetic proteins (BMPs) are a family of potent, multifunctional growth factors belonging to transforming growth factor-β (TGF-β). They are highly conservative in structures. Over 20 members of BMPs with varying functions such as embryogenesis, skeletal formation, hematopoiesis and neurogenesis have been identified in human body. BMPs are unique growth factors that can induce the formation of bone tissue individually. BMPs can induce the differentiation of bone marrow mesenchymal stem cells into osteoblastic lineage and promote the proliferation of osteoblasts and chondrocytes. BMPs stimulate the target cells by specific membrane-bound receptors and signal transduced through mothers against decapentaplegic (Smads) and mitogen activated protein kinase (MAPK) pathways. It has been demonstrated that BMP-2, BMP-4, BMP-6, BMP-7, and BMP-9 play an important role in bone formation. This article focuses on the molecular characterization of BMPs family members, mechanism of osteogenesis promotion, related signal pathways of osteogenic function, relationships between structure and osteogenetic activity, and the interactions among family members at bone formation.

Keywords: Bone morphogenetic proteins; Osteogenic activity; Signal pathways; Structure; Interaction

It is estimated that there are 200 million patients with osteoporosis in the world, and that over 10 billion dollars have been expended on the medicines used for therapeutic treatment of osteoporosis. Milk has long been regarded as a good stimulator for bone growth and development. In addition to containing rich calcium (Ca^{2+}), other functional components and multiple biologically active polypeptides from bovine milk protein also play important roles. In 1985, Snow Brand Milk Products Co., Ltd. in Japan firstly reported the role of milk basic proteins in prevention of bone loss. Since then, a growing number of fundamental researches on the relationships between the bioactive milk proteins and osteoporosis have increasingly drawn widespread attentions.

Bone morphogenetic proteins (BMPs) are a group of bioactive proteins that were firstly isolated from the extract of bone matrix by Urist in 1965 [1]. Cell proliferation, differentiation and matrix biosynthesis during reconstruction process of human bone are coordinately regulated by multiple growth factors, among which, BMPs, as the important inducing factors during embryonic development, are the most closely related to osteoinduction. As of to date, BMPs are a group of the most well studied functional proteins that can be used to postpone or therapeutic treatment of osteoporosis, which have provided important references for a large number of researchers to explore the similar mechanisms of bioactive proteins from the milk.

1. General outline of BMPs family

1.1. Types and structural characteristics

To date, over 20 BMPs family members have been recognized. BMP-1, which is procollagen C proteinase containing 730 amino acid residues with rich cysteine residue, is a regulatory factor for bone growth and belongs to the family of metalloproteinases. The remaining BMPs belong to the multifunctional growth factors, transforming growth factor (TGF-β) superfamily

* Corresponding author at: School of Food Science and Engineering, Harbin Institute of Technology, Harbin 150090, PR China. Tel.: +86 15945192686; fax: +86 451 86282906.
E-mail address: duming121@163.com (M. Du).
Peer review under responsibility of Beijing Academy of Food Sciences.

http://dx.doi.org/10.1016/j.fshw.2014.12.002

[2]. TGF-β superfamily mainly includes four sub-families [3], *i.e.* TGF-β subfamily, BMPs subfamily, growth and differentiation factors (GDFs) subfamily, and activins/inhibins subfamily. Based on the sequence homology and the known functions, BMP family members are generally classified into four categories: BMP-2/4, BMP-5/6/7/8a/8b, BMP-9/10 and BMP-12/13/14 [4].

The precursors of bone morphogenesis proteins, which contain 400–500 amino acid residues, are consisted of three parts, *i.e.* the amino-terminal secretory signal peptide, the correctly folding pro-domain and the carboxyl terminal (contains 100–125 amino acid residues) [5]. Arg-X-X-Arg sequence motif in the pro-domain is hydrolyzed by serine proteinase to form the carboxyl terminal mature protein [6]. The structures of BMP family members are highly conserved; the C-terminal of a large majority of the BMP family members contains seven cysteine residues, among which, cysteine residues form intra-chain disulfide bonds, the other cysteine residue is involved in the formation of a disulfide bond with cysteine residue in other polypeptide chain, thus, linking two protein monomers into dimers with biological activity. This typical local inter-polypeptide disulfide bond is known as cysteine knot, which forms the core of the monomer [7]. Because BMP-3 and BMP-15 do not contain the seventh cysteine residue, their monomers have the biological activity. Except for them, all the other BMP family members are capable of forming either homodimers or heterodimers. The heterodimers of BMP-2/5, BMP-2/6, BMP-2/7 and BMP-4/7 have been found both *in vitro* and *in vivo* and they are all the activated factors playing more effective roles in signal pathway than their corresponding homodimers do [8,9].

1.2. Biological activities of BMPs

With the development of gene engineering technologies, the biological activities of a part of the BMPs family members have been known and confirmed. During the embryonic development, BMPs are involved in the regulation of neurogenesis and erythropoiesis and induction of metamerization. After birth, BMPs play important roles in maintaining bone mass and can induce the directional differentiation of mesenchymal stem cells (MSCs) into osteoblasts and thus, increase the number of mature osteoblasts and enhance the differentiation capability of osteoblasts. BMPs confer their effects on osteoblasts through activating the membrane receptors of BMPs and their signal pathways [5]. Clinical studies have indicated that BMPs have functions of stimulating bone fracture healing, controlling the formation of different morphological characteristics of mammal bone system [10]. It has been confirmed that the biological effects of various BMPs are different. Among over 20 BMPs family members, BMP-2, BMP-4, BMP-6, and BMP-9 play more important roles in ossification, of which, the effects of BMP-9 on ossification are the strongest, followed by those of BMP-2 and BMP-7 [11]. Binding of BMPs to their corresponding receptors is needed for playing their biological roles. The receptors for BMPs are serine/threonine kinase receptors, which are consisted of extracellular region containing 10–12 cysteine reissues, a trans-membrane domain and a serine/threonine kinase cytosolic domain. BMPs receptors are divided into two subfamilies, type-I and type-II. There are five type-I BMP receptors including ALK1(Acvrl1), ALK2(ActRI), ALK3(BRIa), ALK4(ActrIb) and ALK6(BRIb); there are three type-II BMP receptors including BRII, ActRIIa and ActRIIb [7].

1.3. Signal transduction pathways for BMP-induced osteoinductive differentiation

BMPs signal pathways include two regulatory pathways: the classical Smads-dependent pathways and the non-classical Smads-independent mitogen activated protein kinase (MAPK) pathway.

The BMPs signal transduction is mainly mediated *via* the classical BMPs–Receptor–Smads signal pathway [12]. Smads can be divided into three categories: the first category can activate the receptors of BMPs to regulate Smads (R-Smads), including Smad-1, Smad-5 and Smad-8; the second category is co-mediator Smads (Co-Smads), *i.e.* Smad-4; the third category is the inhibitory Smads (I-Smads) including Smad-6 and Smad-7. Fig. 1 shows BMPs firstly bind to the pre-formed type-I and type-II receptor complexes, then type-II receptor is activated and subsequently phosphalyzates the GS-domain of type-I receptor. Afterwards the phosphorylated type-I receptor recognizes the corresponding R-Smads within the cytoplasm and phosphorylates it. The phosphorylated R-Smads, in turn, form complex with Smad-4 and transport into nucleic where it cooperates with other transcriptional factor and accessory factors to direct the transcription of the target genes and to fulfill their roles in differentiation of osteoblasts [13,14].

BMPs bind with either type-I or type-II receptors to form homo-complex or hetero-complex, that can activate BMPs–MAPKs signal pathway, and in turn can transduce the signal into nuclei *via* JNK-1 and 2/3, ERK1/2, NF-B and p38 signal pathways where they regulate the expression of target genes and play their biological roles. This signal transduction pathway plays important role in BMP-induced osteogenesis [7]. Gallea et al. [15] found that MAPK (Erk1/2) and p38 signal pathways play decisive roles in the induction of differentiation of C2C12 cells into osteoblasts.

2. Molecular characteristics of BMPs family members and their roles in osteogenesis

It has been demonstrated that BMP-2, BMP-6 and BMP-9 are the most potent BMP proteins involved in the induction of the differentiation of osteogenic stem cells into osteoblasts. BMP-9 acts during the entire process of the induced differentiation (Fig. 2) [16].

2.1. BMP-2

The mature human BMP-2 is an acidic glycoprotein with molecular weight of approximately 32 kDa. It contains 114 amino acid residues, is rich in glutamate and can be dissolved in neutral salt solution with an isoelectric point between 4.8 and 5.1. There is certain inter-species homology of BMP-2 among bovine, rabbit, mice and human. In 1999, Scheufler et al. [17]