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Review

Bone Morphogenetic Proteins: Structure, biological function and therapeutic applications

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ABSTRACT

Bone Morphogenetic Proteins (BMPs) are multifunctional secreted cytokines, which belong to the TGF-β superfamily. These glycoproteins act as a disulfide-linked homo- or heterodimers, being potent regulators of bone and cartilage formation and repair, cell proliferation during embryonic development and bone homeostasis in the adult. BMPs are promising molecules for tissue engineering and bone therapy. The present review discusses this family of proteins, their structure and biological function, their therapeutic applications and drawbacks, their effects on mesenchymal stem cells differentiation, and the cell signaling pathways involved in this process.

Introduction

The mechanism of bone tissue mineralization is complex event, involving pre-osteoblastic cells, physico-chemical events and a framework constituted by molecules present in the organic matrix [1,2]. Matrix proteins also play an important role in this process, as regulatory and/or nucleating factors in the deposition of hydroxyapatite crystals, formed by calcium and phosphate ions present in plasma and extracellular fluids, in the space between the collagen molecules [3]. Reparative regeneration occurs when tissues are lost due to injuries or diseases, therefore, bone defect repair constitutes an adequate model to study bone regeneration. Unlike fractures, bone defects are less prone to mechanical factors and vascular system obstruction. Johner et al. [4] evaluated the regeneration of bone defects of rabbit tibia, observing that bone formation started within a few days without prior osteoclastic bone resorption, with this regeneration being dependent not only on the size of the defect, but, also, on the activity of pre-osteoblastic cells, and mechanisms which regulate their proliferation, differentiation and function.

In fact, it is widely known that different growth factors act locally to modulate bone formation by stimulating pre-osteoblasts proliferation and activity [5]. A number of bone-derived growth factors have been isolated and characterized from bone matrix, such as Bone Morphogenetic Proteins (BMPs),[1] which display mitogenic, differentiating, chemotactic, and osteolytic activities, allowing these molecules to act as potential determinants of local bone formation. BMPs are potent mediators of cell proliferation and mesenchymal stem cells (MSCs) differentiation, which have been shown to be essential molecules involved in bone repair. In this

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[1] *Abbreviations used:* BMPs, Bone Morphogenetic Proteins; MSC, mesenchymal stem cells; IGFs, Insulin-like Growth Factors; PDGF, platelet-derived growth factor; FGFs, Fibroblast Growth Factors; EGF, Epidermal Growth Factor; TGF-β, Transforming Growth Factor Beta; MIS, Mullerian Inhibiting Substance; GDFS, Growth and Differentiation Factors; Cer1, Cerberus; SOST, Sclerostin; Tsg, Twisted gastrulation; BMPR-IA, type IA BMP receptor; BMPR-IB, type IB BMP receptor; MAPK, mitogen activated protein kinase; PK A, Protein Kinase A; SHH, sonic hedgehog; BMPRs, BMP receptors; bFGF basic fibroblast growth factor; GM-CSF, granulocyte-macrophage colony stimulating factor; BEVS, baculovirus/insect cells system; iPSCs, induced pluripotent stem cells.

review, we discuss BMPs structural and functional aspects, their signaling pathways, their involvement in the differentiation of MSCs into osteoblasts and their possible rople as therapeutic agents.

Growth factors related to bone formation and repair

Osteogenesis involves migration and mitosis of mesenchymal stem cells (MSCs), as well as their differentiation into osteoprogenitor cells, and their differentiation and maturation into osteocytes (Fig. 1). The growth factors involved in the biological events of bone and other connective tissues formation and repair may be grouped, according to their biological activities, into: Insulin-like Growth Factors (IGFs), platelet-derived growth factor (PDGF), Fibroblast Growth Factors (FGFs), Epidermal Growth Factor (EGF) and proteins from the Transforming Growth Factor Beta (TGF-β) superfamily (Table 1) [6,7].

The TGF-β family includes activins, nodal proteins, Mullerian Inhibiting Substance (MIS) and other Growth and Differentiation Factors (GDFS), while BMPs represent the largest subgroup of this family [8]. After identifying the role of BMPs in the initiation of endochondral ossification [9,10], its role in embryonic development and cellular function has been extensively studied, with about 20 BMP family members being characterized, highlighting the role of this group as one of the main growth factors related to bone repair [11].

Bone Morphogenetic Proteins (BMPs)

In 1965, Urist demonstrated that demineralized, lyophilized segments of bone were capable of inducing new bone formation when implanted into ectopic sites, namely, rabbit muscle pouches [12]. In 1971, proposing the name "Bone Morphogenetic Protein" [13]. In 1972, Reddi and Huggins showed that demineralized bone matrix is also capable of inducing bone formation in ectopic sites [14]. Since then, several BMPs were isolated, with these low molecular weight bone glycoproteins being proven to be the responsible for promotion of this ectopic bone formation [15]. BMPs are synthesized by osteoprogenitor cells, osteoblasts, chondrocytes and platelets [16,17] but their production is not restricted to bone, since they also play an essential role in development cell functions.

These proteins play a critical role in the development of many cell types in various tissues, acting in cell proliferation and differentiation, tooth morphogenesis, organogenesis, embryonic development, apoptosis, chemotaxis and repair of a wide variety of tissues [18,19], in addition to glucose homeostasis and modulation of iron homeostasis (Table 2). BMPs induce endochondral/intramembranous ossification and chondrogenesis, by inducing mesenchymal stem cells differentiation towards the osteoblastic lineage [20], being critical for maintenance of skeletal integrity and in bone fracture healing.

BMP classification and structure

To date, around 20 different human BMPs have been found and grouped into subfamilies, based on their sequence similarity and known functions, even though not all members are truly osteogenic (Table 2). Thus, BMP1 does not belong to the TGFβ superfamily, being a metalloprotease that cleaves the C-terminus of procollagen I, II and III and being capable of inducing cartilage formation *in vivo* [21].

TGF-β superfamily proteins are classified according to their protein sequence similarity in humans (Fig. 2) and other species. The BMP family may be divided into four subfamilies according to their amino acid sequence (Table 2 and Fig. 2): (a) BMP2 and 4 (80% homology); (b) BMP3, BMP3B (GDF10); (c) BMP5, 6, 7, 8a and 8b (78% homology); (d) GDF5, 6, 7 [22]. Curiously, BMP3 and BMP13 act either as a BMP negative regulator or as an inhibitor of bone formation, respectively [23].

BMPs are dimeric molecules, constituted by about 120 amino acids, including seven conserved cysteine residues, from which six are highly conserved, comprising a cysteine knot motif linked by three intramolecular disulfide bonds (Fig. 3). Another cysteine is involved in stabilization of the dimer through an intermolecular

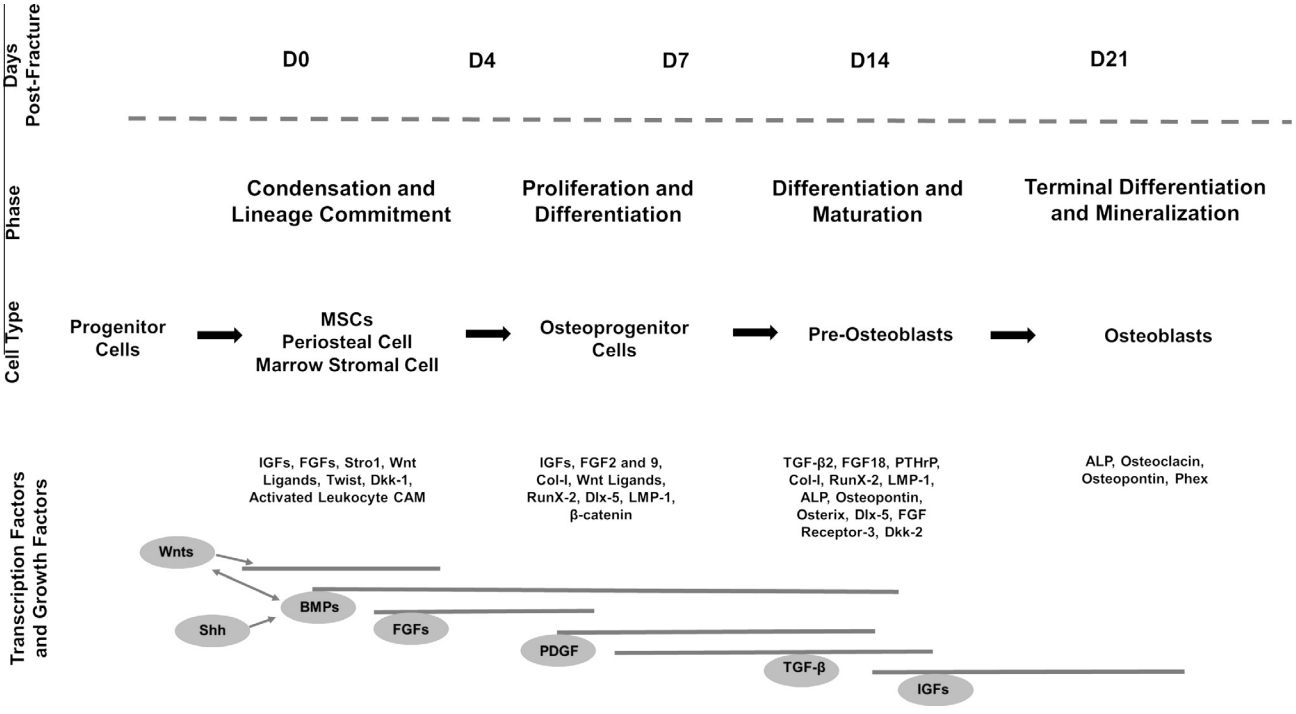

Fig. 1. Osteoblast differentiation. Temporal pattern of growth factors and transcription factors expression during post-fracture osteoblast differentiation.