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Bone morphogenetic proteins and skeletal development: the kidney-bone connection

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Abstract The bone morphogenetic proteins (BMPs) are a family of pleiotropic morphogens isolated and cloned from the demineralized extracellular matrix of bone. BMPs and related cartilage-derived morphogenetic proteins (CDMPs) initiate, promote and maintain bone and cartilage. The pleiotropic effects of BMPs are based on concentration-dependent thresholds. Targeted disruption of gene action by homologous recombination has demonstrated the role of BMP 7 in kidney, eye and skeletal development. BMP 7 is critical for kidney tubulogenesis, retinal pigmented epithelium differentiation and skeletal pattern. BMP 7 is also synthesized by the kidney and is detectable in serum; hence BMP 7 is both an autocrine and endocrine morphogen. It is likely renal BMP 7 may influence skeletal development and growth in children although there may be sources of other BMPs with skeletogenic actions. In conclusion, we are beginning to unravel the mysteries of kidney-bone connection with special reference to pediatric nephrology.

Key words Morphogens · Cytokines · Cartilage · BMP receptors

Introduction

The origin of the mammalian skeleton from three distinct lineages is well known to most students of developmental biology. The three sources of skeletal cells are the neural crest, the somite derived sclerotome and the lateral plate mesoderm giving rise to craniofacial, axial and appendicular skeleton respectively. The initiation and commitment of cartilage and bone is at the crux of the problem of developmental biology of skeleton. The axial and appendicular skeleton is predominantly formed by endochondral sequence, that is a temporary growth plate cartilage serves as a template for the more permanent bone and articular cartilage. The entire craniofacial skeleton is formed by the intramembranous sequence, which consists of the direct transformation of mesenchymal cells to bone. Morphogenesis of skeleton is the cumulative processes in continuum of pattern formation, body plan establishment, organogenesis and the mirror-image bilateral symmetry of skeletal structures of the appendicular skeleton and the medial location of axial skeletal structures such as spine. The primordial premier signals for cartilage and bone initiation and induction are bone morphogenetic proteins (BMPs). The downstream responses that are regulated by BMPs include, but are not limited to, a plethora of transcriptional activators and coactivators of both general and tissue-specific nature. This article focuses on BMPs, as a single protein (or gene) can initiate the entire cascade of bone formation.

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Bone morphogenetic proteins

It is now well known that demineralized bone matrix induces new cartilage differentiation and the cartilage is replaced by bone by the endochondral sequence [1, 2]. The sequential cascade is reminiscent of endochondral bone development in the embryo and includes mesenchymal progenitor migration by chemotaxis, condensation, cartilage matrix calcification, vascular invasion, bone formation and hematopoiesis. This sequence recapitulates limb morphogenesis in the limb bud [3, 4].

The bioactive morphogens in the demineralized extracellular bone matrix were dissociatively extracted and purified [5, 8]. A family of BMPs were identified, isolated and cloned. There are three distinct subfamilies including BMP 2 and 4; BMP 3 and BMP 3B; BMPs 5, 6, 7 and 8 (Table 1). The BMPs are related to members of the TGFβ superfamily. The TGFβ superfamily includes

Table 1 Bone and cartilage morphogenetic proteins

Morphogen	Other names	Potential function
Bone morphogenetic proteins		
BMP 2	BMP 2A	Cartilage and bone morphogenesis/heart
BMP 4	BMP 2B cartilage and bone morphogenesis	
BMP 3	Osteogenesis bone formation/brain	
BMP 3B	GDF-10	Craniofacial bones
BMP 5	–	Bone morphogen
BMP 6	–	Hypertrophy of cartilage/skin
BMP 7	Osteogenic protein 1	Bone differentiation, eye and kidney development
BMP 8	Osteogenic protein 2	Bone formation
BMP 9	–	??
BMP 10	–	??
BMP 11	GDF-11	??
Cartilage-derived Morphogenetic proteins (CDMPs)	Growth/differentiation factor (GDF)	
CDMP-1	GDF-5	Mesenchymal condensation, chondrogenesis
CDMP-2	GDF-6	Cartilage development and hypertrophy
CDMP-3	GDF-7	Ligament and tendon development

activins, inhibins, Mullerian duct inhibitory substance (MIS), nodal, glial-derived neurotrophic factor (GDNF) and growth/differentiation factors (GDFs) [8, 9, 10]. The BMP superfamily members are synthesized as larger precursors with hydrophobic signal sequence, a conserved carboxy-terminal domain with canonical 7-cysteines of which one cysteine is involved in an intermolecular disulfide linkage per dimer. There are three intramolecular disulfide bonds in each monomer. The dimeric confirmation is critical for biological function.

Cartilage-derived morphogenetic proteins

The isolation and cloning of a family of bone morphogenetic proteins (Table 1) from bone prompted us to search for cartilage morphogenetic proteins from articular cartilage. We undertook a systematic study to examine for the presence of chondrogenic proteins from bovine epiphyseal and articular cartilage. Bovine articular cartilage slices were extracted in buffered 1.2 M guanidine hydrochloride. The extract was exchanged with 6.0 M urea in 0.05 M TRIS-HCl, pH 7.4, and further purified by heparin affinity chromatography and preparative gel electrophoresis. An active cartilage morphogenetic protein activity was identified based on a reconstitution with insoluble collagenous matrix. A simultaneous complementary approach using reverse transcription-polymerase chain reaction (RT-PCR) was used. Degenerate oligonucleotide primers were used to examine for BMP-like molecules. Two novel cartilage-derived morphogenetic proteins (CDMPs), CDMP-1 and CDMP-2, were cloned. The bovine CDMP-1 sequence was used to get a full-length human CDMP-1. A 3-kb transcript of CDMP-1 was expressed in articular cartilage [11]. The CDMP-2 was found in articular cartilage, skeletal muscle and placenta. It is of interest that in situ hybridization using CDMP-1 antisense RNA revealed that sites of mesenchymal condensation were strongly positive. CDMP-2 was localized in the hypertrophic chondrocytes of the epiphyseal growth plate. The elegant work of Lee and colleagues [12] identified expression of GDF-5, also called CDMP-1. In brachypodism in mouse there is a mutation in the GDF-5 (CDMP-1) gene as demonstrated by Kingsley and coworkers [12].

CDMP-1 (GDF-5) stimulates chondrogenesis both in vitro and in vivo [13, 14]. CDMP-1 and CDMP-2 stimulated synthesis of aggrecan, the aggregating proteoglycans. The CDMPs preferentially stimulated chondrogenesis. On the other hand, CDMPs were not as active as BMP-7 in the expression of alkaline phosphatase activity of MC 3T3-E1 osteoblastic cell line and ROB-C26 osteoprogenitor cells. In humans, patients with Hunter-Thompson chondrodysplasia exhibit mutations in CDMP-1 gene locus. Recent work has demonstrated the potential role of GDF-7 in tendon and ligament morphogenesis [15]. Thus the CDMPs/GDFs may be critical in joint morphogenesis.

During limb morphogenesis there is an intricate dynamic reciprocal interaction between ectodermally derived apical ectodermal ridge and mesodermally derived mesenchyme. BMP-2 is expressed in the mouse limb bud [16]. BMP 3 (osteogenin) and BMP 4 stimulate chondrogenesis in vitro by the pre-chondrogenic mesodermal cells of the chick limb bud [17–19]. Very recently we have demonstrated chondrogenesis by recombinant BMP-3 in vivo in rats (unpublished observations). BMP-7 is localized in perichondrium and chondrocytes in the early phases of cartilage morphogenesis in humans [20].

The cartilage pattern formation in the limb bud progressively culminates in the epiphyseal growth plate at the two poles of the bone shaft. The presumptive articular cartilage and the underlying subchondral bone formation occur in the secondary centers of ossification. The growth plate is the organizer center of the longitudinal growth of the skeleton and the spatial and temporal transitions are not well understood [21, 22]. There is a programmed chondrocyte hypertrophy and apoptosis [23]. The hypertrophy of cartilage has received considerable attention [24–26]. An elegant pellet culture model was developed

by Kato [27] to study the terminal hypertrophy of chondrocytes. In chemically defined serum-free media, cartilage hypertrophy is a default pathway [26] as it can proceed in the absence of any added growth or morphogenetic proteins. At optimal concentrations of serum (0.1–0.01%), but not at 1–10%, a profound morphogenesis of growth plate-like structure was observed in vitro. A systematic candidate molecule approach revealed that thyroxine is the critical serum factor in the regulation of columnar architecture morphogenesis in vitro. The hypertrophy is inhibited by TGFβ_1, TGFβ_2 and basic FGF [25].

The stability of the phenotype of articular chondrocytes is critically dependent on cell shape and cell density. The geometry of the cell culture is critical for chondrocyte gene expression [28]. In monolayer cell cultures of chondrocytes there is a progressive loss of cartilage phenotype with passage of time. This can be delayed or avoided by high-density micromass cultures or pellet cultures. Alternatively, use of explant cultures of articular cartilage permits the chondrocytes to be encased with their own extracellular matrix. Recombinant human BMP 4, activin and TGFβ can maintain the articular cartilage phenotype [29]. Very recently BMP-7, at concentrations of 30 and 100 ng/ml, was shown to maintain and stimulate the biosynthesis of sulfated proteoglycans [30]. The hydrodynamic size and composition of the glycosaminoglycan chains were identical in both BMP-7-treated and control explants. Thus BMPs may initiate chondrogenesis in vivo and maintain articular cartilage in vitro in chemically defined medium.

BMP and CDMP receptors

Recombinant human BMP 4 and BMP 7 bind to BMP receptor IA (BMPR-IA) and BMP receptor IB (BMPR-IB) [8, 31]. CDMP-1 also binds to both BMPR-IA and -IB. There is collaboration between type I and type II BMP receptors [8, 9] and they are both membrane-bound serine/threonine kinases. The BMP type II receptors phosphorylate BMP type I receptor. The phosphorylated BMP type I receptor in turn phosphorylates a signal-transducing acceptor protein Smad, a term derived from fusion of *Drosophila* Mad and nematode genes Sma 2, 3 and 4. There are eight different Smads. Phosphorylated Smads 1 and 5 are functional mediators of BMP signaling in partnership with Smad 4 [9]. Smads 6 and 7 are inhibitory to phosphorylation of Smads 1 and 5 catalyzed by BMP type I receptor. The transcription of BMP-response genes is initiated by the signaling complex of Smad 1 and Smad 4. BMPs and CDMPs may also regulate cell cycle progression. Cytoskeletal compartmentation of signaling complexes such as Smads may regulate the differentiation of chondroprogenitor cells into chondrocytes. The downstream targets of BMP and CDMPs are almost certainly homeobox genes. In vertebrates there are four clusters of homeobox genes: *a, b, c* and *d* [32]. There is a temporal colinearity during homeobox gene expression. There is considerable excitement about the presence of homeodomain-containing proteins in chondrocytes [33–35].

BMP antagonists

The termination of cartilage and bone morphogenetic protein actions may be modulated by other binding proteins and antagonists. Noggin is a BMP antagonist secreted by Spemann organizer, which was initially suspected as an inducer of neural tissues. Thus a specific "inducer" may terminate a dominant morphogen's (BMP) action and promote a default pathway of neural cell lineage. Recently when noggin gene was knocked out by homologous recombination, joint morphogenesis was profoundly impaired [36]. However, it is not clear whether absence of noggin influenced the survival, longevity, and apoptosis of chondrocytes. Additional experiments using conditional mutants generated by the cre-lox system with regulated tissue-specific noggin expression might alleviate the defects in joint morphogenesis in noggin null mice. There was no effect on homeobox gene Hoxd and Indian hedgehog expression, which are known to be involved in cartilage morphogenesis. Since cartilage is damaged in osteoarthritis and juvenile rheumatoid arthritis, the current progress in cartilage and bone morphogenetic proteins may aid in regeneration of articular cartilage. After all regeneration is a recapitulation of embryonic development and morphogenesis. The emerging discipline of tissue engineering strives to restore function to diseased or damaged tissues based on morphogens including bone morphogenetic proteins [9].

The kidney-bone connection

A powerful approach to determine the function of individual BMPs is by gene knockouts by homologous recombination. During the course of investigations on the knockout of BMP 7, results revealed that the epithelial-mesenchymal interactions during kidney tubulogenesis were disrupted. Thus BMP 7 is critical for kidney morphogenesis [37–39]. Kidney is furthermore a source of BMP 7 production. In view of this it is likely that BMP 7, a known bone morphogen, may regulate bone mass. Thus there is a potential possibility that, in renal osteodystrophy, BMP 7 could be involved in the pathogenesis of the disease, posing a challenge for pediatric nephrologists.

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