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Bone Morphogenetic Proteins: From Origin To Application

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Abstract

Introduction : Bone morphogenetic proteins (BMPs) are the family of ligands which belong to the transforming growth factor ß superfamily. BMPs are the inherent component of the extracellular matrix which are osteoinductive in nature.Apart from osteoinduction, they are responsible for differentiation, proliferation and apoptosis of various cell lineages, thereby playing a role in the homeostasis of vital organs like kidney, heart etc.[1]

Synonyms: Cartilage-derived morphogenetic proteins (CDMPs), Growth Differentiating Factors, Osteogenic proteins (OPs), Osteogenin, Vg-related (Vgr).

History:BMPs are known to induce bone formation in both vertebrates and in vertebrates. Their discovery can be dated back to 1889, in which Senn revealed that the aseptic bone cavities can be healed with the help of decalcified bone.[2] In 1930's, Levander proved that crude alcohol extracts of bone lead to the new bone formation when injected into muscle tissue. In 1961, Sharrard and Collins reported that the use of ethylene diamine tetra acetic acid (EDTA) in combination with decalcified allograft bone can be used for spinal fusion in children. [3]

In 1965, Marshall R. Urist reported that the implantation of demineralized bone matrix into the muscular tissues induces formation of cartilage and bone tissues with bone marrow in the injected site. The factor(s) responsible for this bone formation was named as "bone morphogenetic protein," as its activity was dissolved by digestion with trypsin, a natural protease.[4]

Furthermore, the identification of BMPs mode of action was revealed by Wang and colleagues through the isolation of BMP from extracts of bovine bone in the form of single gel band followed by sequencing the peptide chain obtained from the digestion of the band by trypsin. Later, Wozney et.al did a study and cloned the cDNAs for human BMP-1 through BMP-4 using the peptide sequence information obtained from wang et.al. [5]

With time, various scientists found the coding sequences of other BMPs were cloned based on amino acid homology sequence. (Celeste et al. 1990; Ozkaynak et al. 1990; Sampath et al. 1990).[6]

Breakthrough was the first human clinical trial for BMP which was conducted in 1992. In this study, the subjects had non-union fractures that did not heal for 9 months.

The devices used to demonstrate healing - BMP-7 protein embedded on a collagen matrix (Stryker), BMP-2 protein embedded in collagen (Genetics institute).

The final landmark is the FDA approval in 2002 for OP-1 /BMP-7 for long bone defects and BMP-2 in a collagen carrier within a cage for anterior lumbar inter-body fusions.

Classification of BMPs

A. According to wozney et.al in 1995:[7]

BMP-2 and BMP-4	BMP-5, BMP-6, BMP-7	BMP-3
80% homology	78% homology	significantly different from the other members

BMPs are divided into 3 groups

BMP- 2 and BMP-4 has similar seven-cysteine domains but varies in amino-terminal regions- share 92% of homology.BMP-5, BMP-6, BMP-7, BMP-8 share sequence homology. BMP-7 is OP-1osteoprotegrin 1 and BMP-8 is OP-2 osteoprotegrin 2.BMP-3 (osteogenin), differs from these two subgroups, form a different entity.

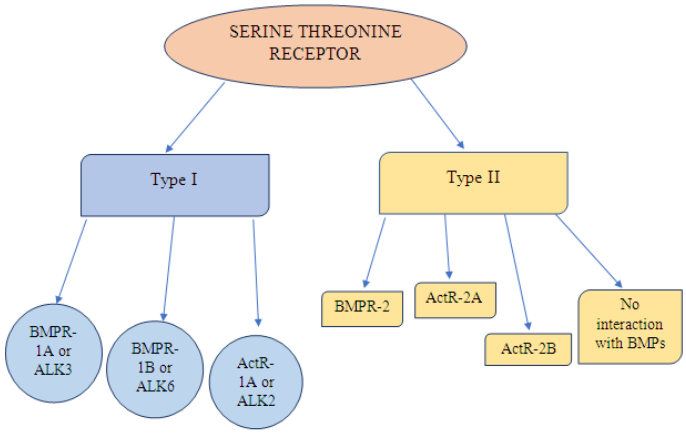
According to the peptide sequence

Till now about 20 BMPs have been identified. All of them belong to Transforming Growth Factor β superfamily except BMP1 which is a metalloproteinase.

Type Of Bmp	Action	Chromosome Linked
BMP-1	Not part of TGF-b family (metalloproteinase)	8
BMP-2	Osteoinductive, osteoblast differentiation, apoptosis	20
BMP-3	Most abundant BMP in bone, inhibits osteogenesis	4
BMP-4	Osteoinductive, lung & eye development	14
BMP-5	Chondrogenesis	6
BMP-6	Osteoblast differentiation, chondrogenesis	6
BMP-7 (Osteogenic Protein-1)	Osteoinductive, development of kidney & eye	20
BMP-8 (Osteogenic Protein-2)	Osteoinductive	
BMP-9	Nervous system, hepatic reticuloendothelial system	
BMP-10	Cardiac development	
BMP-11 (Growth/differentiation factor-8)	Neuronal Tissues	
BMP-12 (Growth/differentiation factor-7)	Tendon-iliac tissue formation	
BMP-13 (Growth/differentiation factor-6)	Tendon & ligament-like tissue formation	
BMP-14 (Growth/differentiation factor-5)	Enhances tendon healing & bone formation	
BMP- 15	Follicle-stimulating hormone activity	

Receptors of BMPS

BMPs, like other TGF-b family members, elicit their effects through two types of serine—threonine kinase transmembrane receptors, type I and type II receptors. Unlike TGF-beta, BMP are capable of binding to type I receptors I the absence of type II receptors. Both receptor types have a short extracellular domain, a single transmembrane domain, and an intracellular domain with serine/threonine kinase activity. 9There is a total of seven types of receptors for both type I and type II (as given in figure below).



Type I receptors (ALK1-7) for the TGF-b family of ligands, three of which bind BMPs: type 1A BMP receptor (BMPR-1A or ALK3), type 1B BMP receptor (BMPR-1B or ALK6), and type 1A activin receptor (ActR-1A or ALK2).

There is a total of four type II receptors for the TGF-b family, three of which are known to interact with BMPs: type 2 BMP receptor (BMPR-2), type 2 activin receptor (ActR-2A), and type 2B activin receptor (ActR-2B). While BMPR-1A, BMPR- 1B, and BMPR-2 are specific to BMPs, ActR-1A, ActR-2A, and ActR-2B can function as receptors for activins, which are also members of the TGF-b superfamily.[10,11]

Quantity of BMPS

Normally, high concentrations of BMPs are required (i.e. 100-1000 ng/ml) at the local site to produce periodontal regeneration (Yamaguchi A). Approximately 10 kg of bovine bone yields only 2 µg of BMP. It consists of complex mixture of BMPs along with various other proteins. But the recombinant BMPs produced by several cellular systems were tested for regeneration. The partially purified recombinant BMPs consist of 0.5-115 µg to produce cartilage formation within 7 days and bone formation within 14 days.[12]

Source of BMPS[13]

1. Extraction from animal or human bone matrix by extensive purification.
2. Production by cellular host by recombinant technology.
3. Direct delivery to cells at the site of desired bone formation of the DNA encoding for the factor-gene therapy.

Isolation of BMPs[14]

- Bone decalcified in cold 20% dilute (0.6N) HCl for less than 3 to 5 days followed by washing in 0.15M NaCl or 70% alcohol produced the most positive results.
- Frozen bone ground to 1-3µm and demineralised in 0.6 HCl for 48 hrs followed by Defattening in 1:1 Chloroform methanol.
- Using different concentration of $CaCl_2$, non-collagenous proteins were extracted.

Purification of BMPs[15]

Purification of BMPs from the demineralized bone matrix can be carried out by four distinct methods:

1) Enzymatic digestion, since they resist collagenase;

2) Ethylene glycol extraction, due to the hydrophobic nature of the BMP molecule;

3) 6 M urea plus 0.5 M CaCl2, since BMPs can be dissociated from other non-collagen proteins in chaotropic solvents;

4) Concanavalin A affinity chromatography due to their hydrophobic nature and to carbohydrate present in their structure.

BMP Signaling Pathways:[16]

BMPs can signal through both canonical and noncanonical pathways.

1. **Canonical pathway**

In the canonical signaling pathway, BMP initiate the signal transduction cascade by binding to cell surface receptors and forming a heterotetrameric complex comprised of two dimers of type I and type II serine/threonine kinase receptors.Eight Smad proteins (Smad1 through Smad8) have been identified.

- Activated BMP type I receptors phosphorylate receptor-regulated Smads (R-Smads), that is, Smad1, Smad5, and Smad8 (BMP-specific R-Smads) at their carboxy-terminal S-S-X-S motifs.
- The phosphorylated and activated R-Smad proteins form complexes with Smad4 (common partner Smad: co- Smad) and move into the nucleus.
- Smad complexes containing two R-Smads and one Smad4 associate with various transcriptional coactivators (p300, CBP, Runx2, and/or GCN5) or co-repressors (c-Ski, SnoN, Tob, or SIP1), and bind to regulatory elements of target genes to regulate their transcription.[17]

2. **Noncanonical pathway:**

BMPs also activate Smad-independent signalling pathways such as mitogen-activated protein kinases (MAPKs), c-Jun amino-terminal kinase (JNK), phosphoinositol-3 kinase (PI3K), Akt, and small GTPases. These non-Smad pathways cooperate with Smad pathways to regulate various cellular responses.[1]

Several non-canonical, Smad-independent signalling pathways for BMPs have been identified. BMP4, for example, was found to activate TAK-1, a serine threonine kinase of the MAPKKK family.

In addition to the MAPK pathway, BMP signaling has been found to affect PI3K/Akt, P/kc, Rho-GTPases, and others. Interestingly, BMPs can have temporal regulation of signaling via the canonical Smad pathway or non-canonical pathways.

Regulators of BMP Signaling

BMP signaling is regulated at multiple levels from the extracellular space to the nucleus. In extracellular compartments, BMP signaling is limited by BMP antagonists, which function through direct binding to BMP, thus preventing their binding to specific receptors. Various extracellular BMP antagonists, such as noggin, chordin, chordinlike 1, chordin-like 2, Gremlin, Cerberus, follistatin, ectodin/uterine sensitization-associated gene-1 (USAG-1), and DAN family members, have been identified in various animal species. Expression of some antagonists such as noggin and Gremlin is up-regulated by BMPs, suggesting that the antagonists establish a negative feedback loop.[18,19]

BMP signaling is also negatively regulated at the cell membrane by BAMBI (BMPand activin membrane-bound inhibitor), a pseudoreceptor for the TGF-b family. BAMBI lacks the intracellular domain of the serine–threonine kinase receptors, and inhibits ligand-induced signalling by preventing the formation of signalling receptor complexes. The expression of BAMBI is induced by BMP and TGF-b, which is another example of negative feedback of TGF-b family signals. Intracellularly, BMP signalling is negatively regulated by I-Smads (Smad6 and Smad7), the E3 ubiquitin ligases Smurf1 and Smurf2, and transcriptional co-repressors, such as c-Ski, SnoN, and Tob.[20,21]

Target Genes for BMPS

During osteoblast differentiation of early mesenchymal cells (e.g., C2C12 cells), numerous genes that regulate transcription and signal transduction were identified as immediate early genes (regulated within 2 h after BMP stimulation), including the inhibitor of differentiation or inhibitor ofDNA-binding (Id) proteins Id1, Id2, and Id3, Smad6, Smad7, OASIS, Prx2, TIEG, and Snail.[22]

In contrast, the intermediate (regulated up to 6 h afterBMP-2 stimulation) and late (regulated up to 24 h after BMP stimulation) response genes are related to processes of osteoblastic differentiation, including the genes for transcription factors Hey1 and Tcf7, which mediate Notch and Wnt signaling, respectively. During angiogenesis, BMP-4 induces the expression of Id1, which is a common target of BMP signals, and the expression of angiogenesis-related genes including vascular endothelial growth factor receptor 2 (VEGFR2) and Tie2, which are receptors for VEGF and angiopoietins, respectively, and stimulates the proliferation of endothelial cells.[23]

Immune Response to Bone Morphogenetic Proteins

A single application of allogeneic BMPs and non-collagenic proteins provides a moderate immune response through the production of immunoglobulins G, but does not decrease the osteoinductive capacity of BMP. On the other hand, a single dose of BMP-non-collagenic protein stimulates a high concentration of anti-BMP antibody, which could inhibit the osteoinductive potential of BMP.2[4]

Carrier Systems

Carriers play an important part in bone induction by contributing to the following functions:

• Localisation and retention of BMPs at the site of application (reduces the dosage)

• Providing a matrix for mesenchymal cell infilteration

• Providing substrate for cell growth and differentiation

• Shapes the new bone formation

• Degradation rate that does not inhibit bone growth and remodelling.[25]

Carriers can be classified as:[26]

1. **According to antigenicity**

- Solid xenogenic (HA)
- Solid alloplastic (polyethylene polymers)
- Gels of

o autogenous/

o allogenic

o alloplastic origin

- Combinations of the above.

2. **According to composition**

A. Natural Polymers

B. Synthetic polymers

A. Natural Polymers:

These include collagen, silk, alginate, agarose, chitin and chitosan and are developed from substances naturally present in extracellular matrix, cartilage and bone.

B. **Synthetic polymers**

1. Ceramic: Hydroxyapatite and other types of calcium phosphate materials can promote formation of bone like mineral surface leading to increased interface between bone and the implanted material.

2. **Nonceramic**

Polylactic acid (PLA) - PLA-based synthetic poylmers, including polylactic acid-p-dioxanone-polyethylene glycol (PLA-DX-PEG) and polylactic acide-polyehylene glycol (PLA-PEG). Due to its versatile temperature dependent liquid-semi solid transition, PLA-PEG allows percutaneous injection after heating.

Polyglycolic acid (PGA) - Polyglycolic acid (PGA) which has superior mechanical strength when combined with PLA results in Polylactic-co-glycolic acid (PLGA).

The main drawback of using synthetic polymers is the risk of potential inflammatory response due to acidic by-

products because of polymer degradation which may interfere with the stability of adsorbed BMPs.

ROLE IN PERIODONTAL REGENERATION

The ability of rhBMPs to induce intramembranous bone formation without endochondral formation has created interest in role of BMPs in periodontal regeneration.

BMPs can stimulates periodontal regeneration through

• Residual cells in periodontal ligament

• Blood clot in the wound of periodontal wound

• Adjacent endosteal spaces and beyond the defect.

Two pathways may be proposed to re-establish tissue relationship in the periodontium following periodontal reconstructive surgery:

1) Growth and migration of the differentiated cells into the wound site from surrounding tissues (i.e., alveolar bone and periodontal ligament),

2) Growth and differentiation of the mesenchymal stem cells.

BMP Used	Study By	Results
rhbmp-2/ACS	Sorensen RG[27]	Accelerated enhanced bone formation
rhBMP-2	Saito E[28]	Sites treated with rhBMP-2/PGS showed a greater degree of bone formation
rhBMP-2	Takahashi et al.[29]	Resolution of ankylosis during periodontal regeneration
rhBMP-12	Ulf M. E. Wikesjö[30]	may have significant effects on regeneration of the PDL
rhOP-1(BMP7) and rhBMP-2	Ripamonti[31]	A temporal enhancement of alveolar bone regeneration and remodelling.
BMP-6	Kuo-Kuang Huang[32]	An increase in new bone and cementum formation
rhBMP-4	Kim CS[33]	The stimulatory effect of rhBMP-4 on osteoblastic differentiation
rhbmp-2/ACS in sinus augmentation	Boyne et al[34]	Aided in implant placement
rhBMP in distraction osteogenesis	Rachmiel et al[35]	Minimised the consolidation period, allowing early placement of implants.
rhbmp-2	Howell[36] and Cochran[37] et al	Bone formation at the extracted site was observed, which helped in endosseous implant placement.

Conclusion

Though The Discovery And Usage Of Bmps Are Like The 'Light In The Darkness' In Terms Of Periodontal Regeneration, More Experimental Studies And Research Are Needed To Fully Tap Their Potential For Regeneration.

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