

Osteogenic protein and its peptide derivatives

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Abstract:

The role of tissue engineering in the field of bone regeneration has been one of the most studied topics in the past half century. To this end, the development of new biocompatible materials and scaffolds can be considered as an essential research purpose. Recently more attention has been focused on using bone morphogenetic proteins (BMPs). Current advances in the development of compatible derivatives of BMP motivated us to summarize them. This review focuses on several classes of short peptide sequences derived from BMP, and highlights *in vitro* and *in vivo* studies that demonstrate the potential of these materials as novel candidates for bone generation engineering and repair.

Key words: Bone morphogenetic proteins (BMP), tissue engineering, peptide

Introduction

Bone formation as a complicated procedure, has been reported to be affected by a variety of hormones, cytokines and growth factors [1, 2]. The osteogenic growth factors such as bone morphogenetic proteins (BMPs, including BMP-2, BMP-6, BMP-7 and BMP-9), transforming growth factors β (TGF- β), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), NELL-1 and other related factors are involved in bone formation process [1, 2]. As a consequence, the surface functionalization with growth factors or direct incorporation of growth factors into the materials has been demonstrated to be effective in improving the cell adhesion and osteoblast differentiation on materials [3-5]. In this review, we first outline the basic aspects of

osteogenic protein and then introduce its peptide derivatives which have same potentials in bone formation with a special focus on their characterization and application in *in vivo* and *in vitro*.

Bone morphogenetic proteins (BMPs) have been reported to act as crucial regulators of cell differentiation and proliferation during development while playing an important role in stem cell biology [6]. BMPs have been identified as osteo-inductive growth factors which are expressed in a variety of cells and tissues with the ability to control new bone formation [7]. BMP, as the most effective osteoinductive growth factor, is a member of TGF- β superfamily [8] that can be produced using recombinant DNA technology.

Specially, recombinant human bone morphogenetic protein-2 (BMP-2), has been demonstrated to stimulate new bone formation *in vivo* effectively [9]. On the other hand, recombinant BMP-2 protein is degraded rapidly in the body by proteinases, while the use of BMP-2, in high doses might cause adverse effects, such as ectopic bone formation, immunological reaction, and even tumorigenesis [10]. Thus, to reduce BMP-2 diffusion from the integration and enhance its efficacy, immobilization of BMP-2 onto nanoparticles has *been introduced* [11]. For example, BMP-2 entrapped in dextran-derived microspheres has been reported to stimulate higher expression of alkaline phosphatase in human periodontal ligament cells, resulting in longer action time compared to the aqueous solution with equivalent BMP-2 concentration [12]. It was also shown that BMP-2 incorporated hydroxyapatite nanoparticles as controlled-release carriers could represent a sustained release profile of BMP-2 [13]. Neumann *et al.* showed that BMP-2 loaded onto nanoporous silica nanoparticles could comprise higher osteoinductive effect in human mesenchymal stem cells in comparison with BMP-free nanoparticles [14]. BMP-2 protein could be immobilized onto chitosan-functionalized Mesoporous Silica Nanoparticles (MSNs) that was able to stimulate osteoblast differentiation [15], resulting in more than 80% of BMP-2 release within 24 h and thus yielded a much higher localized concentration of protein on the cell surface causing specific side effects, which can be reduced by the use of synthesized osteogenic BMP peptide [16, 17]. Saito *et al.* reported that the synthetic peptide KIPKASSVPTELSAISTLYL corresponding to residues 73–92 of the knuckle epitope of BMP-2 could enhance alkaline phosphatase (ALP) activity

compared to other residue sequences in C3H10T1/2 cells [18]. They also demonstrated that 73–92 peptide-conjugated alginate gel remained active at the implanted site and activated osteoblasts that could promote ectopic calcification [19]. Other studies showed that incorporating the BMP-2 peptide in biomaterials could stimulate both *in vitro* osteogenic differentiation and *in vivo* bone regeneration [20–23] (Table 1).

Table 1. a series of osteogenic peptides derived from recombinant proteins.

Peptide sequence and Nomenclature	Protein derivatives	Ref.
KIPKASSVPTELSAISTLYL	73–92 of the knuckle epitope of BMP-2	[18]
SNVILKKYRN [SNV] KPSSAPTQLN [KPS] KAISVLYFDDS [KAI]	short peptides of BMP7	[28]
GQGFSYPYKAVFSTQ [BFP-1] VEHDKEFFHPRYHHR [BFP-2]	derived from the immature region of BMP-7	[31]
RKKNPNCRRH [HBD]	residues 15–24 of BMP-4	[32]

Xiaojun Zhou *et al* demonstrated the synthesis of bioactive BMP-2 peptide grafted MSNs by covalently conjugation of BMP-2 peptide and aminated MSNs. They clearly showed the improvement of dispersibility and biocompatibility of functionalized MSNs having high cellular uptake of nanoparticles against bone marrow stromal cells (BMSCs). Their experimental results also indicated that the prepared MSNs-pep could promote the osteogenic differentiation of BMSCs *in vitro*, after

determination of ALP activity, expression of osteo-related proteins and calcium deposition. Moreover, the loading of dexamethasone (DEX) in MSNs-pep further enhanced the osteogenic differentiation of BMSCs and ectopic bone formation *in vivo*. Altogether, BMP-2 peptide and DEX-incorporated MSNs apparently showed an advanced capacity for osteogenic differentiation with high possibility for bone tissue engineering applications [24].

It has been reported that bone morphogenetic protein-7 (BMP7, also called osteogenic protein-1) had significant effects on a degenerated disc [25, 26]. BMP7 clearly regulated nucleus pulposus cells to secrete aggrecan and collagen II *in vitro* and disc degeneration *in vivo* [25, 27]. Moreover, Chen *et al* [28] constructed three different short peptides (SNVILKKYRN [SNV], KPSSAPTQLN [KPS], and KAISVLYFDDS [KAI]) of BMP7 that clearly presented specific functionalities. Other researchers [29] could conjugate SNV, KPS, and KAI to the C-terminus of RADA16-I that resulted in three functionalized self-assembling peptides [30]. RADA16-I was designed by Zhang; it can easily self-assemble to a nano-fiber network. All of their functionalized peptides demonstrated outstanding biocompatibilities for human degenerated NP cells (hdNPCs) *in vitro*, while RAD-KPS represented suitable compatibility *in vivo* [29].

Kim *et al* have identified the peptide sequences with osteogenic activity isolated from the immature region of BMP-7 that was named BFP-2 (VEHDKEFFHPRYHHR). It showed greater osteogenic activity compared to mature BMP-7 having the ability of osteogenesis induction [31]. They showed that BFP-2 treatment could increase ALP activity, CD44, CD51 expression levels and calcium concentration in BMSCs compared with

BMP-7. BFP-2 also represented a potential bone-forming activity. Their results clearly indicated that BFP-2 could increase osteogenic differentiation of BMSCs and upregulate biological markers of osteogenesis, capable of being used as an osteogenic stimulator in further clinical experiments associated with bone generation engineering [31].

Choi *et al* in 2010 [32] synthesized a peptide sequence corresponding to residues 15-24 of BMP-4 (HBD, RKKNPNCRRH), as a potential heparin binding sequence. Based on their result, HBD peptide could regulate osteogenic differentiation by activating extracellular signal-regulated kinase (ERK1/2) and its related signaling, that play an important role in organizing human mesenchymal stem cells (hMSC). The *in vivo* results also demonstrated that HBD as a form of a complex with alginate gel regulated bone formation in the bone defect and served as a bioactive object for bone regeneration processes.

Another protein is NELL-1, NELL1 has been indicated as a secretory protein that presents potent osteoinductive activity [33]. The NELL1 gene was initially identified in craniosynostosis patients [34]. The human NELL1 gene encodes a polypeptide of 810 amino acids with structural similarities to thrombospondin-1. It contains several structural motifs, including one N-terminal thrombospondin-1-like (TSPN) domain (overlapping with a laminin G domain), one coiled-coil (CC) domain, four von Willebrand factor type C (VWC) domains, and six epidermal growth factor (EGF)-like domains [33]. It has been shown that NELL1 stimulates osteogenic signals via MAPK-pathway and Runx2 and acts as ECM component [35, 36]. It prompted osteoblastic

cell adhesion through at least three cell-binding sites localized to its C-terminal region.

Conclusion

It is clear that Bone morphogenetic proteins are vital regulators of cell differentiation and proliferation. A growing body of literature now suggests the use of BMP *derivatives* for bone generation engineering and repair. Combination and conjugation of BMP derivatives to a variety of matrix indicate their high potentials as bioactive materials that can be applied for bone regeneration procedures. The biocompatibility and extensive versatility of the *derivatives* are currently obvious. Although, much works remain to be done to elucidate cytotoxicity, safety, *in vitro* and *in vivo* immune-reactivity of each derivative, the potential is high for translating them into the treatment of human disorders. However, further studies characterizing the peptide relations with primary or progenitor cells and the precise roles of BMP derivatives in *in vitro* and *in vivo* applications are certainly required.

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Conflict of interests

The authors have no conflicting financial interest.

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