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Review

Bone morphogenetic proteins for articular cartilage regeneration

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SUMMARY

Degeneration of articular cartilage (AC) tissue is the most common cause of osteoarthritis (OA) and rheumatoid arthritis. Bone morphogenetic proteins (BMPs) play important roles in bone and cartilage formation. This article reviews the experimental and clinical applications of BMPs in cartilage regeneration. Experimental evidence indicates that BMPs play an important role in protection against cartilage damage caused by inflammation or trauma, by binding to different receptor combinations and, consequently, activating different intracellular signaling pathways. Loss of function of BMP-related receptors contributes to the decreased intrinsic repair capacity of damaged cartilage and, thus, the multifunctional effects of BMPs make them attractive tools for the treatment of cartilage damage in patients with degenerative diseases. However, the development of BMP therapy as a treatment modality for cartilage regeneration has been hampered by certain factors, such as the eligibility of participants in clinical trials, financial support, drug delivery carrier safety, availabilities of effective scaffolds, appropriate selection of optimal dose and timing of administration, and side effects. Further research is needed to overcome these issues for future routine clinical applications. Research and development leading to the successful application of BMPs can initiate a new era in the treatment of cartilage degenerative diseases like OA.

Introduction

Cartilage is a highly specialized tissue in the human body that covers and protects the ends of long bones from mechanical damage. It mainly consists of hyaluronic acid, type II collagen (Col 2), and a rich proteoglycan matrix with organized chondrocytes. Because it is resilient and smooth, it helps with load-bearing function and lubrication[1,2]. Adult cartilage tissue consists of four zones: superficial, intermediate, deep, and calcified cartilage which may vary with respect to biochemical composition, cell density, and morphology[3,4]. Homeostasis of articular cartilage (AC) is maintained by a normal balance between aggrecan and collagen

Abbreviations: AC, articular cartilage; Ad., adenoviral; ADSCs, adipose-derived stem cells; BMP, bone morphogenetic protein; BAMBI, BMP and Activin membrane combined inhibitors; BMSCs, bone marrow mesenchymal stem cells; BMPR, BMP receptor; Col 2, type II collagen; Co-Smad, common partner Smads; DCBM, decalcified cortical bone matrix; ECM, extracellular matrix; FDCs, fascia-derived cells; GAG, glycosaminoglycans; HAp/Col, transplantation of porous hydroxyapatite collagen; HA-TA, hydrogel based on a tyramine derivative of hyaluronan crosslinked by hydrogen peroxidase; rhBMP, recombinant human bone morphogenetic protein; hBMSCs, human bone marrow mesenchymal stem cells; hMDSCs, human skeletal muscle-derived stem cells; IL-1β, interleukin-1 beta; IPFP-ASCs, infrapatellar fat pad adipose stem cells; IHH, Indian hedgehog; I-Smads, inhibitory Smads; MC-GAG, nanoparticulate mineral content to collagen glycosaminoglycan; MIA, mono-iodoacetate; MSCs, mesenchymal stem cells; NELL-1, NEL-like molecule-1; OA, osteoarthritis; PCL, polycaprolactone; PDCs, periosteum-derived mesenchymal stem cells; PDLSCs, periodontal ligament stem cells; PLGA, L-lactide-co-glycolide; rhBMP, recombinant human bone morphogenetic protein; R-Smads, receptor-regulated Smads; sFlt-1, soluble Flt-1; Smurfs, Smad ubiquitination regulatory factors; SOX-9, sex-determining region Y box gene 9; TGF-β, transforming growth factor-beta.

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contents to maintain structural integrity and strength for joint load and mobility. During normal conditions, articular chondrocytes and subchondral osteoblasts receive mechanical load and strain[5,6]. However, in abnormal conditions, such as mechanical overload or injury, an imbalance between chondrocyte anabolism and catabolism leads to the secretion of pro-inflammatory cytokines and matrix metalloproteinases (MMPs), and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), which inhibit angiogenesis. It is known to all that AC consists of a thin layer of chondrocytes, as well as a relatively dense extracellular matrix (ECM) that restricts the mobility of the chondrocytes. Furthermore, the lack of blood supply, neural, and lymphatic networks, and various local progenitor cells, as well as the dense ECM of cartilage, prevent self-repair by inhibiting the migration of chondrocytes to the injured site[7–9].

Degeneration of cartilage tissue is the most common cause of osteoarthritis (OA), autoimmune conditions like rheumatoid arthritis, osteochondritis dissecans, osteonecrosis, and other significant cartilage-related diseases[6,10]. Due to the predisposition of disease-causing factors, homeostasis cannot always sufficiently compensate for the mechanical load and strain on the body[11]. Consequently, there is an urgent need to improve our understanding of why cartilage injury does not heal, and to develop better treatment for cartilage regeneration. Currently, researchers are striving to develop new concepts and techniques to regenerate normal cartilage that will allow patients to return to a fully active lifestyle.

Bone morphogenetic proteins (BMPs) are molecules secreted by various cells, and are members of the transforming growth factor beta (TGF-β) superfamily. BMPs were discovered by Marshall Urist in 1965 and named for their ability to induce the formation of bone and cartilage[12,13]. At least 15 different BMPs have been identified to date, and the most widely studied of these are BMP-2, BMP-3 (osteogenin), BMP-4, BMP-6, and BMP-7 (also known as osteogenic protein-1) as well as BMP-9[14,15]. BMPs are synthesized inside the cell in precursor form, with a hydrophobic secretory leader and pro-peptide sequences joined to the mature region. BMPs are distinguished from other members of the TGF-β superfamily by the presence of conserved cysteines in the mature region. After dimerization, these proteins are cleaved proteolytically at a consensus Arg-X-X-Arg site to generate mature dimers. BMPs consist of dimers whose chains are connected by disulfide bonds, and this dimerization is a prerequisite for bone induction[16,17].

BMPs play important roles in bone and cartilage formation, including various aspects of embryonic development, such as skeletogenesis, and hematopoietic and epithelial cell differentiation. BMPs signal through cell-surface receptor complexes that consist of two distinct transmembrane serine/threonine kinase receptors, BMP receptor type I (BMPRI) and BMP receptor type II (BMPRII)[18,19]. Dysregulated BMP signaling is involved in pathological processes, such as bone metabolism disorders that lead to heterotopic bone formation, and skeletal and cartilage deformation. Modulation of BMP signaling may be a major therapeutic target for the reconstruction of bone defects, rather than changing the growth rate of preexisting bone[12,14]. This article reviews the experimental and clinical applications of BMPs in cartilage regeneration.

Role of endogenous BMPs in cartilage injury repair

It is evident that chondrocytes are activated to proliferate and migrate towards the injury surface when cartilage is injured. Cartilage injury triggers cytokines and chemokines, such as interleukin (IL)-1α, IL-6, and C–C motif chemokine 2 (CCL2), to induce chondrocyte activation, expression of proteases, and the release of ECM components from the matrix[8,20]. Activated chondrocytes express vascular endothelial growth factor (VEGF), Runt-related transcription factor 2 (RUNX2), collagen X, ADAMTS-5, and MMP13, which leads to subchondral bone alteration along with a thickening of the cortical plate. Reactive oxygen radical production by activated chondrocytes may also facilitate the breakdown of matrix[21,22]. Once the damaged tissue is degraded, chondrogenic progenitors gather at the injured site and initiate the regenerative process. The regenerative process locally activates inflammation-responsive genes in cartilage without angiogenesis or migration of inflammatory cells[5,23]. Cartilage is thought to have limited regenerative potential, and the mechanism by which cartilage growth occurs in diarthrodial joints is not clearly known[24]. Transcription co-activators, such as Yes-associated protein (YAP), and transcriptional co-activators with a PDZ-binding motif (TAZ) are known to play roles in organ size control and cartilage regeneration. There is some evidence indicating that new cartilage tissue formed in distracted joints exhibits cartilage-associated properties, and is able to resist compression and exhibit tensile properties[25,26].

Locally produced BMPs play an important role in protection against cartilage damage or stimulation of regenerative processes during and after inflammatory or traumatic damage[27]. BMPs function in cartilage regeneration by binding to different receptor combinations and consequently activating different intracellular signaling pathways. Moreover, different BMPs may have differential effects on cells[28].

The activity of BMPs is regulated by a number of specific inhibitors at multiple levels. At the extracellular level, BMPs interact with binding proteins such as Noggin and chordin, and binding to these proteins blocks the binding of BMP to its own receptor (BMPR)[29,30]. Intracellularly, the BMP signaling pathway is regulated by inhibiting inhibitory Smads (I-Smads i.e., Smad6 and Smad7) and Smurfs (Smad ubiquitination regulatory factors). I-Smads antagonize the TGF-β signaling pathway either by interacting with phosphorylated type I receptors and thereby preventing the activation of receptor-regulated Smads (R-Smads), or through competition with common partner Smads (Co-Smad or Smad4) for the formation of R-Smad/Co-Smad complexes[31]. Smurfs are able to specifically degrade BMPR-I and Smad proteins to inhibit the BMP signaling pathway[32]. At the level of the cell membrane, transmembrane protein BMP and activin membrane combined inhibitor (BAMBI) plays an important role in the BMP signaling pathway. BAMBI encodes a TGF-β pseudoreceptor, the structure of which is similar to that of the TGF-β receptor, but which differs in biological activity. These two receptors compete each other to bind to BMPR-II receptor, consequently preventing the activation of Smads proteins in the cytoplasm, blocking signal transduction, and influencing the expression of a series of downstream genes[33]. An elevated ratio of BMPs to inhibitors results in increased BMP activity and stimulation of chondrocyte differentiation. During terminal differentiation, chondrocytes become hypertrophic and die due to apoptosis, and are replaced by bone through the production of MMP-13. In the formation of AC, terminal differentiation is prevented, which results in permanent cartilage residing at the end of the long bones[34–36]. It has been reported that blockade of BMP activity causes proteoglycan depletion, which in turn contributes to decreased intrinsic repair capacity in damaged cartilage. Loss of BMP-related Smad proteins (Smad1 and 5) in the Smad signaling pathway leads to terminal differentiation and severe cartilage defects. Furthermore, BMP antagonists and catabolic cytokines contribute to the feeble innate regeneration capacity of the AC[37–39].

BMPs as a tissue engineering growth factor for cartilage regeneration

Damaged cartilage has poor regenerative potential. Moreover, the identification of proper target cells and growth factors for cartilage regeneration remains challenging. Therefore, it is essential to improve our understanding of the causes of poor cartilage healing, and to identify and investigate new disease models in order to develop better treatment strategies[1]. BMPs have been identified as excellent bone formation inducers, but it has become evident that their capacity is not limited to skeletal development. They have been shown to possess the potential for cartilage regeneration in pre-clinical studies. The multifunctional effects of BMPs make them attractive for the treatment of cartilage damage in patients with bone, and these have been reviewed elsewhere (Table I)[40]. We have summarized recent progress in the use of BMPs for cartilage repair below.

BMP-2 for cartilage regeneration

BMP-2 has been extensively used to promote both bone and cartilage regeneration *in vitro* and *in vivo*. Schmal *et al.* measured the concentrations of BMP-2 and other cytokines in the lavage fluids of knee joints collected from patients with circumscribed cartilage defects. High levels of BMP-2 were detected in a normal control group, and BMP2 was found to be the only intra-articular growth factor that correlated with clinical outcome, indicating that it might play an important role in surgically induced cartilage repair and regeneration[41]. Zhang *et al.* found that fibroblast growth factor 2 (FGF2) induces the upregulation of BMP-2, BMP-3, and BMP-4, resulting in satisfactory effects in the process of rabbit AC repair[42]. Severe disorganization of chondrocytes within the growth plate region and profound defects in chondrocyte proliferation, differentiation, and apoptosis were observed in Bmp2 knockout mice. Canonical Wnt/β-catenin signaling may stimulate chondrocyte differentiation, partially through a BMP-2/BMP-4-dependent mechanism, as BMP-2 affects the transcription of RUNX2 in a dose-dependent manner, and subsequently affects the ubiquitination of proteins in chondrocytes[43].

BMP-2 alone or combined with other materials has been widely used to treat bone defects and cartilage degeneration. Claus *et al.* found that the addition of exogenous BMP-2 to human chondrocytes immediately after their isolation from cartilage stimulated collagen type II expression and synthesis. Because BMP-2 can support the expansion of the chondrogenic phenotype of human articular chondrocytes, it may act as a useful therapeutic method in autologous chondrocyte transplantation[44]. When exposed to BMP-2, bovine synovium-derived mesenchymal progenitor cells were induced to express chondrocyte-specific genes[45].

BMP-2 interacts directly or indirectly with matrilin-3, an ECM protein, and enhances the expression of type II collagen and aggrecan, which are required to maintain the tensile strength and elasticity of cartilage[4]. The osteoinductive capacity of recombinant human BMP (rhBMP) in the regeneration of cartilage has been confirmed by *in vitro* studies and evaluated in clinical trials[46]. Lópiz-Morales *et al.* confirmed that rhBMP-2 shows better restoration of subchondral bone than that seen with rhBMP-4 for hyaline cartilage repair in a rabbit osteochondral defect model[47]. Taniyama *et al.* evaluated the effect of transplantation of porous hydroxyapatite collagen (HAp/Col) impregnated with rhBMP-2, and found it to be effective in cartilage repair[48].

Wang *et al.* investigated the effect of a combination of demineralized bone matrix (DBM) and bone marrow mesenchymal stem cells (BMSCs) infected with adenovirus-mediated-BMP-2 (Ad.BMP-2) and TGF-β3 (Ad.TGF-β3) in a pig model. They found that the new type of tissue engineering scaffold produced hyaline cartilage morphology containing a stronger type II collagen and induced cartilage regeneration to repair the defects of joint cartilage[49]. Sieker *et al.* tested the efficacy of direct BMP-2 and Indian hedgehog (IHH) gene delivery via a bone marrow coagulate in combination with adenoviruses carrying complementary DNA (cDNA) of the respective transgene, and suggested the use of these regulators as an improved treatment option for AC defects[50]. In another study, a conically graded scaffold of chitosan-gelatin hydrogel/poly(L-lactide-co-glycolide) (PLGA) was prepared and loaded with BMP-2. The graded scaffold exhibited beneficial effects for cartilage–bone interface reconstruction[51]. Cha *et al.* created a new method of co-delivery of BMP-2 and sex-determining region Y box gene 9 (SOX-9) to dedifferentiated chondrocytes using a microporator transfection system, and found improved efficiency of recovery of normal chondrogenic properties in dedifferentiated chondrocytes, followed by enhanced cartilage formation[52]. Furthermore, it was found that 3D polycaprolactone (PCL) scaffolds with chemically-conjugated BMP-2 can promote significantly greater cartilage regeneration without accelerated osteochondral ossification[53]. Yang *et al.* found that the long-term delivery of BMP-2 to cartilage defects subjected to microfracture could be more favorable for hyaline cartilage regeneration[54]. The delivery of BMP-2 or BMP-6 via Ad vector and direct injection, to treat large weight-bearing osteochondral defects, provided evidence of support to cartilage and subchondral bone regeneration; however, it was insufficient to provide long-term quality osteochondral repair in a pony model[55]. Therefore, much progress is needed before a satisfactory treatment for human cartilage defects can be developed.

BMP-3 for cartilage regeneration

BMP3, previously referred to as osteogenin, has been found to stimulate osteogenic and chondrogenic phenotypes *in vitro* when tested on osteoblasts, periosteal cells, chondrocytes, and BMSCs[56]. It is apparently different from other BMPs, not only by virtue of being the most abundant BMP, accounting for around 65% of the total BMPs in demineralized bone[57], but also because it has been shown by several studies to lack osteogenic ability[58–60]. BMP3 was found to be an antagonist of osteogenic BMPs, such as BMP-2 and 4. It actually acts as a negative regulator of bone density via activating the TGF-β/activin pathway and antagonizing the BMP pathway[59,60]. Previous studies mainly focused on its role in skeletal development; however, it was found that BMP-3 could regulate chondrocyte proliferation through the type II receptor activin receptor type 2b (Acvr2b)[28,61]. Several studies have investigated its function in the repair of cartilage defects[42,62]. The expression of BMP-3 in a certain layer of the AC is associated with mechanical loading[62]. In a recent study of the application of BMP-3 in rabbit AC repair, it was found that BMP-3 inhibits the repair of both partial and full thickness cartilage defects. Gene expression assays showed that BMP-3 impairs the survival of chondrocytes and inhibits the differentiation of BMSCs[42]. Taken together, these observations indicate that BMP-3 may be a negative regulator in the process of AC regeneration via the regulation of the BMP signaling pathway, and further studies are needed to reveal the exact mechanism.

BMP-4 for cartilage regeneration

BMP-4 plays a crucial role in maintaining a chondrogenic phenotype, enhancing matrix production, suppressing hypertrophy, and accelerating the chondrogenesis of stem cells *in vitro*. It was found that adenoviral BMP-4 gene transfer (Ad.BMP-4) efficiently induces the chondrogenic differentiation of human primary mesenchymal stem cells (hMSCs) as effectively as does BMP-2 gene

Table I
Applications of Bone morphogenetic proteins (BMPs) in cartilage regeneration

BMPs	Experimental models	Functional mechanisms	Ref.
BMP-2	Human chondrocytes	Supports expansion of the chondrogenic phenotype of human articular chondrocytes	[44]
BMP-2	Bovine synovium-derived mesenchymal progenitor cells	Turns on the chondrogenic pathway in the appropriate chondrogenic precursor cell pool	[45]
BMP-2 + matrilin-3	Chondrocytes	Maintains tensile strength and elasticity of cartilage by downregulating the expression of matrix-degrading enzymes, and enhancing collagen II and aggrecan expression	[4]
rhBMP-2/rhBMP-4 + alginate gels	Rabbit osteochondral defect model	Obvious restoration of subchondral bone in hyaline cartilage repair; alginate gels act as a perfectly biocompatible carrier for BMPs.	[47]
rhBMP-2 + HAp/Col	Rabbit osteochondral defect model	Repairs cartilage and subchondral bone defects	[48]
Ad.BMP-2 + Ad.TGF-β3	Porcine BMSCs	Promotes the repair of the full-thickness cartilage lesions	[49]
BMP-2 + IHH	Rabbit osteochondral defect model	Repairs AC defects via bone marrow coagulates	[50]
BMP-2 + PLGA	Rat BMSCs	Repairs cartilage–bone interface tissue defects	[51]
BMP-2 + SOX-9	Human chondrocytes	Induces cartilage formation by increasing the expression of type II collagen and aggrecan	[52]
BMP-2 + PCL	Porcine chondrocytes	Promotes cartilage regeneration without accelerated endochondral ossification	[53]
BMP-2 + microfracture	Rabbit osteochondral defect model	Induces hyaline cartilage regeneration by increasing the expression of GAG as well as type II collagen	[54]
Ad.BMP-2 + Ad.BMP-6	Porcine osteochondral defects model	Supported cartilage repair and denser subchondral bone in healing osteochondral defects,	[55]
Ad.BMP-2/Ad.BMP-4	Human MSCs	Promotes chondrogenesis and hypertrophy	[63]
BMP-4	Mice cranial base structures	Promotes cartilage growth, matrix deposition, and chondrocyte proliferation	[64]
Ad.BMP-4	Rabbit osteochondral defect model	Induces redifferentiation of dedifferentiated chondrocytes	[65]
Ad.BMP-4 + DCBM	Rabbit osteochondral defect model	Rapidly repairs large areas of cartilage defects with regeneration of native hyaline AC	[67]
BMP-4 transfected rabbit ADSCs + PLGA scaffold	Rabbit osteochondral defect model	Promotes AC repair	[68]
BMP-4–transduced mice MDSCs	Nude rat osteochondral defect model	Enhances chondrogenesis and significantly improves AC repair	[69]
sFlt-1 + BMP-4–transduced mice MDSCs	Nude rat MIA–induced OA model	Prevents angiogenesis and cartilage resorption	[70]
BMP-4	Rat FDCs	Induces chondrogenic differentiation	[73]
BMP-6	Chicken chondrocytes from embryos	Expressed by maturing chondrocytes and acts in an autocrine manner to accelerate chondrocyte maturation	[74]
BMP-6	Human OA and normal cartilage chondrocytes	Increases the biosynthesis of proteoglycan in stimulated chondrocyte cultures	[77]
BMP-6	Human MSCs	Increases mRNA levels and secretion of factors associated with chondrogenesis and hypertrophic differentiation	[78]
BMP-6	Healing of tooth extraction sockets in the streptozotocin diabetic rat model	Causes chondrogenic differentiation in the subperiosteal bone surrounding extraction socket	[79]
BMP-6	Murine ADSCs	Induces osteogenic gene expression and robust matrix mineralization	[80]
BMP-6	Human ADSCs	Upregulation of chondrogenic genes and downregulation of genes associated with chondrocyte hypertrophy and endochondral ossification	[81]
BMP-6 + HA-TA	Human MSCs	Promotes the expression of chondrogenic marker and enhances collagen type X and osteopontin expression	[82]
BMP-6 + BMP-2	Human PDCs	Induces GAG deposition and contribute to enhanced matrix mineralisation	[83]
BMP-6 + TGF-β3	Human PDLSCs	Increased Sox9, aggrecan, and Col 2 expression; promote very early events in chondrogenesis and directly or indirectly maintain their regulation during the differentiation and maturation of chondrocytes	[84]
BMP-6 + TGF-β3	Sheep BMSCs/ADSCs	Increases chondrogenic gene expressions	[85]
BMP-6 + TGF-β3	Human IPFP-ASCs	This combination has a profound effect on chondrogenic differentiation and the stimulation of collagen type II production in the IPFP-ASCs	[86]
BMP-6 + TGF-β3	Human BMSCs/ADSCs	Increases GAG content, type X collagen, and elicits matrix deposition	[87]
BMP-6 + TGF-β3 + NELL-1	Human PSCs	Promotes chondrogenic differentiation and limits hypertrophic, fibrotic, osteogenic, and apoptotic progression	[89]
plasmid DNA (pShuttlerBMP6)	Rat BMSCs	Enhances cell proliferation and proliferation; strongly affects cell morphology which may be critical in influencing cell differentiation	[91]
Achilles tendon treated with BMP-7	Rat model of massive meniscal defect	Induces ectopic cartilage formation by changing the collagen gene profile of tendon tissue	[93]
BMP-7 (knee injection)	Rat strenuous running induced OA model	Increases cartilage matrix synthesis and reduces OA progression; enhances levels of BMP-7 in cartilage, and suppresses IL-1β in synovium	[94,95]
rhBMP-7 (knee injection)	Sheep osteochondral defect model	Increases the survival of chondrocytes that are able to participate in the repair process	[96]
Chondrocyte modified with plasmid encoding BMP-7	Rabbit osteochondral defect model	Repairs AC	[97]
Rabbit chondrocytes transfected with Ad.hBMP7-GFP	Rabbit chondrocytes	Promotes repair of cartilage; increases collagen II and hyaluronic acid	[98]
rhBMP-7 + microfracture	Rabbit osteochondral defect model	Induces cartilage differentiation, enhances the effects of microfracture	[100]
rhBMP-7 + collagen I	Mini pig osteochondral defect model	Enhances homogeneous cellular distribution, ECM production, cellular organization, and mechanical properties	[101]
rhBMP-9	Human MSCs	Maintains the expression of chondrocyte-specific ECM molecules in the presence of OA-related physiological levels of IL-1β	[104]
rhBMP-9	Calf articular chondrocytes	Stimulates cellular proliferation and ECM deposition; induced hypertrophic chondrocyte formation and mineralization	[105]
BMP-9 + MC-GAG scaffold	Human MSCs	Activation of the non-canonical BMP receptor signaling pathway and upregulation of Sox-9.	[106]

transfer (Ad.BMP-2)[63]. BMP-4, when added into the endochondral cranial base, could enhance cartilage maturation and induce ectopic chondrocyte hypertrophy, indicating that endogenous BMP-4 are required to maintain cartilage growth, matrix deposition, and chondrocyte proliferation[64]. BMP-4 was able to induce the redifferentiation of dedifferentiated chondrocytes *in vitro* and *in vivo* in a rabbit model of AC defects[65]. Another study established a detailed localization of BMPs and BMP signaling components, and BMP-4 expression in callus chondrocytes was detected at the fracture site, indicating that BMP could regulate the bone healing process via influencing chondrocyte activity[66]. Zhang *et al.* created a biomaterial scaffold of perforated decalcified cortical bone matrix (DCBM) and utilized an Ad. BMP-4 gene therapy method to treat full-thickness defects in a rabbit model, and found that this composite biotechnology application rapidly repaired large areas of cartilage defect with the regeneration of native hyaline AC[67]. Nanoparticle delivery of the BMP-4 gene with adipose-derived stem cells (ADSCs) could accelerate AC repair *in vitro* and *in vivo*[68]. BMP-4 gene therapy based on retroviral transduction was also able to promote chondrogenesis *in vitro* and significantly enhanced AC repair *in vivo*[69].

Muscle-derived stem cells (MDSCs) isolated from mouse skeletal muscle have been found to exhibit long-term proliferation, high self-renewal, and multipotent differentiation. It was found that the combination of MDSCs with BMP-4 and soluble Flt-1, an antagonist of VEGF, could efficiently regenerate AC when they were intraarticularly injected in a rat monoiodoacetate (MIA)-induced OA model[69–72]. This MDSC-BMP4 based therapy exerted a beneficial effect by both promoting chondrogenesis and blocking angiogenesis, and resulted in persistent cartilage regeneration and repair. Fascia-derived cells (FDCs), isolated from gluteus maximus muscle fascia (epimysium), have also been shown to undergo chondrogenic differentiation *in vitro* after treatment with BMP-4[73].

BMP-6 for cartilage regeneration

BMP-6 belongs to the TGF-β superfamily and has been reported to play a role in chondrocyte differentiation both *in vivo* and *in vitro*. A previous study found that BMP-6 was expressed by maturing chondrocytes and acts in an autocrine manner to accelerate chondrocyte maturation[74–76]. BMP-6 was detected in both normal and OA adult human AC; the endogenous expression of BMP-6 in cartilage independent of the presence of OA indicates its functional role in the maintenance of joint integrity and might represent a therapeutic molecule for cartilage repair[77]. The addition of BMP-6 increased the weight of the pellets about 10-fold, and the content of proteoglycans, type II procollagen and type X collagen, significantly improved the chondrogenic differentiation of hMSCs in the pellet culture system[78]. The application of BMP-6 in tooth extraction sockets of the diabetic rat induced cartilage production in the subperiosteal region of the socket extra-alveolar bone[79]. Kemmis *et al.* found that BMP-6 drives both the osteogenic and chondrogenic differentiation of murine ASCs, indicating that BMP-6 is a promising growth factor that may be effective in directing the osteogenic and chondrogenic lineages commitment of ASCs[80]. Likewise, human ADSCs are also induced to undergo chondrogenesis by BMP-6[81].

The combination of BMP-6 and other growth factors have proven effective in improving the chondrogenic differentiation of various kinds of stem cells[82–90]. The addition of BMP-6 revealed a tendency to potentiate both chondrogenic and osteogenic differentiation in hMSCs cultivated in a hydrogel, based on a tyramine derivative of hyaluronan crosslinked by hydrogen peroxidase (HA-TA)[82]. Mendes *et al.* found that BMP-6 combined with BMP-2 can promote the chondrogenic differentiation of human periosteum-derived mesenchymal stem cells (hPDCs) *in vitro*, and play pivotal roles in GAG deposition and matrix mineralization[83]. TGF-β3 and BMP-6 are both well-known chondrogenic growth factors for stem cells; when combined, their ability to induce chondrogenesis is enhanced[84]. Several studies found that this combination showed a synergistic effect and could induce the chondrogenesis of ADSCs[85–88], BMSCs[85], and periodontal ligament stem cells (PDLSCs)[84]; therefore, this combination might be the ideal cocktail for chondrogenic induction in these cells. Another study found that the combinatorial application of NEL-like molecule-1 (NELL-1), TGF-β3, and BMP-6 with human perivascular stem cells (hPSCs) remarkably enhanced and accelerated cartilage repair, and might possess stronger chondrogenic potential than the currently accepted TGF-β + BMP regimen in AC repair, providing the basis of a new viable chondral graft suitable for *in vivo* implantation[89,90].

Efforts have also been undertaken to apply BMP-6 based gene therapy to promote cartilage repair. Engineered constructs composed of BMP-6 gene-modified rat BMSCs and 3D chitosan scaffolds were designed, and this gene-activated matrix (GAM) was able to modify rat BMSCs to continuously induce chondrogenic differentiation, indicating that the BMP-6 gene-activated chitosan scaffold has a potential application in cartilage regeneration[91].

BMP-7 for cartilage regeneration

BMP-7 not only has great potential in bone repair applications, but also exhibits features as a cartilage anabolic factor due to its ability to induce matrix synthesis and promote cartilage repair. Data collected thus far suggest a significant role of BMP-7 in cartilage repair, for both articular and disc cartilage applications[92]. Ozeki *et al.* injected BMP-7 into the Achilles tendon of rats and reported that BMP-7 induced ectopic cartilage formation and prevented cartilage degeneration in a rat model by changing the collagen gene profile of tendon tissue[93]. Periodic knee injections of BMP-7 were found to delay AC degeneration induced by strenuous running in rats[94]. Likewise, this treatment also proved to be effective in protecting zymosan-induced arthritis, and cytokines in the synovium may play a role[95]. In another study, Mark *et al.* found that small focal lesions at the injury site that did not progress into the surrounding cartilage were observed in the knees that received rhBMP-7 injection immediately after injury, whereas the cure effect declined if the treatment was postponed[96]. In another study, primary chondrocytes were genetically modified with plasmid-encoding BMP-7 via the commercially available non-viral Turbofect vector and the result showed significantly better cartilage healing with BMP-7-transfected cells than the control[97]. In a recent study, a specific cytokine and 3D culture system were combined by growing adenovirus containing human BMP-7 and green fluorescent protein (Ad.hBMP7-GFP)-transfected chondrocytes with Matrigel, which was shown to heal a cartilage defect in the rabbit knee[98].

It has been reported that BMP-4 is more potent than BMP-7 in inducing cartilage differentiation, and the delivery of BMP-4 protein in a bilayer collagen scaffold yielded the largest amounts of cartilage tissue, which restored a greater surface area of the defect and achieved higher International Cartilage Repair Society scores[99]. Kuo *et al.* used microfracture combined with BMP-7 to treat full-thickness AC injury in adolescent rabbits, and found that this method acted synergistically to improve cartilage repair. The regenerated tissue closely resembled native hyaline AC, leading to an improvement in the quality and quantity of cartilage regenerated[100]. Karsten *et al.* used cell-free type I collagen gel combined with BMP-7 loaded poly (lactic-co-glycolid acid) microspheres to treat full-thickness chondral defects, and found signs of obvious cartilage healing[101].

BMP-9 for cartilage regeneration

BMP-9 is one of the least studied members of the BMP family. However, great attention has been paid to BMP-9 recently because it may be the most osteogenic BMP, as evidenced by *in vitro* and *in vivo* studies[102–104]. BMP-9, also known as growth differentiation factor 2 (GDF-2), is predominantly expressed in the central nervous system and liver during fetal development, and in the adult liver[102,103]. The ability of BMP-9 to induce the osteogenic differentiation of stem cells was found to be more potent than that of other BMPs[16]. Majumdar *et al.* cultured hMSCs with BMP-2 and BMP-9, and found elevated expression of Col 2 mRNA and increased expression of aggrecan and cartilage oligomeric matrix protein, indicating chondrogenic differentiation of the cells. BMPs may play an important role in chondrogenesis, and the effect of BMP-9 is greater than that of BMP-2[104]. In another study, chondrocytes cultured with different concentrations of rhBMP-9 exhibited increases in the total mass of the constructs, absolute cell numbers, glycosaminoglycans (GAG), and collagen, as well as a tendency towards increased GAG percentage and decreased collagen percentage[105]. In a recent study, hMSCs treated with BMP-9 on nanoparticulate mineralized collagen GAG scaffolds differentiated into a stable composite of mineralized and cartilaginous content[106]. All these studies suggested that BMP-9 is a potent modulator of cartilage development *in vitro*.

Limitation and perspective

The use of BMPs in treatment strategies for cartilage repair has been extensively studied, with promising results; however, there are a number of questions that remain to be investigated before these studies can be translated into new therapies for the treatment of cartilage injury and osteoarthritis. The first and most critical issue to resolve is the efficient delivery of BMP proteins to enable successful cartilage repair. BMP proteins applied in isolation have only short-term efficacy due to their rapid degradation after injection. The delivery of BMPs within a sustained-release scaffold is a modality to be investigated in the future. The use of coacervate as a sustained delivery system has shown promise in the delivery of many growth factors for tissue repair, including bone repair, and is injectable[107–109]. Second, although BMPs alone are effective in cartilage repair, the combination of stem cells with BMPs is more effective. Therefore, the choice of suitable stem cells for use in BMP-mediated cartilage repair is very important, because stem cells can directly differentiated into chondrocytes, and additionally can serve as a gene delivery vehicle for BMPs. This is especially attractive, as it allows for the potential use of patients' own stem cells that can be injected directly into the AC space. Local intra-articular injection has a significant advantage as it decreases the systemic effects of BMPs and stem cells, particularly in the case of gene therapy-mediated BMP delivery, thereby alleviating common safety concerns with gene therapy. This approach is also clinically translatable with minimum difficulty, as invasive surgery is not required. The delivery of a combination of BMPs and other growth factors that could recruit host progenitor cells to repair cartilage is another important future direction, because this strategy aims to use the hosts's own stem cells to repair cartilage under the stimulation of BMPs[110].(4) Finally, co-delivery of BMPs and anti-inflammatory factors such as IL-1R will further enhance the role of BMPs *in vivo*, by decreasing the detrimental effects of factors that are destructive to cartilage. Co-delivery of BMPs with angiogenesis blockers such as sFlt1 or VEGF antibody will also achieve better cartilage repair outcomes. BMP delivery combined with the microfracture technique, which has been reported to increase the migration of bone marrow stem cells to the area of cartilage injury, will also enhance cartilage repair.

Conclusion

Cartilage is easily injured, and difficult to repair. Cartilage degeneration leads to compromised daily activities. Therefore, research into and development of new technologies to solve this challenging clinical problem are critical. The use of bioactive growth factors such as BMPs in combination with stem cells and effective scaffolds, as summarized in this review, represent promising new therapeutic modalities for cartilage repair. The successful application of BMPs may initiate a new era in the treatment of cartilage degenerative diseases such as OA.

Author contributions

GHL and JH contributed to the conception and design of this review article. ZHD and YSL performed searches, analyses, and interpretations. ZHD and YSL drafted the paper, XG substantially revised the paper. GHL and JH gave final approval of the version to be submitted.

Conflicts of interest

The authors declare no conflicts of interest.

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