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The Spine Journal 5 (2005) 250S–258S

THE  
SPINE  
JOURNAL

## Gene therapy for spinal fusion

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### Abstract

Spinal fusion will continue to be an important part of the surgical treatment of spinal pathology for the foreseeable future. Traditional challenges to successful spinal fusion surgery include autograft donor site morbidity and pseudoarthrosis. Recent advances in the understanding of the biology of bone formation have allowed the development of therapeutic biologics. Although recombinant bone morphogenetic proteins delivered to the arthrodesis site will stimulate fusion, these proteins have been less successful in more challenging fusion situations (posterolateral), require supraphysiologic doses to promote fusion in humans, and are quite expensive. Gene therapy may represent the easiest method for the application of bone-forming biologic agents to promote spinal fusion. Both in vivo and ex vivo techniques of delivery of therapeutic genes have been used effectively to promote fusion in lower animals. Considerable research is required to identify gene therapy techniques and vectors with acceptable safety profiles and high fusion rates. © 2005 Elsevier Inc. All rights reserved.

**Keywords:** Spinal fusion; Gene therapy; Bone morphogenetic protein; Adenovirus

### Introduction

Spinal arthrodesis is a fundamental treatment of spinal pathology and is one of the most common spinal procedures with more than 200,000 performed in the United States each year. Autograft remains the standard for achieving spinal fusion. However, its procurement is associated with significant morbidity [1–3] and pseudoarthrosis rates exceeding 40% have been reported [4]. These issues underscore the need to understand the biomechanical and cellular events critical to bone formation so as to develop innovative strategies to enhance bone regeneration and spinal fusion. Gene therapy presents a novel method for maximizing successful spinal fusion through local delivery of cellular mediators that stimulate bone formation, while avoiding the complications associated with autograft.

### Spinal fusion biology

Spinal fusion requires surgical preparation of the intended site and a method for stimulating new bone formation. In general, successful bone formation rests on the presence of at least four crucial elements: osteoinduction, osteoconduction, production of an osteoid matrix and mechanical stimulation. Osteoinduction is the activation of various cytokines or growth factors to attract osteoblasts or their progenitor cells to the site of new bone formation or repair and induce them to produce bone. Another requirement for bone formation is osteoconduction, referring to the three-dimensional structure, or scaffold, that permits the ingrowth of bone. Osteoconductive materials provide the architecture for osteogenic cells to congregate, adhere and interact. Examples of osteoconductive materials include freeze-dried bone allograft, ceramics, collagen sponges, and natural and synthetic polymers [5]. These materials can also act as carriers to contain and limit osteoinductive materials to a desired location. The production of an osteoid matrix, which is the precursor to bone formation, can proceed once the proper cells have reached the proper location. Because the production of osteoid is a function of osteoblasts, lineage specific differentiation of mesenchymal stem cells (MSCs) into

FDA device/drug status: approved for this indication (P-1; BMP-2).

Supported in whole or in part by research grants from the Aircast Foundation, North American Spine Society, Orthopaedic Research and Education Foundation, and the National Institutes of Health. Nothing of value received from a commercial entity related to this research.

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osteoblasts is an essential step in the process of bone formation [6–8]. Recent studies have explored possible sources of osteoblasts, including MSCs [9,10], muscle-derived stem cells [11–14], peripheral blood buffy-coat cells [15], bone marrow cells [16,17] and connective tissue [18]. The initial formation of bone tends to be disorganized and robust. The new bone must undergo remodeling to become functional and durable. Osteoblasts and osteoclasts are stimulated to remodel along force lines of strain. Mechanical forces induce remodeling to better resist the strain, which is known as Wolff's law [19]. If there is not adequate strain across a fusion mass, disuse-mode remodeling will remove bone [20]. Recent advances in understanding cellular mechanisms and bone biology have enabled surgeons to influence the biology of spinal fusion with osteoconductive scaffolds and carriers as well as osteoinductive factors such as bone morphogenic proteins (BMPs). Gene therapy approaches can provide the cellular elements as well as the growth factors necessary to induce bone formation and spinal fusion.

### **Bone biology**

Bone is unique among human tissues in that it not only continually remodels, but it also retains the potential for regeneration throughout life. There are two distinctive pathways of osteogenesis, endochondral and intramembraneous. Intramembraneous ossification (eg, the flat bones of the skull, the mandible, part of the clavicle) occurs when mesenchymal progenitor cells differentiate directly into osteoblasts [21–23]. Endochondral ossification is hallmarked by a cartilaginous template provided by mesenchymal cells, vascular invasion of the site, and subsequent new bone formation by osteoblasts.

Bone formation in spinal fusion progresses through sequential phases similar to endochondral ossification, starting with attraction and multiplication of MSCs. At approximately 3 days, these cells have become adherent and have reached maximal proliferation [24]. By day 5, chondroblasts begin to differentiate. Maximal chondrogenesis is achieved at days 7 and 8. Day 9 brings cartilage hypertrophy and mineralization. Angiogenesis begins during the 10th and 11th days. The new vascularity assists osteoblast differentiation, and the process of remodeling is then begun on the nascent endochondral bone. Although the molecular events underlying bone formation in spinal fusion remain undefined, it is thought that osteogenesis is a multistep cascade that recapitulates most, if not all, of the cellular events occurring during embryonic skeletal development [23,25].

### **Biological factors in spinal fusion**

New bone formation is modulated by a variety of osteogenic factors, including platelet-derived growth factor (PDGF)

[26,27], fibroblast growth factors (FGFs) [26–30], insulin-like growth factor-1 (IGF-1) and IGF-2 [31–33], transforming growth factor- (TGF- $\beta$ ) [34], vascular endothelial growth factor (VEGF) [35,36] and BMPs [37]. These factors affect different points along the bone formation process, including chemotaxis, proliferation and differentiation. However, BMPs seem to be distinct in that they are the only group that can initiate and sustain the entire bone formation cascade [38–40]. BMPs comprise a large and diverse family, and many may have a distinct role in the cascade of bone morphogenesis. At least 15 BMPs have been identified in humans and rodents [24]. To date, most attempts at gene therapy to achieve spinal fusion have involved transfection with various BMPs.

BMP-2 and BMP-7 have been most extensively studied, both in animal studies and in clinical trials of spinal fusion. The recombinant forms of these BMPs are commercially available. Although BMPs are quite effective in promoting anterior interbody fusion, posterolateral arthrodesis represents a more challenging environment for achieving fusion, and direct application of recombinant human BMP-2 (rhBMP-2) and rhBMP-7 in this location have met with inconsistent success rates. Further limitations of the use of rhBMPs include the supraphysiologic doses of recombinant protein required to achieve fusion and the high cost associated with the use of this technology. Gene therapy approaches to achieving spinal fusion offer an alternative, less expensive technique for the delivery and sustained expression of osteoinductive proteins.

### **Approaches of gene delivery**

Gene therapy (Table 1) has been a recent focus of research in medicine, including in orthopaedic surgery. One of the biggest obstacles to the use of gene therapy in modern medicine has been the lack of sustained release of the target gene to treat genetic disorders. However, gene therapy could be ideal for orthopaedic use because most gene deliveries are local, and only the transient expression of osteogenic factors is necessary for bone formation [41].

Gene delivery requires a vector to act as a vehicle for gene transfer to the site of fusion. Vectors may be either viral or nonviral. Various nonviral approaches of gene transfer that have been investigated include delivery of naked DNA or plasmids by direct injection, liposome-mediated transfection, particle-mediated delivery (eg, the gene gun), microseeding, electroporation and polymer-DNA complex implantation [42–48]. Direct, nonviral delivery of genes is of interest because compared to delivery of recombinant proteins, these techniques are often less costly and sustain expression of the target protein longer than a one-time dose. However, nonviral vectors have a relatively low efficiency of gene transfer and a lower level of transgene expression. Current investigations are attempting to overcome this limitation.

Table 1  
Glossary of gene therapy terms

Gene therapy	Use of a gene and/or gene product to treat disease or influence human physiology.
Transfection	A method of introducing genetic material to target cells by infection of the target cells generally by a virus. The transgenes may either integrate with host DNA or remain cytoplasmic (episomal).
Transduction	Transfer of genetic material from one cell to another.
Plasmid	An extrachromosomal unit of genetic material.
Viral vector	A virus that has been genetically altered to transfect selected DNA into target cells.
Adenovirus (Type V)	A human-based adenovirus, a non-enveloped, icosahedral virus, 75–80 nm diameter, with a double stranded, linear DNA genome. The recombinant form has been genetically altered to deliver non-integrating transgenes to target (both dividing and non-dividing) cells and is generally unable to replicate.
Retrovirus	RNA viruses which integrate their complementary DNA form and desired transgenes into the host cell chromosomes, generally without significant inflammatory response, but may only target dividing cells. They are also capable of producing a pathologic insertional mutation.
In vivo gene therapy	Transfer of genetic material directly into the receiving organism.
Ex vivo gene therapy	Cells removed from organism, genetic material insert in vitro and transplanted back into organism.
Gene expression	Measurable effect of a gene.
Transgene	A therapeutic gene that is inserted or taken up into target cells.
Recombinant	Genetic material or protein that has received or is the result of genetic material from different sources.
Mesenchymal stem cells	Undifferentiated cell which retains the ability to differentiate into any cell in the mesenchymal line (e.g., vascular, muscular, adipose, osseous, and cartilaginous tissues).

Viral vectors in gene transfer have received more attention in recent studies. There are five principal types of viral vectors, and they can be divided into two groups depending on whether their genomes persist in a nonintegrated form (ie, adenovirus and herpes virus) or become integrated into host chromosomes (ie, retrovirus, adeno-associated virus, lentivirus) [49]. The distinction between integrating and non-integrating viruses is important because viruses that integrate their DNA tend to cause persistent and stable genetic alterations [49]. However, in the case of spinal fusion, gene expression is only required until fusion is achieved. Therefore, transient transgene expression is not necessarily problematic. Consequently, particular attention has been paid to recombinant adenoviruses, and there are only a small number of studies that used retroviral vectors.

Adenoviral vectors present several advantages over the more conventionally used recombinant proteins. First, adenoviral vectors mediate the highest level of transgene production [50]; thus, they are highly efficient for gene transfer in a broad range of cell types, including osteoblasts and osteoblastic progenitor cells. In addition, as the choice of many gene therapy strategies, BMPs are especially advantageous in combination with adenoviruses because they are produced by eukaryotic cells and retain full biological activity in vivo. Most importantly, BMPs can be continuously produced by virus-infected cells so that the action of BMPs should not be significantly affected by protein degradation.

Retroviral vectors introduce genetic material reliably into the host DNA and have the potential for long-term expression. However, the retrovirus has a limited capacity of genetic material and is unable to infect cells that do not divide. In addition, the viral insertion of genetic material directly into host DNA raises the specter of mutagenesis and oncogenesis. The theoretical risk is that the insertion of the viral genome into host chromatin produces a disruption of a cellular sequence associated with malignancy. These risks have been

manifested in two separate incidents. The first involved retrovirally transduced bone marrow cells inducing leukemia in mice [51]. In the second case, 2 of 11 patients treated successfully for severe combined immunodeficiency (SCID) disease developed a leukemia-like disorder apparently caused by retroviral genome integration causing a disruption in the oncogene LMO2 [52,53].

Researchers are now debating whether similar risks apply to other applications of retroviruses and integrating viruses. Nevertheless, current efforts are focused on improving the safety profile of existing vectors as well as developing new vector systems with predetermined insertion sites. One such viral vector under investigation is adeno-associated virus. The adeno-associated virus is a parvovirus that integrates its payload of genetic material to a specific portion of chromosome 19. The specific insertion site limits concerns about oncogenesis, but the virus may lose the site-specific ability when repackaged to deliver recombinant DNA. This virus also lacks the capacity for genetic material that an adenovirus possesses. Further improvement of current vectors, development of novel vectors, or both would undoubtedly benefit various gene therapy approaches to bone regeneration.

Adenoviral and retroviral vector-mediated BMP gene therapy is currently in the preclinical study phase [54]. An adenovirus can carry nearly four times as much genetic material as the retrovirus and is not dependent on cell division for replication [55]. As stated previously, the adenoviral vector is episomal and nonintegrating, which limits the oncogenetic potential by decreasing the likelihood of a tumor-suppressor gene being inactivated by viral insertion. However, the episomal location limits the gene expression to the life of the infected cell, thereby limiting the time course of expression. In addition, concerns have been raised about the immunogenic potential of an adenovirus [56,57].

Although most viral vectors induce some host immune response, adenovirus vectors are highly immunogenic and induce multiple mechanisms of the immunity. Cytotoxic T cells (CTLs) respond to hosts cells displaying viral gene products or unknown transgene products through the cell-mediated immune response. This leads to elimination of the transduced cells. Meanwhile, the humoral antibody response will become activated with repeated administration of the vector. This immunogenicity is problematic because an adenovirus's nonintegrating genome becomes diluted with time by DNA degradation and cell division. Consequently, some adenovirus-mediated gene therapy may require periodic repeat infection. New formulations of recombinant adenovirus have been stripped of viral genes. This significantly reduces the CTL response to infected cells and also blunts the antibody-mediated response. Other viral vectors under investigation such as retrovirus, lentivirus and adeno-associated virus do not tend to induce such vigorous CTL responses, and, because they integrate into the host genome, they also result in less antibody-mediated immunogenicity.

#### *Routes of gene transfer*

It is important to choose an appropriate route of gene delivery. In the case of spinal fusion, local gene delivery is the preferred method. The virus can be introduced directly to infect local cells in the target region (in vivo therapy). Alternatively, MSCs could act as the vehicle for ex vivo or cell-based gene transfer, in which genes for bioactive factors that promote osteoblastic differentiation could be introduced into the MSCs in vitro and implanted at the desired regeneration sites. Various sources of MSCs that could be used for ex vivo gene therapy include bone marrow, peripheral blood cells, muscle and fat. Both in vivo and ex vivo approaches to gene transfer have been studied in spinal fusion models. In vivo techniques are simpler and less time-consuming, but, reports of successful fusion using these techniques have been reported only in athymic rats. An ex vivo method for gene transfer is considered safer because the host is not directly exposed to the virus. In addition, ex vivo manipulations are more controlled and can be verified before application. The ex vivo technique also allows a desirable carrier material to be added to the infected cells. The carrier serves to localize the converted cells to the desired site and may also provide an osteoconductive scaffold. In summary, the in vivo approach tends to be straightforward, faster and less costly, whereas the ex vivo method is theoretically safer and more effective because genetic manipulations take place outside the patient's body, and the transduced osteoblasts or progenitor cells can serve not only as a source of osteogenic factor production but also as a primer site for bone formation.

#### *BMP2 gene therapy*

BMP-2 has been one of the most studied BMPs in spinal fusion, and clinical trials have produced promising results

[58–60]. The recombinant version of BMP-2 has been approved by the Food and Drug Administration (FDA) for anterior spinal interbody fusion. Sandhu et al. [61,62] studied rhBMP-2 in a dog model and found it to be superior to even autograft at 3 months. Although BMP-2 has been more extensively studied as a recombinant protein, BMP2-mediated gene therapy has also been investigated. Lieberman et al. [63] found noticeable differences in the new bone formation of rhBMP and adenovirus-mediated BMP-2. RhBMP-2 was seen to create lacelike bone-spanning defects, even with larger quantities of protein, whereas BMP-2-transduced cells formed robust, coarse trabecular bone [63]. Park et al. [48] directly compared adenoviral vectors and liposomal-delivery on bone healing in a rat femur model with cell-mediated BMP2 gene transfer. Although both treatment groups healed the critical-sized defect, the adenoviral group healed within 4 weeks, compared with 6 weeks for the liposomal group. These studies suggest that the time course and the quality of repair can be modulated with different gene transfer techniques and that gene therapy may improve on recombinant proteins for bone regeneration.

Several studies have shown that ex vivo BMP-2 transduction can be used with different lines of stem cells, including bone marrow [10,63], muscle [13,14] and, more recently, adipose tissue [64,65]. These stem cell transductions all resulted in healing of critical-sized bone defects in small animal models. In a spinal fusion study, Riew et al. [66] expanded bone marrow-derived MSCs and then transduced the stem cells for 1 day with adenoviral BMP-2 (AdBMP-2). They were then implanted in a posterior spinal fusion model at the L5–L6 intertransverse processes in rabbits. At 7 weeks after implantation, only one of five rabbits showed histologic and radiographic evidence of new bone formation. In view of these disappointing results, the researchers increased the period of ex vivo cell transduction from 1 to 7 days before the fusion procedure. With this change, new bone formation was noted at the lumbar intertransverse process in all three experimental rabbits [66]. This group also recently reported using ex vivo AdBMP-2 transduction to perform anterior spinal fusion on pigs [67]. Bone marrow cells were harvested from a resected rib and were expanded. Cells were then incubated overnight with AdBMP-2. Anterior arthrodeses were performed by a thoracoscopic technique, and the researchers reported a 100% fusion success rate by histologic and radiologic evaluation for six of six disc spaces treated with AdBMP-2, whereas none of the controls fused [67]. Wang et al. [68] have compared single-level posterolateral spine fusion rates between rhBMP-2 with various carriers, bone marrow transduced for 48 hours with AdBMP-2 and autograft in rats. They reported higher fusion rates with ex vivo BMP-2 transduction as compared with autogenous iliac crest bone graft (ICBG). All of the animals treated with AdBMP-2 and rhBMP-2 achieved solid fusion masses at 4 weeks, whereas none of the control groups fused. Qualitatively, the ex vivo AdBMP-2-treated rats produced abundant trabecular fusion masses,

whereas the rhBMP-2–treated rats exhibited thinner, lacelike trabecular fusion masses. The researchers concluded that ex vivo AdBMP-2 produced solid posterolateral spinal fusions in rats and was superior to ICBG alone [68].

In vivo delivery of adenoviral BMP-2 induced ectopic bone formation both in injections into thigh muscles of rats [69–71] and in rat spinal fusion models [72]. Alden et al. [72] injected AdBMP-2 at the lumbosacral junction in 12 athymic nude rats. All sites that received injections showed evidence of new bone formation both radiographically and histologically, although fusions were not noted [72]. These studies were carried out in immunocompromised animals. In similar studies in immunocompetent rats, inflammation was seen at the injection site [69]. Okubo et al. [73] induced transient immunosuppression in rats with cyclophosphamide and stimulated ectopic bone formation. Interestingly, when a BMP-2 adenoviral vector was directly injected into the fracture site in a rat fracture model, transgene expression was detected in various cell types, including chondrocytes, osteoblasts and osteoclasts, many of which were incorporated into the newly mineralized bone [74]. The development and refinement of in vivo techniques is enticing because it allows for direct injection of viral vectors into the fusion site, avoiding the costly and time-consuming steps of harvesting, transducing, and implanting stem cells associated with ex vivo approaches.

#### BMP7 gene therapy

BMP-7 is another osteogenic factor with a significant body of research. It was FDA-approved (available as a recombinant protein form and marketed as OP-1 [osteogenic protein-1]) to be used for recalcitrant nonunion of long bones. BMP-7 evidences new bone growth in vivo, promotes the proliferation of osteoblasts and maintains the osteoblast phenotype in vitro [75–77]. It has been used in clinical trials for spinal fusion [78–80] and for treatment of critical defects [81] and tibial nonunions [82,83]. More recent research has been directed toward BMP7 gene therapy to augment bone regeneration. Krebsbach et al. [84] showed that BMP-7–transduced fibroblasts exhibit an osteoblast phenotype and observed the subsequent formation of clinically evident bone. Hidaka et al. [85] recently reported successful posterolateral spinal fusion in athymic rats in a ex vivo study using bone marrow cells that were expanded for 4 weeks, then treated with AdBMP-7 and combined with an allograft osteoconductive scaffold. At 8 weeks after the index procedure, radiographic and mechanical fusion rates were 70% and 80%, respectively, in the experimental group, with no fusion seen in the control groups [85]. However, the amount of time required for cell expansion and transduction in preparation for the spinal fusion procedure in that study would make these strategies difficult and cumbersome to apply in clinical situations.

#### BMP6 and BMP9 gene therapy

Although much attention has been directed toward BMP-2– and BMP-7–mediated bone regeneration, it is not clear

whether they represent the most osteogenic BMPs. We have recently conducted a comprehensive analysis of both in vitro and in vivo osteogenic activity of adenoviruses expressing 14 types of human BMPs (BMP-2 through BMP-15) [86,87]. Recombinant adenoviruses expressing the 14 types of human BMPs were constructed to infect pluripotent mesenchymal progenitor C3H10T1/2 cells, preosteoblastic C2C12 cells and osteoblastic TE-85 cells [54,88]. Subsequently, their respective osteogenic activities were determined. Adenoviruses expressing BMP2, BMP6 and BMP9 showed the most potent induction of osteoblast differentiation of MSCs, while most of the BMPs (with the exception of BMP3) are able to stimulate osteogenesis in mature osteoblasts (Fig. 1). Interestingly, in our unpublished studies we have also found that several combinations of adenovirus-BMPs (such as BMP5 plus BMP10, BMP5 plus BMP12, BMP5 plus BMP13, BMP7 plus BMP10, BMP7 plus BMP12 and BMP7 plus BMP13) exhibited significant synergy in their ability to induce alkaline phosphatase activity. These findings are consistent with the notion that some BMPs may act as promoters at different nodal points in the process of osteogenesis.

One of the most effective stimulators of in vitro alkaline phosphate activity, BMP-6 has not received the attention of BMP-2 and BMP-7. Jane et al. [89] used adenoviral vectors to deliver BMP-2, BMP-4 and BMP-6 and compared their ability to induce new bone in athymic nude rats. Their results indicated that AdBMP-4 produced ectopic bone through mechanisms similar to endochondral ossification, whereas AdBMP-6 seemed to induce bone by way of mechanisms similar to both intramembranous and endochondral ossification. Interestingly, AdBMP-2 induced no bone growth at all [89]. Ultimately, the density of ectopic bone formed by AdBMP-4 and BMP-6 was comparable, but BMP-6 produced more rapid tissue calcification. Recently, Laurent et al. [90] confirmed the efficacy of in vivo AdBMP-6 therapy in percutaneous spinal fusion in rabbits. New Zealand white rabbits underwent fluoroscopically assisted lumbar intertransverse process injections of AdBMP-6. At 14 weeks, solid fusions were noted bilaterally at the levels injected with AdBMP-6 in four of the five rabbits, and the remaining rabbit had a unilateral fusion at the AdBMP-6 level. The controls showed no evidence of bone formation. These results suggest that percutaneously injected AdBMP-6 can induce solid spinal fusions in immunocompetent animals [90].

BMP-9 is one of the least studied BMPs despite recent evidence of its potent osteogenic activity [86]. The recent in vitro studies of BMP-9 [91,92] have been supported by several in vivo studies. Helm et al. [93] has shown that solid spinal fusion can be achieved at 16 weeks after injection of AdBMP-9 directly into the posterolateral region of mice. Similar results have been obtained by Dumont et al. [94] with ex vivo therapy as human mesenchymal cells transduced with AdBMP-9 successfully induced posterolateral spinal fusion in an athymic rat model. At 8 weeks, the sites treated with ex vivo AdBMP-9 showed copious bone formation

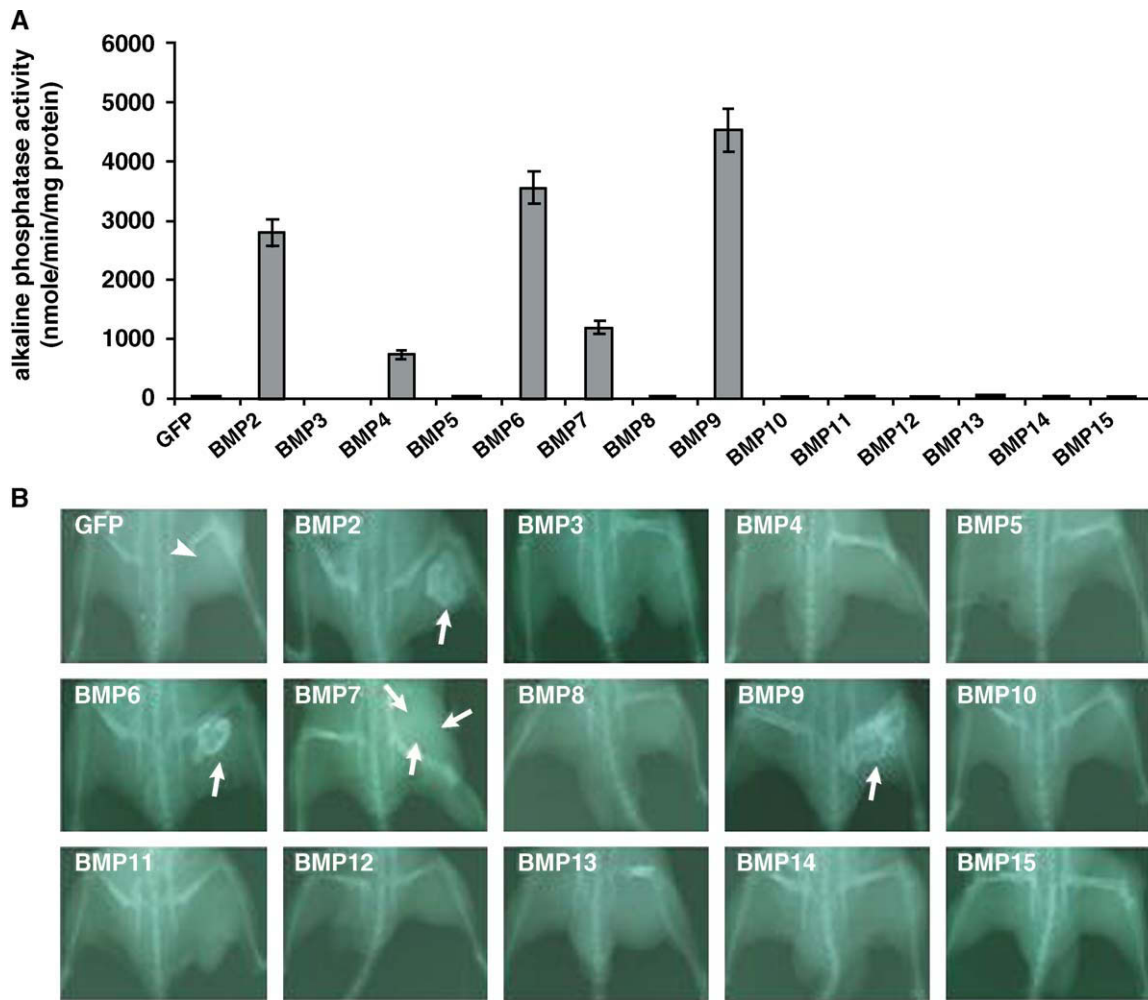


Fig. 1. Distinct in vitro and in vivo osteogenic activity of the 14 types of human adenoviral bone morphogenetic proteins (AdBMPs). (Top) Osteoblastic precursor C2C12 cells were infected with AdBMPs and the control adenoviral green fluorescent protein (AdGFP). Cells were lysed at 4 days after infection for colorimetric assays of the osteogenic marker alkaline phosphatase activity using *p*-nitrophenyl phosphate as a substrate. (Bottom) Orthotopic bone formation induced by AdBMP-transduced C2C12 in athymic nude mice. Exponentially growing C2C12 cells were infected with AdBMPs or the control AdGFP for 15 hours. Approximately  $5 \times 10^6$  of the infected cells were injected into the right quadriceps of athymic nude mice (a triangle indicated in the GFP group as an example). At 5 weeks after injections, mice were killed and subjected to x-ray radiography. Representative results from at least three independent experiments are shown. Adapted from Breyer et al. [54].

and solid spinal fusions, whereas the control injections showed no osteogenic activity [94]. We have recently used AdBMP-2, AdMBP-6 and AdBMP-9 in an ex vivo rabbit posterior spinal fusion model, in which bone marrow aspirate was mixed with the recombinant adenoviruses for 20 minutes. Successful posterolateral fusions were induced in all treatment groups (Phillips and He, unpublished data, 2003). Although more research is required, the current in vitro and in vivo studies strongly suggest that BMP-6 and BMP-9 may represent more potent osteogenic factors than the prototypic BMPs currently used for bone regeneration.

#### *LMP-1 and LMP-3 gene therapy*

Although BMPs represent an important group of biologic factors that promote bone formation, other factors have also

recently been shown to enhance bone formation. One example is LIM mineralization protein-1 (LMP-1), a novel osteoinductive intracellular protein. LMP-1 is a member of the heterogeneous LIM domain family of proteins whose functions include a variety of fundamental processes, such as transcriptional regulation [95], organization of the cytoskeleton [96] and cell type development [97]. Unlike the BMPs, which are secreted proteins that use receptor-ligand interactions, LMP-1 is thought to be an intracellular signaler involved in osteoblast differentiation [98]. Recently, it has been shown that increased concentrations of BMP-2, BMP-4, BMP-6, BMP-7 and TGF- $\beta$ 1 have been detected in LMP-1-transduced cells [98,99]. Although the exact mechanism of LMP-1 remains unclear, solid spinal fusions have been achieved in rat and rabbit models using ex vivo LMP-1-transduced bone marrow cells and buffy-coat blood cells.

Boden et al. [100] reported solid posterior fusion in an athymic rat model using bone marrow cells transduced for 2 hours with AdLMP-1 in a demineralized bone matrix carrier. Viggewarapu et al. [15] showed bone marrow or buffy-coat cells transduced with AdLMP-1 for 10 minutes combined with demineralized bone matrix or collagen-ceramic-composite sponges induced posterolateral lumbar fusion in rabbits.

Another LIM mineralization protein, LMP-3, has recently been shown in mice to promote new bone formation more vigorously than BMP-2 both *in vitro* and *in vivo*. In this investigation, LMP-3 was transduced by plasmid and by adenoviral vectors, producing increased alkaline phosphatase production *in vitro* and large amounts of ectopic bone after intermuscular injections in mice [101].

#### *Use of PTH1-34 peptide and GAM in bone regeneration*

While not yet extensively tested in spinal fusion models, recent studies have developed a gene-activated matrix (GAM) consisting of a collagen sponge that provides the scaffolding to promote cell ingrowth and deliver plasmid DNA directly to the cells involved in fracture repair [42,102]. The plasmid DNA is taken up by local granulation tissue, fibrocytes and capillaries that have migrated into the degradable matrix. These cells then transiently express the plasmid DNA that stimulates the bone regeneration cascade in the area. Fang et al. [102] reported that a 5-mm critical femoral defect in Sprague-Dawley rats healed when treated with GAMs impregnated with BMP-4 complementary DNA (cDNA) or a fragment of parathyroid hormone (PTH1-34) cDNA. Although both cDNAs induced healing of the defects, the bone regeneration occurred more rapidly when both cDNAs were included in the GAM, occurring at 4 weeks instead of 9 weeks. This suggests a possible synergistic effect between BMP-4 and PTH1-34, which is attributed to induction of PTH-dependent cascades [17]. A canine model of a tibial critical defect produced similar results that showed retention and expression of the plasmid for 6 weeks, and bone induction was observed in a dose- and time-dependent manner, although the critical-sized defects did not heal [42]. This type of gene therapy has theoretical advantages because it uses plasmid DNA, so that there are no gene size limitation or any safety concern about the immunogenicity of viral vectors (especially adenoviral vectors) and is inexpensive to produce in large quantities.

#### **Conclusion and future directions**

Spinal fusion will continue to be an important part of the surgical treatment of spinal pathology for the foreseeable future. Challenges to successful spinal fusion surgery include autograft donor site morbidity and pseudoarthrosis. Recent advances in the understanding of the biology of bone formation have allowed the development of therapeutic

biologics. Gene therapy may represent the easiest method for the application of bone-forming biologic agents to spinal fusion. Viral vectors, particularly the adenovirus, may serve as transports for the BMP-encoding genetic material. Advantages of adenoviral vectors include transient gene expression, large gene capacity and compatibility with a wide range of host cells. The primary disadvantage of the adenoviral vector is the high degree of immunogenicity. An adenovirus may be used directly *in vivo* at the fusion site or in an *ex vivo*, cell-based manner. Viral vectors for gene therapy for spinal fusion in humans will need to have a strong biosafety profile and low immunogenetic potential before they become widely used.

#### **Acknowledgment**

We thank Dr. Hue H. Luu of The University of Chicago Hospitals for his critical comments on the manuscript.

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