PIG ANTERIOR SPINE FUSION INDUCED BY ADV-BMP2 TRANSDUCED AUTOLOGOUS BONE MARROW DERIVED MESENCHYMAL STEM CELLS

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Relevance to Musculoskeletal Conditions

BMP-2 gene transfer into bone marrow derived mesenchymal stem cells via an adenoviral vector could induce pig anterior spine fusion.

Introduction

Our previous studies (1,2,3) demonstrated that BMP-2 gene transfer via adenoviral vector into mesenchymal progenitor cells could effectively induce bone formation in both immunodeficient animal and immunocompetent animal. Results from these studies indicated that BMP-2 gene transferred mesenchymal progenitor cells could sustained release BMP to affect the cell differentiation in autocrine and paracrine fashion. In this paper, we further report that natural pig bone marrow mesenchymal stem cells (MSC), which are feasible for clinical applications, could induce the pig anterior spine fusion after transduce with recombinant adenovirus carrying human BMP-2 gene (Adv-BMP2). The purpose of the present study is to develop a minimally invasive surgical technique for fusing the anterior spine by injecting gene-transferred mesenchymal stem cells into the thoracic disc spaces.

Materials and Methods

Bone marrow derived mesenchymal stem cells were isolated from pigs’ ribs and expanded in culture in vitro. The MSC cells were transduced with either Adv-BMP2 or the control virus Adv-ßgal at 50 pfu/cell. In Vitro: BMP-2 protein expression in conditioned media was tested by immunoprecipitation and Western blot analysis using specific monoclonal antibody h4b2/5.10.24 (gift from Genetic Institute). Alkaline phosphatase activity (ALP) and matrix mineralization (Von Kossa and Alizarin Red stainings) were examined to detect the in vitro differentiation effect of transduced MSC. Protein expression of Type I collagen, osteopontin, and bone sialoprotein were analyzed by Western blot. In Vitro: Three pigs underwent general anesthesia and thoracoscopic visualization of the anterior thoracic spine was obtained. Three non-contiguous disc spaces were prepared by a 5-7 mm puncture of the annulus with a small curved curette followed by minimal penetration of the endplates such that some bleeding was obtained. The prepared discs were then injected with autologous MSC transduced with either Adv-BMP2 or Adv-ßgal (control 1) or non-transduced MSC (control 2). Pigs were sacrificed on week 6 post implantation. Harvested specimens underwent radiographic and histologic examination to observe the effect of anterior spine fusion.

Results

A human BMP-2 protein band at molecular weight 20 Kd was detected by specific antibody in the conditioned medium of Adv-BMP2 transduced pig MSC. No such band was observed in the conditioned media of Adv-ßgal transduced MSC or untreated MSC. ALP activity of Adv-BMP2 transduced MSC increased five fold when compared to those of Adv-ßgal transduced MSC or untreated MSC. Matrix mineralization was induced in Adv-BMP2 transduced MSC but not in Adv-ßgal treated cells or untreated cells. Consistent with the expression of functional BMP-2 protein, Adv-BMP2 transduced protein expression of Type I collagen, osteopontin, and one sialoprotein. In vivo study, anterior spine fusion was demonstrated by radiographic examination in all 3 pigs. Moreover, anterior spine fusion was observed only in the disk space where Adv-BMP2 transduced MSC were autologously implanted. Histology examination further confirmed new bone formation in the disk space implanted with Adv-BMP2 transduced MSC. No spine fusion or new bone formation was evidenced in the disk space where control virus Adv-ßgal transduced MSC or untreated MSC were autologously implanted.

Discussion

In this study, we demonstrated that recombinant adenoviral vector mediated BMP-2 gene transfer into pig MSC could induce the cell to produce BMP-2 protein. The transduced MSC differentiated into osteoblast cell type in vitro, demonstrated by marked increase in ALP activity and characteristic matrix mineralization. Furthermore, autologously implanted Adv-BMP2 transduced MSC into the disk space induced anterior spine fusion. Our results suggest the possibility of developing a minimal invasive procedure based on our strategy for anterior spine fusion.

Acknowledgment

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References


Figure 1. Pig anterior spine fusion induced by autologous implantation of Adv-BMP2 transduced MSC. Solid Arrow: Adv-BMP2; Outline Arrow: Control.

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