BMP 12 GENE TRANSFERRED MESENCHYMAL PROGENITOR CELLS COULD FORM TENDON LIKE TISSUE IN VIVO

+Lou J, Ludwig FJ, Zhang JF, Manske PR Department of Orthopaedic Surgery Washington University School of Medicine St. Louis, MO 63110

+Department of Orthopedic Surgery, Washington University School of Medicine
One Barnes Hospital Plaza, STE 11300 St. Louis, MO 63110
phone: (314)362-8484 fax: (314)362-0334 e-mail: louj@msnotes.wustl edu

Relevance to Musculoskeletal Condition

The new member of Bone Morphogenetic Protein (BMP) family , BMP 12, may have function related to tendon.

Introduction

A new member of human BMP family has been recently cloned and named BMP-12. The sequence analysis indicates that BMP-12 is human homologue of mouse growth/differentiation factor (GDF)-7 and belongs to a new subgroup in the TGF-b superfamily. Functionally, BMP-12 appears different from other BMP proteins. In *in vitro* studies, BMP-12 has little affect on AlP activity of myoblasts and osteoblasts. And no AlP activity was induced by BMP-12 in the cell lines which responded to both BMP-2 and TGF-b1. In *in vivo* experiments, subcutaneous BMP-12 protein implants did not induce bone formation but rather in the formation of tendon and ligament tissue. These experiments infer that BMP-12 may be the growth factor whose function relates to tendon and ligament but not bone. This study reports that delivery of human BMP12 gene transduced mesenchymal progenitor cells forms tendon like tissue *in vivo*.

Materials and Methods

A replication deficient adenovirus carrying human BMP-12 (Adv-BMP12) was constructed. The adenovirus mediated BMP-12 gene transfer and expression was detected by immunoprecipitation and western blot with specific monoclone antibody F2B12/9.5.13 (gift from Genetic Institute). The mesenchymal progenitor cell line C3H 10T 1/2 was transduced with Adv-BMP12, as well as with a control adenovirus carrying bgal gene (Adv-bgal), *in vitro* at 50 plague formation unit (pfu)/per cell. The transduced cells were trypsinized the following day and adjusted to 10^7 cell /ml with PBS. Eight nude mice Crl:NU/NU-nuBR received transduced cell injections to both legs. In each mouse, the right thigh received 10^6cell/0.1ml Adv-BMP12 cells, and the left thigh received 10^6cell/0.1ml Adv-bgal cells as control. The legs were harvested at 2 weeks and 4 weeks following cell injection. Harvested legs were processed with standard histology procedures and examined microscopically.

The experimental design was approved by the Animal Studies Committee, Washington University School of Medicine.

Results

Secreted BMP-12 protein was detected in conditioned media from C3H 10T 1/2 cell transduced with Adv-BMP12. Immunoprecipitation and western blot assay with specific antibody demonstrated a band at 19 Kd, which fit the size of the BMP-12. No such band was observed in the media from non-treated cells or Adv-bgal transduced cells. No significant cell proliferation was seen in the Adv-BMP12 transduced cells compared to control cells. Microscopy showed that all right legs (Adv-BMP12 cell injected) presented new tissue formation in thigh muscles. There were two new structures observed: one was tendon-like tissue and the other was cartilage-like tissue. The tendon-like tissues appear at the outer layer of the new tissue and surrounded the cartilage-like tissue core. No significant difference was observed in these tissue structures between the 2 weeks and 4 weeks samples. No new tissue structures were found in any of the left thighs muscles that received Adv-bgal cell injections. The anatomy of the left thigh muscles show no obvious change.

Discussion

Our data indicated that BMP12 gene transfer into mesenchymal progenitor cell can induce tendon-like tissue formation *in vivo*. This result give the new evidence that BMP-12 may be functionally related to tendon. In addition to the tendon-like tissue, a cartilage-like tissue core has also been found in the new tissue structure. This indicates that BMP12 still has the function to induce osseous lineage, which may participate in tendon-bone junction repair. BMP-12 is a new member of the BMP family and its specific function still needs to be investigated.

Acknowledgments

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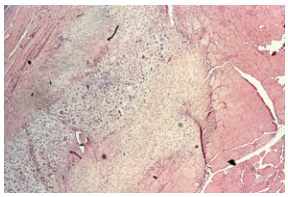

Fig. 1. Histologic examination of harvested nude mice leg injected with Adv-BMP12 transduced C3H/10T 1/2 cells.

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