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

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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 AIP activity of myoblasts and osteoblasts. And no AIP 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 BMP-12 gene transduced mesenchymal progenitor cells forms tendon like tissue in vivo.

Materials and Methods
A replication deficient adeno virus 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 monoclonal 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 plaque formation unit (pfu)/per cell. The transduced cells were trypsinized the following day and adjusted to 10^5 cell /ml with PBS. Eight nude mice CrI:NU/NU-nuBR received transduced cell injections to both legs. In each mouse, the right thigh received 10^7 cell/0.1ml Adv-bgal cells as control. The legs were harvested at 2 weeks and 4 weeks following cell injection. Harvested legs were conditioned 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.

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.

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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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