USE OF DIRECT BMP-2 GENE DELIVERY TO ENHANCE THE HEALING OF SEGMENTAL BONE DEFECTS: EVALUATION IN THE RAT MODEL

Introduction
There is a pressing clinical need for improved methods for repairing osseous defects. Previous studies have shown that the delivery of genes encoding osteogenic proteins lead to the healing of osseous defects that otherwise would not heal. In many of these studies ex vivo gene transfer approaches were used which are more cumbersome and more expensive than the direct in vivo gene delivery approach (1-3). The direct in vivo method does not require the use of osteoconductive matrices or scaffolds like collagen, which was found to impede in vivo cell transduction by adenoviral vectors (4). In an earlier preliminary, experimental study an adenoviral vector was used to transfer a cDNA encoding human bone morphogenetic protein-2 (BMP-2) to critical sized femoral defects in rabbits (5). In order to expedite development of a clinically applicable gene therapy for the treatment of bone defects in humans we evaluated the use of direct BMP-2 gene delivery in a well established rat critical sized defect model.

Materials and Methods
Vector Production
We previously constructed first generation adenoviruses carrying human BMP-2 cDNA and the LacZ gene. These viruses lack the E1 and E3 regions of the adenoviral genome. The transgenes were cloned into the E1 domain, with expression driven by the cytomegalovirus early promoter. The virus was grown and purified by routine methods using 293 cells.

Critical sized defect model
5 mm segmental, critical sized defects were created in the right femora of Sprague–Dawley rats with a dental burr. The bones were stabilized by an external fixator with 1.1 mm Kirschner wires as pins using the method of Einhorn et al. (6). The surrounding muscle was closed around the lesion, producing a closed chamber between the cut ends of the bone. The rats were divided into three groups. The first group received the vector containing the BMP-2 cDNA (Ad-BMP-2), the second group received the vector containing the LacZ gene (Ad-LacZ) as a negative control. The defect of the third group remained untreated.

A dose of 4 x 10⁹ viral particles was administered 24 hours after surgery by a single injection into the defect. A hole was manufactured in the middle of the fixator in order to guide the needle into the middle of the defect. Healing was monitored by weekly X-ray. On day 56 the rats were euthanized and femora evaluated for bone healing histologically, using hematoxylin and eosin staining, and by micro-computed tomography (µCT). Additional sections were stained for expression of the LacZ marker gene.

Results
Immediately after surgery the defects between the distal and the proximal femur had the same size in all groups (Fig.1A). Healed defects were defined as those with bone bridging of at least 75% of the defect side. After 8 weeks, all defects treated with the BMP-2 gene were healed (Fig. 1B, Fig. 2A), whereas none of the control rats healed within this time (Fig. 2B).

The histological evaluation confirms ossification across the entire defect in response to the BMP-2 gene, whereas the control defects were predominantly fibrotic and sparsely ossified. The µCT evaluation confirms complete bridging for the Ad-BMP-2 group, but incomplete healing for the controls. Even 12 weeks after surgery the untreated defects did not heal.

Three days after injection, staining for the LacZ marker gene showed high gene expression in the surrounding muscle tissue (data not shown).

Discussion
The experiments presented here demonstrate the ability of direct BMP-2 gene delivery by an adenoviral vector to enhance healing of a critical sized defect in a long bone in rats. The radiographic, histological and µCT results showed that complete bridging of the defect was achieved after 8 weeks in the BMP-2 treated group. The study revealed high gene expression of the transgene by the skeletal tissue surrounding the defect. The muscle surrounding the defect might guide bone healing and also serve as a possible local source of osteoprogenitor cells.

The results suggest that this model is suitable for development of genetic approaches to enhance bone healing.

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References:

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Figure 1: X-ray of a rat femur with a critical sized defect (5mm) after surgery (A) and defect treated with Ad–BMP-2 after 8 weeks (B).

Figure 2: µCT image of an Ad-BMP-2 treated bone defect (A) and an untreated control (B) after 8 weeks.