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Dual Roles of Ligamentum Flavum for Spinal Fusion: As an Osteoinductive Agent and Carrier for Ex-vivo Gene Transfer

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– Abstract –

Study Design: An in- vitro experiment using human ligamentum flavum (LF) and the adenovirus- BMP- 2 construct, Ad/BMP- 2.

Objectives: To determine the dual roles of LF as an osteoinductive agent and carrier for ex- vivo gene transfer.

Summary of Literature Review: LF is known to have osteogenic potential. Pathologically, ossified LF may cause myelopathy and radiculopathy. BMP- 2 is known as an important factor in the differentiation, and maintenance, of osteoblast phenotypes. Ex- vivo gene transfer, using human LF for spinal fusion, has never been attempted before.

Materials and Methods: The LF cells were cultured from the degenerated LF of spinal stenosis patients. An adenovirus construct, containing BMP- 2 cDNA (Ad/BMP- 2), was also produced. The LF cell cultures were exposed to the adenoviral construct. The Osteocalcin expression was analysed by Western blot analysis. The osteocalcin and BMP- 2 mRNA expressions were analysed by RT-PCR. Bone formation was assessed by alkaline phosphatase and Von Kossa stains.

Results: The LF cell cultures, with Ad/BMP- 2, showed transgene expression in the Western blot analysis. Also, the cultures exhibited the mRNA expressions of both osteocalcin and BMP- 2, in a dose- dependent manner. The LF cultures, with Ad/BMP- 2, demonstrated alkaline phosphatase expression and bone nodule formations from the Von Kossa staining.

Conclusion: The genetically modified LF strongly induced osteogenesis, which can be used during a spinal fusion, as an osteoinductive agent and carrier, for ex- vivo gene transfer.

Key Words: Ligamentum flavum, BMP- 2, Adenovirus, Gene transfer

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plaque forming unit (PFU) MOI 1 Von

PFU 1 PFU 100 virus particles Kossa, 3% silver nitrate 30 가

3. Reverse Transcriptase-Polymerase Chain Reaction

BMP-2 osteocalcin mRNA

D-PBS RNA TRIzol reagent(Life Technologies)

RNA 1 µg Oligo d(T)16 primer 2.5 µM, dNTP mixture 20mM, 10x buffer 2 µl, 20U/µl RNase inhibitor, 25mM MgCl2 4 µl, MuLV Reverse transcriptase 2.5U/µl 20 µl 가 , 42 30 , 95 5 , 4 5 cDNA . 1.

cDNA , 20 µl Ad/BMP-2 BMP-2 mRNA 가 Ad/BMP-2 BMP-2 mRNA 가 (50, 100, 150 MOI) BMP-2 mRNA 가 (Fig. 1). BMP-2 가

4. Western Blot analysis

osteocalcin

Lysis buffer (0.5% Triton X-100, 10Mm HEPES, 150Mm NaCl, 0.02% sodium azide, protease inhibitor mixture) (Sigma) cell lysate 13% tricine-SDS gel . Osteocalcin 1x TBST with 5% block blotted membrane rabbit anti-osteocalcin antibody (1:10,000 dilution) (Chemicon international, Temecula, CA) 1 . Membrane 1x TBST secondary antibody (1:10,000 dilution of goat anti-rabbit IgG, horseradish peroxidase conjugated, Santa Cruz, CA) 45 . Immuno-reactive bands 1xTBST 3 ECL kit(Amersham Pharmacia, Piscataway, NJ) .

2. Ad/BMP-2 mRNA 가 Ad/BMP-2 osteocalcin mRNA 가 (50, 100, 150 MOI) osteocalcin mRNA 가 (Fig. 1). Osteocalcin Blot analysis Chinese hamster ovary osteocalcin 16kDa osteocalcin

5. Alkaline phosphatase, Von Kossa

Alkaline phosphatase buffered acetone (Sigma) citrate osteocalcin Ad/BMP-2 osteocalcin 가 (Fig. 2).

30 , 45 alkaline-dye mixture (Sigma) well 30 2 3. Ad/BMP-2 Ad/BMP-2 (50, 100, 150

Mayer 's Hematoxylin 10

MOI) 14 (bone nodule)
 . Ad/BMP-2 가
 () ,
 Ad/BMP-2 7), 가 alkaline phosphatase
 alkaline phosphatase Von
 Kossa alkaline phos-
 phatase (Fig. 3). alkaline phosphatase
 collagen type I III
 cytoplasm extracellu-
 lar matrix osteonectin , non-collagenous
 cytoplasm 7),
 (BMP-2)
 BMP-2 가
 5), ,
 , BMP-2 가 1,2,4,6,7,12)
 가 가
 가 가 2 BMP-가
 in-vivo (,)
 ex-vivo 가 가
 , 9,13), 가 가
 , alkaline phosphatase , Von Kossa
 BMP-2
 BMP-2 가
 BMP-2
 가

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: adenovirus-BMP-2 (Ad/BMP-2)
 :
 : Ad/BMP-2
 cin mRNA Western Blot analysis BMP-2 mRNA osteocal-
 phosphatase, Von Kossa osteocalcin alkaline
 : Ad/BMP-2 BMP-2 mRNA osteocalcin mRNA
 osteoclastin alkaline phosphatase, Von
 Kossa
 : BMP-2
 : , BMP-2, ,