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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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5).

2, 12).

가  
가

1,2,6,12).

transforming growth factor- 1 (TGF- 1)  
genetic protein (BMP)가

bone morpho-

BMP-2

가 가

TGF- superfamily

가

가

가

1).

가

가

BMP-2

8,10). BMP-2

1.

7

BMP-2

BMP-2  
가

BMP-2

D-PBS  
, 5%

가

2,6).

Dulbecco's Modified Eagle's medium

(DMEM )

가

250 U/ml

가

1A collagenase가

DMEM

2

5%

,

가

가 가

7).

10%  
streptomycin  
5% CO<sub>2</sub>

100 unit/ml penicillin, 100 µg/ml  
DMEM

3

가

가 2

trypsin/EDTA

가

2.

E1 E3

5

가

가

. E1

BMP-2 cDNA (Ad/BMP-2)  
cytomegalovirus promoter

BMP-2

human embryonic kidney 293 cell

Multiplicity of infection (MOI)

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( Li-fe 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

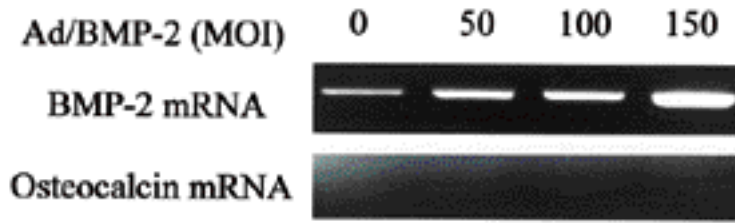
osteocalcin

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) .

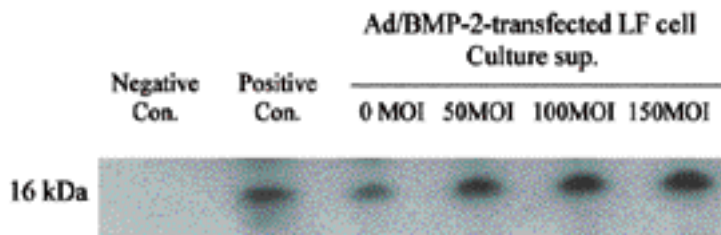
2. Ad/BMP-2 mRNA 가 Ad/BMP-2 osteocalcin mRNA 가 (50, 100, 150 MOI) osteocalcin mRNA 가 (Fig. 1). Osteocalcin Blot analysis Chinese hamster ovary osteocalcin 16kDa osteocalcin

### 5. Alkaline phosphatase, Von Kossa

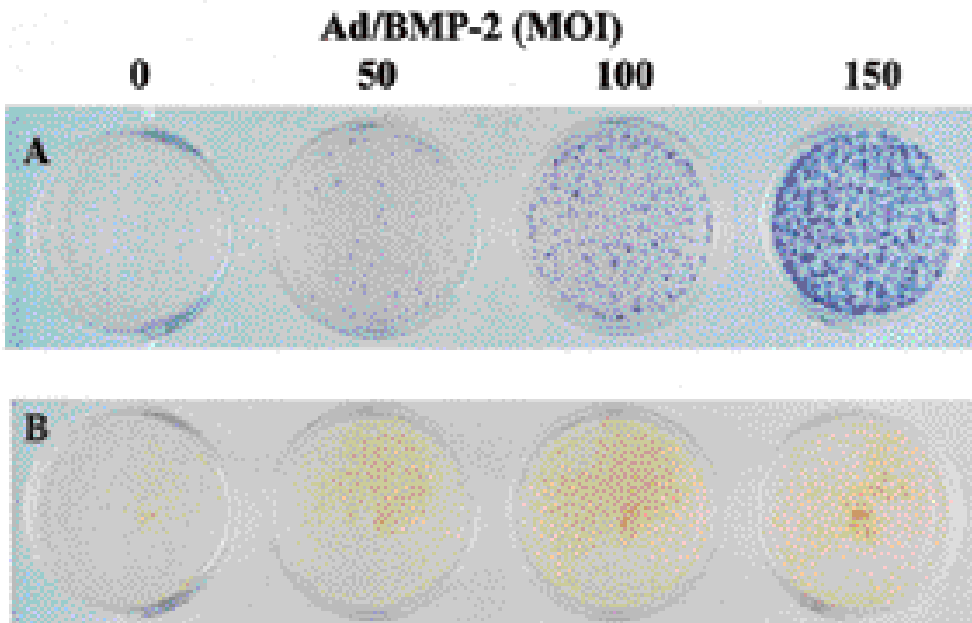
Alkaline phosphatase citrate osteocalcin buffered acetone (Sigma) 30 , 45 (Fig. 2). alkaline-dye mixture (Sigma) well 30 2 3. Ad/BMP-2 Mayer 's Hematoxylin 10 Ad/BMP-2 (50, 100, 150



**Fig. 1.** Transgene expression (BMP-2 mRNA) and expression of osteogenic phenotype (osteocalcin mRNA) in cell culture of ligamentum flavum with an Ad/BMP-2 (MOI of 50, 100, 150). Expression was detected by reverse transcriptase polymerase chain reaction.



**Fig. 2.** Expression of osteocalcin protein in supernatant of ligamentum flavum cell culture with an Ad/BMP-2 (MOI of 50, 100, 150). Expression was detected by Western Blot analysis. Negative control denotes CHO cell without Ad/BMP-2 and positive control denotes saline with osteocalcin protein.



**Fig. 3.** Ligamentum flavum cell culture with various dose of Ad/BMP-2 (MOI of 50, 100, 150) showed dose dependent increase of reactivity with (A) alkaline phosphatase stain and (B) Von Kossa stain, while culture with saline exhibited negative stain.

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

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adenovirus-BMP-2 (Ad/BMP-2)

Ad/BMP-2

BMP-2 mRNA

osteocalcin

osteocalcin mRNA

alkaline phosphatase, Von Kossa

Western Blot analysis

Ad/BMP-2

BMP-2

osteocalcin mRNA

alkaline phosphatase, Von Kossa

, BMP-2,