

Bone Morphogenetic Protein-2 cDNA :

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Osteogenesis by Transfer of Bone Morphogenetic Protein-2 cDNA in Ligamentum Flavum Cells : Approach Toward Tissue Engineering

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– Abstract –

Study Design : In- vitro experiment.

Objectives : To determine the effect of bone morphogenetic protein- 2 in the osteogenesis of human ligamentum flavum cells and test the feasibility of gene transfer to these cells.

Summary of literature review : Bone morphogenetic protein- 2 (BMP- 2) is known to be an important factor in the differentiation and maintenance of the osteoblastic phenotype. Tissue engineering for osteogenesis in ligamentum flavum by BMP- 2 and gene transfer has not been previously studied.

Materials and Methods : Ligamentum flavum cells were harvested and cultured from surgical patients with spinal stenosis. BMP- 2 was produced by transfecting pcDNA3.1/Hygro/BMP- 2 into CHO cells using Lipofectamine 2000. Adenovirus- lacZ (Ad/lacZ) was also produced, and administered with BMP- 2 to cell culture. The expression of lacZ was analyzed by X- gal staining. Bone formation was assessed by alkaline phosphatase, von Kossa, and alizarin Red- S staining, and the expression of osteocalcin was determined immunocytochemically.

Results : Ligamentum flavum cell cultures with Ad/lacZ showed marker gene expression. BMP- 2 induced osteogenesis in ligamentum flavum cells as evidenced by alkaline phosphatase, von Kosa, and alizarin Red- S staining. Also, cell culture with BMP- 2 showed strong positivity with osteocalcin by immunocytochemistry.

Conclusion : BMP- 2 more strongly induced the osteogenesis of ligamentum flavum, and also its gene transfer to ligamentum flavum was found to be feasible. These results may open a new era of ligamentum flavum tissue engineering.

Key Words : BMP- 2, adenovirus, osteogenesis, Tissue engineering

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3.

E1 E3
 lacZ
 cytomegalovirus promotor
 kidney 293 cell
 Multiplicity of infection (MOI) plaque forming unit (PFU)
 100 virus particles

6. Osteocalcin

1 × 10⁴ / well
 chamber slide 16
 37 °C, 5% CO₂
 5% human embryonic kidney 293 cell
 1000 ng/ml BMP-2
 7, PBS
 H₂O₂ 5
 3, Anti-Osteocalcin Antibody (Chemicon, Temecula, CA)
 3% BSA
 slide 4 16
 PBS 3, Biotinylated Link (Dako, Glostrup, Denmark)
 PBS 3 Streptavidin-HRP (Dako, Glostrup, Denmark)
 PBS 3, AEC
 Substrate-Chromagen (Dako, Glostrup, Denmark)
 15 3
 Mayer's Hematoxylin 10
 mounting slide

4.

-galactosidase (lacZ)
 1 0.5% glutaraldehyde
 4 15 PBS
 X-Gal substrate 37 2
 eosin
 hema-toxylin-eosin 37 (Nikon, Labophot-2, Japan)
 LacZ

5. Alkaline phosphatase, Von Kossa, Alizarin Red-S

Alkaline phosphatase buffered acetone (Sigma, St Louis, Mo)
 30 45
 alkaline-dye mixture (Sigma, St Louis, Mo) well
 30
 2 Mayer's Hematoxylin 1
 10 Von Kossa, 3% silver nitrate 30
 가
 2 Alizarin red-S, 40 mM Alizarin red(pH 4.2) 10
 2, PBS
 10

1.

Ad/lacZ galactosidase (Fig. 1).

2.

BMP-2 BMP-2 14 BMP-2 (bone nodule) BMP-2 osteocalcin 2000 ng/ml BMP-2A 2000 ng/ml Osteocalcin (Fig. 2). BMP-2 Alkaline phosphatase, Von Kossa

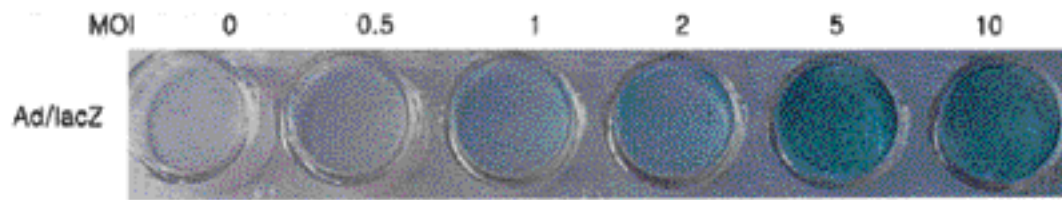


Fig. 1. Expression of marker gene (lacZ) in cell culture of ligamentum flavum with an MOI of 0.5, 1, 2, 5, 10.

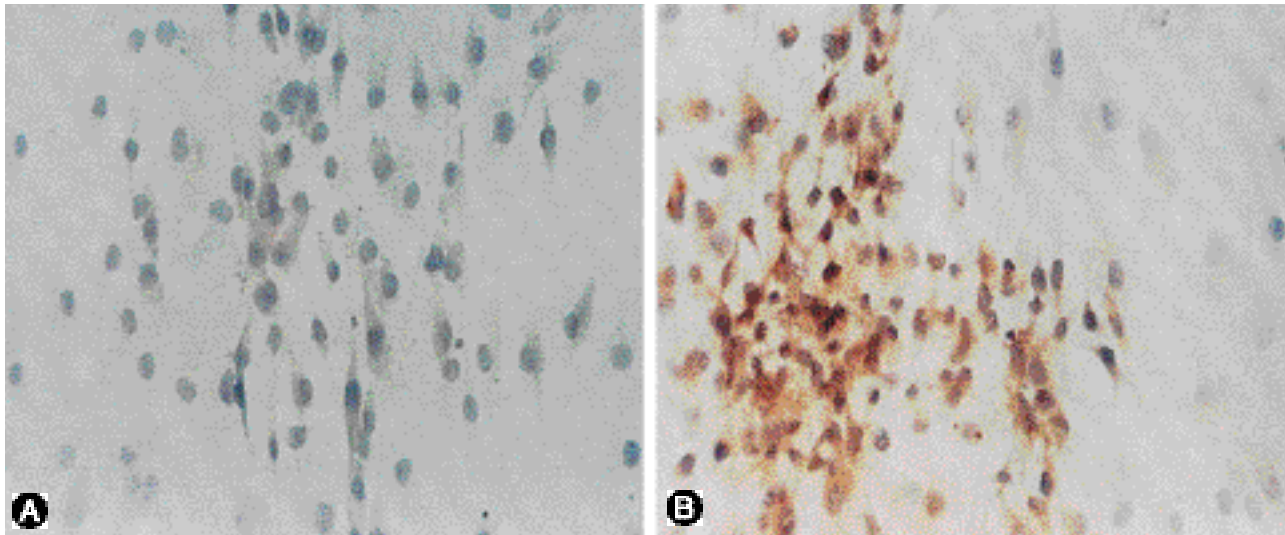


Fig. 2. Ligamentum flavum cell culture with high dose of BMP-2 (2000 ng/ml) expresses osteocalcin in immunocytochemistry. A: control culture without BMP-2, B: culture with BMP-2.

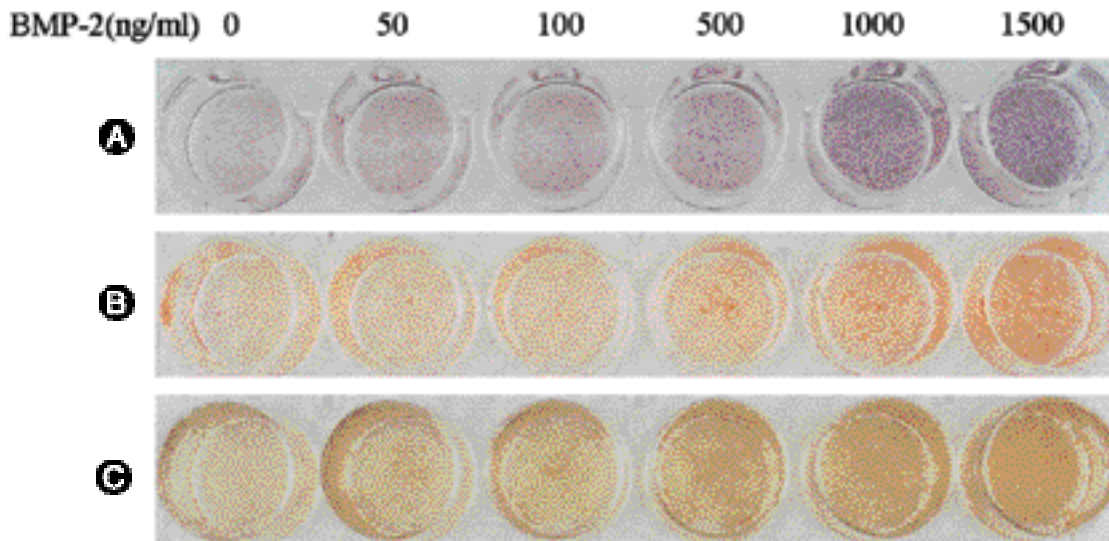


Fig. 3. Ligamentum flavum cell culture with various dose of BMP-2 showed dose dependent increase of reactivity with (A) alkaline phosphatase stain, (B) von Kossa stain, (C) Alizarin Red-S stain.

Alizarin Red-S
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mineralization

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(Fig. 3).

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^{1,2,4,5,6,10)}

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BMP-2
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BMP-2

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BMP-2

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, BMP-2

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alkaline phosphatase

alkaline phosphatase
collagen type

BMP-2

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I III
cytoplasm extracellular matrix
collagenous osteonectin
plasm

, non-
cyto-

⁶⁾

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bone morphogenetic protein-2 가

: BMP-2 BMP-2 bone morphogenetic protein-2(BMP-2) 가

CHO pcDNA3.1/Hygro/BMP-2 Ad/lacZ BMP-2 BMP-2 Lipofectamine 2000 -lacZ (Ad/lacZ) X-gal alkaline phosphatase, von Kossa, alizarin Red-S osteocalcin

: Ad/lacZ , BMP-2 alkaline phosphase, von Kosa, alizarin Red-S osteocalcin 가

: BMP-2, ,