

: TGF- 1

Matrix Synthesis of Human Intervertebral Disc Cells According to Grade of Degeneration: Under the Basal State and TGF- 1 Stimulation

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– Abstract –

Study Design : *In vitro* experiment to determine the matrix synthesis of human intervertebral disc (IVD) cell according to grade of degeneration.

Objectives : To quantify proteoglycan synthesis of human IVD cells in various grade of degeneration under the stimulation of TGF- 1.

Summary of Literature Review : Sophisticated method to delivery of growth factors, in continuous manner, is the genetic modification of disc cells through gene transfer. Poor responsiveness of degenerated disc to anabolic stimuli can mitigate potential application of growth factor or therapeutic gene transfer in the management of degenerative disc disease

Materials and Methods : IVD tissue was obtained from nineteen patients during surgical disc procedure. Grade of degeneration was confirmed by preoperative MRI. Isolation and three dimensional culture of disc cells were performed. Disc cells were treated with exogenous TGF- 1. Newly synthesized proteoglycans were assessed by ³⁵S- sulfate incorporation using chromatography on Sepadex G- 25 in PD- 10 columns. One- way analysis of variance with Fishers protected LSD post- hoc test was performed to compare amount of newly synthesized proteoglycans and power analysis was also conducted. Significance level was set p<0.05.

Results : Difference between cultures of control (grade I) and degenerated (grade II- V) discs in proteoglycan synthesis in basal condition was statistically insignificant (p=0.35, power=0.21- 0.63). Difference between cultures of control and degenerated disc in proteoglycan synthesis under the stimulated condition with TGF- 1 was also statistically insignificant (p=0.54, power=0.24- 0.47). However cultures in stimulated condition showed increased amount of newly synthesized proteoglycans compared to those of basal condition regardless of the grade of degeneration (p<0.05).

Conclusion : Anabolic response of human intervertebral disc cells is relatively insensitive to grade of disc degeneration, which facilitate application of gene therapy in various conditions of disc degeneration.

Key Words : Disc degeneration, TGF- 1, Proteoglycan

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(TGF- β 1) 가 .

가 ^{1,4)} 2 ^{5,13,15)} 1. ⁶⁾ , , 19 (31 ~50) 가 (transforming growth factor- β 1, osteogenic protein-1, insulin like growth factor-1) ¹⁰⁾ . Grade I () 3 , Grade II 3 , Grade III 6 , Grade IV, 3 , Grade V 4 . Geys balanced salt solution (GBSS, GIBCO-BRL, Grand Island, NY) 20 ⁷⁾ . ⁹⁾ 5% heat-inactivated fetal bovine serum (FBS, GIBCO-BRL, Grand Island, NY), 0.2% pronase (Calbiochem, La Jolla, CA), 0.004% deoxyribonuclease II type IV (DNase, Sigma, St. Louis, MO) Hams F-12 medium and Dulbeccos Modified Eagle Medium (F12/DMEM, GIBCO-BRL, Grand Island, NY) 37°C 60 . F12/DMEM pronase 0.02% collagenase type II (Sigma, St. Louis, MO) 2 37°C 12 . F12/DMEM nylon (pore size 75 μ m) ¹⁷⁾ 5×10^5 /ml 24 well plate (Falcon, Franklin Lakes, NJ) . 10% FBS, 1% v/v penicillin, streptomycin, nystatin (all antibiotics from GIBCO-BRL, Grand Island, NY) F12/DMEM 3 37°C 5% CO₂ .

2.

3

0.15M NaCl (Kelco, Chicago, IL)

1.2% low viscosity alginate gel

Trypsin

mililiter

alginate gel

102 mM CaCl₂

alginate gel

CaCl₂

10

polymerization

0.15 M NaCl

F12/DMEM

3

Alginate bead

24well culture plate

well

10

1% v/v penicillin, streptomycin, nystatin

Serumless medium (Newman-Tytell)

48

37°C

5% CO₂

8).

3. TGF- 1

TGF- 1

가

3

1, 2, 3, 4

4.

3

TGF- 1

Grade II-V

가

3

ng/ml)

3)

TGF- 1

Grade I-V

TGF- 1 (2

TGF- 1

3)

5.

4

가

35S

8M guanidine hydrochloride, 20

mM EDTA

가

4°C

48

proteinase inhibitors

Sephadex G-25M

PD-10 col-umn (Pharmacia Biotech, Uppsala, Sweden)

scintillation mixture (Ultima Gold, Packard, Meriden, CT)

가

PD-10 column

2, 3, 4

scintillation

2).

Scintillation

TGF- 1

Grade I

(

6.

±

SPSS (SPSS Inc. Chicago IL)

Analysis of variance

Fisher 's protected LSD post-hoc test, power analysis

p<0.05

1.

10⁶/g

10⁶/ml

4 × 3

Trypan blue exclusion test

95~100%

3

95%

90~

2. TGF- 1

TGF- 1

2

1

(

3

)

가

2

(Fig. 1).

3.

TGF- 1

Grade I

100%

Grade II

95.1 ± 8.3%,

Grade III

91.1 ± 16.1%

Grade IV

86.2 ± 13.2%,

Grade V

88.2 ± 14.6%

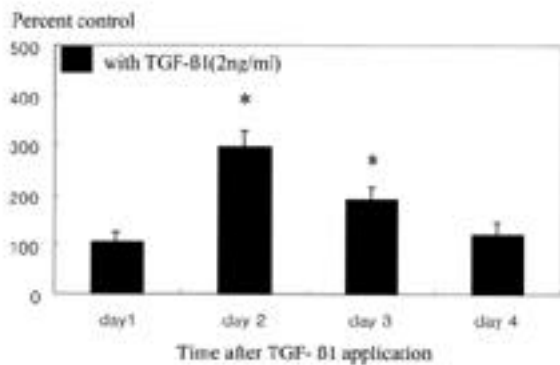


Fig. 1. Content of newly synthesized proteoglycan over duration of culture as assayed by incorporation of ^{35}S -sulfate. Human intervertebral disc cells cultured in alginate beads, treated by TGF-1 (2ng/ml) showed no effect at day 1, 3 fold increase at day 2, and 2 fold increase at day 3. and 1.2 fold increase at day 4. *: $p < 0.05$.

($p=0.35$, power 0.21~0.63)(Fig. 2).

4. TGF- 1

TGF- 1 (2 ng/ml)

TGF- 1

100%

Grade

I 304.0 ± 47.3%, Grade II 297.6 ± 55.4%, Grade III

310.2 ± 50.4%, Grade IV	280.2 ± 86.2%	Grade V
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270.3 ± 88.7%	가가	TGF-
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1	Grade I	Grade V
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가 가 Grade

($p=0.54$, power 0.24-0.47)(Fig. 2).

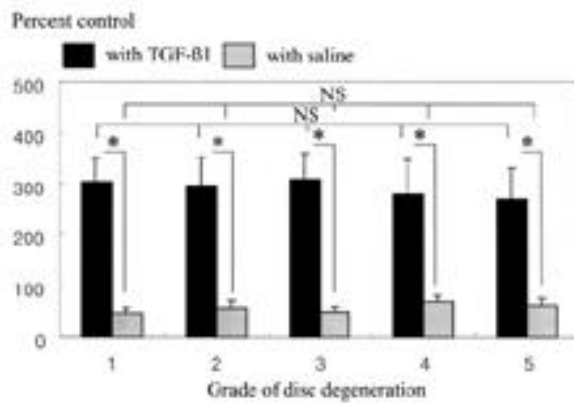


Fig. 2. Content of newly synthesized proteoglycan over grade of degeneration as assayed by incorporation of ^{35}S -sulfate. Human intervertebral disc cells cultured in alginate beads, treated by TGF- β_1 (2ng/ml) showed 3 fold increase in newly synthesized proteoglycan compared to those treated with normal saline, while degeneration grade failed to affect proteoglycan synthesis ($p=0.54$, power 0.24-0.47 in TGF- β_1 treated group, $p=0.35$, power 0.21-0.63 in basal group). *: $p<0.05$) NS: statistically non-significant.

가

가

가

가

TGF- 1

TGF- 1

3

가

가

가

가

가

13)

가

19,20, 22-24)

3)

14)

0.21~0.64
(0.8)

31 50

가

가

가

가

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