: 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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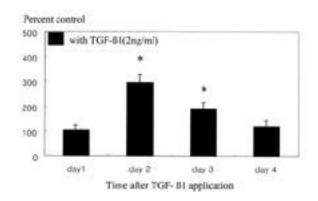


Fig. 1. Content of newly synthesized proteoglycan over duration of culture as assayed by incorporation of ³⁵S-sulfate. Human intervertebral disc cells cultured in alginate beads, treated by TGF- 1 (2ng/ml) showed no effect at day 1, 3 fold increase at day 2, and 2 fold increase at day 3. and 1.2 fold 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 $304.0 \pm 47.3\%$, Grade II $297.6 \pm 55.4\%$, Grade III $310.2 \pm 50.4\%$, Grade IV $280.2 \pm 86.2\%$ Grade V $270.3 \pm 88.7\%$ 가가 TGF-1 Grade V Grade I 가 가 Grade

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

13)

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Percent control

with TGF-81 with saline

500

NS

NS

100

1 2 3 4 5

Grade of disc degeneration

Fig. 2. Content of newly synthesized proteoglycan over grade of degeneration as assayed by incorporation of ³⁵S-sulfate. Human intervertebral disc cells cultured in alginate beads, treated by TGF- 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- 1 treated group, p=0.35, power 0.21-0.63 in basal group). *: p<0.05) NS: statistically non-significant.

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