Eur Spine J (2002) 11 : 452–458
DOI 10.1007/s00586-001-0383-5

ORIGINAL ARTICLE

Jukka Tolonen
Mats Grönblad
Johanna Virri
Seppo Seitsalo
Tapio Rytömaa
Erkki Karaharju

Oncoprotein c-Fos and c-Jun immunopositive cells and cell clusters in herniated intervertebral disc tissue

Received: 1 November 2000
Revised: 9 October 2001
Accepted: 21 December 2001
Published online: 19 March 2002

J. Tolonen · M. Grönblad (✉) · J. Virri
E. Karaharju
Research Laboratory, Spine Research Unit,
Department of Orthopaedics and
Traumatology,
Helsinki University Central Hospital,
Helsinki, Finland
e-mail: mats.gronblad@hus.fi,
Tel.: +358-9-47172500,
Fax: +358-9-47172354

M. Grönblad
Department of Physical Medicine
and Rehabilitation,
Helsinki University Central Hospital,
P. O. Box 340, 00029 HUS, Helsinki,
Finland

S. Seitsalo
Orton Hospital, Helsinki, Finland

T. Rytömaa
Finnish Centre for Radiation
and Nuclear Safety,
Helsinki, Finland

Abstract The oncoproteins c-Fos and c-Jun create a transcriptional site early response activating protein (AP-1) mediating the regulation of gene expression in response to extracellular signalling by, for example, cytokines. These proteins are important in the signalling pathway from the cell membrane to the nucleus. Previously, oncoproteins have been located in articular synovium and in chondrocytes, participating in transcription. There is, however, no such study of intervertebral disc tissue. In disc degeneration and after herniation, cell proliferation markers have been demonstrated. In the present study we visualize the AP-1 transcriptional site factors c-Fos and c-Jun in 38 human herniated intervertebral disc tissue samples by immunohistochemical staining with monoclonal antibodies. No immunoreactivity could be observed in control disc tissue, indicating that after herniation, disc cells are entering from the resting stage to the cell cycle. Furthermore, c-Jun immunoreactivity was also observed in disc cell clusters, thus demonstrating them to be active transcriptional sites in disc tissue. c-Fos immunoreactivity was seen in 15/38 and c-Jun in 28/38 herniated discs (39% and 74% respectively). Immunopositive *groups* of disc cells were noted in 7/28 (25%) of the oncoprotein-immunopositive samples. We did not see any difference in immunoreactivity between female and male patients. Furthermore, we did not notice any statistical difference regarding the immunoreaction for proto-oncogenes c-Fos and c-Jun in extrusions, sequesters and protrusions. Nor did immunostaining show any significant relationship with preoperative pain duration. We concluded that, in herniated disc tissue, the oncoproteins c-Fos and c-Jun are activated in disc cells and cell clusters. In the future, learning more about this transcriptional signal pathway may result in new specific treatments for intervertebral disc pathology.

Keywords Oncogene proteins · c-Fos protein · c-Jun protein · Disc herniation

Introduction

Proto-oncogenes encode proteins with three main sites of action: the cell-surface membrane, the cytoplasm and the nucleus [2]. The Jun oncogene is expressed as a 65-kd protein [5]. It was first detected in cancer research [4], which explains why it is called an "oncogene". It is a member of the early activating protein (AP-1) family of transcription factors, which mediate the regulation of gene expression in response to extracellular signaling [3]. For example, stimulation of quiescent murine fibroblasts by growth factors and by phorbol esters results in a rapid and transient transcriptional activation of proto-oncogenes [7].

Furthermore, transient inhibition of protein synthesis induces expression of proto-oncogenes and stimulates resting cells to enter the cell cycle [18].

The proto-oncogene products c-Fos and c-Jun connect or heterodimerize through their leucine zippers to form the AP-1 transcription factor [15]. The transcriptional activity of the heterodimer is regulated by signal-dependent phosphorylation and dephosphorylation events.

In human chondrocytes, c-Fos and c-Jun genes are expressed both in normal and osteoarthritic articular cartilage [26]. Osaki et al. have demonstrated that in rat chondrocytes the cell growth inductive and the mitogenic effect of transforming growth factor β1 (TGF-β1) are mediated by the c-Fos gene [13]. In an experimental osteoarthritis (OA) model, oncoproteins were detected predominantly in the synovial lining cells [16], whereas in normal synovial lining cells such reactivity was low. Furthermore, in OA cartilage chondrocytes at the superficial and middle layers were found to participate in the synthesis of oncoproteins [16].

Overexpression of the c-Fos proto-oncogene has recently been shown to inhibit chondrocyte differentiation [21]. Interestingly, however, the rates of proliferation and apoptosis were unaffected. Nodule formation was inhibited if induction of c-Fos was only at early stages of differentiation [21].

The level of expression of c-Jun is lower in maturing or hypertrophic chondrocytes than in proliferating chondrocytes [9]. This suggests that the c-Jun family negatively regulates the maturation process of chondrocytes.

Activation of AP-1, a heterodimeric complex of Fos and Jun proteins, is required for chondrocyte matrix metalloproteinase production and cell proliferation [10]. Cytokines and growth factors induce reactive oxygen species production, which is a signal pathway for activation of AP-1. In herniated disc tissue, matrix metalloproteinase activity is prevalent [17], and there is a change in the balance between degradative enzymes and endogenous inhibitors.

Clusters of disc cells have been observed in degenerated intervertebral disc tissue [8]. Earlier studies have reported similar clusters of chondrocytes in osteoarthritic cartilage, where they have been linked to a possible production of extracellular matrix components involved in matrix repair [8]. In intervertebral disc tissue, such clusters could perhaps participate in the repair of tissue damage, but very little is so far known about such clusters of disc cells.

We analyzed the transcription promoters c-Fos and c-Jun in herniated disc tissue in order to locate potential cell transcription and cell activation, thereby identifying possible differences in oncoprotein expression between normal and herniated disc tissue. Previously there has been no research concerning oncoprotein expression in intervertebral disc tissue. These proteins are essential in the signal pathway from the cell membrane to the nucleus. Further knowledge regarding this signal pathway may bring forth new specific treatments for disc pathology.

Materials and methods

We analyzed tissue material from 38 discectomy operations. As a normal control we used nucleus pulposus material from a disc tissue bank (–70°C) of four organ donors, none of whom had any history of low back pain. The control tissue was obtained by an anterior approach. A detailed description of the clinical data of the disc tissue material from the herniated discs and normal controls is given in Table 1. The preoperative pain duration was reported by the patients in a routine low-back pain patient questionnaire, including items on preoperative radicular pain duration, a pain drawing, the Oswestry Disability Index and patient demographics.

After removal, tissue material was immediately frozen to –70°C in the operating theater, and 8-μ-thick cryostat sections were fixed in ice-cold acetone. Immunoreactions were detected using an avidin biotin complex- (ABC-) peroxidase staining kit (Vectastain Elite, Vector Laboratories, Burlingame, Calif., USA). Hematoxylin was used for counterstaining.

We used monoclonal c-Fos and c-Jun antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, Calif., USA), both at the dilution 1:50. As a positive control we used rheumatoid arthritic synovia and human dermal skin samples. As a method control for monoclonal immunostaining, we also stained sections omitting the primary antibody. We also did immunostaining as above following preabsorption of the two monoclonal antibodies with the corresponding antigen at 1:10.

Three sections from each disc specimen were stained with both antibodies. The presence or absence of immunostaining was compared with clinical data (age, gender, preoperative pain duration and prolapse type).

Statistical analyses were done using the SOLO statistical software program (BMDP, Los Angeles, Calif., USA). Groups were compared using either chi-square analyses or Fisher's exact test, as applicable. Level of statistical significance was set at $P<0.05$.

Table 1 Clinical data of 38 herniated disc samples from discectomy operation and five normal controls (*DHT* disc herniation tissue, *DNT* normal disc control tissue, *S* sequester[a], *E* extrusion[b], *P* protrusion[c])

Tissue type	Sex(M/F)	Prolapse type	Radicular pain	Age (years)
DHT (*n*=38)	22/16	S=12, E=24, P=2	3wks–36mths; mean=6.9mths	20–74; mean 41.6
DNT (*n*=4)	3/1			31–53; mean 43

[a] *Protrusion* is an abnormal bulging of the annulus fibrosus, which remains continuous
[b] *Extrusion* is where tissue is exposed to the epidural space, but remains continuous
[c] *Sequester* is free disc tissue material in epidural space [20]

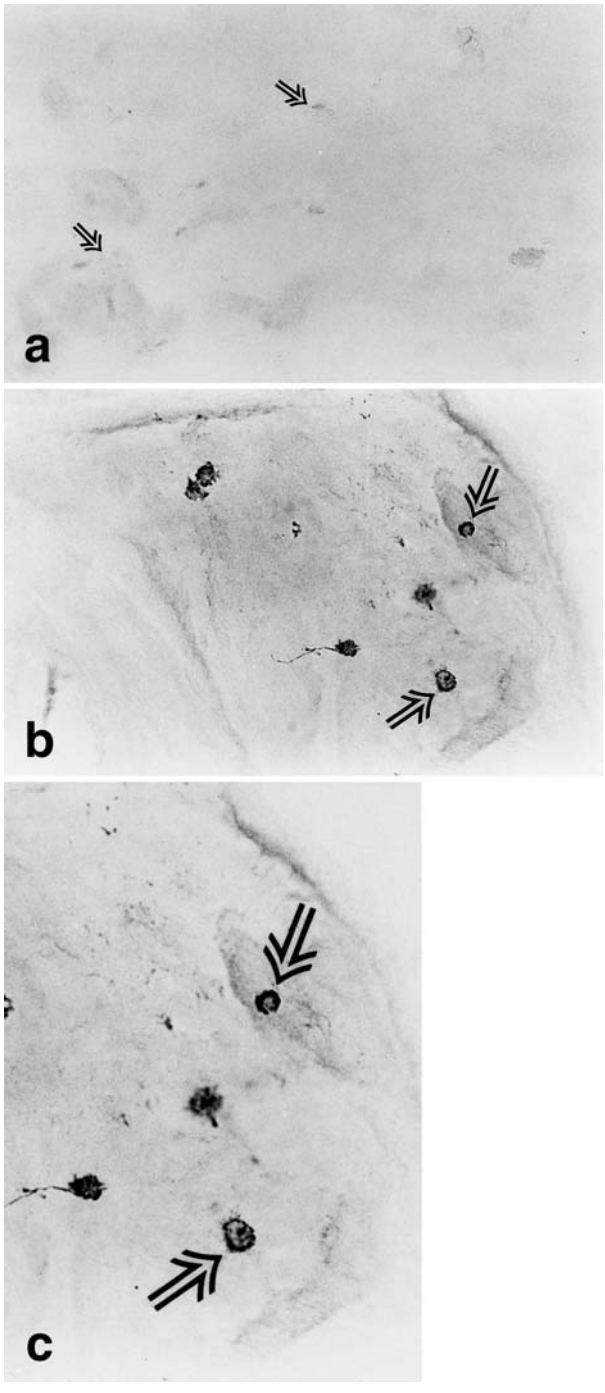

Fig. 1 **a** Control disc from a 13-year-old female organ donor. Monoclonal c-Fos oncoprotein antibody at dilution 1:50 using the avidin-biotin complex- (ABC-) immunostaining method, with hematoxylin counterstaining (original magnification ×370). Note the lack of immunoreactivity (*open arrows*) **b** Oncoprotein c-Fos immunopositive disc cells (*open arrows*) in an extruded disc herniation sample from a 39-year-old male patient (ABC-immunostaining, hematoxylin counterstaining, original magnification ×370). **c** Higher magnification of the oncoprotein c-Fos immunopositive cells (*open arrows*) seen in **b**. Observe the perinuclear location of the immunoreaction (ABC-immunostaining, hematoxylin counterstaining, original magnification ×550)

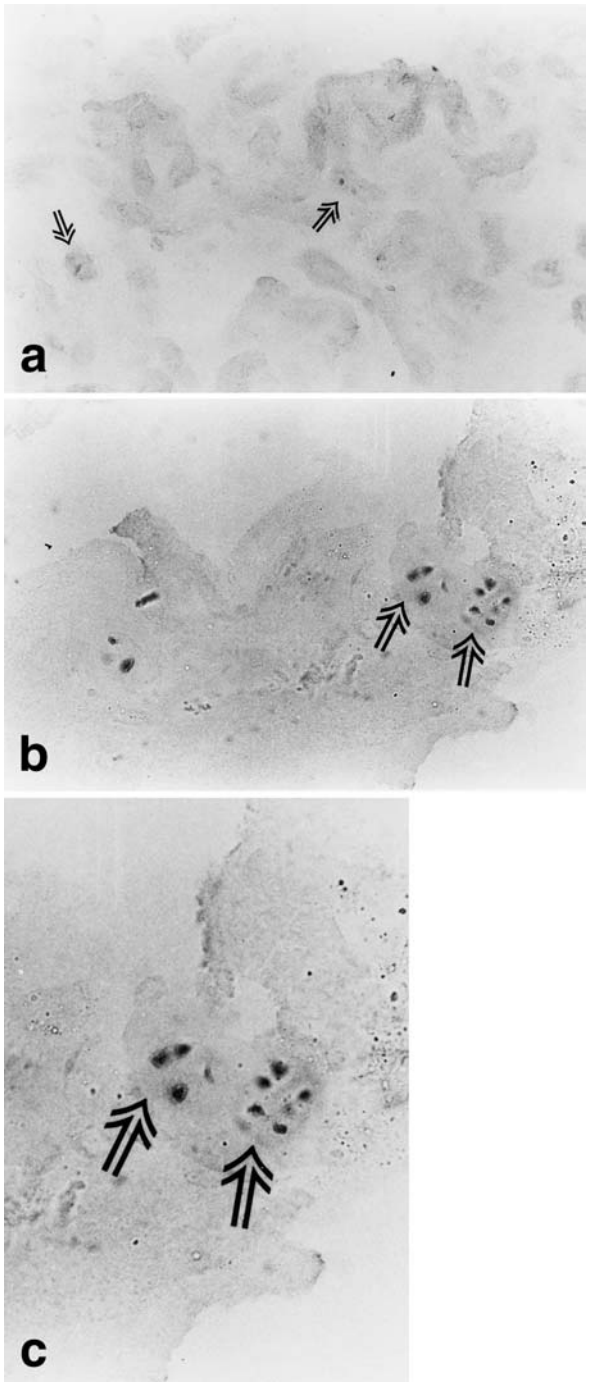

Fig. 2 **a** The same control disc as in Fig. 1a, now stained with oncoprotein c-Jun monoclonal antibody at dilution 1:50 (ABC-immunostaining method, hematoxylin counterstaining, original magnification ×250). Note the total lack of immunoreactivity. Pale hematoxylin counterstained nuclei can be seen (*open arrows*). **b** Oncoprotein c-Jun immunopositive disc cell groups (conglomerates) (*open arrows*) in an extruded disc herniation sample from a 45-year-old female patient (monoclonal antibody at dilution 1:50, ABC-immunostaining method, hematoxylin counterstaining, original magnification ×370). **c** Higher magnification of the c-Jun immunopositive cells (*open arrows*) seen in **b**. Observe the nuclear location of the immunoreaction (ABC-immunostaining, hematoxylin counterstaining, original magnification ×550)

Table 2 Clinical data of the patients and immunohistochemical staining results (ABC-peroxidase immunostaining method, monoclonal antibodies at dilution 1:50) for oncoproteins c-Fos and c-Jun. Samples are arranged according to prolapse type [*Co* Immunoreactivity in disc cell conglomerates (groups of cells)]

No. (age, years)	Sex (M/F)	Pain duration (months)	Prolapse type	Operation level	Staining result c-Fos/c-Jun
1 (63)	F	4	P	L3/4	–/+
2 (40)	M	36	P	L4/5	–/+
3 (74)	M	3	E	L3/4	–/+
4 (39)	M	2.5	E	L3/4	+/+(Co)
5 (34)	M	1.75	E	L4/5	+/+
6 (55)	F	3	E	L2/3	–/–
7 (40)	M	2.5	E	L5/S1	+/+
8 (58)	M	2.5	E	L4/5	+/+
9 (61)	M	3	E	L5/S1	–/–
10 (20)	M	3	E	L5/S1	–/+
11 (44)	M	12	E	L4/5	–/+(Co)
12 (34)	M	30	E	L4/5	+/+
13 (36)	F	6	E	L5/S1	–/+(Co)
14 (45)	F	6	E	L5/S1	–/–
15 (49)	F	5	E	L4/5	–/+
16 (41)	F	3.5	E	L4/5	+/+
17 (43)	M	4	E	L4/5	+/+(Co)
18 (39)	F	9	E	L4/5	+/+
19 (34)	M	5	E	L4/5	+/+
20 (47)	F	3.5	E	L4/5	–/+(Co)
21 (33)	F	12	E	L5/S1	+/+
22 (29)	M	7	E	L3/4	–/+
23 (61)	M	4	E	L5/S1	–/–
24 (23)	F	6	E	L5/S1	–/–
25 (52)	M	3	S	L4/5	–/+(Co)
26 (33)	F	2	S	L5/S1	+/+
27 (33)	F	0.75	S	L4/5	–/–
28 (32)	M	2.5	S	L5/S1	+/+
29 (34)	F	3	S	L5/S1	–/+
30 (55)	M	2	S	L4/5	–/+
31 (34)	F	13	S	L4/5	–/–
32 (38)	M	24	S	L5/S1	+/+
33 (25)	M	3.5	S	L5/S1	–/–
34 (26)	M	5	S	L4/5	–/–
35 (43)	M	6	S	L4/5	–/–
36 (43)	M	6	S	L4/5	–/+(Co)

Results

The clinical data of herniated discs are shown in Table 1. Herniated disc tissue samples were classified by the operating surgeon as previously described [20, 22]. Control discs showed a normal morphology macroscopically; no signs of autolysis were observed, i.e. all disc cells looked intact. All control discs were immunonegative for both c-Fos and c-Jun (Fig. 1a, Fig. 2b). Rheumatoid arthritic synovia and dermal samples, studied as positive controls, showed c-Fos and c-Jun immunopositive cells. Sections stained omitting the primary antibody did not show any immunoreaction for either of the oncoproteins. Neither did sections preabsorbed with the corresponding antigen.

Oncoprotein c-Fos immunoreaction was noted in 15/38 herniated samples (39%) (Fig. 1b,c) and for c-Jun in 28/38 (74%) (Fig. 2b,c; Table 2). For each of these disc samples studied, when immunopositivity was detected, it was observed in all three sections cut through the respective specimen. When a disc sample was immunonegative, none of the three sections studied showed any immunoreactivity. Immunopositive clusters of disc cells (conglomerates, i.e., ≥3 cells) were seen in 25% of the total immunopositive samples (7/28) (Table 2). The immunoreaction in these clusters of disc cells was mainly of the c-Jun type (Fig. 2b,c).

None of the studied herniated discs showed immunoreactivity only to c-Fos. Immunoreactivity only to c-Jun was detected in 13 disc herniation samples and reactivity to both oncoproteins in 15 disc herniation samples (Table 2).

Twenty-three of the herniated samples were from men and 15 from women. The age of the patients varied from 20 to 74 years (mean 41.6 years). Twenty-four of the her-

Table 3 Immunohistochemical staining results for oncoproteins c-Fos and c-Jun in subgroups of herniated disc tissue samples (n=38)

Subgroups	Immunoreactivity*		
	c-Fos	c-Jun	Conglomerates[a]
Males (n=23)	10/23	18/23	5/18
Females (n=15)	5/15	10/15	2/10
Extrusions (n=24)	12/24	19/24	5/19
Sequesters (n=12)	3/12	7/12	2/7
Protrusions (n=2)	0/2	2/2	0/2

*Group differences nonsignificant (Fisher exact/Chi-square tests, as applicable)
[a] Number of samples with c-Jun immunoreactive disc cell *groups* of all samples with c-Jun immunoreactivity

Table 4 The relationship of immunoreactivity for oncoproteins c-Fos and c-Jun with pain duration before the disc herniation operation. In the acute/subacute group pain duration was ≤3 months, in the chronic group pain duration was >3 months

Groups	Immunoreactivity*			
	c-Fos	c-Jun	Conglomerates[a]	
Acute/subacute group (n=14)		6/14	11/14	2/11
Chronic group (n=24)	9/24	17/24	5/17	

*Group differences were nonsignificant (Fisher exact/Chi-square tests, as applicable)
[a] Number of samples with c-Jun immunoreactive disc cell *groups* of all c-Jun immunoreactive samples

niation samples were extruded, 12 sequestrated and 2 protruded. Detailed immunoreaction in different subgroups is shown in Table 3 and Table 4.

We did not note any gender-related differences in the immunoreactivity. Nor were there any statistically significant differences regarding proto-oncogene immunoreactions in extrusions compared to sequestrations (Table 3). The total number of protrusions (n=2) was too small to make any statistical analysis.

Comparing the pain duration before operation, there were no statistically significant differences between the acute/subacute and chronic group regarding the immunoreaction for the proto-oncogenes c-Fos and c-Jun (Table 4). Nor did the presence of immunoreaction in cell groups (conglomerates) show any statistical difference between these two groups (Table 4).

Discussion

Nuclear oncoproteins c-Fos and c-Jun (or its related proteins JunB or JunD) represent the transcription factor AP-1 (activating protein-1), and they may act as an intracellular messenger converting short-term signals generated by extracellular stimulators into long-term changes in cell phenotype. This is done by regulating the expression of downstream genes that possess an AP-1 binding site [1].

The importance of AP-1 transcription factors in chondrocyte differentiation has been highlighted in an in vitro study by Thomas et al. [21]. In particular, their study found that the induction of c-Fos resulted in a concomitant increase in the expression of fra-1 and c-Jun. Furthermore, the overexpression of c-Fos was found to directly inhibit chondrocyte differentiation.

It has previously been shown that the growth-stimulative effect of transforming growth factor β1 in chondrocytes is mediated by the activation of the oncoprotein c-fos gene [13]. This is mediated by protein kinase activation [25]. In the present study, we show the expression of the oncoproteins c-Fos and c-Jun in herniated disc tissue. We have previously reported expression of TGF-β and induction of TGF-β receptor in these same tissues [23]. This may suggest that the proliferative effect of TGF-β is at least partly mediated by these oncoproteins in herniated disc tissue as well.

In rheumatoid cartilage, the matrix metalloproteinase-1 (MMP-1) promoter is demonstrated to be activated by Jun-related proteins as well as Fos/Jun-related protein heterocomplex [24]. Furthermore, c-Fos combined with any of the Jun-related proteins failed to stimulate the tissue inhibitor of metalloproteinases-1 (TIMP-1) promoter, although it was activated by Jun-related protein heterocomplexes [24]. In the present study, we did not see c-Fos immunopositivity alone (Table 2), whereas c-Jun immunoreactivity was noted as well as the immunoreactivity to both oncoproteins (Table 2). This may suggest that the proteolytic activity is controlled by the expression of the c-Fos and c-Jun oncoproteins in herniated disc tissue as well.

Interestingly, the oncoprotein expression in disc cell clusters was mainly of the c-Jun type. This may indicate that in disc cell clusters c-Jun may be more important than c-Fos. Turnover of the matrix components is established with an intricate balance between synthesis and degradation of the associated molecules, such as MMP and TIMP. c-Fos has been demonstrated to be degradative [24], whereas Jun-related proteins stimulate both MMP and TIMP [24]. This may indicate that disc cell clusters participate in the turnover of extracellular matrix components. Marked degradative enzyme activity in disc tissue after disc herniation has been noted previously [12]. Furthermore, disc tissue has a tendency to decrease in size after herniation [11, 19]. Matrix metalloproteinase activation in herniated disc tissue has previously been shown [17]. There was a change in the balance between degradative enzymes (MMPs) and endogenous inhibitors (TIMPs) [17]. Regulation of this balance could be partly mediated by AP-1 protein in disc tissue as well.

The duration of pain before operation had no significant association with the expression of oncoproteins. The results did not show any statistical difference between acute/subacute and chronic herniations.

Previously it has been shown by Paajanen and coworkers that the proliferation potential drops in recurrent herniations [14]. That study did not show any correlation between proliferative disc cells and the degree of disc degeneration on magnetic resonance imaging.

The formation of clusters of disc cells is associated with degenerative disc disease [8]. These clusters may produce certain extracellular matrix components and they may also function to repair damaged tissue. The pattern of proliferation cell nuclear antigen (PCNA) and proliferation-associated antigen Ki-67 positivity in degenerated disc tissue samples suggests that disc cell clusters arise through increased cell proliferation [8]. Furthermore, colony formation in vitro by annular intervertebral disc cells from patients with degenerative disc disease and young controls has previously been demonstrated [6].

Conclusions

Taken together, our findings of oncoprotein c-Fos and c-Jun expression in disc cells and cell clusters may indicate that disc cells respond to disc herniation. This might mean that disc cells participate in a reaction cascade, where they are actively in contact with the surrounding tissue. This also implies that more information about disc cells will be required before we can better understand mechanisms of disc degeneration and herniation, and the tissue remodelling that follows upon a disc herniation. Furthermore, in the future, we believe that disc cell research regarding the signal pathway from the cell membrane to the nucleus will provide more information on how disc cells function in different stages of the cell cycle, and in different pathological conditions. Pathological conditions of interest include disc degeneration and disc herniation. This may provide us with new types of treatment, e.g., gene therapy or perhaps blockage of the signal pathway.

Acknowledgements Financial support from the Paulo Foundation and the Yrjö Jahnsson Foundation and research funding from Helsinki University Central Hospital is gratefully acknowledged.

References

1. Angel P, Karin M (1991) The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. Biochim Biophys Acta 1072:129–157
2. Angel P, Allegretto EA, Okino ST, Hattori K, Boyle WJ, Hunter T, Karin M (1988) Oncogen jun encodes a sequence-specific trans-activator similar to AP-1. Nature 332:166–171
3. Bohmann D, Tjian R (1989) Biochemical analysis of transcriptional activation by Jun: differential activity of c- and v-Jun. Cell 59:709–717
4. Bohmann D, Bos TJ, Admon A, Nishimura T, Vogt PK, Tjian R (1987) Human proto-oncogene c-jun encodes a DNA binding protein with structural and functional properties of transcription factor AP-1. Science 238:1386–1392
5. Bos TJ, Bohmann D, Tsuchie H, Tjian R, Vogt PK (1988) v-jun encodes a nuclear protein with enhancer binding properties of AP-1. Cell 52:705–712
6. Desai BJ, Gruber HE, Hanley EN Jr (1999) The influence of Matrigel or growth factor reduced Matrigel on human intervertbral disc cell growth and proliferation. Histol Histopathol 14: 359–368
7. Jahner D, Hunter T (1991) The stimulation of quiescent rat fibroblasts by v-src and v-fps oncogenic protein-tyrosine kinases leads to the induction of a subset of immediate early genes. Oncogene 6:1259–1268
8. Johnson WEB, Menage J, Evans EH, Eisenstein SM, Roberts S (1999) Increased intervertebral disc cell proliferation and novel proteoglycan production: evidence of cellular repair responses to degenerative tissue damage? 26th Annual Meeting of the International Society for the Study of the Lumbar Spine, 21–25 June, Kona, Hawaii
9. Kameda T, Watanabe H, Iba H (1997) C-Jun and JunD suppress maturation of chondrocytes. Cell Growth Differ 8: 495–503
10. Lo YY, Cruz TF (1995) Involvement of reactive oxygen species in cytokine and growth factor induction of c-fos expression in chondrocytes. J Biol Chem 270:11727–11730
11. Maigne J-Y, Rime B, Deligne B (1992) Computer tomography follow-up study of forty-eight cases of non-operatively treated intervertebral disc herniation. Spine 17:1071–1074
12. Ng SCS, Weiss JB, Quennel R, Jayson MIV (1986) Abnormal connective tissue degrading enzyme patterns in prolapsed intervertebral discs. Spine 11: 695–701
13. Osaki M, Tsukazaki T, Yonekura A, Miyazaki Y, Iwasaki K, Shindo H, Yamashita S (1999) Regulation of c-fos gene induction and mitogenic effect of transforming growth factor-beta1 in rat articular chondrocyte. Endocr J 46: 253–261
14. Paajanen H, Haapsalo H, Kotilainen E, Aunapuu M, Kettunen J (1999) Proliferation potential of human lumbar disc after herniation. J Spinal Disord 12: 57–60
15. Papavassiliou AG, Treier M, Chavrier C, Bohmann D (1992) Targeted degradation of c-Fos, but not v-Fos, by a phosphorylation-dependent signal on c-Jun. Science 258:1941–1944
16. Pelletier JP, Faure MP, DiBattista JA, Wilhelm S, Visco D, Martel-Pelletier J (1993) Coordinate synthesis of stromelysin, interleukin-1, and oncogene proteins in experimental osteoarthritis. An immunohistochemical study. Am J Pathol 142:95–105
17. Roberts S, Caterson B, Menage J, Evans EH, Jaffray DC, Eisenstein SM (2000) Matrix metalloproteinases and aggrecanase: their role in disorders of the human intervertebral disc. Spine 25:3005–3013
18. Rosenwald IB, Setkov NA, Kazakov VN, Chen JJ, Ryazanov AG, London IM, Epifanova OI (1995) Transient inhibition of protein synthesis induces expression of proto-oncogenes and stimulates resting cells to enter the cell cycle. Cell Prolif 28:631–644
19. Saal JA, Saal JS, Herzog RJ (1990) The natural history of lumbar intervertebral disc extrusion treated nonoperatively. Spine 15:683–686

20. Spengler D, Oullette EA, Battie M, Zeh J (1990) Elective discectomy for herniation of a lumbar disc. J Bone Joint Surg Am 72:230–237
21. Thomas DP, Sunters A, Gentry A, Grigoriadis AE (2000) Inhibition of chondrocyte differentiation in vitro by constitutive and inducible overexpression of the c-fos proto-oncogene. J Cell Sci 113:439–450
22. Tolonen J, Grönblad M, Virri J, Seitsalo S, Rytömaa T, Karaharju E (1995) Basic fibroblast growth factor immunoreactivity in blood vessels and cells of disc herniations. Spine 20:271–276
23. Tolonen J, Grönblad M, Virri J, Seitsalo S, Rytömaa T, Karaharju E (2001) Transforming growth factor beta receptor induction in herniated intervertebral disc tissue. An immunohistochemical study. Eur Spine J 10:172–176
24. Tsuji M, Hirakawa K, Kato A, Fujii K (2000) The possible role of c-fos expression in rheumatoid cartilage destruction. J Rheumatol 27:1606–1621
25. Yonekura A, Osaki M, Hirota Y, Tsukazaki T, Miyazaki Y, Matsumoto T, Ohtsuru A, Namba H, Shindo H, Yamashita S (1999) Transforming growth factor-beta stimulates articular chondrocyte cell growth through p44/42 MAP kinase (ERK) activation. Endocr J 46:545–553
26. Zafarullah M, Martel-Pelletier J, Cloutier JM, Gedamu L, Pelletier JP (1992) Expression of c-fos, c-jun, jun-b, metallothionein and metalloproteinase genes in human chondrocyte. FEBS Lett 306:169–172