J. Tolonen
M. Grönblad
J. Virri
S. Seitsalo
T. Rytömaa
E. O. Karaharju

Platelet-derived growth factor and vascular endothelial growth factor expression in disc herniation tissue: an immunohistochemical study

Received: 6 May 1996
Accepted: 26 June 1996

J. Tolonen (✉) · M. Grönblad
J. Virri · S. Seitsalo · E. O. Karaharju
Research Laboratory,
Spine Research Unit,
Department of Orthopaedics
and Traumatology, University of Helsinki
Topeliuksenkatu 5,
FIN-00250 Helsinki, Finland
Tel. + 358-0-4717287;
Fax + 358-0-4717587

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

Abstract Angiogenesis is essential in tissue growth and regeneration. There are several factors that are able to stimulate vascular endothelial cell growth, including platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF). Disc herniation tissue (DHT) contains vascular ingrowth, which promotes granulation tissue formation. In this study we observed 50 disc herniations for PDGF and VEGF immunoreactivity. PDGF immunopositivity was detected in 38 samples (78%). In 28 samples (56%) there were PDGF immunopositive capillaries, PDGF immunopositive disc cells were detected in 19 samples (38%) and PDGF immunopositive fibroblasts in 6 DHT samples (12%). VEGF immunopositive capillaries were identified in 44 DHT samples (88%). For neither growth factor was immunopositivity dependent on preoperative radicular pain duration. In extrusions ($n = 25$) VEGF immunopositive capillaries were detected in 23 samples (92%) and PDGF immunopositivity in 21

samples (84%). PDGF immunopositivity was more commonly associated with capillaries than with nuclei of disc cells. In sequesters ($n = 20$) VEGF immunopositive capillaries were identified in all samples and PDGF immunopositivity in 16 (80%). As in extrusions, PDGF immunoreaction was more prevalent in capillaries than in disc cells. Patient age did not relate to VEGF expression. In all age groups it was higher than 80%. Thus capillaries in disc herniation tissue are evidently newly formed and our results demonstrate that PDGF and VEGF participate in the neovascularization process. The presence of PDGF in fibroblasts and in disc cells suggests that this growth factor regulates the function of these cells, possibly the proliferation of the cells and the production of extracellular matrix components.

Key words Intervertebral disc · Disc herniation · Platelet-derived growth factor · Vascular endothelial growth factor

Introduction

Angiogenesis involves proliferation of endothelial cells, chemotaxis and enzymatic degradation of the basement membrane of local blood vessels [8, 12, 33]. This process is essential in tissue growth and regeneration. Several factors are able to stimulate vascular endothelial cell growth,

including fibroblast growth factor (FGF) [10], angiogenin [7], transforming growth factor beta (TGF-beta) [25], platelet derived growth factor (PDGF) [26] and vascular endothelial growth factor (VEGF) [2, 4, 11, 16].

Platelet-derived growth factor (PDGF) is structurally a dimer of A chains (17 kD) and B chains (16 kD). It appears either in the form of an A-A or a B-B homodimer or

as an A-B heterodimer [15]. It stimulates the growth of vascular endothelial cells [26] and fibroblasts [29]. It also acts as a chemotactic signal for fibroblasts, even at very low concentrations [22]. In cartilage it has been demonstrated to increase the level of intracellular free calcium ions in chondrocytes [9]. Furthermore, it stimulates DNA and proteoglycan synthesis in cartilage tissue [9] and chondrocyte proliferation [13].

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF), is a dimeric, heparin-binding protein with a molecular weight of 45 kD [4, 5]. Its expression has previously been demonstrated in brain, kidney, pituitary gland, lung, adrenal gland, heart, liver, stomach mucosa and ovary, as well as in some tumours [1, 6]. VEGF is actually a family composed of four different species of VEGF [6]. It regulates endothelial differentiation, blood vessel growth and vascular repair [21], and it shares homologies of about 21% and 24%, respectively, with the A and B chains of PDGF [30]. VEGF also regulates plasminogen activators and plasminogen activator inhibitor-1 in microvascular endothelial cells [20]. It promotes blood vessel hyperpermeability, endothelial cell growth, angiogenesis and enhanced glucose transport [3]. Hypoxia has been shown to induce VEGF, which may in turn mediate hypoxia-initiated angiogenesis [28].

Intervertebral disc tissue may herniate in three basic patterns. In protrusions it bulges posteriorly, but the annulus fibrosus remains intact. In extrusions disc material is exposed to the epidural space; there is still, however, a continuity with disc tissue. In sequesters there is a separate fragment of disc tissue material in the epidural space.

Disc herniation tissue (DHT) contains vascular ingrowth [14, 17, 32]. This neovascularization promotes granulation tissue formation. In a previous study we demonstrated basic FGF expression in DHT [31]. The aim of this study was to investigate PDGF and VEGF expression in DHT and compare it with vascularization.

Materials and methods

Our disc material was obtained from 50 discectomy operations. The age of the patients ranged from 24 to 71 years. There were 26 male and 24 female patients (Table 1). All the tissue material was rapidly frozen in the operating theatre and 8 μ thick cryostat sections were cut (2800 Frigocut, Reichert-Jung). The sections were fixed in ice-cold acetone and stained by the avidin biotin complex- (ABC-) peroxidase immunohistochemical staining method (Vectastain, Vector Lab, Burlingame, Calif.). Disc material was also obtained from organ donor patients as a control. In these specimens there was no sign of autolysis.

We used a polyclonal platelet-derived growth factor antibody (1 mg/ml) at a dilution 1:100 (R & D Systems, Minneapolis, Minn.) and a polyclonal vascular endothelial growth factor antibody (1 mg/ml) at a dilution 1:500 (Santa Cruz Biotechnology, Santa Cruz, Calif.). Antigen absorption (1:10) was made to test the specificity of the antibodies. We used a polyclonal von Willebrand factor antibody (1:20000) to visualize vascular endothelium (Dakopatts, Copenhagen). In addition to studying control discs, sections were also stained omitting the primary antibody. The immunohisto-

Table 1 Clinical characteristics of the patients sampled for disc herniation tissue

Patient no.	Age (years)	Sex	Preoperative pain duration (months)
1	39	M	2.5
2	32	F	2
3	55	F	3
4	37	M	2.5
5	44	F	0.5
6	27	F	6
7	28	M	6
8	37	F	3.5
9	71	M	3
10	68	M	3
11	45	M	2.5
12	37	M	2.5
13	42	F	2
14	47	M	2
15	47	F	3
16	39	F	2
17	56	M	0.75
18	49	F	5
19	63	F	4
20	31	M	2
21	45	M	3.5
22	39	M	12
23	30	M	1
24	46	F	6
25	45	F	0.5
26	46	F	0.75
27	56	F	6
28	33	F	12
29	29	M	5
30	29	M	6
31	58	F	0.25
32	31	M	6
33	55	F	0.5
34	47	M	12
35	53	M	8
36	52	F	2
37	44	F	6
38	24	F	1
39	40	F	3.5
40	31	M	2.5
41	42	M	4
42	39	M	2.5
43	34	F	3
44	39	F	9
45	47	M	24
46	42	M	12
47	46	F	4
48	58	M	2.5
49	31	M	6
50	56	M	4

Table 2 Platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) immunohistochemical staining results (*nr* not reported)

Patient (no.)	Prolapse (type)	PDGF (immunopositivity)			VEGF (immunopositivity)
		Nuclear	Blood vessel	Fibroblast	
1	nr	—	—	—	—
2	nr	—	—	—	—
3	Extrusion	+	—	—	—
4	Extrusion	—	—	+	+
5	Sequester	—	—	—	+
6	Protrusion	—	+	—	—
7	Sequester	—	+	—	+
8	Sequester	—	+	—	+
9	Extrusion	—	—	—	+
10	Sequester	—	—	—	+
11	Extrusion	—	+	—	+
12	Sequester	—	—	—	+
13	Extrusion	—	—	—	+
14	Sequester	—	+	—	+
15	Extrusion	—	+	—	+
16	Extrusion	—	+	—	+
17	Sequester	—	+	—	+
18	Extrusion	—	+	—	—
19	Protrusion	—	—	—	+
20	Sequester	—	+	—	+
21	Extrusion	—	+	—	+
22	Extrusion	—	+	+	+
23	Extrusion	—	+	—	+
24	Extrusion	—	+	—	+
25	Sequester	—	+	—	+
26	Sequester	—	+	—	+
27	Sequester	—	+	—	+
28	Extrusion	—	+	—	+
29	Extrusion	—	—	—	+
30	Protrusion	—	—	—	—
31	Extrusion	+	+	—	+
32	Extrusion	+	+	—	+
33	Sequester	+	—	+	+
34	Sequester	+	—	—	+
35	Sequester	+	—	—	+
36	Sequester	+	+	+	+
37	Sequester	+	—	—	+
38	Sequester	+	+	—	+
39	Extrusion	+	+	+	+
40	Sequester	+	+	+	+
41	Extrusion	+	—	—	+
42	Extrusion	+	+	—	+
43	Sequester	—	—	—	+
44	Extrusion	—	—	—	+
45	Sequester	+	—	—	+
46	Extrusion	+	+	—	+
47	Extrusion	+	—	—	+
48	Extrusion	+	—	—	+
49	Extrusion	+	+	—	+
50	Extrusion	+	+	—	+

chemical results were then compared with the patient clinical data (Tables 1–4).

Results

Immunohistochemical staining results are described in detail in Table 2. Altogether, 38 DHT samples (78%) showed PDGF immunopositivity. In 27 DHT samples (54%) this immunoreactivity was detected in blood vessels (Fig. 1 A) and in 19 (38%) it was found in nuclei of disc cells (Fig. 2). Six samples (12%) also showed PDGF immunopositive fibroblasts (Fig. 3). Forty-four DHT samples (88%) showed VEGF immunopositive capillaries (Fig. 1 B). In Fig. 1 C capillary endothelial cells are identified with von Willebrand factor antibody. The five studied control discs did not show any PDGF or VEGF immunoreactivity (Fig. 4). Sections stained omitting the primary antibody did not show any immunoreactivity.

Mean preoperative pain duration for all patients was 4.12 months (median 3 months). VEGF and PDGF immunopositivity did not show any dependence on the duration of preoperative pain. Mean pain duration in the VEGF immunopositive group was 4.51 months (median 3 months). In the VEGF immunonegative group it was 4.08 months (median 4 months). Mean preoperative pain duration in the PDGF immunonegative group was 3.54 months (median 3 months); in the PDGF immunopositive group it was 4.16 months (median 3 months). In the group where disc cell nuclei showed PDGF immunoreactivity the mean preoperative pain duration was 5.46 months (median 4 months). In the group where capillaries were immunoreactive for PDGF the mean preoperative pain duration was 4.13 months (median 3.5 months). Finally, in the group where fibroblasts stained for PDGF the mean preoperative pain period was 3.83 months (median 2.5 months). However, there were only six such DHT samples in the present material (Table 2).

Table 3 compares PDGF and VEGF immunoreactivity in the different prolapse types. Of the 25 extruded disc herniation samples studied, 23 (92%) showed VEGF immunoreactivity and 21 (84%) PDGF immunoreactivity. PDGF immunoreaction was located in disc cell nuclei in 11 samples (44%); in 16 samples (64%) it was associated with blood vessels and in 3 samples (12%) fibroblasts were PDGF immunopositive. Twenty of the DHTs were sequesters, VEGF immunopositive capillaries were detected in all of them. PDGF immunopositivity was detected in 16 samples (80%); in 8 of these immunoreaction was localized in disc cell nuclei, 10 (50%) showed PDGF immunopositive capillaries, and three (15%) PDGF immunopositivity in fibroblasts. In the three disc protrusions studied, one showed vascular PDGF immunoreactivity and VEGF immunoreactivity. For two of the tissue samples the prolapse type had not been defined by the operating spine surgeon.

Fig. 1 **A** Platelet-derived growth factor (PDGF) immunopositivity in capillaries (*open arrows*) in an extrusion disc herniation tissue sample from a 39-year-old man. (Operation level L5–S1, avidin biotin complex- (ABC) immunostaining, haematoxylin counterstaining, original magnification $\times 370$) **B** Vascular endothelial growth factor (VEGF) immunopositive capillaries (*black arrow*) in an extrusion from a 31-year-old man (Operation level L4–L5, ABC-immunostaining, haematoxylin counterstaining, original magnification $\times 250$). **C** von Willebrand factor immunopositive capillaries (*black arrows*) in an extrusion disc herniation tissue sample from the same 39-year-old male patient as the sample in **A** (ABC-immunostaining, haematoxylin counterstaining, original magnification $\times 370$)

Fig. 2 An extrusion from a 40-year-old woman. Note PDGF immunopositivity in the nuclei of disc cells (*black arrows*). (ABC-immunostaining, haematoxylin counterstaining, original magnification $\times 370$)

Fig. 3 PDGF immunopositive fibroblasts (*black arrows*) in a sequester from a 52-year-old woman (Operation level L5–S1, ABC-immunostaining, haematoxylin counterstaining, original magnification $\times 370$)

Fig. 4 A normal control disc from a 50-year-old man. Note total lack of immunoreaction. (Level L4–L5, PDGF antibody, ABC-immunostaining, haematoxylin counterstaining, original magnification $\times 370$)

Table 3 PDGF and VEGF immunoreactivity by prolapse type

Prolapse type ^a	PDGF immunopositivity				VEGF immunopositivity
	Total	Nuclear	Blood vessel	Fibroblast	
Protrusions (<i>n</i> = 3)	1/3	0/1	1/1	0/1	1/3
Extrusions (<i>n</i> = 25)	21/25 (84%)	11/21 (52%)	16/21 (76%)	3/21 (14%)	23/25 (92%)
Sequesters (<i>n</i> = 20)	16/20 (80%)	8/16 (50%)	10/16 (63%)	3/16 (19%)	20/20 (100%)

^aTwo samples were not classified

Table 4 PDGF and VEGF immunoreactivity by patient age group

Age group	PDGF immunopositivity				VEGF immunopositivity
	Total	Nuclear	Blood vessel	Fibroblast	
Below 40 years (<i>n</i> = 21)	14/21 (66.7%)	5/14 (23.8%)	13/14 (61.9%)	3/14 (14.2%)	17/21 (81.0%)
40–50 years (<i>n</i> = 17)	15/17 (88.2%)	7/15 (41.2%)	10/15 (58.8%)	1/15 (5.9%)	16/17 (94.1%)
Over 50 years (<i>n</i> = 12)	9/12 (75.0%)	7/9 (50.8%)	5/9 (41.6%)	2/9 (16.7%)	11/12 (91.7%)

Table 4 compares immunoreactivity for PDGF and VEGF among different age groups. VEGF immunoreactivity showed no clear age dependence and was present in more than 80% of all types of DHT (Table 4). Blood vessel associated PDGF immunopositivity was clearly more common than disc cell nuclear immunoreactivity in the youngest patients (under 40 years old). However, in the oldest age group PDGF immunoreaction appeared to be slightly more common in disc cells than in blood vessels (Table 4).

Discussion

DHT contains vascular ingrowth promoting granulation tissue formation [14, 17], PDGF is a potent chemotactic agent for fibroblasts [22] and it induces cellular growth of fibroblasts and stimulates their collagenase production [29]. PDGF is liberated from alpha-granules of platelets and macrophages [26, 29]. One finding of the present study was the demonstration of PDGF immunopositive fibroblasts in DHT, suggesting a regulative role for PDGF in fibroblast function, including in disc herniations. PDGF immunopositive disc cells were also demonstrated, the nuclei exhibiting strong immunoreactivity. The number of disc cells is greater in disc herniations than in normal discs (unpublished data). This could suggest that PDGF also regulates the function of disc cells and that in DHT PDGF stimulates extracellular matrix component production as has been observed for cartilage tissue [9, 23, 24]. PDGF may also be stimulative for disc cell proliferation. In a previous study we demonstrated disc cell cytoplasmic fibroblast growth factor (FGF) immunoreactivity [31], while in this study we noted disc cell nuclear PDGF immunoreactivity. This suggests that these growth factors are part of a greater network of growth factors and cytokines that contribute to overall disc tissue responses, governing repair of tissue damage.

DHT has a tendency to disappear or to decrease in size with time [18, 27]. Furthermore, it has been demonstrated that there is ongoing proteolytic enzyme activity in prolapsed discs [19]. PDGF stimulates collagenase production in fibroblasts [29] and has a chemotactic effect on them [22]. Our results may suggest a possible role for PDGF in regulation of proteolysis including in DHT. This proteolytic process is part of regeneration and may also act as a stimulator for angiogenesis.

Disc tissue is normally avascular and the appearance of capillaries is a sign of aging or tissue degeneration [32]. A neovascularization process also takes place following disc tissue injury and in disc herniation. The blood vessels in herniated disc tissue are mainly newly formed [32]. Several factors are able to stimulate such vascular endothelial cell growth. In this study we demonstrated PDGF and VEGF immunopositivity in disc herniation tissue capillaries. In a previous study we located FGF in these same capillaries [31]. These growth factors act synergistically in angiogenesis, and our results suggest that these factors also participate in the neovascularization process in DHT.

No relationship between VEGF or PDGF expression and the duration of preoperative pain before operation was noted. In the different prolapse types PDGF immunoreaction was detected slightly more often in capillaries than in nuclei of disc cells, but the difference was not marked (Table 3). VEGF immunopositive capillaries were highly prevalent. VEGF was expressed in all sequesters and in more than 90% of extrusions. In both sequesters and extrusions blood vessels were more often immunoreactive for VEGF than PDGF (Table 3).

VEGF expression in capillaries was similar across all age groups, whereas PDGF expression varied by age group, with a higher prevalence in capillaries than in disc cells being particularly characteristic of patients younger than 40 years (Table 4). In a previous study cellular FGF immunoreactivity was detected more often in younger pa-

tients and vascular FGF expression was found to be similar across all age groups [31].

Our results confirm that PDGF and VEGF participate in the neovascularization process. Furthermore, PDGF expression in disc cells and in fibroblasts suggests that this growth factor regulates the cellular function of these cells, mainly the production of the extracellular matrix components and proliferation of the cells.

Conclusion

The immunolocalization of the angiogenic agents PDGF and VEGF in capillaries of the herniated intervertebral

disc supports the active neovascularization process. Furthermore, the presence of PDGF in disc cells suggests that this growth factor may be regulative for the production of extracellular matrix components and the proliferation of these cells. This disc cell activation is important in the pathogenesis of the intervertebral disc and needs further examination.

Acknowledgements This study was supported by grants from the Paulo Foundation and the Yrjö Jahnsson Foundation and by research funding from Helsinki University Central Hospital.

References

- Berse B, Brown LF, Van de Water L, Dvorak HF, Senger DR (1992) Vascular permeability factor (vascular endothelial growth factor) gene is expressed differentially in normal tissues, macrophages and tumors. *Mol Biol Cell* 3: 211–220
- Conn G, Soderman DD, Schaeffer M-T, Wile M, Hatcher VB, Thomas KA (1990) Purification of a glycoprotein vascular endothelial cell mitogen from a rat glioma-derived cell line. *Proc Natl Acad Sci USA* 87: 1323–1327
- Connolly DT (1991) Vascular permeability factor: a unique regulator of blood vessel function. *J Cell Biochem* 47: 219–223
- Ferrara N, Henzel WJ (1989) Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochem Biophys Res Commun* 161: 851–858
- Ferrara N, Leung DW, Cachianes G, Winer J, Henzel WJ (1991a) Purification and cloning of vascular endothelial growth factor secreted by pituitary folliculostellate cells. *Methods Enzymol* 198: 391–404
- Ferrara N, Houck KA, Jakeman LB, Winer J, Leung DW (1991b) The vascular endothelial growth factor family of polypeptides. *J Cell Biochem* 47: 211–218
- Fett JW, Strydom DS, Lobb RR et al (1985) Isolation and characterization of angiogenin, an angiogenic protein from human carcinoma cells. *Biochemistry* 24: 5480–5486
- Folkman J, Haudenschild DD, Zetter BR (1979) Long-term culture of capillary endothelial cells. *Proc Natl Acad Sci USA* 285: 41–43
- Fukuo K, Morimoto S, Kaji K et al (1989) Association of increased intracellular free Ca^{2+} by platelet-derived growth factor with mitogenesis but not with proteoglycan synthesis in chondrocytes – effect of suramin. *Cell Calcium* 10: 29–35
- Gospodarowicz D, Bialecki H, Greenburg G (1978) Purification of the fibroblast growth factor activity from bovine brain. *J Cell Biochem* 253: 3736–3743
- Gospodarowicz D, Abraham JA, Schilling J (1989) Isolation and characterization of a vascular endothelial cell mitogen produced by pituitary-derived folliculostellate cells. *Proc Natl Acad Sci USA* 86: 7311–7315
- Gross JL, Moscatelli D, Rifkin DB (1983) Increased capillary endothelial cell protease activity in response to angiogenic stimuli in vitro. *Proc Natl Acad Sci USA* 80: 2623–2627
- Guerne PA, Sublet A, Lotz M (1994) Growth factor responsiveness of human articular chondrocytes: distinct profiles in primary chondrocytes, subcultured chondrocytes and fibroblasts. *J Cell Physiol* 15: 476–484
- Hirsch C (1957) Studies on the pathology of low back pain. *J Bone Joint Surg [Am]* 41: 237–243
- Johnson A, Heldin C-H, Wasteson Å et al (1984) The C-sis gene encodes a precursor of the B chain of platelet-derived growth factor. *EMBO J* 3: 921–928
- Leung DW, Cachianes G, Kuang W-J, Goeddel DW, Ferrara N (1989) Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 246: 1306–1309
- Lindblom K, Hultqvist C (1950) Absorption of the protruded disc tissue. *J Bone Joint Surg [Am]* 32: 557–560
- Maigne J-Y, Rime B, Deligne B (1992) Computer tomography follow-up of forty-eight cases of nonoperatively treated lumbar intervertebral disc herniation. *Spine* 17: 1071–1074
- Ng SCS, Weiss JB, Quenell R, Jayson MIV (1986) Abnormal connective tissue degrading enzyme patterns in prolapsed intervertebral discs. *Spine* 11: 695–701
- Pepper MS, Ferrara N, Orci L, Montesano R (1991) Vascular endothelial growth factor (VEGF) induces plasminogen activators and plasminogen activator inhibitor-1 in microvascular endothelial cells. *Biochem Biophys Res Commun* 181: 902–906
- Peters KG, De Vries C, Williams LT (1993) Vascular endothelial growth factor expression during embryogenesis and tissue repair suggests a role in endothelial differentiation and blood vessel growth. *Proc Natl Acad Sci USA* 90: 8915–8919
- Postlethwaite AE, Keski-Oja J, Moses HL, Kang AH (1987) Stimulation of the chemotactic migration of human fibroblasts by transforming growth factor beta. *J Exp Med* 165: 251–256
- Prins APA, Lipman JM, McDevitt CA, et al (1982a) Effect of purified growth factors on rabbit articular chondrocytes in monolayer culture. II. Sulfated proteoglycan synthesis. *Arthritis Rheum* 25: 1228
- Prins APA, Lipman JF, Sokoleff L (1982b) Effect of purified growth factors on rabbit articular chondrocytes in monolayer culture. I. DNA synthesis. *Arthritis Rheum* 25: 1217

-
25. Roberts AB, Sporn MB, Assoian RK et al (1986) Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen production in vitro. *Proc Natl Acad Sci USA* 83:4167–4171
26. Ross R, Glomset J, Kariya B, Harker L (1974) A platelet-dependent serum factor that stimulates the proliferation of arterial smooth muscle cells in vitro. *Proc Natl Acad Sci USA* 71:1207–1210
27. Saal JA, Saal JS, Herzog RJ (1990) The natural history of lumbar intervertebral disc extrusion treated nonoperatively. *Spine* 15:683–686
28. Shweiki D, Itin A, Soffer D, Keshet E (1992) Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-induced angiogenesis. *Nature* 359:843–845
29. Stiles CD (1983) The molecular biology of the platelet-derived growth factor. *Cell* 33:653–655
30. Tischer E, Gospodarowicz D, Mitchell R et al (1989) Vascular endothelial growth factor: a new member of the platelet-derived growth factor gene family. *Biochem Biophys Res Commun* 165:1198–1206
31. Tolonen J, Grönblad M, Virri J et al (1995) Basic fibroblast growth factor immunoreactivity in blood vessels and cells of disc herniations. *Spine* 20:271–276
32. Yasuma T, Kouichi A, Yamauchi Y (1993) The histology of lumbar intervertebral disc herniation: the significance of small blood vessels in the extruded tissue. *Spine* 18:1761–1765
33. Zetter B (1980) Migration of capillary endothelial cells is stimulated by tumour-derived factors. *Nature* 285:41–43