Altered Distribution of Heparan Sulfate Proteoglycans in Psoriasis

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Heparan sulfate proteoglycans (HSPGs) are components of the basement membrane (BM) of various tissues. They consist of a core protein and negatively charged glycosaminoglycan side chains: the heparan sulfate (HS) moieties.

In psoriasis, uninvolved skin of psoriasis patients and in normal skin, the distribution of HSPGs was studied immuno-histochemically by means of three different monoclonal antibodies: JM-72, directed against the HSPG-core protein, JM-13 against the sulfated domains of HS and JM-403 against the unmodified/low sulfated parts of HS.

In psoriasis JM-13 staining was consistently absent in the tips of the dermal papillae, whereas JM-13 showed a continuous staining in the BM of uninvolved and normal skin. JM-403 staining was present in BM of all specimens. In addition, a honeycomb-like staining was found in epidermis of normal skin and to a lesser extent in uninvolved skin, due to binding with plasma membrane-associated HS. In psoriasis this JM-403 staining of the epidermis was invariably absent. JM-72 showed a continuous staining of BM in all biopsies.

In conclusion, normal human skin and involved psoriatic skin show clear differences in expression of HS. These data may provide insight into the role of HSPGs in psoriasis, which remains further to be elucidated. *Key words: dermo-epidermal junction; basement membrane; immunohistochemical; normal skin; uninvolved psoriasis.*

Psoriasis is a hyperproliferative skin disease, the aetiology of which is still unknown. In the epidermis of uninvolved skin of psoriasis patients, various changes have been described, but none of these have until now been shown to play the major role in the pathogenesis of psoriasis (1). Investigations of incipient psoriasic lesions and spreading psoriatic plaques have shown that even in very early lesions and in adjacent uninvolved skin, changes can be detected in the stroma. Using laser Doppler flowmetry, Macdonald Hull et al. showed increased blood flow that could be demonstrated before clinical lesions appeared (2). In addition, the endothelial marker enzyme alkaline phosphatase was distinctly increased 1 cm outside spreading psoriatic plaques (3), and the extracellular matrix (ECM) protein tenascin was already found to be increased before clinical lesions appeared (4).

In recent studies, the dermo-epidermal junction has received little attention. The basement membrane (BM) of the epidermis is known to influence a series of important functions, such as keratinocyte differentiation, growth and migration. In addition, due to the assembly the BM-components and the

presence of anionic sites in the BM, the BM plays a role as a selective filtration barrier (5–8).

In psoriasis, the role of some BM-components was studied in detail (4, 9–12). An important component of the BM, which has never been studied before in psoriatic lesions, is heparan sulfate proteoglycan (HSPG). This member of the heterogeneous family of the proteoglycans is composed of glycosaminoglycan side chains, the negatively charged heparan sulfate (HS) moieties, that are covalently bound to a core protein (13, 14). There are two classes of HSPGs: one class is present on the cell membrane of many cell types, the other class is expressed in the ECM and more specifically in BM (14). The BM-associated HSPGs bind to laminin, fibronectin, collagen type IV and nidogen and therefore play an important role in BM assembly (15). Because of the negatively charged shield they block passage of anionic macromolecules through the BM. The storage of growth factors is considered to be an important function of HSPGs (5, 16). This storage of growth factors, the important role in BM assembly and the barrier function of HSPGs, combined with the fact that abnormal proliferation, differentiation and increase in transepidermal water loss are important characteristics of a psoriatic lesion (17, 18), pointed towards a possible change of HSPGs in psoriasis.

Recently, three monoclonal antibodies directed against the HSPGs have become available: monoclonal antibody JM-13, directed against the highly sulfated domains of HS, mAb JM-403, directed against the unmodified/low sulfated parts of HS, and mAb JM-72, an antibody directed against the core protein of BM HSPGs. These antibodies enabled us to study the distribution of HSPG in detail. The aim of the present study was to investigate immunohistochemically the localisation and distribution of HSPG-core protein and HSPG side chains in psoriatic lesions and to compare them to normal skin and uninvolved psoriatic skin.

MATERIALS AND METHODS

Patients and biopsies

Twenty patients with psoriasis and 20 healthy volunteers participated in this study. The 20 patients suffering from psoriasis (7 women and 13 men, age range 24–72 years; mean 48.2 years) had used no topical treatment for at least 2 weeks, and no systemic anti-psoriatic therapy for at least 1 year. After local anaesthesia, a biopsy (φ4 mm) from a psoriatic lesion was taken in 20 patients. In 6 patients, an additional biopsy was taken from a clinically uninvolved part of the skin, at a distance of at least 20 cm from a lesion.

In addition, biopsies were taken from the normal skin of 20 healthy volunteers without history or sign of skin disease (19 men and one woman, age range 25–56 years; mean 29,9 years).

Biopsies were embedded in Tissue Tek OCT compound (Miles Scientific, Naperville, U.S.A.), snap frozen in liquid nitrogen and stored at −80°C. Tissues were sectioned at 7 μm and fixed in acetone (0°C) for 10 min and again stored at −80°C until use.

Permission to conduct this study was obtained from the local Ethics Committee of the University Hospital Nijmegen. Informed consent was given by all subjects in this study.

Antibodies

For immunohistochemical staining, three monoclonal antibodies against HSPG were used. mAb JM-72 (19), which is directed against the BM- associated HSPG-core protein, and two mAbs, designated as JM-13 (20) and JM-403 (21) against the HS-side chains. The production, characterisation and demonstration of specificity of these monoclonal antibodies were carried out by van den Born et al., as previously published (19–21). The two mAbs JM-13 and JM-403 recognise different epitopes along the HS polysaccharide chain: JM-13 binds to N-sulfated epitopes in HS and heparin (which is closely related to HS but much more sulfated); JM-403 preferentially binds to low-sulfated HS preparations. Moreover, the epitope is fully dependent on the presence of an N-unsubstituted glucosamine residue, thus having a free amino group. These units are not found in hyaluronic acid, thereby excluding binding of JM-403 to hyaluronic acid (22). In conclusion: mAb JM-13 binds to an epitope in the highly sulfated domains of HS and heparin, whereas mAb JM-403 binds to unmodified/low sulfated regions of HS.

Immunohistochemical staining

For all three antibodies an indirect peroxidase technique was used. Control stainings were carried out using nonimmune mouse IgG. After thawing at room temperature for 30 min, slides were fixed again in acetone (0°C) for 10 min, washed in phosphate-buffered saline (PBS) and incubated for 1 h at room temperature with the primary antibodies [anti HSPG-core mAb JM-72 (ascites, 1:250 in PBS), anti HS mAb JM-403 (ascites, 1:250) and anti HS mAb JM-13 (undiluted culture supernatant)].

After having been washed twice in PBS the slides were incubated for 30 min with a solution of rabbit-anti-mouse-immunoglobulins (DAKOPATTS, Copenhagen, Denmark) conjugated with peroxidase, diluted 1:50 in phosphate buffer containing 5% human AB-serum. After two further washes in PBS and a preincubation with sodium acetate buffer (pH 4.9), slides were stained in a solution of 3-amino-9-ethyl-carbazole (AEC) in sodium acetate buffer containing 0.01% H_2O_2 for 10 min. Slides were counterstained with Mayer's haematoxylin (Sigma, St Louis, U.S.A.) and mounted in glycerin gelatin (Sigma, St Louis, U.S.A.).

Histological examination

The distribution of the staining in dermis, epidermis and basal membrane was assessed by two independent investigators using a 3-point scale: 0 = staining not present, 1 = discontinuously distributed staining, 2 = continuously distributed staining. In addition, staining of BM of bloodvessels in the dermis was scored as being either present or absent and the presence of cell membrane-associated staining was recorded as well.

RESULTS

All control stainings using nonimmune mouse IgG were negative.

JM-13

All 20 specimens of psoriatic skin showed a striking staining pattern, completely different from the staining pattern in normal and uninvolved skin. JM-13 staining was consistently absent in the tips of the dermal papillae. A clear, linear staining was present invariably in the lower parts of the dermal papillae (Fig. 1b).

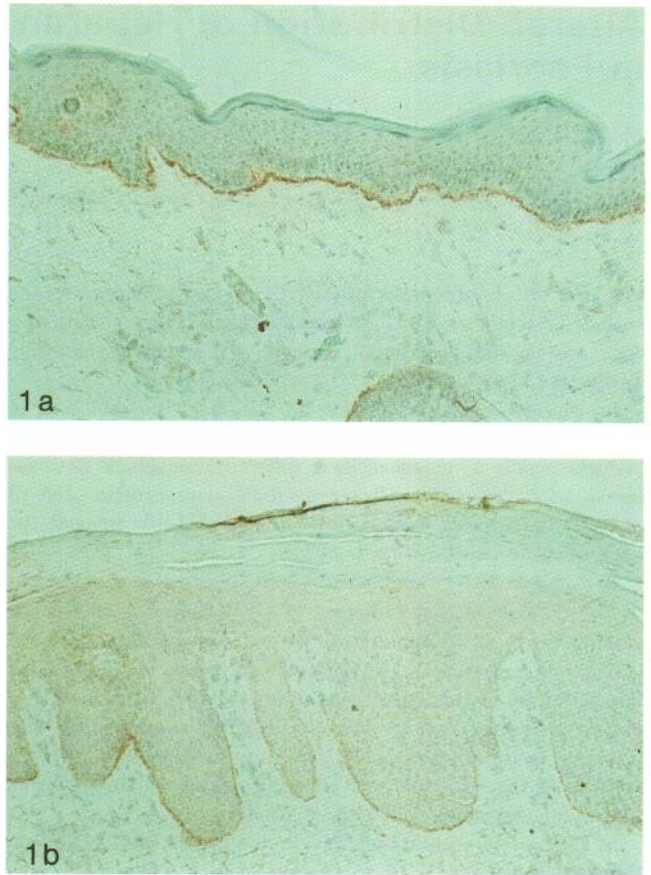The JM-13 staining in all 20 specimens of normal skin showed a continuous staining of the BM at the dermo-

Fig. 1. Immunohistochemical detection of HS by mAb JM-13. *(a)* Normal skin. *(b)* Psoriasis. Note absence of staining in the tips of the dermal papillae.

epidermal junction (Fig. 1a). The clinically uninvolved skin of psoriatic patients showed a pattern that was similar to that observed in healthy volunteers. In contrast to the antibodies JM-403 and JM-72, mAb JM-13 only stained the BM of the dermo-epidermal junction and not the BM of the bloodvessels.

JM-403

In all specimens from normal skin, JM-403 staining, directed against the unmodified/low sulfated parts of HS, displayed a strong honeycomb-like staining in the epidermis (Fig. 2a). The staining pattern suggested staining of membrane-bound structures or intercellular substances. This staining pattern was most pronounced in the stratum spinosum and stratum granulosum and was less prominent in the stratum corneum and basal layers. In uninvolved epidermis of psoriatic patients the cell membrane associated staining was seen more focally and was less conspicuous, whereas this staining pattern was invariably absent in the lesional skin of all psoriatic patients (Fig. 2b).

In the BM of skin and bloodvessels the JM-403 staining was bright, continuous and present in all specimens.

JM-72

Staining with the antibody JM-72, directed against the core protein of the HSPG, was positive in all specimens. In normal

Fig. 2. Immunohistochemical staining of HS-side chains by mAb JM-403. *(a)* Normal skin. Note the honeycomb-like staining in the epidermis. *(b)* Psoriasis. Note the absence of honeycomb-like staining.
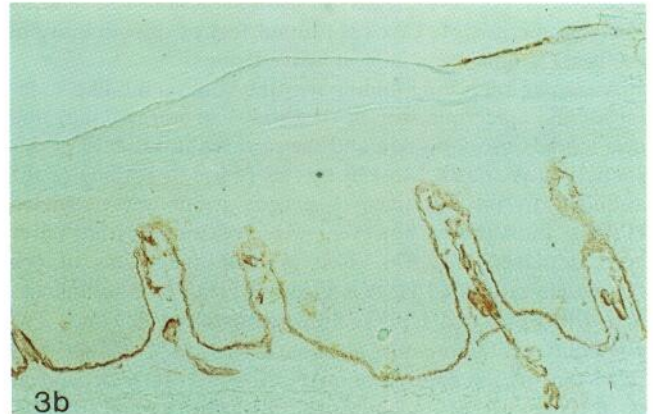
Fig. 3. Immunohistochemical detection of HSPG-core protein by mAb JM-72. *(a)* Normal skin. *(b)* Psoriasis.

skin, uninvolved psoriasis and psoriatic lesions the staining was restricted to the BM of the dermal-epidermal junction and the BMs of the blood vessels in the dermis. There was a strong and continuous linear staining (Fig. 3). No additional structures were stained.

DISCUSSION

At present little is known about the distribution of HSPGs in human skin. In normal human epidermis, Tammi et al. localised keratinocyte surface-associated HSPGs by means of electron microscopy (23), and recently, van den Born et al. described the expression of BM-associated HSPGs in normal human skin (19).

In this study we analysed the distribution of HSPGs in psoriasis, uninvolved psoriasis and normal skin by means of immunohistochemical staining. Three new monoclonal antibodies were used, two directed against the HS-side chains (JM-13 and JM-403) and one against the HSPG-core protein (JM-72) (19–21). mAb JM-72 gave a continuous, similar staining in all specimens, indicating that the core protein of HSPG is present in normal skin, uninvolved psoriasis and psoriatic skin without a difference in distribution. This also applies to the staining of the BM by the mAb JM-403, directed against the HS-side chains, which also gave a bright, continuous staining in all specimens.

However, staining with JM-13, directed against a different

epitope on the HS-side chain, showed a clearly different pattern in all specimens. It was absent in the tips of the dermal papillae of all psoriasis specimens. The heterogeneity in staining of the HS-side chains by the two antibodies JM-13 and JM-403 can be explained by the different epitopes recognised. The epitope of JM-403 is located in the unmodified/low sulfated domains of HS. This is in contrast with the epitope of JM-13, which is located in the N-sulfated parts of HS-side chain.

Several mechanisms can explain the absence of JM-13 staining in the tips of the dermal papillae. This might be the result of decreased accessibility of HS due to epitope masking by cationic molecules or immune complexes, or alternatively the epitopes are lost due to enzymatic cleavage of HS. A third possibility could be a steric hindrance of BM components caused by changed molecular interactions. These three possibilities are discussed below.

In psoriasis, neutrophil polymorphonuclear leukocytes (PMNs) and plasma proteins move from the tip of the capillary loops in the dermal papillae into the epidermis (24, 25). It is striking to observe that in this part of the basal membrane, where the PMNs and plasma proteins pass, the JM-13 staining is absent. It is tempting to assume a connection between these events. Maybe the JM-13 epitope is blocked by plasma and/or PMN-derived cationic proteins, remaining the unmodified/low sulfated regions in HS accessible for JM-403 binding. This mechanism has been described in previous studies on SLE-nephritis, showing a binding of cross-reactive anti-nuclear antibodies to HS (26, 27). Recently, the adherence of leukocyte

integrin Mac-1 to heparan sulfate has been described (28). The adhesion to heparan sulfate of this integrin, which co-ordinates adhesive functions including leukocyte migration, strengthens this explanation for the absence of JM-13 staining. A second explanation might be the lytic action of inflammatory cells, such as PMNs, that may destroy the JM-13 epitope. Thirdly, many interactions between the HS-side chain and the BM components fibronectin, laminin and collagen type IV are known. Battaglia et al. describe the formation of ternary complexes between laminin, nidogen and proteoglycan, suggesting a key role for nidogen in basement membrane assembly (15, 29). It could thus be that one of these interactions is changed in the tips of the dermal papillae in such a way that the accessibility of the JM-13 epitope is altered by steric hindrance of BM components. A previous study already found a general loss of BM integrity in psoriatic lesions (30) and by electron microscopy, Cox (31) found foci of discontinuity in the basal lamina, especially in active lesions.

A second interesting finding was the honeycomb-like staining of the epidermis with mAb JM-403 in normal skin and non-lesional psoriatic skin and the remarkable absence of this staining in psoriasis. Most likely this is due to binding of the antibody to the keratinocyte plasma membrane-associated HSPGs. In literature, however, such crossreactivity has never been described (32, 33). Two possible candidates of cell membrane-associated HSPGs in skin which share homologies with BM-associated HSPGs are syndecan and CD44v3. The possibility of staining of hyaluronic acid by mAb JM-403 was ruled out, as the epitope of mAb JM-403 is fully dependent on the presence of an N-unsubstituted glucosamine residue, thus having a free amino group. These units are not found in hyaluronic acid (22).

Both CD44v3 and syndecan-1 are known to play an important role in cell-cell interactions, cell-ECM interactions and the binding of growth factors (34–36). These biological functions are important in the regulation of cell growth and migration. Absence of either syndecan-1 or CD44v3 in the epidermis of a psoriatic lesion could contribute to changes in proliferation, differentiation and migration in psoriasis.

In conclusion, an altered HSPG expression in psoriasis was described compared to normal skin, namely the absence of high sulfated HS-side chains (JM-13) in the tips of the dermal papillae in psoriatic patients and the different staining pattern with mAb JM-403 against low sulfated HS-side chains in psoriasis compared to uninvolved and normal skin.

To the best of our knowledge, these changes in HSPG expression in psoriasis have never been described before. These data may provide insight into the role of HSPGs in psoriasis, which remains further to be elucidated.

REFERENCES

1. van de Kerkhof PCM, Bos JD. Pathogenetic aspects of psoriasis. Clin Dermatol 1995; 13:97–190.(Abstract.)
2. Macdonald Hull S, Goodfield M, Wood EJ, Cunliffe WJ. Active and inactive edges of psoriatic plaques: identification by tracing and investigation by laser–Doppler flowmetry and immunocytochemical techniques. J Invest Dermatol 1989; 92: 782–785.
3. van de Kerkhof PC, van Rennes H, de Grood R, Bauer FW, Mier PD. Metabolic changes at the margin of the spreading psoriatic lesion. Br J Dermatol 1983; 108: 647–652.
4. De Jong EMGJ, Schalkwijk J, van de Kerkhof PCM. Epidermal proliferation and differentiation, composition of the inflammatory infiltrate and the extracellular matrix in the margin of the spreading psoriatic lesion. Eur J Dermatol 1991; 1: 221–227.
5. Timpl R. Structure and biological activity of basement membrane proteins. Eur J Biochem 1989; 180: 487–502.
6. Briggaman RA. Biochemical composition of the epidermal-dermal junction and other basement membrane. J Invest Dermatol 1982; 78: 1–6.
7. Woodley DT. Importance of the dermal-epidermal junction and recent advances. Dermatologica 1987; 174: 1–10.
8. Yurchenco PD, Schittny JC. Molecular architecture of basement membranes. FASEB J 1990; 4: 1577–1590.
9. Mondello MR, Califano L, Cannavo SP, Di Mauro D, Guarneri B, Magaudda L, et al. Psoriasis and cyclosporin: immunohistochemical aspects of the basement membrane. Acta Derm Venereol (Stockh) 1994; Suppl 186: 96–98.
10. el Sherif AI, Krupinski A, Majewski S, Jablonska S. Leukocyte-extracellular matrix interactions in psoriasis. Int J Dermatol 1994; 33: 371–373.
11. Fyrand O. Studies on fibronectin in the skin. II. Indirect immunofluorescence studies in psoriasis vulgaris. Arch Dermatol Res 1979; 266: 33–41.
12. Glinski W, Jarzabek Chorzelska M, Kuligowski M, Pierozynska Dubowska M, Glinska Ferenz M, Jablonska S. Basement membrane zone as a target for human neutrophil elastase in psoriasis. Arch Dermatol Res 1990; 282: 506–511.
13. Hardingham TE, Fosang AJ. Proteoglycans: many forms and many functions. FASEB J 1992; 6: 861–870.
14. Gallagher JT, Lyon M, Steward WP. Structure and function of heparan sulphate proteoglycans. Biochem J 1986; 236: 313–325.
15. Battaglia C, Mayer U, Aumailley M, Timpl R. Basement-membrane heparan sulfate proteoglycan binds to laminin by its heparan sulfate chains and to nidogen by sites in the protein core. Eur J Biochem 1992; 208: 359–366.
16. Ruoslahti E. Proteoglycans in cell regulation. J Biol Chem 1989; 264: 13369–13372.
17. Rajka G, Thune P. The relationship between the course of psoriasis and transepidermal water loss, photoelectric plethysmography and reflex photometry. Br J Dermatol 1976; 94: 253–261.
18. Motta S, Monti M, Sesana S, Mellesi L, Ghidoni R, Caputo R. Abnormality of water barrier function in psoriasis. Role of ceramide fractions. Arch Dermatol 1994; 130: 452–456.
19. van den Born J, van den Heuvel LP, Bakker MA, Veerkamp JH, Assmann KJ, Berden-JH. Monoclonal antibodies against the protein core and glycosaminoglycan side chain of glomerular basement membrane heparan sulfate proteoglycan: characterization and immunohistological application in human tissues. J Histochem Cytochem 1994; 42: 89–102.
20. van den Born J, van den Heuvel LP, Bakker MA, Veerkamp JH, Assmann KJ, Berden JH. Production and characterization of a monoclonal antibody against human glomerular heparan sulfate. Lab Invest 1991; 65: 287–297.
21. van den Born J, van den Heuvel LP, Bakker MA, Veerkamp JH, Assmann KJ, Berden JH. A monoclonal antibody against GBM heparan sulfate induces an acute selective proteinuria in rats. Kidney Int 1992; 41: 115–123.
22. van den Born J, Gunnarsson K, Bakker MA, Kjellen L, Kusche Gullberg M, Maccarana M, et al. Presence of N-unsubstituted glucosamine units in native heparan sulfate revealed by a monoclonal antibody. J Biol Chem 1995; 270: 31303–31309.
23. Tammi RH, Hyyrylainen AM, Maibach HI, Tammi MI. Ultrastructural localization of keratinocyte surface associated heparan sulphate proteoglycans in human epidermis. Histochemistry 1987; 87: 243–250.
24. Pinkus H, Mehregan AH. The primary histologic lesion of seborrheic dermatitis and psoriasis. J Invest Dermatol 1966; 46: 109–116.
25. Verschoore M, Kowalewski C, Chorzelska MJ, Bernard BA, Darmon YM. Intraepidermal leakage of plasma proteins after

tape stripping of normal skin and uninvolved psoriatic skin. Br J Dermatol 1990; 122: 391–397.

26. Termaat RM, Brinkman K, van Gompel F, van den Heuvel LP, Veerkamp JH, Smeenk RJ, et al. Cross-reactivity of monoclonal anti-DNA antibodies with heparan sulfate is mediated via bound DNA/histone complexes. J Autoimmun 1990; 3: 531–545.

27. Kramers C, Hylkema MN, van Bruggen MC, van de Lagemaat R, Dijkman HB, Assmann KJ, et al. Anti-nucleosome antibodies complexed to nucleosomal antigens show anti-DNA reactivity and bind to rat glomerular basement membrane in vivo. J Clin Invest 1994; 94: 568–577.

28. Diamond MS, Alon R, Parkos CA, Quinn MT, Springer TA. Heparin is an adhesive ligand for the leukocyte integrin Mac-1 (CD11b/CD18). J Cell Biol 1995; 130: 1473–1482.

29. Aumailley M, Battaglia C, Mayer U, Reinhardt D, Nischt R, Timpl R, et al. Nidogen mediates the formation of ternary complexes of basement membrane components. Kidney Int 1993; 43: 7–12.

30. Gay S, Kresina TF, Gay R, Miller EJ, Montes LF. Immunohistochemical demonstration of basement membrane collagen in normal human skin and in psoriasis. J Cutan Pathol 1979; 6: 91–95.

31. Cox AJ. The dermal-epidermal junction in psoriasis. J Invest Dermatol 1969; 53: 428–435.

32. Stow JL, Farquhar MG. Distinctive populations of basement membrane and cell membrane heparan sulfate proteoglycans are produced by cultured cell lines. J Cell Biol 1987; 105: 529–539.

33. Jalkanen M, Rapraeger A, Bernfield M. Mouse mammary epithelial cells produce basement membrane and cell surface heparan sulfate proteoglycans containing distinct core proteins. J Cell Biol 1988; 106: 953–962.

34. Sanderson RD, Hinkes MT, Bernfield M. Syndecan-1, a cell-surface proteoglycan, changes in size and abundance when keratinocytes stratify. J Invest Dermatol 1992; 99: 390–396.

35. Jackson DG, Bell JI, Dickinson R, Timans J, Shields J, Whittle N. Proteoglycan forms of the lymphocyte homing receptor CD44 are alternatively spliced variants containing the v3 exon. J Cell Biol 1995; 128: 673–685.

36. Bennett KL, Jackson DG, Simon JC, Tanczos E, Peach R, Modrell B, et al. CD44 isoforms containing exon V3 are responsible for the presentation of heparin-binding growth factor. J Cell Biol 1995; 128: 687–698.