Expression of proteoglycan core proteins in human bone marrow stroma

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Heparan sulphate proteoglycans (HSPGs) present on the surface of bone marrow stromal cells and in the extracellular matrix (ECM) have important roles in the control of adhesion and growth of haemopoietic stem and progenitor cells. The two main groups of proteoglycans which contain heparan sulphate chains are members of the syndecan and glypican families. In this study we have identified the main surface membrane and matrix-associated HSPGs present in normal human bone marrow stroma formed in long-term culture. Proteoglycans were extracted from the adherent stromal layers and treated with heparitinase and chondroitinase ABC. The core proteins were detected by Western blotting using antibodies directed against syndecans-1–4, glypican-1 and the ECM HSPG, perlecan. Stromal cell expression at the RNA level was detected by Northern blotting and by reverse transcription PCR. Glypican-1, syndecan-3 and syn-

decane-4 were the major cell-membrane HSPG species and perlecan was the major ECM proteoglycan. There was no evidence for expression of syndecan-1 protein. Syndecan-3 was expressed mainly as a variant or processed 50–55 kDa core protein and in lower amounts as the characteristic 125 kDa core protein. These results suggest that syndecan-3, syndecan-4 and glypican-1 present on the surface of marrow stromal cells, together with perlecan in the ECM, may be responsible for creating the correct stromal ‘niche’ for the maintenance and development of haemopoietic stem and progenitor cells. The detection of a variant form of syndecan-3 as a major stromal HSPG suggests a specific role for this syndecan in haemopoiesis.

Key words: extracellular matrix, haemopoiesis, heparan sulphate, syndecans.

INTRODUCTION

Interactions between haemopoietic progenitor cells and the bone marrow stroma in which they reside are recognized to be of physiological importance not only for normal proliferation and differentiation but also for the maintenance of haemopoietic stem cells [1]. Heparan sulphate proteoglycans (HSPGs) present on bone marrow stromal cells and in the extracellular matrix (ECM) have increasingly defined roles in haemopoiesis. The positioning of haemopoietic stem and progenitor cells within discrete areas of marrow stroma results in access to locally available growth factors which can themselves be sequestered by heparan sulphate [2]. Basic fibroblast growth factor acts at an early stage of haemopoiesis and requires heparan sulphate as a co-receptor, which enables it to bind to and activate its high-affinity receptor [3,4]. The haemopoietic growth factors granulocyte macrophage-colony-stimulating factor (GM-CSF) and interleukin-3 can bind to heparan sulphate and can then be presented in a biologically active form to haemopoietic progenitor cells [2,5]. Macrophage inflammatory protein-1 α (MIP-1 α) is a chemokine that has an inhibitory effect on the proliferation of haemopoietic stem cells and also binds to heparan sulphate [6,7]. The ability of primitive haemopoietic progenitor cells to bind to stroma is partly dependent on the presence of HSPGs in the ECM [8] and bone marrow stromal-derived heparan sulphate has been shown to have an important role in the maintenance *in vitro* of human long-term culture-initiating cells [9].

Expression of HSPGs by progenitor cells is variable; the murine factor-dependent cells Paterson (FDCP)-mixed haemopoietic progenitor cell line lacks surface-membrane heparan

sulphate and synthesizes a chondroitin 4-sulphate proteoglycan, whereas the human TF-1 cell line contains an HSPG as a major proteoglycan [10,11]. The malignant plasma cells of myeloma express heparan sulphate-bearing syndecan-1 [12], and recent work has shown that Reed–Sternberg cells of classical Hodgkin’s Disease also express syndecan-1 [13]. In these cases, the cell-membrane heparan sulphate may play a part in cell-matrix adhesion by binding to stromal ECM ligands, such as fibronectin [14].

Heparan sulphate-bearing proteoglycans, probably of stromal cell origin, thus have major roles in the control of both growth and adhesion of haemopoietic stem and progenitor cells and their characterization on the stromal cells and in the ECM is important. The marrow environment *in vivo* is mimicked by the adherent stromal layer that forms in long-term culture which is able to support the growth of haemopoietic progenitor cells [1]. A recent study has characterized several different HSPGs from the murine bone marrow stromal cell line MS-5 that can support the growth of human progenitor cells [15], but although the molecular structure of the glycosaminoglycan chains of heparan sulphate has been analysed in human long-term bone marrow cultures [16], the associated proteoglycan core proteins have hitherto been uncharacterized.

The two major groups of cell-membrane proteoglycans that contain heparan sulphate chains are members of the syndecan and glypican families. There are four syndecans (syndecans-1–4) and five glypicans, the latter being linked to the cell membrane by a glycosylphosphatidyl inositol anchor [17,18]. Most cells and tissues express at least one syndecan family member; most express multiple syndecans [19] with characteristic expression

Abbreviations: RT-PCR, reverse transcription PCR; HSPG, heparan sulphate proteoglycan; ECM, extracellular matrix; GM-CSF, granulocyte macrophage-colony-stimulating factor; MIP-1 α , macrophage inflammatory protein-1 α .

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patterns in individual cell types and tissues, e.g. syndecan-1 is expressed by epithelial cells [20] and malignant plasma cells [12], syndecan-3 (*N*-syndecan) is found predominantly in the central nervous system [21], syndecan-2 is produced mainly by mesenchymal cells [22] and syndecan-4 is found in many cell types. Glypican-1 is also a widely expressed proteoglycan and commonly occurs on cell surfaces with one or more of the syndecans [18]. Other cell-surface HSPGs are betaglycan, which binds basic fibroblast growth factor via its heparan sulphate chains [23], and an isoform of CD44 (the CD44E variant or epican), which can also occur as a heparan sulphate-bearing protein in some types of cultured cell [24].

In the present study we set out to identify and characterize the main surface-membrane HSPGs present in normal human bone marrow stroma together with the matrix-associated HSPG, perlecan [25].

MATERIALS AND METHODS

Human long-term cultures

Marrow was obtained with informed consent from normal donors undergoing bone marrow harvests and collected into Iscoves medium/10% foetal calf serum with 10 units/ml preservative-free heparin. Red cells were removed by sedimentation with a 1% methylcellulose solution. Mononuclear cells $[(1.5-2.0) \times 10^7]$ were cultured in 10% foetal calf serum, 10% donor horse serum, 5×10^{-7} M hydrocortisone and Iscoves medium at an osmolarity of 320 mosmol/kg in a total volume of 10 ml in a flat-bottomed flask (Falcon, 4×8 cm). Flasks were gassed with 5% CO₂ in air and placed at 33 °C. Cultures were fed weekly by removing half the medium and replacing it with fresh medium. Stromal layers from 18 flasks were harvested after reaching confluence at around 3–4 weeks. Prior to harvesting (3 days) the flasks were irradiated with 1500 cGy of gamma radiation from a ¹³⁷Cs source to destroy endogenous haemopoietic cells. For isolation of proteoglycans prior to Western blotting, the stromal layer of each flask was labelled with 50 μ Ci ³⁵Na₂SO₄ of specific activity 1200–1400 Ci/mmol for 24 h before extraction.

Isolation of proteoglycans from stromal layers

The culture medium was removed and retained for further analysis. The adherent stromal layers were rinsed three times with PBS at 4 °C prior to adding 5 ml of 1% Triton X-100 buffer containing the protease inhibitors 10 mM EDTA, 5 mM *N*-ethylmaleimide, 5 mM benzamidine and 1 mM PMSF. The stromal layers were then scraped from the bottoms of the flasks and centrifuged at 3200 *g* for 60 min at 4 °C to separate the Triton extract (containing membrane proteoglycans) from the insoluble residue. The detergent extract was added to a DEAE-Sepharose column that was washed through with 1% Triton X-100 buffer to retain proteoglycans on the Sepharose beads. The Triton-insoluble residue was washed three times with 1% Triton X-100 buffer, resuspended in OG buffer (60 mM octylglucoside substituted for Triton X-100) and centrifuged as above, leaving a final detergent-insoluble residue (matrix). The media, Triton extracts and octyl glucoside extracts were added to DEAE-Sepharose columns, which were washed through with 0.1% Triton X-100/200 mM NaCl/20 mM Tris/HCl, pH 7.4. The proteoglycan fractions were eluted from the beads with 0.1% Triton X-100/1 M NaCl/20 mM Tris/HCl, pH 7.4, and dialysed against 0.1% Triton X-100/100 mM NaCl/1 mM CaCl₂/50 mM 6-aminohexanoic acid/50 mM Hepes, pH 7.0. After dialysis the samples were supplemented with 50 μ g/ml leupeptin, 2.5 μ g/ml

pepstatin and 1 mM PMSF and treated with enzyme as described below.

Enzyme treatments, electrophoresis and Western blotting

Samples of resuspended matrix, media or cell extracts were incubated for 3 h at 37 °C either with or without 5 m-units of heparitinase and 25 m-units of chondroitinase ABC in 100 mM NaCl/1 mM CaCl₂/50 mM Hepes, pH 7.0, in the presence of protease inhibitors and 100 μ g/ml BSA. Both enzymes were obtained from Seikagaku Kogyo (Tokyo, Japan). Electrophoresis of enzyme-treated samples was performed in 6–20% SDS at 120 V until the dye track reached the bottom of the gel. Electrotransfer to Zeta-probe membrane (Bio-Rad) was performed for 3 h at 70 V. After transfer the membranes were inactivated with 0.5% casein/0.6 M NaCl/10 mM phosphate buffer, pH 7.4. The membranes were incubated for 2 h with 10 μ g/ml primary antibody in PBS/0.5% casein, and then for 1 h with alkaline phosphatase-conjugated goat anti-mouse antibody (Promega), diluted 1:5000 in PBS/0.5% casein. The bands were detected by chemiluminescence, using CSPD (Tropix, Bedford, MA, U.S.A.) as substrate, and recorded on Hyperfilm (Amersham). Control membranes were incubated with the secondary antibody alone. A control lane was set up with material extracted from normal foetal human lung fibroblasts [22].

Antibodies

Monoclonal antibodies directed against the following core proteins were used: syndecan-1 ectodomain (B-B4; Serotec, Oxford, U.K.), syndecan-2 cytoplasmic domain (6G12), syndecan-4 ectodomain (8G3), syndecan-3 ectodomain (1C7), glypican-1 (S1), perlecan (mixture of four monoclonal antibodies which recognize four different epitopes in the core protein) and an antibody (3G10, anti-stub) against a common epitope generated from all heparitinase-digested HSPGs. All the antibodies except for B-B4 were prepared and supplied by G. David, University of Leuven, Leuven, Belgium.

Northern-blotting analysis

Total RNA was extracted from adherent stromal layers with RNazol (Biotech) and 10–20 μ g was loaded on to formaldehyde/1.2% agarose gels and run at 70 V in Mops/formaldehyde buffer. The distance of migration of the ribosomal RNA bands was measured by staining the gel in 10 μ g/ml ethidium bromide in water for 30 min. The separated RNA was transferred from the gel to Hybond N membranes (Amersham) in 20 $\times$ SSC buffer (where 1 $\times$ SSC is 0.15 M NaCl/0.015 M sodium citrate) overnight. The membrane was washed in 2 $\times$ SSC/1% SDS, exposed to UV illumination and stored at –20 °C. It was then washed in hybridization buffer (1% BSA/7.5% SDS in phosphate/NaCl) for 1–2 h and the radioactive probe was added and allowed to hybridize at 65 °C overnight. The membrane was then washed in 2 $\times$ SSC/1% SDS at 65 °C for 30 min followed by a second 30-min wash with 0.2 $\times$ SSC/0.1% SDS for cross-species sequences or 0.1 $\times$ SSC/0.1% SDS for homologous gene sequences. The membrane was wrapped in Saran wrap, inserted into an X-ray cassette and exposed to XAR 5 film (Kodak).

Probes for syndecan genes

Probes were made to rat syndecans-2, -3 and -4 and to human syndecan-1 by restriction-enzyme digestion of the non-homologous ectodomains from the respective full-length cDNAs. Probe length for syndecan-1 digested with *Pst*I was 406 bp, for syndecan-2 digested with *Bam*HI and *Hind*III was 145 bp, for

Figure 1 Western blots of proteoglycan core proteins from marrow stromal cells grown in long-term culture

Stromal layers were extracted with Triton X-100 leaving a residue from which proteoglycans were additionally extracted with octyl glucoside to give a final detergent-insoluble residue (matrix, M). The different fractions [Triton X-100 (TX), octyl glucoside (OG) and matrix] were digested with heparitinase and chondroitinase ABC, separated by electrophoresis in 6–20% SDS and transferred to nitrocellulose membranes. These were incubated with antibodies directed against the different core proteins and positive bands were detected by enhanced chemiluminescence. Control membranes were incubated with secondary antibody alone. A control lane contained material extracted from normal human lung fibroblasts. Results are shown using antibodies against glypican-1 (top left panel), syndecan-4 (top right panel), syndecan-3 (1C7; middle, left panel), syndecan-2 (middle, right panel) and syndecan-1 (B-B4; bottom panel). HA, double digest with heparitinase and chondroitinase ABC; N, not digested; HLF, normal human lung fibroblasts.

syndecan-3 digested with *Ava*I was 143 bp and for syndecan-4 digested with *Bgl*II was 219 bp. The double-stranded DNA (25–50 ng) was denatured at 100 °C for 2 min and random primed using 50 μ Ci 32 P according to the manufacturer's instructions (Boehringer Mannheim random-prime kit).

Reverse transcription PCR (RT-PCR) analysis of syndecan core proteins from human marrow stroma

Total RNA was extracted from the stromal layer with RNazol and mRNA was isolated with an oligo(dT)₂₅ magnetic separation

system (DynaL, Merseyside, U.K.). Reverse transcription was performed with avian reverse transcriptase (Promega) primed with oligo-dT (Boehringer). The cDNA was purified using GeneClean (Strattech, Luton, Herts, U.K.) and amplified by primers complementary to sequences within the 3' untranslated regions of the human *syn-1*, *syn-2* and *syn-4* genes. Each primer (30 pmol) was added to the reaction mix. The primer sequences were as follows: syndecan 1, 5' = GCATCGCACCATTCTGAC, 3' = CTCCCGACCATAGATTAG; syndecan 2, 5' = CAGATTTGCAACAGCAAA, 3' = TTAACACCAGTCTGC-AAC; and syndecan 4, 5' = ATCTAGGTCCGCCCTCAA, 3' = GGCAGAGATATACACTCT. The correct-sized transcript for syndecan-1 was 376 bp, for syndecan 2 was 329 bp and for syndecan-4 was 430 bp. PCR cycling conditions were 94 °C for 1 min, 50 °C for 1 min and 72 °C for 1 min for 35 cycles. The PCR products were run on a TBE/2% agarose gel (where 1 × TBE = 45 mM Tris/borate/1 mM EDTA) together with control samples in which reverse transcriptase was omitted.

The respective PCR products were electro-eluted from a Tris/acetate/EDTA/1% agarose gel and then cloned using the TA cloning technique (Invitrogen). The identity of the inserts was checked by sequencing using the dideoxy chain-termination method (Sequenase, USB, Cleveland, OH, U.S.A.).

RESULTS

Western-blotting analysis

The expected 64 kDa band for glypican-1 was present in the Triton X-100 extract after heparitinase enzyme digestion but was not expressed in the octyl glucoside extract or in the residue (Figure 1, top left panel). Syndecan-4 was detectable with monoclonal antibody 8G3 as a 37 kDa core protein in the enzyme-digested Triton extract and as a stronger band in the fibroblast control (Figure 1, top right panel). Detection of syndecan-3 with the ectodomain-specific 1C7 antibody showed a band between 100 and 200 kDa, characteristic for this protein, but mostly a strong 55 kDa band in the Triton X-100 extract following heparitinase treatment (Figure 1, middle, left panel). Detection of syndecan-2 with the 6G12 antibody resulted in a weak 48 kDa band in the enzyme-digested Triton extract and was hard to

Figure 2 Western blot of proteoglycan core proteins from different marrow stromal fractions prepared as described in Figure 1

Membranes were incubated with an antibody directed against a common epitope generated from all heparitinase-digested HSPGs (anti-stub antibody). For abbreviations, see Figure 1.

Figure 3 Western blot to detect perlecan in different marrow stromal fractions prepared as described previously

Membranes were incubated with an antibody against perlecan (a mixture of four antibodies recognizing different epitopes in the core protein). For abbreviations, see Figure 1.

Figure 4 Western blot of media from stromal cultures

Membranes were incubated with anti-stub (3G10), anti-glypican-1 (Gly) and anti-perlecan (Per) antibodies. 2°; secondary antibody alone. HA, double digest with heparitinase and chondroitinase ABC; N, not digested.

detect compared with the others, requiring longer exposures (Figure 1, middle, right panel). There was no evidence for syndecan-1 using the B-B4 antibody against the syndecan-1 ectodomain. Even long exposures remained negative, whereas a clearly positive 80 kDa syndecan-1 band was detected in the lung fibroblast extract (Figure 1, bottom panel). The 3G10 antibody against all HSPG cores (anti-stub antibody) indicated that the octyl glucoside extract and residue contained only a very large HSPG core which was probably perlecan (Figure 2). The corresponding Triton extract contained several bands with four major ones. Two strong bands were detected: one between 100 and 200 kDa and one just above 50 kDa, together with two lesser bands, one around 60 kDa and one (or a doublet) around 35 kDa.

The ECM proteoglycan, perlecan, was present in the enzyme-treated residue as a large HSPG core and was virtually absent from the other extracts (Figure 3). Examination of the medium showed positive bands for perlecan and also for two HSPGs detected using the 3G10 antibody, one of 100–200 kDa and another of around 60 kDa (Figure 4). Further testing of the medium showed a faint 64 kDa band for glypican-1 but showed no evidence of the syndecan-3 55 or 125 kDa bands (results not shown).

Figure 5 Northern blots of syndecan RNA transcripts expressed in marrow stromal cells grown in long-term culture

Total RNA was extracted from stromal layers and separated on a formaldehyde/1.2% agarose gel. RNA was transferred to Hybond N membranes, which were incubated with radiolabelled DNA probes to human syndecan-1 (top panel) and rat syndecan-3 (bottom panel). (Top panel) lane 1, rat brain; lane 2, MRC5 cell line; lanes 3 and 4, different marrow stromas. (Bottom panel) lane 1, MRC5; lane 2, rat brain; lanes 3 and 4, different marrow stromas.

Northern-blotting analysis

RNA transcripts for glypican-1 and for syndecan-4 were readily detectable in all marrow stromal samples. Signals for syndecan-2 were not detectable with the probe made from rat sequences, although repeat analysis with a probe to human syndecan-2 resulted in weak signals following long exposure times (results not shown). RNA transcripts for the two syndecans in which protein expression was either absent (syndecan-1) or expressed as a variant form (syndecan-3) are shown in Figure 5. The human syndecan-1 ectodomain probe resulted in a weak signal consistent with two transcripts of 2.6 and 3.4 kb in the stromal lanes with a stronger signal in the control MRC5 cell line (loading equal with ethidium bromide staining; Figure 5, top panel). A band consistent with an expected transcript size of 5.6 kb with the rat syndecan-3 ectodomain probe was seen in the stromal samples and a more strongly positive signal in the rat brain control (loading equal with ethidium bromide staining; Figure 5, bottom panel).

RT-PCR analysis

RT-PCR was performed to confirm expression of syndecans-1, -2 and -4 at the RNA level in marrow stroma. The correct-size transcripts for syndecan-1 (376 bp), syndecan-2 (329 bp) and syndecan-4 (430 bp) were detected in all stromal samples, with negative results in controls without reverse transcriptase. The specific identity of each band was confirmed by sequencing the respective cloned RT-PCR products.

DISCUSSION

One important role of marrow stroma in normal haemopoietic development is to provide anchorage sites for co-localization of stem and progenitor cells and heparan sulphate-binding growth factors that may stimulate proliferation and differentiation of progenitors, e.g. interleukin-3 and GM-CSF, or may act as stem-cell inhibitors, e.g. MIP-1 α . It is likely that the heparan sulphate chains that mediate these activities have their origins in cell-membrane proteoglycans of the stromal cells rather than the haemopoietic cells themselves, which may lack syndecan-bearing heparan sulphate. The different syndecans may fulfil particular functions, e.g. syndecan-4 is localized to focal adhesions where it may function in cell signalling [26], whereas syndecan-1 may have a role in regulating cell proliferation, as the level of its expression is associated with the clinical outcome of some carcinomas [27] and the addition of syndecan-1 ectodomain results in apoptosis and growth inhibition of myeloma cells [28] and in suppression of tumour cell growth [29]. It was therefore our intention to identify the core proteins of the stromal cell membrane-bound HSPGs, which may have particular roles in haemopoiesis.

We show here that glypican-1, syndecan-3 and syndecan-4 are the major cell-membrane HSPG species in human marrow stroma and that perlecan is the major ECM proteoglycan. Glypican has recently been shown to regulate the biological activity of fibroblast growth factors [30] and may therefore have a similar role in marrow stroma. Expression of syndecan-4 is consistent with its generally widespread tissue distribution.

Analysis of syndecan-3 in marrow stroma revealed expression of a 55 kDa protein. This was detected with the syndecan-3 ectodomain antibody 1C7 as a strong band in the stroma, whereas the expected 125 kDa core protein showed much weaker expression, although it was expressed strongly in the control fibroblasts (Figure 1, middle, left panel). The 55 kDa protein bears heparan sulphate as it was generated by heparitinase treatment and was detected by the 3G10 antibody; it bears an epitope known to be present in the ectodomain of syndecan-3 but is unlikely to be intact syndecan-3. We think it is likely that this is a variant form of syndecan-3, lacking at least part of the cytoplasmic domain. The large size difference between 'intact' syndecan-3 (125 kDa) and processed syndecan-3 (55 kDa) may be because processing cleaves between the glycosaminoglycan-attachment site and 1C7 epitope on one side and structures that are known to promote self-aggregation, i.e. the motif present in the transmembrane domain and ectodomain flanking region [31], on the other side. According to this reasoning, the 125 kDa protein would represent aggregated syndecan-3 core protein and the 55 kDa protein would be the non-aggregated, monomeric syndecan-3 ectodomain. There was no direct evidence for syndecan-3 shedding to the media (Figure 4), but processing may occur intracellularly and does not necessarily have to lead to shedding. Alternatively we may not have been able to detect the shed products in the media. Regulated shedding of syndecan-1 and -4 ectodomains by thrombin and growth-factor-receptor activation has been described and it has been suggested that the soluble ectodomains may have physiological roles [32]. This may be similar to the role of syndecan-3 in the nervous system, where a significant fraction of the *N*-syndecan molecules are present in a soluble form, presumably as a result of proteolytic membrane shedding. This may act as a control mechanism for terminating the association of syndecan-3 with the cytoskeleton [33]. Syndecan-3 in marrow stroma may thus be susceptible to degradation by similar or by different mechanisms and the processed products may have specific roles in haemopoiesis.

Table 1 Expression of syndecans and other HSPGs in human bone marrow stroma

Relative strength of expression of protein and RNA for different HSPGs. ++, strong expression; +, weak expression; —, not expressed; nd, not done.

HSPG	Western analysis		Northern analysis	RT-PCR
	Cell membrane	ECM		
Syndecan-1	—	—	+	Present
Syndecan-2	+	—	+	Present
Syndecan-3	++	—	+	nd
Syndecan-4	++	—	++	Present
Glypican-1	++	—	++	nd
Perlecan	—	++	nd	nd

Syndecan-2 was difficult to detect, requiring long exposures to develop the 48 kDa core protein (Figure 1, middle, right panel) and also to detect the RNA transcripts by Northern-blot analysis. Syndecan-2 was easily detected, however, using human-based primers in the RT-PCR reaction. The weak expression of syndecan-2 at both protein and RNA levels was perhaps surprising in view of the 'fibroblast' component of marrow stroma. There was no evidence for stromal expression of syndecan-1 protein using the B-B4 antibody, although the human lung fibroblast control was clearly positive, giving the expected band size of 80 kDa (Figure 1, bottom panel). The absence of syndecan-1 protein may reflect the lack of epithelial cells in the adherent stromal layer. The detection of syndecan-1 RNA would however be consistent with post-transcriptional regulation of syndecan-1 expression in stromal cells, as is the case in peritoneal macrophages [34].

The 3G10 antibody, acting against all heparitinase-digested HSPGs, gives an indication of the relative abundance of different HSPGs. The octyl glucoside extract and residue contained only a very large HSPG core, which was consistent with the presence of perlecan (Figure 2). The Triton extract contained four bands, consistent with core proteins corresponding to those of syndecan-4 (35 kDa), the shorter form of syndecan-3 (55 kDa), glypican (64 kDa) and the expected 125 kDa core of syndecan-3.

A summary of the results is shown in Table 1. Comparison of expression at the protein and mRNA levels generally showed good correlation for all the syndecans, suggesting that regulation is mainly at the level of transcription. Our results suggest that syndecan-4, syndecan-3 and glypican-1, present on the surface of marrow stromal cells, together with perlecan in the ECM, may be responsible for creating the correct stromal niche for the maintenance and development of haemopoietic stem and progenitor cells. These findings differ from those of a recent study of a murine stromal cell line in which syndecan-1 and syndecan-2 were the most abundant HSPGs, followed by syndecan-3 and syndecan-4 [15]. It is possible that the differences in human and murine expression of proteoglycans may relate to the ability of human stromas to more efficiently maintain haemopoietic stem cells.

The different functions of individual HSPGs in normal marrow stroma are unknown. A limitation of our study is the relatively long period of growth in the presence of high serum concentrations, which are required for the formation of an adequate stromal layer. Although the stroma supplies the correct environment to promote the growth of haemopoietic progenitor

cells, the expression pattern of matrix proteins could change during the culture period. However, our detection of a variant form of syndecan-3 as a major HSPG suggests a specific role for this syndecan in haemopoiesis. Further characterization of syndecan-3 in freshly isolated cells together with its localization within particular areas of stroma, e.g. near to developing haemopoietic foci, will be the focus of future studies.

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