Molecular Biology of the Cell
Vol. 5, 797–805, July 1994

Members of the Syndecan Family of Heparan Sulfate Proteoglycans Are Expressed in Distinct Cell-, Tissue-, and Development-specific Patterns

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Submitted March 21, 1994; Accepted June 13, 1994
Monitoring Editor: Erkki Ruoslahti

The syndecans are a gene family of four transmembrane heparan sulfate proteoglycans that bind, via their HS chains, diverse components of the cellular microenvironment. To evaluate the expression of the individual syndecans, we prepared cDNA probes to compare mRNA levels in various adult mouse tissues and cultured mouse cells representing various epithelial, fibroblastic, endothelial, and neural cell types and B cells at various stages of differentiation. We also prepared antibody probes to assess whether the extracellular domains of the individual syndecans are shed into the conditioned media of cultured cells. Our results show that all cells and tissues studied, except B-stem cells, express at least one syndecan family member; most cells and tissues express multiple syndecans. However, each syndecan family member is expressed selectively in cell-, tissue-, and development-specific patterns. The extracellular domain of all syndecan family members is shed as an intact proteoglycan. Thus, most, if not all, cells acquire a distinctive repertoire of the four syndecan family members as they differentiate, resulting in selective patterns of expression that likely reflect distinct functions.

INTRODUCTION

Heparan sulfate is present at the cell surface in the form of heparan sulfate proteoglycans (HSPGs) where it is found in abundance on most, if not all, adherent cells (Kraemer, 1971). A large number of molecules, such as growth factors, extracellular matrix components, proteases, protease inhibitors, and viral coat proteins bind heparan sulfate and/or heparin, its related glycosaminoglycan (for review see Bernfield *et al.*, 1992). In the best studied examples, such as FGF-2 and antithrombin III, the binding of heparan sulfate greatly potentiates the function of the ligand. HSPGs are presented at the cell surface either as glycosyl phosphatidylinositol–linked proteins, such as glypican (David *et al.*, 1990) and cerebroglycan (Stipp *et al.*, 1994), or as integral membrane proteins, such as betaglycan (Lopez-Casillas *et al.*, 1991), CD44E (Brown *et al.*, 1991), and the syndecans.

After the original cloning of syndecan-1 from mouse mammary epithelia (Saunders *et al.*, 1989a), cDNAs for three other proteoglycan core proteins have been cloned. High sequence identity of their cytoplasmic and transmembrane domains, together with conservation of GAG attachment sites in their extracellular domains, indicate they are an evolutionarily related family of proteoglycans (Bernfield *et al.*, 1992). These are syndecan-2 (fibroglycan) (Marynen *et al.*, 1989; Pierce *et al.*, 1992), syndecan-3 (N-syndecan) (Carey *et al.*, 1992; Gould *et al.*, 1992), and syndecan-4 (ryudocan, amphiglycan) (David *et al.*, 1992; Kojima *et al.*, 1992; Baciu *et al.*, 1994) based on the order of their discovery and the tissue of their original cloning.

Emerging data suggest that the expression of the individual syndecan family members is highly regulated. Syndecan-1 (Saunders *et al.*, 1989a; Kiefer *et al.*, 1990; Mali *et al.*, 1990; Cizmeci-Smith *et al.*, 1992; Kojima *et al.*, 1992), the most extensively studied integral membrane HSPG, is abundant in epithelial cells and embryonic mesenchyme and can bind, via its heparan sulfate chains, a wide variety of matrix molecules and growth factors such as collagens I, III, V, fibronectin, and FGF-2. Furthermore, the proteoglycan core protein

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appears to associate intracellularly with F-actin, thus bridging the extracellular matrix with the cytoskeleton (Rapraeger and Bernfield, 1982).

Syndecan-1 expression is regulated at multiple levels. Transcriptional control has been observed in vivo during development at sites of epithelial-mesenchymal interactions (Vainio *et al.*, 1992) and in vitro during induction by the antimicrobial peptide PR-39 (Gallo *et al.*, in press). The syndecan-1 promoter contains multiple consensus transcription factor binding sites including Antennapedia, MyoD (E box), NF-kB, Sp1 (GC box), CAAT, and TATA boxes (Hinkes *et al.*, 1993; Vihinen *et al.*, 1993). In addition, syndecan-1 expression is regulated posttranscriptionally (Sanderson *et al.*, 1992a, 1992; Vainio *et al.*, 1992; Yeaman and Rapraeger, 1993) and posttranslationally (Sanderson and Bernfield, 1988). These multiple levels of regulation precisely control the expression of syndecan-1 during various developmental processes, beginning as early as the four-cell stage (Sutherland *et al.*, 1991) as well as during cutaneous wound repair (Elenius *et al.*, 1991). The shedding of the extracellular domain of syndecan-1 from the cell surface as an intact proteoglycan provides an additional potential control (Jalkanen *et al.*, 1987).

Syndecan-2, isolated from human lung fibroblasts (Marynen *et al.*, 1989) and rat liver (Pierce *et al.*, 1992), is found in abundance in fibroblasts, endothelia, and hepatocytes (Pierce *et al.*, 1992; David *et al.*, 1993) but, in contrast to syndecan-1, is deficient in epithelia (David *et al.*, 1993). During development, syndecan-2 is expressed at sites of cell-cell and cell-matrix interactions (e.g., epithelial-mesenchymal interfaces at prechondrogenic and preosteogenic mesenchymal condensations) and persists in perichondrium, periostium, and connective tissue cells (David *et al.*, 1993). Syndecan-3, isolated from chick embryo limb buds (Gould *et al.*, 1992) and rat newborn Schwann cells (Carey *et al.*, 1992), is found in abundance in these tissues as well as in brain, heart, and aortic smooth muscle (Carey *et al.*, 1992; Gould *et al.*, 1992). During mouse development, syndecan-3 expression complements the expression of syndecan-1 and is seen at sites of neural crest migration (Gallo *et al.*, 1993). Syndecan-4, isolated from chick 14-d embryo (Baciu *et al.*, 1994), rat microvascular endothelia (Kojima *et al.*, 1992), and human lung fibroblasts (David *et al.*, 1992), has been detected in multiple cell types (David *et al.*, 1992).

This study aims to evaluate the expression and shedding of the four mouse syndecan family members. Using cDNA probes for each syndecan family member, we examined mRNA levels of the syndecans in adult mouse tissues, cultured cells, and B cell lines representing various stages of differentiation. Polyclonal antisera specific for syndecans-2, -3, or -4 were produced to evaluate each syndecan in the conditioned media (CM) of cultured cells. Our results show that all cells and tissues studied, except B-stem cells, express at least one syndecan family member with most cells and tissues expressing multiple syndecans. However, for each syndecan family member, the level of mRNA differed between specific cell types, tissues, and B cells at various stages of differentiation. The extracellular domain of each syndecan family member was shed intact from cells in culture and, to an extent, varied between the various cell types and B cells studied. Distinct cell-, tissue-, and development-specific patterns of expression and shedding likely reflect distinct functions for the individual members of the syndecan gene family.

MATERIALS AND METHODS

Cloning of Mouse Syndecan-2, -3, and -4

Partial clones of murine syndecan-2, -3, and -4 were obtained by the polymerase chain reaction (PCR) using oligonucleotides flanked at the 5′-end with *Bam*HI restriction sites and at the 3′-end with *Eco*RI restriction sites and a translational stop codon (5′-TTA-3′) to prevent read through in the pGEX-2T expression system (described below). Oligonucleotide primers encompass the extracellular domains (Figure 1) that are the most divergent in sequence between the individual syndecans (Bernfield *et al.*, 1992).

For syndecan-2, the 5′-oligonucleotide (5′-GCGGATCCGAGACGAGAACAGAGCTGACATCC-3′) spans positions 578–596 of the previously determined mouse sequence (David *et al.*, 1992). The 3′-oligonucleotide (5′-GCGAATTCTTACCGTTTAAACAGATTGTCTGA-3′) spans positions 931–952.

For syndecan-3, oligonucleotides sequences were derived from the rat *N*-syndecan sequence (Carey *et al.*, 1992). The 5′-oligonucleotide spans positions 1–23 (5′-GCGGATCCCTTCGAGAGACAGCCATGCGGTT-3′). The 3′-oligonucleotide spans 858–882 (5′-GCGAATTCTTACTTCCGCTCTAGTATGCTCTTCTG-3′).

For syndecan-4, oligonucleotides sequences were derived from the rat ryudocan sequence (Kojima *et al.*, 1992). The 5′-oligonucleotide spans positions 85–104 (5′-GAGTCGATTCGAGAGACTGA-3′). The 3′-oligonucleotide spans 434–456 (5′-GCGAATTCTTATCTTTCAAAAATGTTGCTGCCCTG-3′).

Each set of oligonucleotides were used as primers in PCR reactions using cDNA derived from mouse 15–18-d embryos. Single products were obtained for each PCR reaction corresponding to ~460 base pairs (bp) for syndecan-2, ~870 bp for syndecan-3, and ~370 bp for syndecan-4.

PCR products were isolated from 1% SeaKem agarose gels and purified by centrifugation through SpinX columns (Costar, Cambridge, MA). These DNAs were used as templates for radiolabeled probes used in Northern blot analyses as well as for construction of fusion protein antigens for production of polyclonal antisera (described below).

Construction of Mouse Syndecan-Glutathione S-Transferase Fusion Proteins for Production of Polyclonal Antibodies

PCR-generated fragments of syndecan-2, -3, and -4 were digested with *Eco*RI and *Bam*HI to obtain cohesive ends suitable for subcloning into the pGEX-2T vector (Pharmacia, Piscataway, NJ). Partial DNA sequencing confirmed the syndecan-2 mouse sequence. For syndecan-3 and syndecan-4, sequences were similar but not identical to the corresponding rat syndecan sequences. Subclones produced fusion proteins linked to glutathione S-transferase containing the thrombin cleavage site. Fusion proteins were separated from crude bacterial extract proteins by binding to glutathione Sepharose 4B and eluting by thrombin cleavage as described by the manufacturer.

Purified proteins were used for immunization of rabbits at the Pine Acres Rabbitry/Farm (Norton, MA). Polyclonal antisera, preadsorbed with acetone powder derived from *Escherichia coli* strain DH5alpha containing the pGEX-2T vector, were tested in Western blots of CM from NMuMG and C27-3 cells. The nomenclature assigned to the antisera is MSE (for mouse syndecan extracellular domain) followed by the number corresponding to the specific syndecan member. Therefore, MSE2 denotes antiserum against mouse syndecan-2, MSE3 antiserum for mouse syndecan-3, and MSE4 antiserum for mouse syndecan-4. In addition, monoclonal antibody 281-2 (Jalkanen *et al.*, 1985) was used to detect syndecan-1 proteoglycan.

Immunoblot Analyses

Proteoglycans were isolated from CM of NMuMG and C27 cells as described previously (Rapraeger and Bernfield, 1985) and used to test the specificity of preadsorbed antisera by Western blot analysis. To analyze core proteins, glycosaminoglycan side chains were removed from proteoglycans by treatment with heparan sulfate lyase (heparitinase) and chondroitin sulfate ABC lyase (Seikgaku Kogyo, Rockville, MD) as described previously (Sanderson and Bernfield, 1988).

Proteoglycans were purified from the conditioned media of various cell lines to determine the relative accumulation of the syndecan extracellular domains. In some cases syndecans were prepared from whole cells by overnight agitation at 4°C in 4 M urea, 150 mM NaCl, 25 mM tris (hydroxymethyl) aminomethane pH 7.4, 1% Triton X-100, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM N-ethylmaleimide. Proteoglycans were purified from the extraction solution as described for CM.

Slot blot analyses were done on proteoglycan applied to Immobilon-N membranes (Millipore, Bedford, MA) using the Minifold II Slot-blot apparatus (Schleicher and Schuell, Keene, NH). The syndecans were detected using the electrochemiluminesence (ECL) detection system (Amersham Life Sciences, Amersham, UK) as described by the manufacturer. For Western blots, proteoglycans and core proteins were separated on gradient sodium dodecyl sulfate (SDS)-polyacrylamide gels containing 2.7 M urea. The gradients ranged from 22 to 3.5% acrylamide (7.5% crosslinking) or 17.5 to 3.5% acrylamide (7.5% crosslinking). Proteins were transferred to Immobilon-N membranes, and the syndecans were detected by immunoblot analyses using the ECL detection system.

Isolation of Total RNA from Mouse Tissues and Cultured Mouse Cell Lines for Northern Analyses

Total cellular RNA was extracted from homogenized adult mouse (C57BL) tissues and cultured mouse cells by the guanidinium isothiocyanate method (Chirgwin *et al.*, 1979). For Northern blot analyses, ~10 μg RNA per lane was separated on 1% agarose-formaldehyde gels and transferred to Genescreen Plus membranes (Du Pont-New England Nuclear, Wilmington, DE) by capillary action. Probes were prepared with random hexamer oligonucleotides using the PCR-generated templates of syndecan-2, -3, and -4 (described above), mouse syndecan-1 *Eco*RI-*Hin*dIII fragment (Saunders *et al.*, 1989a), or the 800-bp *Pst* I fragment of murine β-actin gene. Hybridizations were performed at 65°C in QuikHyb solution as described by the manufacturer (Stratagene, La Jolla, CA). Membranes were washed twice in 2× SSPE, 0.1% SDS for 15 min each at room temperature and 0.2× SSPE, 0.1% SDS for 15 min at 55°C. Blots were analyzed with the Molecular Dynamics Phosphorimager (Sunnyvale, CA). Blots were stripped in 0.1× SSPE, 0.1% SDS at 100°C for 15 min and reprobed as above. Band intensities were quantitated using the ImageQuant v3.0 software as described by the manufacturer (Molecular Dynamics, Sunnyvale, CA).

Cell Culture

Cell lines 38B9 (Alt *et al.*, 1981), PD-31 (Lewis *et al.*, 1982), 70Z/3 (Paige *et al.*, 1978), P3X63 Ag8.653 (Karasuyama and Melchers, 1988), and MPC-11 (Laskov and Scharff, 1970) were grown in RPMI-1640 (Mediatech, Washington, DC) with 10% fetal calf serum (FCS) (TC Biologicals, Tulare, CA), 0.584 g/L L-Gln (Mediatech), and 50 μM 2-mercaptoethanol (Biorad, Richmond, CA).

Cell lines 32D (Greenberger *et al.*, 1983) and Ba F3 (Mathey-Prevot *et al.*, 1986) were grown in RPMI-1640 with 10% FCS, 0.584 gm/L L-Gln media supplemented with 10% P3X63-IL3 conditioned media.

Cell lines ABE-8.1/2 (Burchiel and Warner, 1980) and WEHI-279 (Warner *et al.*, 1979) cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Mediatech), 10% FCS, 0.584 g/L L-Gln, and 50 μM 2-mercaptoethanol. 2PK-3 (Warner *et al.*, 1979) were grown in the same media supplemented with 4.5 g/L glucose.

Cell lines NIH/3T3 (Jainchill *et al.*, 1969), C17-2 (Snyder *et al.*, 1992), and C27-3 (Snyder *et al.*, 1992) were grown in DMEM with 4.5 g/L glucose, 10% FCS, 0.584 L-Gln. BALB/3T3 (Aaronson and Todaro, 1968), MAE (Hoak *et al.*, 1981), MBE (Hoak *et al.*, 1981), and MHE (Hoak *et al.*, 1981) cells were grown in DMEM with 1.0 g/L glucose, 10% FCS, and 0.584 g/L L-Gln. NMuMG (Owens, 1974) cells were grown in DMEM with 4.5 g/L glucose, 7.5% FCS, 0.584 g/L L-Gln, nonessential amino acids (Mediatech), and 0.01 mg/ml insulin.

Primary keratinocytes were obtained from minced skin of newborn Swiss Webster mice and cultured in Eagle's minimal essential medium (Whittaker Bioproducts, Walkersville, MD), 10% Chelex-treated FCS (TC Biologicals), 0.584 g/L L-Gln (Mediatech), 50 μM calcium chloride, and 5.36 mM potassium chloride. Cell lines BK-1 (Yuspa *et al.*, 1981) and BALB/MK-2 (Makino and Weissman, 1991) were grown in the same media supplemented with 10 ng/ml epidermal growth factor.

Cell line C3H 10T1/2 (Reznikoff *et al.*, 1973) was grown in Eagle Basal medium with Earles's balanced salts (GIBCO, Grand Island, NY) with 10% FCS.

RESULTS

The members of the syndecan family of HSPGs are encoded by distinct genes whose sequences are sufficiently similar in their transmembrane and cytoplasmic domains to indicate common ancestry. To evaluate the expression of each member of this gene family, we have prepared both cDNA and antibody probes to compare mRNA levels in various mouse cell lines and tissues and to assess the proteoglycans in CM.

Specific Probes for Members of the Syndecan Gene Family

Using PCR primers and C57BL mouse whole embryo cDNA, probes corresponding to regions of the extracellular domains of syndecan-2, -3, and -4 were obtained (Figure 1). The extracellular domains were chosen because these regions show the greatest sequence diversity among the syndecans (Bernfield *et al.*, 1992). These probes, together with the *Eco*RI-*Hin*dIII restriction fragment of the mouse syndecan-1 cDNA (Saunders *et al.*, 1989a), were used to analyze C57BL mouse liver and brain RNA and to prepare constructs with the pGEX vector for generation of fusion proteins to be used as immunogens. Sequencing of the cDNAs confirmed the identity of mouse syndecan-2 (David *et al.*, 1993) and established partial sequences for mouse syndecan-3 and -4.

The syndecan-1 probe hybridized to two distinct bands, a minor band with a calculated molecular size

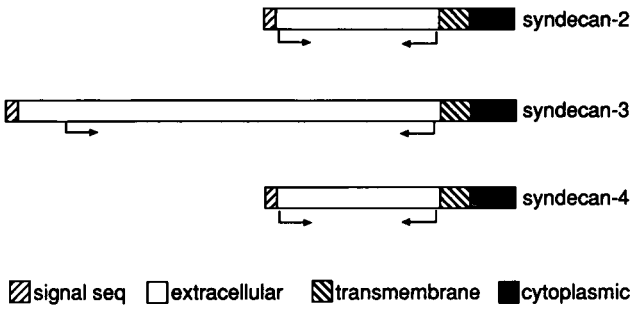

Figure 1. Generation of specific cDNA probes for the syndecan extracellular domains. The arrows below each cDNA representation correspond to primers used for PCR amplification of the extracellular domains of syndecan-2, -3, and -4. The PCR-generated probes were used for Northern blot analyses and fusion protein construction as described in MATERIALS AND METHODS.

of 3.4 kilobase (kb) and a major band at 2.6 kb (Figure 2), identical to previous observations (Saunders *et al.*, 1989a). These two RNA species are products of processing at alternate polyadenylation sites (Hinkes *et al.*, 1993). The syndecan-2 probe identified three distinct bands corresponding to 3.5, 2.4, and 1.2 kb, as in human and rat tissues (Marynen *et al.*, 1989; Pierce *et al.*, 1992). In human tissues, these different size transcripts are produced by differential polyadenylation (Marynen *et al.*, 1989). The syndecan-3 probe hybridizes to a single 5.9-kb band from brain RNA, as observed in tissues from chick (Gould *et al.*, 1992) and rat (Carey *et al.*, 1992). The syndecan-4 probe hybridizes to a single 2.6-kb band, as observed in various human cultured cells (David *et al.*, 1992) and several rat tissues (Kojima *et al.*, 1992), but distinct from the three transcripts for syndecan-4 in chicks (Baciu *et al.*, 1994). These observations indicate that the PCR probes generated are specific and recognize the appropriate number and size of syndecan transcripts.

Syndecan Transcripts Are Expressed in a Cell- and Tissue-specific Manner

Comparison of the abundance of syndecan mRNA levels, relative to β-actin mRNA levels, between the various cell types and adult mouse tissues shows that the syndecans are expressed selectively. Syndecan-1 mRNA is abundant in fibroblastic and epithelial cells, being especially high in keratinocytes, but very low in endothelial and neural cells (Figure 3). This corresponds well with expression in adult tissues where syndecan-1 mRNA is most abundant in tissues rich in epithelia and fibroblasts such as skin, liver, kidney, and lung, less abundant in brain and small intestine, and undetectable in cardiac and skeletal muscles (Figure 4). Prior studies show similar mRNA distributions in human cells (Lories *et al.*, 1992) and mouse tissues (Saunders *et al.*, 1989a).

In contrast, syndecan-2 mRNA is low or undetectable in epithelial cells but abundant in endothelial, neural, and fibroblastic cells (Figure 3). In tissues, it is most abundant in liver, which may reflect expression in hepatocytes (Pierce *et al.*, 1992) as well as in endothelia and fibroblasts (Figure 4). It is intermediate in amount in brain, heart, skeletal muscle, kidney, and lung and low in skin and small intestine.

Syndecan-3 mRNA is also low or undetectable in epithelial cells and most abundant in neural cells (Figure 3). It is also in C3H10T1/2 fibroblastic cells, primitive mesenchymal cells, and in some endothelial cells. Brain tissue contains the greatest amount of syndecan-3 mRNA (Figure 4). Skin contains low levels whereas liver, kidney, lung, and small intestine do not contain detectable levels. Intermediate levels of syndecan-3 mRNA in the heart, but not skeletal muscle, may reflect neural elements in this tissue.

Syndecan-4 mRNA is abundant in both epithelial and fibroblastic cells with intermediate levels in neural cells and lower amounts in endothelial cells. In tissues, it is abundant in liver and kidney, intermediate in amount in brain and lung, and low in heart, skeletal muscle, skin, and small intestine.

These results show that all cells and tissues tested express at least one syndecan family member. Furthermore, each family member is expressed in cell-specific patterns: syndecan-1 mainly in epithelial and fibroblastic cell types, syndecan-2 mainly in nonepithelial cell types, syndecan-3 mainly in neural cells, and syndecan-4 in most cell types. The selective expression of the syndecans by cultured cells and tissues is consistent with the regulation and selective expression of syndecan-1 and -2 during mouse embryogenesis (Solursh *et al.*, 1990; Sutherland *et al.*, 1991; Trautman *et al.*, 1991; David, 1993). The expression patterns of these four syndecan family members differ from that expected from the tissues of their initial cloning (Bernfield *et al.*, 1992).

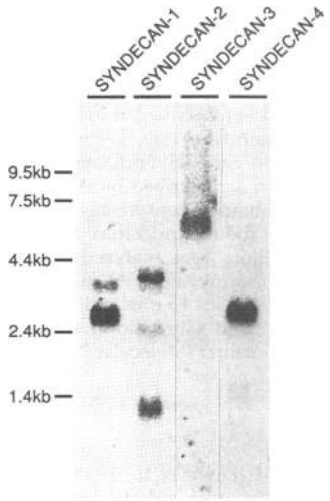

Figure 2. Northern blot analysis of syndecan mRNA. Total RNA isolated from adult mouse liver (for lanes 1, 2, and 4) and brain (for lane 3) was subjected to Northern blot analyses with the PCR-generated probes for each syndecan family member. Molecular weight standards are denoted at left.

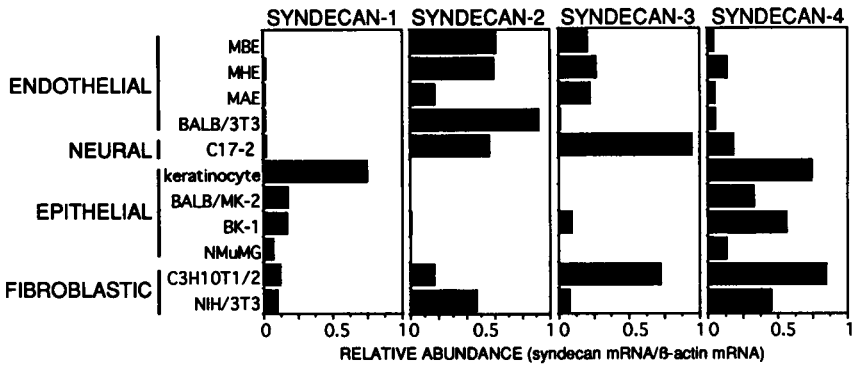

Figure 3. Selective expression of syndecan mRNA in cultured mouse cells. Total RNA isolated from cultured mouse cells was analyzed by Northern blots for each syndecan family member and for mouse β-actin. Relative abundance of each syndecan was determined by Phosphorimager analysis of all transcripts normalized to mouse β-actin mRNA levels. Because of differences in probe specific activity, values can be used only to compare relative abundance of syndecans between cell types. Cells are grouped according to cell type as indicated.

B Cells Acquire a Distinctive Syndecan Repertoire as They Differentiate

The expression of each syndecan was examined in cell lines that represent B cells at various stages of differentiation (Figure 5). Syndecan-1 mRNA was undetectable at the B-stem cell stage, in low amounts at the pre-B cell stage, again undetectable at the B-lymphocyte stage, and abundant in amount at the plasma cell stage. These results correspond well with prior stainings of cells from bone marrow, blood, and lymph nodes (Sanderson *et al.*, 1989). This alternating pattern of syndecan-1 expression is consistent with the idea that syndecan-1 is involved in binding cells of the B lineage to the interstitial matrix (Saunders and Bernfield, 1988; Sanderson *et al.*, 1989; Ridley *et al.*, 1993). Neither syndecan-2 nor syndecan-3 mRNA was detected in any cells of the B lineage, except for low levels of syndecan-3 mRNA in MPC-11 cells.

The pattern of syndecan-4 mRNA expression differs from that of the other syndecans. Although syndecan-4 mRNA is undetectable at the B-stem cell stage, it is abundant in B cells at all subsequent stages of differentiation (Figure 5).

These results extend the finding of the cell- and tissue-type specific expression of the syndecans. Of all the B cells tested, only those at the most undifferentiated stage fail to express a syndecan transcript. This is similar to the lack of syndecan-1 expression in the zygote and two-cell embryo (Sutherland *et al.*, 1991), reinforcing the idea that the pattern of syndecan expression may depend on differentiation into specific cell types.

Intact Extracellular Domains of Syndecan-2, -3, and -4 Are Present in Conditioned Media in Cell Type-specific Patterns

Because mRNA levels may not necessarily reflect levels of proteoglycan, we developed antibodies specific for each syndecan family member. The syndecan-1 extracellular domain is readily shed from the cell surface into the CM as an intact proteoglycan (Jalkanen *et al.*, 1987). Western blot analyses showed that each antiserum recognized a high molecular weight proteoglycan in the CM from NMuMG and C27-3 cell lines (Figure 6).

When the proteoglycans were treated with heparitinase and chondroitinase ABC to remove GAG chains, each antiserum recognized a single core protein. In the CM from NMuMG cells, monoclonal antibody 281-2, specific for syndecan-1, recognized a core protein migrating at ~56 kDa. The antiserum against mouse syndecan-2 extracellular domain, MSE2, recognized a core protein migrating at ~28 kDa, and the antiserum against mouse syndecan-4 extracellular domain, MSE4, recognized a protein migrating at ~26 kDa. In C27-3 cells, the antiserum against mouse syndecan-3 extracellular domain, MSE3, recognized a large core protein migrating at ~102 kDa as well as a nonspecific band

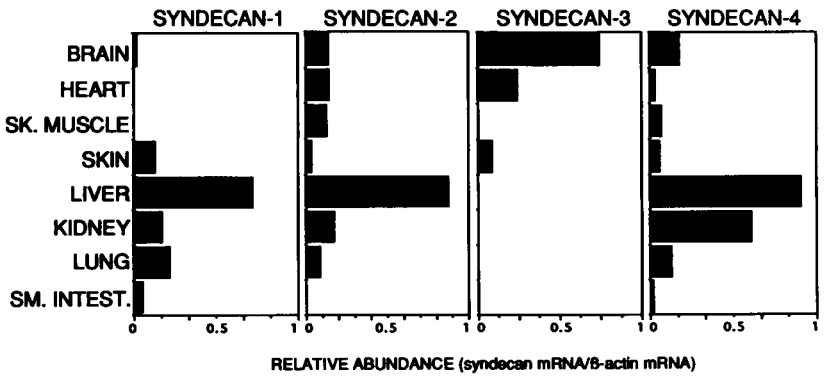

Figure 4. Selective expression of syndecan mRNA in adult mouse tissues. Total RNA isolated from adult mouse tissues was analyzed as in Figure 3.

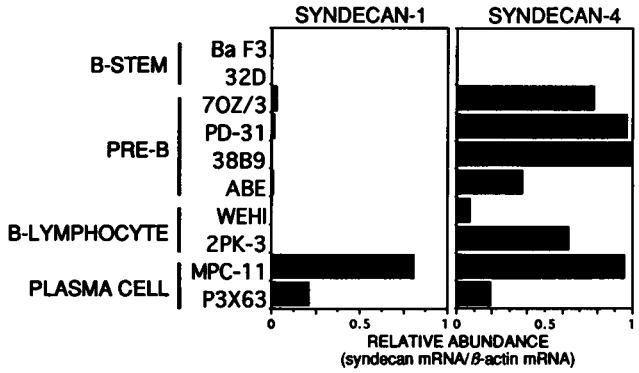

Figure 5. Selective expression of syndecan mRNA in B cells at various stages of differentiation. Total RNA isolated from cultured mouse cells representing B cells at various stages of differentiation was analyzed as in Figure 3.

at ~67 kDa that was seen inconsistently. As previously shown for the syndecan-1 extracellular domain, the syndecan-2, -3, and -4 core proteins from CM all migrate at molecular sizes that are slightly smaller than those reported for their corresponding full length core proteins (Jalkanen *et al.*, 1987; Carey *et al.*, 1992; David *et al.*, 1992; Kojima *et al.*, 1992). Unlike the other family members, syndecan-4 lacks a dibasic sequence adjacent to the plasma membrane. Thus the size of the syndecan-4 core protein from PD-31 CM was compared directly by Western blot to the syndecan-4 core protein prepared from PD-31 whole cell extracts. The syndecan-4 core protein from the cell extract migrated at ~30 kDa, larger in size than the core protein derived from CM. Thus, like syndecan-1, the immunoreactive syndecans in CM appear to correspond solely to the intact extracellular domains. These results confirm that the PCR probes encode the mouse syndecans, demonstrate the monospecificity of each antiserum, and indicate that the extracellular domain of each syndecan family member is shed intact from cultured cells.

The proteoglycans from CM of each cell line were partially purified, adsorbed onto cationic membranes, and probed to evaluate the extent of shedding of each syndecan extracellular domain. Each cell line shed at least one syndecan family member. Furthermore, each syndecan is shed in a cell-specific manner (Figure 7). The extracellular domain of syndecan-1 predominates in the CM of epithelial cells and neural cells, syndecan-2 in the CM of most cells, syndecan-3 in the CM of neural cells, and syndecan-4 in the CM of neural, epithelial, and fibroblastic cells. When the CM from cells of the B lineage were tested, the extracellular domain of syndecan-1 was seen only with pre-B and plasma cell lines (Figure 8). The extracellular domain of syndecan-4 accumulates in greatest abundance in the CM of pre-B cells. In general, these patterns of proteoglycan accumulation in CM correspond with the steady-state mRNA levels of the syndecans. The few disparities observed between antibody reactivity and mRNA abundance may reflect variations in proteoglycan synthesis, transport to the cell surface, shedding, stability in the CM, and/or recovery during purification.

DISCUSSION

This study evaluates the expression and shedding of the four syndecan family members in a variety of mouse tissues and cell lines. All cell types, except progenitor B cells, express at least one syndecan family member. This expression may reflect a requirement of all differentiated cells for a syndecan. Nevertheless, as cells differentiate, they acquire a distinctive repertoire of the four syndecans, resulting in cell type–specific syndecan expression. This selective expression likely reflects distinct functions, as suggested previously (Cizmeci-Smith *et al.*, 1992; Lories *et al.*, 1992; David *et al.*, 1993). For example, syndecan-1 is expressed predominantly in epithelial and fibroblastic cells where it binds collagens and heparin-binding growth factors and is likely involved in epithelial cell shape and organization (Rapraeger *et al.*, 1987; Saunders *et al.*, 1989b; Kato and Bernfield, 1990; Leppä *et al.*, 1991, 1992). In contrast, syndecan-3 predominates in neural tissues where it binds pleiotrophin (Raulo *et al.*, 1994) and may bind collagens less avidly than it binds the heparin-binding growth factor FGF-2 (Cher-

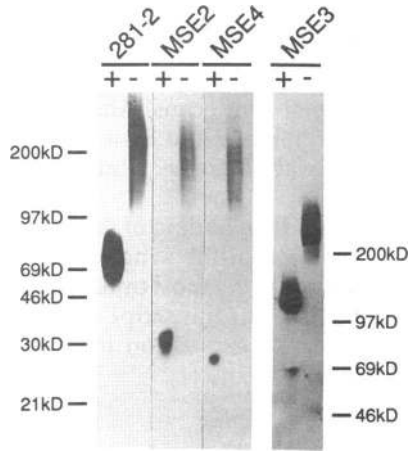

Figure 6. Western blot analyses of syndecan extracellular domains. Purified proteoglycans from CM of NMuMG cells (for syndecans-1, -2, and -4) and C27-3 cells (for syndecan-3) were subjected to SDS-polyacrylamide gel electrophoresis and analyzed by Western blots as described in MATERIALS AND METHODS. For syndecans-1, -2, and -4, a 3.5–22% polyacrylamide gradient was used. For syndecan-3, a 3–17% polyacrylamide gradient was used because of its large size. Proteoglycans were digested with heparitinase and chrondroitinase ABC to remove glycosaminoglycan chains (+) and compared to intact proteoglycans (−). Antibodies were used as follows: 281-2 mAb for mouse syndecan-1, MSE2 for mouse syndecan-2, MSE3 for mouse syndecan-3, and MSE4 for mouse syndecan-4. Molecular weight standards are as indicated.

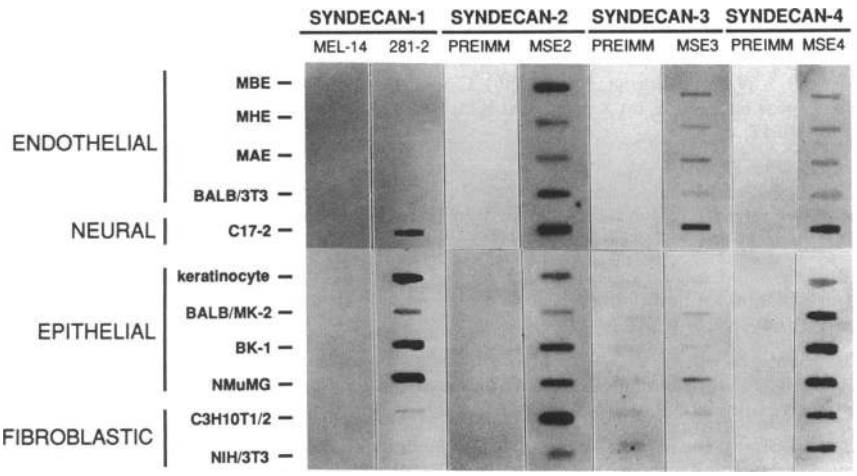

Figure 7. Selective accumulation of syndecan extracellular domains in CM from various mouse cell lines. Proteoglycans were purified from CM and analyzed in slot blots using antibodies 281-2, MSE2, MSE3, and MSE4 as in Figure 6. As controls, an irrelevant mAb (MEL-14) (Gallatin *et al.*, 1983) and preimmune sera (PREIMM) were used.

nousov and Carey, 1993). Similarly, syndecan-2 that is most abundant in endothelial cells may interact with plasma proteins such as antithrombin III, lipoprotein lipase, and lipoprotein-associated coagulation inhibitor (Novotny *et al.*, 1993).

Most cells and tissues express several syndecans. This multiplicity may reflect the large variety of roles in cellular physiology that involve cell surface heparan sulfate. *Drosophila melanogaster* apparently has only one syndecan-like HSPG, and it is expressed in epithelial, neural, lymphoid, and mesenchymal cell types (Spring *et al.*, 1994). The four syndecan family members in the mouse may have evolved from a single ancestral gene to accommodate the increasingly complex cellular microenvironment of mammalian cells.

Finally, each mouse syndecan can be shed into the CM as an intact extracellular domain. The extracellular domains of syndecan-1, -2, and -3 are predicted to be shed via cleavage at dibasic residues in the core protein external to the cell membrane. Syndecan-4 lacks this dibasic residue but is also shed as is demonstrated by its presence as a smaller extracellular domain in CM. *Drosophila* syndecan is also shed despite the absence of a protease-susceptible site near the plasma membrane (Spring *et al.*, 1994). The function of this shedding is not clear. It is possible that cell surface and shed syndecans have distinct functions, as suggested for membrane anchored growth factors (Ansorge *et al.*, 1991).

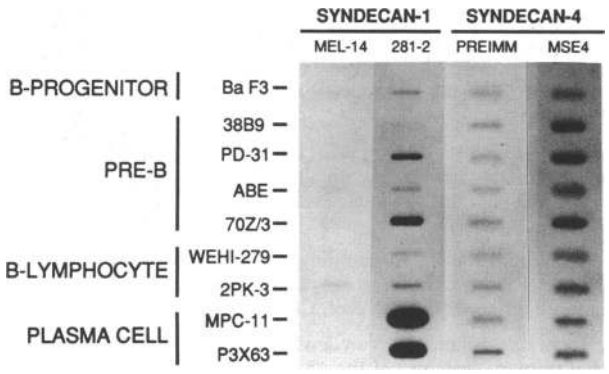

Figure 8. Selective accumulation of syndecan extracellular domains in CM from B cell lines at various stages of differentiation. Proteoglycans were purified from CM of B cells representing various stages of differentiation and analyzed in slot blots using antibodies 281-2 and MSE4 as in Figure 7. The low levels of syndecan-1 in BaF3 CM is because of the presence of 10% P3X63 conditioned medium in the BaF3 cell medium (see MATERIALS AND METHODS).

ACKNOWLEDGMENTS

We thank the Office of Enrichment Programs at Harvard Medical School (C.W.K.), National Institutes of Health grants AR-01874 (R.L.G.), CA-28735, HL46491, and HD-06763 (M.B.), and the Lucille P. Markey Charitable Trust for financial support.

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