Developmental expression of syndecan, an integral membrane proteoglycan, correlates with cell differentiation

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Summary

Syndecan is an integral membrane proteoglycan that behaves as a matrix receptor by binding cells to interstitial matrix and associating intracellularly with the actin cytoskeleton. Using immunohistology, we have now localized this proteoglycan during the morphogenesis of various derivatives of the surface ectoderm in mouse embryos. Syndecan is expressed on ectodermal epithelia, but is selectively lost from the cells that differentiate into the localized placodes that initiate lens, nasal, otic and vibrissal development. The loss is transient on presumptive ear, nasal and vibrissal epithelia; the derivatives of the differentiating ectodermal cells that have lost syndecan subsequently re-express syndecan. In contrast, syndecan is initially absent from the mesenchyme underlying the surface ectoderm, and is transiently expressed when the surface ectoderm loses syndecan. These results demonstrate that expression of syndecan is developmentally regulated in a distinct spatiotemporal pattern. On epithelia, syndecan is lost at a time and, location that correlates with epithelial cell differentiation and, on mesenchyme, syndecan is acquired when the cells aggregate in proximity to the epithelium. This pattern of change with morphogenetic events is unique and not duplicated by other matrix molecules or adhesion receptors.

Key words: heparan sulfate and chondroitin sulfate proteoglycan, syndecan, cell surface, ectoderm, epithelial-mesenchymal interaction.

Introduction

The formation of organs during development requires changes in the behavior of cells that result, in part, from changes in the expression of various adhesion molecules. These include the cell–cell adhesion molecules or CAMS, the matrix receptors and the extracellular matrix molecules. The resulting changes in adhesion can lead to changes in cell shape, movement and function. Changes in expression of a variety of these adhesion molecules have been correlated with morphogenetic events in various organs but the cause of the changes in expression is unclear (Takeichi, 1988; Edelman, 1988).

We have identified and cloned syndecan, an integral membrane protein that bears both heparan sulfate and chondroitin sulfate chains (Saunders et al. 1989) and that behaves as a receptor for interstitial matrix components (Bernfield and Sanderson, 1990). Syndecan binds cells via its heparan sulfate chains to collagen types I, III and V (Rapraeger et al. 1985; Koda et al. 1985), fibronectin (Saunders and Bernfield, 1988), and thrombospondin (Sun et al. 1989) and associates intracellularly with the actin-containing cytoskeleton when its extracellular domain is cross-linked at the cell surface (Rapraeger et al. 1986). Syndecan polarizes to the basolateral surface of simple epithelia in mature tissues and on cells in culture (Jalkanen et al. 1987; Hayashi et al. 1987), but is rapidly lost by shedding of the extracellular domain when the cells round up following their release from culture dishes (Jalkanen et al. 1987). These findings have led us to propose that syndecan anchors epithelial cells to the matrix and stabilizes the form of epithelial sheets (Saunders and Bernfield, 1988).

We have now assessed syndecan expression during eye, ear, nose and vibrissal development from the surface ectoderm and underlying mesenchyme of mouse embryos. The results show that syndecan expression changes similarly during the development of each organ. Syndecan is expressed on the surface ectoderm but is lost when and where the epithelial cells
form a placode and is generally re-expressed on epithelia upon completion of the morphogenetic sequence. Syndecan is initially absent from the mesenchyme but is transiently expressed when the mesenchymal cells condense, and is then lost following completion of the morphogenesis. These changes in expression occur in association with the epithelial–mesenchymal interactions of these tissues. These data, which extend our prior observations on syndecan expression during tooth and limb development, indicate that syndecan expression is regulated in a highly coordinated manner. A preliminary report of this work has appeared in abstract form (Trautman et al. 1987).

Materials and methods

Embryos from timed pregnancies of Swiss-Webster mice (day of vaginal plug is day 0) were placed in alpha minimum essential media. Prior to day 10, embryonic age was established by the number of somites. Tissues were fixed in Carnoy’s fluid at 4°C, dehydrated, embedded in paraffin and sectioned (~5 μm) and deparaffinized in a standard fashion. Stained sections were dehydrated, cleared, mounted in Permoun, viewed on a Zeiss Photomicroscope II and were recorded on Panatomic X film at ASA 32.

Preparation and properties of monoclonal antibody 281-2 and a rabbit serum antibody, both directed against determinants on the core protein of the cell surface proteoglycan, have been reported previously (Jalkanen et al. 1985; Jalkanen et al. 1988). Control antibodies were MEL-14, another rat IgG2A monoclonal (Gallatin et al. 1983), and non-immune rabbit serum. For peroxidase staining, sections were immersed in methanol containing 0.3% H2O2 for 30 min at room temperature to block endogenous peroxidatic activity. Sections were rehydrated through graded ethanol and washed three times for 15 min in modified phosphate-buffered saline (mPBS; 120 mM NaCl, 10 mM Na2HPO4, 4 mM HPO4). Sections were incubated with either rabbit serum (100 μg ml⁻¹) or swine serum (10%) for 30 min, washed three times in mPBS for 5 min and incubated overnight at 4°C with monoclonal (36 or 356 μg ml⁻¹) or serum (1:100) antibodies.

Peroxidase staining

After washing three times for 5 min in mPBS, sections were incubated with either 50 μg ml⁻¹ of a biotinylated rabbit anti-rat IgG, no cross reactivity to mouse IgG (Vector Laboratories Inc., Burlingame, CA), or swine anti-rabbit IgG conjugated with horseradish peroxidase (HPO; 100 μg ml⁻¹; Accurate Scientific, Westbury, NY). After washing three times for 5 min with mPBS, sections were then incubated for 30 min with Vector ABC HPO kit consisting of avidin DH and biotinylated HPO (Vector Laboratories Inc., Burlingame, CA), or peroxidase activity was demonstrated directly as previously described (Jalkanen et al. 1988).

Alkaline phosphatase staining

After washing with mPBS, sections were incubated with a goat anti-rabbit Fab conjugated with alkaline phosphatase (Promega Biotec, Madison Wis). Phosphatase activity was demonstrated by incubating sections with a mixture of 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (Promega Biotec, Madison, Wis). Competition studies were performed by preincubating sera with the purified proteoglycan ectodomain (4 μg ml⁻¹) overnight at 4°C and using the 15 000 g supernatant as described for the sera (Hayashi et al. 1987).

To attempt to unblock antigenic sites, sections were preincubated with chondroitinase ABC (chondroitin sulphate ABC lyase, Miles) in 50 mM Tris (pH 7.4), 5 mM calcium acetate, 10 mM N-ethylmaleimide, 1 mM phenylmethylsulfonyl fluoride and 5 μg ml⁻¹ pepstatin A. Digestion with 0.1 U ml⁻¹ of the enzyme was carried out for 90 min at 37°C then for another 90 min following addition of an equal amount of fresh enzyme. Controls were adjacent sections preincubated in buffer without enzyme.

Results

We have assessed syndecan expression during the embryogenesis of various derivatives of the surface ectoderm, specifically the optic, otic, nasal and vibrissal epithelia. Each developmental sequence is initiated by an interaction of the surface ectoderm with an underlying tissue, usually mesenchyme, and each begins with the elongation of a localized region of surface ectodermal cells and with the condensation of the mesenchyme cells associated with this discrete site. Each organ then undergoes its characteristic morphogenesis (Kolega, 1986; Ettenson, 1985; Gurdon, 1987).

Optic development

In the day 8 mouse embryo, the neuroectoderm that forms the optic stalk comes into close proximity with the lateral surface ectoderm. At this stage, syndecan is expressed at the basolateral surfaces of the single layer of surface ectoderm cells and over the entire surfaces of the neuroectoderm cells. Faint stain is seen on the intervening mesenchyme cells (Fig. 1A). During the next 24 h, the optic stalk contacts the surface ectoderm. At the site of contact, the surface ectoderm cells begin to differentiate by elongating along their apical–basal axis to become columnar, forming the lens placode. The cells of the forming lens placode show markedly reduced stain while the surface ectoderm cells adjacent to the forming placode retain stain. The neuroectoderm no longer stains while the adjacent mesenchyme cells condense and become intensely positive for syndecan (Fig. 1B). At day 10, the lens placode and optic vesicle invaginate, forming the lens cup and the double-layered optic cup, respectively. Neither structure stains, while the surface ectoderm and mesenchyme cells retain stain at their surfaces (Fig. 1C). At day 11, the lens cup forms the lens vesicle but remains attached to the overlying epithelium. The lens vesicle also does not show stain; the mesenchyme cells lose syndecan stain while the surface ectoderm retains stain. The cells of the surface ectoderm that remain in contact with the lens vesicle do not show stain (Fig. 1D). These results indicate that syndecan is initially expressed on the surface ectoderm, and is lost when the ectoderm cells become a placode, while the associated mesenchyme cells transiently express syndecan.

By day 15, the basic eye structures are formed. The stratified epidermis covering the eyelid shows abundant stain, as do the palpebral and bulbar conjunctivae, the
Fig. 1. Distribution of syndecan during mouse eye development. (A) A region of the head in the day 8 embryo prior to lens placode formation demonstrates stain on the surface ectoderm (SE) and neuroectoderm (NE) cells. Minimal stain is seen on the intervening cranial mesenchyme cells that are lateral to the optic vesicle. (B) The optic region of the day 9 embryo demonstrates loss of this syndecan stain from the surface ectoderm cells that elongate to form the lens placode (LP). (C) The optic region of the day 10 embryo demonstrates the absence of syndecan stain in the invaginating lens and optic cups (LC, OC). Stain remains on the surface ectoderm and on the mesenchyme cells. (D) The optic region of the day 11 embryo demonstrates the unstained lens vesicle (LV). The surface ectoderm retains stain, but the mesenchyme cells show decreased stain. The area of contact between the developing lens vesicle and the surface ectoderm does not show stain (*). (E) The optic structures of the day 15 embryo demonstrate staining of the stratified epidermis epithelium of the eyelid. The dermal stroma (DS), corneal stroma (CS), lens epithelium (LE), and lens fiber cells (LF) do not show stain. (F) The optic region of a day 9 embryo stained with MEL-14, an irrelevant monoclonal antibody, demonstrates the absence of stain. SE, surface ectoderm; NE, neuroectoderm; M, mesenchyme; LP, lens placode; LC, lens cup; LV, lens vesicle; OC, optic cup; LE, lens epithelium; LF, lens fiber cells; CS, cornea stroma; DS, dermal stroma (Bars=25 μm in A–D and F and bar=62.5 μm in E).
corneal epithelium and periderm, each derived from the surface ectoderm. These latter layers are separated by two mesenchymally derived layers, the corneal stroma and the corneal endothelium, neither of which show stain. The lens epithelium and the lens fiber cells derived from the lens placode do not show stain (Fig. 1E). Thus, syndecan is not re-expressed during optic development. A section of this region from a day 9 embryo demonstrates lack of staining with the control antibody (Fig. 1F).

**Placode development**

Cranial surface ectoderm at other sites was examined to assess whether a similar change in syndecan expression occurs during the formation of the nasal and otic placodes. The nasal placodes form at day 8.5 by elongation of the surface ectoderm cells and, like the lens placode, the cells forming the nasal placode lose stain during this differentiation (data not shown). By day 10, these placodes invaginate to form the olfactory or nasal pits which persist in not showing stain (Fig. 2A,B). Later, the cells lining the nasal passages regain stain (Fig. 2C).

Otic placodes form by day 8 from the surface ectoderm of the head. As with the nasal placode, these ectodermal cells lose their stain when they elongate, and derivatives of the placode, such as the epithelium of the semi-circular canals, re-express syndecan (data not shown).

**Vibrissal development**

At day 10, the maxillary process shows well-stained surface ectoderm cells overlying unstained mesenchyme (data not shown). When the surface ectoderm cells begin to elongate at day 11.5, their syndecan stain is reduced (Fig. 3A). When this epithelium invaginates to form vibrissal rudiments, it loses stain completely whereas the adjacent ectoderm remains stained. Concomitant with this loss of stain, the underlying mesenchyme condenses and acquires stain (Figs 3A and 3B). With further development, these mesenchymal cells lose their stain and the ectodermal derivative, the external root sheath of the hair, re-expresses syndecan (Fig. 3C). Thus, vibrissae also show loss of syndecan expression with epithelial cell elongation, reappearance with later development and transient staining of the associated mesenchyme.

**Masking of cell surface proteoglycan in tissue sections**

Developing optic, nasal and vibrissal structures were assessed for artifacts that could result in the localized loss of stain without an actual loss of syndecan. Identical changes in expression were seen following Bouin's, Saint Marie's and Carnoy's fixatives (data not shown).

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**Fig. 2. Distribution of syndecan during nasal development.** (A) A region of the head in the day 10 embryo immediately after nasal placode formation. The invaginating placode which constitutes the forming nasal vesicle does not show stain, but the adjacent surface ectoderm (SE) shows intense stain. (B) The nasal region of the day 11 embryo shows a deepened nasal vesicle, forming the nasal pit. The surface ectoderm stains while the ectoderm forming the nasal pit does not. (C) The nasal region of the day 15 embryo shows re-expression of syndecan in the epithelium constituting the nasal passages. The basal cell layer stains at its apical and lateral surfaces. The more superficial cells in the distal region show stain over their entire surface while these cells are devoid of stain near the nasopharynx. SE, surface ectoderm; NEP, nasal epithelium; bar=25 μm in A, C, and bar=62.5 μm in B.
Developmental expression of syndecan

A SE shown. Masking by hyaluronic acid or chondroitin sulfate chains was excluded by finding no difference in staining after pre-treating sections with chondroitinase ABC (data not shown) (Trelstad et al. 1974). The possibility that the loss of staining is due to alteration or masking of the single epitope recognized by monoclonal 281–2 was excluded by finding nearly identical staining with an anti-syndecan serum antibody (Fig. 4A). Finally, staining with the serum antibody was completely prevented by preincubating the serum with the purified extracellular domain of syndecan, indicating that the antigen detected in the embryonic tissues is authentic syndecan (Fig. 4B) (Jalkanen et al. 1985; Jalkanen et al. 1988).

Discussion

Syndecan expression is consistently and reproducibly regulated during the development of various organs derived from the surface ectoderm and its underlying mesenchyme. Syndecan is expressed on surface ectodermal epithelia, is selectively lost from those cells differentiating into placodes and then is generally re-expressed by epithelia during subsequent development. The associated mesenchyme does not express syndecan initially, expresses it as the cells condense and loses syndecan upon subsequent development. These spatio-temporal patterns of syndecan expression consistently follow morphogenetic rather than histologic boundaries and appear to be induced by epithelial–mesenchymal interactions. These changes in expression, seen during lens, nasal, otic and vibrissal development, are unique and are not duplicated precisely by changes in expression of matrix components, cell adhesion molecules or other matrix receptors (Edelman, 1986; Takeichi, 1988).

The antibodies used here for histochemical localization of syndecan recognize epitopes on the syndecan core protein. Syndecan in tissues exists in polymorphic forms in which the number and size of its GAGs chains vary with the cell type and cellular organization (Saunders and Bernfield, 1988). These polymorphic forms are, therefore, not distinguished by the antibodies. Thus, a form of syndecan may change during these developmental sequences without being detected by the antibody.

Syndecan expression correlates with differentiation

The pattern of syndecan expression seen here is similar to the pattern of syndecan expression observed in the developing tooth and limb (Thesleff et al. 1988; Solursh et al. 1990). In each of these organ rudiments, syndecan is lost from epithelia at a discrete region that is
Syndecan expression is dictated by embryonic tissue interactions

The pattern of syndecan expression seen here tracks the epithelial–mesenchymal interactions known to occur in these developing organs. In general, the surface ectoderm is influenced by the underlying mesenchyme to form a placode, and, in turn, the differentiating epithelium influences the mesenchymal cells to condense. This reciprocal interaction likely continues as the epithelium further differentiates under the influence of the condensed mesenchyme. During these interactions, syndecan-deficient mesenchymal tissues become syndecan-expressing when the syndecan-expressing surface ectodermal cells become syndecan-deficient. Analogous switching of syndecan expression as a consequence of reciprocal tissue interactions is also seen during tooth and kidney development (Thesleff et al. 1988; Vanio et al. 1989).

The changes in cellular organization and differentiation that result from these epithelial–mesenchymal interactions undoubtedly involve changes in expression of various adhesion molecules. Indeed, some undergo a similar spatiotemporal variation in expression as syndecan. For example, tenasin (cytotactin, hexabrachion, J1, myotendinous antigen) is expressed and distributed in embryonic mesenchyme in a similar fashion as syndecan (Chiquet et al. 1986; Tan et al. 1987; Aufderheide et al. 1987). Although tenasin is found associated with a proteoglycan, this proteoglycan differs from syndecan in several respects, including size of core protein, nature of glycosaminoglycan chains and lack of hydrophobic transmembrane domain (Hoffman et al. 1987, 1988). The cadherins, E, N, and P, have been extensively studied during morphogenesis, and comparison with syndecan expression during optic and hair follicle development indicates that these molecules show similar but not identical changes (Nose and Takeichi, 1986; Hatta et al. 1986; Hatta et al. 1987; Takeichi et al. 1988; Hirai et al. 1989).

Functional consequences of changes in syndecan expression

In vitro studies have shown that syndecan binds matrix components with high affinity and appears to stabilize epithelial cells in sheets (Saunders and Bernfield, 1988). Its loss with epithelial differentiation and its appearance in condensing mesenchyme are consistent with these results. The surface ectodermal cells losing syndecan are changing their shape and organization. The mesenchymal cells expressing syndecan are condensing...
and presumably associating with the matrix components known to be accumulating within condensed mesenchyme.

Syndecan, like other heparan sulfate proteoglycans, (Saksela et al. 1988; Burgess and Maciag, 1989), binds to basic fibroblast growth factor (bFGF) (Sanderson and Bernfield, 1990). This peptide stimulates proliferation, migration and matrix production of mesenchymal cells and remains active and protected from proteolysis when bound to heparan sulfate (Saksela et al. 1988). A possible mechanism for syndecan loss is shedding of its extracellular domain from the cell surface. This shedding presumably occurs by cleavage at the protease-susceptible site that is immediately adjacent to its transmembrane domain (Saunders et al. 1989). In cultured epithelial cells, this shedding is markedly accelerated by suspending the cells, thus altering their adhesion and shape (Saunders and Bernfield, 1988).

Thus, loss of the ectodomain from the epithelium could conceivably be a mechanism by which active bFGF could locally diffuse to influence the adjacent mesenchmal cells, which would then condense.

We would like to thank John Lee for his technical assistance and Hendrik van Straaten for his support. This work was supported by NIH HD 07673, CA 28735, EY 00262 and a grant from the Lucille P. Markey Charitable Trust.

References


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(Accepted 22 September 1990)