Syndecan and Tenascin Expression Is Induced by Epithelial–Mesenchymal Interactions in Embryonic Tooth Mesenchyme

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Abstract. Morphogenesis of embryonic organs is regulated by epithelial–mesenchymal interactions associating with changes in the extracellular matrix (ECM). The response of the cells to the changes in the ECM must involve integral cell surface molecules that recognize their matrix ligand and initiate transmission of signal intracellularly. We have studied the expression of the cell surface proteoglycan, syndecan, which is a matrix receptor for epithelial cells (Saunders, S., M. Jalkanen, S. O’Farrell, and M. Bernfield. J. Cell Biol. In press.), and the matrix glycoprotein, tenascin, which has been proposed to be involved in epithelial–mesenchymal interactions (Chiquet-Ehrismann, R., E. J. Mackie, C. A. Pearson, and T. Sakakura. 1986. Cell. 47:131-139) in experimental tissue recombinations of dental epithelium and mesenchyme. Our earlier studies have shown that in mouse embryos both syndecan and tenascin are intensely expressed in the condensing dental mesenchyme surrounding the epithelial bud (Thesleff, I., M. Jalkanen, S. Vainio, and M. Bernfield. 1988. Dev. Biol. 129:565–572; Thesleff, I., E. Mackie, S. Vainio, and R. Chiquet-Ehrismann. 1987. Development. 101:289–296). Analysis of rat–mouse tissue recombinants by a monoclonal antibody against the murine syndecan showed that the presumptive dental epithelium induces the expression of syndecan in the underlying mesenchyme. The expression of tenascin was induced in the dental mesenchyme in the same area as syndecan. The syndecan and tenascin positive areas increased with time of epithelial–mesenchymal contact. Other ECM molecules, laminin, type III collagen, and fibronectin, did not show a staining pattern similar to that of syndecan and tenascin. Oral epithelium from older embryos had lost its ability to induce syndecan expression but the presumptive dental epithelium induced syndecan expression even in oral mesenchyme of older embryos. Our results indicate that the expression of syndecan and tenascin in the tooth mesenchyme is regulated by epithelial–mesenchymal interactions. Because of their early appearance, syndecan and tenascin may be used to study the molecular regulation of this interaction. The similar distribution patterns of syndecan and tenascin in vivo and in vitro and their early appearance as a result of epithelial–mesenchymal interaction suggest that these molecules may be involved in the condensation and differentiation of dental mesenchymal cells.

Reciprocal tissue interactions regulate morphogenesis and cell differentiation in the embryo. During these interactions, cells send and respond to generally unknown inductive signals and differentiate according to their developmental history and position in the embryo (Wessells, 1977; Saxén et al., 1980). The extracellular matrix (ECM) appears to play an important role in these developmental events by providing the positionally correct microenvironment for cell attachment and gene regulation (Hay, 1983; Ekblom et al., 1986; Watt, 1986). During morphogenesis, epithelial–mesenchymal interactions are accompanied by molecular changes in the ECM. For example, changes have been described in the distribution patterns of the interstitial collagens, proteoglycans, and fibronectin during organ development (Grobstein and Cohen, 1965; Thesleff et al., 1979; Bernfield and Banerjee, 1982; Ekblom et al., 1986). Of special interest in this respect is the accumulation of the matrix glycoprotein tenascin (also known as cytotactin) in the early organ-specific mesenchyme during morphogenesis of hair follicles, teeth, and mammary gland, and the proposal that tenascin is involved in epithelial–mesenchymal interactions during organogenesis (Chiquet-Ehrismann et al., 1986). The response of the cells to the changes in the ECM must involve integral cell surface molecules that recognize their matrix ligand and initiate transmission of signal intracellularly. One of the well-characterized matrix receptors is the
The antibodies used were polyclonal rabbit antibodies against laminin (Gibco of Dr. R. Chiquet-Ehrismann, Friedrich Miescher Institute, Basel, Switzerland) with PBS (pH 7.4) at 25°C for 5 × 15 min. The mAb 281-2 against the core of tenascin are involved in cell-matrix interactions during mesenchymal cell condensation in the developing tooth.

The tissues in the region of mandibular molar tooth germs from 11-, 13-, and 17-d-old mouse embryos were used. Embryonic age was timed in mice according to the vaginal plug (day 0) and in rats by morphological criteria. The first branchial arch (mandibular arch) of 11-d mouse embryos and 13-d rat embryos was removed under a stereo microscope in PBS supplemented with Ca++. The presumptive molar tooth area was carefully cut from the rest of the jaw (Fig. 1 b). Molar tooth rudiments with some surrounding tissues were fixed with cold 94% ethanol, embedded in paraffin wax (Tissue Prep, Fisher Scientific Co., Pittsburgh, PA), and serially sectioned at 5 µm.

In this study, we show that epithelial–mesenchymal tissue interactions control the expression of the cell surface PG, syndecan, and the matrix glycoprotein, tenasin, in embryonic tooth mesenchyme. The presumptive dental epithelium induces expression of syndecan even in the otherwise negative oral mesenchyme. We suggest that syndecan and tenasin are involved in cell–matrix interactions during mesenchymal cell condensation in the developing tooth.

Materials and Methods

Preparation and Culture of Tissues

The tissues in the region of mandibular molar tooth germs from 11-, 13-, and 17-d-old mouse embryos (CBAxC57BL) and 13-d-old rat embryos (Wistar) were used. Embryonic age was timed in mice according to the vaginal plug (day 0) and in rats by morphological criteria. The first branchial arch (mandibular arch) of 11-d mouse embryos and 13-d rat embryos was removed under a stereo microscope in PBS supplemented with Ca++, pH 7.3, and the presumptive molar tooth area was carefully cut from the rest of the jaw (Fig. 1 b). Molar tooth germs with some surrounding tissues were dissected from 13- and 17-d-old mouse embryos. For separation of the epithelium from the mesenchyme, the tissues were incubated for 2 min in 2.25% trypsin/0.75% pancreatin on ice. The tissues were microsurgically separated in culture medium consisting of MEM (Orion Diagnostica, Espoo, Finland) supplemented with 10% FCS (Gibco Laboratories, Paisley, Scotland) with PBS (pH 7.4) at 25°C for 5 × 15 min. The mAb 281-2 against the core protein of the cell surface PG, syndecan, has been described earlier (Jalkanen et al., 1987). The antibodies against chick and rat tenasin were gifts of Dr. R. Chiquet-Ehrismann, Friedrich Miescher Institute, Basel, Switzerland, and Dr. E. Mackie, Sandoz, Basel, Switzerland. Other antibodies used were polyclonal rabbit antibodies against laminin (Gibco Laboratories, Bethesda Research Laboratories, Gaithersburg, MD) fibronectin (DAKOPATT, Copenhagen, Denmark) and type III collagen (a gift of Dr. L. Peltonen, National Health Laboratory, Helsinki). The mAb Hermes-1, which recognizes a human lymphocyte homing receptor (a gift of Dr. S. Jalkanen, Department of Medical Microbiology, University of Turku; Jalkanen et al., 1987), as well as normal rabbit serum were used in control stainings. The FITC-conjugated secondary antibodies were from DAKOPATT.

The tissues were incubated with the primary antibody (50 µg/ml) for 3–5 h, washed three to five times in PBS at 25°C for 1 h, incubated with the secondary antibody (1:40) for 2 h, washed again, and mounted. In some experiments, the samples were first incubated with normal serum (50 µg/ml) according to the primary antibody to block possible unspecific binding of the antibodies.

Tooth rudiments of 13- and 17-d-old mouse embryos and whole heads of 10- and 11-d-old mouse embryos, as well as some recombinant and cultured tissues, were fixed with cold 94% ethanol, embedded in paraffin wax (Tissue Prep, Fisher Scientific Co., Pittsburgh, PA), and serially sectioned at 5 µm. The deparaffinized sections were incubated with the primary and secondary antibodies at 25°C for 30 min, washed 3 × 15 min with PBS at 25°C, and mounted. For immunoperoxidase staining, the Vectastain Avidin Biotinylated horseradish peroxidase complex kit was used (Vector Laboratories, Inc., Burlingame, CA).

Metabolic Labeling and Immunosoliation of 35SO4-labeled Syndecan

The freshly recombined mesenchyme (20–50 pieces/experiment) and epithelia (3–4 pieces/embryos) were preincubated at 37°C in sulfate-free MEM (without antibiotics) supplemented with 10% dialyzed FCS for 2 h. Therefore, the medium was changed and 100 µCi/ml 35SO4 (American International, Amersham, UK) was added. After 18 h of incubation at 37°C, the tissues were collected and sonicated in 500 µl extraction buffer (PBS containing 1% NP-40, 0.1% deoxycholate, 0.1% SDS, and 1 mM PMFS). The samples were stored at −20°C until analyzed.

Each tissue extract was subjected to immunosoliation of syndecan (Jalkanen et al., 1987; 1988). mAbs Hermes-1 and 281-2 were bound to 2 ml of CNBr-activated Sepharose C1-4B column (antibody concentration 0.5 mg/ml). Each sample, diluted to 20 ml with PBS (pH 7.4) containing 1% Triton X-100, was first passed through plain Sepharose C1-4B (2 ml), which was discarded, and then run through Hermes-1 and 281-2 columns. These columns were then washed with 20 ml of PBS–Triton X-100 and eluted separately with 3 ml of 50 mM triethylamine, pH 11.5. Fractions of 1 ml were collected at the speed of 6–8 ml/h, and those containing triethylamine were neutralized with 1% Triton X-100, washed with 10% TCA and 95% ethanol and counted by liquid scintillation, as described earlier (Jalkanen et al., 1985). Fractions containing PG were pooled, and ethanol was precipitated and used for further analysis.

Size Analysis

PGs eluted and precipitated from 281-2 columns were subjected to SDS-PAGE analysis in 4–22% gradient gels (O'Farrel, 1975). PGs were visualized by autoradiography. The molecular mass of syndecan was estimated on parallelly run 14C-labeled myosin (200 kD), phosphorylase B (92.5 kD), and albumin (67 kD) standards.

Results

Expression of Syndecan and Tenascin in Presumptive Dental Mesenchyme Results from Interaction with Epithelium

Immunoperoxidase staining of sagittal sections through the head and neck of 10- and 11-d mouse embryos with the mAb 281-2 against syndecan indicated that in vivo the epithelium of the frontonasal process as well as the first (mandibular) and second branchial arches expressed syndecan. The underlying mesenchyme was also stained, but the intensity de-


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creased significantly towards deeper mesenchymal cell layers (Fig. 1, a and b). The entodermal lining of the second branchial arch as well as its underlying mesenchyme were negative. Upon formation of the epithelial tooth bud in a 13-d embryo, the condensed dental mesenchyme acquired intense stain. At this developmental stage also, tenascin was intensely expressed by the condensed dental mesenchyme (Fig. 1, e and f).

When the presumptive dental epithelium and mesenchyme from the mandibular arch of 11-d-old mouse embryos (Fig. 1 b) were enzymatically separated and cultured in recombination, intense syndecan staining appeared in the epithelium as well as in a restricted part of the mesenchyme in the epithelial contact area. The peripheral mesenchyme that was not in contact with epithelial tissue was negative or only weakly positive for syndecan (Figs. 1 c and 2 a). The syndecan positive area corresponded to a translucent zone seen in phase-contrast microscope (Fig. 2 b). Because of the intense expression of syndecan in the presumptive mouse dental epithelium, the initiation and spreading of the syndecan positivity in the mesenchyme could not be well demonstrated. Therefore, corresponding epithelial tissue from 13-d-old rat embryos was used as inductor instead of mouse tissue. The mAb 281-2 has been produced in rats against a mouse mammary epithelial cell line (Jalkanen et al., 1985). Neither rat tissues in vivo nor recombinants of rat epithelium and mesenchyme were stained with the mAb 281-2, indicating that the mAb 281-2 does not react with rat antigen (data not provided).
Figure 2. Immunofluorescent localization of syndecan in experimental recombinants of presumptive dental epithelium and mesenchyme. The explants were cultured for either 6 or 24 h, fixed with methanol, and stained as whole mounts, as described in Materials and Methods. Rat epithelium (c and d) that is not stained with the mAb 281-2 was used to demonstrate the initiation of syndecan expression in the mesenchyme. (a) Recombination of epithelium and mesenchyme from 11-d mouse embryonic mandible (see Fig. 1 b) has resulted in intense expression of syndecan in the mesenchyme during 24 h in culture. (b) The syndecan positive area in mesenchyme corresponds to a translucent zone seen in phase-contrast microscope. (c) After 6 h of recombination culture, syndecan positivity is restricted to mesenchymal cells that are in close proximity with the epithelium. (d) After 24 h of culture, the mesenchymal syndecan positive areas underlying two unstained rat epithelia have increased in size and partly united. (E) Presumptive dental epithelium; (M) presumptive dental mesenchyme. Bars, (a) 80 μm; (b) 100 μm; (c and d) 200 μm.

Figure 3. Tenascin in recombinant explants. Tenascin appeared in similar recombinant explants also in the mesenchyme that directly underlined the epithelium (Fig. 3, a–c). Tenascin was first seen as thin fibrils perpendicular to the epithelial–mesenchymal interface and the tenascin positive area in the mesenchyme increased during time of contact (Fig. 3 b). Tenascin appeared later than syndecan in the mesenchyme and the tenascin positive zone spread with a delay as compared to the spreading of the translucent area seen in phase-contrast microscope that corresponded to the syndecan positive area (Fig. 3, c and d). The mesenchyme that was cultured without the epithelium did not express tenascin (not shown). Tenascin, induced by the epithelium, was detected with antibodies against both rat and chick tenasin.

Laminin, Fibronectin, and Type III Collagen Do Not Co-distribute with Syndecan and Tenascin in the Mesenchyme

The cell surface PG, syndecan, has been shown to interact with molecules in the ECM (Koda et al., 1985; Saunders and Bernfield, 1988). Therefore, its distribution pattern was compared with some other ECM components that have been proposed to play important roles during epithelial–mesenchymal interactions in morphogenesis of various organs, including the tooth. The expression of laminin, type III collagen, and fibronectin was studied by immunostaining recombinant explants of 11-d mouse embryonic epithelium and mesenchyme. These matrix molecules did not show a similar pattern of expression as syndecan and tenascin. After 24 h of recombination culture, laminin, type III collagen, and fibronectin were all present at the epithelial–mesenchymal interface. This obviously was indicative of the formation of a basement membrane between the two tissues. The basal laminae of capillaries were also laminin positive (Fig. 4 a). Type III collagen and fibronectin were localized throughout the presumptive dental mesenchyme and did not show accumulation under the epithelial contact area (Fig. 4, b and c).

Syndecan in the Mesenchyme Is Induced and Not Transferred from the Epithelium

To exclude the possibility that syndecan in the presumptive dental mesenchyme would have originated from the syndecan positive dental epithelium by shedding of proteolytically cleaved ectodomain (Rapraeger and Bernfield, 1985; Jalkanen et al., 1987), interspecies recombinants between presumptive dental tissues from 13-d-old rat and 11-d-old mouse
embryos were prepared. When mouse dental epithelium was combined with rat mesenchyme, no mouse syndecan was detected in the rat mesenchyme during 24 h of culture (Fig. 5 a). This indicated that syndecan in the mesenchyme was not epithelial in origin. In these experiments, the appearance of tenasin positivity in the mesenchyme indicated that the two tissues did interact (Fig. 5 c).

On the other hand, in reciprocal recombinants where rat epithelium was used as an inductor and mouse presumptive dental mesenchyme as the responding tissue, an intense syndecan as well as tenasin positive area appeared in the mesenchyme during 24 h of culture. This indicated again that the epithelium controlled syndecan expression in the mesenchyme, and also that epithelial–mesenchymal interaction had taken place across the two species (Fig. 5, b and d).

**Epithelial–Mesenchymal Interaction Regulates the Biosynthesis of Syndecan in the Mesenchyme**

The influence of epithelial–mesenchymal tissue interaction on syndecan biosynthesis in the mesenchyme was studied by metabolic labeling and immunoisolation of syndecan. The levels of syndecan synthesis between presumptive dental mesenchymes cultured either in isolation or in combination with rat epithelium were compared. The sulfate-labeled syndecan of rat tissues failed to bind to the mAb 281-2 immunoaffinity column that confirmed that the 281-2 did not recognize syndecan of rat origin (not shown). The Hermes-1 immunoaffinity column was used as control. When uninduced and induced mesenchymes were labeled with sulfate and total PG of the tissue extracts were analyzed by SDS-PAGE, no clear differences between the samples were detected (Fig. 6, lanes A and B). However, if these extracts were passed through immunocolumns, the extracts of induced mesenchymes yielded a 5–10-fold higher amount of sulfate-labeled material bound to the mAb 281-2 column than those of uninduced mesenchymes (not shown). This was reflected also in a preliminary analysis of these samples by SDS-PAGE, which revealed a typically smeary PG band (Rapraeger et al., 1985), present in higher amounts in induced than in uninduced samples (Fig. 6, lanes C and D). Thus, the induction of syndecan expression in the mesenchymes, as revealed earlier by immunostainings, was also evident at the biosynthetic level.

**The Induction of Syndecan Expression Depends on the Developmental Stage of the Tissues**

The presumptive dental epithelium is known to possess the tooth forming odontogenic potential before day 11 of mouse embryonic development (Mina and Kollar, 1987; Lumsden, 1988). To examine whether the potential to induce syndecan expression and the competence to respond to the inductive signal depends on the developmental stage of the tissues, recombinations of epithelium and mesenchyme between 11- and 17-d-old embryos were made. The epithelial component of the 17-d embryonic tooth consists of several different cell types (see Fig. 1 d) that cannot be microsurgically dissected.
Figure 4. Immunofluorescent localization of laminin, collagen type III, and fibronectin in recombinants of presumptive dental epithelium and mesenchyme cultured for 24 h and stained as whole mounts. (a) Laminin expression is evident in the epithelial–mesenchymal interface, which is probably indicative of formation of the basement membrane between the two tissues. Also, the basal laminae of capillaries are laminin positive. (b) Collagen type III and fibronectin (c) localize throughout the mesenchyme and they are accumulated in the interface between epithelium and mesenchyme indicating formation of the basement membrane. (E) presumptive dental epithelium; (M) presumptive dental mesenchyme. Bar, (a–c) 200 μm.

Therefore, we used oral epithelium, which originates from the same region in the mandibular arch epithelium as the tooth bud. In vivo, this oral epithelium was syndecan positive whereas the underlying mesenchyme was negative (Fig. 1 d). When these tissues were separated and recombined for culture, syndecan expression was not induced in the mesenchyme during 24 h in culture (Fig. 7 a). Recombination of the 17-d embryonic oral epithelium with the 11-d presumptive dental mesenchyme did not result in induction of syndecan expression in the mesenchyme either (Fig. 7 b). The presumptive dental epithelium from an 11-d embryo did, however, induce syndecan expression in the 17-d oral mesenchyme, which in vivo did not express syndecan. In these recombinants, the syndecan positive area appeared first at the epithelial–mesenchymal interface, and it increased during time of contact (Fig. 7, c and d). These results indicated that the developmentally advanced epithelium had lost its ability to induce syndecan expression in mesenchyme, whereas the oral mesenchyme had remained competent to respond to epithelial induction.

Figure 5. Immunofluorescent localization of syndecan by mAb 281-2 in reciprocal recombinant explants of rat and mouse presumptive dental epithelium and mesenchyme. Note that the mAb 281-2 does not stain syndecan of rat origin. The explants were cultured for 24 h, fixed, and stained as whole mounts. (a) A recombinant of 11-d mouse embryonic epithelium and 13-d rat embryonic mesenchyme. The epithelium is intensely stained, but mouse syndecan is not detected in rat mesenchyme indicating that the ectodomain of syndecan has not been proteolytically cleaved and shedded from the epithelium to the mesenchyme. (b) A recombinant of rat epithelium and mouse mesenchyme. Intense syndecan expression has been induced in the mesenchyme. Syndecan in rat epithelium is not stained. (c and d) Similar explants to those in a and b but stained with tenascin antibodies. The induction of tenascin positive zones in the mesenchyme indicates that tissue interactions take place between mouse and rat tissues. (E) presumptive dental epithelium; (M) presumptive dental mesenchyme. Bars, (a and b) 225 μm; (c and d) 85 μm.
Expression of Syndecan and Tenascin in the Mesenchyme Is Induced by Epithelium

The cell surface PG, which was recently named syndecan (Saunders et al., 1989) and which is recognized by the mAb 281-2, was originally extracted from a mouse mammary epithelial cell line (Jalkanen et al., 1985). The mapping of the distribution patterns of this PG in various tissues of adult mouse by immunocytochemistry has indicated that it is present predominantly in epithelial tissues (Hayashi et al., 1987). The present results show that the mAb 281-2 stains also mesenchymal tissue in the frontonasal process and in the branchial arches of 10- and 11-d-old embryos, and our recent observations have indicated that this PG is transiently expressed in mesenchyme during tooth morphogenesis (Thesleff et al., 1988). The stage-specific and restricted distribution pattern of syndecan in embryonic mesenchymal tissue and its loss from adult connective tissue suggests that this PG has important functions during embryogenesis.

Our experiments on interspecies recombinants of mouse and rat presumptive dental epithelium and mesenchyme showed conclusively that epithelial tissue induced a syndecan positive zone in the mesenchyme that was in immediate contact with the epithelium. Also, the monitoring of syndecan biosynthesis, by metabolic labeling and immunoisolation, indicated that the dental epithelium induced syndecan expression in mesenchyme. Although tissue interactions have been shown to induce specific molecular changes in several epithelial–mesenchymal organs (Heuberger et al., 1982; Bernfield and Banerjee, 1982; Ekblom et al., 1986), induction of syndecan expression is the first molecular change identified so far in the differentiating dental mesenchyme.

Based on the distribution of the matrix glycoprotein tenascin during early organ morphogenesis, it was proposed earlier that tenascin is involved in epithelial–mesenchymal interactions (Chiquet-Ehrismann, 1986; Aufderheide et al., 1987). We have now shown directly that tenascin expression is induced by the epithelium in the dental mesenchyme. Similar results have been recently reported on developing gut where tenascin expression in the mesenchyme was induced by an epithelial cell line (Aufderheide et al., 1988), and on mammary gland where embryonic and neoplastic epithelium induce tenascin synthesis in their surrounding mesenchyme (Inaguma et al., 1988).

The molecular mechanisms of the transmission of inductive signals are generally not known but mediation by close cell–cell contacts, by cell–matrix interactions, as well as by soluble mediators have been proposed (Saxén et al., 1980; Gurdon, 1987). It is known of some embryonic interactions that the responding tissue starts to produce the same ECM molecules as the inducing tissue (Lash and Vasan, 1979; Watt, 1986). The cell surface PG, syndecan, that was induced in the mesenchyme in our experiments is a predominantly epithelial molecule (Hayashi et al., 1987; Jalkanen, 1987), and it is expressed also by the branchial arch epithelium that was used as inductor tissue. It is possible that syndecan in the inductive epithelium is involved in the control of expression of a similar molecule in the responding mesenchyme. However, the 17-d embryonic epithelium, which was syndecan positive, did not induce syndecan expression in the mesenchyme. Since the mAb 281-2 recognizes the core of the ectodomain of this PG, it is still possible that the glycosaminoglycan composition of the oral epithelial proteoglycan changes during development. Such molecular polymorphism of syndecan has been recently reported in different epithelia (Sanderson and Bernfield, 1988).

We do not know at present what mechanisms are operating in the induction and spreading of syndecan and tenascin positive zones, and whether the signals regulating the expression of the two molecules are related. Because tenascin is an exclusively mesenchymal molecule and syndecan (as we have shown) is not transferred from the epithelium to the mesenchyme, the time dependent spreading of the syndecan and tenascin positive zones must have resulted either from migration of the induced cells, from diffusion of morphogen(s), or from a chainlike transfer of the inductive capacity from induced to uninduced mesenchymal cells. The involvement of different molecular mediators (e.g., cell surface molecules such as PGs or gangliosides [Mugnai et al., 1988; Sariola et al., 1988]) or diffusible morphogens (Gurdon, 1987; Robertson, 1987; Slack, 1987) can be explored in our model system in the future.

Our results showed that the presumptive dental epithelium induces syndecan expression also in 17-d-old mouse embryonic oral mesenchyme that did not express syndecan in vivo. This indicates that the oral mesenchyme had remained...
Figure 7. Immunofluorescent localization of syndecan in heterochronal epithelial–mesenchymal recombinants of oral and dental tissues. The explants were cultured in recombination for 24 h, fixed, and stained as whole mounts. (a) Syndecan expression has not been induced in mesenchyme after separation and recombination of oral epithelium and mesenchyme from 17-d-old embryo (see Fig. 1 d). (b) 17-d embryonic oral epithelium has not induced syndecan expression in 11-d mesenchyme. (c) The presumptive dental epithelium of 11-d embryo has induced syndecan expression in 17-d embryonic oral mesenchyme, which in vivo is negative (see Fig. 1 d). (d) A similar explant to that in c, except that rat epithelium has been used as an inductor. Since rat epithelium is not stained with the mAb 281-2, the intense syndecan expression induced in mouse mesenchyme is clearly visible. (OE) 17-d mouse embryonic oral epithelium; (OM) 17-d mouse embryonic oral mesenchyme; (E) 11-d mouse or 13-d rat embryonic presumptive dental epithelium; (M) 11-d mouse embryonic presumptive dental mesenchyme. Bars, (a) 75 μm; (b) 175 μm; (c and d) 165 μm.

syndecan; (E) 11-d mouse or 13-d rat embryonic presumptive dental epithelium; (M) 11-d mouse embryonic presumptive dental mesenchyme. Bars, (a) 75 μm; (b) 175 μm; (c and d) 165 μm.

The In Vivo and In Vitro Co-distribution of Syndecan and Tenascin Suggest Morphogenetic Functions

In vivo, syndecan and tenascin are accumulated in the condensed mesenchyme under the invaginating epithelial tooth bud (Chiquet-Ehrismann et al., 1986; Thesleff et al., 1987, 1988). The condensation of the mesenchymal cells is induced by the presumptive dental epithelium that was also used as inductor in the present studies (Mina and Kollar, 1987). The syndecan and tenasin positive zone that was induced by the epithelium in the present experiments appeared as a translucent area in the phase-contrast microscope. This area probably represents a mass of similarly determined cells and may be analogous to condensing dental mesenchyme in vivo. Hence, important morphogenetic roles can be suggested for syndecan and tenasin during early tooth development. A role for tenasin rather than for other ECM molecules is further supported by our findings that other matrix molecules, namely laminin, type III collagen, and fibronectin, were not induced in the same syndecan-positive mesenchymal area as tenasin. These molecules neither show accumulation in the condensed dental mesenchyme in vivo (Thesleff et al., 1979; Chiquet-Ehrismann et al., 1986).

Syndecan is a matrix receptor in epithelial cells (Koda and Bernfield, 1984; Koda et al., 1985; Saunders and Bernfield, 1988), and may translate the changes in the ECM composition into cellular behavior (Rapraeger et al., 1986). Whether syndecan in mesenchymal tissue is, in fact, an integral cell surface molecule with similar matrix binding properties to those in epithelial cells is not known at present. Our recent molecular analysis of syndecan, synthesized by both dental epithelium and mesenchyme, revealed the same molecular sizes of 200–250 kD by SDS-PAGE, and showed that the main glycosaminoglycan bound to this PG in the dental mesenchyme was heparan sulfate (our unpublished results). Thus, it is reasonable to assume that the ectodomain recognized by the mAb 281-2 in mesenchymal tissue has also properties of a matrix receptor.

Syndecan may also have the capacity to self-associate as has been reported for some PGs (Fransson and Havsmark, 1982; Dietrich et al., 1983) and be involved in direct cell–cell interactions during condensation. Its association with the cytoskeleton (Rapraeger et al., 1986) may also regulate...
changes in cell shape. Moreover, since syndecan bears predominantly heparan sulfate glycosaminoglycan (Rapraeger et al., 1985), it is possible that it binds heparin binding growth factors (Mercola and Stiles, 1988; Slack et al., 1987). In this respect, it is noteworthy that intense syndecan expression in dental mesenchyme is followed by a period of active cell proliferation (Thesleff et al., 1988), and that the distribution of epidermal growth factor binding cells in the tooth germ shows correlation with syndecan distribution (Partanen and Thesleff, 1987).

Tenascin interferes with fibronectin-mediated cell attachment and affects cell shape (Mackie et al., 1987; Chiquet-Ehrismann et al., 1988). Induction of tenasin expression in the dental mesenchyme may therefore be associated with rounding and condensation of these cells. The cell binding domain of tenascin has been localized to its distal arms, but the cell surface receptor for tenasin has not yet been identified (Chiquet-Ehrismann et al., 1988). Based on our present findings and on our recent preliminary experiments indicating that syndecan extracted from embryonic tooth mesenchyme binds tenasin (our unpublished observations), we can speculate that tenasin and syndecan represent a couple of a cell surface receptor and a matrix ligand. In conclusion, the similar distribution patterns of syndecan and tenascin in vivo and in vitro and their early appearance as a result of epithelial–mesenchymal interaction suggest that these molecules may be involved in the condensation and differentiation of dental mesenchymal cells.

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