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*Short Contribution*

# Simultaneous loss of expression of syndecan-1 and E-cadherin in the embryonic palate during epithelial-mesenchymal transformation

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**ABSTRACT Epithelial-mesenchymal transformation (EMT) is the key mechanism for fusion and confluence of the rodent palate. During this process, medial edge epithelia (MEE) form a midline seam that subsequently transforms to mesenchymal cells. We studied syndecan-1 and E-cadherin, two molecules which have been shown to promote the epithelial phenotype, to determine their fate during palatal EMT. We found that both syndecan-1 and E-cadherin are expressed on basolateral surfaces of the MEE at day 14. Twelve hours later, when a midline seam has formed, syndecan-1 and E-cadherin are still present on its basal and lateral epithelial surfaces and they persist after the seam breaks up into epithelial islands. Then, expression of both molecules is lost simultaneously and abruptly when EMT occurs. On the contrary, previous *in vitro* studies of cell lines transfected with antisense cDNAs suggested that loss of syndecan-1 would lead to loss of E-cadherin or vice versa. We conclude that *in vivo*, synthesis of both E-cadherin and syndecan-1 is downregulated synchronously by the initiation of EMT, leading to an effective and correctly timed conversion of the epithelial cells to mesenchyme.**

KEY WORDS: *syndecan-1, E-cadherin, epithelial-mesenchymal transformation, murine palatogenesis, craniofacial development*

The tissue phenotype called epithelium is the first to form in vertebrate phylogeny and ontogeny (Hay, 1990). The contiguous cells are joined by cell-cell adhesion molecules and junctions that seal off the embryo from the outside. The major cell-cell adhesion molecules are E-cadherin, an integral membrane glycoprotein (Takeichi, 1995), and syndecan-1, a heparan sulfate proteoglycan (Bernfield and Sanderson, 1990). The major junctions are zonulae occludentes and adherentes, and maculae adherentes (desmosomes). E-cadherin is distributed as a complex with α-and β-catenin on lateral epithelial surfaces where it interacts with E-cadherin complexes on adjacent cells (Cowin and Burke, 1996). E-cadherin also occurs on basal surfaces, where its function is not understood. Syndecan-1 has a nearly identical distribution, but there is evidence that on basal epithelial surfaces, it mediates cell-substratum interactions with collagen and/or other matrix molecules (Bernfield *et al.*, 1992).

During embryogenesis, epithelia give rise to motile mesenchymal cells that invade the underlying ECM. The mesenchymal cell is an elongated cell type, typified by the fibroblast, with no cell junctions and greatly reduced cell-cell adhesion. No E-cadherin is present, β-catenin is distributed diffusely in the cytoplasm, and syndecan-1 is usually lost. When epithelial tumors give rise to metastases, they transform to mesenchyme-like cells that have a capacity similar to that of fibroblasts for invading ECM (Behrens *et al.*, 1989; Takeichi, 1995).

There is a major literature comparing the effects of E-cadherin and syndecan-1 on the epithelial and mesenchymal phenotype. Transfection of fibroblastic cell lines with E-cadherin c-DNA transforms them to simple squamous or cuboidal epithelia (see Takeichi, 1995) and recently, E-cadherin has been shown to transform primary embryonic fibroblasts to stratified epithelium with desmosomes (Vanderburg and Hay, 1996). Syndecan-1

*Abbreviations used in this paper:* ECM, extracellular matrix; EMT, epithelial-mesenchymal transformation; MEE, medial edge epithelium/epithelia

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imposes an epithelial-like morphology on tumorigenic mammary cell lines as does E-cadherin transfection, which upregulates syndecan-1 expression (Leppä *et al.*, 1996). Antisense E-cadherin transfection of these mammary cell lines suppresses syndecan-1 (Leppä *et al.*, 1996). Turn off of E-cadherin activity by antisense E-cadherin cDNA (Vleminckx *et al.*, 1991) or by antibodies against E-cadherin (Behrens *et al.*, 1989) results in loss of the epithelial phenotype concomitant with transformation to the mesenchymal phenotype. Antisense syndecan-1 cDNA also converts epithelial cells to mesenchyme-like cells (Kato *et al.*, 1995). Where it has been studied, syndecan-1 expression has been observed to be suppressed by antisense E-cadherin (Leppä *et al.*, 1996). On the other hand, transfection with antisense syndecan-1 leads to downregulation of E-cadherin, presumably at the transcriptional level as its mRNA is reduced (Kato *et al.*, 1995). Thus, in assessing the relative roles of syndecan-1 and E-cadherin in EMT, one is left with examples from the *in vitro* literature supporting the idea that syndecan-1 downregulation suppresses E-cadherin and/or E-cadherin downregulation suppresses syndecan.

Here, we ask what would the situation be in a naturally occurring EMT? Would syndecan-1 turn off first, followed by E-cadherin, or vice versa? We chose to study the *in vivo* transformation of the embryonic palatal medial edge epithelia (MEE). During development of the rodent palate, the MEE of the palatal shelves adhere and transform to mesenchyme under the influence of TGFβ3 (see Sun *et al.*, 1998). This EMT was discovered by electron microscopy (Fitchett and Hay, 1989) and confirmed by cell lineage studies (see Hay, 1995). It is one of the late EMT (E14-15 in the mouse) and so the MEE are well developed epithelia with prominent desmosomes and keratin fibrils, which they lose during the transformation while acquiring vimentin and mesenchymal morphology. N-cadherin and syndecan-1 have been shown to downregulate at about this time (Brinkley *et al.*, 1992; Janet A. Davies, personal communication), but the fate of E-cadherin in the embryonic rodent palate during transformation of the MEE to mesenchyme has not yet been described. Moreover, this is the first study to map the distribution of E-cadherin in frozen sections that parallel those examined for syndecan-1, avoiding all the potential artifacts (Brinkley *et al.*, 1992) of wax embedding on antigen epitopes.

In the mouse embryo, secondary palate formation occurs between days E12 and E15. On E12, the two palatal shelves arise from the medial walls of the maxillary processes. Initially, they grow vertically, and by E14, rotate to a horizontal position above the tongue and grow toward each other (Fig. 1A). At this time, the MEE is two cell layers thick, consisting of basal cells and superficial periderm cells. Over the next 12 h, the MEE contact and adhere, forming a midline epithelial seam in the posterior part of the palate (Fig. 1B). Most of the midline periderm cells slough off prior to the adhesion (*, Fig. 1A) and desmosomes form between opposing basal cells, holding the seam together (Fitchett and Hay, 1989). With growth of the palate, the seam thins to one cell layer, then breaks into small islands (arrow, Fig. 1C). The basal lamina disappears at the same time and the epithelial cells extend filopodia into the adjacent ECM (Fitchett and Hay, 1989). This process is followed by transformation of the MEE seam and islands into mesenchymal cells that elongate and migrate through the connective tissue, resulting in confluence of the intact palate by E15 (Fig. 1D).

In order to visualize E-cadherin, it was necessary to use frozen sections because the antigen does not survive paraffin embedding. We stained adjacent frozen sections of the same palates with syndecan-1 and E-cadherin antibodies, in order to show their localization and follow their expression precisely. Frozen sections thus permit E-cadherin to be stained in parallel with syndecan-1, and they are also free of the artifacts noted above (Brinkley *et al.*, 1992). At E14, both E-cadherin and syndecan-1 are expressed intensely along the basolateral surfaces of the medial edge, oral, and nasal epithelia of each palatal shelf and the connective tissue is seen to be free of stain (data not shown), whereas in wax sections some staining of mesenchyme is observed.

At E14, 12 h, when opposed MEE form a midline seam containing two layers of basal cells, both syndecan-1 and E-cadherin express in the midline seam where EMT transformation will take place (Fig. 1E and G; arrows indicate same area at higher magnification in Fig. 1F and H). The staining for both molecules is mainly along cell-cell contact areas, but the basal surfaces of the seam cells also stain for syndecan-1 and E-cadherin to varying degrees. Thus, epithelia in the seam maintain epithelial characteristics and express essentially identical patterns of E-cadherin and syndecan-1.

At day E14, 18 h, after the seam breaks into epithelial islands, syndecan-1 and E-cadherin can still be detected on the cell-cell contact areas and basal surfaces of the epithelial islands (arrows, Fig. 1I-L), but neither are present in the transformed mesenchyme (asterisks, Fig. 1I,K). This demonstrates that as soon as the island becomes mesenchymal, both molecules rapidly and abruptly disappear at the same time. No transitional mesenchymal cells expressing either or both molecules are seen.

Examination of numerous sections of embryonic palates undergoing MEE transformation to mesenchyme confirmed that E-cadherin and syndecan-1 are regulated together during embryonic EMT. This is in contrast to the situations discussed in the introduction where in mammary epithelial cell lines the syndecan core protein is upregulated by transfection of E-cadherin cDNA and downregulated by antisense E-cadherin (Leppä et al., 1996), and E-cadherin is downregulated by transfection with antisense syndecan-1 cDNA (Kato *et al.*, 1995). These results suggest that E-cadherin regulates syndecan-1 and vice versa in epithelial cell lines transfected *in vitro*. It was important to study the correlation of syndecan-1 and E-cadherin expression in an unmanipulated embryonic EMT in order to come to a more generalized conclusion about the relative roles of syndecan and E-cadherin in epithelial determination *in vivo*. Indeed, our data do not suggest a sequence of events in which loss of syndecan leads to loss of E-cadherin or vice versa during palatal EMT. Experimental manipulations of the levels of syndecan-1 and E-cadherin *in vitro* may stimulate compensation mechanisms, in some cases not involving transcriptional control (Leppä *et al.*, 1996), whereby one molecule can downregulate or upregulate the other by a decrease or increase in its expression. These mechanisms may differ from those operating in embryonic EMT.

Studying a very dramatic example of normal EMT in the palate, thus, we come to the conclusion that in the embryo, syndecan-1 and E-cadherin can disappear simultaneously during loss of the epithelial phenotype *in vivo*. Moreover, a number of other events occur simultaneously with E-cadherin/ syndecan-1 disappearance in transforming palatal MEE. Keratin fibrils and desmosomes are upregulated in the epithelial seam, presumably to strengthen the bond between the newly adherent MEE cells, and they persist until the seam is replaced by vimentin-rich connective tissue lacking

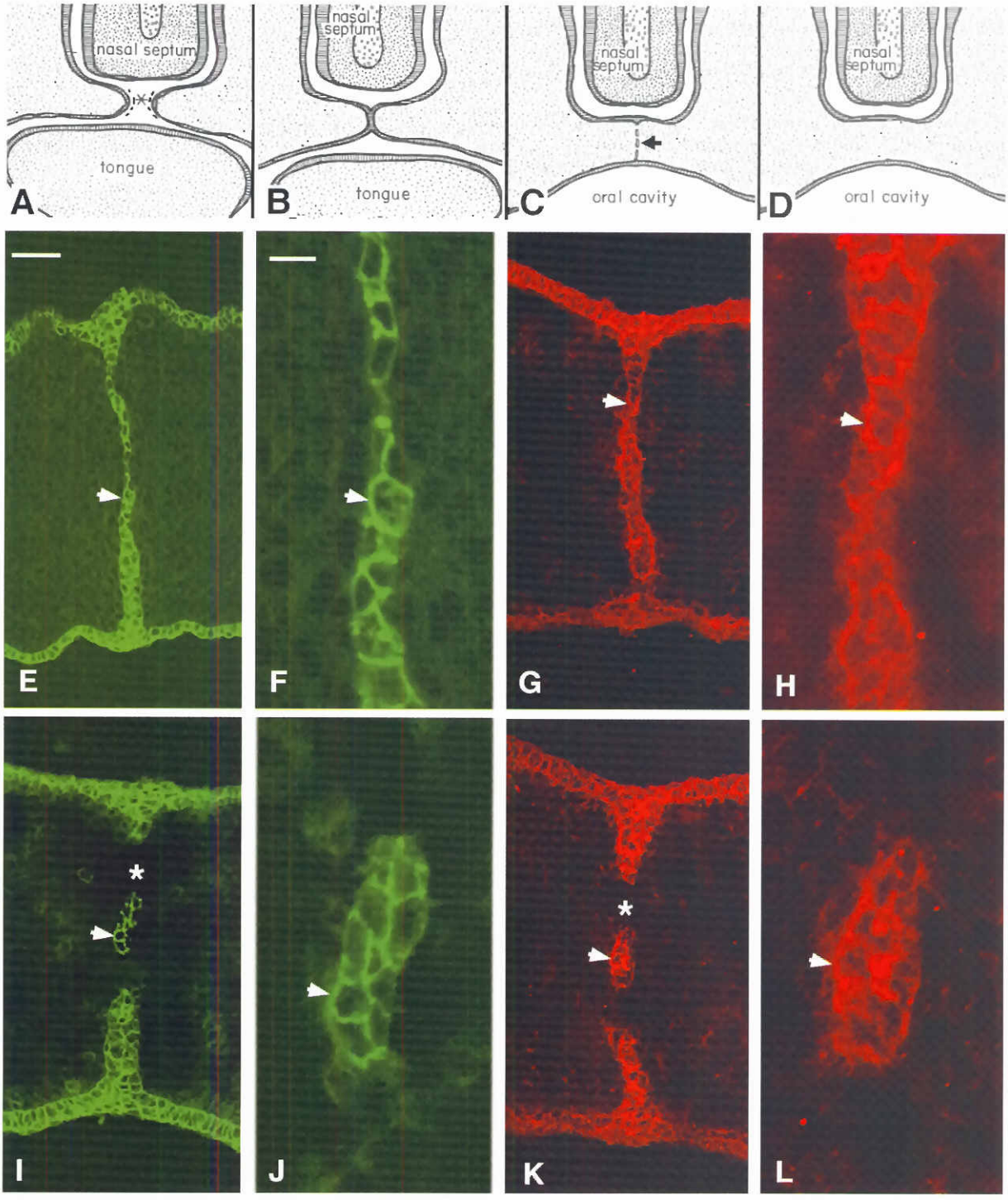


**Fig. 1. Developmental sequence of palate morphogenesis and epithelial adhesion molecules. (A-D)** *Diagrams showing the successive stages of the fusion and confluence of the posterior palatal shelves. On E14 (A), the palatal shelves rotate to a horizontal position and approach each other. The periderm cells slough off (*, A), and the basal epithelial cells adhere and form a midline seam during the next 12 h. (B) Six hours later, the epithelial seam breaks into epithelial islands (arrow, C) that transform into mesenchymal cells. Finally (E15), the seam disappears and transformed mesenchymal cells migrate through the connective tissue compartment, resulting in palatal confluence (D).* **(E-L)** *Immunostaining of adjacent frozen sections at E14, 12 h (E-H) and E14, 18h (I-K). E and I (E-cadherin) and G and K (syndecan-1) are shown at higher magnification in F,J,H and L, respectively. E-cadherin and syndecan-1 are simultaneously expressed during the formation and break up of the MEE seam. Within adjacent sections of the islands, E-cadherin (arrows, I,J) and syndecan-1 (arrows, K,L) can both be detected on basolateral surfaces. However, in the area where the epithelia have transformed into mesenchymal cells, no staining for either is seen (*, I,K). Bars, 100 μm (E,G,I and K); 25 μm (F,H,J and L).*

desmosomes (Fitchett and Hay, 1989). It is possible that in the embryo, keratin, desmosomes, E-cadherin, and syndecan-1 are downregulated at exactly the same time in the MEE, leading to an effective and correctly timed EMT. Thus, the adherent epithelial state of the opposed MEE is maintained until connective tissue confluence can be achieved. We have found that during EMT when cadherins (and presumably also syndecan-1) are lost, β-catenin is released to take up a diffuse location in the cytoplasm of neural crest mesenchyme (Kim *et al.*, 1997). This fibroblastic β-catenin probably signals the nucleus via cytoplasmic pathways, as it is not transported into the nucleus. It will be interesting in the future to investigate further the role of syndecan-1 and E-cadherin in such signaling pathways, and to explore the possible overall control of epithelial-mesenchymal transformation by genes upstream to syndecan-1 and E-cadherin.

## Experimental Procedures

Embryos from timed pregnancies of Albino Swiss Webster (Taconic Animal Lab., CT) mice (midnight before day of vaginal plug is day 0, time 0) were removed from day 14 to day 15 at 6 h intervals. The embryos were rinsed in phosphate buffered saline (PBS, 150 mM NaCl, 2.5 mM KCL, 10 mM $Na2HPO_4$, 2 mM $KH_2PO_4$, pH 7.4), then fetal heads were removed. They were fixed in 2% periodate-lysine-paraformaldehyde at room temperature for 1 h, rinsed in PBS, infiltrated in 15% Glucose/PBS at 4°C overnight, embedded in OCT compound (Tissue-TEK, Elkhart, IN) and frozen in liquid nitrogen. They were sectioned at 15 μm thickness (Microtome model HM 500 OM, Microm Inc., Germany).

Antibodies used in this study were: monoclonal rat-anti mouse E-cadherin (ECCD2, Zymed Laboratories. Inc, CA) and monoclonal rat-anti mouse syndecan-1 (281-2, Jalkanen *et al.*, 1985). Frozen sections were infiltrated with acetone at -20°C for 4 min and rehydrated in PBS. The sections were then incubated with 2% goat serum in PBS at 4°C overnight to block nonspecific staining. Antibodies to E-cadherin (1:100) and syndecan-1 (1:100) were added and incubated at room temperature for 3 h. The sections were washed in PBS, incubated with rabbit anti-rat rhodamine labeled antibodies (1:200, Cappel) in PBS at room temperature for 1 h, then washed again in PBS. The sections were viewed by a Zeiss confocal microscope (M185). Photographs for E-cadherin were taken using the green channel and syndecan-1 with the red channel.

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