**Int. J. Dev. Biol. 45 (S1)**: S101-S102 (2001)

*Short Report*

# Adhesion of medial edge epithelium cells is altered in the absence of Transforming Growth Factor $\beta_3$

CONSUELO TUDELA, ROSA BONELLI, CONSUELO ORTEGA, MIGUEL A. FORMOSO, TAMARA MARTÍNEZ, RAQUEL PÉREZ, CARMEN MAESTRO, AURORA DEL RÍO, JORGE MURILLO, CARMEN BARRIO and CONCEPCIÓN MARTÍNEZ-ÁLVAREZ*

*Departamento de Ciencias Morfológicas I. Facultad de Medicina, Universidad Complutense de Madrid, Spain*

**ABSTRACT** Mutations of the gene of Transforming growth factor beta 3 (TGF-$\beta_3$) lead to cleft palate in both humans and mice. This cleft seems to be produced by a defect in the adhesion of opposing medial edge epithelia (MEE) when contact between palatal shelves occurs. Using immunohistochesmistry, we demonstrate here that intercellular adhesion is altered in MEE cells of TGF-$\beta_3$ null mutant mice. The most striking features observed in these palates are the irregular distribution of β-catenin in the cytoplasm, in contrast to the regular and circumferential location found in wild type embryos, and the different expression of vinculin and β-actin, which is similar to the observed in the wild type oral palatal epithelium, that does not participate in palatal fusion.

The mutation of the gene of *TGF-$\beta_3$* is amongst the causes of cleft palate in mammals (Proetzel *et al.,* 1995). The mechanisms producing this cleft are not well known, but observation of the epithelium covering the tips of the palatal shelves (MEE) has revealed the existence of several differences between TGF-$\beta_3$ null mutant mice (TGF-$\beta_3$ -/-) and their wild type litter mates (TGF-$\beta_3$ +/+), that point to a defect of the MEE differentiation (Taya *et al.,* 1999).

During palate development, just prior to contact between palatal shelves, the MEE becomes multilayered, its most superficial cells bulge (bulging cells) (Martinez-Alvarez *et al.,* 2000a), and microvilli and lamellipodia develop on its apical surface (Taya *et al.,* 1999). A five to ten cell layer thick epithelial "seam", the midline epithelial seam (MES), is formed just after the initial adhesion, that, through a mechanism of cellular intercalation (Tudela *et al.,* in preparation), narrows and extends, becoming two and, finally, one cell layer thick. Soon after, by programmed cell death, epithelial-mesenchymal transformation and migration to the oral and nasal palatal epithelia, the MES disappears and the palatal mesenchyme becomes confluent in the midline. In the TGF-$\beta_3$ null mutants, although palatal shelves grow, approach each other and contact, opposing MEE adhesion fails and a cleft palate is produced (Proetzel *et al.,* 1995). Unlike in the wild type, just prior to contact between the palatal shelves, the TGF-$\beta_3$ null mutant MEE is only one cell layer thick and the MEE surface does not show the characteristic bulging cells (Martínez-Alvarez, 2000a and b), nor microvilli or lamellipodia (Taya *et al.,* 1999). Furthermore, when the palatal shelf contact is established, MEE cells do not intercalate (Tudela *et al.,* in preparation), nor transform into mesenchyme, as wild type MEE cells do. The simultaneous alteration of all these processes in the TGF-$\beta_3$ -/- palates strongly suggests a failure in the cytoskeleton - extracellular enviroment relationship of MEE cells, which is essential to the establishment of correct adhesions and movements amongst cells (Hay, 1995). In an attempt to determine whether such a relationship is altered in the TGF-$\beta_3$ -/- palates, we aimed to compare the expression pattern of some cellular adhesion and cytoskeletal molecules between TGF-$\beta_3$ +/+ and -/- MEE cells, as part of a larger study oriented to investigate the role of TGF-$\beta_3$ in the MEE cells differentiation and fate.

We analyzed the expression of several cell adhesion and cytoskeletal molecules in both TGF-$\beta_3$ -/- and +/+ MEE just prior to the contact between palatal shelves and soon after. We immunolabelled embryonic day 14.5 (E14.5) TGF-$\beta_3$ +/+ and -/- mouse palate sections with monoclonal antibodies directed against E-cadherin (Takara), α and β-catenin (Alexis), β-actin (Sigma), vinculin (Sigma) and LFA-1 integrin (Novocastra). To ensure that the multilayered organization of MEE cells observed prior to palatal shelf adhesion in the wild type palates is not due to cellular proliferation, we also labelled similar sections with an anti-PCNA monoclonal antibody (Dako). Secondary antibodies were Cy3 conjugated anti-rat (Jackson Lab), FITC conjugated anti-mouse (Vector) and monoclonal envision POD (Dako).

Our results show important differences between the wild type and the TGF-$\beta_3$ null mutant MEE. E14.5 TGF-$\beta_3$ +/+ preadhesion MEE is multilayered, but this arrangement is not due to cellular proliferation, since PCNA labelling demonstrated infrequent positive anti-PCNA cells in the MEE that were abundant in the palatal mesenchyme (Fig. 1). E13 MEE has a large number of positive anti-PCNA cells, that clearly decreases at E14 (Tudela *et al.,* in preparation). These results are in accordance with earlier studies showing inhibition of the DNA synthesis 24 to 36 hours prior to palatal shelf adhesion. As suggested by Brinkley (1984), a cellular redistribution might occur in the MEE at this time point that leads to this multicellular organization. This arrangement is needed to ensure a strong adhesion when the contact between palatal shelves takes place.

In the wild type palates, the pattern of expression of all E-cadherin, α and β-catenin was regular and circumferentially located in the basal cells, and basolateral in the superficial cells. All MEE cells were rounded. The actin cytoskeleton was circumferentially distributed unless in the bulging cells, where actin filaments are perpendicular to the apical surface (Martínez-Álvarez *et al.,* 2000b). Interestingly, all MEE cells were anti-vinculin positive, but different labelling intensities gave a "patched" appearance to the epithelium (Fig. 2a). This observation suggests different adhesion situations amongst MEE cells at the same time point. The zones of more adhesion could correspond to cells more actively involved in the movements needed to cause the cellular redistribution suggested by Brinkley (1984).

***Address correspondence to:** Concepción Martínez-Álvarez. Departamento de Ciencias Morfológicas I. Facultad de Medicina, Universidad Complutense de Madrid, Spain. e-mail: calvarez@eucmax.sim.ucm.es

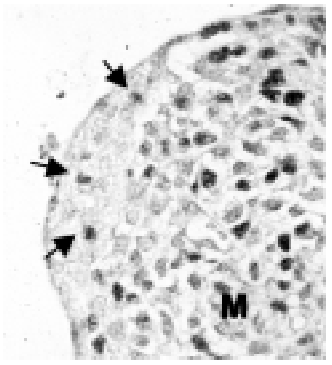


**Fig. 1. Immunollabeling of an E14.5 TGF-$\beta_3$ +/+ palatal shef with an anti-PCNA monoclonal antibody.** *Arrows indicate anti-PCNA positive cells in the medial edge epithelium. M, mesenchyme.*

The expression of these molecules in the MEE was remarkably different in the TGF-$\beta_3$ -/- palates. The MEE was monolayered and cells expressed E-cadherin and α-catenin all around. Unlike in the TGF-$\beta_3$ +/+ palates, the β-catenin immunolabelling formed positive accumulations irregularly distributed in the cytoplasm. Important changes were observed in the expression of vinculin and β-actin in the TGF-$\beta_3$ -/ - palates, as they were only concentrated in the region of apical-lateral contacts between cells (Fig. 2b), similar to the pattern presented by the oral palatal epithelial cells in both wild type (Fig. 2a) and TGF-$\beta_3$ -/- palates. This arrangement could correspond to the presence of these molecules in adherent junctions (zonula adherens) established between these cells, where vinculin co-localizes with actin (Yap *et al.,* 1997).

These results indicate that MEE cell adhesion is greatly modified under the TGF-$\beta_3$ influence. The *TGF-$\beta_3$* gene is expressed in the MEE just prior to contact of palatal shelves and its expression continues until the MES disappearance (Pelton *et al.,* 1990), coinciding with the most dramatic changes suffered by MEE cells. However, in the absence of TGF-$\beta_3$, the MEE resembles the oral palatal epithelium (that does not participate in palatal fusion), does not adhere to the opposing MEE and its cells neither intercalate nor transform into mesenchyme. It is likely that the most important alteration suffered by the TGF-$\beta_3$ -/- MEE cells is the defect in the junction of β-catenin to the cytoplasmic tail of E-cadherin, causing the irregular accumulation of β-catenin in the cytoplasm observed in these palates. Cell adhesion mediated by the E-cadherin-β-catenin complex might be necessary for the movements amongst MEE cells required to become a multilayered epithelium, to intercalate and to transform into mesenchyme, and the role of TGF-$\beta_3$ could be to maintain this complex. If the E-cadherin-β-catenin complex is altered in the absence of TGF-$\beta_3$, MEE cells would behave as the oral epithelial cells (that do not receive the TGFβ 3 influence), adhering amongst them through zonula adherens, junction that conferes stability to epithelia (Yap *et al.,* 1997), thus impeding their mobility.

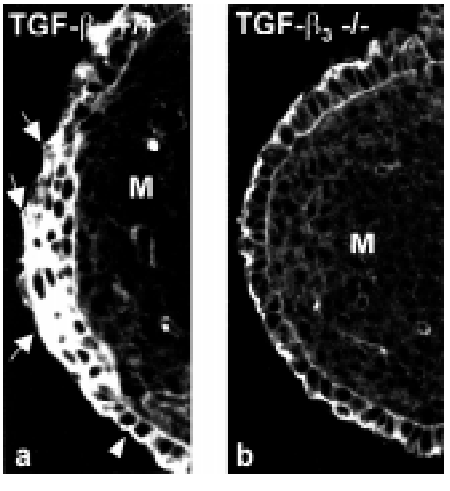


**Fig. 2. Immunolabelling with an anti-vinculin monoclonal antibody of E14.5 TGF-$\beta_3$ +/+ (a) and -/- (b) palatal shelves.** *Arrows in (a) indicate patches of increased labelling intensity in the medial edge epithelium. The arrowhead points to the oral palatal epithelium. M, mesenchyme.*

The comparative study of the expression of the integrin LFA-1 in the MES of TGF-$\beta_3$ +/+ and -/- palates brought about very interesting information. First, it showed that the adhesion of MEE cells to the underlying mesenchyme changes when the contact between TGF-$\beta_3$ +/+ palatal shelves is established and that this does not occur in the null mutants. This finding is in accordance with the previously reported persistence of the basal lamina under the TGF-$\beta_3$ -/- MEE observed by Kaartinen *et al.* (1997). It also demonstrated that, unlike in the wild type palates, TGF-$\beta_3$ -/- contacting MEE express this integrin on their apical surfaces. This molecule could be present in substitution of chondroitin sulphate proteoglycan, that mediates the adhesion between opposing MEE in the wild type embryos and is never expressed in the TGF-$\beta_3$ -/- mice (Gato *et al.,* in preparation). However, this integrin is not useful to palatal shelf adhesion, as these mice have cleft palate. It is also likely that its presence interferes with the recognition amongst opposing MEE cells needed to start the normally existing cell intercalation that begins as soon as the MES is formed. This cell intercalation is abolished in the TGF-$\beta_3$ -/- mice (Tudela *et al.,* in preparation).

The results shown here lead to the conclusion that in the absence of TGF-$\beta_3$ there are significant changes in the intercellular adhesion of MEE cells that might be responsible for the altered palatal shelf adhesion occuring in the TGF-$\beta_3$ null mutant mice. Which mechanisms underlye these adhesion defects is still to be determined.

*This work has been supported by a grant: 08.6/0042.1/2000, from the Comunidad de Madrid (Spain).*

## References

BRINKLEY, L. (1984). Changes in cell distribution during mouse secondary palate closure *in vivo* and *in vitro*. I. Epithelial cells. *Dev. Biol.* 102: 216-227.

HAY, E.D. (1995). An Overview of epithelio-mesenchymal transformation. *Acta Anat.* 154: 8-20.

KAARTINEN, V., CUI, X.M., HEISTERKAMP, N., GROFFEN, J. and SHULER, C.F. (1997). Transforming growth factor-$\beta_3$ regulates transdifferentiation of medial edge epithelium during palatal fusion and associated degradation of the basement membrane. *Dev. Dyn. 209*: 255-260.

MARTÍNEZ-ALVAREZ, C., TUDELA, C., PÉREZ-MIGUELSANZ, J., O´KANE, S., PUERTA, J. and FERGUSON, M.W.J. (2000a). Medial edge epithelial cell fate during palatal fusion. *Dev. Biol.* 220(2): 343-357.

MARTÍNEZ-ALVAREZ, C., BONELLI, R., TUDELA, C., GATO, A., MENA, J., O´KANE, S. and FERGUSON, M.W.J. (2000b). Bulging medial edge epithelial cells and palatal fusion. *Int. J. Dev. Biol.* 44: 331-335.

PELTON, R.W., HOGAN, B.L., MILLER, D.A. and MOSES, H.L. (1990). Differential expression of genes encoding TGFs β1, β2 and β3 during murine palate formation. *Dev. Biol.* 141: 456-460.

PROETZEL, G., PAWLOWSKI, S.A., WILES, M.V., YIN, M., BOIVIN, G.P., HOWLES, P.N., DING, J., FERGUSON, M.W.J. and DOTSCHMAN, T. (1995). Transforming growth factor-β3 is required for secondary palate fusion. *Nat. Genet.* 11: 409-414.

TAYA, Y., O´KANE, S. and FERGUSON, M.W.J. (1999). Pathogenesis of cleft palate in TGF-β3 Knockout mice. *Development.* 126 (17): 3869-3879.

YAP, A.S., BRIEHER, W.M. and GUMBINER, M. (1997). Molecular and functional analysis of cadherin-based adherens junctions. *Annu. Rev. Cell Dev. Biol.* 13: 119-146.