PALATE DEVELOPMENT IN THE TGF-β_3 KNOCKOUT MOUSE. LOW VACUUM SCANNING ELECTRON MICROSCOPY REVEALS CHANGES IN THE MEDIAL EDGE EPITHELIUM

Concepción MARTÍNEZ-ÁLVAREZ[1], Sharon O'KANE[2], Yuji TAYA[2], Mark W.J. FERGUSON[2]
[1]*Departamento de Ciencias Morfológicas I, Facultad de Odontología, Universidad Complutense de Madrid, España and*
[2]*Division of Cells, Inmunology and Development, School of Biological Sciences, University of Manchester, United Kingdom*

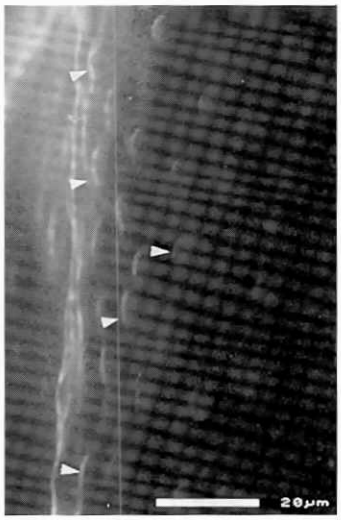

Figure 2: Medial edge epithelium of an E-14 TGF-β_3 +/+ mouse left palatal shelf. Arrowheads indicate many cellular protrusions in the superficial layer.

TGF-β_3 is a growth factor of the TGF-ß superfamily, which includes inhibition of cellular proliferation amongst its actions (Moses, 1992). The mRNA for TGF-β_3 has been detected in the epithelium of the tip of mouse palatal shelves from the stage E-12, when they are in a vertical position, until E-15, when the shelves fuse, the midline epithelial seam formes and disrupts (Fitzpatrick et al, 1990). TGF-β_3 inhibition in E-13 mouse palate cultures, by antisense oligodeoxynucleotides or neutralizing antibodies, leads to a failure of fusion, what suggests an important role for TGF-β_3 in this process (Brunet et al, 1995). Morphological studies of TGF-β_3 knockout (TGF-β_3 KO) mice reveal cleft palate in the homozygous -/- mice (Proetzel et al., 1995), but reasons for the fusion to be disturbed are still unknown. We have studied, using Low Vacuum Scanning Electron Microscopy (LVSEM), the surface of the medial edge epithelium (MEE) of E-14 (immediately before fusion in normal mice) and E-15 (fusion time in normal mice) mice derived from mating TGF-β_3 heterozygotes (+/-), in order to detect any differences in the MEE amongst the TGF-β_3 homozygous KO (-/-), heterozygous (+/-) and homozygous normal +/+ mice. LVSEM allows for the study of biological tissues fixed for only a very short time before visualization, without requiring any previous dehydration or gold coating, as in conventional scanning electron microscopy. This means that the tissue is observed in almost its natural state.

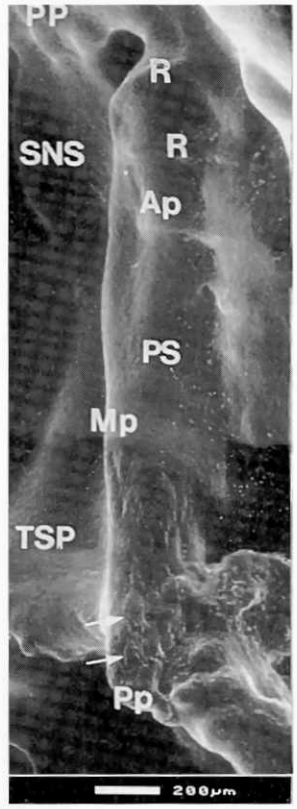

Figure 1: Oral cavity of an E-14 TGF-β_3 knockout mouse. Left palatal shelf (PS) has a vertical position. Anterior (Ap), middle (Mp) and posterior (Pp) parts of the palate can be distinguished. R: ruga. PP: primary palate. SNS: secondary nasal septum. TSP: tectal septal process. Arrows indicate bulges in the future soft palate.

We have examined the developing palates of litters consisting of 12 E-14 and 20 E-15 mice. Genotyping was performed as described in Proetzel et al. (1995) and showed 2 (16.6 %) TGF-β_3+/+, 4 (33.3 %) -/- and 6 (50 %) +/- E-14 and 6 (30 %) TGF-β_3 +/+, 3 (15 %) -/- and 11 (55 %) +/- E-15 mice. Time mated mice were killed by an overdose of chloroform and the embryos removed by caesarian section, placed in cold Hank's balanced salt solution and decapitated. After the jaws and tongues were removed, heads were fixed in 0.1M sodium cacodylate buffered 1% glutaraldehyde (pH 7'3), for between one and forty eight hours. Inmediately prior to scanning, samples were rinsed in distilled water in order to eliminate from the surfaces any components of the previously used solutions. Scanning electron microscopy was performed using a low vacuum scanning electron microscope ESEM (Electro Scan), with water vapour at a pressure of 6'4 torr at a temperature of 8ºC, in order to maintain 100% humidity on the surface of the sample.

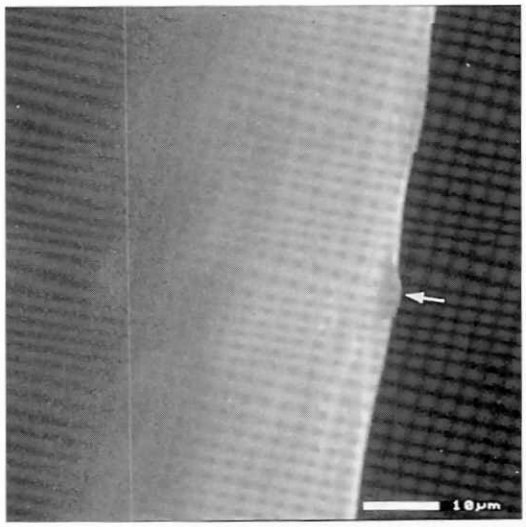

Figure 3: Medial edge epithelium of an E-14 TGF-β_3 -/- mouse right palatal shelf. Its superficial layer is almost totally flat and only one cellular protrusion can be observed (arrow).

Palatal shelves in 11 of 12 E-14 mice were still vertical and looked morphologically normal and similar in all three differently genotyped mice groups, showing from three to five rugae and division in anterior, middle and posterior palate (Fig. 1). The posterior palate had started to show the characteristic bulges which are usually seen on the soft palate in later stages. Likewise, primary palate, secondary nasal septum and tectal septal process did not reveal any differences amongst all three types of genotypes and E-14 control mice. In 1 TGF-β_3 +/- embryo, the palatal shelves had partially fused in

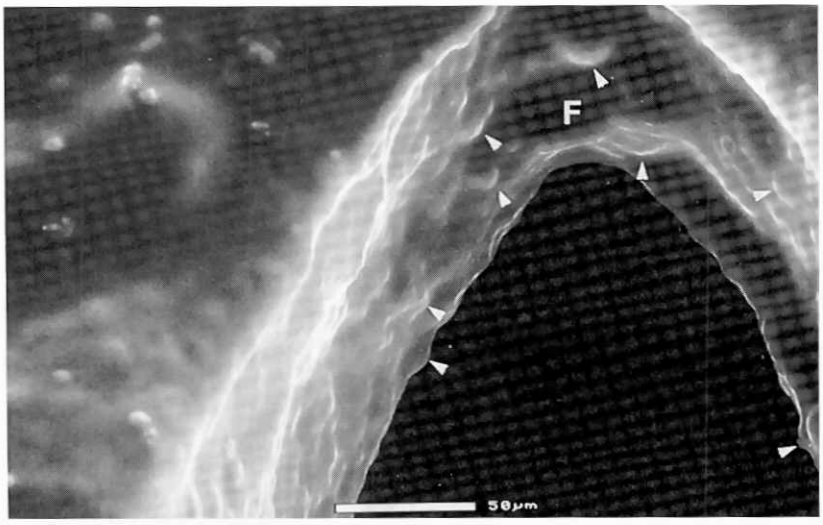

Figure 4: Unfused posterior part of an E-15 TGF-β_3 +/+ palate. Arrowheads point to cellular bulges in the medial edge epithelium of both palatal shelves. F: Posterior part of the fusion.

the middle third, as sometimes seen in E-14 control mice. However, there were differences in the MEE of TGF-β_3 +/+ and +/-, and TGF-β_3 -/-. TGF-β_3+/+ and +/- mice (Fig. 2) showed many of the characteristic cellular bulges previously observed by Martínez-Álvarez and Ferguson (1996) in the palatal shelves of E-14 normal mice, and thought to be cells undergoing desquamation process, possibly as part of an apoptotic phenomenon. On the contrary, the MEE of the TGF-β_3 -/- mice was almost totally flat, with only one or two of these protruding cells observed troughout its length (Fig. 3).

Palatal shelves of all 20 E-15 mice studied had partially or completely fused. Fusion was complete in the palate of 5 TGF-β_3 +/+ and 9 +/- mice, while 1 +/+ and 2 +/- showed a very small unfused region in the posterior palate (Fig. 4), which was considered normal. The MEE of this unfused part of the palatal shelves showed a large number of the cellular bulges (Fig. 4). All 3 TGF-β_3 -/- mice had anterior and posterior cleft palate, with only the middle third of the palate fused (Fig. 5).The unfused parts of these palatal shelves showed almost no cellular bulges or protrusions in the MEE, the epithelium being almost completely flat (Fig. 5).

Cell death and desquamation of the superficial layer of the palatal MEE has been hypothesised as a prerequisite for palatal fusion (Fitchet and Hay, 1989). The almost complete absence of protruding cells in the MEE of the TGF-β_3 -/- mice reported here is in contrast to the large numbers of protruding bulges seen in the TGF-β_3 +/+ and +/- MEE. This suggests that in the palates of the TGF-β_3 -/- mice there are morphological changes in the MEE, resulting in a disturbance of palatal fusion. These alterations may be indicating underlying abnormalities in the normal cell death process in the superficial layer of the MEE or in the adhesion potential of these cells.

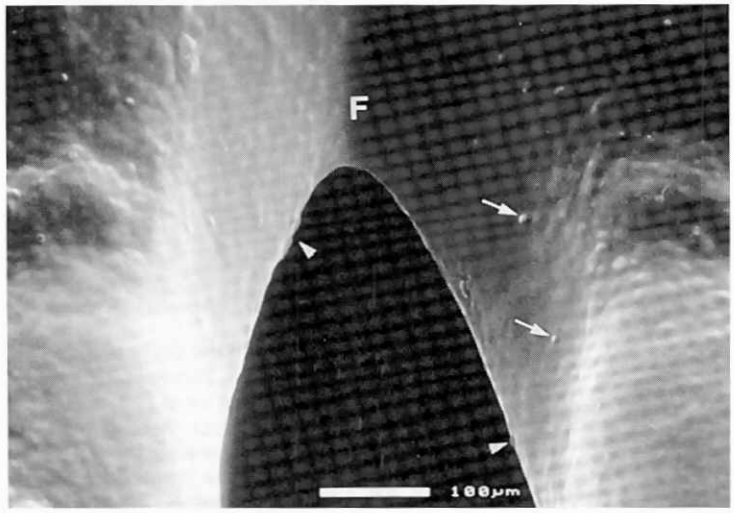

Figure 5: Unfused posterior part of an E-15 TGF-β_3 -/- palate. The medial edge epithelium in both palatal shelves is almost flat and only very few cellular bulges (arrowheads) are observed. Arrows indicate cellular debris settled over the epithelial surface. F: posterior part of the fusion.

References

Brunet, C.L., Sharpe, P.M. and Ferguson, M.W.J. (1995). Inhibition of TGF-β_3 (but not TGF-β_1 or TGF-β_2) activity prevents normal mouse embryonic palate fusion. Int. J. Dev. Biol. 39: 345-355.

Fittchet, J.E., Hay, E.D. (1989). Medial edge epithelium transforms to mesenchyme after embryonic palatal shelves fuse. Dev.Biol. 131: 455-474.

Fitzpatrick, D.R., Denhez, F., Kondaiah, P. (1990). Differential expression of TGF-β isoforms in murine palatogenesis. Development 109: 585-595.

Moses, H.L. (1992). TGF-β regulation of epithelial cell proliferation. Mol. Reprod. Dev. 32: 179-184.

Martínez-Alvarez, C., Ferguson, M.W.J. (1996). Morphological study of the developing mouse palate using low vacuum scanning electron microscopy. Sent to the Journal of Anatomy.

Proetzel, G., Pawlowski, S.A., Wiles, M.V., Yin, M., Boivin, G.P., Howles, P.N., Ding, J., Ferguson, M.W., Doetschman, T. (1995). Transforming growth factor β_3 is required for secondary palate fusion. Nature Genetics. 11 (4): 409-414.