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Original Article

Expression of transforming growth factor alpha (TGFα), epidermal growth factor receptor (EGF-R) and cell proliferation during human palatogenesis: an immunohistochemical study

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ABSTRACT Transforming growth factor alpha (TGFα) is not only a potent mitogen for several cell types, it interferes with cell differentiation. To investigate the possible role of TGFα in the fusion of the palatal processes in humans, the distribution of TGFα and its receptor (epidermal growth factor receptor= EGF-R) were studied using immunohistochemistry. 23 human palates were obtained from embryos and fetuses at 6 to 12 weeks of gestation and embedded in paraffin. In each case, the degree of cell proliferation was assessed using an antibody reacting with the nuclear antigen Ki-67. The epithelial and mesenchymal cell phenotypes were studied with anti-cytokeratin and anti-vimentin antibodies. TGFα and its receptor were detected in all the human palates, regardless of the stage of fusion. They were more highly expressed in the epithelial cells than in the mesenchymal cells of the palatal shelves. At first, proliferative activity was intense in both the mesenchyme and the epithelia and was later principally limited to the nasal or oral epithelia and also to the degenerating epithelial seam. At 10 weeks, when the midline palatal seam broke up into epithelial islands, the epithelial cells remained immunolabeled for TGFα, EGF-R and showed an increased number of proliferating cells. Programmed cell death (PCD) of medial edge epithelia (MEE) has been well documented, however other mechanisms must be considered during palatogenesis. Complex interactions between different growth factors have a probable role in epithelial mesenchymal transformation (EMT) and migration as well as in extracellular matrix synthesis.

KEY WORDS: *palate development, transforming growth factor alpha, oral epithelium, cell proliferation, human fetus*

Introduction

The palate arises as bilateral outgrowths from the maxillary processes (embryonic week 6 in humans) (Diewert, 1985, 1986). The shelves initially grow vertically down the sides of the tongue (embryonic week 6.5 in humans). Each palatal shelf consists of a central core of neural-crest derived mesenchyme, surrounded by a layer of undifferentiated epithelial cells (Greene and Pratt, 1976; Ferguson, 1988). At embryonic week 7, the shelves elevate to horizontal position above the tongue and contact each other, to separate the oral and nasal cavities.

The medial edge epithelia (MEE) fuse together to form a midline epithelial seam which degenerates, allowing mesenchymal continuity across the palate (Ferguson, 1988). This fusion mechanism is associated with the processes of both modeling and proliferation. The three alternative fates proposed for the MEE during palatogenesis are: programmed cell death (PCD) (Hudson and Shapiro, 1973; Pratt and Martin, 1975; Greene and Pratt, 1976), epithelial-mesenchymal transformation (EMT) (Fitchett and Hay, 1989; Shuler *et al.*, 1991, 1992; Griffith and Hay, 1992) and MEE cell migration (Carette and Ferguson, 1992a). Regional palatal epithelial differentiation is induced by the mesenchyme (Ferguson and Honig, 1984), probably through the interaction of extra cellular matrix (ECM) molecules and soluble factors (Ferguson, 1987, 1988).

Several growth factors are present during mammalian palatogenesis. Genetic analysis and tissue-specific expression studies support a role for transforming growth factor alpha (TGFα) in craniofacial development (Ardinger *et al.*, 1989; Shiang *et al.*, 1993). TGFα is considered as the embryonic homologue of EGF (Derynck, 1986). These two growth factors share considerable

Abbreviations used in this paper: ECM, extra cellular matrix; EGF, epidermal growth factor; EGF-R, EGF (or TGFα) receptor; EMT, epithelial mesenchymal transformation; MEE, medial edge epithelium; PBS, phosphate buffer saline; PCD, programmed cell death; TGFα, transforming growth factor alpha.

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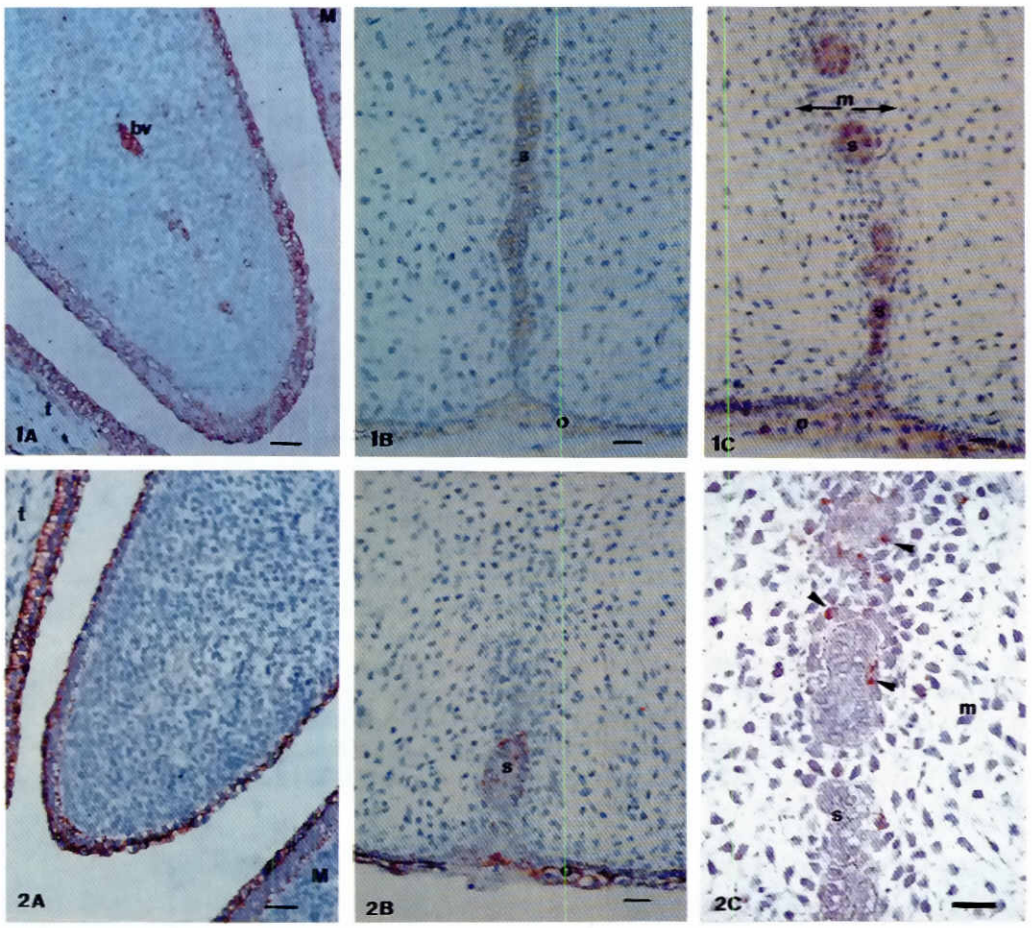

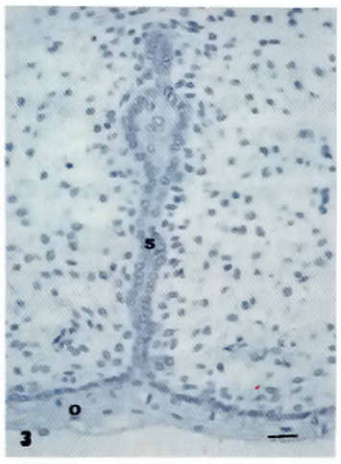

Fig. 1. Expression of TGFα during MEE fusion: frontal sections. *Contact of opposing palatal shelves results in a midline bilaminar epithelial seam which breaks up into isolated epithelial islands.* **(A)** *Unrotated precontact single shelf at 7 weeks. TGFα is present in the epithelia covering the tongue (t), the lateral maxilla (M) and the entire palatal shelf. TGFα is less intensely expressed in the mesenchyme, but intense staining is present in the blood vessels (bv).* **(B)** *At 8 weeks, opposed palatal shelves contact showing bilaminar epithelial seam (s) which strongly expresses TGFα. Intense staining is also present in oral epithelium (o).* **(C)** *At 9 weeks, disappearance of portions of the seam leaves islands of epithelial clumps (s) allowing mesenchymal confluence (m). Some of the epithelial remnants keep connections to the overlying epithelium (arrow). The epithelial islands and the oral epithelium (o) strongly express TGFα. Bars, 20 μm.*

Fig. 2. MEE cells phenotype during palatal shelves fusion. *Frontal sections stained for keratin* **(A,B)** *or vimentin* **(C)**. *Bars, 20 mm.* **(A)** *At 7 weeks the shelf is covered by a layer of epithelium which is immunostained with the anticytokeratins antibody as well as the tongue (t) and the lateral maxilla (M).* **(B)** *At 10 weeks only an epithelial remnant of the MEE keeps connection to the overlying oral epithelium (o) and continues to express keratin.* **(C)** *At 9 weeks, the level of staining for vimentin appears greatest in a few cells closest to the area of confluence and near the isolated epithelial islands (arrowed).*

Fig. 3. Frontal negative control section through a human embryonic palate at 8 weeks. *Bar 20 μm.*

homology and appear to have a single receptor: EGF receptor (EGF-R) (Torado *et al.*, 1980). EGF or TGFα binding to this receptor induce an autophosphorylation of the EGF-R which may somehow be involved in triggering the action of the growth factors.

EGF-R and TGFα have been localized in the embryonic mouse palate (Adamson and Meek, 1984; Abbott *et al.*, 1988; Shiota *et al.*, 1990; Dixon *et al.*, 1991). TGFα has been immunolocalized in MEE cells, together with its receptor around the time of palatal epithelium seam formation and degeneration (Pratt, 1987; Abbott *et al.*, 1988; Dixon *et al.*, 1991) and its interaction with the EGF-R appears to play a role in palatogenesis, particularly in MEE seam disruption. However results reported were somehow different and were purported to support a role for TGFα either in the programmed apoptotic death of MEE cells (Pratt, 1987; Abbott, 1988) or in EMT

of the basal cells of the midline epithelial seam (Fitchett and Hay, 1989; Dixon *et al.*, 1991).

Fetal palatal shelves were successfully cultured in a serum-free medium containing EGF, and selective removal of EGF from the medium resulted in reduced growth and death of palatal epithelial cells (Pratt, 1987). TGFα has been shown to stimulate cell proliferation in the developing palate (Yoneda and Pratt, 1981), although a few studies have assessed the epithelial proliferative activity during the palatal processes fusion *in vivo* (Hudson and Shapiro, 1973; Pratt and Martin, 1975; Luke, 1989). In this study, we have used MIB-1, a new monoclonal antibody which recognizes recombinant parts of the Ki-67 antigen (Cattoretti *et al.*, 1992). Ki-67 is a human nuclear cell proliferation-associated antigen that is expressed in proliferating and dividing cells during all active parts of the cell cycle, i.e., G1-, S-, G2- and M phases, although absent in the G0- phase (Key *et al.*, 1992). MIB-1 detects proliferating cells in microwave-processed formalin-fixed paraffin sections.

While the role of TGFα in mitogenesis and morphogenesis has been well documented (Lee *et al.*, 1993), its specific role during palatal shelf fusion is still unclear. Moreover, only a few studies have described TGFα and R-EGF expression and midline epithelial seam formation and disruption in the developing human palate. These processes have been essentially studied in murine or rodent models. We have therefore immunolocalized TGFα, EGF-R, Ki-67 protein, cytokeratin and vimentin on serial sections during MEE degeneration in the normally developing human palate in order to investigate, *in vivo*, their distribution changes during development.

Results

TGFα and EGF-R immunoreactivity was detected in all the human palates examined at each stage of development. Distributions of TGFα and EGF-R were almost identical at every stage examined. They were expressed predominantly on the epithelial cells especially in epithelial cells of the MEE and less intensely in the mesenchymal tissue of the palatal shelves. (Fig. 1).

Cytokeratins were immunolocalized in all epithelia at all stages of palatal development, particularly in the epithelial seam and even in the remnants at the end of the epithelial fusion. (Fig. 2A-B). At this time, vimentin appeared most strongly expressed in a few cells near the isolated epithelial islands (Fig. 2C). No other epithelia on oral and nasal surfaces of the palate expressed vimentin.

No immunoreactivity was detected in any of control sections (Fig. 3).

At 7 weeks

The palatal shelves grew vertically and were covered by a layer of epithelium which was immunostained with the anticytokeratins antibody (Fig. 2A). EGF-R and TGFα were present in the epithelia covering the nasal cavities, the tongue, the floor of the mouth, the lateral maxilla, the mandible and the MEE. TGFα and R-EGF were homogeneously expressed in the mesenchyme but less intensely. Both epithelial and mesenchymal cellular compartments showed extensive proliferation with a high number of cells stained by the MIB-1 antibody. Strong staining for vimentin was present throughout the palatal mesenchyme, but was absent from all epithelial cells.

At 8 weeks

At 8 weeks the anterior portions of the shelves assumed a horizontal orientation above the tongue, while the posterior portions remained vertical. The palatal shelves approached the nasal septum and entered into contact with them. The fusion progressed posteriorly. On the frontal serial sections, the fusion was not homogeneous. In some sections, contact but no fusion could be seen, whereas in some other sections the MEE of opposing shelves were fused. Horizontal sections provided a better representation of this spatially and temporally variable situation (Fig. 4). The progression of palatal fusion was associated with the reduction of the MEE into a single layer of cells (Figs. 1B, 4A-D).

The labeling for TGFα and EGF-R was high in all epithelia and specially in the MEE, whatever the degree of fusion. The mesenchyme exhibited more intense staining than before. At this stage the mesenchyme maintained a high proliferative activity (Fig. 4B,D) whatever the degree of fusion. By contrast, a shutdown in proliferative activity appeared in the epithelial basal layer with the simultaneous disappearance of the superficial layers and fusion. Although in uncontacted posterior sections (Fig. 4B) intense immunoreactive MIB-1 staining was recognized in many cells of the MEE basal layer, a few MIB-1 positive cells were still present in the MEE seam after the moment when palatal shelves had made contact (Fig. 4D).

At 9 weeks

(Figs. 1C, 4E-F). There was a general increase in epithelial staining for TGFα and EGF-R. The fused basal layers formed the midline palatal epithelial seam. The cells forming the epithelial seam were positive for TGFα and EGF-R. Staining was present throughout the palatal epithelia, less intense in mesenchyme, and persisted in the epithelial seam. The amount of proliferating cells in the MEE seemed highly increased as compared with 8 weeks, while the mesenchyme showed a dramatic decrease of proliferating activity. The level of staining for vimentin appeared greatest in the seam cells closest to the area of confluence (Fig. 2C).

At 10 weeks

(Fig. 4G-H). During the later stages of palatal fusion, the midline palatal seam broke up into isolated epithelial islands, where a number of proliferating cells were observed. Most of these proliferating cells were located in the periphery of the island, but some of them were included inside the heart of island. Epithelial islands were surrounded by a poorly proliferative mesenchyme. Immunolabeling for TGFα, EGF-R and keratin (Fig. 2B) continued to be observed inside these epithelial clumps. Rather intense immunoreactive TGFα and EGF-R stainings were observed in the mesenchyme. Moreover, at this stage of development, the cells in the midline fused mesenchyme could not be distinguished from the surrounding mesenchymal cells.

Discussion

We have shown the immunolocalization of TGFα and its receptor throughout the palatal fusion processes in humans as well as its association with cell proliferation and differentiation. TGFα and EGF-R were highly expressed in epithelial cells and less intensely in the mesenchymal tissue of palatal shelf. As the epithelial seam degenerated there was a general increase in epithelial staining for TGFα and EGF-R, particularly in the seam remnants. Proliferative activity was very high in both the mesenchyme and the epithelia during early palatal development. It gradually decreased in mesenchyme, whereas it persisted in oral and nasal epithelia, in

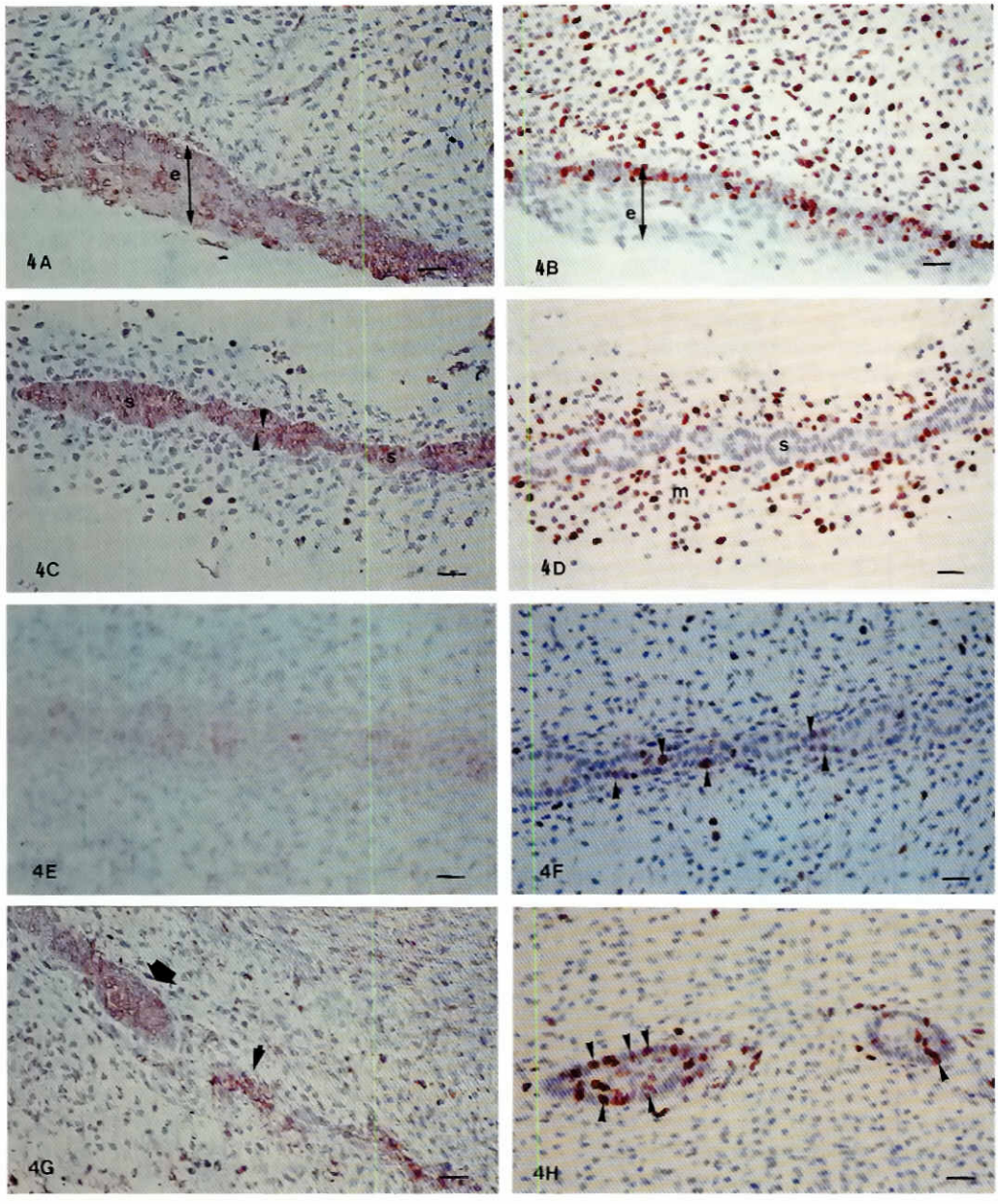

Fig. 4. Expression of TGFα (A,C,E,G) and Ki-67 protein (B,D,F,H) at different stages of palatal fusion. (A-D) *Week 8.* **(A)** *Unelevated posterior portion is covered by a layer of epithelium which highly expresses TGFα. The growth factor is less intensely expressed in the mesenchyme.* **(B)** *Both epithelial and mesenchymal compartments show extensive proliferation.* **(C)** *In anterior portion the MEE of opposing shelves fuse together. The palatal fusion is associated with the reduction of the MEE seam (s) into a single layer of cells (arrowed) highly expressing TGFα.* **(D)** *A shutdown in the proliferative activity appears in the MEE basal layer. The mesenchyme maintains a high proliferative activity.* **(E,F)** *Week 9.* **(E)** *Immunostaining for TGFα persists in the epithelial seam and increases in the mesenchyme.* **(F)** *The mesenchyme shows a dramatic decrease of proliferative activity, while the amount of proliferating cells in the MEE (arrowed) seams greatly increased as compared to Fig. 4D.* **(G,H)** *Week 10.* **(G)** *Immunolabeling for TGFα continues to be observed in the isolated epithelial islands (arrowed) and staining increases throughout the mesenchyme.* **(H)** *Epithelial islands are surrounded by a poorly proliferative mesenchyme. A number of MIB-1 positive cells are present in these epithelial clumps. Most of these proliferating cells are located in the periphery of the island, but some of them are included inside the heart of island. Bars, 20 μM.*

the enamel organ and also in the degenerating epithelial seam. Later, the isolated epithelial islands of the midline seam were surrounded by a poorly proliferating mesenchyme and showed an extensive amount of cell proliferation.

TGFα has been shown to be mitogenic for a variety of epithelia and other tissues. In addition, TGFα is capable of promoting not only epithelial cell growth but also cell differentiation (Lee *et al.*, 1993). The growth and differentiation of the oral, nasal and MEE are probably very dependent upon TGFα and its receptor, but the precise role of this growth factor is not fully understood at present.

Pratt (1987) proposed a model to account for the role of

TGFα in palatal shelves fusion in support of the MEE PCD mechanism. However, his theory was based on immunohistochemical results reported by Abbott *et al.* (1988), who found a decrease in EGF-R expression between embryonic days 12 and 15 in mouse. Based on this data, Pratt concluded there had been a cessation of TGFα synthesis by MEE cells during fusion, resulting in cell death. However Abbott *et al.* used a polyclonal antibody to EGF-R and it is probable that it did not react well with ligand-occupied receptors. In our study we have used monoclonal antibodies, which specifically recognize EGF-R or TGFα regardless of whether they are liganded or free. Our data, which conform to those reported in mouse by Dixon *et al.* (1991) and Shiota *et al.* (1990), share a persistence and even an increase in TGFα and EGF-R in MEE cells during seam formation and, later on, in the seam remnants.

A few studies have assessed the palatal epithelial proliferation *in vivo* during critical period of the palatal fusion processes (Hudson and Shapiro, 1973; Luke, 1989), principally through autoradiographic techniques. More recently, Carette and Ferguson (1992b) have used bromodeoxyuridine to study the distribution of S phase cells in palatal embryonic epithelial sheets in culture. The technique described in the present study provides a nuclear labeling in all the cells in proliferative cycle out of G0 phase. Our study fundamentally differs from previous investigations, which are limited to S and M phases.

We found a pattern of decrease in cell proliferation, since there are a few MIB-1 positive cells in the MEE when palatal shelves have just contacted each other. Carette and Ferguson (1992b) reported, by bromodeoxyuridine incorporation in palatal epithelial sheets culture, that MEE cells became very slowly divided after 19 hours. However, we were able to demonstrate the persistence of cell proliferation in the MEE cell islands. To our knowledge, this cell activity has never been reported. Indeed the occurrence of cell death within the medial epithelial lamina between the fusing palatal processes has been well documented (Greene and Pratt, 1976). If the proliferation of the MEE cells increases at later stages of fusion, then previous results about the incorporation of triated thymidine in MEE, which were purported to support PCD, could be interpreted very differently. During the migration of processes a significant reduction of DNA synthesis was reported in epithelial cells at the tip (edge) of the vertical shelf prior to elevation (Hudson and Shapiro, 1973). Pratt and Martin (1975) also described a cessation of DNA synthesis in the same region before fusion. This reduction may represent the initial resumption of MEE cell proliferation described in PCD mechanism. However, the cessation of DNA synthesis does not necessarily mean that a cell is destined to die and may be related to the induction of a new pattern of gene transcription which is required for the cell differentiation. The cessation of DNA synthesis in the MEE could be considered to be the indication of phenotype transformation.

Luke (1989) did not specifically examine proliferation at the edge "partly because the epithelium at the edge after elevation is not the same epithelium that was there before elevation". In 1988 Ferguson observed that seam degeneration involved EMT and not only cell death, as previously imagined. Scanning electron microscopy observations have previously revealed changes in the shape of the epithelial cells along the free edges of processes preparatory to their fusion (Waterman and Meller, 1974; Babiarz *et al.*, 1979). At the time of the midline adhesion, when the cells of the MEE superficial layer are dying and sloughing off, the basal cell layer of the MEE switches from the epithelial intermediate filament type (keratin) to the mesenchymal type (vimentin) (Fitchett and Hay, 1989; Shuler *et al.*, 1991, 1992). Griffith and Hay (1992) traced labeled mesenchymal cells originating in the midline seam through their differentiation into fibroblasts, as judged by their ultrastructure. The cell lineage of the MEE was characterized by DiI labeling (Shuler *et al.*, 1991) and demonstrated that these cells originally present as MEE contained vimentin in the latter stage of palatal fusion. In our study, the level of staining for vimentin at 9 weeks appears greatest in the seam cells closest to the area of confluence which could be interpreted as their EMT.

If the MEE cells in isolated islands which are no longer connected to the adjacent epithelia are undergoing cell proliferation (Fig. 4H), the proliferation of these cells could support their subsequent EMT and add an additional data set supporting this fate for the MEE. However, frontal sections show that some of the epithelial remnants keep connections to the overlying epithelium (Figs. 1C, 2B). Lineage analysis of midline epithelial seam disruption in mouse palatal organ culture suggests that most seam cells migrate orally and nasally into the epithelial triangles and subsequently into the oral and nasal epithelia (Carette and Ferguson, 1992a). The role of EGF or TGFα in cell migration has been demonstrated in several models *in vitro* using either keratinocytes (Chen *et al.*, 1993) or respiratory epithelial cells (Zahm *et al.*, 1993). However, our experiment is not suitable for studying cell migration; an organ culture model would need to be investigated.

We demonstrated the presence of TGFα throughout all the steps of fusion and with the same intensity of immunolabeling. Simultaneously, the epithelia facing the nasal and oral cavities differentiate respectively into pseudostratified, ciliated columnar and stratified, squamous keratinizing epithelia (Greene and Pratt, 1976). Our study confirms that TGFα remains present in those epithelial cells (data not shown). At the same time we also demonstrated a variation in the degree of epithelial cell proliferation. This may suggest that TGFα not only plays a role in cell growth, but interferes in other processes, such as cell differentiation. *In vitro*, EGF was first known to inhibit the cessation of DNA synthesis and also the subsequent degeneration of embryonic palatal MEE (Hassell and Pratt, 1977; Abbott and Pratt, 1987a,b). More recently, Dixon and Ferguson (1992) found that EGF and TGFα did not inhibit MEE degeneration in palatal shelves cultured under serum-free conditions. These organ cultures under serum-free conditions show additional interaction between many factors, e.g. TGFα, EGF, platelet derived growth factor (PDGF) during MEE degeneration. An intense expression of the basic fibroblast growth factor (FGFb) in the MEE during seam formation as well as disruption was also reported (Sharpe *et al.*, 1993). Sharpe *et al.* (1992) demonstrated that activity of EGF/TGFα in the developing mammalian palate may be modulated by endogenous growth factors at the EGF-R and post-receptor level. Moreover, Dixon *et al.* (1993) suggested that palatal MEE cells synthesized ECM molecules, e.g. tenascin, and that this synthesis could be stimulated by TGFα and serum. Physiological effects of TGFα during normal palatal development are likely to be the result of complex interactions between differing growth factors and ECM molecules at differing developmental times. It remains for us to investigate this. We also have to determine whether the proliferating cells inside isolated epithelial clumps are in direct contact with the underlying mesenchymal extracellular matrix through breaks in the basement membrane, which have been described by Fitchett and Hay (1989) and Shuler *et al.* (1991).

Materials and Methods

Material

23 human palates were selected from normal non-infected embryos ranging in developmental age between 6 and 11 weeks as following:

at 7 weeks: 5 palates
at 8 weeks: 5 palates
at 9 weeks: 6 palates
at 10 weeks: 7 palates

They were collected within 15 to 50 minutes following medical inductions or voluntary interrupted pregnancies by pneumatic suction. Palates were dissected free of debris in a 0.9% saline solution and fixed in 10% formalin. Specimens were aged initially according to usual morphological criteria such as foot-length (Streeter, 1920). Specimens whose palatal development did not correlate with the embryonic mensurations and gestational age (Waterman and Meller, 1974) were excluded.

Immunohistochemical method

Serial sections of 4 to 6 μm were cut either in a frontal plane or in a horizontal plane, and mounted on chrome-alum-gelatin precoated slides and dried at room temperature for 48 hours before being stained. Then, sections were dewaxed with xylene, passed through descending alcohols and hydrated.

Some antigenic determinants were demasked using a treatment with saponin, a micro-wave pretreating or an enzymatic digestion.

TGFα was exposed by pretreating with 0.05% saponin in distilled H_2O, for 50 minutes at 37°C.

To allow the nuclear antigen Ki-67 to react with the MIB-1 monoclonal antibody the slides were placed in a plastic tray containing 10 mM citrate buffer (10 mM citric acid monohydrate, pH 6.0 adjusted with 2N NaOH), and heated for 5 minutes in a microwave oven at the highest power (700 W). The level of the buffer solution was checked after heating, and, if necessary, citrate buffer was added to prevent slide dehydration. The whole procedure was repeated four more times (i.e., total microwave incubation: 4x5 min).

For the expression of anti-human cytokeratin antibody, an enzymatic predigestion with 0.1% pepsin in 0.01N HCL was carried out for 60 minutes at 37°C.

Endogenous peroxidase activity was blocked by 0.3% hydrogen peroxide in PBS (phosphate buffered saline, pH 7.4) for 5 minutes. The labeled avidin-streptavidin technique was applied with a Kit DAKO-LSAB (K680). Sections were treated with a serum blocking solution (normal goat serum 6% for 5 minutes) then the primary antibody, a biotinylated secondary antibody (biotinylated goat anti mouse immunoglobulin for 10 minutes), an enzyme-labeled-streptavidin complex for 10 minutes, the substrate chromogen mixture (AEC= 3-amino-9-ethylcarbazole), and finally counterstained with diluted hematoxylin. A positive reaction was indicated by the presence of a local red precipitation.

Negative control slides were carried out using the same procedure and either omitting the primary antibody or adding a non-immune serum.

The avidin-biotin complex (ABC) method was only applied for TGFα with the OSI* Immunohistochemistry System. Blocking normal horse serum was applied for 20 minutes then the biotinylated secondary antibody was applied for 30 minutes and followed by a preformed avidin-biotinylated horseradish peroxidase macromolecular complex for 30 minutes. A specific negative control was obtained by substituting the primary antibody by a trpE(ab-1)IgG mouse antibody at the same concentration.

The primary antibodies used were:

- monoclonal mouse anti-human TGFα (Ab-2) (Oncogene Science, Cat. No. HCS05, Inc., USA) reacting with the -COOH terminal 34-50 residues (Sorvillo *et al.*, 1990) was diluted to 1:80 in PBS and incubated overnight at 4°C.
- monoclonal mouse antibody against EGF-R was obtained from Amersham (mAb, Amersham, Buckinghamshire, UK) and used at a concentration of 1:15 in PBS for 30 minutes at 37°C.
- monoclonal mouse antibody MIB-1 (mAb, Immunotech. Cat. No. 0505), reacting with the Ki-67 nuclear antigen associated with cell proliferation, is found throughout the cell cycle (G1, S, G2, M phases) and is absent in resting (G0) cells (Key *et al.*, 1992). MIB-1 was diluted to 1:50 in PBS and incubated 10 minutes at room temperature.
- monoclonal mouse antibody DAKO-CK, MNF 116 (Code No. M 821-Lot No. 061), reacts with an epitope which is present in a wide range of cytokeratins, including keratins Nos. 10, 17 and 18. Following pepsin predigestion, the antibody was used at a dilution of 1:50 for 10 minutes at room temperature.
- monoclonal mouse anti-swine vimentin (DAKO-Vimentin Code No.M725) recognizes human vimentin, but does not react with other closely related intermediate filament proteins, including desmin and glial fibrillary acid protein. The antibody was incubated for 10 minutes at room temperature at a dilution of 1:10.

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