Spatiotemporal Localization of Periostin and Its Potential Role in Epithelial-Mesenchymal Transition during Palatal Fusion

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Key Words

Periostin • Palatal fusion • Epithelial-mesenchymal transition • Mesenchymal matrix • Epithelial matrix

Abstract

The medial epithelial seam (MES) between the palatal shelves degrades during palatal fusion to achieve the confluence of palatal mesenchyme. Cellular mechanisms underlying the degradation of MES have been proposed, such as apoptosis, epithelial-mesenchymal transition (EMT) and migration of medial edge epithelia (MEE). Extracellular matrix components have been shown to play an important role in EMT in many model systems. Periostin (also known as osteoblast-specific factor-2) is a secreted mesenchymal extracellular matrix component that affects the ability of cells to migrate and/or facilitates EMT during both embryonic development and pathologic conditions. In this study, we evaluated the spatiotemporal expression patterns of periostin during mouse palatal fusion by in situ hybridization and immunofluorescence. Periostin mRNA and protein were present in the palatal mesenchyme, the protein being distributed in a fine fibrillar network and in the basement membrane, but absent from the epithelium. During MES degradation, the protein was strongly expressed in the basement membrane

underlying the MES and in some select MEE. Confocal microscopic analysis using an EMT marker, twist1, and an epithelial marker, cytokeratin 14, provided evidence that select MEE were undergoing EMT in association with periostin. Moreover, the major extracellular matrix molecules in basement membrane, laminin and collagen type IV were degraded earlier than periostin. The result is that select MEE establish interactions with periostin in the mesenchymal extracellular matrix, and these new cell-matrix interactions may regulate MEE transdifferentiation during palatal fusion.

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Abbreviations used in this paper

CK14	cytokeratin 14
ColIV	collagen type IV
DAPI	4',6-diamidino-2-phenylindole
E	embryonic day
EMT	epithelial-mesenchymal transition
LN	laminin
MES	medial epithelial seam
MEE	medial edge epithelia
PBS	phosphate-buffered saline
TGF- β	transforming growth factor- β

Introduction

Formation of the secondary palate is a complex and critical event involving coordinated outgrowth, reorientation, adhesion and fusion of the bilateral palatal shelves. Disturbance in any stage during palatal fusion may result in cleft palate, one of the most common birth defects in humans [Ferguson, 1988]. In the mouse, bilateral palatal shelves arise from the maxillary process of the first branchial arch at embryonic day 12 (E12). At first, the two palatal shelves grow vertically along the sides of the tongue, but at E14.0, they reorient to a horizontal position above the dorsum of the tongue. The medial edge epithelia (MEE) of the opposing palatal shelves then adhere with each other to form a medial epithelial seam (MES). At E14.5, the MES degrades to achieve the confluence of palatal mesenchyme and to complete the process of palatal fusion [Ferguson, 1987; Shuler et al., 1991].

Epithelial-mesenchymal transition (EMT) is considered to be one of the mechanisms involved in the degradation of MES [Fitchett and Hay, 1989; Shuler et al., 1991; Griffith and Hay, 1992]. EMT is a process that involves basal lamina degradation, formation of new cell-extracellular matrix interactions, acquisition of cell motility and loss of intercellular junctions [Boyer et al., 2000]. Previous studies suggest that extracellular matrix components are important determinants to the cellular response to transforming growth factor- β (TGF- β), which is involved in many models of EMT. Epithelial cells cultured on mesenchymal matrix such as fibronectin and collagen type I undergo TGF- β -mediated EMT. These same cells when cultured on matrices containing laminin (LN) and collagen type IV (ColIV) are resistant to EMT and maintain an epithelial phenotype even when stimulated with exogenous TGF- β [Menke et al., 2001; Kim et al., 2006]. TGF- β_3 null mice with cleft palate retain the LN-containing basement membrane that is correlated inversely with the ability of MEE to transdifferentiate [Karttinen et al., 1997], suggesting that contact of MEE with mesenchymal extracellular matrix during palatal fusion has an important role in promoting TGF- β_3 -mediated EMT.

Periostin is a secreted mesenchymal extracellular matrix molecule belonging to the fasciclin family and is expressed in cells undergoing ECM remodeling and/or EMT during both embryonic development [Kruzynska-Frejtag et al., 2001, 2004] and pathologic conditions [Oka et al., 2007; Kikuchi et al., 2008]. Periostin can interact with other ECM scaffold proteins, such as fibronectin, tenascin C, collagen type I, collagen type V and heparin [Sugiura et al., 1995; Takayama et al., 2006; Norris et al.,

2007]. It serves as a ligand for select integrins, such as $\alpha V\beta_3$, $\alpha V\beta_5$, $\alpha_6\beta_4$ and β_1 and modulates cell-matrix interactions affecting the ability of cells to adhere, migrate, proliferate, survive and/or undergo EMT [Horiuchi et al., 1999; Gillan et al., 2002; Bao et al., 2004; Baril et al., 2007; Butcher et al., 2007; Li et al., 2010]. Previous studies have shown that periostin facilitates EMT and induces metastatic behavior by upregulating vimentin, fibronectin and matrix metalloproteinase 9 via integrin $\alpha V\beta_5$ in an epithelium-derived tumor cell line [Yan and Shao, 2006]. Periostin has also been reported to promote the migration and proliferation of epithelial cancer cells which was accompanied by inducing vimentin and N-cadherin and downregulating E-cadherin [Hong et al., 2010]. However, it has not been determined whether periostin has a role in the degradation of MES during palatal fusion.

In this study, we evaluated the spatiotemporal expression patterns of periostin by in situ hybridization and immunofluorescence to examine the biological functions of this protein during palatal fusion. Moreover, we performed confocal microscopic analysis to clarify whether MEE associated with periostin were undergoing EMT. Lastly, we investigated temporal changes of basement membrane components to demonstrate cell-mesenchymal matrix contact to enable some select MEE to undergo EMT during palatal fusion.

Materials and Methods

Specimen Preparation

Swiss-Webster and C57BL/6 mice heads were dissected on the indicated gestational days, fixed in 4% paraformaldehyde/phosphate-buffered saline (PBS) at 4°C overnight, washed with PBS, dehydrated through graded ethanol series and embedded in paraffin. Serial frontal sections were prepared along the anterior-posterior axis for in situ hybridization, immunofluorescence and confocal microscopic analysis.

In situ Hybridization

We amplified a 954-bp fragment of mouse periostin from E14.0 palate cDNA using the following primers: forward, 5'-CCA ACC AGC AGA GAA ATC CCT, and reverse, 5'-CTG AGA ACG GCC TTC TCT TGA TC. This fragment was cloned into pGEM-Teasy using TA cloning. The ^{35} S-labelled antisense RNA probe for periostin transcripts was synthesized using the cDNA clone as a template after linearizing with *Xmn*I. The probe contained sequences complementary to those encoding the C-terminal domain of periostin isoform 2. Our probe differs from that used in Kruzynska-Frejtag et al. [2001, 2004] but still recognizes all isoforms. We also cloned other probes that recognized a subset of isoforms including a new isoform A (sequence structure of C-terminal domain: a + c + d + f), but no differences in expression were noted between these and the pan-periostin probe (data not

shown). Radioactive in situ hybridization with ^{35}S -labeled probes was carried out as described [Rowe et al., 1992]. After washing, the sections were dehydrated and dipped in photographic emulsion for autoradiography. The slides were exposed for 2–3 weeks. Dark- and bright-field images were captured with a Hitachi camera mounted on a Zeiss Axiophot.

Immunofluorescence

After deparaffinization, rehydration and antigen retrieval using EDTA buffer, 5- μm -thick sections were blocked with 3% bovine serum albumin/PBS for 30 min at room temperature to reduce background staining and incubated with the primary antibody against periostin (1:100, BioVender), LN α -1 (1:100, Santa Cruz), ColIV (1:100, Abcam), twist1 (1:50, Abcam), integrin β_1 (1:100, R&D Systems) and integrin β_5 (1:50, Abcam) at 4°C overnight, followed by 3 rinses in PBS. Frozen sections were used for integrin α_V (1:100, Chemicon) and integrin β_3 (1:100, Chemicon). Fluorescence-labeled secondary antibodies (1:100, Invitrogen) were added for 1 h at room temperature. After washing with PBS 3 times, coverslips were mounted with mounting medium including 4',6-diamidino-2-phenylindole (DAPI; Vector Labs). To confirm the specificity of antibodies, additional slides were incubated without the primary antibodies. No fluorescence staining was found in these sections.

Confocal Microscopic Analysis

Spatial relationships between periostin and MEE were analyzed using confocal laser scanning microscopy. Sections were deparaffinized in xylenes and rehydrated with descending ethanol series. After antigen retrieval, sections were blocked with 3% bovine serum albumin/5% Triton X-100/PBS blocking solution at 37°C for 30 min and incubated with the primary antibody against periostin, twist1, cytokeratin 14 (CK14; 1:100, Abcam) at 4°C overnight and with fluorescence-labeled secondary antibody (1:100, Invitrogen) for 1 h at room temperature, washed with PBS, coverslipped with mounting media including DAPI. All sections were examined with a Nikon Laser Scanning Confocal microscope (C1) that was equipped with an argon (488 nm) and 2 He-Ne lasers (543 and 633 nm). Sections were scanned with a Plan Fluor 40 $\times$ NA0.75 lens and a Plan Apo VC 60 $\times$ NA1.4 oil lens as individual images or z-stack images. z-stack series were obtained with 0.2- μm increments to the entire section thickness (20 μm , approximately 100 images in the z-stack). Digital data of frame-mode images were recorded. DeltaViewer 2.1.1 (<http://delta.math.sci.osaka-u.ac.jp/DeltaViewer/>) and NIH ImageJ (<http://rsbweb.nih.gov/ij/>) were used for 3D data visualization.

Results

In situ Hybridization Analysis of Periostin mRNA during Palatal Fusion

Periostin transcripts were localized in the palatal mesenchyme with strong signals in the medial tip as well as in the oral side of the palate, but not in the epithelium at E14.0 (fig. 1a–c). After contact of the shelves, intense periostin mRNA expression was observed in the mesen-

chyme directly adjacent to the MES (fig. 1d–f, j). However, as the degradation of MES proceeded, this distribution of periostin mRNA extended inside the MES at E14.5 and E15.0 (fig. 1g–i, k). Other areas of abundant periostin expression include the dental papillae and tongue as described by others [Kruzynska-Freitag et al., 2004].

Spatiotemporal Distribution Pattern of Periostin Protein and Integrins during Palatal Fusion

Periostin protein was localized in a distribution that mirrored the expression pattern of periostin mRNA. Fluorescence was mainly localized in the palatal mesenchyme with strong labeling on the oral side while the signal was absent in the ossification center of the maxillary bones (fig. 2a–l). We noted that the far anterior palate and the soft palate showed stronger reactivity compared with other parts (fig. 2a–c, j–l). High magnification images showed that periostin was present in the mesenchyme distributed with a fine fibrillar network architecture and in the basement membrane, but not in the epithelium at E14.0 (fig. 3a, b). However, as palatal fusion proceeded, periostin immunoreactivity was also detected inside the MES at E14.5. Furthermore, there was relatively stronger fluorescence in the basement membrane region of MES undergoing degradation (fig. 3c). There appeared to be active remodeling of periostin in some positions of the MES (fig. 3d). The periostin-containing basement membrane was reorganized during the process of MES degradation, and this distribution was maintained even at late stages (E15.0) of palatal fusion (fig. 3e). During this late period, remodeling of periostin fibrillar networks in the mesenchyme was far advanced, and by E16.0, the periostin-containing basement membrane of MES was barely detectable (fig. 3f). The MEE express select integrins (α_V , β_1 , β_3 and β_5) (fig. 4a–d), which were reported to be receptors for periostin [Kuhn et al., 2007].

Select MEE Transdifferentiate into Mesenchyme in Association with Periostin

To investigate whether MEE associated with periostin were undergoing EMT, we performed confocal microscopic analysis using an EMT marker, twist1, and an epithelial marker, CK14. Twist1 is a transcription factor that induces EMT by loss of E-cadherin-mediated cell-cell adhesion and induction of mesenchymal markers, such as fibronectin and N-cadherin [Hong et al., 2010]. CK14 is an intermediate filament keratin expressed only in epithelial cells [Schweizer et al., 2006]. To distinguish MEE undergoing EMT from other mesenchymal cells in the region, we used periostin-containing basement

Fig. 1. a–i In situ hybridization analysis of periostin mRNA during palatal fusion. ns = Nasal septum; pb = palatine bone; ps = palatal shelf; t = tongue; tg = tooth germ. Arrows indicate strong signals in the medial tip as well as in the oral side of the palate. Arrowheads indicate MES. **j, k** High-magnification images of

MES at E14.0 (**j**) and E14.5 (**k**). No signals in MES at E14.0 late. The distribution of periostin mRNA extended inside the MES after E14.5 is indicated by an arrow. Scale bars = 175 μm (**a–i**) and 40 μm (**j, k**).

membrane as a marker to separate MES from mesenchyme.

Twist1 was not expressed in MES at E14.0 (data not shown), but as the degradation of MES occurred, select MEE became twist1 positive. As shown in figure 5a–c, the integrity of periostin-containing basement membrane was completely retained. Twist1-positive cells, indicated by the arrowheads (fig. 5a–c), were present between these basement membranes representing MEE undergoing EMT. As the process proceeded, intense labeling of periostin was detected around these twist-positive cells representing transdifferentiation from MEE (fig. 5d–f). We obtained 3D images to show MEE undergoing EMT by demonstrating that they were 3-dimensionally surrounded by the mesenchymal matrix component periostin. Figure 5g–i shows confocal images projected from a z-stack series at three different z-positions extending from the top to the bottom of the cell, represented by an arrowhead. These results further indicate that the twist1-positive cells were located between intact periostin-containing basement membrane. This MEE

cell was surrounded by mesenchymal matrix (periostin) at all three z-positions. 3D reconstruction of periostin (fig. 5k) showed that the fine fibrillar architecture of periostin formed inside the MES while no periostin was observed inside MES before MES degradation, as shown in figure 5j.

CK14 showed strong intensity in MEE at E14.0 (data not shown). Figure 6 shows confocal images of MEE projected from a z-stack series at three different z-positions at E14.5. During degradation of MES, CK14 immunoreactivity gradually diminished. Cells marked with a dotted circle in figure 6a were located between periostin-containing basement membrane and they were CK14 negative, indicating that MEE had lost the epithelial phenotype. These cells were surrounded by periostin (fig. 6a). Thus, MEE undergoing EMT exhibited a loss of keratin intermediate filaments while associated with periostin. CK14 labeling in an epithelial island, indicated with two white arrowheads (fig. 6a), was fading out as the z-position was changed (fig. 6b, d). These cells in the epithelial island indicated by the two arrowheads (fig. 6a) were ob-

Fig. 2. Fluorescent images of periostin during palate development at E14.0–E16.0. Fluorescence was mainly localized in the palatal mesenchyme with strong labeling in the far anterior and soft palate (arrows) and with absent signal in the ossification center of the maxillary bones (asterisks). Arrowheads indicate MES. Scale bars = 175 μ m.

served to have intact nuclear morphology throughout each z-position (fig. 6c, e; online suppl. video 1, www.karger.com/doi/10.1159/000320178), indicating that no signs of apoptosis were present. The cells remained vital, were undergoing EMT, and were also directly associated with the mesenchymal matrix, periostin.

Early Degradation of Epithelial Matrix in Basement Membrane and Newly Established Contact with the Underlying Mesenchymal Matrix, Periostin

Our data revealed prolonged retention of periostin-containing basement membrane underlying the MES during palatal fusion. Therefore, we examined the spatiotemporal relationship between periostin and major epithelial matrix components of basement membrane, LN and ColIV. At E14.0, intact periostin and LN or ColIV-containing basement membrane were observed (fig. 7a, b, fig. 8a, b). However, it was clear that LN in the basement membrane had already started to degrade (fig. 7c, e), as a clear line of basement membrane was no longer present, and that periostin was still completely retained in the same position (fig. 7d, f). Therefore, the MEE established new contacts with the underlying mesenchymal matrix

expressing periostin (fig. 7d, f). A similar relationship was found between ColIV (fig. 8a, c) and periostin (fig. 8b, d). MEE in the region were in contact with periostin (fig. 8d, e, i). Moreover, these cells were twist1 positive, indicating that they were in the process of EMT (fig. 8i, j).

Discussion

In this study, we have demonstrated the spatiotemporal localization of periostin during the palatal fusion process. There were no major differences in the distributions of periostin mRNA and protein, suggesting that periostin expression is regulated by transcriptional mechanisms. It has been reported that regulation of palatal fusion may have different mechanisms in the anterior and posterior regions of the palatal shelf. Thus, comparisons of the mid-palatal region during palatal fusion with the anterior and posterior regions were an important objective of our study. The anterior and posterior regions of the palate have been reported to fuse mainly by tissue remodeling, whereas the mid-palate closes mainly by medial elongation of the shelf [Chou et al., 2004; Okano et al., 2006].

Fig. 3. High-power views of periostin at E14.0 (**a, b**), E14.5 (**c, d**), E15.0 (**e**) and E16.0 (**f**). The protein showed intense labeling in the basement membrane (arrows) underlying the MES (arrowheads) at E14.5. As seam disruption proceeded, protein was also observed inside the MES. Scale bars = 40 μ m.

Fig. 4. Double-staining confocal images with integrins and DAPI. Integrin α V (**a**), integrin β_3 (**b**), integrin β_5 (**c**) and integrin β_1 (**d**) at E14.0 late. Frozen sections (**a, b**) and paraffin-embedded sections (**c, d**) were used. All of the integrins were expressed in MEE (arrowheads). Scale bars = 10 μ m. Full-color images are given in the online version of the figure.

Fig. 5. **a–f** Triple-staining confocal images with periostin (red), the EMT marker twist1 (green) and DAPI (blue) at E14.5. Lines and arrowheads indicate basement membrane and twist1-positive MEE, respectively. **g–i** z-stack confocal images at 3 different z-positions indicated with arrows, covering from the surface to the bottom of the cell (arrowheads). Periostin is given in red and twist1 in green. Dotted circles indicate the same cell surrounded

by periostin. **j** Periostin 3D reconstruction before MES disintegration (E14.0 late). No periostin was observed inside MES. **k** Periostin 3D reconstruction at E14.5. Inside MES, a fibrillar architecture of periostin was formed. The arrowhead indicates the site where the same cells with arrowheads shown in **g** and **h** are located. **j, k** The yellow line represents the basement membrane. Scale bars = 15 μm.

Periostin was present in the mesenchyme with higher levels of expression in the far anterior and posterior region compared with other regions. Since periostin is involved in cell proliferation and migration [Gillan et al., 2002; Kuhn et al., 2007; Hong et al., 2010; Li et al., 2010], regional heterogeneity in the expression of periostin may play a role in the localized remodeling of the palatal shelf.

In contrast to the connective tissue mesenchyme, periostin was absent or diminished from ossification centers. Previous reports show that twist1, a bone differentiation transcription factor, can directly bind to and activate periostin gene expression [Oshima et al., 2002]. In our study, the expression of both twist1 and periostin is highest in

undifferentiated mesenchyme. The possibility that periostin controls osteoblast fate is supported in mice with a germline deletion of periostin. In these embryos, ectopic ossification in heart valve mesenchymal cells was observed [Tkatchenko et al., 2009]. This indicates that twist-mediated loss of periostin in select regions of EMT-derived mesenchyme may promote the differentiation of osteoblasts.

Our data showed that strong periostin expression occurred in the mesenchyme immediately adjacent to MES, but not in the epithelium. The protein showed intense labeling in the basement membrane underlying the MES during the seam disruption stage. As seam disruption

Periostin/CK14/DAPI

Fig. 6. z-stack confocal images of MEE at 3 different z-positions staining for periostin (red), the epithelial marker CK14 (green) and DAPI (blue) at E14.5. Cells in the yellow dotted circle represent MEE undergoing EMT (CK14 negative). White arrowheads indicate MEE undergoing EMT (diminished CK14). Both were associated with periostin. **b–e** Wide-field images. Scale bars = 25 μ m.

Periostin/LN/DAPI

Fig. 7. **a–d** Triple-staining images with LN, periostin and DAPI at E14.0 and E14.5. **e, f** Wide-field images of **c** and **d**, respectively. **c–f** Lines with arrowheads indicate the part where the LN-containing basement membrane has already started to degrade but the periostin-containing basement membrane was still completely retained. **d, f** Arrowheads show MEE allowed to establish new contact with periostin. Scale bars = 20 μ m.

Fig. 8. **a–d** Triple-staining images with periostin, ColIV and DAPI at E14.0 late and E14.5. ColIV and DAPI (**a, c**), periostin and DAPI (**b, d**). **c, d** Wide-field images. Lines with arrowheads indicate the part where the ColIV-containing basement membrane has already started to degrade but the periostin-containing basement membrane was retained; arrowheads indicate MME. **e–g** Triple-staining confocal images with periostin, ColIV and twist1 at E14.5. Periostin, ColIV and twist1 (**e**), ColIV (**f**) and peri-

ostin (**g**). **h–j** High-power images of the rectangle indicated in **e**. Periostin and ColIV (**h**), periostin and twist1 (**i**) and ColIV and twist1 (**j**). Lines with arrowheads (**f–h**) indicate the part where the ColIV-containing basement membrane has already started to degrade. **i, j** MEE were associated with periostin and undergoing EMT (arrowheads). Scale bars = 20 μm (**a–g**) and 10 μm (**h–j**). Full-color images are given in the online version of the figure.

proceeded, periostin mRNA and protein were also observed inside the MES. These findings are consistent with previous studies reporting that high levels of periostin mRNA and protein were localized to sites of epithelial-mesenchymal interactions (tooth germs) [Kruzynska-Frejtag et al., 2004]. Periostin is also highly expressed in pathological conditions such as cancer invasion [Fukushima et al., 2008; Kikuchi et al., 2008] and fibrosis [Takayama et al., 2006; Oka et al., 2007]. Periostin is well known to promote cellular motility and facilitate EMT [Gillan et al., 2002; Yan and Shao, 2006; Butcher et al., 2007; Li et al., 2010]. Moreover, periostin is reportedly regulated by several important signaling molecules in-

involved in EMT such as TGF- β_3 [Norris et al., 2009], TGF- β_1 [Horiuchi et al., 1999] and twist [Oshima et al., 2002]. Therefore, periostin may create an EMT-supportive microenvironment enabling MEE to transdifferentiate in order to achieve complete mesenchymal confluence.

Identification of MEE undergoing EMT has used many methods. Several labeling methods such as physical labeling (Dil and CCFSE) [Shuler et al., 1991; Griffith and Hay, 1992], molecular biology-based labeling (vector carrying lacZ) [Martinez-Alvarez et al., 2000] and genetic labeling (Cre-Loxp system) [Martinez-Alvarez et al., 2000] have been used to trace the fate of MEE. Here, we simply took advantage of periostin-containing basement

membrane to separate MES from the mesenchyme/mesenchymal cells since the structural integrity of periostin was retained late into the stages of palatal fusion. We demonstrated the association of MEE undergoing EMT (twist1-positive and CK14-negative MEE) with periostin 3-dimensionally. In addition, it was apparent that MEE undergoing EMT (diminished CK14) were also associated with periostin. The presence of strong periostin labeling around a select subset of MEE supports the hypothesis that periostin may play an important role in altering cell response favorable for EMT during palatal fusion. Furthermore, it was noted that MEE undergoing EMT (twist1-positive MEE) were surrounded by intense periostin expression as palatal fusion advanced. Previous work showed that knockdown of twist mRNA using siRNA decreased palatal fusion, and twist mRNA was upregulated by TGF- β_3 [Yu et al., 2008]. These findings suggest that the acquisition of periostin expression in MEE undergoing EMT may contribute to the changed microenvironment and may promote the mesenchymal phenotype induced by TGF- β_3 though twist1.

Basal lamina degradation and new formation of cell-mesenchymal matrix interactions play a role in EMT during palatal fusion [Kaartinen et al., 1997]. Our study revealed that LN and ColIV in the basement membrane were degraded much earlier than the mesenchymal matrix, periostin. In instances where both epithelial-specific and mesenchymal matrices coexisted in the basement membrane, the epithelial phenotype in MEE was maintained. This suggests that integrin engagement with epithelial-specific matrices in the basement membrane has a dominant effect on maintaining epithelial phenotype and suppressing EMT. However, during MES degradation, epithelial-specific matrices were degraded, and new interactions were established with underlying mesenchymal matrix. It was previously reported that selective loss of the basement membrane correlated with EMT occurred during both embryonic development [Fujiwara et al., 2007] and pathologic conditions [Spaderna et al., 2006]. Our data provided evidence for selective loss of epithelial matrix ColIV in the basement membrane underlying MES correlated with MEE transdifferentiation, indicated by expression of the mesenchymal marker twist1. Moreover, we could link the association with the mesenchymal matrix periostin to EMT. It is currently known that periostin serves as a ligand for some members of the integrin family including $\alpha V\beta_3$, $\alpha V\beta_5$ and β_1 [Butcher et al., 2007; Kuhn et al., 2007; Li et al., 2010], which were expressed in MEE. Therefore, interaction between periostin and integrins may occur at epithe-

lial-mesenchymal interfaces after degradation of epithelial-specific matrices in the basement membrane. Focal adhesion kinase, phosphoinositide 3-kinases and integrin-linked kinase are intracellular signaling components of the integrin complex, and all of these are activated by periostin [Bao et al., 2004; Baril et al., 2007]. These kinases function as important regulators of the EMT response [Kang and Svoboda, 2002; Guarino et al., 2007]. Thus, our findings support a hypothesis that periostin alters the MEE response by affecting signaling in MEE through alterations in their engagement of integrins and promotes EMT initiated by TGF- β_3 .

In summary, we have described the spatiotemporal distribution of periostin during palatogenesis at the sites of mesenchymal-epithelial interaction during the onset of EMT. The differential patterns of epithelial-specific matrices and mesenchymal matrix during MES degradation suggest novel roles, namely that periostin may play a role in creating the environment favorable for EMT through interactions with integrins.

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