MINIREVIEW

Regulation of Epithelial-Mesenchymal Transition in Palatal Fusion

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During palatal fusion, the midline epithelial seam between the palatal shelves degrades to achieve mesenchymal confluence. Morphological and molecular evidence support the theory that the epithelial-mesenchymal transition is one mechanism that regulates palatal fusion. It appears that transforming growth factor (TGF)-β signaling plays a role in palatal EMT. TGFβ3 is the main inducer in palatal fusion and activates both Smad-dependent and -independent signaling pathways, including the key EMT transcription factors, Lef1, Twist, and Snail1, in the MEE prior to the palatal EMT program. The roles and interactions among these transcription factors will be discussed. Exp Biol Med 234:483–491, 2009

Key words: EMT; cleft; Snail; Twist; TGFβ; palate fusion

Introduction

The secondary palate initiates as an outgrowth of the maxillary prominences at approximately embryonic day (E) 11.5 in the mouse (Fig. 1). The palate shelves initially grow vertically along the sides of the tongue (E13.5) and then rise above the tongue as the latter drops in the oral cavity due to the forward and downward growth of the mandible (E14.0). With continued growth, the shelves appose at the midline (E14.5) and eventually fuse (E15.5) (1). Numerous genes are expressed during palatal development, which are summarized in Figure 1B. In this review, we will focus only on the genes related to epithelial-mesenchymal transition (EMT) during palatal fusion.

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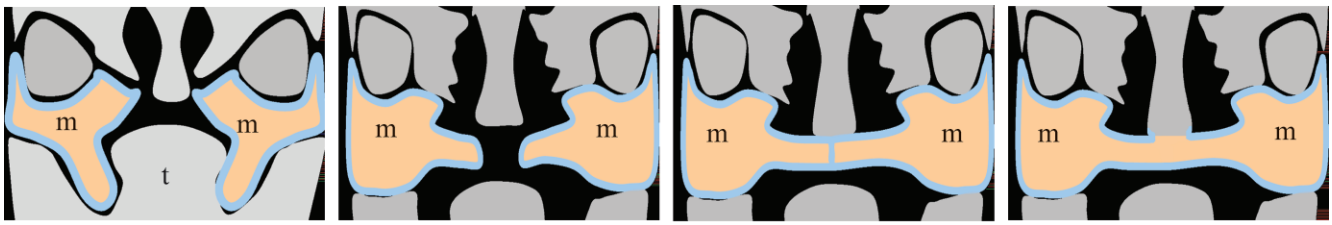
During fusion, the epithelium that covers the tip of opposing palatal shelves adheres, intercalates, and thins into a single-layer midline epithelial seam (MES) (2). The disintegration of this seam results in the confluence of the palatal mesenchyme. Tremendous interest has arisen in the area of the cellular mechanisms underlying MES degradation. EMT is one of the proposed models that regulate the medial edge epithelial (MEE) cell fate (2–10). However, other mechanisms have been proposed, such as apoptosis (11–14), which supports the theory that all MEE cells die during fusion. Alternatively, MES cells have been suggested to disappear by migrating along the midline toward the nasal and oral epithelia (15, 16). Others suggested that all events, including apoptosis, migration, and EMT, are likely to occur (14, 17). Evidences supporting these theories, especially of EMT and apoptosis, will be presented and further discussed.

Evidence of Palatal EMT. EMT is a well-documented phenomenon occurring during embryonic development, tumorigenesis, metastasis, and wound healing (4). It was first named by Gary Greenburg and Elizabeth Hay (18) and defined as the process that produces a complete loss of epithelial traits by the former epithelial cells accompanied by the acquisition of mesenchymal characteristics *in vitro* (19). Turning an epithelial cell into a mesenchymal cell requires alterations in morphology, cytoskeleton, adhesion, and migration capacity (20). During palatal fusion, the MEE cells at the tip of the degrading epithelial seam display these characteristics in transmission electron micrographs (21, 22). The transforming epithelial cells extend filopodia and pseudopodia through breaks in the surrounding basement membrane (2, 21). Several fluorescent dyes, such as DiI (1, 1′-dioctadecyl-3, 3, 3′, 3′-tetramethylindo-carbocyanine perchlorate) and CCFSE (carboxy dichlorofluorescein diacetate succinimidyl ester), were used to track the MEE cells *in vivo* and *in vitro* (3, 23). After the dyes were specifically incorporated into the MEE cells prior to palatal fusion, they were observed in the mesenchyme after the



Vertical Growth | Elevation | Adhesion | Fusion

Mesenchyme	Epithelium
EphB2/B3[10]	pERK[5]
Fgf10[22]	Fgfr2b[22]
Fgf7[22]	pMEK[5]
Fz4[14]	PI3K[5]
Gli1, 2, 3[24]	pPTEN[5]
Id2[23]	Shh[24]
Lhx8[28]	TBX22[2]
Msx1[18]	TBX1[29]
Osr2[16]	
Pax9[16]	
Pdgfrα[25]	
PI3K[5]	
Ror2[14]	
SATB2[9]	
Shox2[18,26]	
Snail[23,19]	
TBX22[2]	
Twist[23]	
Wnt5a[14]	

Mesenchyme	Epithelium
EphB2/B3[10]	pERK[5]
Fgf10[22]	Fgfr1[3]
Fgfr1, 2b[22]	Fgfr2b[22]
$GABA_A\beta3$[13]	$GABA_A\beta3$[13]
Lhx8[28]	pMEK[5]
Msx1[18]	PI3K[5]
Osr2[16]	pPTEN[5]
PI3K[5]	Ptc2[24]
Shox2[18, 26]	Shh[24]
Snail[23,19]	Shox2[26]
Twist[23,27]	TBX1[29]
TGFβ1,2[3]	TGFβ1,2[21]
	Wnt11[17]

Mesenchyme	Epithelium
Bmp2, 3, 4[20]	BMP3[9]
EphB2/B3[10]	CS-PG[12]
Fgf10[21]	EphB2/B3[10]
Lhx8[28]	Fgf2[3]
MMP2, 13[1,4]	Fgfr2[3]
Msx1[18]	$GABA_A\beta3$[13]
PI3K[5]	IRF6[15]
Shox2[23, 26]	MMP-13[1]
Snail[23, 19]	MT1-MMP[1]
Twist[23, 27]	pMEK[5]
	PI3K[5]
	pPTEN[5]
	Ptc2[24]
	Shh[24]
	Shox2[26]
	Smad2[8]
	Snail[19]
	STAT[3]
	TBX1[29]
	TIMP-2[1]
	Tgfβ3[3]
	TgfbrII[6], III[7]
	Twist[27]
	Wnt11[17]
	Zeb1(Zfhx1a)[9]

Mesenchyme
Bmp2,3,4[20]
Lhx8[28]
MMP2[4]
Osr2[16]
Snail[19]

Figure 1. Gene expression during different stages of palatal development. The schematic drawing shows coronal view of normal palate shelf and key stages of palatal development. At about E12–E13 days, the palatal shelves grow downward along the tongue (t.). At E13–E13.5 days, the palatal shelves elevated above the tongue when it drops. At E 14.5, the palatal shelves adhere to each other in the midline. After E15.5 days, the MES completely degrades and palate fuses. Main genes that express and function at each palatal developmental stages are summarized. Genes are separated according to the location of its expression pattern (mesenchyme vs. epithelium). 1: Blavier L. et al, 2001, Mol Biol Cell; 2: Braybrook C. et al, 2002, Hum Mol Genet; 3: Britto JA. et al, 2001, Cleft Palate Craniofac J; 4: Brown NL. et al, 2002, J Dent Res; 5: Cho KW. et al, 2008, Gene Expr Pattern; 6: Cui XM et al, 1998, Int J Dev Biol; 7: Cui XM. et al, 2000, Int J Dev Biol; 8: Cui SM. et al, 2003, Dev Biol; 9: Dean D. et al, 2008, Development; 10: McLean W. et al, 2008, Mech Dev; 11: FitzPatrick DR. et al, 2003, Hum Mol Genet; 12: Gato A. et al. 2002. Dev Biol; 13: Hagiwara N. et al, 2003, Dev Biol; 14: He F. et al, 2008, Development; 15: Kondo S. et al, 2002, Nat Genet; 16: Lan Y, et al, 2004, Development; 17: Lee J-M. et al, 2008, Dev Biol; 18: Li Q. et al, 2007, Int J Dev Biol; 19: Martínez-Álvarez C. et al, 2004, Dev Biol; 20: Nie XG. et al, 2006, Int J Dev Biol; 21: Pelton RW. et al, 1990, Dev Biol; 22: Rice R. et al, 2004, J Clin Invest; 23: Rice R. et al, 2005, Dev Dyn; 24: Rice R. et al, 2006, Gene Expr Patterns; 25: Xu X. et al, 2005, Dev Dyn; 26:Yu L., et al, 2005 Development; 27: Yu W. et al, 2008, Dev Dyn; 28: Zhao YG. et al, 1999, PNAS; 29: Zoupa M., 2006, Int J Dev Biol. A color version of this figure is available in the online version of the journal.

shelves fused. The presence of CCFSE in the mesenchyme indicated that those MEE cells underwent EMT (21).

These cell-lineage tracing studies furnished remarkable insight into the fate of MEE cells by providing the pilot evidence of EMT occurrence during palatal fusion. Further, several investigators used genetic labeling to trace the fate of MEE cells. Transgenic mice carrying the Shh (sonic hedgehog)-Cre allele or K14 (Keratin-14)-Cre allele were cross-bred with the R26R reporter line (16, 24, 25). Cre-recombinase mediated the activation of the reporter LacZ gene (encoding β-galactosidase) in the MEE cells as well as in their descendants allowing fate mapping. Two groups of researchers ruled out EMT in their studies (24, 25). However, their data showed incomplete penetrance of the Cre-recombinase activity, as many epithelial cells escaped, and the pattern of β-gal staining was "patchy." The absence of incomplete labeling was likely due to the chimeric nature of the Cre-loxP system (26). Further, their conclusion was challenged by another study using the same technique in which the Cre-recombinase activity was more uniform in the palatal epithelium (16). In this study, β-gal positive cells were found in the mesenchyme at a distance from the midline in late E14.5 and E15 embryonic palates. Staining patterns in other tissue indicate that these cells are not an

artifact resulting from the ectopic expression of the transfected genes. A large portion of the cells were β-gal positive, indicating that EMT occurred during seam degeneration.

The recent development in modern microscopy and image analysis of living cells provides exciting new capabilities for visualizing the dynamic process of palatal fusion. Our group has successfully cultured the palatal shelves in an environment chamber using live confocal microscopy to capture the palatal fusion process *in vitro*. MEE cells were labeled with CCFSE dye and recorded actively moving throughout the 24 hr in culture (27). 4D-imaging (3 dimensions over time) of the palatal fusion process was reconstructed. Our early results suggest that a portion of MEE cells survive and undergo EMT (27). These results correlate with those previously reported using genetic markers (16) and TEM (21, 22).

Molecular Signaling Events in Palatal EMT. Transforming growth factor-beta (TGFβ) possesses a vast number of functions, including controlling proliferation, survival, and EMT (28). Recent evidence indicates that the TGFβ pathway plays a major role in stimulating EMT in cancer cells (29). All TGFβ isoforms (TGFβ 1–3) bind the same receptors (TGFβr1 and TGFβr2 dimers) and have similar cellular effects *in vitro*, but they are differentially expressed during embryogenesis, suggesting they may exert different functions (30). Their actual function depends upon which member of the TGFβ family is activated, as well as the responding cell specificity. In the case of EMT, usually either TGFβ1 (cancer) or TGFβ3 (development) is involved. TGFβ1 and 3 isoforms and the receptors are expressed in palatal tissue, especially during the disintegration of the MES (31–33). Their roles and functions have been investigated and summarized in previous studies and reviews (24, 34, 35). TGFβ3 has been identified as a possible candidate gene related with non-syndromic cleft palate (36). The only craniofacial anomaly present in homozygous TGFβ3-deficient mice is cleft of the secondary palate (37, 38). The fusion is rescued by adding exogenous TGFβ3 in cultures of the mutant palate (39), demonstrating that TGFβ3 is essential for MEE fate in palatal fusion. In chickens, TGFβ3 is not expressed in the palatal epithelium, and the palate does not normally fuse. Addition of TGFβ3 to cultured palatal shelves allows them to fuse, but fusion fails in the absence of TGFβ3. Taken together, these results indicate the essential role played by TGFβ3 in palatal fusion but do not reveal the function of other TGFβ isoforms. Interestingly, TGFβ1 expressed in the TGFβ3 domain partially rescues the cleft palate phenotype of TGFβ3 null mutants (40), demonstrating an isoform-specific role for TGFβ3 in the palatal epithelium during fusion that cannot be fully compensated by TGFβ1.

TGFβ signaling is able to induce both apoptosis and EMT in the same type of cells simultaneously (40). The different response to TGFβ stimulation in cells has been related to cell-cycle state. TGFβ induced EMT in both unsynchronized cells and synchronized at G1/S phase, whereas TGFβ did not induce EMT in cells synchronized at G2/M phase (41). In addition, TGFβ activates RhoA-dependent pathway to induce cell-cycle arrest and EMT at the same time in NmuMG epithelial cells (42). This TGFβ-induced growth inhibition of epithelial cells is associated with effects on G1 phase cyclins, cyclin-dependent kinases (CDKs), and CDK inhibitors (43, 44) via p21 and p15 (45). Attenuating cell proliferation seems to be a conserved characteristic associated with EMT (46). It is possible that EMT requires a cell-cycle arrest necessary for the cytoskeletal changes to occur before cell migration (42).

In palatal fusion, whether TGFβ induces apoptosis or EMT in MEE cells remains a conundrum. However, experimental evidence agrees that MEE cells stop DNA synthesis prior to their disappearance from the midline (47, 48). In TGFβ3 knockout mice, MEE cells increase BrdU incorporation, indicating that they are still proliferating (48). However, disrupted TGFβ signaling results in less apoptosis in MEE cells *in vivo* (24, 34), when cultured *in vitro*, MEE cells undergo cell-cycle arrest shortly after TGFβ3 treatment, followed by EMT. Apoptosis occurs at a relatively later stage (60 hr after treatment) (49). These results agree with the findings in AML-12 cells that the apoptotic response is only apparent after 24 hr (50). The cell-cycle state of MEE right before normal palatal fusion remains unclear. Further, depletion TGFβ signaling in transgenic mice (24, 34) may change the MEE cell-cycle state, thus leading to an alternative cell fate. In summary, cell-cycle progression is closely related to the TGFβ-controlled MEE cell fate. TGFβ may induce cell-cycle arrest, cell transformation, and apoptosis in MEE cells in a temporally tightly controlled and highly integrated manner.

After binding the receptors, TGFβ propagates the signal by phosphorylating many downstream effectors, such as Smad proteins (Fig. 2) (51). Generally, Smad2 and Smad3 are phosphorylated by TGFβs upon binding to their receptors. During palate fusion, Smad2—but not Smad3—is phosphorylated (48). Phosphorylated Smad2 is selectively missing in the MEE in TGFβ3 knockout mice, indicating that no other signaling compensates for the absence of TGFβ3, although TGFβ1 is expressed. In addition, overexpression of Smad2 partially rescues the cleft palate of TGFβ3 null mutants (48, 52). These results suggest that Smad signaling is one of the central mediators of TGFβ signaling during palatal fusion. Other Smad-independent signals, including PI-3 kinase, MAPK, MEK, and RhoA, may also play a role during palatal fusion and have been investigated (6, 10). Although MAPK, MEK, and RhoA signaling can induce EMT in epithelial cell lines *in vitro* (53), they have no effect on palatal fusion individually (6). A recent study using the Cre/flox transgenic system to deplete Smad 4 (the co-smad necessary for the nuclear transport of Smad 2/3) expression in epithelial cells demonstrated that signaling through the Smad pathway alone was not sufficient for blocking palate fusion. The

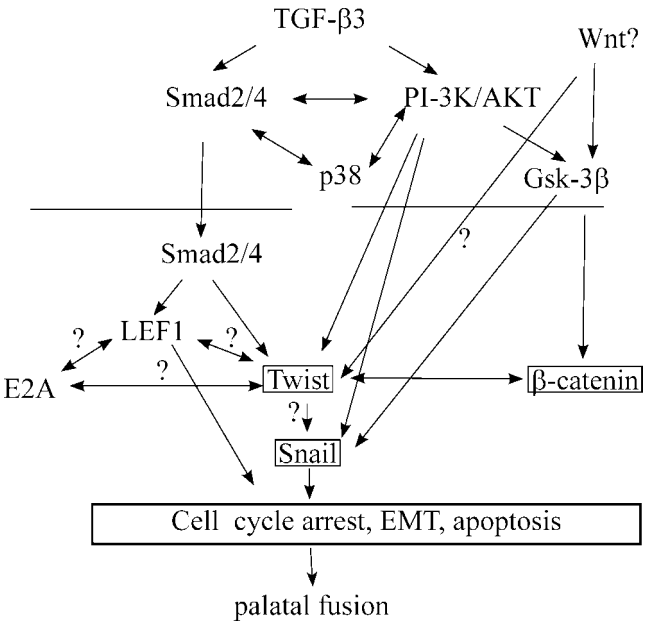


Figure 2. Proposed signaling events in palatal EMT during fusion. During palatal EMT, TGFβ3 signals via Smad2/4 and PI3K. On transcription level, palatal EMT may be regulated by cooperations between multiple transcription factors, such as Lef1, Twist, and Snail. Other suspicious signaling and cooperation (Wnt, E2A) are proposed with question mark.

additional downregulation of p38 MAPK was required to achieve the complete block of MEE degradation similar to animals with the TGFβr2 conditional knockout (54). This study suggested that Smad4 and p38 MAPK are functionally redundant in mediating TGFβ3 signaling during palatal fusion. The mechanisms of p38 MAPK activation by TGFβ signaling in the palate are unknown. However, it is known that TGFβ signaling can activate PI-3 kinase (PI3K) during EMT (55) and that PI3K can activate the MAPK pathway through its p85 regulatory subunit and activation of the Rho-Ras proteins (56). Our study found that inhibition of PI3K activity by LY-294002 in murine palatal culture decreased palatal fusion *in vitro* (10). Collectively, extensive crosstalk between signaling pathways may serve in TGFβ-mediated MEE transition, which increases the obstacles in studying the function of a single gene.

Extracellular matrix (ECM) turnover is another important step in EMT (57). Several matrix metalloproteinases (MMPs) were expressed in palatal fusion (57, 58), and palates cultured in MMP inhibitors had a higher level of palatal fusion failures (59). During mouse palatogenesis, the pro and active forms of MMP-2 are increased *in vivo* (59). The inhibition of MMP-2 *in vitro* resulted in blocked palatal fusion. In addition, microarray analysis revealed that MMP-11, 12, and 14 are also up-regulated during palatogenesis (9). The finding that MMP knockout mice do not exhibit a phenotype of cleft palate highlights the complexity of the MMP family and their redundant functions (59). If one enzyme is knocked out, another may compensate for the loss (60).

Transcriptional Regulation in Palatal EMT. Repression of E-cadherin is the hallmark of the EMT program in both embryogenesis and tumorigenesis (61). The demonstration that several transcriptional repressors can control E-cadherin expression in epithelial cells has provided a new avenue of research in the field of EMT (62). Other E-cadherin repressors include Zinc finger E-box binding homeobox1 (Zeb1; also known as Zfhx1A, dEF1, Tcf8, and Zfhep) (63) and Smad-interacting protein1 (Zeb2; also known as Sip1) (64), the basic-loop-helix factors Twist1, E12/E47, Snail1 (65), and Wnt signaling mediator Lef1 (8).

Twist is considered a master regulator in both embryonic morphogenesis and EMT. It controls mesoderm formation in *Drosophila* (66, 67) and is associated with metastasis in several cancers (68, 69). Mutations of the Twist gene are associated with Saethre-Chotzen's syndrome (70). Palatal anomalies were reported in this syndrome, including high-arched palate, cleft palate, and bifid uvula (71). The heterozygous Twist mutant mouse, which resembles the phenotype of Saethre-Chotzen's syndrome, has several craniofacial defects, but cleft palate was not reported (72). However, Twist mRNA transcripts and protein are expressed in palatal mesenchyme (73) and briefly in the epithelial cells at the tip of palatal shelves (74), indicating Twist functions in palatogenesis. In our lab, we found that Twist protein was expressed in the mesenchyme and intensively in the MEE before fusion and was quickly down-regulated after fusion (Fig. 3) (75). When we downregulate Twist expression *in vitro* using siRNA, palatal fusion was delayed. Further, Twist mRNA levels in whole palatal shelves decreased in the presence of TGFβ3 neutralizing antibody or PI3K inhibitor treatment and increased 3–6 hr after TGFβ3 stimulation (75). This suggested that Twist may regulate palatal fusion by TGFβ3 via PI3K pathway activation. In addition, Twist has been implicated in many signaling pathways, such as SHH, Gli3, FGFs and BMP (76). Its function of integrating different signaling pathways may explain the variable penetrance of the phenotype observed in both humans and mice carrying heterozygous Twist mutations. A critical threshold for Twist activity may be required for normal morphogenesis. Thus its role in specific temporal-spatial window of palatogenesis needs to be further explored.

The zinc-finger transcription factor Snail1 and Slug (also known as Snail2) function in gastrulation and neural-crest EMT in different species (77). The functional analysis of Snail has highlighted its roles in the EMT and in epithelial cell migration by suppressing E-cadherin expression (44). Snail1 controls the expression of E-cadherin directly by binding to the consensus sequences CAGGTG in its promoter (78). In palate, Snail1 is expressed in a few cells in the MES, compatible with its role in triggering EMT in a small MES subpopulation (17). Microarray analysis (both *in vivo* and *in vitro*) demonstrated that the up-regulation of Snail1 was detected at E14 (*in vivo*). It was

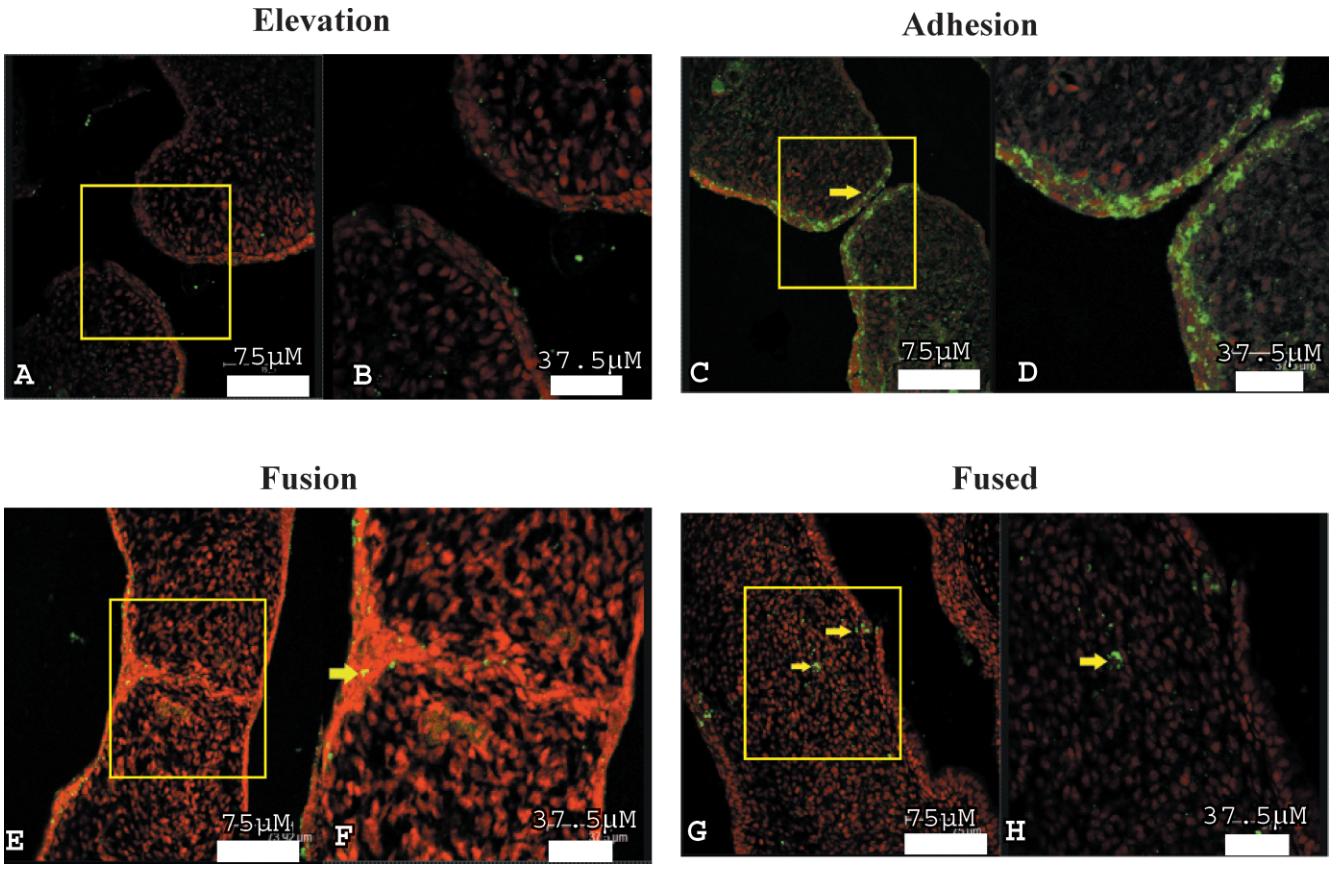


Figure 3. Twist protein expression increased prior to palatal EMT. A and B: at E 13, the palatal shelves were still open, scattered green staining using the anti-Twist antibody were found in both mesenchyme and epithelium. The tissues were counterstained with ToPro-3 (red) to label nuclei. C and D: at E 14–14.5 days, the palatal shelves just made contact in the midline. Increased staining for Twist protein was detected at the tip of the palatal shelves (arrow) where the MES (midline epithelial seam) would form. E and F: at E 14.5–15 days the MES formed and started transformation. Twist protein expression was downregulated. Some Twist staining was observed in the epithelial triangle (arrow). G and H: at E 15.5 days, the palate completely fused. Epithelial islands disappeared and the mesenchyme achieved confluence. A few cells in the mesenchyme expressed Twist protein (arrows). B, D, F, and H are enlarged views of the A, C, E, and F, respectively. Twist protein (green), nuclei (red). Scale bars (in A, C, E, and G) = 75 μM, scale bars (in B, D, F, H) = 37.5 μM.

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strongly expressed at 30 hrs in culture when the shelves were actively fusing and is down-regulated after fusion (75). Specific deletion of the Snail1 gene in neural crest cells did not cause any obvious defects; however, epithelial expression is not affected in these experiments (79). In an organ culture system, we down regulated Snail1 in the whole palate with siRNA and delayed palatal fusion (80). This suggests that Snail1 may regulate palatal EMT rather than mesenchymal proliferation although its mRNA expression was found mainly in the mesenchyme (73). Slug/Snail2, a Snail1 homolog, is also involved in palatogenesis. Fifty percent of Slug null mice have a secondary cleft palate, and the phenotype penetrance increases to 100% on a Snail1 heterozygous background (79). These results indicate that Snail homologs have redundant functions regarding neural crest cell proliferation in the palate.

Different pathways use Snail1 to trigger EMT, thus strict regulation of Snail1's expression, stability, and intracellular localization is essential (81). TGFβ induces EMT in lens epithelial cells via Snail1 to activate the PI3K/Akt pathway. Snail1 can also be activated by the cleavage of E-cadherin by MMP-3 via the Rac1/ROS pathway in mammary cells (82). An integrin-linked kinase (ILK)–dependent pathway has also been proposed to activate Snail1 in colon carcinoma cells (83). Snail1 localization to the nucleus is controlled by phosphorylation of a nuclear export motif and a proteosomal degradation motif, which are each phosphorylated by Gsk-3β, a regulator of β-catenin activity (84, 85). Interestingly, Gsk-3β activity is also required in a specific temporal window during palatogenesis. The Gsk-3β null mice with CD1 background exhibit cleft palate, and restoring Gsk-3β activity partially rescues the phenotype (86). Gsk-3β activity is repressed by both canonical Wnt (87) and PI3K/Akt pathway (88), presenting another example of crosstalk between signaling pathways in regulating palatal fusion.

Lef1 is a member of the HMG (high mobility group) box family and a nuclear mediator of Wnt signaling (89).

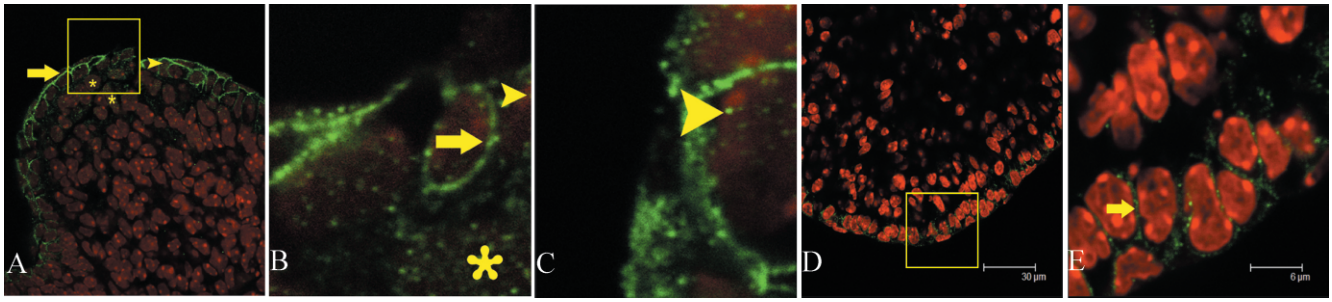

Figure 4. β-Catenin only showed membrane staining after treatment of Twist siRNA. In control groups (A, B, C), the β-catenin (green) had both cytoplasmic (arrowheads) and membrane-staining (arrows). β-Catenin was also expressed in some mesenchymal cells right underneath the epithelium (stars). In palates treated with 200 nM Twist siRNA (D, E), the cytoplasmic β-catenin decreased dramatically, β-catenin stained only cell membranes where the tight junctions between epithelial cells form (arrow, K). No overlapping staining pattern of the β-catenin and nuclei (red) were observed. B, C, and E are views of β-catenin staining in the yellow boxes of A, D, respectively.

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Previous work showed that the activation of LEF1 by β-catenin is associated with c-Fos-induced EMT in mammary cells (90) and with ILK-mediated EMT (91) as well as with metastasis in carcinomas (92). Lef1 mRNA was found in epithelium of maxilla process and its levels increased during palatal culture in the MEE seam with a peak at 36 hr (9). However, it appears that Lef1 activity in response to TGFβ3 signaling is through the activated Smad2/4 complex instead of β-catenin (6) during palatal EMT.

Zeb1, another zinc finger E-box binding homeobox transcription factor, is implicated in EMT and binds a set of E-box-like elements that overlap with those bound by Zeb2. Zeb1 is important for the TGFβ-induced repression of E-cadherin and expression of vimentin (63). Zeb1 knockout mice have developmental defects, including cleft palate (93). The overexpression of Zeb1 in cancer is associated with the repression of E-cadherin (94). It is possible that in the Zeb1 knockout mice, E-cadherin is not repressed, which may explain the palatal phenotype.

Possible Interactions Between Different Signaling Pathways in Palatal EMT. The development of vertebrate embryos is regulated by a number of different signaling pathways. These pathways are frequently dependent on each other, being connected by crosstalk between cells and tissues at different molecular levels (76). It was suggested both PI3K and p38 MAPK can modify the Smad protein activity (56, 95). In addition, PI3K through Akt modulates Gsk-3β activity and β-catenin level, the same as canonical Wnt signaling (96). The abundance of β-catenin is regulated by phosphorylation-dependent proteosomal degradation, unless GSK-3β is silenced. Wnt signaling can also crosstalk with TGFβ through Twist and β-catenin. β-Catenin exerts dual roles as a lateral membrane component of adherens junctions and also as a transducer and transcriptional activator of Lef1. In renal proximal tubular cells, TGFβ-induced β-catenin activity is required for the synthesis of α-SMA as an EMT marker (97, 98). The repression of E-cadherin by Snail1, Twist or other repressors leads indirectly to the expression of vimentin and other mesenchymal gene products, partly because of the β-catenin/Lef1 activation. But TGFβ also directly activates the TCF/Lef1 transcription complex through the tyrosine phosphorylation of Smad2 (20). Twist can form a complex with Smad4 in osteoblast differentiation (99). Work in osteoblast cells suggests that Twist may interact with Smad4, thus providing another mechanism to repress epithelial genes. During normal palatal fusion, β-catenin remained in the cytoplasm (6). In our work, when cultured with Twist siRNA, cytoplasmic β-catenin is dramatically reduced (Fig. 4), indicating that downregulating Twist expression changes the gene expression profile in MEE cells in palatal EMT (75).

In our experiments, down regulation of Twist does not completely block palatal fusion (75), suggesting that other factors are also involved in palatal EMT. During the mesoderm development in *Drosophila*, Twist and Snail1 are expressed in neighboring regions and play distinct roles in this process. Twist is required for the activation of downstream mesodermal genes, for the full expression of Snail1 and for the maintenance of its own expression (66). Only the absence of both Twist and Snail1 results in the complete loss of all mesodermal characteristics (66). Although the phenotypes of Twist and Snail1 mutants differ partially, the double mutant of both genes has a much stronger phenotype than that of either single mutant alone. In addition, Snail suppressed the activation of p21 expression induced by E2A in a Twist-dependent manner (44), and Twist1 stability is markedly enhanced when bound to E2A proteins (99). The E2A gene encodes two distinct bHLH transcription factors, E12 and E47, through alternative splicing. E2A proteins, belonging to the class I HLH proteins, are capable of forming homo- or heterodimers (100) and also play crucial roles in EMT. E2A represses E-cadherin expression and induces EMT by binding to the E-pal elements in E-cadherin promoter, a manner similar to Snail (101). It is intriguing that we also found E-pal elements on the mouse Snail promoter. It is, therefore, tempting to speculate that the members of the bHLH family may bind

and regulate Snail's expression. These transcription repressors of E-cadherin either work in cooperation or competition in EMT and the functional networks among Snail1, E2A, and Twist remain to be elucidated (Fig. 4).

Conclusion

In vivo and *in vitro* evidence support EMT during palatal fusion. TGFβ3 is a major player in palatal EMT. It regulates several pathways through Smad2/Smad4 signaling. However, other downstream mediators such as PI3K and p38 MAPK are also contributing to this process. TGFβ and PI3K may regulate key transcription factors such as Twist and Snail, regulating this transition to achieve complete palatal fusion. In addition, Twist and Snail may cooperate in the process of MEE transition.

We thank Jeanne Santa Cruz for editing the manuscript. The gene list (Fig. 1) is a work in progress, and new contributions can be sent to ksvoboda@bcd.tamhsc.edu.

1. Murray JC, Schutte BC. Cleft palate: players, pathways, and pursuits. J Clin Invest 113:1676–1678, 2004.
2. Nawshad A. Palatal seam disintegration: to die or not to die? That is no longer the question. Dev Dyn 237:2643–2656, 2008.
3. Shuler CF, Halpern DE, Guo Y, Sank AC. Medial edge epithelium fate traced by cell lineage analysis during epithelial-mesenchymal transformation in vivo. Dev Biol 154:318–330, 1992.
4. Hay ED. An overview of epithelio-mesenchymal transformation. Acta Anat (Basel) 154:8–20, 1995.
5. Kaartinen V, Cui XM, Heisterkamp N, Groffen J, Shuler CF. Transforming growth factor-beta3 regulates transdifferentiation of medial edge epithelium during palatal fusion and associated degradation of the basement membrane. Dev Dyn 209:255–260, 1997.
6. Nawshad A, Hay ED. TGFbeta3 signaling activates transcription of the LEF1 gene to induce epithelial mesenchymal transformation during mouse palate development. J Cell Biol 163:1291–1301, 2003.
7. Sun D, Griffith CM, Hay ED. Carboxyfluorescein as a marker at both light and electron microscope levels to follow cell lineage in the embryo. Methods Mol Biol 135:357–363, 2000.
8. LaGamba D, Nawshad A, Hay ED. Microarray analysis of gene expression during epithelial-mesenchymal transformation. Dev Dyn 234:132–142, 2005.
9. Nawshad A, LaGamba D, Olsen BR, Hay ED. Laser capture microdissection (LCM) for analysis of gene expression in specific tissues during embryonic epithelial-mesenchymal transformation. Dev Dyn 230:529–534, 2004.
10. Kang P, Svoboda KK. PI-3 kinase activity is required for epithelial-mesenchymal transformation during palate fusion. Dev Dyn 225:316–321, 2002.
11. Cuervo R, Valencia C, Chandraratna RA, Covarrubias L. Programmed cell death is required for palate shelf fusion and is regulated by retinoic acid. Dev Biol 245:145–156, 2002.
12. Cuervo R, Covarrubias L. Death is the major fate of medial edge epithelial cells and the cause of basal lamina degradation during palatogenesis. Development 131:15–24, 2004.
13. Taniguchi K, Sato N, Uchiyama Y. Apoptosis and heterophagy of medial edge epithelial cells of the secondary palatine shelves during fusion. Arch Histol Cytol 58:191–203, 1995.
14. Mori C, Nakamura N, Okamoto Y, Osawa M, Shiota K. Cytochemical identification of programmed cell death in the fusing fetal mouse palate by specific labelling of DNA fragmentation. Anat Embryol (Berl) 190:21–28, 1994.
15. Carette MJ, Ferguson MW. The fate of medial edge epithelial cells during palatal fusion in vitro: an analysis by DiI labelling and confocal microscopy. Development 114:379–388, 1992.
16. Jin JZ, Ding J. Analysis of cell migration, transdifferentiation and apoptosis during mouse secondary palate fusion. Development 133: 3341–3347, 2006.
17. Martinez-Alvarez C, Blanco MJ, Perez R, Rabadan MA, Aparicio M, Resel E, Martinez T, Nieto MA. Snail family members and cell survival in physiological and pathological cleft palates. Dev Biol 265: 207–218, 2004.
18. Greenburg G, Hay ED. Epithelia suspended in collagen gels can lose polarity and express characteristics of migrating mesenchymal cells. J Cell Biol 95:333–339, 1982.
19. Hay ED. The mesenchymal cell, its role in the embryo, and the remarkable signaling mechanisms that create it. Dev Dyn 233:706–720, 2005.
20. Lee JM, Dedhar S, Kalluri R, Thompson EW. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. J Cell Biol 172:973–981, 2006.
21. Griffith CM, Hay ED. Epithelial-mesenchymal transformation during palatal fusion: carboxyfluorescein traces cells at light and electron microscopic levels. Development 116:1087–1099, 1992.
22. Mato M, Uchiyama Y, Aikawa E, Smiley GR. Ultrastructural changes in rat palatal epithelium after beta-aminopropionitrile. Teratology 11: 153–167, 1975.
23. Shuler CF, Guo Y, Majumder A, Luo RY. Molecular and morphologic changes during the epithelial-mesenchymal transformation of palatal shelf medial edge epithelium in vitro. Int J Dev Biol 35: 463–472, 1991.
24. Xu X, Han J, Ito Y, Bringas P, Jr., Urata MM, Chai Y. Cell autonomous requirement for Tgfbr2 in the disappearance of medial edge epithelium during palatal fusion. Dev Biol 297:238–248, 2006.
25. Vaziri Sani F, Hallberg K, Harfe BD, McMahon AP, Linde A, Gritli-Linde A. Fate-mapping of the epithelial seam during palatal fusion rules out epithelial-mesenchymal transformation. Dev Biol 285:490–495, 2005.
26. Sauer B. Inducible gene targeting in mice using the Cre/lox system. Methods 14:381–392, 1998.
27. Svoboda KK, Yu W, Visualizing palate fusion and exploring the roles of regulatory proteins. Craniofacial Genetics Meeting, 2008, http://craniofacialgenetics.org/forum/index.php?action=dlattach;topic=28.0;attach=56.
28. Yue J, Mulder KM. Transforming growth factor-beta signal transduction in epithelial cells. Pharmacol Ther 91:1–34, 2001.
29. Valcourt U, Kowanetz M, Niimi H, Heldin CH, Moustakas A. TGF-beta and the Smad signaling pathway support transcriptomic reprogramming during epithelial-mesenchymal cell transition. Mol Biol Cell 16:1987–2002, 2005.
30. Roberts AB, Sporn MB. Differential expression of the TGF-beta isoforms in embryogenesis suggests specific roles in developing and adult tissues. Mol Reprod Dev 32:91–98, 1992.
31. Cui XM, Shuler CF. The TGF-beta type III receptor is localized to the medial edge epithelium during palatal fusion. Int J Dev Biol 44:397–402, 2000.
32. Cui XM, Warburton D, Zhao J, Crowe DL, Shuler CF. Immunohistochemical localization of TGF-beta type II receptor and TGF-beta3 during palatogenesis in vivo and in vitro. Int J Dev Biol 42:817–820, 1998.
33. Fitzpatrick DR, Denhez F, Kondaiah P, Akhurst RJ. Differential expression of TGF beta isoforms in murine palatogenesis. Development 109:585–595, 1990.
34. Dudas M, Kim J, Li WY, Nagy A, Larsson J, Karlsson S, Chai Y, Kaartinen V. Epithelial and ectomesenchymal role of the type I TGF-beta receptor ALK5 during facial morphogenesis and palatal fusion. Dev Biol 296:298–314, 2006.
35. Gritli-Linde A. Molecular control of secondary palate development. Dev Biol 301:309–326, 2007.
36. Murray JC. Gene/environment causes of cleft lip and/or palate. Clin Genet 61:248–256, 2002.
37. Proetzel G, Pawlowski SA, Wiles MV, Yin M, Boivin GP, Howles

PN, Ding J, Ferguson MW, Doetschman T. Transforming growth factor-beta 3 is required for secondary palate fusion. Nat Genet 11: 409–414, 1995.
38. Kaartinen V, Voncken JW, Shuler C, Warburton D, Bu D, Heisterkamp N, Groffen J. Abnormal lung development and cleft palate in mice lacking TGF-beta 3 indicates defects of epithelial-mesenchymal interaction. Nat Genet 11:415–421, 1995.
39. Taya Y, O'Kane S, Ferguson MW. Pathogenesis of cleft palate in TGF-beta3 knockout mice. Development 126:3869–3879, 1999.
40. Yang LT, Kaartinen V. Tgfb1 expressed in the Tgfb3 locus partially rescues the cleft palate phenotype of Tgfb3 null mutants. Dev Biol 312:384–395, 2007.
41. Yang Y, Pan X, Lei W, Wang J, Song J. Transforming growth factor-beta1 induces epithelial-to-mesenchymal transition and apoptosis via a cell cycle-dependent mechanism. Oncogene 25:7235–7244, 2006.
42. Denicourt C, Dowdy SF. Another twist in the transforming growth factor beta-induced cell-cycle arrest chronicle. Proc Natl Acad Sci U S A 100:15290–15291, 2003.
43. Pardali K, Kowanetz M, Heldin CH, Moustakas A. Smad pathway-specific transcriptional regulation of the cell cycle inhibitor p21(WAF1/Cip1). J Cell Physiol 204:260–272, 2005.
44. Takahashi E, Funato N, Higashihori N, Hata Y, Gridley T, Nakamura M. Snail regulates p21(WAF/CIP1) expression in cooperation with E2A and Twist. Biochem Biophys Res Commun 325:1136–1144, 2004.
45. Matsuyama S, Iwadate M, Kondo M, Saitoh M, Hanyu A, Shimizu K, Aburatani H, Mishima HK, Imamura T, Miyazono K, Miyazawa K. SB-431542 and Gleevec inhibit transforming growth factor-beta-induced proliferation of human osteosarcoma cells. Cancer Res 63: 7791–7798, 2003.
46. Acloque H, Thiery JP, Nieto MA. The physiology and pathology of the EMT. Meeting on the epithelial-mesenchymal transition. EMBO Rep 9:322–326, 2008.
47. Gehris AL, Greene RM. Regulation of murine embryonic epithelial cell differentiation by transforming growth factors beta. Differentiation 49:167–173, 1992.
48. Cui XM, Chai Y, Chen J, Yamamoto T, Ito Y, Bringas P, Shuler CF. TGF-beta3-dependent SMAD2 phosphorylation and inhibition of MEE proliferation during palatal fusion. Dev Dyn 227:387–394, 2003.
49. Ahmed S, Liu CC, Nawshad A. Mechanisms of palatal epithelial seam disintegration by transforming growth factor (TGF) beta3. Dev Biol 309:193–207, 2007.
50. Song J. EMT or apoptosis: a decision for TGF-beta. Cell Res 17:289–290, 2007.
51. Derynck R, Zhang Y. Intracellular signalling: the mad way to do it. Curr Biol 6:1226–1229, 1996.
52. Cui XM, Shiomi N, Chen J, Saito T, Yamamoto T, Ito Y, Bringas P, Chai Y, Shuler CF. Overexpression of Smad2 in Tgf-beta3-null mutant mice rescues cleft palate. Dev Biol 278:193–202, 2005.
53. Nawshad A, LaGamba D, Hay ED. Transforming growth factor beta (TGFbeta) signalling in palatal growth, apoptosis and epithelial mesenchymal transformation (EMT). Arch Oral Biol 49:675–689, 2004.
54. Xu X, Han J, Ito Y, Bringas P, Jr., Deng C, Chai Y. Ectodermal Smad4 and p38 MAPK are functionally redundant in mediating TGF-beta/BMP signaling during tooth and palate development. Dev Cell 15:322–329, 2008.
55. Nawshad A, Lagamba D, Polad A, Hay ED. Transforming growth factor-beta signaling during epithelial-mesenchymal transformation: implications for embryogenesis and tumor metastasis. Cells Tissues Organs 179:11–23, 2005.
56. Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. Nat Rev Mol Cell Biol 7:85–96, 2006.
57. Blavier L, Lazaryev A, Groffen J, Heisterkamp N, DeClerck YA, Kaartinen V. TGF-beta3-induced palatogenesis requires matrix metalloproteinases. Mol Biol Cell 12:1457–1466, 2001.
58. Morris-Wiman J, Burch H, Basco E. Temporospatial distribution of matrix metalloproteinase and tissue inhibitors of matrix metalloproteinases during murine secondary palate morphogenesis. Anat Embryol (Berl) 202:129–141, 2000.
59. Brown NL, Yarram SJ, Mansell JP, Sandy JR. Matrix metalloproteinases have a role in palatogenesis. J Dent Res 81:826–830, 2002.
60. Woessner JF, Jr. Role of matrix proteases in processing enamel proteins. Connect Tissue Res 39:69–73; discussion 141–149, 1998.
61. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol 7:131–142, 2006.
62. Thiery JP. Epithelial-mesenchymal transitions in development and pathologies. Curr Opin Cell Biol 15:740–746, 2003.
63. Liu Y, El-Naggar S, Darling DS, Higashi Y, Dean DC. Zeb1 links epithelial-mesenchymal transition and cellular senescence. Development 135:579–588, 2008.
64. Wakamatsu N, Yamada Y, Yamada K, Ono T, Nomura N, Taniguchi H, Kitoh H, Mutoh N, Yamanaka T, Mushiake K, Kato K, Sonta S, Nagaya M. Mutations in SIP1, encoding Smad interacting protein-1, cause a form of Hirschsprung disease. Nat Genet 27:369–370, 2001.
65. Pungchanchaikul P, Gelbier M, Ferretti P, Bloch-Zupan A. Gene expression during palate fusion in vivo and in vitro. J Dent Res 84: 526–531, 2005.
66. Leptin M. twist and snail as positive and negative regulators during Drosophila mesoderm development. Genes Dev 5:1568–1576, 1991.
67. Leptin M, Grunewald B. Cell shape changes during gastrulation in Drosophila. Development 110:73–84, 1990.
68. Puisieux A, Valsesia-Wittmann S, Ansieau S. A twist for survival and cancer progression. Br J Cancer 94:13–17, 2006.
69. Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, Savagner P, Gitelman I, Richardson A, Weinberg RA. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell 117:927–939, 2004.
70. Kress W, Schropp C, Lieb G, Petersen B, Busse-Ratzka M, Kunz J, Reinhart E, Schafer WD, Sold J, Hoppe F, Pahnke J, Trusen A, Sorensen N, Krauss J, Collmann H. Saethre-Chotzen syndrome caused by TWIST 1 gene mutations: functional differentiation from Muenke coronal synostosis syndrome. Eur J Hum Genet 14:39–48, 2006.
71. Stoler J, Rogers G, Mulliken JB. The frequency of palatal anomalies in Saethre-Chotzen syndrome. The Cleft Palate-Craniofacial Journal, 2008.
72. Bourgeois P, Bolcato-Bellemin AL, Danse JM, Bloch-Zupan A, Yoshiba K, Stoetzel C, Perrin-Schmitt F. The variable expressivity and incomplete penetrance of the twist-null heterozygous mouse phenotype resemble those of human Saethre-Chotzen syndrome. Hum Mol Genet 7:945–957, 1998.
73. Rice R, Thesleff I, Rice DP. Regulation of Twist, Snail, and Id1 is conserved between the developing murine palate and tooth. Dev Dyn 234:28–35, 2005.
74. Bloch-Zupan A, Hunter N, Manthey A, Gibbins J. R-twist gene expression during rat palatogenesis. Int J Dev Biol 45:397–404, 2001.
75. Yu W, Kamara H, Svoboda KK. The role of twist during palate development. Dev Dyn 237:2716–2725, 2008.
76. Hornik C, Brand-Saberi B, Rudloff S, Christ B, Fuchtbauer EM. Twist is an integrator of SHH, FGF, and BMP signaling. Anat Embryol (Berl) 209:31–39, 2004.
77. Barrallo-Gimeno A, Nieto MA. The Snail genes as inducers of cell movement and survival: implications in development and cancer. Development 132:3151–3161, 2005.
78. Cano A, Perez-Moreno MA, Rodrigo I, Locascio A, Blanco MJ, del Barrio MG, Portillo F, Nieto MA. The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. Nat Cell Biol 2:76–83, 2000.
79. Murray SA, Oram KF, Gridley T. Multiple functions of Snail family genes during palate development in mice. Development 134:1789–1797, 2007.
80. Yu WS, Svoboda, KKH Role of Gsk3b in murine palatal development. FASEB J 22:582–584, 2008.
81. Nieto MA. The snail superfamily of zinc-finger transcription factors. Nat Rev Mol Cell Biol 3:155–166, 2002.

82. Radisky DC, Levy DD, Littlepage LE, Liu H, Nelson CM, Fata JE, Leake D, Godden EL, Albertson DG, Nieto MA, Werb Z, Bissell MJ. Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. Nature 436:123–127, 2005.
83. Tan C, Costello P, Sanghera J, Dominguez D, Baulida J, de Herreros AG, Dedhar S. Inhibition of integrin linked kinase (ILK) suppresses beta-catenin-Lef/Tcf-dependent transcription and expression of the E-cadherin repressor, snail, in APC-/- human colon carcinoma cells. Oncogene 20:133–140, 2001.
84. Zhou BP, Deng J, Xia W, Xu J, Li YM, Gunduz M, Hung MC. Dual regulation of Snail by GSK-3beta-mediated phosphorylation in control of epithelial-mesenchymal transition. Nat Cell Biol 6:931–940, 2004.
85. Schlessinger K, Hall A. GSK-3beta sets Snail's pace. Nat Cell Biol 6: 913–915, 2004.
86. Liu KJ, Arron JR, Stankunas K, Crabtree GR, Longaker MT. Chemical rescue of cleft palate and midline defects in conditional GSK-3beta mice. Nature 446:79–82, 2007.
87. Yook JI, Li XY, Ota I, Hu C, Kim HS, Kim NH, Cha SY, Ryu JK, Choi YJ, Kim J, Fearon ER, Weiss SJ. A Wnt-Axin2-GSK3beta cascade regulates Snail1 activity in breast cancer cells. Nat Cell Biol 8:1398–1406, 2006.
88. Mercado-Gomez O, Hernandez-Fonseca K, Villavicencio-Queijeiro A, Massieu L, Chimal-Monroy J, Arias C. Inhibition of Wnt and PI3K signaling modulates GSK-3beta activity and induces morphological changes in cortical neurons: role of tau phosphorylation. Neurochem Res 33:1599–1609, 2008.
89. Zhang YD, Chen Z, Song YQ, Liu C, Chen YP. Making a tooth: growth factors, transcription factors, and stem cells. Cell Res 15:301–316, 2005.
90. El-Tanani MK, Barraclough R, Wilkinson MC, Rudland PS. Regulatory region of metastasis-inducing DNA is the binding site for T cell factor-4. Oncogene 20:1793–1797, 2001.
91. Novak A, Hsu SC, Leung-Hagesteijn C, Radeva G, Papkoff J, Montesano R, Roskelley C, Grosschedl R, Dedhar S. Cell adhesion and the integrin-linked kinase regulate the LEF-1 and beta-catenin signaling pathways. Proc Natl Acad Sci U S A 95:4374–4379, 1998.
92. Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, Kinzler KW. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science 275:1787–1790, 1997.
93. Jin JZ, Li Q, Higashi Y, Darling DS, Ding J. Analysis of Zfhx1a mutant mice reveals palatal shelf contact-independent medial edge epithelial differentiation during palate fusion. Cell Tissue Res 333:29–38, 2008.
94. Eger A, Aigner K, Sonderegger S, Dampier B, Oehler S, Schreiber M, Berx G, Cano A, Beug H, Foisner R. DeltaEF1 is a transcriptional repressor of E-cadherin and regulates epithelial plasticity in breast cancer cells. Oncogene 24:2375–2385, 2005.
95. Taniguchi CM, Tran TT, Kondo T, Luo J, Ueki K, Cantley LC, Kahn CR. Phosphoinositide 3-kinase regulatory subunit p85alpha suppresses insulin action via positive regulation of PTEN. Proc Natl Acad Sci U S A 103:12093–12097, 2006.
96. Cardona-Gomez P, Perez M, Avila J, Garcia-Segura LM, Wandosell F. Estradiol inhibits GSK3 and regulates interaction of estrogen receptors, GSK3, and beta-catenin in the hippocampus. Mol Cell Neurosci 25:363–373, 2004.
97. Masszi A, Fan L, Rosivall L, McCulloch CA, Rotstein OD, Mucsi I, Kapus A. Integrity of cell-cell contacts is a critical regulator of TGF-beta 1-induced epithelial-to-myofibroblast transition: role for beta-catenin. Am J Pathol 165:1955–1967, 2004.
98. Lewandoski M. Conditional control of gene expression in the mouse. Nat Rev Genet 2:743–755, 2001.
99. Hayashi M, Nimura K, Kashiwagi K, Harada T, Takaoka K, Kato H, Tamai K, Kaneda Y. Comparative roles of Twist-1 and Id1 in transcriptional regulation by BMP signaling. J Cell Sci 120:1350–1357, 2007.
100. Massari ME, Murre C. Helix-loop-helix proteins: regulators of transcription in eucaryotic organisms. Mol Cell Biol 20:429–440, 2000.
101. Perez-Moreno MA, Locascio A, Rodrigo I, Dhondt G, Portillo F, Nieto MA, Cano A. A new role for E12/E47 in the repression of E-cadherin expression and epithelial-mesenchymal transitions. J Biol Chem 276:27424–27431, 2001.
102. Braybrook C, Lisgo S, Doudney K, Henderson D, Marcano AC, Strachan T, Patton MA, Villard L, Moore GE, Stanier P, Lindsay S. Craniofacial expression of human and murine TBX22 correlates with the cleft palate and ankyloglossia phenotype observed in CPX patients. Hum Mol Genet 11:2793–2804, 2002.
103. Britto JA, Evans RD, Hayward RD, Jones BM. From genotype to phenotype: the differential expression of FGF, FGFR, and TGFbeta genes characterizes human cranioskeletal development and reflects clinical presentation in FGFR syndromes. Plast Reconstr Surg 108: 2026–2039; discussion 2040–2026, 2001.
104. Nie XG. [Differential expression of Bmp2, Bmp4 and Bmp3 in embryonic development of mouse anterior and posterior palate.]. Chin Med J (Engl) 118:1710–1716, 2005.
105. Gato A, Martinez ML, Tudela C, Alonso I, Moro JA, Formoso MA, Ferguson MW, Martinez-Alvarez C. TGF-beta(3)-induced chondroitin sulphate proteoglycan mediates palatal shelf adhesion. Dev Biol 250: 393–405, 2002.
106. Hagiwara N, Katarova Z, Siracusa LD, Brilliant MH. Nonneuronal expression of the GABA(A) beta3 subunit gene is required for normal palate development in mice. Dev Biol 254:93–101, 2003.
107. Kondo S, Schutte BC, Richardson RJ, Bjork BC, Knight AS, Watanabe Y, Howard E, de Lima RL, Daack-Hirsch S, Sander A, McDonald-McGinn DM, Zackai EH, Lammer EJ, Aylsworth AS, Ardinger HH, Lidral AC, Pober BR, Moreno L, Arcos-Burgos M, Valencia C, Houdayer C, Bahuau M, Moretti-Ferreira D, Richieri-Costa A, Dixon MJ, Murray JC. Mutations in IRF6 cause Van der Woude and popliteal pterygium syndromes. Nat Genet 32:285–289, 2002.
108. Lan Y, Ovitt CE, Cho ES, Maltby KM, Wang Q, Jiang R. Odd-skipped related 2 (Osr2) encodes a key intrinsic regulator of secondary palate growth and morphogenesis. Development 131:3207–3216, 2004.
109. Lee JM, Kim JY, Cho KW, Lee MJ, Cho SW, Kwak S, Cai J, Jung HS. Wnt11/Fgfr1b cross-talk modulates the fate of cells in palate development. Dev Biol 314:341–350, 2008.
110. Li Q, Ding J. Gene expression analysis reveals that formation of the mouse anterior secondary palate involves recruitment of cells from the posterior side. Int J Dev Biol 51:167–172, 2007.
111. Rice R, Spencer-Dene B, Connor EC, Gritli-Linde A, McMahon AP, Dickson C, Thesleff I, Rice DP. Disruption of Fgf10/Fgfr2b-coordinated epithelial-mesenchymal interactions causes cleft palate. J Clin Invest 113:1692–1700, 2004.
112. Rice R, Connor E, Rice DP. Expression patterns of Hedgehog signalling pathway members during mouse palate development. Gene Expr Patterns 6:206–212, 2006.
113. Xu X, Bringas P, Jr., Soriano P, Chai Y. PDGFR-alpha signaling is critical for tooth cusp and palate morphogenesis. Dev Dyn 232:75—84, 2005.
114. Yu L, Gu S, Alappat S, Song Y, Yan M, Zhang X, Zhang G, Jiang Y, Zhang Z, Zhang Y, Chen Y. Shox2-deficient mice exhibit a rare type of incomplete clefting of the secondary palate. Development 132: 4397–4406, 2005.
115. Zhao Y, Guo YJ, Tomac AC, Taylor NR, Grinberg A, Lee EJ, Huang S, Westphal H. Isolated cleft palate in mice with a targeted mutation of the LIM homeobox gene lhx8. Proc Natl Acad Sci U S A 96:15002–15006, 1999.
116. Zoupa M, Seppala M, Mitsiadis T, Cobourne MT. Tbx1 is expressed at multiple sites of epithelial-mesenchymal interaction during early development of the facial complex. Int J Dev Biol 50:504–510, 2006.