ORAL DISEASES

Oral Diseases (2011) 17, 733–744 doi:10.1111/j.1601-0825.2011.01806.x
© 2011 John Wiley & Sons A/S
All rights reserved
www.wiley.com

INVITED MEDICAL REVIEW

The mechanism of TGF-β signaling during palate development

J Iwata, C Parada, Y Chai

Center for Craniofacial Molecular Biology, Ostrow School of Dentistry, University of Southern California, Los Angeles, CA, USA

Cleft palate, a malformation of the secondary palate development, is one of the most common human congenital birth defects. Palate formation is a complex process resulting in the separation of the oral and nasal cavities that involves multiple events, including palatal growth, elevation, and fusion. Recent findings show that transforming growth factor beta (TGF-β) signaling plays crucial roles in regulating palate development in both the palatal epithelium and mesenchyme. Here, we highlight recent advances in our understanding of TGF-β signaling during palate development.

Oral Diseases (2011) **17**, 733–744

Keywords: Cranial neural crest cells; TGF-β signaling mechanism; Cleft palate

Introduction

Cleft lip with or without cleft palate (CL/P) is among the most common craniofacial deformities in humans, with ethnic and geographic variations in prevalence, and affects feeding, digestion, speech, hearing, middle-ear ventilation, respiration, appearance, and more (Goudy *et al*, 2006; Berbert-Campos, 2007; Chen *et al*, 2008; Cooper-Brown *et al*, 2008). Patients with cleft palate require care from birth to adulthood from many disciplines, including nursing, plastic surgery, maxillofacial surgery, otolaryngology, speech therapy, audiology, psychological and genetic counseling, orthodontics, and dental treatment (De Bodt and Van Lierde, 2006; Prahl-Andersen and Ju, 2006; Arosarena, 2007; Aminpour and Tollefson, 2008; Costello *et al*, 2008; Kasten *et al*, 2008; Panetta *et al*, 2008; Sitzman *et al*, 2008). The care for patients with cleft palate remains a cause for concern, particularly in developing countries where cleft palate is an economic burden, and the quality of care still varies substantially (Wehby and Cassell, 2010). Therefore, prevention of cleft palate is the ultimate objective. Here, we introduce some new ideas regarding the mechanisms of cleft palate, with the hope that we can prevent these congenital birth defects.

Correspondence: Yang Chai, Center for Craniofacial Molecular Biology, Ostrow School of Dentistry, University of Southern California, 2250 Alcazar Street, Los Angeles, CA 90033,USA. Tel: +1 323 442 3170, Fax: +1 323 442 2981, E-mail: ychai@usc.edu
Received 10 January 2011; revised 31 January 2011; accepted 31 January 2011

Palate formation

Etiology of cleft lip with or without cleft palate

CL/P has an incidence of 1/500–1/1000 birth worldwide (Wong and Hagg, 2004; Mossey *et al*, 2009). Cleft palate is usually classified into the following four categories: (1) complete cleft palate with cleft lip; (2) cleft of primary (anterior) palate, in which the cleft is limited to the anterior incisive fossa and may or may not involve cleft lip; (3) cleft of the secondary (posterior) palate, in which the cleft defect is limited to the posterior incisive fossa; and (4) submucosal cleft including a bifid uvula (Chai and Maxson, 2006). The causes of these various cleft palate types are being dissected through studies of human populations and the use of animal models. The etiology of cleft palate correlates with ethnic, racial, and geographic variations, socio-economic status, single-gene disorders, chromosome aberrations, and exposure to teratogens (alcohol, tobacco, anti-convulsants etc.), suggesting that multiple genetic and environmental factors are involved in cleft palate (Cobourne, 2004; Little *et al*, 2004; Lidral *et al*, 2008; Scapoli *et al*, 2008; Vieira, 2008; Meng *et al*, 2009; Zhu *et al*, 2009). Almost 70% of human cleft palates are regarded as non-syndromic, whereas the remaining approximately 30% of cleft palate cases are syndromic (Jugessur *et al*, 2009). Recent advances in our understanding of the molecular mechanisms of cleft palate have therefore resulted from the combination of genetic studies in humans and analysis of targeted mutations in mice.

Non-syndromic cleft lip with or without cleft palate (NSCL/P)

The genetics of NSCL/P is complex, involving variations in numerous genes together with epigenetic factors, such as maternal smoking, alcohol consumption, and expo-

sure to certain drugs or toxins resulting in defects in normal palate development (Carinci *et al*, 2007; Yu *et al*, 2009). Mutations in *TGFB3* (see Table 1 for description of all genes abbreviated and cited herein), *IRF6*, *CYP*, *MSX1*, and *TBX10* have been associated with NSCL/P (Vieira *et al*, 2007; Yu *et al*, 2009). In addition, polymorphic variants associated with NSCL/P were identified by a genome-wide association scan (GWAS) in *IRF6*, chromosome 8q24.21, *ABCA4* on 1p22.1, and *MAFB* on 20q12 (Beaty *et al*, 2010). In parallel, several studies have shown that folic acid may have a protective effect on cleft palate and neural tube defects (Peer *et al*, 1958a,b; Czeizel and Dudas, 1992; Tolarova and Harris, 1995; Hartridge *et al*, 1999; Mostowska *et al*, 2010; Zhu *et al*, 2009; Wehby and Murray, 2010).

Syndromes with cleft lip with or without cleft palate

Approximately 400 known human syndromes have been described in which a cleft palate is part of the disorder (Jugessur *et al*, 2009). Several clefting factors have been implicated by studies in mouse models and genetic screening in humans: *IRF6* in Van der Woude syndrome, *TGFBR1* or *TGFBR2* in Loeys–Dietz syndrome, *PHF8* in Siderius X-linked mental retardation, *DHCR7* in Smith-Lemli-Opitz syndrome, *DHCR24* in desmosterolosis, *HSD3B7* in CDPX2 (X-linked dominant chondrodysplasia punctata type 2), *SC5D* in lathosterolosis (Engelking *et al*, 2006; Porter, 2006; Jugessur *et al*, 2009). Some of the biological mechanisms underlying cleft palate may be similar among these disorders. For example, inborn errors of lipid

Table 1 Summery of genes cited

Gene	*Official name*	*Function*	*References*
ABCA4	ATP-binding cassette, sub-family A, member 4	ATP-binding cassette transporters	Beaty *et al* (2010)
BMP2	Bone morphogenetic protein 2	TGF-β superfamily ligand	Zhang *et al* (2002) Hilliard *et al* (2005)
BMP4	Bone morphogenetic protein 4	TGF-β superfamily ligand	Zhang *et al* (2002) Hilliard *et al* (2005)
CRKL	v-crk sarcoma virus CT10 oncogene homolog (avian)-like	Protein kinase containing SH2 and SH3 domains	Vitelli and Baldini (2003)
CYP	Cytochrome P450	Monooxygenase, and involved in drug metabolism and cholesterol or steroid synthesis	Yu *et al* (2009)
DHCR7	7-dehydrocholesterol reductase	Enzyme which removes the C7–C8 double bond of 7-dehydrocholesterol and produces cholesterol	Fitzky *et al* (2001) Porter (2006)
DHCR24	24-dehydrocholesterol reductase	A flavin adenine dinucleotide (FAD)-dependent oxidoreductase	Porter (2006)
FBN1	Fibrillin 1	Extracellular calcium-binding microfibrils	Brooke *et al* (2008) Habashi *et al* (2006) Kalluri and Han (2008)
FGF10	Fibroblast growth factor 10	FGF family ligand	Rice *et al* (2004) Hosokawa *et al* (2010)
FGFR2	Fibroblast growth factor receptor 2	FGF receptor	Rice *et al* (2004)
HSD3B7	Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7	Enzyme of the short-chain dehydrogenase/reductase superfamily	Porter (2006)
Insig1/2	Insulin induced gene 1 and 2	A cholesterol synthesis regulator	Engelking *et al* (2006) Porter (2006)
IRF6	Interferon regulatory factor 6	Transcriptional factor	Vieira *et al* (2007) Jugessur *et al* (2009)
MAFB	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B	Basic leucine zipper transcriptional factor	Beaty *et al* (2010)
MSX1	msh homeobox 1 Muscle segment homeobox 1	Transcriptional factor	Hilliard *et al* (2005) Yu *et al* (2009)
MYF5	Myogenic factor 5	Mitogenic factor	Hosokawa *et al* (2010)
OSR1	Odd-skipped related 1	Transcriptional factor	Lan *et al* (2004)
OSR2	Odd-skipped related 2	Transcriptional factor	Lan *et al* (2004) Hilliard *et al* (2005)
PAX9	Paired box 9	Transcriptional factor	Peters *et al* (1998) Hilliard *et al* (2005)
PHF8	PHD finger protein	Histone lysine demethylase	Jugessur *et al* (2009)
SC5D	Sterol-C5-desaturase homolog	Enzyme for cholesterol biosynthesis	Krakowiak *et al* (2003) Porter (2006)
SHH	Sonic hedgehog	Signal molecule	Rice *et al* (2004) Hilliard *et al* (2005)
SHOX2	Short stature homeobox protein 2	Homeobox gene	Hilliard *et al* (2005) Yu *et al* (2005)
TBX1	T-box 1	Transcriptional regulator	Lindsay *et al* (2001) Portnoi (2009)
TBX10	T-box 10	Transcriptional factor	Yu *et al* (2009)
TGFB3	Transforming growth factor, beta	TGF-β ligand	Yu *et al* (2009)
TGFBR1	Transforming growth factor, beta receptor 1	TGF-β receptor	Jugessur *et al* (2009)
TGFBR2	Transforming growth factor, beta receptor 2	TGF-β receptor	Jugessur *et al* (2009)

metabolism cause human malformation syndromes, such as Smith-Lemli-Opitz syndrome, lathosterolosis, desmosterolosis, X-linked dominant chondrodysplasia punctata type 2, congenital hemidysplasia with ichthyosiform erythroderma and limb defects (Engelking *et al*, 2006; Porter, 2006). All of these syndromes involve craniofacial anomalies including cleft palate. Moreover, animal models for a cholesterol synthesis defect, such as *Dhcr7*, *Sc5d*, and *Insig1/-2* conventional knockout mice, have cleft palate (Fitzky *et al*, 2001; Krakowiak *et al*, 2003; Engelking *et al*, 2006). Interestingly, these null mutant mice have severe malformations specifically in the craniofacial region. Another example is transforming growth factor beta (TGF-β) signaling (see Table 2 for description of all proteins abbreviated and cited herein), which is crucial in regulating organogenesis during embryonic development (Ito *et al*, 2003; Sasaki *et al*, 2006; Oka *et al*, 2007a,b; Hosokawa *et al*, 2010; Iwata *et al*, 2010). For instance, heterozygous mutations in *TGFBR1* or *TGFBR2* are associated with Loeys–Dietz syndrome, which can manifest with craniofacial malformations such as cleft palate, craniosynostosis, and hypertelorism; skeletal defects such as scoliosis, arachnodactyly, and joint laxity; and vascular problems including arterial tortuosity with the potential for aneurysms and dissections (Mizuguchi *et al*, 2004; Loeys *et al*, 2005, 2006; Loeys and De Paepe, 2008). In addition, heterozygous mutations in *FBN-1*, which encodes an elastic extracellular matrix protein called fibrillin-1, lead to excessive TGF-β signaling and cause Marfan syndrome, which exhibits a clinical phenotype similar to Loeys–Dietz syndrome (Habashi *et al*, 2006; Brooke *et al*, 2008; Kalluri and Han, 2008). DiGeorge syndrome, which has a variably sized deletion on chromosome 22 (del22q11) including about 30 genes such as the transcription factor *TBX1* and the signal adaptor protein *CRKL*, exhibits a phenotype similar to that resulting from mutation in *TGFBR2* and shows altered TGF-β signaling (Lindsay *et al*, 1999, 2001; Lindsay, 2001; Vitelli and Baldini, 2003; Wurdak *et al*, 2005; Portnoi, 2009).

Table 2 TGF-β signaling molecules

Protein	*Official name*	*Function*	*References*
β-spectrin/ELF	Embryonic liver fodrin	Membrane cytoskeletal protein	Mishra *et al* (1999) Tang *et al* (2003)
CTGF/CCN2	Connective tissue growth factor/CCN family member 2	Mitogen	Grotendorst (1997) Oka *et al* (2007a)
FKBP12	FK506 binding protein 1A, 12 kDa	Immunophilin protein family	Wang and Donahoe (2004)
SMAD2	Smad family member 2	Transcriptional modulator	Nomura and Li (1998) Weinstein *et al* (1998) Hoot *et al* (2008)
SMAD3	Smad family member 3	Transcriptional modulator	Yang *et al* (1999)
SMAD4	Smad family member 4	Transcriptional modulator	Yang *et al* (1998) Ko *et al* (2007) Xu *et al* (2008)
TAK1	Transforming growth factor (TGF)-beta-activated kinase 1	Serine/threonine protein kinase family	Kimura *et al* (2000) Sorrentino *et al* (2008) Yamashita *et al* (2008)
TGF-β1	Transforming growth factor beta 1	TGF-β ligand	Fitzpatrick *et al* (1990) Letterio *et al* (1996) McCartney-Francis *et al* (1996) Sudarshan *et al* (1998) Goumans *et al* (1999) Li *et al* (2007) Mu *et al* (2008)
TGF-β2	Transforming growth factor beta 2	TGF-β ligand	Sanford *et al* (1997)
TGF-β3	Transforming growth factor beta 3	TGF-β ligand	Sanford *et al* (1997)
TβRI/ALK5	Transforming growth factor beta receptor I/Activin receptor-like kinase 5	TGF-β receptor	Larsson *et al* (2001) Dudas *et al* (2006) Li *et al* (2008) Zhao *et al* (2008)
TβRII	Transforming growth factor beta receptor II	TGF-β receptor	Oshima *et al* (1996) Chai *et al* (2003) Ito *et al* (2003) Wurdak *et al* (2005) Sasaki *et al* (2006) Xu *et al* (2006) Oka *et al* (2007a) Oka *et al* (2007b) Hosokawa *et al* (2010) Iwata *et al* (2010)
TβRIII	Transforming growth factor beta receptor III	TGF-β receptor	Stenvers *et al* (2003)
TRAF6	Tumor necrosis factor (TNF) receptor-associated factor 6	A ubiquitin ligase	Sorrentino *et al* (2008) Yamashita *et al* (2008)

Developmental time course of palate formation

The formation of a continuous secondary palate separating the oral and nasal cavities is a complex process involving multiple events, including palatal shelf growth, elevation, and fusion. In humans, the palate develops from two primordiums, which are the primary and secondary palate. The primary palate represents only a small part of the adult hard palate. The secondary palate contributes to the development of the hard and soft parts of the palate. Palatal shelves begin to develop early in the 6th week from the internal aspect of the maxillary primordiums. The initial palatal shelves are positioned vertically along each side of the tongue. Subsequently, as the result of jaw growth and tongue descent, the palatal shelves re-orient into a horizontal position, expand, and fuse. In the hard palate, mesenchymal cells are replaced by intramembranous bone formation. In contrast, the posterior part of the secondary palate, which is the soft palate, does not undergo ossification. It contains five muscles, the tensor veli palatine, levator veli palatine, palatoglossus, palatopharyngeus, and musculus uvula, and extends posteriorly beyond the nasal septum, with the uvula at the end of the posterior border of the secondary palate. The fusion of palatal shelves is marked by the median palatine rephe. In mice, palate development is similar to that of humans (Chai and Maxson, 2006). Mouse palatal development also starts from two primordiums. During mouse development, mouse palatogenesis initiates at embryonic day 11.5 (E11.5) as marked by the formation of palatal shelves extending from the internal aspects of the maxillae. Following initiation, the two developing palatal shelves first grow vertically along the lateral sides of the tongue by E13.5. During E13.5–E14.5, because of the growth of the jaws and descent of the tongue, space develops to accommodate the horizontal apposition of the palatal shelves above the tongue, thus allowing the two vertical palatal shelves to re-orient to a horizontal position above the dorsal side of the tongue, a process termed palatal elevation. The two elevated palatal shelves grow horizontally toward each other to meet along the facial midline and form the medial edge epithelial (MEE) seam. The MEE seam will soon undergo degradation, a process referred to as palatal fusion, which gives rise to mesenchymal confluence and the formation of a single continuous palate (Ferguson, 1988). Fusion of the palatal shelves starts at E14.5, with completion of a continuous palate by E16.5 (Xu *et al*, 2006). Both the elevation and fusion of the palatal shelves occur in an anterior to posterior sequence (Figure 1).

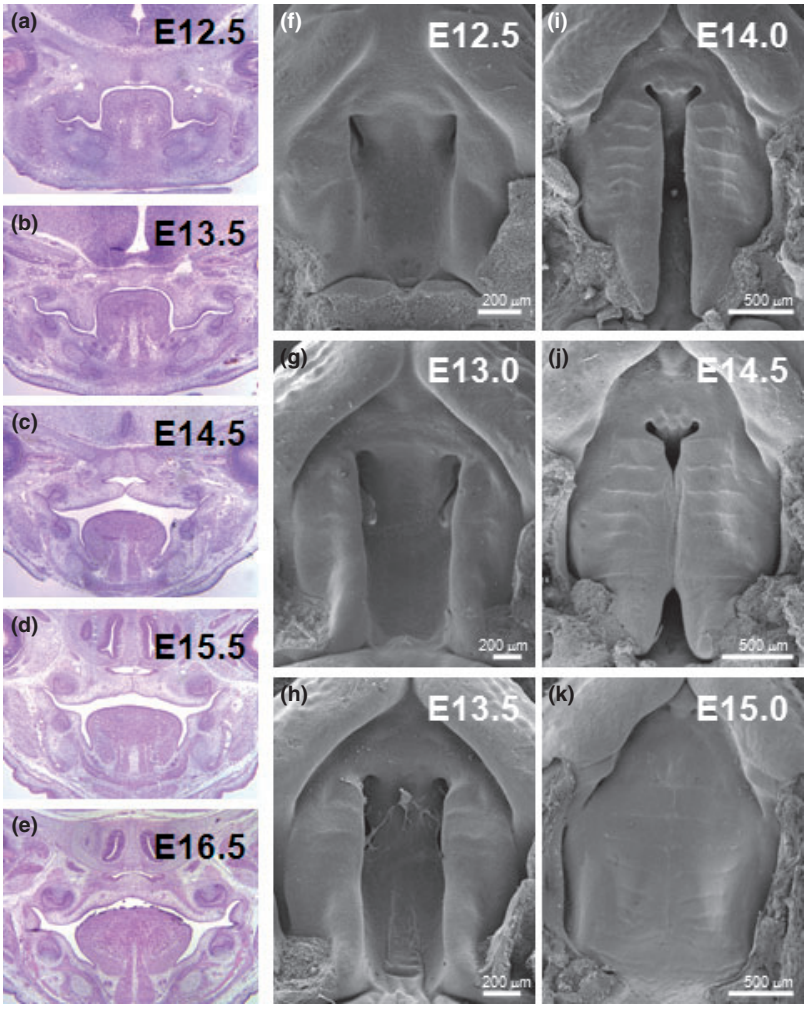

Figure 1 Time course of palate formation during embryonic development in mice. (**a-e**) Hematoxylin and eosin staining at E12.5 (**a**), E13.5 (**b**), E14.5 (**c**), E15.5 (**d**), and E16.5 (**e**). (**f-k**) Scanning electron microscopic (SEM) images at E12.5 (**f**), E13.0 (**g**), E13.5 (**h**), E14.0 (**i**), E14.5 (**j**), and E15.0 (**k**)

Cell lineage in the palate

The palatal structures are composed of cranial neural crest cell (CNCC)-derived ectomesenchymal cells, mesoderm-derived cells, and pharyngeal ectoderm-derived epithelial cells (Ferguson, 1984; Shuler *et al*, 1992; Ito *et al*, 2003). The epithelia that cover the palatal shelves are regionally divided into oral, nasal, and medial edge MEE. The nasal and oral epithelia differentiate into pseudostratified and squamous epithelia, respectively, whereas the MEE is removed from the fusion line via programed cell death, cell migration, or epithelial-mesenchymal transformation (EMT) (Fitchett and Hay, 1989; Shuler *et al*, 1992; Shuler, 1995; Martinez-Alvarez *et al*, 2000). Recent advances in cell fate analysis during palate formation have occurred using transgenic animal models that express a reporter gene. For example, using the *K14-Cre;R26R* model, it has been shown that MEE cells mainly disappear because of apoptosis and migration, but not EMT, during palatal fusion (Xu *et al*, 2006). Using the *Wnt1-Cre;R26R* model, it has been shown that more than 90% of palatal mesenchymal cells are derived from CNCC (Ito *et al*, 2003; Iwata *et al*, Submitted). Neural crest cells (NCC) are a transient population of cells created during higher vertebrate early embryonic development. During neurulation, NCC undergo successive epithelial-to-mesenchymal transformations along the cranial-caudal embryonic axis (Serbedzija *et al*, 1992), delaminate and migrate along defined pathways to contribute to the formation of a wide variety of target tissues, including peripheral neurons and glia, melanocytes, smooth muscle cells, connective tissues, secretory cells, most craniofacial cartilages and bones, and the developing aortic arch arteries and outflow track of the heart (Nichols, 1981; Kirby and Waldo, 1995; Sela-Donenfeld and Kalcheim, 1999; Chai *et al*, 2000; Jiang *et al*, 2000; Snider *et al*, 2007).

TGF-β signaling

SMAD-dependent TGF-β signaling pathway

The TGF-β superfamily includes activins, BMP, and TGF-β cytokines (Derynck and Zhang, 2003). The TGF-β pathway stimulates various signaling networks to control cell fate determination, growth, and differentiation (Massague and Wotton, 2000). TGF-β initiates signaling by assembling receptor complexes that activate Smad transcription factors (Massague and Wotton, 2000; Feng and Derynck, 2005). The extracellular region of the receptors is a small, tightly folded globular domain whereas the cytoplasmic region is formed by a short juxtamembrane segment, a protein kinase domain, and little else (Derynck and Feng, 1997; Derynck *et al*, 1998). Initially, the TGF-β1, -β2, or -β3 isoform binds to the TGF-β type II receptor (TβRII). With the binding of the ligand, TβRII is phosphorylated and activated. Both TGF-β1 and TGF-β3 have a high affinity for TβRII. In contrast, TGF-β2 has a low affinity for TβRII and requires an accessory receptor, TβRIII (also known as β-glycan), for high-affinity interaction with the heteromeric-signaling complex. Subsequently, the TGF-β type I receptor (TβRI; also known as ALK5, activin receptor-like kinase 5) is recruited into the complex and is activated by TβRII-mediated phosphorylation in the glycine–serine (GS) region (a GSGS sequence). Thus, TGF-β stimulation brings together heterotetrameric active receptor complexes. Receptor-associated SMADs (R-SMAD), SMAD2 and SMAD3, are recruited into the activated receptor complex in association with adaptor proteins and are specifically phosphorylated at the carboxyl terminus by TβRI. This phosphorylation event results in dissociation of R-SMAD from the receptor complex, association with the co-mediator SMAD (co-SMAD) SMAD4, nuclear translocation and subsequent transcriptional activation or repression (Figure 2). Each TGF-β ligand may have a choice of several receptors and adaptor proteins, and a given cell may express different receptor complexes and downstream signaling molecules or a given cell may express different receptor complexes resulting in divergent signaling outcomes.

SMAD-independent TGF-β signaling pathway

Transforming growth factor beta also activates SMAD-independent signaling cascades, including MAPK pathways such as the ERK, JNK, and p38 MAPK kinase pathways (Kang *et al*, 2009; Zhang, 2009). Studies using SMAD4-deficient cells, or dominant-negative SMADs, support the model that MAPK pathway activation is independent of SMADs (Figure 2). JNK or p38 MAPK activation by TGF-β is accompanied by Tβ RI-kinase [Correction added after online publication 2 June 2011: TGF-β RI-kinase was changed to Tβ RI-kinase] activity-independent TRAF6-TAK1 phosphorylation (Sorrentino *et al*, 2008; Yamashita *et al*, 2008). SMAD6 can bind to TAK1 and down-regulate its activity, whereas SMAD7 can enhance and sustain JNK activation (Kimura *et al*, 2000). However, it is still largely unknown how SMAD-independent pathways are specified and function. The balance between SMAD-dependent and SMAD-independent pathways may define cellular-specific responses to TGF-β.

Adapter proteins and modifiers

β-spectrin. β-spectrin (also known as ELF, embryonic liver fodrin) was first isolated from mouse E11 libraries and plays a crucial role in the propagation of TGF-β signaling (Mishra *et al*, 1999). $Elf^{-/-}$ mice exhibit a phenotype similar to $Smad2^{+/-}$;$Smad3^{+/-}$ mutant mice in that they undergo midgestational death because of gastrointestinal, liver, neural, and heart defects. Specifically, β-spectrin associates with SMAD3 and the TGF-β receptor complex and leads to their translocation to the nucleus. Disruption of β-spectrin expression in $Elf^{-/-}$ mice results in mislocalization of SMAD3 and SMAD4 and disruption of TGF-β signaling following cell cycle disruption (Tang *et al*, 2003).

FKBP12. Adjacent to the kinase domain of TβRI is a conserved 30-amino acid segment known as the juxtamembrane GS domain. In the basal state, the GS

domain forms a wedge that presses against the catalytic center. The immunophilins FKBP12 and FKBP12.6 bind to the GS domain and stabilize this inactive conformation (Wang and Donahoe, 2004).

Connective tissue growth factor (CTGF). CTGF (also known as CCN2) is an extracellular protein that belongs to the CCN family. CTGF has been demonstrated to regulate a wide spectrum of cellular functions including proliferation, migration, adhesion, survival, differentiation, and synthesis of ECM proteins in various cell types, in both normal and pathological conditions (Chen and Lau, 2009). CTGF acts by modulating and mediating diverse signaling pathways, such as TGF-β, BMP, and WNT (Abreu *et al*, 2002). *Ctgf*$^{-/-}$ mice exhibit skeletal dysmorphology that is caused by impaired chondrocyte proliferation and altered ECM composition within the hypertrophic zone of long bones during development. *Ctgf*$^{-/-}$ mice also exhibit cleft palate because of failure of palatal shelf elevation (Ivkovic *et al*, 2003). Studies in diverse cell types and organisms have shown that TGF-β1 is a strong inducer for *Ctgf* expression and that CTGF acts as a downstream mediator of the TGF-β signaling pathway (Grotendorst, 1997; Shi-wen *et al*, 2006; Arnott *et al*, 2007; Woods *et al*, 2009). In addition, CTGF modulates TGF-β activation and signal transduction by the binding of TGF-β through its von Willebrand type C/cysteine-rich (vWC/CR) domain, which enhances the affinity of TGF-β ligands for their receptors (Abreu *et al*, 2002; Chen and Lau, 2009).

TGF-β and cleft palate

Human syndromes related to TGF-β signaling

Human linkage studies have shown that point mutations in the fibrillin-1 gene (*FBN-1*) cause Marfan syndrome, in which patients are typically abnormally tall with long limbs and long thin fingers and have defects of the heart valves and aorta, lungs, eyes, the dural sac surrounding the spinal cord, skeleton, and the hard palate (Neptune *et al*, 2003; Dietz *et al*, 2005; Judge and Dietz, 2005; Singh *et al*, 2006). Fibrillin-1 binds to latent TGF-β ligands to inhibit TGF-β activation, and patients with Marfan syndrome usually have up-regulated TGF-β signaling (Dietz *et al*, 2005; Judge and Dietz, 2005; Habashi *et al*, 2006; Brooke *et al*, 2008; Kalluri and Han, 2008). Patients with Loeys–Dietz syndrome, who have heterozygous mutations in *TGFBR1* or *TGFBR2*, exhibit a similar, but more severe, clinical phenotype than Marfan syndrome, with craniofacial malformations such as cleft palate, craniosynostosis, and hypertelorism, skeletal defects such as scoliosis, arachnodactyly, and joint laxity, and vascular problems including arterial tortuosity, with the potential for aneurysms and dissections (Mizuguchi *et al*, 2004; Loeys *et al*, 2005, 2006).

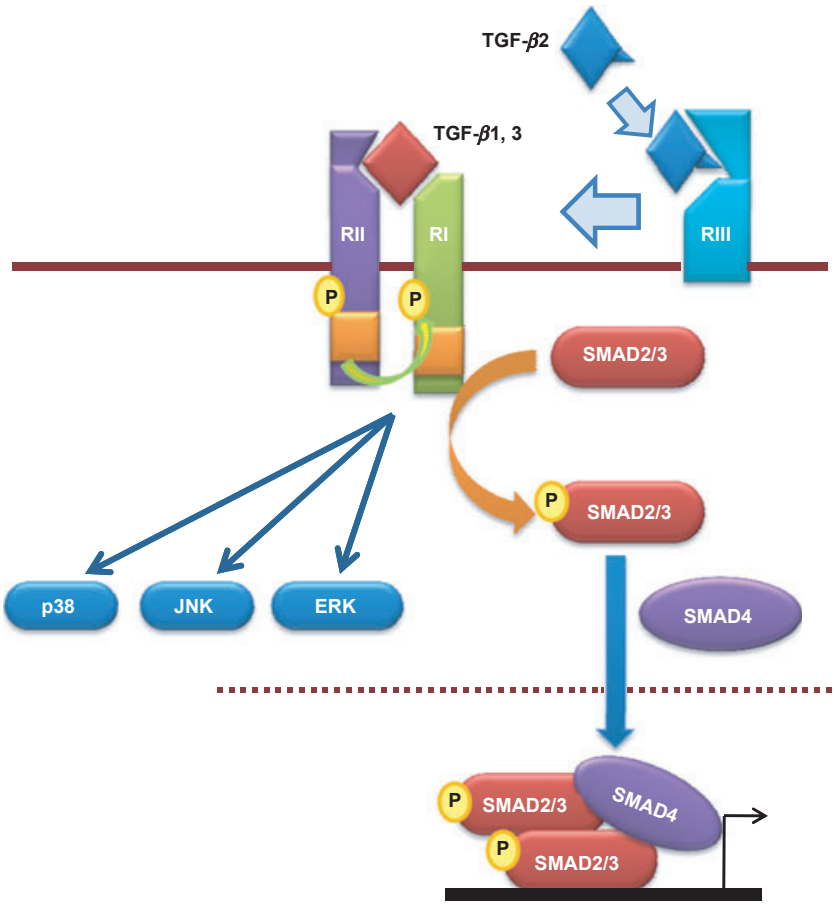

Figure 2 Schematic diagram of TGF-β signaling. Schematic diagram indicates the mechanism of TGF-β signaling. TGF-β initiates signaling by assembling receptor complexes that activate SMAD transcription factors. Initially, the TGF-β1, -β2, or -β3 isoform binds to the TGF-β type II receptor (TβRII). With the binding of the ligand, TβRII is phosphorylated and activated. Both TGF-β1 and TGF-β3 have a high affinity for TβRII. In contrast, TGF-β2 has a low affinity for TβRII and requires TβRIII. Subsequently, the TβRI is recruited into the complex and is activated by TβRII-mediated phosphorylation. Receptor-associated SMADs (R-SMAD), SMAD2 and SMAD3, are recruited into the activated receptor complex and are specifically phosphorylated by TβRI. This phosphorylation event results in dissociation of R-SMAD from the receptor complex, association with the SMAD4, nuclear translocation, and subsequent transcriptional activation or repression. TGF-β also activates SMAD-independent signaling cascades, including MAPK pathways, such as the ERK, JNK, and p38 MAPK kinase pathways, in some physiological and pathological conditions

Mouse craniofacial phenotypes after deletion of genes related to TGF-β signaling

Tgfb1 *null* (Tgfb1$^{-/-}$) *mice*. Transforming growth factor beta 1 (TGF-β1) is strongly expressed in MEE cells prior to adherence of the opposing palatal shelves (Fitzpatrick *et al*, 1990). Following adherence of the palatal shelves, TGF-β1 gradually ceases to be expressed in mesenchymal cells (Li *et al*, 2007; Mu *et al*, 2008). *Tgfb1* null newborn mice grow normally for the first 2 weeks and then develop a rapid wasting syndrome with diffuse inflammatory infiltrates and die by 3–4 weeks of age (Letterio *et al*, 1996; McCartney-Francis *et al*, 1996; Sudarshan *et al*, 1998). Moreover, about half of *Tgfb1* homozygous embryos die at E10 because of hematopoietic and vasculogenetic defects in the yolk sac (Goumans *et al*, 1999). Heterozygous *Tgfb1* knockout mice are developmentally normal.

Tgfb2 *null* (Tgfb2$^{-/-}$) *mice*. Transforming growth factor beta 2 is expressed in palatal mesenchymal cells during adherence of the opposing palatal shelves. *Tgfb2* null mice exhibit perinatal mortality and a wide range of developmental defects including cardiac, lung, craniofacial, limb, spinal column, eye, inner ear, and urogenital defects (Sanford *et al*, 1997). Heterozygous *Tgfb2* knockout mice are developmentally normal.

Tgfb3 *null* (Tgfb3$^{-/-}$) *mice*. Transforming growth factor beta 3 begins to be expressed strongly in the MEE cells prior to adherence of the opposing palatal shelves and continues to be expressed during palatal fusion (Tudela *et al*, 2002; Dudas *et al*, 2004; Yang and Kaartinen, 2007; Martinez-Sanz *et al*, 2008; Yang *et al*, 2008). TGF-β3 plays an essential role in MEE cell degradation and palatal fusion (Kaartinen *et al*, 1995; Proetzel *et al*, 1995). *Tgfb3* homozygous mice have cleft palate caused by defects in MEE seam degeneration and palate fusion (Kaartinen *et al*, 1995; Proetzel *et al*, 1995; Taya *et al*, 1999), and this defect can be rescued by *Smad2* overexpression (Cui *et al*, 2005). The loss of TGF-β3 function leads to changes in metalloproteinase 13 (MMP-13) expression and loss of cell surface filopodia (Taya *et al*, 1999; Blavier *et al*, 2001). Heterozygous *Tgfb3* knockout mice are developmentally normal.

Tgfbr1 *null* (Tgfbr1$^{-/-}$/Alk5$^{-/-}$) *mice*. *Tgfbr1* conventional knockout mice die around embryonic day 10.5 (E10.5) because of defects in vascular development of the yolk sac and placenta and an absence of circulating red blood cells (Larsson *et al*, 2001). Heterozygous *Tgfbr1* knockout mice are developmentally normal.

Tgfbr2 *null* (Tgfbr2$^{-/-}$) *mice*. *Tgfbr2* conventional knockout mice have defects of yolk sac hematopoiesis and vasculogenesis, resulting in embryonic lethality around E10.5 (Oshima *et al*, 1996). Heterozygous *Tgfbr2* knockout mice are developmentally normal.

Tgfbr3 *null* (Tgfbr3$^{-/-}$) *mice*. *Tgfbr3* homozygous mice die around E13.5 with proliferative defects in the heart and increased apoptosis in the liver (Stenvers *et al*, 2003). Heterozygous *Tgfbr3* knockout mice are developmentally normal.

Smad2 *null* (Smad2$^{-/-}$) *mice*. Homozygous *Smad2* mice fail to form an organized egg cylinder and lack mesoderm (Nomura and Li, 1998; Weinstein *et al*, 1998). About 20 percent of heterozygous *Smad2* mice have severe gastrulation defects and lack mandibles or eyes (Nomura and Li, 1998).

Smad3 *null* (Smad3$^{-/-}$) *mice*. Homozygous Smad3 null mice die between 1 and 8 months because of a primary defect in immune function (Yang *et al*, 1999). Heterozygous *Smad3* knockout mice are developmentally normal.

Smad4 *null* (Smad4$^{-/-}$) *mice*. Homozygous *Smad4* mice die between E6.5 and E8.5. All *Smad4*$^{-/-}$ mice are developmentally delayed at E6 and show little or no elongation in the extraembryonic portion of late egg-cylinder-stage embryos (Yang *et al*, 1998). Heterozygous *Smad4* knockout mice are developmentally normal.

Alk5$^{fl/fl}$;K14-Cre *mice*. Epithelial-specific deletion of the TGF-β type I receptor gene (*Alk5$^{fl/fl}$;K14-Cre*) causes clefts in the posterior part of the palate (soft palate) and the anterior portions of the hard palate (Dudas *et al*, 2006). *Alk5$^{fl/fl}$;K14-Cre* mice also have submucous cleft palate because of failure to undergo apoptosis during MEE disappearance between E14.5 and E16.5 (Dudas *et al*, 2006).

Tgfbr2$^{fl/fl}$;K14-Cre *mice*. Epithelial-specific deletion of the TGF-β type II receptor gene (*Tgfbr2$^{fl/fl}$;K14-Cre*) causes clefts in the posterior part of the palate (soft palate) and the anterior portions of the hard palate (Xu *et al*, 2006). *Tgfbr2$^{fl/fl}$;K14-Cre* mice also exhibit submucous cleft palate because of failure to undergo apoptosis during MEE disappearance between E14.5 and E16.5 (Xu *et al*, 2006).

Smad2$^{fl/fl}$;K5-Cre *mice*. Mice with an epithelial-specific conditional knockout of *Smad2* (*Smad2$^{fl/fl}$;K5-Cre*) survive, but exhibit accelerated formation and malignant progression of chemically induced skin tumors. They exhibit no detectable phenotype during palatal formation (Hoot *et al*, 2008).

Smad4$^{fl/fl}$;K14-Cre *mice*. Mice with epithelial-specific deletion of *Smad4* (*Smad4$^{fl/fl}$;K14-Cre*) die at birth, but exhibit no detectable phenotype during palatal formation (Xu *et al*, 2008).

Alk5$^{fl/fl}$;Wnt1-Cre *mice*. Mice with neural crest cell–specific deletion of *Tgfbr1/Alk5* (*Alk5$^{fl/fl}$;Wnt1-Cre*) are born alive, but severely disfigured, and die soon after birth with a hypoplastic cranium, split snout, small mandible, cleft lip and palate, and small tongue (Dudas *et al*, 2006; Li *et al*, 2008; Zhao *et al*, 2008).

Tgfbr2$^{fl/fl}$;Wnt1-Cre *mice*. Mice with neural crest cell–specific deletion of *Tgfbr2* (*Tgfbr2$^{fl/fl}$;Wnt1-Cre*) are born alive, but die soon after birth with cardiovascular defects, and thymic, parathyroid, and craniofacial anomalies, such as complete cleft palate, small mandible, small tongue, small maxilla, and calvaria defects (Chai *et al*, 2003; Ito *et al*, 2003; Hosokawa *et al*, 2010; Iwata *et al*, 2010). The defects in *Tgfbr2$^{fl/fl}$;Wnt1-Cre* embryos mimic defects in patients with Loeys–Dietz and DiGeorge syndromes (Ito *et al*, 2003; Wurdak *et al*, 2005).

Smad4$^{fl/fl}$;Wnt1-Cre *mice*. *Smad4$^{fl/fl}$;Wnt1-Cre* embryos are arrested at E11.5–E12.5 because of decreased blood circulation to the yolk sacs by E12.0. *Smad4$^{fl/fl}$;Wnt1-Cre* embryos show defective phenotypes by E10.5, including underdevelopment of the first branchial arch and failure of fusion in the middle of the frontonasal process and the mandibular process of the first branchial arch (Ko *et al*, 2007).

Alk5$^{fl/fl}$;Nestin-Cre *mice*. *Alk5$^{fl/fl}$;Nestin-Cre* mice die at E15 with unilateral or bilateral cleft lip because of increased cell death and retarded development of palatal shelves (Li *et al*, 2008).

Tgfbr2$^{fl/fl}$;Myf5-Cre *mice*. Mesoderm-derived cells in the palate consist of less than 10% of all palatal mesenchymal cells. Mesoderm-derived cell-specific deletion of *Tgfbr2* (*Tgfbr2$^{fl/fl}$;Myf5-Cre*) causes defects of the supraoccipital bone with meningoencephalocele and discontinuity of the neural arch of the C1 vertebra, but no apparent phenotype in palatal formation (Hosokawa *et al*, 2007; and our unpublished data).

Gene expression patterning and tissue-tissue interaction

Recent discoveries have highlighted the existence of genetic heterogeneity along the anterior–posterior (A–P) and medial–lateral axes of the developing palate (Hilliard *et al*, 2005; Han *et al*, 2009). Constant and reciprocal interactions between the palatal epithelium and the mesenchyme are responsible for setting up this genetic heterogeneity along the A–P axis and may provide differential regulatory mechanisms for the fusion of the anterior versus posterior region of the palate (Zhang *et al*, 2002; Murray and Schutte, 2004; Rice *et al*, 2004). Multiple genes have been found to be critical for the development of the anterior region of the palate. For example, *Msx1*, *Bmp4*, *Bmp2*, *Fgf10*, and *Shox2* show restricted expression patterns in the anterior region of the palate (Rice *et al*, 2004; Hilliard *et al*, 2005). Loss of *Msx1* results in a cell proliferation defect in the CNCC-derived palatal mesenchyme in the anterior region of the secondary palate, followed by a reduction in *Bmp4* and *Bmp2* gene expression in the mesenchyme (Zhang *et al*, 2002). *Fgf10* is expressed in the anterior palatal mesenchyme and functions in the palatal epithelium to mediate *Shh* expression, which in turn regulates *Bmp2* expression in the mesenchyme to promote cell proliferation. *Shox2* is expressed in the anterior palatal mesenchyme, and loss of *Shox2* results in an incomplete cleft of the anterior palate, whereas fusion of the posterior palate is normal (Yu *et al*, 2005). In contrast, we know less about the specific gene expression patterns in the posterior region of the palate. *Fgfr2* is expressed in the epithelium and the mesenchyme in the middle and posterior palate. FGF8 induces the expression of *Pax9* in the posterior region of palatal mesenchyme, and loss of *Pax9* results in cleft palate (Peters *et al*, 1998; Hilliard *et al*, 2005). In addition to the differential gene expression patterns along the A–P axis of the developing palate, there is also mesenchymal heterogeneity between the medial and lateral regions of the palatal shelf. For example, the *odd skipped-related genes Osr1* and *Osr2* are expressed in a medial–lateral gradient in the palate. Mutation in *Osr2* results in the compromised development of the medial aspect of the palatal shelf and retards palatal shelf elevation (Lan *et al*, 2004). The expression of *Fgfr2* is limited in the medial aspect of the developing palatal shelf, suggesting a possible functional significance in regulating the development and elevation of palatal shelves. There are also oral–nasal gene expression patterns that control the heterogeneity of palatal mesenchyme and determine the fate of CNC-derived cells during palatogenesis (Han *et al*, 2009). These studies demonstrate that there are different regulatory mechanisms that control the development and fusion of various regions of the palate.

Conclusion

The utility of animal models with cleft palate is impressive as we have begun to discover the cellular and molecular mechanisms of palate formation. To date, over 200 genetically mutated mice are reported to exhibit cleft palate, and approximately 400 known human syndromes exhibit cleft palate as a part of their clinical symptoms. However, the molecular and cellular mechanisms associated with cleft palate are still not completely defined. We propose that rescue experiments using genetic and pharmacological approaches may lead to a greater understanding of the gene–gene and gene–environment interactions in craniofacial development.

Acknowledgements

We thank Julie Mayo for critical reading of the manuscript. This study was supported by grants from the National Institute of Dental and Craniofacial Research, NIH (DE020065, and DE012711) to Yang Chai.

Author contributions

J. Iwata made substantial contributions to the conception and design of the paper, drafted and revised the article critically for important intellectual content and was involved in the final approval of the version to be published. C. Parada revised it for intellectual content and was involved in the final approval of the version to be published. Y. Chai made substantial contributions to the conception and design of the paper, revised the article critically for important intellectual

content, and was involved in the final approval of the version to be published. All authors read and approved the final manuscript.

References

Abreu JG, Ketpura NI, Reversade B, De Robertis EM (2002). Connective-tissue growth factor (CTGF) modulates cell signalling by BMP and TGF-beta. *Nat Cell Biol* **4:** 599–604.

Aminpour S, Tollefson TT (2008). Recent advances in presurgical molding in cleft lip and palate. *Curr Opin Otolaryngol Head Neck Surg* **16:** 339–346.

Arnott JA, Nuglozeh E, Rico MC *et al* (2007). Connective tissue growth factor (CTGF/CCN2) is a downstream mediator for TGF-beta1-induced extracellular matrix production in osteoblasts. *J Cell Physiol* **210:** 843–852.

Arosarena OA (2007). Cleft lip and palate. *Otolaryngol Clin North Am* **40:** 27–60. vi.

Beaty TH, Murray JC, Marazita ML *et al* (2010). A genome-wide association study of cleft lip with and without cleft palate identifies risk variants near MAFB and ABCA4. *Nat Genet* **42:** 525–529.

Berbert-Campos C (2007). Legal considerations in the management of cleft lip and palate. *Cleft Palate Craniofac J* **44:** 223–225.

Blavier L, Lazaryev A, Groffen J, Heisterkamp N, DeClerck YA, Kaartinen V (2001). TGF-beta3-induced palatogenesis requires matrix metalloproteinases. *Mol Biol Cell* **12:** 1457–1466.

Brooke BS, Habashi JP, Judge DP, Patel N, Loeys B, Dietz HC III (2008). Angiotensin II blockade and aortic-root dilation in Marfan's syndrome. *N Engl J Med* **358:** 2787–2795.

Carinci P, Becchetti E, Baroni T *et al* (2007). Extracellular matrix and growth factors in the pathogenesis of some craniofacial malformations. *Eur J Histochem* **51**(Suppl 1)**:** 105–115.

Chai Y, Maxson RE Jr (2006). Recent advances in craniofacial morphogenesis. *Dev Dyn* **235:** 2353–2375.

Chai Y, Jiang X, Ito Y *et al* (2000). Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. *Development* **127:** 1671–1679.

Chai Y, Ito Y, Han J (2003). TGF-beta signaling and its functional significance in regulating the fate of cranial neural crest cells. *Crit Rev Oral Biol Med* **14:** 78–88.

Chen CC, Lau LF (2009). Functions and mechanisms of action of CCN matricellular proteins. *Int J Biochem Cell Biol* **41:** 771–783.

Chen JL, Messner AH, Curtin G (2008). Newborn hearing screening in infants with cleft palates. *Otol Neurotol* **29:** 812–815.

Cobourne MT (2004). The complex genetics of cleft lip and palate. *Eur J Orthod* **26:** 7–16.

Cooper-Brown L, Copeland S, Dailey S *et al* (2008). Feeding and swallowing dysfunction in genetic syndromes. *Dev Disabil Res Rev* **14:** 147–157.

Costello BJ, Edwards SP, Clemens M (2008). Fetal diagnosis and treatment of craniomaxillofacial anomalies. *J Oral Maxillofac Surg* **66:** 1985–1995.

Cui XM, Shiomi N, Chen J *et al* (2005). Overexpression of Smad2 in Tgf-beta3-null mutant mice rescues cleft palate. *Dev Biol* **278:** 193–202.

Czeizel AE, Dudas I (1992). Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* **327:** 1832–1835.

De Bodt M, Van Lierde K (2006). Cleft palate speech and velopharyngeal dysfunction: the approach of the speech therapist. *B-ENT* **2**(Suppl 4)**:** 63–70.

Derynck R, Feng XH (1997). TGF-beta receptor signaling. *Biochim Biophys Acta* **1333:** F105–F150.

Derynck R, Zhang YE (2003). Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature* **425:** 577–584.

Derynck R, Zhang Y, Feng XH (1998). Smads: transcriptional activators of TGF-beta responses. *Cell* **95:** 737–740.

Dietz HC, Loeys B, Carta L, Ramirez F (2005). Recent progress towards a molecular understanding of Marfan syndrome. *Am J Med Genet C Semin Med Genet* **139C:** 4–9.

Dudas M, Nagy A, Laping NJ, Moustakas A, Kaartinen V (2004). Tgf-beta3-induced palatal fusion is mediated by Alk-5/Smad pathway. *Dev Biol* **266:** 96–108.

Dudas M, Kim J, Li WY *et al* (2006). Epithelial and ectomesenchymal role of the type I TGF-beta receptor ALK5 during facial morphogenesis and palatal fusion. *Dev Biol* **296:** 298–314.

Engelking LJ, Evers BM, Richardson JA, Goldstein JL, Brown MS, Liang G (2006). Severe facial clefting in Insig-deficient mouse embryos caused by sterol accumulation and reversed by lovastatin. *J Clin Invest* **116:** 2356–2365.

Feng XH, Derynck R (2005). Specificity and versatility in tgf-beta signaling through Smads. *Annu Rev Cell Dev Biol* **21:** 659–693.

Ferguson WJ (1984). Epithelial-mesenchymal interactions during vertebrate palatogenesis. *Curr Top Dev Biol* **19:** 137–164.

Ferguson MW (1988). Palate development. *Development* **103**(Suppl)**:** 41–60.

Fitchett JE, Hay ED (1989). Medial edge epithelium transforms to mesenchyme after embryonic palatal shelves fuse. *Dev Biol* **131:** 455–474.

Fitzky BU, Moebius FF, Asaoka H *et al* (2001). 7-Dehydrocholesterol-dependent proteolysis of HMG-CoA reductase suppresses sterol biosynthesis in a mouse model of Smith-Lemli-Opitz/RSH syndrome. *J Clin Invest* **108:** 905–915.

Fitzpatrick DR, Denhez F, Kondaiah P, Akhurst RJ (1990). Differential expression of TGF beta isoforms in murine palatogenesis. *Development* **109:** 585–595.

Goudy S, Lott D, Canady J, Smith RJ (2006). Conductive hearing loss and otopathology in cleft palate patients. *Otolaryngol Head Neck Surg* **134:** 946–948.

Goumans MJ, Zwijsen A, van Rooijen MA, Huylebroeck D, Roelen BA, Mummery CL (1999). Transforming growth factor-beta signalling in extraembryonic mesoderm is required for yolk sac vasculogenesis in mice. *Development* **126:** 3473–3483.

Grotendorst GR (1997). Connective tissue growth factor: a mediator of TGF-beta action on fibroblasts. *Cytokine Growth Factor Rev* **8:** 171–179.

Habashi JP, Judge DP, Holm TM *et al* (2006). Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. *Science* **312:** 117–121.

Han J, Mayo J, Xu X *et al* (2009). Indirect modulation of Shh signaling by Dlx5 affects the oral-nasal patterning of palate and rescues cleft palate in Msx1-null mice. *Development* **136:** 4225–4233.

Hartridge T, Illing HM, Sandy JR (1999). The role of folic acid in oral clefting. *Br J Orthod* **26:** 115–120.

Hilliard SA, Yu L, Gu S, Zhang Z, Chen YP (2005). Regional regulation of palatal growth and patterning along the anterior-posterior axis in mice. *J Anat* **207:** 655–667.

Hoot KE, Lighthall J, Han G *et al* (2008). Keratinocyte-specific Smad2 ablation results in increased epithelial-mesenchymal transition during skin cancer formation and progression. *J Clin Invest* **118:** 2722–2732.

Hosokawa R, Oka K, Yamaza T *et al* (2010). TGF-beta mediated FGF10 signaling in cranial neural crest cells controls development of myogenic progenitor cells through tissue-tissue interactions during tongue morphogenesis. *Dev Biol* **341:** 186–195.

Hosokawa R, Urata M, Han J, *et al* (2007). TGF-beta mediated Msx2 expression controls occipital somites-derived caudal region of skull development. *Dev Biol* **310:** 140–153.

Ito Y, Yeo JY, Chytil A *et al* (2003). Conditional inactivation of Tgfbr2 in cranial neural crest causes cleft palate and calvaria defects. *Development* **130:** 5269–5280.

Ivkovic S, Yoon BS, Popoff SN *et al* (2003). Connective tissue growth factor coordinates chondrogenesis and angiogenesis during skeletal development. *Development* **130:** 2779–2791.

Iwata J, Hosokawa R, Sanchez-Lara PA, Urata M, Slavkin H, Chai Y (2010). Transforming growth factor-beta regulates basal transcriptional regulatory machinery to control cell proliferation and differentiation in cranial neural crest-derived osteoprogenitor cells. *J Biol Chem* **285:** 4975–4982.

Jiang X, Rowitch DH, Soriano P, McMahon AP, Sucov HM (2000). Fate of the mammalian cardiac neural crest. *Development* **127:** 1607–1616.

Judge DP, Dietz HC (2005). Marfan's syndrome. *Lancet* **366:** 1965–1976.

Jugessur A, Farlie PG, Kilpatrick N (2009). The genetics of isolated orofacial clefts: from genotypes to subphenotypes. *Oral Dis* **15:** 437–453.

Kaartinen V, Voncken JW, Shuler C *et al* (1995). Abnormal lung development and cleft palate in mice lacking TGF-beta 3 indicates defects of epithelial-mesenchymal interaction. *Nat Genet* **11:** 415–421.

Kalluri R, Han Y (2008). Targeting TGF-beta and the extracellular matrix in Marfan's syndrome. *Dev Cell* **15:** 1–2.

Kang JS, Liu C, Derynck R (2009). New regulatory mechanisms of TGF-beta receptor function. *Trends Cell Biol* **19:** 385–394.

Kasten EF, Schmidt SP, Zickler CF *et al* (2008). Team care of the patient with cleft lip and palate. *Curr Probl Pediatr Adolesc Health Care* **38:** 138–158.

Kimura N, Matsuo R, Shibuya H, Nakashima K, Taga T (2000). BMP2-induced apoptosis is mediated by activation of the TAK1-p38 kinase pathway that is negatively regulated by Smad6. *J Biol Chem* **275:** 17647–17652.

Kirby ML, Waldo KL (1995). Neural crest and cardiovascular patterning. *Circ Res* **77:** 211–215.

Ko SO, Chung IH, Xu X *et al* (2007). Smad4 is required to regulate the fate of cranial neural crest cells. *Dev Biol* **312:** 435–447.

Krakowiak PA, Wassif CA, Kratz L *et al* (2003). Lathosterolosis: an inborn error of human and murine cholesterol synthesis due to lathosterol 5-desaturase deficiency. *Hum Mol Genet* **12:** 1631–1641.

Lan Y, Ovitt CE, Cho ES, Maltby KM, Wang Q, Jiang R (2004). Odd-skipped related 2 (Osr2) encodes a key intrinsic regulator of secondary palate growth and morphogenesis. *Development* **131:** 3207–3216.

Larsson J, Goumans MJ, Sjostrand LJ *et al* (2001). Abnormal angiogenesis but intact hematopoietic potential in TGF-beta type I receptor-deficient mice. *EMBO J* **20:** 1663–1673.

Letterio JJ, Geiser AG, Kulkarni AB *et al* (1996). Autoimmunity associated with TGF-beta1-deficiency in mice is dependent on MHC class II antigen expression. *J Clin Invest* **98:** 2109–2119.

Li Y, Yang J, Luo JH, Dedhar S, Liu Y (2007). Tubular epithelial cell dedifferentiation is driven by the helix-loop-helix transcriptional inhibitor Id1. *J Am Soc Nephrol* **18:** 449–460.

Li WY, Dudas M, Kaartinen V (2008). Signaling through Tgf-beta type I receptor Alk5 is required for upper lip fusion. *Mech Dev* **125:** 874–882.

Lidral AC, Moreno LM, Bullard SA (2008). Genetic Factors and Orofacial Clefting. *Semin Orthod* **14:** 103–114.

Lindsay EA (2001). Chromosomal microdeletions: dissecting del22q11 syndrome. *Nat Rev Genet* **2:** 858–868.

Lindsay EA, Botta A, Jurecic V *et al* (1999). Congenital heart disease in mice deficient for the DiGeorge syndrome region. *Nature* **401:** 379–383.

Lindsay EA, Vitelli F, Su H *et al* (2001). Tbx1 haploinsufficieny in the DiGeorge syndrome region causes aortic arch defects in mice. *Nature* **410:** 97–101.

Little J, Cardy A, Munger RG (2004). Tobacco smoking and oral clefts: a meta-analysis. *Bull World Health Organ* **82:** 213–218.

Loeys B, De Paepe A (2008). New insights in the pathogenesis of aortic aneurysms. *Verh K Acad Geneeskd Belg* **70:** 69–84.

Loeys BL, Chen J, Neptune ER *et al* (2005). A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. *Nat Genet* **37:** 275–281.

Loeys BL, Schwarze U, Holm T *et al* (2006). Aneurysm syndromes caused by mutations in the TGF-beta receptor. *N Engl J Med* **355:** 788–798.

Martinez-Alvarez C, Tudela C, Perez-Miguelsanz J, O'Kane S, Puerta J, Ferguson MW (2000). Medial edge epithelial cell fate during palatal fusion. *Dev Biol* **220:** 343–357.

Martinez-Sanz E, Del Rio A, Barrio C *et al* (2008). Alteration of medial-edge epithelium cell adhesion in two Tgf-beta3 null mouse strains. *Differentiation* **76:** 417–430.

Massague J, Wotton D (2000). Transcriptional control by the TGF-beta/Smad signaling system. *EMBO J* **19:** 1745–1754.

McCartney-Francis NL, Mizel DE, Redman RS *et al* (1996). Autoimmune Sjogren's-like lesions in salivary glands of TGF-beta1-deficient mice are inhibited by adhesion-blocking peptides. *J Immunol* **157:** 1306–1312.

Meng L, Bian Z, Torensma R, Von den Hoff JW (2009). Biological mechanisms in palatogenesis and cleft palate. *J Dent Res* **88:** 22–33.

Mishra L, Cai T, Yu P, Monga SP, Mishra B (1999). Elf3 encodes a novel 200-kD beta-spectrin: role in liver development. *Oncogene* **18:** 353–364.

Mizuguchi T, Collod-Beroud G, Akiyama T *et al* (2004). Heterozygous TGFBR2 mutations in Marfan syndrome. *Nat Genet* **36:** 855–860.

Mossey PA, Little J, Munger RG, Dixon MJ, Shaw WC (2009). Cleft lip and palate. *Lancet* **374:** 1773–1785.

Mostowska A, Hozyasz KK, Wojcicki P, Dziegelewska M, Jagodzinski PP (2010). Associations of folate and choline metabolism gene polymorphisms with orofacial clefts. *J Med Genet* **47:** 809–815.

Mu Z, Yang Z, Yu D, Zhao Z, Munger JS (2008). TGFbeta1 and TGFbeta3 are partially redundant effectors in brain vascular morphogenesis. *Mech Dev* **125:** 508–516.

Murray JC, Schutte BC (2004). Cleft palate: players, pathways, and pursuits. *J Clin Invest* **113:** 1676–1678.

Neptune ER, Frischmeyer PA, Arking DE *et al* (2003). Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. *Nat Genet* **33:** 407–411.

Nichols DH (1981). Neural crest formation in the head of the mouse embryo as observed using a new histological technique. *J Embryol Exp Morphol* **64:** 105–120.

Nomura M, Li E (1998). Smad2 role in mesoderm formation, left-right patterning and craniofacial development. *Nature* **393:** 786–790.

Oka K, Oka S, Sasaki T *et al* (2007a). The role of TGF-beta signaling in regulating chondrogenesis and osteogenesis during mandibular development. *Dev Biol* **303:** 391–404.

Oka S, Oka K, Xu X, Sasaki T, Bringas P Jr, Chai Y (2007b). Cell autonomous requirement for TGF-beta signaling during odontoblast differentiation and dentin matrix formation. *Mech Dev* **124:** 409–415.

Oshima M, Oshima H, Taketo MM (1996). TGF-beta receptor type II deficiency results in defects of yolk sac hematopoiesis and vasculogenesis. *Dev Biol* **179:** 297–302.

Panetta NJ, Gupta DM, Slater BJ, Kwan MD, Liu KJ, Longaker MT (2008). Tissue engineering in cleft palate and other congenital malformations. *Pediatr Res* **63:** 545–551.

Peer LA, Bryan WH, Strean LP, Walker JC Jr, Bernhard WG, Peck GC (1958a). Induction of cleft palate in mice by cortisone and its reduction by vitamins. *J Int Coll Surg* **30:** 249–254.

Peer LA, Strean LP, Walker JC Jr, Bernhard WG, Peck GC (1958b). Study of 400 pregnancies with birth of cleft lip-palate infants; protective effect of folic acid and vitamin B6 therapy. *Plast Reconstr Surg Transplant Bull* **22:** 442–449.

Peters H, Neubuser A, Kratochwil K, Balling R (1998). Pax9-deficient mice lack pharyngeal pouch derivatives and teeth and exhibit craniofacial and limb abnormalities. *Genes Dev* **12:** 2735–2747.

Porter FD (2006). Cholesterol precursors and facial clefting. *J Clin Invest* **116:** 2322–2325.

Portnoi MF (2009). Microduplication 22q11.2: a new chromosomal syndrome. *Eur J Med Genet* **52:** 88–93.

Prahl-Andersen B, Ju Q (2006). Quality improvement of cleft lip and palate treatment. *Angle Orthod* **76:** 265–268.

Proetzel G, Pawlowski SA, Wiles MV *et al* (1995). Transforming growth factor-beta 3 is required for secondary palate fusion. *Nat Genet* **11:** 409–414.

Rice R, Spencer-Dene B, Connor EC *et al* (2004). Disruption of Fgf10/Fgfr2b-coordinated epithelial-mesenchymal interactions causes cleft palate. *J Clin Invest* **113:** 1692–1700.

Sanford LP, Ormsby I, Gittenberger-de Groot AC *et al* (1997). TGFbeta2 knockout mice have multiple developmental defects that are non-overlapping with other TGFbeta knockout phenotypes. *Development* **124:** 2659–2670.

Sasaki T, Ito Y, Bringas P Jr *et al* (2006). TGFbeta-mediated FGF signaling is crucial for regulating cranial neural crest cell proliferation during frontal bone development. *Development* **133:** 371–381.

Scapoli L, Martinelli M, Arlotti M *et al* (2008). Genes causing clefting syndromes as candidates for non-syndromic cleft lip with or without cleft palate: a family-based association study. *Eur J Oral Sci* **116:** 507–511.

Sela-Donenfeld D, Kalcheim C (1999). Regulation of the onset of neural crest migration by coordinated activity of BMP4 and Noggin in the dorsal neural tube. *Development* **126:** 4749–4762.

Serbedzija GN, Bronner-Fraser M, Fraser SE (1992). Vital dye analysis of cranial neural crest cell migration in the mouse embryo. *Development* **116:** 297–307.

Shi-wen X, Stanton LA, Kennedy L *et al* (2006). CCN2 is necessary for adhesive responses to transforming growth factor-beta1 in embryonic fibroblasts. *J Biol Chem* **281:** 10715–10726.

Shuler CF (1995). Programmed cell death and cell transformation in craniofacial development. *Crit Rev Oral Biol Med* **6:** 202–217.

Shuler CF, Halpern DE, Guo Y, Sank AC (1992). Medial edge epithelium fate traced by cell lineage analysis during epithelial-mesenchymal transformation *in vivo*. *Dev Biol* **154:** 318–330.

Singh KK, Rommel K, Mishra A *et al* (2006). TGFBR1 and TGFBR2 mutations in patients with features of Marfan syndrome and Loeys-Dietz syndrome. *Hum Mutat* **27:** 770–777.

Sitzman TJ, Girotto JA, Marcus JR (2008). Current surgical practices in cleft care: unilateral cleft lip repair. *Plast Reconstr Surg* **121:** 261e–270e.

Snider P, Olaopa M, Firulli AB, Conway SJ (2007). Cardiovascular development and the colonizing cardiac neural crest lineage. *ScientificWorldJournal* **7:** 1090–1113.

Sorrentino A, Thakur N, Grimsby S *et al* (2008). The type I TGF-beta receptor engages TRAF6 to activate TAK1 in a receptor kinase-independent manner. *Nat Cell Biol* **10:** 1199–1207.

Stenvers KL, Tursky ML, Harder KW *et al* (2003). Heart and liver defects and reduced transforming growth factor beta2 sensitivity in transforming growth factor beta type III receptor-deficient embryos. *Mol Cell Biol* **23:** 4371–4385.

Sudarshan C, Yaswen L, Kulkarni A, Raghow R (1998). Phenotypic consequences of transforming growth factor beta1 gene ablation in murine embryonic fibroblasts: autocrine control of cell proliferation and extracellular matrix biosynthesis. *J Cell Physiol* **176:** 67–75.

Tang Y, Katuri V, Dillner A, Mishra B, Deng CX, Mishra L (2003). Disruption of transforming growth factor-beta signaling in ELF beta-spectrin-deficient mice. *Science* **299:** 574–577.

Taya Y, O'Kane S, Ferguson MW (1999). Pathogenesis of cleft palate in TGF-beta3 knockout mice. *Development* **126:** 3869–3879.

Tolarova M, Harris J (1995). Reduced recurrence of orofacial clefts after periconceptional supplementation with high-dose folic acid and multivitamins. *Teratology* **51:** 71–78.

Tudela C, Formoso MA, Martinez T *et al* (2002). TGF-beta3 is required for the adhesion and intercalation of medial edge epithelial cells during palate fusion. *Int J Dev Biol* **46:** 333–336.

Vieira AR (2008). Unraveling human cleft lip and palate research. *J Dent Res* **87:** 119–125.

Vieira AR, Cooper ME, Marazita ML, Orioli IM, Castilla EE (2007). Interferon regulatory factor 6 (IRF6) is associated with oral-facial cleft in individuals that originate in South America. *Am J Med Genet A* **143A:** 2075–2078.

Vitelli F, Baldini A (2003). Generating and modifying DiGeorge syndrome-like phenotypes in model organisms: is there a common genetic pathway? *Trends Genet* **19:** 588–593.

Wang T, Donahoe PK (2004). The immunophilin FKBP12: a molecular guardian of the TGF-beta family type I receptors. *Front Biosci* **9:** 619–631.

Wehby GL, Cassell CH (2010). The impact of orofacial clefts on quality of life and healthcare use and costs. *Oral Dis* **16:** 3–10.

Wehby GL, Murray JC (2010). Folic acid and orofacial clefts: a review of the evidence. *Oral Dis* **16:** 11–19.

Weinstein M, Yang X, Li C, Xu X, Gotay J, Deng CX (1998). Failure of egg cylinder elongation and mesoderm induction in mouse embryos lacking the tumor suppressor smad2. *Proc Natl Acad Sci U S A* **95:** 9378–9383.

Wong FK, Hagg U (2004). An update on the aetiology of orofacial clefts. *Hong Kong Med J* **10:** 331–336.

Woods A, Pala D, Kennedy L *et al* (2009). Rac1 signaling regulates CTGF/CCN2 gene expression via TGFbeta/Smad signaling in chondrocytes. *Osteoarthritis Cartilage* **17:** 406–413.

Wurdak H, Ittner LM, Lang KS *et al* (2005). Inactivation of TGFbeta signaling in neural crest stem cells leads to multiple defects reminiscent of DiGeorge syndrome. *Genes Dev* **19:** 530–535.

Xu X, Han J, Ito Y, Bringas P Jr, Urata MM, Chai Y (2006). Cell autonomous requirement for Tgfbr2 in the disappearance of medial edge epithelium during palatal fusion. *Dev Biol* **297:** 238–248.

Xu X, Han J, Ito Y, Bringas P Jr, Deng C, Chai Y (2008). Ectodermal Smad4 and p38 MAPK are functionally redundant in mediating TGF-beta/BMP signaling during tooth and palate development. *Dev Cell* **15:** 322–329.

Yamashita M, Fatyol K, Jin C, Wang X, Liu Z, Zhang YE (2008). TRAF6 mediates Smad-independent activation of JNK and p38 by TGF-beta. *Mol Cell* **31:** 918–924.

Yang LT, Kaartinen V (2007). Tgfb1 expressed in the Tgfb3 locus partially rescues the cleft palate phenotype of Tgfb3 null mutants. *Dev Biol* **312:** 384–395.

Yang X, Li C, Xu X, Deng C (1998). The tumor suppressor SMAD4/DPC4 is essential for epiblast proliferation and mesoderm induction in mice. *Proc Natl Acad Sci U S A* **95:** 3667–3672.

Yang X, Letterio JJ, Lechleider RJ *et al* (1999). Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-beta. *EMBO J* **18:** 1280–1291.

Yang LT, Li WY, Kaartinen V (2008). Tissue-specific expression of Cre recombinase from the Tgfb3 locus. *Genesis* **46:** 112–118.

Yu L, Gu S, Alappat S *et al* (2005). Shox2-deficient mice exhibit a rare type of incomplete clefting of the secondary palate. *Development* **132:** 4397–4406.

Yu W, Serrano M, Miguel SS, Ruest LB, Svoboda KK (2009). Cleft lip and palate genetics and application in early embryological development. *Indian J Plast Surg* **42**(Suppl)**:** S35–S50.

Zhang YE (2009). Non-Smad pathways in TGF-beta signaling. *Cell Res* **19:** 128–139.

Zhang Z, Song Y, Zhao X, Zhang X, Fermin C, Chen Y (2002). Rescue of cleft palate in Msx1-deficient mice by transgenic Bmp4 reveals a network of BMP and Shh signaling in the regulation of mammalian palatogenesis. *Development* **129:** 4135–4146.

Zhao H, Oka K, Bringas P, Kaartinen V, Chai Y (2008). TGF-beta type I receptor Alk5 regulates tooth initiation and mandible patterning in a type II receptor-independent manner. *Dev Biol* **320:** 19–29.

Zhu H, Kartiko S, Finnell RH (2009). Importance of gene-environment interactions in the etiology of selected birth defects. *Clin Genet* **75:** 409–423.

Copyright of Oral Diseases is the property of Wiley-Blackwell and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.