Developmental Biology 320 (2008) 19–29

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

TGF-β type I receptor Alk5 regulates tooth initiation and mandible patterning in a type II receptor-independent manner

Hu Zhao [a], Kyoko Oka [a], Pablo Bringas [a], Vesa Kaartinen [b], Yang Chai [a,*]

[a] Center for Craniofacial Molecular Biology (CCMB), School of Dentistry, University of Southern California, 2250 Alcazar Street, CSA 103, Los Angeles, CA 90033, USA
[b] Developmental Biology Program, The Saban Research Institute of Children's Hospital Los Angeles, Los Angeles, CA 90027, USA

ARTICLE INFO

Article history:
Received for publication 30 October 2007
Revised 18 March 2008
Accepted 20 March 2008
Available online 15 April 2008

Keywords:
TGF-β
Alk5
Craniofacial development
Mouse
Mandible
Tooth

ABSTRACT

TGF-β superfamily members signal through a heteromeric receptor complex to regulate craniofacial development. TGF-β type II receptor appears to bind only TGF-β, whereas TGF-β type I receptor (ALK5) also binds to ligands in addition to TGF-β. Our previous work has shown that conditional inactivation of *Tgfbr2* in the neural crest cells of mice leads to severe craniofacial bone defects. In this study, we examine and compare the defects of TGF-β type II receptor (*Wnt1-Cre;Tgfbr2*$^{fl/fl}$) and TGF-β type I receptor/Alk5 (*Wnt1-Cre;Alk5*$^{fl/fl}$) conditional knockout mice. Loss of *Alk5* in the neural crest tissue resulted in phenotypes not seen in the *Tgfbr2* mutant, including delayed tooth initiation and development, defects in early mandible patterning and altered expression of key patterning genes including *Msx1, Bmp4, Bmp2, Pax9, Alx4, Lhx6*/*7 and Gsc*. *Alk5* controls the survival of CNC cells by regulating expression of *Gsc* and other genes in the proximal aboral region of the developing mandible. We conclude that ALK5 regulates tooth initiation and early mandible patterning through a pathway independent of *Tgfbr2*. There is an intrinsic requirement for Alk5 signal in regulating the fate of CNC cells during tooth and mandible development.

Introduction

Transforming growth factor β (TGF-β) belongs to a large superfamily of structurally related proteins including activins, bone morphogenetic proteins (BMPs) and growth differentiation factors (GDFs) (Massague, 1998). TGF-β superfamily members signal through a heteromeric complex consisting of a type I and a type II receptor. Upon ligand binding, type II receptors recruit and phosphorylate type I receptors, which then propagate the signal by phosphorylating Smad proteins. Phosphorylated Smads can form a complex and move into the nucleus, where they act as transcription factors (Shi and Massague, 2003). The number of TGF-β ligands greatly exceeds the number of type II and type I receptors. In humans, there are at least 33 TGF-β ligands; whereas only five type II and seven type I receptors have been found. Combinatorial interactions of different type I and type II receptors in the receptor complexes allow for specificity in ligand binding (Feng and Derynck, 2005). TGF-β ligands bind only to TGF-β RII. TGF-β RI (also known as ALK5) and ALK1 can both function as the type I receptor for TGF-βs and activate different Smad complexes (Feng and Derynck, 2005). In addition, ALK5 can also function as the type I receptor for GDF 8, 9 or 11 (Mazerbourg et al., 2004; Rebbapragada et al., 2003; Oh et al., 2004; Andersson et al., 2006). GDF8 binds ActRIIB and then partners with ALK5 to induce phosphorylation of Smad2/Smad3 (Rebbapragada et al., 2003). GDF9 interacts with BMPRII and ALK5 to phosphorylate Smad2/Smad3 (Mazerbourg et al., 2004). GDF11 interacts with ActRII and ALK5 to phosphorylate Smad2/Smad3 (Andersson et al., 2006). Some alternative downstream pathways for TGF-β have been identified, including MAPK, PI3-kinase, and small Rho-related GTPase (Dudas and Kaartinen, 2005). However, the relationship of these pathways to TGF-β receptors is not clear.

TGF-β is involved in various biological processes including embryonic development, cell proliferation, migration and differentiation, extracellular matrix (ECM) secretion and immunoregulation. During craniofacial development, TGF-β signals play important roles, especially in palatal development. Loss-of-function mutations in *Tgfb2* or *Tgfb3* result in cleft palate (Sanford et al., 1997; Kaartinen et al., 1995; Proetzel et al., 1995). The conventional knockout of *Tgfbr2* results in early embryonic lethality (Oshima et al., 1996), preventing a full phenotypic analysis. The conditional knockout of *Tgfbr2* in cranial neural crest (CNC) cells results in cleft palate, calvaria defect and other craniofacial bone defects (Ito et al., 2003; Sasaki et al., 2006). *Tgfbr1*/*Alk5* conventional knockout mice die around embryonic day 8 (E8) as the result of failed angiogenesis (Larsson et al., 2001).

The first morphological sign of murine tooth development occurs around E11 as a local thickening of the dental epithelium. Incisor initiation begins about one half day earlier than molar initiation. The epithelium invaginates into the underlying condensed CNC-derived mesenchymal cells and forms the tooth bud at E12.5–E13.5. Towards

* Corresponding author.
E-mail address: ychai@usc.edu (Y. Chai).

0012-1606/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2008.03.045

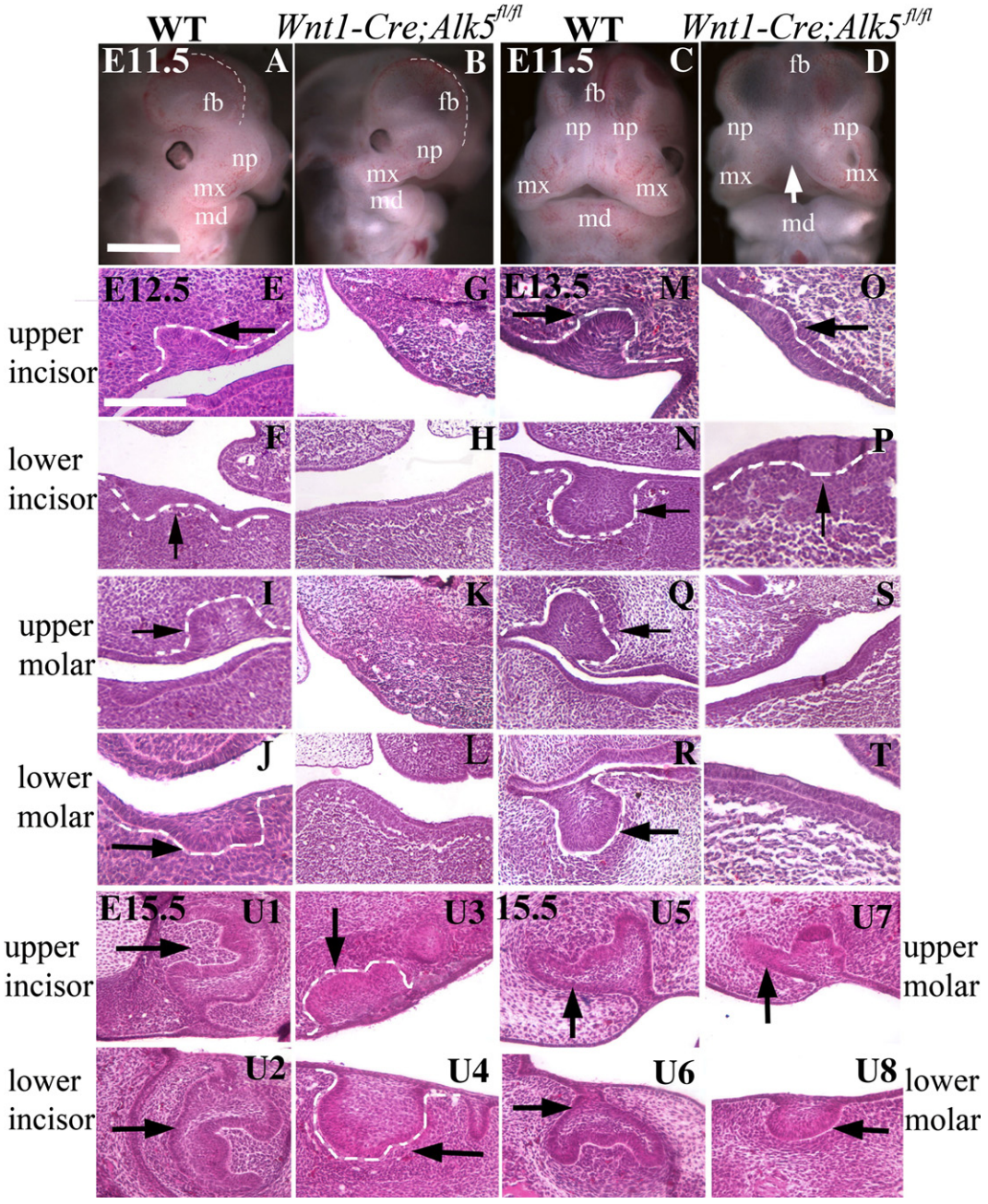

Fig. 1. *Wnt1-Cre;Alk5*$^{fl/fl}$ mutant mice display delayed tooth initiation. (A–D) Side (A, B) and face (C, D) views of E11.5 wild-type and *Wnt1-Cre;Alk5*$^{fl/fl}$ littermate embryos. *Alk5* embryos display abnormal phenotypes including bulging forebrain (outlined with broken lines), facial cleft (indicated by white arrow), small mandible processes and malformed maxilla processes. (E–L) At E12.5, the development of incisors (E, F) and molars (I, J) in both maxilla and mandible processes has reached the lamina stage in wild-type mice. In contrast, no epithelium thickening is detectable in the incisor (G, H) or molar regions (K, L) of *Wnt1-Cre;Alk5*$^{fl/fl}$ embryos. (M–T) At E13.5, incisor (M, N) and molar (Q, R) development has reached the bud stage in wild-type, with condensed mesenchyme tissue surrounding tooth buds. In *Wnt1-Cre;Alk5*$^{fl/fl}$ embryos at E13.5, epithelium thickening is visible at the prospective incisor region (O. P) but not the molar region (S, T). In wild-type embryos at E15.5, incisor (U1, U2) and molar (U5, U6) development has reached the cap stage (U1, U2). In *Wnt1-Cre;Alk5*$^{fl/fl}$ embryos at E13.5, incisor (U3, U4) and molar (U7, U8) development has only reached the bud stage. Arrows indicate position of the tooth germs. Tooth germs are outlined with dotted lines. fb, forebrain; np, nasal process; mx, maxilla process; md, mandible process. Scale bar in panel A=2 mm. Scale bar in panel E=50 μm.

the late bud stage, a signaling center, known as the enamel knot, that controls the morphology of the tooth germ is formed at the tip of the tooth germ (Tucker and Sharpe, 2004). Around E14.5, the tooth bud progresses to the cap stage and tooth morphology is established. Terminal differentiation occurs during the bell stage around E16.5 when the epithelium and mesenchyme differentiate into ameloblasts and odontoblasts, respectively.

The construction of a tooth involves a series of processes including tooth patterning, initiation and morphogenesis. Epithelial–mesenchymal interaction plays a key role in tooth development. The first identified interaction occurs at E9.5–E10.5 in mice, and recombination experiments have defined the oral epithelium as the source of induction signals (Mina and Kollar, 1987). After E11.5–E12.5, the tooth induction ability shifts, such that the mesenchyme then signals back to the epithelium. This shift in inductive ability appears to correlate with a shift of BMP4 expression from the epithelium to the mesenchyme (Tucker and Sharpe, 2004).

The role of TGF-β in early tooth development is not well understood. In part, it is because of the late appearance of TGF-β ligand expression during tooth development and the lack of a tooth phenotype in TGF-β knockout mutants. TGF-β1 expression first appears in the tooth bud at E13 (Vaahtokari et al., 1991). TGF-β2 and