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INVITED REVIEW

# Palatal fusion — Where do the midline cells go? A review on cleft palate, a major human birth defect

**Marek Dudas[a,c], Wai-Yee Li[a], Jieun Kim[a], Alex Yang[a], Vesa Kaartinen[a,b,*]**

[a]*Developmental Biology Program, The Saban Research Institute of Childrens Hospital Los Angeles, Mail Stop 35, 4650 Sunset Blvd., Los Angeles, CA 90027, USA*
[b]*Departments of Pathology and Surgery, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA*
[c]*Institute of Biology and Ecology, P.J. Safarik University in Kosice, Slovakia, EU*

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## Summary

Formation of the palate, the organ that separates the oral cavity from the nasal cavity, is a developmental process characteristic to embryos of higher vertebrates. Failure in this process results in palatal cleft. During the final steps of palatogenesis, two palatal shelves outgrowing from the sides of the embryonic oronasal cavity elevate above the tongue, meet in the midline, and rapidly fuse together. Over the decades, multiple mechanisms have been proposed to explain how the superficial mucous membranes disappear from the contact line, thus allowing for normal midline mesenchymal confluence. A substantial body of experimental evidence exists for cell death, cell migration, epithelial-to-mesenchymal transdifferentiation (EMT), replacement through new tissue intercalation, and other mechanisms. However, the most recent use of gene recombination techniques in cell fate tracking disfavors the EMT concept, and suggests that apoptosis is the major fate of the midline cells during physiological palatal fusion. This article summarizes the benefits and drawbacks of histochemical and molecular tools used to determine the fates of cells within the palatal midline. Mechanisms of normal disintegration of the midline epithelial seam are reviewed together with pathologic processes that prevent this disintegration, thus causing cleft palate.


*Abbreviations:* anoikis, used by some to name the activation of cell death by degradation of the basal lamina; A–P, anterior–posterior; BM, basement membrane (basal lamina, lamina basalis); cataptosis, used by some to name the activation of basal lamina degradation by the cell death within the adjacent epithelium; CL/P, cleft lip and/or palate; CP, cleft palate; GF, GFs, growth factor/factors; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transdifferentiation or transformation; lacZ, $\beta$-galactosidase; ME, medial edge of the palatal shelf; MEE, medial edge epithelium; MED, midline epithelial dysfunction; MES, midline epithelial seam.

*Corresponding author. Tel.: +1 323 669 2212; fax: +1 323 671 3613.
*E-mail addresses:* marek.dudas@upjs.sk (M. Dudas), vkaartinen@chla.usc.edu (V. Kaartinen).

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**Contents**



## Introduction: cleft palate

Cleft palate (CP) is a malformation characterized by a defect in the upper wall of the oral cavity. Nonsyndromic cleft lip and/or palate (CL/P) rank among the most frequent birth defects in humans (Ferguson, 1988; Wyszynski, 2002), occurring in approximately one of 800 live births, with a substantial variation between ethnic groups. In addition, >300 Mendelian disorders have CP within the clinical picture, and multiple nutritional and toxicologic factors have also been identified as causal agents (Hrubec et al., 2006; Murray and Schutte, 2004; Rice, 2005; Wyszynski, 2002). Despite this, the biological mechanisms underlying palatal clefting remain largely unexplained. This paper discusses the current state of our understanding of one of the most important events in formation of the palate – embryonic palatal fusion – failure of which results in CP. In-depth analysis of this topic from the cellular and histochemical aspects is presented, and is accompanied by discussion on challenges that remain in the field.

## Embryonic palatogenesis and the process of palatal fusion

Interestingly, most animals do not possess a palate, thus lacking an anatomical separation between nasal and oral cavities (Chai and Maxson, 2006; Daeschler et al., 2006; Ferguson, 1981; Ferguson et al., 1984; Ferguson, 1988; Shah et al., 1985a, b, 1988, 1990; Shah and Ferguson, 1988). Absence of palate is also initially seen in developing mammalian embryos. During early stages, they have a primitive oral pit, stomodeum, which later becomes an undivided oronasal cavity. Subsequently, embryonic palatogenesis occurs to finish the formation of a separate nose and mouth; this process is unique to higher vertebrates, and is vulnerable to many external and internal influences.

The palate is formed relatively late in organogenesis: during the intrauterine weeks 8–12 in humans (embryonic days E12–E15 in mice). The primary palate (Barteczko and Jacob, 2004) is a very small anterior part of the palate, comprising the anlage of premaxilla (incisive or intermaxillary bone, os Goethei). This part of the palate must fuse with the posterior part, called the secondary palate, which is the major portion of the palate formed by fusion of two maxillary outgrowths named palatal (palatine) shelves. First, palatal shelves appear as protrusions on the lateral walls of the oronasal cavity; both shelves then grow vertically around the tongue (Fig. 1(a)). Later, shelves rapidly (within hours in mice) elevate to a horizontal position above the tongue. Elevation and growth of palatal shelves is mostly driven by changes in the mesenchymal stroma, which is derived largely from neural crest cells that have migrated from the neural tube region into the craniofacial area (Basch et al., 2004; Dudas et al., 2006, 2004b; Farlie et al., 1999, 2004; Jones and Trainor, 2005; Raible and Ragland, 2005; Trainor, 2005).

Further growth of palatal shelves (Fig. 1(b)) is necessary for achieving contact in the midline by their medial edge epithelium (MEE, Fig. 1(c)). This epithelium has a unique ability to recognize the contact, and to become adherent exclusively to the opposite shelf (palatal adhesion). Failure in this respect may result in aberrant adhesion of palatal shelves to improper intraoral structures, thus blocking successful palatogenesis (Alappat et al.,