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Mini Review
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Navigating the Palate Shelf

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Abstract

The separation of oral and nasal cavities by horizontal secondary palate is critical for the formation of mammalian oro-facial complex. This horizontal continuous palate arises from the fusion of two bilateral palate shelves that are originally separate and orientated in vertical direction along both sides of the tongue. In order to form the horizontal palate, the vertical palate shelves have to convert themselves to be horizontal ones that are positioned above the tongue, a process termed palate elevation or palate re-orientation, of which the mechanisms have been poorly understood for decades. However, in the past several years, studies with mouse model system have significantly improves our understanding of the mechanisms controlling palate elevation/re-orientation. This mini review is aiming to summarize the recent progress made in the field focusing on the morphogenetic movements that drive the re-orientation as well as the genes involved.

Keywords: Secondary palate; Palate elevation; Cleft palate; Tissue re-orientation

Overview on Mouse Secondary Palate Formation

The formation of mammalian secondary palate is a multi-step process involving complex embryonic developmental events that must be precisely regulated at molecular, cellular and morphogenetic levels [1-3], and the malformation in palatogenesis could result in cleft palate, a common birth defect, in which the oral and nasal cavities are not separated [1,3,4]. Without special care, cleft palate could be lethal as it may cause a number of severe physiological problems such as breath and feeding. Therefore, researches in this field will have great impacts on both basic developmental biology as well as public health care. In the past decades, studies with model systems, especially mice, have explored significant insights into the mechanisms underlying mammalian palatogenesis and cleft palate.

In mouse development, the first morphological sign of secondary palate formation emerges on embryonic day 11.5 (E11.5). At this stage, a group of mesenchymal cells within the bilateral maxillary processes are specified to be nascent palatal mesenchymal cells that form a region termed palatal outgrowths [1,5]. On E12.5, the nascent palate mesenchymal cells in the two palatal outgrowths move into the oral-nasal cavity to form the mesenchymal block of palatal shelf, and this block is wrapped by epithelial cells [1,3,6]. It is worth to point out that there are two different cell sources for palate shelf, the mesenchymal cells are derived from the neural crests, whereas the epithelial cells are originated from embryonic ectoderm. The resulting palate shelf is positioned along both sides of the tongue and grows vertically until E13.5 as shown in Figure 1. Therefore, the stages from E12.5 to E13.5 can be considered as vertical stages. However, the vertical palatal shelves are converted to be horizontal ones on E14.5 and are positioned above the dorsal side of the tongue (Figure 1) [3,7]. This process has been traditionally called palate elevation, whereas re-orientation is used more and more frequently as it better reflects the process for the reasons elaborated in the sections below. The converted horizontal shelf can be roughly compartmentalized into oral, nasal and medial edge regions and the epithelium also undergo regional specification to form oral epithelium, nasal epithelium and Medial Edge Epithelium (MEE) accordingly. The differentiation of MEE is evident as several genes have been found to be expressed specifically in this area such as MMP13 [8], Cytokeratin 17 and 6 [9]. From E14.5 to E15.0, the two palate shelves continue to grow horizontally towards each until contact along the facial midline (Figure 1). This contact triggers the fusion of two MEEs to form a single seam that will eventually disappear on E15.5 to establish the mesenchymal confluence in the fused palate shelf, a continuous secondary palate that separates the oral and nasal cavities (Figure 1).

From this synopsis, one can already sense the complexity of secondary palate development as it involves tissue initiation, growth, re-orientation, regional specification and cellular fusion, and

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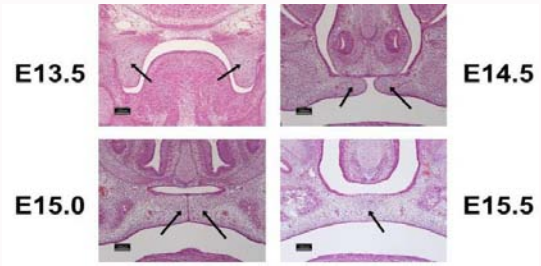

Figure 1: Development of the mouse secondary palate between embryonic day 13.5 (E13.5) and E15.5 showing the palate shelves (arrows) that are vertical on E13.5, horizontal on E14.5, in fusing on E15.0 and fused on E15.5.

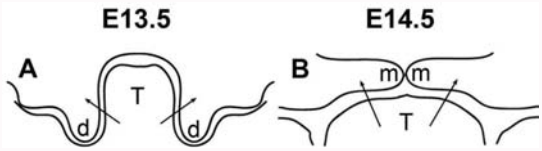

Figure 2: The distal end of vertical palate on E13.5 is traditionally thought to correspond to the future medial edge area on E14.5.
d: Distal end; m: Medial edge.

a comprehensive discussion covering all these aspects is beyond the scope of this mini review. Instead, this article will focus on one particular process that converts a vertical shelf to a horizontal one, namely, palate re-orientation.

Palate Re-Orientation-Morphological Aspect

As briefed above, the conversion of a vertical palate shelf to a horizontal one has been traditionally called palate elevation. When this term was coined, the distal tip of the vertical palate shelf was assumed to be corresponding to the medial edge in the horizontal palate shelf (Figure 2), since these two areas are similar in morphology. Under this assumption, the vertical-horizontal conversion occurs through shelf rotation that flips up the distal end of the vertical shelf to the horizontal position above the tongue [1,3]. In this model, the palate shelf is difficult to overcome the physical obstruction challenged by the tongue, although the forward displacement of the tongue during palate elevation may bring down the level of the tongue [10]. Later studies based on more comprehensive histological observation suggested that the palate tissue may bulge out from the medial side of the vertical shelf instead of "flip-up" or "fold-up" and the palate shelves may "flow" over the tongue during elevation [11,12]. Since these early studies based primarily on morphological observation without regional specific marker at that time, it was difficult to draw definitive conclusions.

One challenge here is that palate elevation occurs very rapidly, and it is difficult to catch up the stages in transition. Our study with mouse *Zeb1* mutant embryos found that palate elevation in this mutant was delayed by 24 to 48 hours, making the palate elevation in slow motion [13]. Using *Zeb1* mutant embryos, we could easily capture a series of images, in which the palate shelves were bulging toward the tongue, supporting the notion that during palate elevation, the tissue grow out from the side of the vertical shelf instead of rotating up. A key question here is to determine the location of prospective medial edge in the vertical palate shelf, since the rotation or "flip up" model assumes that the distal end corresponds to the future medial edge, and if the "bulging of the medial wall" model is correct, the prospective medial

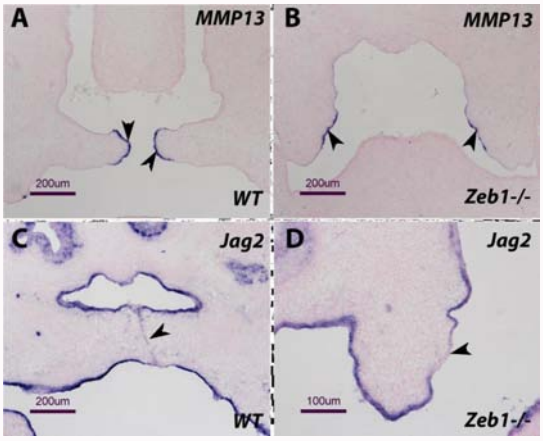

Figure 3: Marker analysis reveals that the prospective MEE is located on the side of the vertical palate shelf rather than the distal end. MMP13 is exclusively expressed in the MEE region in the wild type horizontal palate on E14.5 (arrowheads in A), but in *Zeb1* mutant vertical palate, MMP13 expression domain is located on the side facing the tongue (arrowheads in B). Consistently, Jag2 expression is specifically down-regulated in wild type MEE on E15.5 (arrowheads in C), and in Zeb1 mutant, the loss of Jag2 expression occurs on the side of vertical shelf (arrowheads in D).

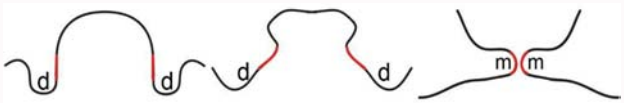

Figure 4: Schemetic illustration of palate re-orientation. The prospective and definitive medial edges are marked by red color.

edge should be located on the side of the vertical shelf instead of distal end. As mentioned above, the MEE region is specified on E14.5 by expressing several MEE specific markers such as *MMP13* [8,14]. In *Zeb1* mutant embryos, the palate elevation is in slow motion, but the epithelial regional specification is apparently in normal pace. On E14.5, *Zeb1* mutant palate shelf is still vertical, the MEE, however, has been specified, as demonstrated by the *MMP13* expression, and interestingly, the *MMP13* expression domain is located on the inner side of shelf (Figure 3), indicating that the prospective MEE is located on the side of the vertical shelf, not the distal end of the shelf [14]. Moreover, the MEE region on E15.5 is also marked by the loss of *Jag2* expression in wild type palate [14], and in *Zeb1* mutant palate, the loss of *Jag2* expression occurs on the inner side of the vertical palate shelf on E15.5 (Figure 3). These molecular evidences, together with previous studies, demonstrated that the prospective MEE is located on the inner side of the vertical palate shelf, not the distal end, and the palate elevation is achieved by outgrowth from the medial wall rather than shelf rotation (Figure 4) [14]. Apparently, the conversion of palate shelf from vertical orientation to horizontal orientation does not involve rotation or elevation, palate re-orientation is therefore a better term to use than palate elevation.

Palate Re-Orientation – Molecular Aspect

Recent studies with mouse genetics have identified an increasing number of genes involving palate re-orientation. As discussed above, loss of *Zeb1* function in mice causes a slower than normal re-orientation, demonstrating its function in controlling the timing of re-orientation [13,15]. Interestingly, the emergence of *Snail* expression is delayed in *Zeb1* mutant palate by 24 hours [14], and loss of *Snail* and *Slug* functions in palate mesenchymal cells leads to failure in palate re-orientation [16], indicating that *Snail* and *Slug*

functions are required for palate re-orientation and are probably responsible for the defects in *Zeb1* mutant mice. The bulge or growth out of the medial wall of vertical shelf must involve cell migration or cell movement, and members of *Snail* gene family are required for mesoderm cell migration during gastrulation [17]. Therefore, it is possible that the function of *Snail* in palate is to promote cells migration and movement during re-orientation. Zeb1 function is associated with TGF-1, 2, [18,19] and consistently, compound mutant embryos of (*Tgf- 1*$^{+/-}$: *Tgf- 2*$^{-/-}$) in C57 or *Tgf- 2*$^{-/-}$ alone in 129 background display re-orientation defects [20]. Similar to *Zeb1* mutant, *Pdgfc* mutant embryos also display vertical palate on E14.5 and only re-orientate after E15.5 [21]. Unlike *Zeb1* mutant mice, the horizontal shelf in *Pdgfc* mutant embryos are truncated indicating an additional growth defect [21]. Wnt5A induces palate mesenchymal cell migration in organ culture, and *Wnt5A* mutant mice suffer defects in palate re-orientation [22]. Also, Gsk-3 function in either mesenchymal or epithelial cells is required for palate re-orientation indicating that palate re-orientation involves epithelial-mesenchymal interactions [23]. In addition, *Prdm16* mutant mice display failed palate re-orientation not due to a secondary defect in the low jaw and tongue [24]. It is foreseeable that the number of the genes identified involving palate re-orientation will grow rapidly, and how to harness these genes together to form pathways and network will be the next major challenge in the field.

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