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Runx1 is involved in the fusion of the primary and the secondary palatal shelves

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ABSTRACT

Runx1 is expressed in medial edge epithelial (MEE) cells of the palatal shelf. Conditionally rescued Runx1$^{-/-}$ mice showed limited clefting in the anterior junction between the primary and the secondary palatal shelves, but not in the junction between the secondary palates. In wild type mice, the fusing epithelial surface exhibited a rounded cobblestone-like appearance, while such cellular prominence was less evident in the *Runx1* mutants. We also found that Fgf18 was expressed in the mesenchyme underlying the MEE and that locally applied FGF18 induced ectopic Runx1 expression in the epithelium of the palatal explants, indicating that Runx1 was induced by mesenchymal Fgf18 signaling. On the other hand, unpaired palatal explant cultures revealed the presence of anterior–posterior (A–P) differences in the MEE fates and fusion mechanism. Interestingly, the location of anterior clefting in *Runx1* mutants corresponded to the region with different MEE behavior. These data showed a novel function of Runx1 in morphological changes in the MEE cells in palatal fusion, which is, at least in part, regulated by the mesenchymal Fgf signaling via an epithelial–mesenchymal interaction.

Introduction

Cleft palate is the most frequent congenital orofacial abnormality. In mammals, the palate is an anatomical structure that forms a barrier between the oral and nasal cavities, which allows breathing and feeding to continue at the same time. The palate is derived from two structures; an anterior portion, the primary palate, and a posterior portion, the secondary palate. The secondary palate also borders the nasal septum superiorly (Ferguson, 1988). The primary palate and the nasal septum are derived from the posterior protrusion of the fronto-nasal process, whereas the secondary palate develops from bilateral outgrowths of the maxillary process (Kaufman, 1992). Palatogenesis consists of multiple steps that require a series of changes in tissue morphology and cell differentiation (Ferguson, 1988; Rice, 2005). These steps begin with the enlargement of the palatal shelves and bilateral outgrowth beside the tongue, then the palatal processes elevate and grow towards the midline. Thereafter, the bilateral palatal processes fuse with each other in the midline and also with the inferior margin of the nasal septum (Ferguson, 1988). In the anterior region, the anteromedial borders of the palatal shelves fuse with the primary palate. Following contact, the medial edge epithelial sheets merge to form the medial edge epithelium (MEE) seam that will soon undergo degeneration and disappear (Ferguson, 1988). Disruption at any step of the process results in the formation of a cleft palate.

The epithelial–mesenchymal interactions during development are essential for the induction of many organs. Early palatal development is one such example, and a recent study has shown that the

Abbreviations: Fgf, fibroblast growth factor; Fgfr, fibroblast growth factor receptor; SEM, Scanning Electron Microscopy; TEM, transmission electron microscopy; Tgf, transforming growth factor; MEE, Medial edge epithelium.

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palatal mesenchyme induces the proliferation of the underlying epithelium, and thus instructs the growth and morphology of the palate (Rice, 2005; Rice et al., 2004). In palatal fusion, early tissue recombination experiments have revealed that the differentiation of the fusing epithelium is also determined by the underlying mesenchyme (Ferguson et al., 1984). However, the nature of this signal remains controversial.

Runx1 is a member of the *Runx* family genes which encode transcription factors that play various important roles in embryogenesis (Ito, 2008). Runx proteins share the 128 amino acid-region termed the Runt domain (RD) required for DNA binding and heterodimerization with Core binding factor beta (CBFβ). Studies of human genetic diseases and an analysis of null allele mice have shown that Runx1 is necessary for hematopoiesis from its definitive stage and it is also the most common target of chromosomal translocations and mutations in human leukemia (Okuda et al., 1996, Okada et al., 1998, Wang et al., 1996; Look, 1997). Runx1 is also involved in the early stage of skeletogenesis (Lian et al., 2003; Yamashiro et al., 2004; Smith et al., 2005) and in the development of a nociceptive neuron subpopulation in the dorsal root ganglion to regulate pain sensitivity (Chen et al., 2006). We have previously found that Runx1 is specifically expressed in the tip of the fusing palatal epithelium during palatogenesis (Yamashiro et al., 2002; Aberg et al., 2004). This finding suggested the possible role of Runx1 in the palatal developmental process. It is interesting to note that patients with a cleft lip and palate also tend to develop acute leukemia more frequently than normal individuals (Nishi et al., 2000; Zhu et al., 2002). Another report has also shown that children with leukemia are more likely to have cleft lip or cleft palate (Zack et al., 1991). These reports support the possible roles of Runx1 in palatal development, however, palatal development in Runx1-deficient mice has not been analyzed because Runx1$^{-/-}$ mice die prior to palatal development at E12.5, due to hemorrhaging in the central nervous system and a complete lack of any definitive hematopoietic cells. To overcome the early embryonic lethality in Runx1$^{-/-}$ mice, we conditionally rescued Runx1 expression under the control of the hematopoietic-specific promoter (Yokomizo et al., 2007). Because of the selective rescue of Runx1, these mice are able to survive until the late embryonic stages.

In the mammals, Fgf signaling regulates various functions during the developmental process. Fgfs act as both epithelial and mesenchymal signals and regulate the epithelial–mesenchymal interactions in the various stages of organogenesis. In early palatogenesis, mesenchymal Fgf10 signals stimulate the epithelium to proliferate via epithelial Ffgr2b and to induce Shh, which signals back to the underlying mesenchyme, thus indicating that Fgf10–Shh signaling regulates the growth of the palatal process, and this signaling is involved in the epithelial–mesenchymal interaction (Rice et al., 2004). Among the several known members of FGFs, *FGF3*, *FGF7*, *FGF10*, *FGF18* have been shown to be associated with their SNPs and nonsyndromic cleft lip and palate (Riley et al., 2007). In fact, *Fgf18*, *Fgf10*, and *Fgfr2b* null-mutant mice present with cleft palate phenotypes resulting from underdevelopment of the palatal shelves (Liu et al., 2002; Rice et al., 2004; Alappat et al., 2005). On the other hand, *Runx2* null mutant mice lack teeth and their tooth development is arrested in an early stage (D'Souza et al., 1999). Runx2 mediates the functions of Fgf signaling via epithelial–mesenchymal interactions. Hair belongs to the group of epidermal appendages, such as the tooth, and its development is also closely regulated by epithelial–mesenchymal interactions. In hair follicles, Runx1 is intensely expressed in the bulge at the telogen stages, as well as in the inner epithelial sheath and its deficiency resulted in zigzag hair (Raveh et al., 2006) and the inactivation of stem cells (Osorio et al., 2008). Various Fgfs are expressed in hair follicles and Fgf18 is specifically expressed in the bulging regions of the telogen hair follicle (Kawano et al., 2005). Interestingly, the subcutaneous administration of FGH18 induced accelerated hair growth (Kawano et al., 2005). From these findings in regard to hair and palatal development, we speculate that the *Runx1* expression might therefore be regulated by Fgfs, especially by Fgf18 via the epithelial–mesenchymal interaction in palatal development.

In the present study, we investigated the functional roles of Runx1 in palatogenesis, and evaluated the upstream signals of Runx1 expression. The palatal analysis of conditionally rescued *Runx1* null mutant mice revealed that Runx1 is involved in the palatal fusion between the primary and the secondary palates. We also found that the Runx1 expression in the palatal epithelium is regulated by mesenchymal Fgf signaling, thus indicating that palatal fusion is regulated by epithelial–mesenchymal interactions via the Fgf–Runx1 pathway. In addition, we explored the anterior–posterior (A–P) difference in morphological changes associated with palatal fusion. Unpaired palatal explants culture revealed the presence of anterior–posterior (A–P) differences in the MEE fates and fusion mechanism. Furthermore, the location of anterior clefting in *Runx1* mutants corresponds to the region with different MEE behavior.

Materials and methods

Animals

Wild type mouse fetuses were obtained from the ICR strain. Runx1$^{-/-}$ mice is lethal due to hemorrhage at about E12.5, when the palatal

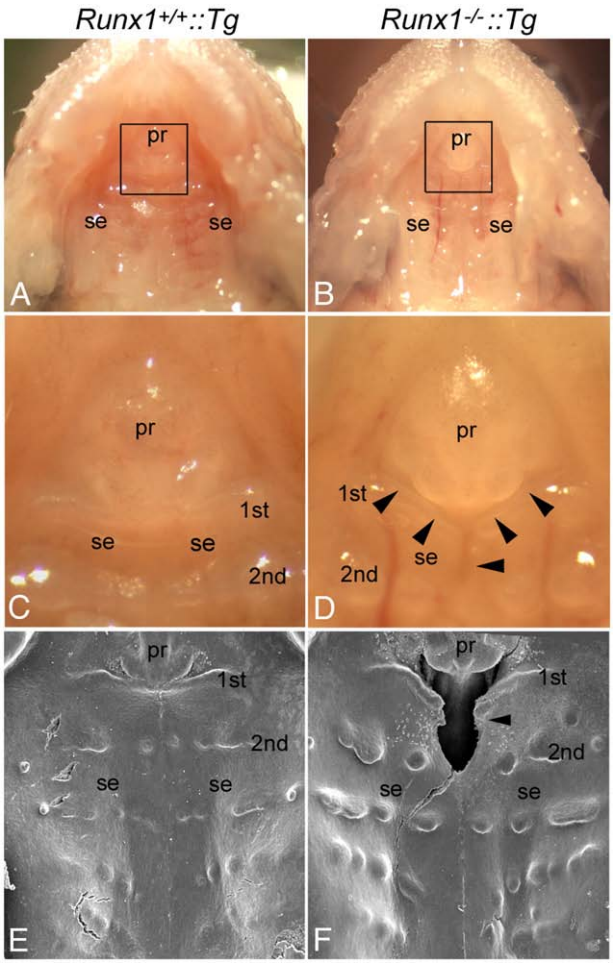

Fig. 1. Phenotype of E17.5 *Runx1*$^{+/+}$*/*::*Tg* control mice (A, C, E), and *Runx1*$^{-/-}$*/*::*Tg* mice (B, D, F). *Runx1*$^{-/-}$*/*::*Tg* mice exhibited partial anterior clefting at the first rugae area and between the primary and the secondary palates (B, D, F), while, control mice showed complete fusion of the palate without any gap between the primary and the secondary palates except at the palatine foramen (A, C, E). (C and D) A higher magnification of the anterior palate (inset in panels A, B). The arrowheads indicate the cleft. pr, primary palate; se, secondary palate; 1st, 1st rugae; 2nd, 2nd rugae; pf, palatine foramen.