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学 位 論 文 名	Runx1 is involved in fusion between the primary and secondary palate （Runx1 は一次口蓋と二次口蓋の癒合に関与する）
論文審査委員	（主査） 教　授　高田　健治 （副査） 岡山大学教授　山城　　隆　　准教授　西村　理行　　講　師　飯田　征二

論 文 内 容 の 要 旨

Introduction

In mammals, the *Runx* gene family consists of three members (*Runx1*, *Runx2* and *Runx3*). They all have a homologous DNA binding site in the promoter area termed the runt domain (RD). For transcriptional regulation, Runx1 forms a heterodimer with Cbf-β required for the DNA binding activity. Runx1 acts as the master regulator of a gene expression in hematopoiesis. *Runx1* is also expressed in the cartilage and epithelium tissues in the craniofacial region. A previous report (Yamashiro *et al.*, 2002) revealed that *Runx1* is expressed in the medial edge epithelium cells of the palatal processes and the expression becomes more intense and localized at the tip of the processes that contact at the midline of the palate. This suggested the possible roles of *Runx1* in palatogenesis.

In the present study, we investigated the functional roles of Runx1 in palatogenesis and the regulatory mechanisms of *Runx1* expression.

Methods

Runx1-deficient mice die prior to palatogenesis due to impaired hematopoiesis. This embryonic lethality was rescued by introducing *Runx1* expression under the control of the GATA-1 promoter in the *Runx1* mutants. *Runx1*$^{-/-}$/∴*Tg* mice survived until late-gestation and the palatal development process can proceed until completion.

Phenotypes of E17.5 *Runx1*$^{-/-}$/∴*Tg* mice were evaluated by direct and histological observations. Ultrastructures of the palatal epitheliums were analyzed by SEM and TEM analysis.

The expression patterns of *Runx1*, *Runx2*, *Runx3* and *Fgf18* mRNA, in the developing palatal tissues of the wild type mice, were identified by *in situ* hybridization.

The possible induction of *Runx1* by Fgf18 protein was identified by the bead experiment. Fgf-18 releasing beads were placed on the palatal explants and cultured. The *Runx1* mRNA expression was then evaluated by *in situ* hybridization.

Results

By direct observation, *Runx1*$^{-/-}$*::Tg* mice resulted in anterior clefting between the primary and the secondary palate, and between the secondary palates at the first ruga region. Histological observation of the anterior clefting showed the palatal processes did not contact each other and the palatal processes did not fuse to the primary palate. In the molar region of the palate, the secondary palate was fused, but the nasal septum did not contact with the secondary palate. Craniofacial phenotypes in bone or cartilage regions were not evident in the *Runx1*$^{-/-}$*::Tg* mice.

In the control mice, an SEM analysis revealed that the fusing epithelial surface exhibited a rounded cobblestone-like appearance. A TEM analysis revealed that the fusing epithelium displayed desquamation of the surface cells and cell blebs prior to fusion. These cell blebs contained large lysosomal bodies in their cytoplasm. In contrast, such changes in the cell surface or in the organelle were not evident in the epithelium of *Runx1*$^{-/-}$*::Tg* mice. These results indicate that the anterior cleft palate in *Runx1*$^{-/-}$*::Tg* mice result from the failure in epithelial fusion.

In situ hybridization showed the strongly expression of *Runx1* mRNA in the fusing epithelium of the primary and secondary palates. *Runx2* mRNA expression was overlapped in the middle part of the secondary palate. While *Runx3* mRNA was not present in the growing palatal processes. It is possible that the anterior clefting in *Runx1* null mutants might be explained by a functional redundancy among *Runx* gene family.

In addition, we also reported that *Fgf18* mRNA was expressed in the mesenchyme underlying the fusing epithelium during palatal fusion. Therefore, we explored the possible induction of Runx1 by Fgf18 protein in bead experiments. Fgf18 beads induced ectopic *Runx1* expression in the epithelium of the palatal explants. These finding indicated that epithelial *Runx1* is induced by mesenchymal Fgf18 signaling.

Conclusions

The palatal phenotypes of conditionally rescued *Runx1* null mutant mice (*Runx1*$^{-/-}$*::Tg*) revealed that *Runx1* is involved in the palatal fusion between the primary and secondary palate. The expression of *Runx1* in the palatal epithelium is regulated by mesenchymal Fgf signaling, thus indicating that palatal fusion between the primary and secondary palate is regulated by epithelial-mesenchymal interactions via the Fgf-Runx1 signaling pathway, at least in part.

論 文 審 査 の 結 果 の 要 旨

本研究の目的は、口蓋形成における転写制御因子 Runx1 の機能的役割と *Runx1* mRNA 発現の調節機構を明らかにすることである。

口蓋形成において *Runx1* mRNA は口蓋癒合部の上皮に発現しており、機能的レスキューを行った *Runx1* ノックアウトマウスを解析した結果、Runx1 が一次口蓋と二次口蓋の上皮の癒合に関与していることが明らかとなった。さらに、器官培養系ならびに *in situ* hybridization 法による検討の結果、上皮の *Runx1* mRNA の発現は、癒合部直下の間葉組織に発現する Fgf18 シグナルによって誘導されることが明らかとなった。これらの結果より、口蓋突起の癒合の分子機構に上皮－間葉相互作用が関与することが明らかとなった。

以上の研究結果は、口蓋裂発生のメカニズムを解明する上で重要な知見を与えるものであり、博士（歯学）の学位を授与するに値するものと認める。