

## WNT11/FGFR1B CROSS-TALK MODULATE THE FATE OF CELLS IN PALATE DEVELOPMENT

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**INTRODUCTION:** Cellular and molecular events like elevation and fusion of the developing palate occur during embryonic development; these complex mechanisms are known to be mediated by cellular modulations<sup>1,2</sup>. In addition, convergent extension, playing an important role in embryogenesis, was observed in palatogenesis<sup>3</sup>. Some signalling molecules, such as *Wnt11* and *Fgfr1*, are closely associated with convergent extension movement during other organogenesis, including mouse kidney development<sup>4</sup>. To investigate the molecular interactions between *Wnt11* and *Fgfr1b*, we employed an over-expression method as a gain of function and treatment with pharmacological inhibitors as a loss of function with an *in vitro* palate culture system. Moreover, we treated *Wnt11* siRNA during *in vitro* palate culture to confirm the functional significance of *Wnt11* in regulating palatal fusion.

**METHODS:** Electroporation of organ cultures; The *Wnt-11* expression construct (1 µg/µl) in PBS buffer was injected into the palatal mesenchyme using a microcapillary needle, and 20 ms current pulses of 25 volts were applied using an electroporator.

Small Interfering RNA (siRNA) treatment; Diluting a siRNA stock (50 µM) with DMEM/F12 medium containing transfection reagent (siPORT<sup>TM</sup>NeoFX<sup>TM</sup>, Ambion) then incubated for 10mins at room temperature. Scrambled control siRNA (Silencer<sup>®</sup> Negative-control siRNA; Ambion) and *Wnt11* siRNA (Silencer<sup>®</sup> Pre-designed siRNA; siRNA ID, 65306; Ambion) were treated at final concentrations of 500 nM<sup>5,6</sup>.

**RESULTS:** Concerning specific morphological phenomenon, we examined expression patterns of *Wnt11* and *Fgfr1b*, which are believed to be key factors in convergent extension, in mouse palate development. *Wnt11* and *Fgfr1b* expression patterns suggest their fundamental importance in palatal growth and fusion. *Wnt-11* over-expression

and SU5402 as a *Fgfr1* inhibitor, containing bead implantation were employed in *in vitro* organ culture. Results revealed that interactions between *Wnt11* and *Fgfr1b* may be important in modulating cellular events, such as cell proliferation for growth and apoptosis for fusion. Moreover, *Wnt11* siRNA results showed that apoptosis, induced by *Wnt11*, was necessary for palatal fusion.

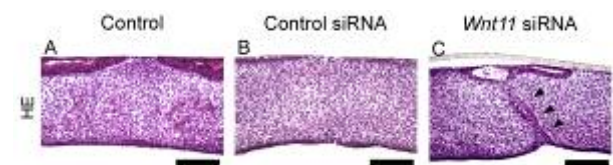


Fig. 1: H-E staining was examined after 500nM siRNA treatment.

**DISCUSSION & CONCLUSIONS:** *Fgfr1b* induced cell proliferation in the developing palate mesenchyme to grow and contact each palatal shelf; negative feedback of Fgfs, triggered by excessive cell proliferation, then inhibited the expression of *Fgfr1b* and activated the expression of *Wnt11* to fuse each palate via activating apoptosis.

**REFERENCES:** <sup>1</sup>Taniguchi, K., Sato, N., Uchiyama, Y. (1995) Arch. Histol. Cytol. 58:191-203. <sup>2</sup>Gritli-Linde, A. (2007) Dev. Biol. 301:309-326. <sup>3</sup>Martinez-Alvarez, C., Tudela, C., Perez-Miguelsanz, J., et al (2000) Dev. Biol. 220:343-357. <sup>4</sup>Majumdar, A., Vainio, S., Kispert, A., et al (2003) Development 130:3175-3185. <sup>5</sup>Shiomi, N., Cui, X.M., Yamamoto, et al (2006) Dev. Dyn. 235:1785-1793. <sup>6</sup>Nakajima, A., Ito, Y., Asano, M., (2007) Dev. Dyn. 236:791-801.