Ex Utero Repair of Cleft Lip Using Targeted Wnt Delivery

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INTRODUCTION: Cleft lip/palate (CLP) is the most common congenital craniofacial anomaly, with a high global incidence. We previously developed a unique compound Pbx-deficient murine model with fully penetrant CLP and demonstrated genetic rescue strategies to reconstitute Wnt signaling and correct midface clefting.[1] We now seek to restore Wnt-mediated craniofacial development programs in our Pbx-deficient embryonic murine model using microsurgical intervention *ex utero***.**

METHODS: Wildtype and Pbx-deficient viable murine embryos were dissected out of the uterus, yolk sac, and amnion pierced open, and the embryos placed in a 37°C modified Whole Embryo Culture (WEC) system. We then fabricated collagen microspheres out of 1% neutralized type I collagen via a double emulsion technique that would serve as a delivery vehicle for Wnt. At gestation day E11.5, collagen microspheres soaked in murine purified Wnt9b protein were microsurgically implanted at the midface lambdoidal (λ)-junction of wildtype and Pbx-compound mutant embryos. Correction of CLP was assessed by gross morphology, histology, and evaluation of the restoration of apoptotic programs. Furthermore, titration assays were conducted to optimize the dose of Wnt by assessing protein content and release kinetics from the microspheres with regard to space and time.

RESULTS: Preliminary results demonstrate that embryos continue to develop normally in this “artificial uterus” for 24 hours, evidenced by normal facial development in cultured embryos. Targeted release of Wnt9b at the λ-junction resulted in augmented Wnt expression compared to the normal endogenous signaling of Wnt. Microsurgical implantation of Wnt soaked microspheres resulted in cleft correction in 25.1% of the Pbx-deficient embryos (an area ratio decrease of more than 50% of clefting on the implanted side when compared to the untreated contralateral side was considered “rescued”). The difference in the area ratio of clefting between implanted and non-implanted embryos was significant ($p<0.05$).

CONCLUSION: Ex utero correction of CLP in our murine model via microsurgical intervention and targeted delivery of Wnt is a promising and innovative strategy. We believe this approach may open new avenues towards unconventional and innovative prenatal interventions for patients with CL/P as well as provide future pre-natal repair approaches for other congenital head and neck disorders.

REFERENCES:

1. Ferretti E., Li B, Zewdu R, et al. A Conserved Pbx-Wnt-p63-Irf6 regulatory module controls face morphogenesis by promoting epithelial apoptosis. Dev Cell. 2011; 21(4): 627-41