Repair of Cleft Lip in Utero by Reactivation of Craniofacial Developmental Programs

Karina A. Hernandez DO, Elisabetta Ferretti PhD, Alyssa J. Reiffel MD, James Hart DVM, Jason A. Spector MD, Licia Selleri MD PhD

Abstract

Background: Cleft lip/palate (CLP) affects 1 in 500-700 live births and is the most common human craniofacial defect. Pbx homeoproteins have been linked to normal craniofacial development through activation of the transcriptional regulators Wnt9-Wnt3, which control apoptotic programs in the embryonic lambdoidal junction of the developing midface. Previous work has demonstrated that compound loss of *Pbx* results in fully penetrant CLP by disruption of *Wnt9b-Wnt3* transcriptional regulation. We sought to restore normal facial morphogenesis in compound *Pbx*-deficient mice via genetic engineering and subsequent reactivation of genetic programs that control apoptosis.

Methods: Mice with conditional Pbx1 inactivation in surface cephalic ectoderm tissues in a Pbx2 deficient background were used. Generation of a Rosa-Wnt1 knock-in allele was achieved via homologous recombination in mouse embryonic stem cells. Pbx1/2 compound mutant mice were crossed to Rosa26-Wnt1 mice and the progeny were then crossed to the *"Crect"* deleter strain (which produces the protein Cre in superficial cephalic ectoderm - SCE - cells) in order to achieve site specific expression of Wnt1 only in the cephalic ectoderm (Figure 1).

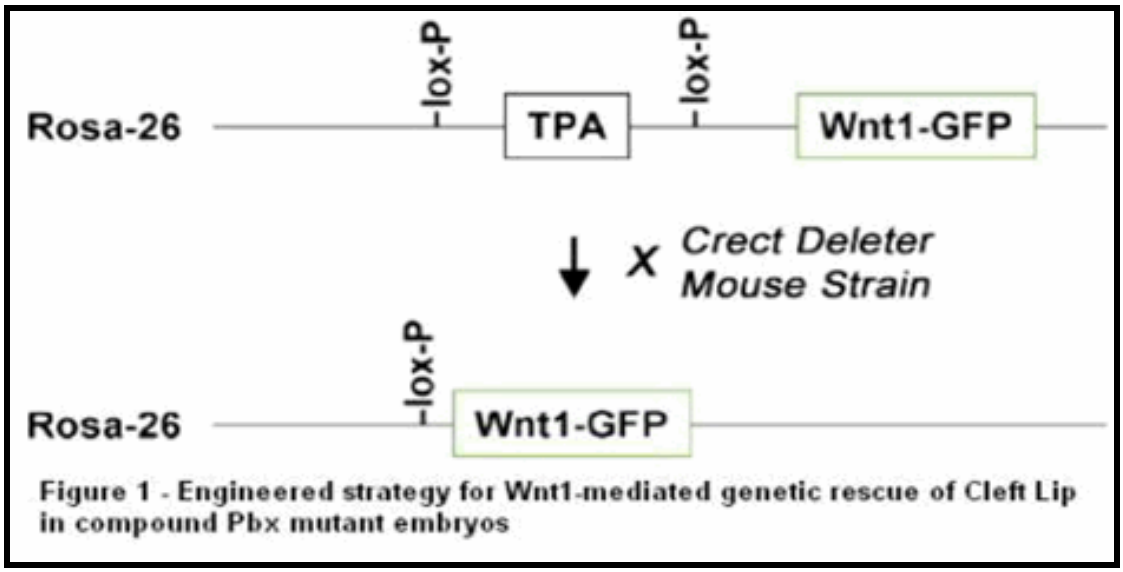

Figure 1 - Engineered strategy for Wnt1-mediated genetic rescue of Cleft Lip in compound Pbx mutant embryos

Results: Progeny with genotype *Pbx1*$^{flox/flox}$*;Pbx2*$^{+/-}$*;Crect*$^{Cre/+}$*;Rosa-Wnt1*, in which *Wnt1* is expressed in SCE cells thus reactivating Wnt signaling at the lambdoidal junction, showed full correction of the cleft lip, while Pbx1/2 compound mutants showed persistent bilateral clefting (Figure 2).

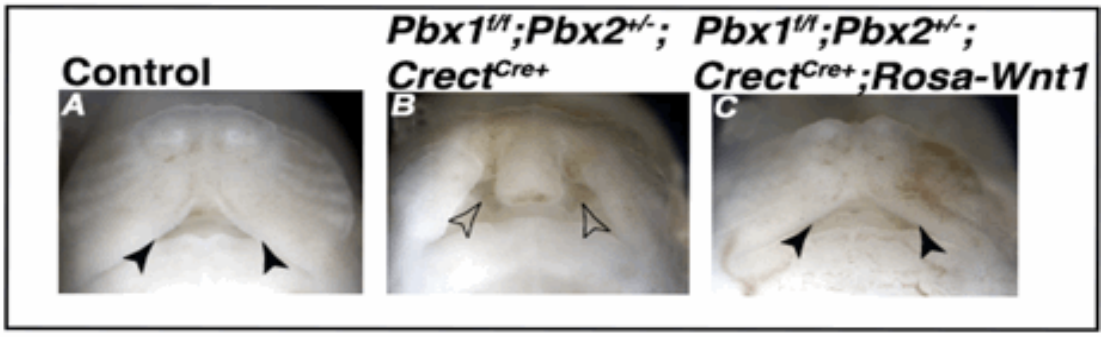

Figure 2: Complete repair of the CL phenotype in Pbx mutant embryo via ectopic expression of Wnt1 in Crect-positive64 SCE cells. Control and rescued lip (A and C; black arrowheads) and CL (B; empty arrowheads).

Conclusion: Our results show it is possible to employ genetic rescue strategies to reconstitute Wnt signaling in *Pbx* compound mutant embryos exhibiting CLP, therefore correcting, at least in part, midfacial clefting. To our knowledge this is the first report of genetic correction of cleft lip in the mouse embryo. Our results will pave the way towards novel approaches for the genetic correction of this disfiguring malformation *in utero*, first in model systems such as mice and sheep, and ultimately in humans.

Disclosure/Financial Support
Supported in part by a Marie Curie Fellowship (OIF-CT-2005-022003) (to Elisabetta Ferreti), March of Dimes and Birth Defects Foundation (6-FY03-071) (to Licia Selleri), National Institutes of Health (RO1 HD43997, 2RO1 HD061403, and R21 DE18031) (to Licia Selleri), Cleft Palate Foundation (to Licia Selleri) and Alice Bohmfalk Trust (to Licia Selleri).

None of the authors have any financial interest with any of the products, drugs or devices mentioned in this manuscript.