Neogenin Regulates Craniofacial Development

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INTRODUCTION

Congenital defects are highly prevalent and are leading causes of infant death. Craniofacial abnormalities make up nearly 20% of all congenital defects occurring at a rate of 3 per 500 births[1]. Craniofacial development is influenced by a wide variety of genetic and environmental factors and although many gains have been made, our knowledge of these factors remains incomplete. Much of the bone and cartilage of the face and skull is derived from a multipotent population of cells termed cranial neural crest cells (CNCCs)[2]. During development, these CNCCs must migrate to the unformed embryonic head and differentiate into cells capable of generating cartilage and bone[3]. Neogenin, a receptor well known for its role in axon guidance, is also capable of influencing cell differentiation, migration, and survival[4]. Interestingly, it has been shown that mice lacking Neogenin exhibit certain craniofacial defects, primarily in formation of the jaw.

RESULTS

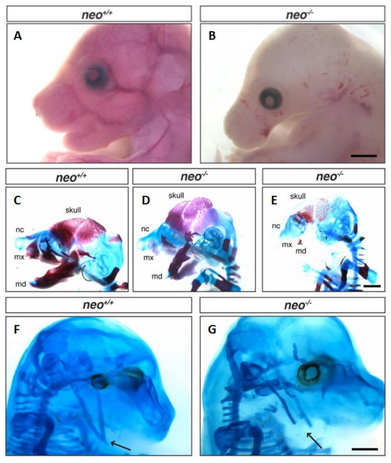

Fig1: Characterizing the craniofacial defects in *neogenin* mutant mice
(A,B) Gross morphology of E17.5 *neo*$^{+/+}$ and *neo*$^{-/-}$ littermates.
(C-E) Analysis of the bone and cartilage formation in control and *neo*$^{-/-}$ littermates with combined staining of Alizarin Red to label bones and Alcian Blue to stain for cartilage.
(F,G) Characterization of the cartilage development of Neogenin littermates at E14.5. Although cartilage formation is mainly not affected, Meckel's cartilage (Arrows) does not fuse. Md: Mandible, MxR: Maxillary Region, NS: Nasal Cavity. Scale bar: 2mm.

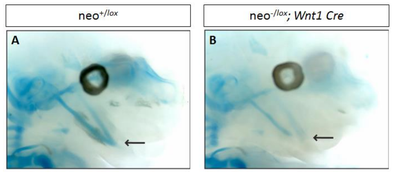

Fig2: Failure in Meckel's cartilage fusion is observed in the *neo*$^{-/lox}$*; Wnt1 Cre* mice conditional mice
(A,B) Characterization of the cartilage development in of *neo*$^{-/lox}$*; Wnt1 Cre* conditional littermates at E14.5. As observed in the *neo*$^{-/-}$, cartilage formation of the cranial skeleton is mostly unaffected, but the Meckel's cartilage (Arrows) fails to fuse.

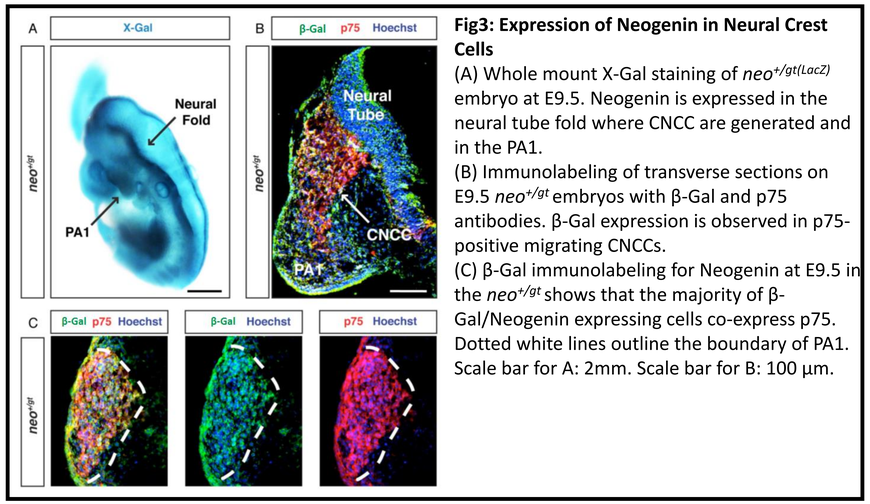

Fig3: Expression of Neogenin in Neural Crest Cells
(A) Whole mount X-Gal staining of *neo*$^{+/gt(LacZ)}$ embryo at E9.5. Neogenin is expressed in the neural tube fold where CNCC are generated and in the PA1.
(B) Immunolabeling of transverse sections on E9.5 *neo*$^{+/gt}$ embryos with β-Gal and p75 antibodies. β-Gal expression is observed in p75-positive migrating CNCCs.
(C) β-Gal immunolabeling for Neogenin at E9.5 in the *neo*$^{+/gt}$ shows that the majority of β-Gal/Neogenin expressing cells co-express p75. Dotted white lines outline the boundary of PA1. Scale bar for A: 2mm. Scale bar for B: 100 µm.

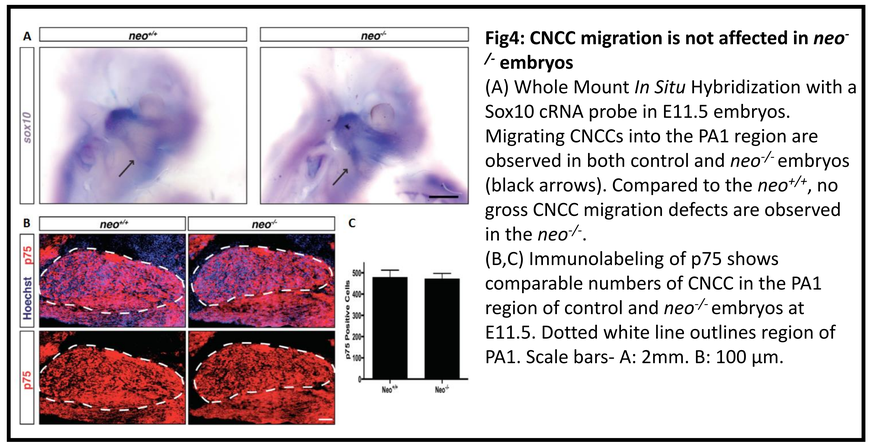

Fig4: CNCC migration is not affected in *neo*$^{-/-}$ embryos
(A) Whole Mount *In Situ* Hybridization with a Sox10 cRNA probe in E11.5 embryos. Migrating CNCCs into the PA1 region are observed in both control and *neo*$^{-/-}$ embryos (black arrows). Compared to the *neo*$^{+/+}$, no gross CNCC migration defects are observed in the *neo*$^{-/-}$.
(B,C) Immunolabeling of p75 shows comparable numbers of CNCC in the PA1 region of control and *neo*$^{-/-}$ embryos at E11.5. Dotted white line outlines region of PA1. Scale bars- A: 2mm. B: 100 µm.

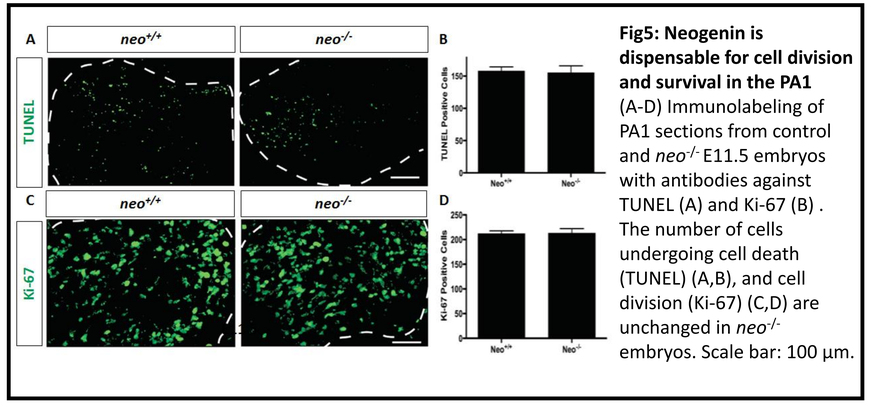

Fig5: Neogenin is dispensable for cell division and survival in the PA1
(A-D) Immunolabeling of PA1 sections from control and *neo*$^{-/-}$ E11.5 embryos with antibodies against TUNEL (A) and Ki-67 (B) . The number of cells undergoing cell death (TUNEL) (A,B), and cell division (Ki-67) (C,D) are unchanged in *neo*$^{-/-}$ embryos. Scale bar: 100 µm.

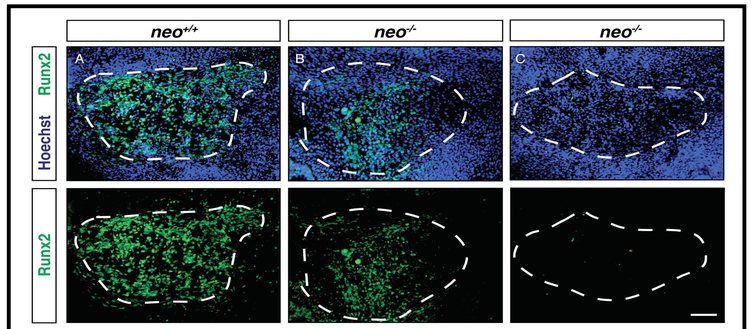

Fig6: Altered Runx2 expression in *neo*$^{-/-}$ embryos
(A,B,C) Immunolabeling for Runx2 at E14.5. (A) Immunohistochemistry labeling for Runx2 in Neogenin littermates. Coronal sections through the mandible show that expression of Runx2 is down regulated in the *neo*$^{-/-}$ (B,C), and *neo*$^{-/-}$ that have more severe mandible phenotypes (C) exhibit a lower level of Runx2 expression compared to the control. White lines show region of volumetric bone mass. Scale bar: 100 µm.

CONCLUSIONS

- Neogenin is involved in craniofacial development
- Defects in craniofacial development are, in part, due to loss of Neogenin from CNCCs
- Neogenin does not seem to be required for migration, survival, or proliferation of CNCCs
- Neogenin mutant mice display decreased Runx2 expression in their developing mandibles, suggesting Neogenin may regulate differentiation of CNCCs into osteoblasts

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