Disruption of ERK/MAPK Pathway in Neural Crest Causes Pierre-Robin Sequence.

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Objectives: Disrupted ERK1/2 signaling is associated with several developmental syndromes in humans. For instance, haploinsufficient ERK2 expression causes conotruncal and craniofacial anomalies arising from perturbation of neural crest development. The goal of this study is to understand the function of Erk2 in the postmigratory neural crest populating the craniofacial region during mouse development.

Methods: We studied *Wnt1-Cre;Erk2*$^{fl/fl}$, *Osr2-Cre*;*Erk2*$^{fl/fl}$, *Col1-CreERT2;Bmpr1a*$^{fl/fl}$, and *Dlx5-CreERT2;Bmpr1a*$^{fl/fl}$ mice.

Results: *Wnt1-Cre*;*Erk2*$^{fl/fl}$ mice exhibited cleft palate (CP), malformed and malpositioned tongues, compromised tendon development, micrognathia and mandibular asymmetry. CP in these mutants was associated with failure of palatal elevation, caused by the tongue malposition and micrognathia as demonstrated by *in vitro* and *in vivo* experiments. *Osr2-Cre*;*Erk2*$^{fl/fl}$ mice in which the mutation is restricted to the palatal mesenchyme did not display CP, confirming that CP in *Wnt1-Cre;Erk2*$^{fl/fl}$ mice is a secondary defect. The tongue phenotypes in *Wnt1-Cre;Erk2*$^{fl/fl}$ mice were significantly rescued after *in vitro* culture in which the mandible was removed,

indicating that the tongue malformation in *Erk2* mutants might be a secondary defect. The primary malformation, i.e., micrognathia with mandibular asymmetry, in the *Wnt1-Cre;Erk2*$^{fl/fl}$ mice was linked to a severe osteogenic differentiation defect occurring right before palatal shelf elevation. Microarray analyses showed that *Bmp6* was downregulated in *Wnt1-Cre-Erk2*$^{fl/fl}$ mandibles at the onset of osteogenesis, with consequent downregulation of BMP downstream targets such as *Msx1* and *Id*s. Accordingly, exogenous BMP6 rescued the osteogenic differentiation defect in mandibular explants from *Wnt1-Cre;Erk2*$^{fl/fl}$ embryos.

Conclusions: Collectively, our study demonstrates that the mandibular defect in *Erk2*-mutant mice leads to Pierre-Robin sequence and that Erk2 regulates *Bmp6* expression in the mandibular primordium to control osteogenesis.

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