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;Erk2β/−, Osr2-Cre;Erk2β/−, Coll1-CreERT2;Bmpr1aα/−, and Dlx5-CreERT2;Bmpr1aβ/− mice.

Results: Wnt1-Cre;Erk2β/− 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;Erk2β/− mice in which the mutation is restricted to the palatal mesenchyme did not display CP, confirming that CP in Wnt1-Cre;Erk2β/− mice is a secondary defect. The tongue phenotypes in Wnt1-Cre;Erk2β/− 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<sup>0/0</sup> 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<sup>0/0</sup> mandibles at the onset of osteogenesis, with consequent downregulation of BMP downstream targets such as Msx1 and Ids. Accordingly, exogenous BMP6 rescued the osteogenic differentiation defect in mandibular explants from Wnt1-Cre;Erk2<sup>0/0</sup> 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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