SIX1 regulates hinge patterning during mandible/maxilla development.

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Six1 is a homeodomain protein that, together with Eya co-activators, promotes proliferation, survival and differentiation of progenitor cells during development and cancer. Mutations in *SIX1* are linked to branchiootic syndrome (BOS3) in humans, though its role in jaw morphogenesis in unclear. To examine this question, we examined facial development in *Six1*$^{-/-}$ mutant embryos. *Six1*$^{-/-}$ embryos die at birth with severe craniofacial malformations that include transformation of the posterior region of the maxilla into a rod-shaped bone. This transformation is preceded by expansion of *Dlx3 and Dlx5*, genes associated with jaw development and induced by Endothelin-A receptor (EDNRA) signaling, into the proximal portion of the first pharyngeal arch and downregulation of maxillary-associated genes *Dlx2* and *Twist1*. EDNRA signaling establishes the identity of neural crest cells (NCCs) in the mandibular portion of first pharyngeal arch, due in part to exclusion of the EDNRA ligand EDN1 from the proximal and maxillary first arch. We found that transgenic overexpression of Edn1 in maxillary NCCs (*CBA-Edn1;Wnt1-Cre*) resulted in similar gene expression changes seen in *Six1*$^{-/-}$ embryos. Indeed, deletion of one allele of *Ednra* in a mutant *Six1* background (*Six1*$^{-/-}$*;Ednra*$^{+/-}$) rescued the *Six1* mutant jaw phenotype. Interestingly, overexpression of Six1 in cell culture resulted in upregulation of *Jagged1* (a mediator of maxillary NCC identity). Additionally, *Six1*$^{-/-}$ embryos show decreased *Jagged1* and *Hey* expression in the “hinge” region in the proximal first arch. These changes in *Six1*$^{-/-}$ embryos cause an expansion of *Prrx1* and *Barx1* expression and decrease of *Pou3f3* expression similarly to changes in *CBA-Edn1;Wnt1-Cre* embryos. Our results suggest that SIX1 by regulating JAG/NOTCH signaling in the “hinge” region of the arch controls DV patterning for proper mandible and maxilla development. Work funded by NIH/NIDCR DE018899 and DE023050.