Role of histone methyltransferase Nsd1 in craniofacial skeletal development

Katherine Shei, Chiaki Arai, DDS, PhD, Noriaki Ono, DDS, PhD

Department of Diagnostic and Biomedical Sciences, UTHealth Houston School of Dentistry

Objectives: Histone methylation plays an important role in regulating gene expression. *NSD1* (nuclear receptor binding SET domain protein 1) encodes a histone methyltransferase that adds methyl groups to histone proteins. Heterozygous loss of function of *NSD1* causes Sotos syndrome (OMIM #117550), which is associated with overgrowth of the central nervous system and craniofacial deformities. However, the role of NSD1 in craniofacial skeletal development remains undefined. We hypothesize that NSD1 inactivation causes craniofacial bone deformities.

Experimental Methods: We analyzed the skull of *Nsd1* conditional knock-out mice at postnatal day (P)21. In these mice, *Nsd1* was specifically deleted in Prrx1-expressing osteochondral progenitor cells using *Nsd1*-floxed alleles with *Prrx1*-cre and a *Rosa26-tdTomato* reporter allele for cell fate mapping. Three littermate samples of *Prrx1-cre; Nsd1*$^{fl/+}$ (Control) and *Prrx1-cre; Nsd1*$^{fl/fl}$ (Nsd1-cKO) mice were analyzed with a dental x-ray (Mobile-X). Measurements of the maxilla and mandible were taken using cephalometric landmarks and ImageJ. The samples were cryosectioned after decalcification, immunostained for chondrocyte marker SOX9, and imaged using an automated fluorescence microscope (Zeiss). The samples were also stained with H&E and Safranin O to highlight structural changes in bone and cartilage. Chromogenic images were captured with a whole-slide scanner (Hamamatsu NanoZoomer).

Results: Nsd1-cKO mice presented a significantly hypoplastic maxilla associated with significantly reduced cartilages in the spheno-occipital synchondrosis (SOS) in the cranial base. Interestingly, *Prrx1-cre*-marked tdTomato^{+} cells were only present in the Nsd1-cKO SOS, indicating a defect in the transition from cartilage to bone.

Conclusion: Inactivation of *Nsd1* results in a hypoplastic maxilla and shorter cranial base, therefore possibly causing the craniofacial deformities seen in Sotos Syndrome. Deficient chondrocyte differentiation in the cranial base synchondrosis likely prevents the bidirectional outward growth of the cartilaginous zones, leading to a hypoplastic smaller cranial base of NSD1 mutants.

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