Role of histone methyltransferase Nsd1 in craniofacial skeletal development

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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 Prx1-expressing osteochondral progenitor cells using Nsd1-floxed alleles with Prx1-cre and a Rosa26-tdTomato reporter allele for cell fate mapping. Three littermate samples of Prx1-cre; Nsd1^fl/+ (Control) and Prx1-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, Prx1-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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