Nobel mice models provide the new insights of pathogenesis for multiple hereditary exostoses
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Introduction: Multiple hereditary exostoses (MHE) results in severe skeletal malformations, including multiple bony tumors, short stature, and limb length inequalities. Genetic linkage by using extended families and polymorphic markers has defined three loci as being associated with the disease: EXT1, EXT2, and EXT3. Although mutations in EXT genes have been linked to MHE, it is poorly understood how they lead to the formation of exostoses. One of the reasons is that heterozygote mice of Ext1 or Ext2 have never shown the limb exostoses. Ext1 has been classified as a tumor suppressor gene and somatic Loss of Heterozygosity (LOH) or secondary mutations in an EXT homolog have been hypothesized to give rise to the isolated exostoses. Here, we successfully establish the two novel mice models for MHE and show the direct evidence that the only a few, but biallelic Ext1 inactivation is necessary to give rise the multiple exostoses. Furthermore, we show one of the possible cellular origins for osteochondromas.

Materials & Methods: Mice. Creation of the loxP-flanked Ext1' allele has been described previously. To produce conditional Ext1 mutant mice, Col2-CreERT and FSP1-Cre transgenic mice were mated.

Radiologic Analysis. 28-days-old mice, dissected fore- and hind-limbs were analyzed by X-ray with a Faxitron MX-20 DC4.

Skeletal analysis. For whole-mount analysis of skeletons, mice were eviscerated and fixed in 95% ethanol overnight. The preparations were stained with Alcian blue and Alizarin red for standard procedure.

Histology and immunohistochemistry. Samples were embedded in paraffin, decalcified in EDTA, and sectioned at 5 µm. For Safranin O/Fast Green staining, sections were stained in 0.02% aqueous Fast Green, followed by 0.1% aqueous Safranin O.

Results

Generation of mice model for MHE. To generate the mice model for MHE, we employed a method of stochastic inactivation of loxP-flanked Ext1 alleles (Ext1') using a tamoxifen-dependent Cre transgene driven by the Col2a1 promoter. Col2-CreERT, Ext1'/' mice developed multiple osteochondromas and other MHE-like skeletal defects without tamoxifen treatment. (Throughout this paper, Col2-CreERT, Ext1'/' mice that were raised without tamoxifen treatment are designated as Ext1-SKO [stochastic knockout] mice.) Multiple bony protrusions involving the wrist, fibula, shoulder, and rib were identified by radiographic and macroscopic observations (Fig. 1A, 1B). Histological examination revealed bony tuberosities with a cartilage cap (Fig. 1C), which is consistent with the histological features of osteochondromas. These Ext1-SKO mice showed short stature, bowing deformity of the forearm, subluxation/dislocation of the radius, and scoliosis. These results show that Ext1-SKO mice phenocopy the skeletal defects of MHE quite faithfully.

Recombination efficiency of Col2-CreERT without tamoxifen during postnatal development. We hypothesized that the phenotypes observed in Ext1-SKO mice are caused by leaky Cre activity. To test this hypothesis, we analyzed the spatiotemporal recombination pattern of the Col2-CreERT transgene using the Rosa26-lacZ reporter. Postnatally, a small number of lacZ' clusters were found in the femoral head cartilage at P0, and the number of lacZ' clusters showed no significant increase during the postnatal period.

Genetic characterization of osteochondromas in Ext1-SKO mice. The fact that the stochastic biallelic inactivation of Ext1 leads to the formation of multiple osteochondromas led us to examine whether osteochondromas are clonal growth of Ext1-null chondrocytes. To address this issue, we first analyzed DNA samples isolated from the cartilage cap region of osteochondromas by laser capture microdissection (LCM). To confirm %Ext1' ratio, we performed semi-quantitative analysis using the standard allele-specific PCR protocol. Surprisingly, this analysis revealed that all 9 osteochondromas examined were heterogeneous, containing both the recombined and intact alleles. The ratio between the recombined Ext1' and intact Ext1 alleles was variable, and in most samples examined, the Ext1' allele represented a minor proportion of the total Ext1 alleles present. FSP1-expressed cells are one of the possible cellular origins for osteochondroma. To understand the role of heparan sulfate in stroma, we crossed Ext1' mice with FSP1-Cre transgenic mice. Surprisingly, these mutant mice showed multiple exostoses in their limbs, and vertebrae postnatally. 3D-CT revealed the multiple bony protuberances at the end of the long bones (Figure 2). These results indicate that FSP1-expressed cells are one of the possible cellular origins for osteochondroma.

DISCUSSION: In this paper, we show that Ext1-SKO mice recapitulate many of the key features of human MHE. Most importantly, this is the first mouse model that develops multiple osteochondromas in long bones, which is the hallmark of MHE and which could not be reproduced previously in Ext1 or Ext2 heterozygous mutant mice. The Ext1-SKO model has provided novel insights into the pathogenic mechanism of MHE. It has been debated that whether the LOH at Ext1 underlies the pathogenesis of MHE. Our present findings lend a strong support to the hypothesis that LOH occurring in a small population of chondrocytes plays a role in the development of osteochondromas in MHE. The requirement for biallelic inactivation is further supported by the total absence of osteochondromas in mice in which only one copy of Ext1 is inactivated either in a small fraction of chondrocytes. Surprisingly, FSP1-Cre; Ext1'/' mice showed the multiple exostoses phenotype. These mice model provides us a critical knowledge to understand the pathogenesis of MHE, but also potentially uncover a novel mode of action of heparan sulfate in tissue development.

REFERENCES: