

G Protein Couple Estrogen Receptor-1 (gper-1) Regulates The Proliferation And Hypertrophy Of Chondrocytes During Mouse Endochondral Ossification

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ABSTRACT INTRODUCTION:

The proliferation and hypertrophy of chondrocytes play important roles in endochondral ossification of growth plate, which is tightly regulated during long bone elongation. This study focused on exploring the impact of the deletion of G protein-coupled estrogen receptor-1 (GPER-1) on the regulation of chondrocyte proliferation and hypertrophy within the growth plate. Previous research has demonstrated that global GPER-1 deletion female mice displayed inhibited bone growth, while male mice experienced enhancement. These results indicate that the specific roles of GPER-1 in bone growth regulation are not fully understood. To overcome the limitations associated with the global GPER-1 knockout mice, which might lead to various developmental issues, we generated mice lacking GPER-1 specifically in chondrocytes. Our hypothesized that GPER-1 might play a role in regulating endochondral ossification within the growth plate and subsequently influence the longitudinal growth of long bones.

METHODS:

We purchased the GPER-1^{tm1c} mice from KOMP and crossed with collagen 2a-Cre mice from Jackson Lab. The offspring intercrossed to obtain Col2a-Cre; GPER-1^{fl/fl} mice (KO, Fig1A). The Institutional Animal Care and Use Committee approved all the animal studies. Sulfated glycosaminoglycan (sGAG) was stained with Safranin O / Fast Green. Detecting protein expression in growth plate cartilage by immunohistochemistry (IHC), including GPER-1, type II collagen, Ki67, type X collagen, and IHH.

RESULTS SECTION:

In the KO mice, Cre was expressed only in chondrocytes, which expressed type II collagen. Genotyping performed by PCR using tail genomic DNA (Fig. 1B). Both the control and KO groups had GPER-1 floxed alleles (507 bp). Only KO mice expressed Cre recombinase under the type II collagen-specific promoter (420 bp). KO mice had a reduced GPER-1 protein expression in the chondrocytes but not in cortical bone tissue (Fig. 1C). In growth plate cartilage, the number of GPER-1 positive cells decreased by approximately 82% in CKO mice. Male KO mice exhibited reduced body length (crown-rump length) at 6 and 8 weeks of age, while female KO mice displayed diminished body length at 4, 6, and 8 weeks of age compared to the control mice (Fig. 2A). In both male and female mice, the tibial length of KO mice exhibited a significant decrease at 8 weeks of age compared to control mice (Fig. 2B). In the structure of growth plate, the thickness of the proliferative zones was decreased and the hypertrophic zones were increased in the KO mice compared to control mice (Fig. 2C). The proliferative chondrocytes stained by Ki67-IHC was significantly reduced in KO mice and the hyperopic chondrocytes stained by type X collagen and IHH-IHC were significantly increased in KO mice (Fig. 3).

DISCUSSION:

During endochondral ossification, a growth plate forms where chondrocytes proliferate, transforming into bone-replacing hypertrophic chondrocytes. The potential for circulating testosterone to convert into estrogen indicated that GPER-1 could be influenced both in males and females. We employed the chondrocyte-specific GPER-1 knockout model to investigate the response of growth plate chondrocytes following GPER-1 deficiency. In both male and female KO mice, we noted a substantial reduction in proliferative chondrocytes alongside a noteworthy expansion of the hypertrophic zone stained with type X collagen. Notably, GPER-1 deficiency heightened the expression of IHH. These findings substantiate the physiological function of GPER-1 in governing chondrocyte proliferation and hypertrophy within the growth plate, consequently influencing bone and body length.

SIGNIFICANCE:

GPER-1 might promote chondrocyte proliferation and inhibit hypertrophy during skeletal development. The effects of GPER-1 regulating growth plate development were effective both in males and females.

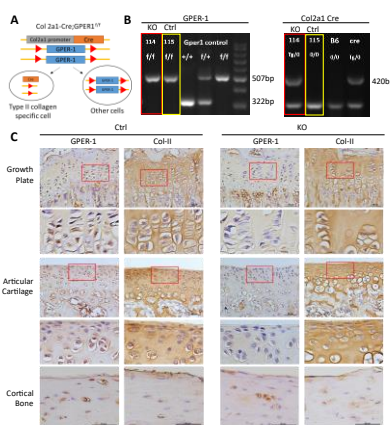


Fig 1. Generation of chondrocyte-specific knockout mice using the Cre-loxP system. (A) Schematic diagram of Col2a1-Cre; GPER-1 KOMP-/- mice. (B) Genotyping of Col2a1-Cre and GPER-1 was showed on control (Ctrl) and GPER-1 knockout (KO) mice. (C) (E) GPER-1 and type II collagen (Col-II) were analyzed in tibia growth plate, articular cartilage, and cortical bone by IHC staining.

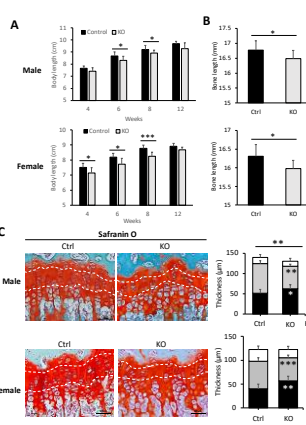


Fig 2. Effects of GPER-1 knockout on phenotype change in male and female mouse. (A) Body length, and (C) bone length were decreased during sexual maturation. (C) The representative microslides and quantification of thicknesses in resting zone (RZ), proliferation zone (PZ) and hyperopic zone (HZ) by Safranin O staining.

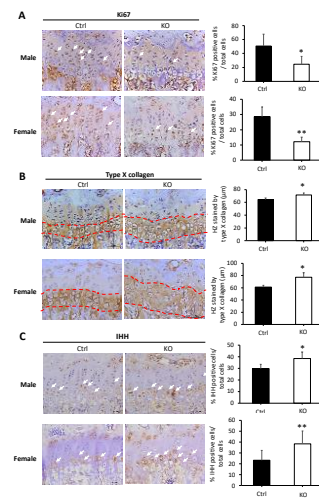


Fig 3. Effects of GPER-1 knockout on regulation of chondrocytes proliferation and hypertrophy. (A) The representative microslides and quantification of proliferative chondrocytes stained by Ki67-IHC. (B) The hyperopic zone stained by type X collagen-IHC. (C) The representative microslides and quantification of IHH-labeled chondrocytes.