Histological Analysis of Cartilage Abnormalities and Therapeutic Effects in Hyp Mice, a Model of XLH

Takuya Ogawa[1,2], Tomohiro Shimizu[1], Tomoka Hasegawa[2], Tomomaya Yamamoto[2], Norio Amizuka[2], Norimasa Iwasaki[1]
[1]Department of Orthopedic Surgery, Hokkaido University Graduate School of Medicine
[2]Department of Ultrastructure of Hard Tissue, Hokkaido University Graduate School of Dental Medicine
Email of Presenting Author: tkyoztf@yahoo.co.jp

Disclosures: Takuya Ogawa (N), Tomohiro Shimizu (N), Tomoka Hasegawa (N), Tomomaya Yamamoto (N), Norio Amizuka (N), Norimasa Iwasaki (N)

INTRODUCTION: X-linked hypophosphatemic rickets (XLH) is caused by PHEX mutations that induce excessive FGF23 secretion, hypophosphatemia, and impaired mineralization. While defective mineralization is well recognized, the contribution of growth plate cartilage abnormalities to skeletal deformities remains insufficiently understood. We hypothesized that XLH-associated growth plate pathology is driven by aberrant IHH–PTHrP signaling, coupled with impaired vascular invasion, resulting in chondrocyte hyperproliferation and disorganized endochondral ossification. This study aimed to characterize these mechanisms in Hyp mice and evaluate therapeutic interventions targeting both mineral metabolism and growth plate signaling.

METHODS: Male Hyp mice and wild-type littermates, aged 8 weeks, were subjected to comprehensive analysis. Femora were examined histologically using hematoxylin-eosin, von Kossa, and immunohistochemistry of PTHrP, IHH, VEGF, endomucin. Bulk RNA sequencing was performed to identify altered signaling pathways, and candidate genes were validated by quantitative PCR. For therapeutic evaluation, additional Hyp mice were randomized into four groups and treated for 4 weeks with vehicle, alfacalcidol (400 pmol/kg/day, 5 times/week), anti-PTHrP neutralizing antibody (75 µg, twice weekly), or a combination regimen. Histomorphometric analysis of growth plate cartilage thickness, chondrocyte column organization, and vascular invasion into the hypertrophic zone was performed, and quantitative differences between groups were assessed using ANOVA followed by Tukey–Kramer post-hoc tests ($p<0.05$ considered significant).

RESULTS SECTION: Hyp mice exhibited markedly thickened growth plate cartilage with disrupted columnar organization and significantly upregulated IHH–PTHrP signaling. VEGF expression in growth plate cartilage was reduced by approximately 40% compared with wild-type, and endomucin staining revealed markedly impaired vascular invasion at the osteo-chondral junction of Hyp mice. In therapeutic cohorts, alfacalcidol improved vascular invasion and partially reduced growth plate hyperplasia in Hyp mice, while anti-PTHrP antibody suppressed excessive chondrocyte proliferation, reducing growth plate thickness by approximately 30% compared with vehicle, but failed to restore vascular invasion. Combination therapy exerted additive effects, reducing growth plate thickness by approximately 50%, partially restoring columnar organization, and significantly enhancing vascular invasion. These improvements resulted in partial normalization of growth plate architecture, exceeding the effects of either monotherapy.

DISCUSSION: Our data demonstrate that the pathology of growth plate cartilage in Hyp mice is characterized by two complementary defects: (1) excessive chondrocyte proliferation mediated by IHH–PTHrP signaling and (2) impaired vascular invasion associated with reduced VEGF expression. The therapeutic experiments directly support this dual mechanism. Alfacalcidol effectively improved vascular invasion but was insufficient to control chondrocyte hyperplasia, whereas anti-PTHrP antibody suppressed proliferation but failed to restore angiogenesis. Only the combination regimen simultaneously corrected both processes, resulting in greater reductions in growth plate thickness and improved organization. These findings indicate that targeting a single pathway is inadequate to restore growth plate integrity in XLH, and that dual modulation of mineral metabolism and proliferative signaling is necessary.

SIGNIFICANCE/CLINICAL RELEVANCE: (1-2 sentences): This study provides direct evidence that growth plate abnormalities in XLH are driven by both proliferative and vascular defects. Combined treatment with active vitamin D3 and anti-PTHrP antibody may represent a novel therapeutic approach to improve skeletal growth in pediatric XLH patients.

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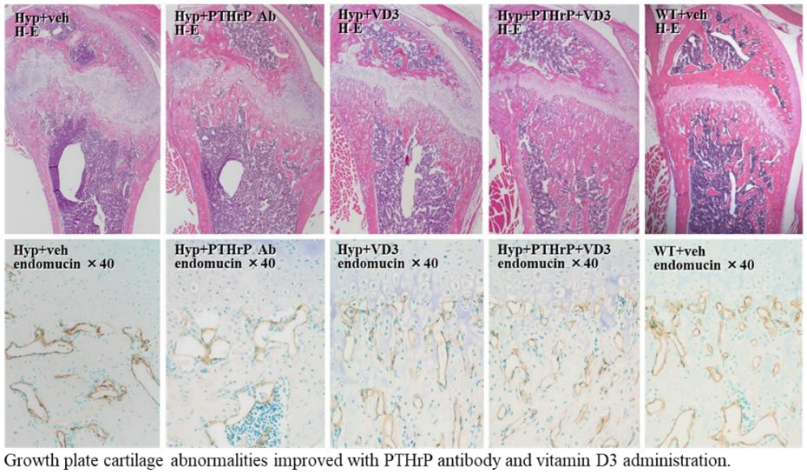
IMAGES AND TABLES:



Growth plate cartilage abnormalities improved with PTHrP antibody and vitamin D3 administration.