Augmented PTHrP forward signal from resting zone chondrocytes induces endosteal hyperostosis.

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Objectives: Parathyroid hormone-related protein (PTHrP) is a paracrine factor crucial in chondrogenesis and osteogenesis. PTHrP analogs are clinically used as an anabolic agent (abaloparatide), while excessive tumor cell-derived PTHrP induces osteolysis in bone metastasis. In the postnatal growth plate, slow-cycling chondrocytes in the resting zone express PTHrP as a skeletal stem cell-derived forward signal, promoting chondrocytes' proliferation in the adjacent layer. We recently reported that Hedgehog signaling activation enhances PTHrP expression in resting zone chondrocytes and accelerates their osteogenic cell fates. However, the long-term impact of augmented PTHrP forward signal from resting zone chondrocytes remains undefined. This study aims to unravel the novel function of PTHrP forward signal from resting zone chondrocytes in endochondral bone growth.

Experimental Methods: To enhance PTHrP expression in a cell type-specific manner, we generated a *cre*-inducible *Rosa26-iPTHrP* allele. We overexpressed PTHrP specifically in PTHrP^{+} resting chondrocytes using a tamoxifen-inducible *PTHrP-creER* line, and their cell fates were traced with a *Rosa26-tdTomato* reporter allele (PTHrP-PTHrP*). To test the autocrine PTHrP function in resting chondrocytes, we further crossed PTHrP-PTHrP* with *PTH/PTHrP receptor* (*PTH1R*) floxed alleles (PTHrP-PTHrP*ΔPTH1R). All mice received a single dose of tamoxifen at postnatal day (P) 6 and were chased for up to P96.

Results: PTHrP-PTHrP* mice developed growth plate hyperplasia due to a substantial expansion of PTHrP-negative chondrocytes at P36. After 3 months of chase, a small number of PTHrP-expressing mutant cells delaminated across the endosteal space and induced massive osteogenesis, resulting in endosteal hyperostosis. Interestingly, PTHrP-PTHrP*ΔPTH1R mice showed an identical phenotype to PTHrP-PTHrP* mice, demonstrating the dispensable roles of PTHrP-PTH1R autocrine signaling in resting chondrocytes.

Conclusion: Augmented PTHrP forward signal from resting zone chondrocytes induces growth plate chondrogenesis and endosteal osteogenesis through cell-non-autonomous mechanisms, unraveling a novel link from growth plate stem cells to trabecular bone formation.