

# Hedgehog activation promotes osteogenic cell fates of growth plate resting zone chondrocytes

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**Objectives:** The resting zone of the postnatal growth plate is organized by slow-cycling chondrocytes expressing parathyroid hormone-related protein (PTHrP), which include a subgroup of skeletal stem cells that contribute to the formation of columnar chondrocytes. The PTHrP–Indian hedgehog (Ihh) feedback regulation is essential for sustaining growth plate activities; however, molecular mechanisms regulating cell fates of PTHrP+ resting chondrocytes and their eventual transformation into osteoblasts remain largely undefined. In this study, we hypothesized that Hedgehog signaling facilitates osteogenic cell fates of PTHrP+ resting chondrocytes through multiple mechanisms.

**Experimental Methods:** We utilized a tamoxifen-inducible PTHrP-creER line that can exclusively mark PTHrP+ chondrocytes in the resting zone of the postnatal growth plate without marking any other cell types in long bones. To specifically activate Hedgehog signaling in PTHrP+ resting chondrocytes and visualize the fate of their descendants within growth plate and bone marrow, we also used Patched-1-floxed (Ptc1 encodes a Hedgehog receptor which inhibits its downstream Smoothened signaling) and R26R-tdTomato reporter alleles. All mice received a single dose of tamoxifen at postnatal day (P) 6 and were chased for up to 3 months. Histological analysis and three-dimensional micro-computed tomography (3D-μCT) analysis were performed at P14, 21, 28, 36, 42, 56, 70, 96.

**Results:** Hedgehog-activated PTHrP+ chondrocytes formed large concentric clonally expanded cell populations within the resting zone ('patched roses') at P21 and generated significantly wider columns of chondrocytes, resulting in hyperplasia of the growth plate at P36. Interestingly, Hedgehog-activated PTHrP+ cell descendants migrated away from the growth plate and eventually transformed into trabecular osteoblasts in the diaphyseal marrow space in the long term.

**Conclusion:** Hedgehog activation drives resting zone chondrocytes into transit-amplifying states as proliferating chondrocytes and eventually converts these cells into osteoblasts, unraveling a novel Hedgehog-mediated mechanism that facilitates osteogenic cell fates of PTHrP+ skeletal stem cells.