

Essential roles of parathyroid hormone-related protein (PTHrP) in the formation of the mandibular condylar cartilage

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Objectives: The mandibular condylar cartilage (MCC) is an essential component of the temporomandibular joint (TMJ), which orchestrates the vertical growth of the mandibular ramus through distinctive modes of cell differentiation as a secondary cartilage. In the epiphyseal growth plate, parathyroid hormone-related protein (PTHrP) is expressed by resting zone chondrocytes and promotes chondrocyte proliferation. The aim of this study was to determine how PTHrP regulates the MCC formation.

Experimental methods: We utilized PTHrP-mCherry knock-in reporter mice in which the PTHrP allele is engineered so that a red fluorescent protein is expressed instead of a functional PTHrP protein. PTHrPmCherry/+ heterozygous mice were used as controls, and PTHrPmCherry/mCherry homozygous mice were used as knockout (PTHrP-KO). Mandibular condyle morphology was evaluated by 3D micro-CT, whole-mount skeletal staining, and safranin O staining. Immunohistochemistry and RNAScope were performed to evaluate expression patterns of relevant proteins and mRNAs, respectively. Cell proliferation was evaluated by EdU.

Results: At postnatal day 3, PTHrP-mCherry, RUNX2, SOX9 and Collagen 10 were expressed in the superficial, polymorphic, flattened and hypertrophic chondrocyte layers of MCC, respectively. Pth1r encoding the PTHrP receptor was expressed by chondrocytes. PTHrP protein was expressed in the polymorphic layer in the control but abrogated in that of PTHrP-KO. At embryonic day 18.5, the MCC of PTHrP-KO mice was significantly smaller than the control, associated with significantly less Safranin-O and SOX9-positive cells. Cell proliferation in the polymorphic layer was significantly reduced in PTHrP-KO mice, associated with significant reduction of PTHrP target genes including Mef2c, Ihh and Runx2.

Conclusion: PTHrP released from cells in the superficial layer acts on RUNX2+ precursor cells in the adjacent layer and promotes their proliferation and differentiation into chondrocytes.