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. *PTHrP*$^{mCherry/+}$ heterozygous mice were used as controls, and *PTHrP*$^{mCherry/mCherry}$ 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 and SOX9 were expressed in the superficial, polymorphic, and flattened 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 *Col2a1*, *Mef2c*, *Ihh*, *Pth1r* 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. Our study identifies a unique mechanism by which PTHrP facilitates the MCC formation, providing a potential target to intervene the continual and adaptive growth of MCC.

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