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Review Article

Hylonome

Extracellular Ca^{2+}-sensing in cartilage

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The development of growth plate cartilage is critical to endochondral bone formation – the process responsible for linear growth in childhood. Cells in the growth plate proliferate and ultimately differentiate into hypertrophic chondrocytes that produce a mineralized matrix and initiate new bone formation. The pace of growth plate development is maintained by systemic hormones and more importantly by local autocrine and paracrine factors produced by chondrocytes. Parathyroid hormone-related protein (PTHrP), Indian hedgehog (Ihh), and their receptors constitute a feedback loop within the growth plate that delays the maturation of chondrocytes[1,2]. In contrast, signaling via insulin-like growth factor-1 (IGF-1) and its receptor promotes the differentiation of cells into hypertrophic chondrocytes[3,4].

The availability of Ca^{2+} in the extracellular fluid affects the formation of calcified growth plate cartilage. Deficiencies of Ca^{2+} can lead to certain forms of rickets in which growth plates are softened and demineralized as results of dysfunctional hypertrophic chondrocytes[5,6]. Rickets seen in children with severely restricted calcium intakes are healed by calcium supplementation[7,8]. In a similar manner, rickets that develop in vitamin D receptor knockout mice can be prevented by high Ca^{2+} diets[9], supporting a role for Ca^{2+} in growth plate development. We previously found that raising extracellular $[Ca^{2+}]$ ($[Ca^{2+}]_e$) activates classic G protein-coupled signaling pathways and promotes cell differentiation in cultured chondrogenic RCJ3.1C5.18 (C5.18) cells[10-12]. This supports the idea that membrane Ca^{2+}-sensing mechanisms may be involved in coupling changes in $[Ca^{2+}]_e$ to subsequent cellular responses.

C5.18 cells express "parathyroid-like" CaRs[10,11]. Blocking the expression and/or function of endogenous CaRs by antisense oligonucleotides or overexpression of dominant-negative mutant CaRs (F707W-CaR) altered proteoglycan (PG) accumulation, mineral deposition, and gene expression in response to changes in $[Ca^{2+}]_e$[10,12], supporting a role for the CaRs in mediating extracellular Ca^{2+}-induced cell differentiation[10,12]. Growth plate chondrocytes (GPCs) also express CaRs[11]. We, therefore, hypothesize that high $[Ca^{2+}]_e$ may promote GPC differentiation by activating CaRs and altering activity of the Ihh/PTHrP pathway and/or IGF-1/IGF-1R signal transduction.

To address this hypothesis, we studied the effects of changes in $[Ca^{2+}]_e$ and PTHrP signaling on PG synthesis, matrix mineralization, and gene expression, by qPCR, of chondrogenic markers and critical components of PTHrP/Ihh and IGF-1 signaling pathways in freshly isolated mouse growth plate chondrocytes (mGPCs). To confirm the role of CaRs in chondrocyte differentiation, we examined the effects of changing $[Ca^{2+}]_e$ on the above parameters in chondrocytes isolated from mice with ablation of full-length CaRs (CaR-/-)[13].

mGPCs in culture recapitulate steps of cell differentiation in growth plates. Beginning in subconfluent cultures, these cells produced PG-containing matrix in a time-dependent manner until they began to deposit minerals. Thereafter, mineral deposition increased and PG accumulation decreased. In association with the morphological changes, expression of matrix genes changes as cell differentiation proceeded over time. Early chondrogenic markers – Agg and the α_1-subunit of type II collagen [α_1(II)] – were expressed at high levels in subconfluent and confluent cultures and decreased in post-confluent cultures. Conversely, RNA levels for markers of terminal differentiation – alkaline phosphatase, osteopontin (OP), and osteocalcin (OC) – were low in subconfluent and confluent cultures and increased in post-confluent cultures.

Raising $[Ca^{2+}]_e$ suppressed PG synthesis, promoted mineralization of matrix, decreased RNA levels of Agg and α_1(II), and increased gene expression of OP and OC in mGPCs. The advanced cell differentiation is accompanied by reduced RNA levels for Ihh and the PTH1R and increased RNA levels for IGF-1 and IGF-1R. High $[Ca^{2+}]_e$

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also suppressed the expression of IGF-binding proteins 2, 3, and 4. Treating chondrocytes with PTHrP(1-34) (10^{-7}M) blunted the ability of high $[Ca^{2+}]_e$ to alter PG accumulation, mineral deposition, and gene expression of differentiation markers, suggesting that PTHrP may delay chondrocyte differentiation by blocking cell responses to Ca^{2+}. Overexpression of a constitutively active human PTH1R in mGPCs partially blocked the high $[Ca^{2+}]_e$-induced cell differentiation. These data support a scheme for how raising $[Ca^{2+}]_e$ promotes chondrocyte differentiation: by reducing the level of PTHrP/Ihh signaling – a pathway that delays cell maturation – and by increasing availability of IGF-1 – a growth factor that facilitates the differentiation of hypertrophic chondrocytes.

In cultures of chondrocytes isolated from CaR-/- mice, high $[Ca^{2+}]_e$ suppressed PG accumulation, promoted mineral deposition, and altered expression of chondrogenic marker genes with comparable potency as in wt control cells. These suggest that deletion of full-length CaRs has no impact on the high $[Ca^{2+}]_e$-induced cell differentiation. Reverse transcription (RT)-PCR amplified a cDNA from RNA isolated from the growth plate of CaR-/- mice. The sequence of this PCR product indicates that it is part of an alternatively spliced CaR transcript missing exon 5 of the CaR gene. The expression of an alternatively spliced CaR is further supported by the detection of CaR immunoreactivity in the CaR-/- growth plate. These observations indicate that ablation of CaR gene in the CaR-/- mice is incomplete. Whether the spliced CaRs, expressed in the CaR-/- chondrocytes, or other Ca^{2+}-sensing molecules are compensating for the loss of full-length CaRs requires further investigations.

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