Examination of proteins involved in the regulation of calcium homeostasis in differentiating mesenchymal cells

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Summary

In vitro cartilage differentiation

In vitro chondrogenesis is a dynamic, multistep process regulated by a variety of molecular processes, many of which involve activation and deactivation of protein kinases and phosphatases sensitive to changes of intracellular [Ca^{2+}]. In chicken HD mesenchymal cell cultures, chondrogenic mesenchymal cells differentiate into chondroblasts and then to chondrocytes during a 6-day-long culturing period. The majority of chondroblasts, characterised by the ability of production of a cartilage specific ECM, appear from culturing day 3. We have previously demonstrated that elevation of free cytosolic [Ca^{2+}] at the time of differentiation of chondroblasts was mainly due to a Ca^{2+}-influx and it was indispensable to cartilage formation in chicken HD mesenchymal cell cultures. In the present work we describe, that chondrogenic cells secreted ATP during the differentiation period. Administration of ATP to the culture medium evoked Ca^{2+}-transients exclusively in the presence of extracellular Ca^{2+} and mostly on day 3 of culturing, when the final commitment of chondroblasts occurs. Moreover, ATP caused elevated protein expression of the chondrogenic transcription factor Sox9 and ATP also stimulated cartilage matrix production. Administration of suramin, which blocks all P2X receptors but not $P2X_4$ did not impede the effects of ATP, furthermore, $P2X_4$ appeared in the plasma membrane fraction and gave signals with immunocytochemistry only from day 3. Expressions of both ionotropic purinergic receptors $P2X_1$, $P2X_4$, $P2X_5$ and $P2X_7$ and metabotropic purinergic receptors $P2Y_1$, $P2Y_3$ and $P2Y_5$ at the mRNA level and $P2Y_1$, $P2Y_2$ and $P2Y_4$ at the protein level were detected. Among the agonists of the metabotropic receptors, ADP and UDP did not evoke any Ca^{2+}-transients in HDC and had no influence on cartilage formation. On the other hand administration of UTP resulted in transient elevation of cytosolic Ca^{2+} concentration in 3-day-old HDC but did not stimulate matrix production

during the chondrogenesis. Based on these data, we suggest a role of $P2X_4$ in the generation of ATP-dependent Ca^{2+}-transients of differentiating chondroblasts.

***In vitro* skeletal muscle cultures**

The 95 kDa triadin (Trisk 95), an integral protein of the sarcoplasmic reticulum membrane in skeletal muscle, interacts with both the ryanodine receptor (RyR) and calsequestrin. While its role in the regulation of Ca^{2+}-homeostasis has been extensively studied, data is not available on whether the overexpression or the interference with the expression of Trisk 95 would affect Ca^{2+} sparks the localized, elementary Ca^{2+}-release events (ECRE). In the present study ECRE and Ca^{2+}-transients were studied using laser scanning confocal microscopy on C2C12 cells and on primary cultures of skeletal muscle. Liposome or adenovirus-mediated Trisk 95 overexpression and shRNA interference with triadin translation were used to modify the level of the protein. Stable overexpression in C2C12 cells significantly decreased the amplitude and frequency of Ca^{2+} sparks, and the frequency of embers. In line with these observations, depolarisation-evoked Ca^{2+}-transients were also suppressed. Similarly, adenoviral transfection of Trisk 95 into cultured mouse skeletal muscle cells significantly decreased both the frequency and amplitude of spontaneous global Ca^{2+}-transients. Inhibition of endogenous triadin expression by RNA interference caused opposite effects. Primary cultures of rat skeletal muscle cells expressing endogenous Trisk 95 readily generated spontaneous Ca^{2+}-transients but rarely produced Ca^{2+} sparks. Their transfection with specific shRNA sequence significantly reduced the triadin-specific immunoreactivity. Functional experiments on these cells revealed that while caffeine-evoked Ca^{2+}-transients were reduced, ECRE appeared with higher frequency. These results suggest that Trisk 95 negatively regulates RyR function by suppressing localized Ca^{2+}-release events and global Ca^{2+}- signals in cultured muscle cells.

Kulcsszavak: *in vitro* porcképződés, vázizom, Ca^{2+}-spark

Key words: *in vitro* cartilage formation, skeletal muscle, calcium spark