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Dynamic expression of VDR and 1-alpha-hydroxylase in differentiated and re-differentiated human articular chondrocytes

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Purpose

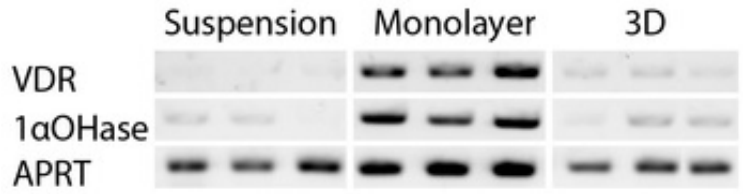
The goal was to investigate potential roles played by vitamin D in the regulation of joint cartilage biology. We studied the expression of two central elements of vitamin D metabolism, namely the vitamin D receptor and its converting enzyme 1-α-hydroxylase in human knee cartilage and chondrocytes.

Methods and Materials

Expression of receptor and enzyme was determined by immunohistochemistry/immunofluorescence, reverse-transcriptase PCR and western blot on differentiated, de-differentiated and re-differentiated chondrocytes. Cartilage was harvested from a macroscopically healthy looking area of the lateral femoral condyle during knee replacement surgery in 4 otherwise healthy patients aged 50-70. Suspension cultures of differentiated chondrocytes were established by short enzymatic digestion of cartilage using Collagenase XI and further incubation in non-adherent vessels. De-differentiated cells were the result of serial expansion of chondrocytes during 4 weeks after isolation in monolayers cultures. Chondrocyte re-differentiation was achieved by propagating cell pellets for 3 weeks in the presence of chondro-inductive morphogens.

Results

Both protein and gene expression of vitamin D receptor appear to be very low or undetectable in native cartilage and/or differentiated chondrocytes. In contrast, receptor expression was upregulated in de-differentiated cells after monolayer expansion, however, this upregulation was lost when cells regained chondrogenic phenotype in 3D pellets. The expression of 1-α-hydroxylase was observed on the superficial layer of chondrocytes in native cartilage, which correlated with weak but detectable outcomes by PCR and western blot on differentiated cultures. Similarly, levels of the enzyme were increased after cell expansion in monolayers and decreased in 3D pellet cultures.



Conclusion

Our study uncover a previously unknown regulation of vitamin D receptor between differentiated and re-differentiated phenotypes in cartilage cells. Furthermore, this study is pioneering on investigating the expression of 1-α-hydroxylase in cartilage tissue and chondrocytes. Further work is needed to ascertain if receptor and enzyme expression is regulated in disease conditions or affected by inflammatory environments.

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