

MAINTENANCE OF CHONDROCYTE PHENOTYPE BY THE VIMENTIN INTERMEDIATE FILAMENTS

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INTRODUCTION: The vimentin cytoskeleton is believed to facilitate extracellular signal transduction inducing transcriptional responses within the chondrocyte. The spatial organisation of vimentin differs in human osteoarthritic chondrocytes, and a reduction in vimentin protein was observed in a rat model of osteoarthritis. The aim of this study was to assess the role of the vimentin filaments on cartilage chondrocyte homeostasis.

METHODS: Chondrocytes from 7-day-old bovine articular cartilage were plated at 1×10^6 cells/24 well plate, and vimentin filaments disrupted with 1-5mM acrylamide for 24, 48 and 72hrs. Disruption of the vimentin filaments was confirmed using a FITC-conjugated vimentin antibody visualized using confocal microscopy. The cytotoxic effects of acrylamide were assessed using the CytoTox® 96 assay. Changes in phenotypic markers of chondrocytes i.e. type II collagen and sGAG were measured by immunoblotting and DMMB assay, respectively. The amount of phosphorylated versus total MAP kinase proteins (ERK 1/2, p38 and JNK) was also conducted by immunoblotting. Specific MAP kinase inhibitors (U0126, SB203580 and SP600125 respectively) were added to chondrocyte cultures in the presence of acrylamide to determine the involvement of each in vimentin-mediated signal transduction.

RESULTS: In the presence of acrylamide, the vimentin filaments were observed to collapse around the nuclei, whereas the filaments in control cells were organized into a dense network throughout the cytoplasm. Acrylamide was cytotoxic in a dose-dependent manner, which was significant after 72 hours in culture. Hence, all other data are normalized to cell number. Levels of sGAG in the media decreased with increasing acrylamide concentrations. This was also evident with pro type II collagen expression; interestingly, disruption of vimentin inhibited the processing of the pro-collagen into the II (α 1) chains. An increase in phosphorylated ERK 1/2 was observed with increasing acrylamide

concentration. Preliminary data indicates that increased phosphorylation of ERK 1/2 correlates with a change in chondrocyte phenotype. There were minimal effects on phosphorylated p38 kinase and JNK in acrylamide treated chondrocytes. We are currently analyzing the effect of blocking ERK phosphorylation using specific inhibitors to determine whether this prevents the change in phenotype.

DISCUSSION & CONCLUSIONS: We have shown that cytoskeletal vimentin element modulation by disruption with acrylamide alters the phenotype of the chondrocyte. ERK phosphorylation is widely reported to inhibit chondrogenesis^{1,2}, therefore we believe that disassembly of the vimentin architecture, by an as yet unknown mechanism, results in phosphorylation of ERK 1/2 which may alter the phenotype of the chondrocyte i.e. inhibits sGAG and type II collagen production. We are currently determining the mechanism(s) involved in mediating these matrix changes, and the role of the chondrocyte cytoskeleton in signal transduction, changes in which may contribute to joint pathologies such as osteoarthritis.

REFERENCES: ¹ B.E. Bobick and W.M. Kulyk. 2004 *J Biol Chem* **279**: 4588-4595. ² T. Tallheden, C. Karlsson, A. Brunner, J. van der Lee, R. Hagg, R. Tommasini, and A. Lindahl . 2004 *Osteoarthritis Cartilage* **12**:525-535.

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