Mechano-regulation of the vimentin cytoskeleton and its role in maintaining chondrocyte phenotype.

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Introduction: The chondrocyte cytoskeleton comprises actin microfilaments, tubulin microtubules and vimentin intermediate filaments. The vimentin cytoskeleton is believed to facilitate extracellular signal transduction promoting chondrocyte transcriptional responses. The chondrocyte cytoskeleton has previously been shown to be highly disorganised or often absent in both human and in a rat model of osteoarthritis. In addition, the reduction in vimentin protein content observed in human osteoarthritis is concomitant with increased cleavage of the N-terminus. The N-terminus of vimentin is essential for the assembly of the intermediate filament network.

The aims of this study were three-fold: [1] to compare vimentin organisation in human normal and osteoarthritic cartilage, [2] to determine whether mechanical load affected vimentin organisation and/or expression and [3] how this might impact on cartilage chondrocyte homeostasis.

Methodology: Human articular cartilage was derived from age-matched normal and osteoarthritic donors. Vimentin network organisation was assessed using immunofluorescence in conjunction with laser scanning confocal microscopy, and transcriptional effects assessed using quantitative PCR. To determine the effect of load on vimentin organisation, cartilage explants from 7-day-old bovine calves were subjected to physiological and abnormal loads over defined time periods, and analysed as above. To determine more specifically whether vimentin network dysregulation or loss of expression affects the chondrocyte phenotype, isolated bovine cartilage chondrocytes were treated with either 5mM acrylamide or vimentin-specific siRNA and analysed for changes in matrix gene and protein expression.

Results: In comparison to human normal cartilage chondrocytes the spatial organisation of vimentin differed in osteoarthritic chondrocytes; in OA cells vimentin mRNA levels were also significantly reduced. Physiological loads induced the re-organisation of the vimentin network (Figure 1).

Further effects on the chondrocyte vimentin network were observed after applying an abnormal load; these effects were also observed at the transcriptional level. Both the disassembly (acrylamide) and translational inhibition (siRNA) of vimentin (Figure 2A) significantly reduced type II collagen synthesis (Figure 2B) and aggrecan synthesis (not shown).

Discussion: We have shown that the vimentin network organisation differs in pathological articular cartilage and in tissue subjected to abnormal loading. Cytoskeletal vimentin element modulation, by either disruption or inhibition of protein synthesis dramatically affects chondrocyte biosynthesis. There is a strong correlation between loss of vimentin organisation/synthesis and a loss of the chondrocyte phenotype. 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.

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References:
3 Lambrecht et al, OA Cartilage. 16:163-73, 2007