Cartilage Hyaluronan and CD44 in the Age of DAMPs
Warren Knudson, Ph.D.

Recent reports have proposed the role of innate immune activation by chondrocytes in the pathogenesis and early development of inflammation within the articular joint during osteoarthritis (OA). It has long been described that LPS and IL-1 treatment results in catabolic activation of chondrocytes. The toll-like receptors (TLRs) constitute one receptor family in innate inflammation responses activated by LPS and other molecules known as damage associated molecular patterns (DAMPs). Extracellular matrix (ECM) breakdown products represent one group of innate DAMPs. Our long term goal was to demonstrate the role of DAMPs, generated during early cartilage degeneration, in the activation of an innate immune response in chondrocytes. In our studies we have demonstrated that CD44 [a receptor for hyaluronan (HA)] is enzymatically shed from the cell surface of OA chondrocytes releasing CD44-extracellular domain peptides (CD44-ECD); these CD44 fragments were present in direct extracts of intact OA cartilage. Like fibronectin fragments, these CD44-ECD peptides have the potential to serve as a DAMP. In our preliminary results, CD44-ECDs generated by human OA chondrocytes exhibited DAMP-like activity *in vitro* and, we could force a selective release of CD44-peptides by adenoviral over-expression of the full-length substrate. However, the activation of target chondrocytes by CD44-ECD and HEK-Blue™-TLR4 cells, a cell line expressly designed for studying the stimulation of TLR4, was not consistent. As such we could not confirm the validity of CD44-ECDs as DAMPs at least using the approach that we originally proposed. As such this work is still ongoing. The other aim in our studies was to examine the contribution of HA production to diminish innate immune responses in chondrocytes. This aim has been the most successful in our studies. In our preliminary results, HAS2 overexpression (HAS2-OE)—via Ad-Tet-On-HAS2 transduction—blocked MMP13 and TSG6 mRNA and protein expression in chondrocytes activated by IL-1β, TNFα, LPS or HA oligosaccharides—the latter two examples of potent DAMPs that initiate signals through TLR4. We have extended this work to include another confirmed DAMP, 30 kD N-terminal fragment of fibronectin (fibronectin fragments, FN-fr) known to be active on chondrocytes, cartilage and rabbit knee joints in vivo. HAS2-OE also blocks the activation of FN-fr. In co-cultures of human OA chondrocytes transduced with Ad-Tet-On-HAS2 with nontransduced bovine chondrocytes or, conditioned media from HAS2-OE bovine chondrocytes, no anti-catabolic inhibitory activity was transferred to the non-transduced target chondrocytes. These results suggest that the high levels of exogenous HA generated by HAS2-OE do not have the capacity to reduce the pro-catabolic phenotype in non-transduced chondrocytes. These results have led us to unravel new mechanisms for blocking OA-like catabolism. What we have found is that HAS2-OE (and likely endogenous HAS2 as well) regulate intracellular metabolism as much or more-so than changes to the extracellular matrix. We determined that this is why HAS2-OE and 4MU, seemingly opposite effects, both exert chondroprotective effects. This unexpected observation will have a large impact on OA as a way to target and regulate abnormal chondrocyte metabolism downstream of the pro-catabolic activation of these cells by cytokines or DAMPs.