EXPRESSION OF CELL DEATH PATHWAY TRANSCRIPTS IN RNA FROM HIGHLY CALCIFIED VERSUS MINIMALLY CALCIFIED MENISCI
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Abstract:
Purpose: Advanced osteoarthritis (OA) of the knee is a debilitating and painful condition that involves not only articular cartilage but often the fibrocartilage of the meniscus. A number of studies have demonstrated that meniscal degradation positively correlates with articular cartilage degradation, and there is mounting evidence to suggest that meniscal cells may play an active role in the pathogenesis of OA. In the diseased meniscus, basic calcium phosphate and calcium phosphate dihydrate crystals are commonly observed. We sought to understand the nature of certain biological processes in meniscal pathology. In particular, we explored the differential expression of cell death pathway transcripts in RNA derived from highly calcified menisci vs. RNA derived from menisci displaying minimal calcification. Such studies could potentially define new therapeutic targets for the treatment of OA and uncover novel markers of disease progression.

Methods: Menisci were obtained from surgical discard tissue of 12 patients undergoing joint replacement surgery. Patients ranged in age from 60 to 72 years and included five males and seven females. All patients were Caucasian. The menisci were processed both for alizarin red staining to assess for deposition of calcium crystals, and for RNA isolation. Scoring for alizarin red staining enabled the stratification of samples into two classes based upon degree of crystal deposition. The minimal crystal deposition class (n=6; mean age: 62 years) was defined as tissue that displayed a limited number of small size calcium deposits at the edges of the meniscus, while tissues classified as highly calcified (n=6; mean age: 64 years) displayed clusters of calcium crystals within the body of the meniscus and widespread clusters of medium and large-sized calcium deposits at the edges of the meniscus.
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Abstract (continued):

RNAs from the two classes of samples were isolated and pooled to create two distinct meniscal populations for expression profiling: minimally calcified RNAs vs. highly calcified RNAs. The RNA populations were reverse transcribed and utilized in a microarray format to determine the relative expression of cell death pathway transcripts for each class of pooled menisci. Average delta Ct and \(2^{(-\text{dCt})}\) values for each transcript were calculated for each pooled class of menisci, and the fold difference in expression between the highly calcified vs. the minimally calcified menisci were reported. Those transcripts that displayed differential expression were validated by RT-PCR.

**Results:** Eighty-four key genes whose expression is important in regulating central mechanisms of cell death were profiled. These included both pro- and anti-apoptotic transcripts, transcripts relevant to autophagy, and transcripts involved in cell necrosis. In the pool of RNAs derived from highly calcified menisci, the major cell death pathway that demonstrated differential expression when compared with the pool of RNAs derived from minimally calcified menisci were those transcripts involved in necrosis. These included DPYSL4 (dihydropyrimidase-like 4), JPH3 (junctophilin 3), MAG (myelin-associated glycoprotein), KCNIP1 (Kv channel interacting protein 1), and S100A7A (S100 calcium-binding protein A7A), all of which were significantly over-expressed (3 to 15-fold) in the pool of RNAs derived from highly calcified menisci. No autophagy-relevant transcripts were over-expressed. Likewise, there were very few apoptosis-relevant transcripts that were differentially expressed in the RNA pool derived from highly calcified menisci, and these included both pro- and anti-apoptotic transcripts.

**Conclusions:** Our study, which is the first to focus on cell death pathway transcripts in the meniscus, strongly suggests that the major cell death event governing the fate of cells in highly calcified menisci is one of necrosis. In contrast to apoptosis or autophagy, the process of necrosis results in cell swelling, lysis, and pro-inflammatory cytokine release. Recent studies demonstrate that necrosis, rather than occurring as a passive process, can occur as an active, adaptive process, although the downstream mechanisms in executing cell death are poorly understood. Of particular interest is the potential involvement of reactive oxygen species in this process. Our studies provide some insight into the physiological processes that lead to meniscal compromise in the osteoarthritic joint and, if reactive oxygen species are involved in the necrotic pathway in meniscal cells, could potentially suggest a path to treatment in the prevention of meniscal cell death.