The Impact of Glycosaminoglycan Loss on Chondrocyte Viability
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Introduction: Chondrocyte death and glycosaminoglycan (GAG) loss have been linked with the degradation of articular cartilage in human and experimental OA1,2. However, it remains unclear whether GAG loss induces chondrocyte death and how this related to progression of OA. This study addresses the direct effect of GAG loss on chondrocyte survival and the susceptibility to cell death in response to mechanical injury.

Materials and Methods: Articular cartilage was obtained from porcine knees (n=8) aged between 6–9 months. Explants were equilibrated by 48 hours incubation in serum-containing media and then treated with protease-free chondroitinase ABC (CABC) for create a GAG loss model or cultured in serum-free DMEM for 24 hours as control. Mechanical injury was applied with a mechanical testing device following the protocol of D’Lima3. Additionally for each explant, the strain level was determined. Live and dead cells are simultaneously viewed in situ by confocal microscopy (LSM510, Zeiss, Germany) using a fluorescent double-stain. The pan-caspase inhibitor Z-VADfmk was added at 10 μM in culture medium to determine the inhibition of chondrocyte apoptosis after mechanical injury. Z-VADfmk was added at 24 hours before, 36 hours and 72 hours after injury. The percent of cell death was analyzed at 24 and 72 hours.

Results: Cartilage explants were subjected to 2-14 MPa injuries to determine impact of CABC treatment on stiffness. Both groups had increased strain as the stress increased, but GAG-depleted explants showed higher strain than control above 8 MPa (Fig. 1A). The largest increase in strain in both groups occurred >8 MPa. With increasing degree of injury, control and GAG loss group had dead cells in superficial zone (*P<0.01, Fig. 1B).

To clarify the correlation between GAG loss and chondrocyte death after injury, cartilage explants were subjected with 8MPa injury immediately after CABC treatment. The results revealed that GAG loss and chondrocyte death are closely correlated; chondrocytes in GAG depleted areas were more susceptible to death after injury compared with GAG rich areas (Fig. 2). Mechanical injury-induced cell death was enhanced in CABC-treated explants.

To determine the mechanisms of cell death after injury, cartilage explants were immunostained for active caspase-3, a marker of apoptotic cell death (Fig. 3). Control explants with 8 MPa injury contained positive cells especially in superficial and top of middle zone. On the other hand the dead cells (as seen in live-dead assay) in CABC-treated explants were not staining positive staining for active caspase-3.

Z-VADfmk did not alter cell viability in non-injury groups. Mechanical injury at 8 MPa did not induce immediate (day 0) chondrocyte death. Subsequently viability in the injury groups decreased to 53.2% in the absence of Z-VADfmk. The caspase inhibitor significantly (P=0.03) improved viability to 64.0%. This immediate cell death was not changed by Z-VADfmk. Cell death increased further during subsequent culture for 3 days. Importantly this additional cell death was almost completely prevented by Z-VADfmk (P=0.006).

Discussion: In cartilage explants where GAG was depleted with CABC, chondrocyte death induced by mechanical injury was increased. Additionally, the size of the structural defects or clefts and the distribution of dead cells in the CABC treated explants were different. In the present study we show that GAG depletion increases strain levels and this change may be one factor contributing to the increased cell death. In our experiment, cell death in control cartilage occurred near the surface cracks, but in CABC-treated cartilage chondrocyte death was distributed diffusely on the surface. The type of cell death indicates that the immediate injury-induced cell death in CABC-treated samples is either not via apoptosis or not susceptible to the effects of Z-VADfmk. The subsequent spreading of cell death is highly susceptible to rescue by Z-VADfmk, indicating an apoptotic mechanism.

References:

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