**The Importance of an Extracellular Matrix in Apoptosis in Chondrocytes**

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**Introduction:** One of the mechanisms for the pathogenesis of osteoarthritis is the induction of apoptosis by chondrocytes, which result in a disturbance, and impaired function of its surrounding extracellular matrix (ECM) and ultimately causes cartilage failure. The presence of an intact ECM has been demonstrated to be crucial for the maintenance and integrity of chondrocyte function and cartilage tissue. To address the importance of the extracellular matrix to the homeostasis of chondrocytes, the induction of apoptosis was assessed while chondrocytes were grown the presence of or on a ECM, including fibronectin, matrigel, and matrigel(-) and purified perlecan. Various conditions were tested including adherent and non-adherent suspension cultures which is a culture model previously shown to preserve the cartilage phenotype. Matrigel, a commercially available defined ECM is considered a basement membrane matrix equivalent that may mimic a natural extracellular environment. The major components of Matrigel are laminin, collagen IV, and heparan sulfate proteoglycan perlecan. Perlecan is a proteoglycan that plays a critical role in the function of the basement membrane and is a component of the pericellular environment of chondrocytes. Matrigel (-) provided a similar matrix as Matrigel, however there is a reduction in the amount of growth factors that are present, including a >50% reduction in the amount of perlecan. The objectives of the study were to study the role of ECM on chondrocyte homeostasis and specifically its importance in protecting chondrocytes from apoptosis. Since chondrocytes grow in vivo in such a specialized environment our goal was to mimic aspects of this in vitro and test their response to a natural inducer of apoptosis.

**Materials and Methods:** Equine chondrocytes were obtained post mortem from the stifle joints of young adult horses utilizing aseptic techniques and were placed in DMEM complete medium with 10% FBS. Chondrocytes were isolated from their matrix by enzymatic digestion with 2 mg/ml collagenase for one hour, followed by an overnight digestion with 0.5 mg/ml collagenase at 37 C. The cells were cultured in DMEM with 10% FBS at 100,000 cells per well (or dish). The cultures were established in chamber slides for some studies where the matrix was added to the medium. Additions (or coatings) were either perlecan (BD Biosciences), Matrigel and growth factor reduced Matrigel (Matrigel-) (BD Biosciences). Some cultures were left to adhere to plastic and others were grown in suspension using poly-HEMA coated wells and dishes. In all cases cultures were allowed to equilibrate with the environment for 24 h prior to treatment for an additional 24-48h with Fas antibody (500 ng/ml/Axxora). Media was collected and cells, if not attached, were centrifuged and cytosins were prepared. All cells (cytospins or on slides) were fixed with 4% paraformaldehyde/PBS and permeabilized in 0.1% Triton X-100, 0.1% sodium citrate. A TUNEL assay (in situ Cell Death Detection Kit, Roche) to identify nick end-labeling of DNA with fluorescein-dUTP was used. Slides were counter stained with propidium iodine to identify all cells and analyzed microscopically to identify the amount of TUNEL positive chondrocytes. To further investigate the potential role of various ECM on apoptosis, equine chondrocytes were prepared in the same manner as described and were plated on plates, including plain plastic, a suspension (polyHEMA) plate, fibronectin and Matrigel wells with and without the presence of the Fas antibody for 24h. The supernatant was analyzed using a Cell Death Detection ELISA (Roche). Photometric analysis generates an absorbance and that value (- background) was used to determine the % increase of apoptosis over the control (no Fas).

**Results:** A TUNEL assay was used to identify the number of live cells as compared apoptotic cells that were induced by Fas activation using fluorescent microscopy. All these assessments were conducted on chondrocytes in suspension with the matrix added to medium. The number of TUNEL positive cells was 40.7 % +/- 19.4 as compared to the control 0.84 % +/- 1.01 in the chondrocytes grown in suspension on a poly-HEMA coated slide chambers.

**Discussion:** In cartilage chondrocytes grown within a unique ECM environment, more recently, have been shown to contain perlecan in their pericellular matrix. In this study we show that certain matrices can alter the response to inducers of apoptosis, as can growing chondrocytes in a phenotypically-correct environment. Specifically matrix containing perlecan and or in some experiments purified perlecan can protect or alter the response of chondrocytes to stimuli of apoptosis. The data suggest that perlecan is involved, but it may be indirect, since many growth factors and molecules bind to perlecan. This finding was demonstrated using the Matrigel and the growth factor reduced (perlecan reduced coated dishes).Matrigel. The importance of maintaining the integrity of the ECM of chondrocytes to maintain their “well-being” in vivo is likely a factor in the integrity and function of articular cartilage. Further understanding of the precise molecules involved, and their ability to interact with the ECM for the protection of cells from apoptosis will provide a viable target for therapeutic intervention in a variety of diseases.

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