FIBRONECTIN FRAGMENTS HAVE DIFFERENT EFFECTS ON PROTEOGLYCAN SYNTHESIS BY CELLS FROM ANNULS FIBROSUS, NUCLEUS PULPOSUS AND ARTICULAR CARTILAGE

*Department of Orthopedic Surgery, Rush Medical College, Chicago, IL. ++Departments of Orthopedic Surgery and Biochemistry, Rush Medical College, Chicago, IL. 312-942-4661, Fax: 312-942-8828, kmasuda@rush.edu

INTRODUCTION: Fibronecin (Fn) is an extracellular matrix protein responsible for cell adhesion and matrix formation. It is synthesized in large amounts in response to injury. In human osteoarthritic joints, intact Fn and proteolytic fragments of Fn (Fn-f) are present in increased amounts in articular cartilage (5,6). It has been shown that Fn-fs can induce the release of catabolic cytokines and matrix metalloproteinases (MMPs) from cartilage. These fragments also markedly suppress proteoglycan (PG) synthesis by articular chondrocytes, and, as a result, cause the PG content of cartilage to drop rapidly (3, 4). A recent study of degenerating intervertebral discs showed that the contents of Fn and often of Fn-f are elevated in these tissues (8). There are no reports about the potential in vitro effects of Fn-f on intervertebral disc cells.

The purpose of this study was to determine what effects Fn-fs have on different populations of intervertebral disc cells (nucleus pulposus, inner annulus fibrosus, and outer annulus fibrosus) and to compare those to the effects they have on cells from articular cartilage.

MATERIALS AND METHODS:

Cell Culture: Cells were harvested from bovine steer (14-16 months old) articular cartilage and intervertebral disc (nucleus pulposus, inner annulus fibrosus and outer annulus fibrosus). Cells were resuspended in 1.2% low viscosity sterile alginate beads at 2 million/ml (1). The resulting alginate beads containing different cell populations were cultured in DMEM/F12 supplemented with 10%FBS, 25µg/ml ascorbic acid with daily changes of medium. After 7 days in culture, the cells were cultured for 7 more days in the presence of various concentrations of Fn-f (fragment mixture, generated by digesting purified Fn with stromelysin) at 0 (Control) and 10^{-8} M through 10^{-5} M.

DNA Content: DNA content was measured using the Hoechst 33258 dye method and fluorometry (1). The rate of PG synthesis was measured during the last 4 hours of culture on day 14 using 35S-sulfate radiolabeling, followed by a rapid filtration assay (7). Total PG content was also assessed using the DMMB method (1) after papain digestion.

Statistical Analysis: Statistical analysis was performed comparing control by one-way ANOVA, using the Fisher’s PLSD test as a post hoc test. Level of p < 0.01 was used to determine statistical significance.

RESULTS:

DNA Content: The DNA content decreased moderately in the articular cartilage and nucleus pulposus groups: this was significant only at the highest concentration (100 nM) of Fn-f (p<0.0001). In contrast, the DNA content increased slightly in the two annulus fibrosus groups.

PG Synthesis: Fn-fs markedly suppressed 35S-incorporation by articular chondrocytes in a dose-dependent manner (p<0.0001). In the case of the nucleus pulposus cells this was less pronounced (Figure 1), gaining statistical significance only at 100 nM (p<0.0001, vs Control). The fragments had no significant effect on PG synthesis by cells from the inner layer of the annulus fibrosus but, at 100 nM, treatment with the Fn-fs led to a significant decrease in the DNA content. Cells from the nucleus pulposus responded to Fn-fs in a similar although less pronounced manner as chondrocytes.

The effects of Fn-fs on cells from the outer layer of the annulus fibrosus were very different; at 10 nM, Fn-fs stimulated PG synthesis significantly while at 100 nm they caused an increase in the DNA content.

Oegema et al showed that the content of Fn is quite high in degenerated discs (8) and suggested that Fn-fs present at elevated levels may induce the cells to degrade the matrix further. Our results suggest that this may be true in the case of the nucleus pulposus cells. The observation that Fn-fs can upregulate PG synthesis by cells from the outer layer of the annulus fibrosus, suggests that the fragments may play a role in repair of annular tears. The mechanisms responsible for the differences in the response of these related cell types are under investigation.


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****Biochem, Rush Medical College, Chicago, IL.**