Characterization of Serine Protease Activity in Human Osteoarthritis Joint Fluid

Sue Yocum1, David Clemmons2, Michael Rowland2, Walker Busby2, Debra Kellner1, Scott Lazerwith1, Frances Sverdrup1, Matt Yates1

1Translational and Molecular Medicine, Pfizer Global Research and Development, New London, CT; 2Dept. of Endocrinology, Univ of North Carolina Medical College, Chapel Hill, NC

sue.yocum@pfizer.com

Introduction: Insulin-like growth factor 1 (IGF-1) stimulates extracellular matrix protein synthesis and cell growth in normal cartilage. IGF-1 is present in articular joint fluid, and chondrocytes express high affinity IGF-1 receptors that mediate IGF-1 stimulated DNA and proteoglycan synthesis. IGF binding proteins (IGFBP's) are also expressed in cartilage and synovium and are present in synovial fluid; IGFBP's are involved in modulation of IGF-1/IGF-1 receptor response. Specifically, IGFBP-3 opposes IGF-1 trophic action in cartilage while IGFBP-5 potentiates IGF-1 effects in chondrocytes. In osteoarthritis (OA), chondrocytes are resistant to IGF-1 stimulation with decreased viability of cells and reduced proteoglycan synthesis. Proteolysis of IGFBP-5 reduces the affinity of IGFBP-5 for IGF-1, and increased IGFBP degradation has been reported in OA joint fluid. Inhibition of IGFBP-5 degradation was shown to limit cartilage destruction in the Pond-Nuki dog (1) and rat MMT (2) models of OA. C1s, a serine protease, is proposed to be the IGFBP-5 protease present in dog OA joint fluid (1). This report examines IGFBP-5 proteolytic activity in human OA synovial fluid.

Materials and Methods: Pooled OA joint fluid was subjected to ammonium sulfate precipitation and the supernatant was loaded onto a butyl sepharose column with active fractions then subjected to sequential separation by wheat germ agglutinin affinity and Concanavalin A columns. Eluted fractions with activity were pooled and subjected to electrophoresis via SDS-PAGE, zymography, and/or immunoblotting. IGFBP-5 proteolytic activity was assessed by cleavage of intact IGFBP-5 followed by SDS-PAGE and immunoblotting. Gel slices corresponding to immunoblotted activity were subjected to protein identification by mass spectrometry. Enzyme inhibition assays were performed using either intact IGFBP-5 (1) or a C1s chromogenic assay (3).

Results: Analysis of human OA joint fluid following the three step purification yielded a single 88 kDa band by zymography that was consistent with C1s via immunoblotting.

Mass spectroscopy was applied to trypsin digested peptides and was searched against the SWISS-Prot mammalian database. C1s was identified as the major serine protease present. To further determine the protease activity in joint fluid, extensive characterization of the effects of several protease inhibitors on IGFBP-5 proteolysis was undertaken and compared to the inhibitory profile of inhibitors on activity of C1s against a peptide substrate. Data was consistent with a serine protease activity as responsible for cleavage and was similar to the rank order of potency against purified C1s. Further metalloproteinase and aggrecanase inhibitors did not have significant inhibitory activity against joint fluid.

Discussion: Several proteases have been reported to cleave IGFBP-5; however, the presence of these in biofluids from diseased tissue has not been reported particularly with respect to OA. We show here that C1s is present in joint fluid and is the IGFBP-5 protease present in OA joint fluid. Inhibition of IGFBP-5 proteolysis has been shown in vivo to reduce cartilage degradation in the rat and dog. IGFBP-5 binding to IGF-1 potentiates the action of IGF-1 in cartilage. Therefore, inhibition of IGFBP-5 proteolysis may be a strategy to modulate reparative responses in articular cartilage.