SPECIFIC IMMUNOLOGICAL DETECTION OF THE (V+C)- FIBRONECTIN ISOFORM

Introduction:

Fibronectins are a part of the repertoire of matrix molecules produced by chondrocytes in order to assemble a functional cartilage matrix. They are encoded by a single gene, but significant protein heterogeneity results from alternative RNA splicing. The population of fibronectins in adult cartilage includes high levels of the cartilage-specific (V+C)- isoform which lacks the V, III-15 and I-10 segments as well as isoforms that include Extra Domain B (ED-B+) (1,2). The synthesis and accumulation of total cartilage fibronectins are increased in osteoarthritis (3,4). The cartilage specific expression of the (V+C)- fibronectin isoform makes it an attractive candidate for a biomarker to monitor osteoarthritis if the following criteria are met: 1. The synthesis and accumulation of (V+C)- fibronectin in cartilage should increase as does total fibronectin. 2. (V+C)- fibronectin should be released from the cartilage to the synovial fluid. 3. (V+C)- fibronectin should be easily distinguished from other fibronectin isoforms. In this study, we report the production of a monoclonal antibody specific for (V+C)- fibronectin without cross-recognition of fibronectin isoforms in plasma. We have used this antibody to confirm that (V+C)- fibronectin accumulation is increased in osteoarthritic cartilage and that (V+C)- fibronectin can be readily detected in the synovial fluid.

Methods:

Full length (V+C)- fibronectin and full length V+,C+ fibronectin were cloned into the pVL1392 baculovirus expression vector and then expressed in SF9 or SF21 insect cells. Recombinant proteins were purified by gelatin affinity chromatography. Purified, recombinant (V+C)- fibronectin was used to immunize BALB/C mice. Splenic lymphocytes were fused with SP2 myeloma cells and hybridomas were screened in an ELISA assay. A monoclonal antibody specific for (V+C)- fibronectin was obtained. Specificity was confirmed by Western analysis.

Synovial fluid and cartilage were obtained at necropsy from the hips of two dogs with spontaneous osteoarthritis as a result of hip dysplasia (four joints) and from the hips of two unaffected dogs (two joints). Cartilage was collected from visible lesions or from the area adjacent to the fovea where lesions would first appear and extracted with 4M urea. Total fibronectin in cartilage extracts and synovial fluid were quantitated in an ELISA assay using a polyclonal antibody to human fibronectin which cross reacts with the dog. Cartilage specific (V+C)- fibronectin in cartilage extracts and synovial fluid were quantitated in ELISA assay using the monoclonal antibody specific for (V+C)- fibronectin. RNA was extracted from cartilage lesions and disease-free areas from two additional dogs. An RNase protection assay, using a riboprobe spanning the I10/I11 exons, was performed to quantitate relative expression of (V+C)- fibronectin RNA as previously described (5).

Results:

A monoclonal antibody was selected for positive reactivity with recombinant (V+C)- fibronectin and the native (V+C)- fibronectin isoform from articular cartilage as well as for the simultaneous failure to react with recombinant V+,C+ fibronectin and native plasma fibronectin. (V+C)- fibronectin forms homodimers with itself but does not heterodimerize with other isoforms (6). In Western analysis, the monoclonal antibody to (V+C)- fibronectin reacted with the small dimers and the monomers corresponding to the (V+C)- isoforms present in cartilage and synovial fluid while all isoforms were recognized by a monoclonal antibody with an epitope in the Hep 2 domain (Fig 1). Total fibronectin content was increased 18 fold in lesion cartilage, while the cartilage-specific (V+C)- fibronectin was increased 11 fold (Table 1). At the RNA level, %/(V+C)- was 52% and 61% in OA and disease-free cartilage, respectively. The total concentration of fibronectin in synovial fluid from the hip joints of dogs with osteoarthritis was 5 fold greater than that of dogs without osteoarthritis. The (V+C)- isoform was detected in synovial fluid (Fig. 1, Table 1) but did not increase to the extent of total fibronectin.

Discussion:

The increase in total fibronectin in the cartilage and synovial fluid of dogs with osteoarthritis is consistent with previous reports (3). As with ED-B(+) fibronectin (2), the (V+C)- isoform is also increased in osteoarthritic cartilage as total fibronectin increases. This is not the case when chondrocytes are removed from the cartilage matrix and placed in monolayer culture where the %/(V+C)- drops dramatically (5) even when total fibronectin production increases. The successful production of a monoclonal antibody specific for (V+C)- fibronectin is consistent with a unique structure for this isoform. It makes it possible to explore the use of fibronectin as a biomarker for cartilage matrix turnover in osteoarthritis.

References:


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