Expression of cartilage oligomeric matrix protein by human synovium

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Abstract Human synovium was analyzed for the possible expression of cartilage oligomeric matrix protein (COMP). Immunostaining with polyclonal antiserum to COMP demonstrated positive staining within the synovial cells and immediately subjacent connective tissue, with less intense staining in the deeper connective tissue. Western blot analysis using either polyclonal or monoclonal antibodies to human COMP confirmed the presence of COMP by immunoreactive bands with the same molecular mass (approximately 110 kDa) as purified articular cartilage COMP. PCR using oligonucleotides that span human COMP exons 7–13 revealed identical amplification products from cDNA prepared from either human chondrocytes or synovium. Northern blot analysis using a biotinylated-probe to human COMP, spanning exons 12–13, also reveal an identical hybridization product to either human chondrocyte or synovium total RNA. Human synovium should be considered as a potential tissue source of COMP in any investigation of biological markers of cartilage metabolism.

1. Introduction

Cartilage oligomeric matrix protein (COMP) is an abundant, homopentameric, non-collagenous component of articular cartilage with a bouquet-like molecular structure [1–3]. Recent reports suggest that measurements of COMP in serum or synovial fluid serve as a marker of cartilage metabolism. Saxne and Heinegård were the first to report elevated levels of COMP, as measured by enzyme-linked immunoassay, in the knee joint synovial fluids of patients with reactive rheumatoid arthritis [4]. In the same study, they noted a significant increase in serum COMP levels in patients with juvenile chronic arthritis compared to healthy children. Sharif et al. reported that serum COMP levels increased during the first year in patients with radiographic progression of arthritis compared to no increase in serum COMP levels in patients without radiographic progression [5]. Using discriminate analysis, they demonstrated that serum levels of COMP greater than 3.17 μg/ml had a 70% sensitivity and a 78% specificity in predicting disease progression. In both human osteoarthritic and rheumatoid arthritic joints, COMP has been observed to degrade in the cartilaginous matrix, and intact COMP and degraded fragments are released into the synovial fluid [6]. More recently, it has been shown that COMP fragments rather than total COMP content, may be more indicative of cartilage degradation, since increased levels of COMP fragments have been detected in synovial fluids from patients with either osteoarthritis or rheumatoid arthritis [6,7]

Although initially believed to be a cartilage-specific molecule, COMP has also been detected in tendon, albeit in much lower amounts [8]. Critical for any marker of cartilage metabolism is the knowledge of its tissue distribution and the rate of the marker's secretion, degradation, and clearance from synovial fluid [9]. Below we describe the expression of COMP by another non-cartilage source that may have important ramifications for synovial fluid and/or serum COMP measurements.

2. Material and methods

Human COMP was purified from normal human articular cartilage as described previously [6]. Protein concentrations were determined using the bicinchoninic acid protein assay (Pierce Chemical Co.) with BSA as a standard. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on gradient gels of 4–15% polyacrylamide according to the protocol of Laemmli [10]. Reduction of disulfide bonds was achieved by addition 2.5% (v/v) 2-mercaptoethanol in the sample buffer. Gels were either stained with Coomassie Brilliant Blue or subjected to electrophoretic transfer to nitrocellulose as described by Towbin et al. [11]. Polyclonal antiserum to COMP, purified from bovine and human articular cartilage, was raised in rabbits as described previously [6]. Monoclonal antibodies to human COMP were raised in rats; cell culture supernatant from the hybridomas were used for immunological assays. Immunoblots were developed with either monoclonal or polyclonal antiserum to human COMP, followed by a secondary antirat or antirabbit horseradish peroxidase conjugated antibody and ECL chemiluminesence (Amersham).

Human synovium from five patients undergoing knee replacement surgery for non-inflammatory arthritis were divided into three portions. One portion of the material was fixed for 24 h in fresh 4% paraformaldehyde in 50 mM phosphate, 0.15 M NaCl, pH 7.4 (PBS) at 4°C for immunostaining. Specimens were dehydrated, embedded in paraffin, sectioned (5 μm), adsorbed to silanized oven-dried glass slides, deparaffinized, and hydrated to 0.1% bovine serum albumin in PBS. Sections were treated with either polyclonal antiserum to human COMP, preimmune rabbit serum (control), rat monoclonal antibody to human COMP, or rat monoclonal antibody to an irrelevant antigen (control), followed by incubation with either a biotinylated goat antirabbit or rabbit antirat antibody (Biogenex), respectively, for 30 min at room temperature. After washing with PBS, sections were incubated at room temperature with alkaline phosphatase-conjugated streptavidin (Biogenex) for 30 min, developed with Vector Red I (Vector) in 0.01 M Tris-HCl, pH 8.2, and counterstained with Mayer's Hematoxylin (Sigma).

A second portion of the tissue, approximately 1 g wet weight, was frozen to −70°C and then homogenized (Polytron) in 10 ml of ice-cold 50 mM Tris-HCl, 0.15 M NaCl, pH 7.4, containing 10 mM EDTA, 1 mM PMSF, 2 mM NEM. Samples were centrifuged for 30 min at 8000 rpm, 4°C. The supernatants were subjected to gel electrophoresis and western blotting.

A third portion of the samples (approximately 100 mg) was homogenized (Polytron) in 1 ml TRIzol reagent and total RNA-isolated (mRNA Isolation System, Life Technologies) according to the man-

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ufacturer's specifications. Total RNA then served as a template for first-strand cDNA synthesis through reverse transcriptional (RT) reaction using the superscript preamplification system (Life Technologies). The cDNA then was used as a template for PCR amplification of an internal sequence of COMP mRNA from 742–1363 base pairs using oligonucleotides GCA TGC AGA CTG CGT CCT AG and GTC CCG AGA CTC CTG ATG TC. PCR products were analyzed by agarose gel electrophoresis and stained with ethidium bromide. Isolated adult and fetal human chondrocytes also had total RNA isolated by the same method. Briefly, human chondrocytes were isolated from articular cartilage slices by sequential enzymatic digestion with trypsin (2 mg/ml) (Worthington Biochemical Co.), hyaluronidase (0.5 mg/ml) (Sigma), then collagenase (1.5 mg/ml) (Worthington Biochemical Co.) in Ham's F-12 media containing gentamycin (50 μg/ml). The isolated cells were allowed to recover in spinner culture for 24 h, and then total RNA-isolated [1].

For Northern blot analysis, both synovium and cartilage total RNA were resolved on 1% agarose gels electrophoresis and transferred to nylon membrane by capillary transfer. A double-stranded human COMP 84-base pair biotinylated-labeled nucleic acid probe

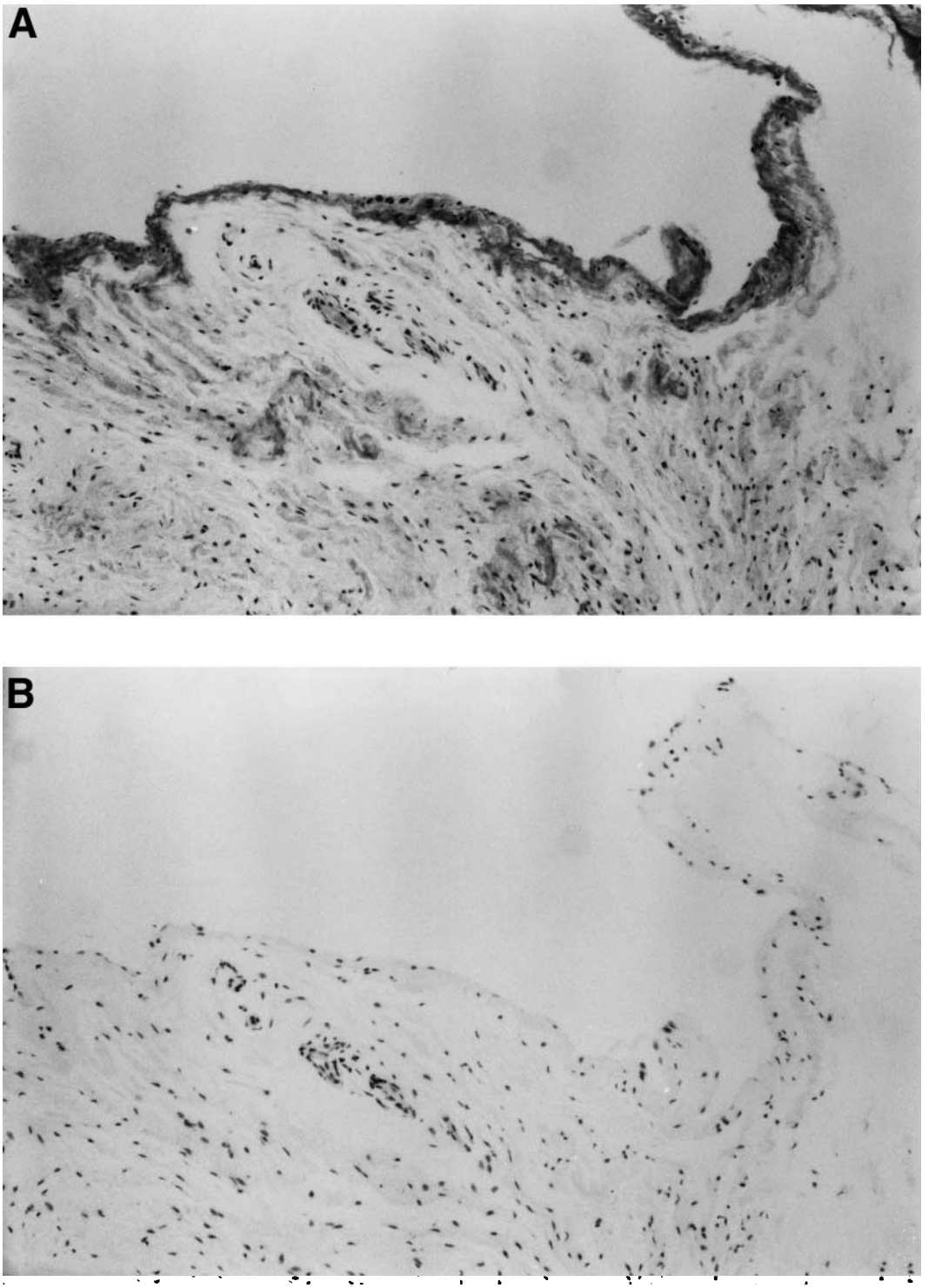

Fig. 1. Localization of COMP from human synovium using immunostaining with polyclonal antiserum to COMP (A) demonstrated positive staining within the synovial cells and immediately subjacent connective tissue, with less intense staining in the deeper connective tissue. Control sections with preimmune serum showed no staining (B).

was synthesized, corresponding to the human COMP sequence from 1280–1363, using PCR with biotin-14-dCTP (PCR Nonradioactive Labeling System, Life Technologies) and oligonucleotides GCG GAT GTG GAC CAC GAC and GTC CCG AGA CTC CTG ATG TC. Nucleic acids were crosslinked to the nylon membranes with ultraviolet light (Stratalinker), blocked with herring sperm DNA, probed with the heat-denatured 84-base pair biotinylated-labeled probe to human COMP, and allowed to hybridize overnight at 42°C. After washing, bound probe was detected using the Photogene Nucleic Acid Detection System (Life Technologies) according to the manufactures protocol.

3. Results and discussion

Among the potential advantages of COMP as a biological marker of arthritis is its abundance in articular cartilage and lack of abundant expression in other tissues [12]. As is true for most molecules that are initially believed to be tissue-specific, COMP has proven to have a wider tissue distribution. Critical for the development of a cartilage marker is a clear understanding of its distribution in sources other than articular cartilage.

In this study we examined the expression of COMP by human synovium. Immunostaining with either monoclonal or polyclonal antisera to human COMP revealed the presence of intensely positive COMP staining within synovial cells and the immediately subjacent connective tissue with less intense staining in the deeper connective tissue. There was no staining with rabbit preimmune serum or irrelevant rat monoclonal antibodies (controls) (Fig. 1). Synovium was also extracted with buffer containing EDTA. This method of extraction should preferentially extract COMP from the matrix, since it has been shown that COMP or its interaction partners are bound by a mediation with divalent cations [1–3]. Western blot analysis using either polyclonal or monoclonal antibodies to human COMP confirmed the presence of COMP by immunoreactive bands with the same molecular mass (approximately 110 kDa) as purified articular human COMP (Fig. 2). PCR used on cDNA generated from articular cartilage chondrocytes and synovium mRNA revealed an amplification product of the same size (approximately 0.6 kb), corresponding to the 622 base pairs of an internal COMP sequence (Fig. 3). This region spans exons 7–13, thereby eliminating the possibility of contaminating DNA [13]. Northern blot analysis using either total RNA revealed identical hybridization products from synovium and articular chondrocytes, corresponding to a product of the appropriate size (approximately 2.2 kb) using a biotinylated-labeled double-stranded COMP DNA probe that also spans exons 12–13, also eliminating the possibility of contaminating DNA (Fig. 4). Human synovium contains mRNA for COMP and produces protein, as noted by Western blot and immunostaining.

The synovial membrane lines diarthroidal joints and consists of an inner cellular layer (usually 2–4 cells thick) and an outer layer of irregularly arranged connective tissue that are not separated by a basement membrane [14]. It has been previously shown that synovial cells have chondrogenic potential and can express cartilaginous extracellular matrix proteins and degradative enzymes [15–25].

The results of the present study indicate that synovium should be included as another tissue source of COMP, and may contribute to either synovial fluid or serum COMP levels. If COMP is to be developed as an accurate biological marker of cartilage metabolism, further studies are needed to determine the synthetic rate and deposition of COMP by human synovium and how these may change in various arthritic states.

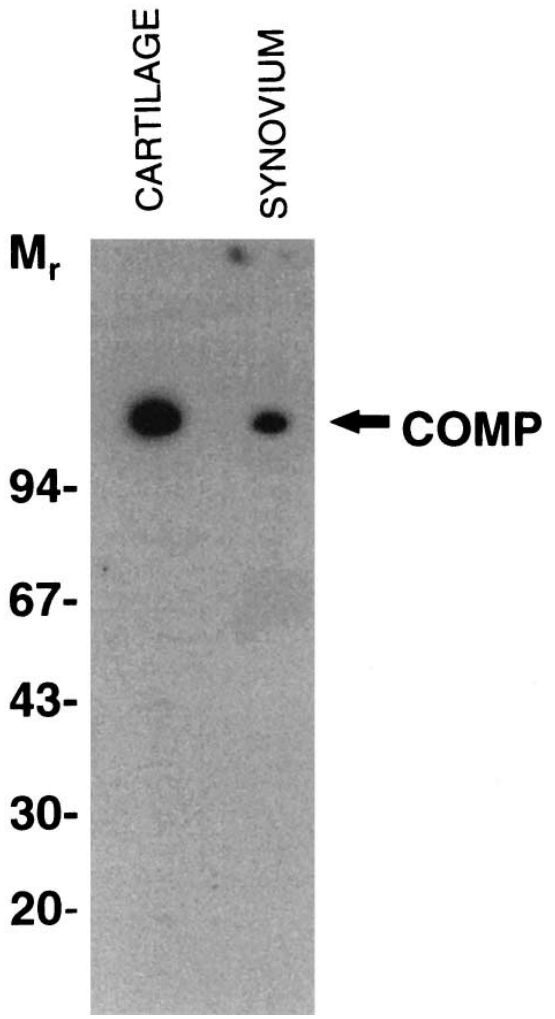

Fig. 2. Western blot analysis of reduced purified articular cartilage COMP (cartilage) and synovial tissue extract (synovium) after 5–15% gradient SDS-polyacrylamide gel electrophoresis using a rat monoclonal antibody to human COMP revealed a single identical molar mass band (approximately 110 kDa).

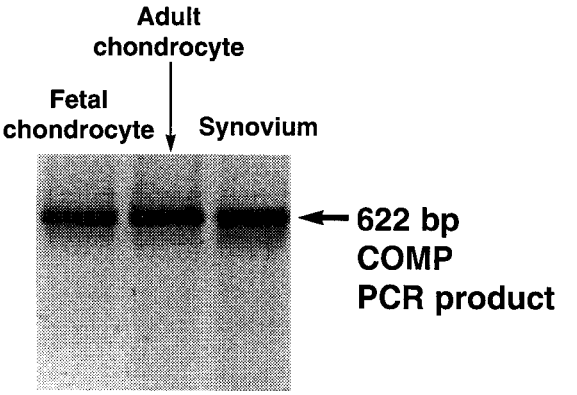

Fig. 3. PCR using oligonucleotides that span human COMP exons 7–13 revealed identical amplification products from cDNA prepared from either human chondrocytes (either fetal or adult) or synovium.

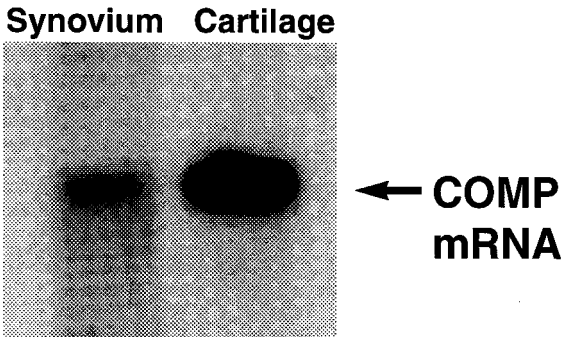

Fig. 4. Northern blot analysis using a biotinylated-probe to human COMP, spanning exons 12–13, reveal an identical hybridization product to either human chondrocyte (cartilage) or synovium total RNA.

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