DISTRIBUTION OF SUPERFICIAL ZONE PROTEIN (SZP) IN OSTEOARTHRITIC CARTILAGE

INTRODUCTION:

Superficial zone protein (SZP) is a 345 kD glycoprotein, which contains glycosaminoglycan chains (1). Immunohistochemical and biochemical studies have demonstrated that this molecule displays a highly restricted distribution in normal articular cartilage (2,3). It is present at the very surface of the cartilage in the lamina splendens. The molecule is synthesized by chondrocytes in the superficial zone, but not by chondrocytes in the middle or deep zones of normal articular cartilage.

Recent studies have shown SZP is homologous to megakaryocyte stimulating factor (MSF) (4) and lubricin (5), which is the major lubricating glycoprotein in synovial fluid. Human mutations in SZP cause the disorder camptodactyly-arthropathy-coxa vara-pericarditis (6). Monoclonal antibodies to human SZP have been generated (7). In this study the monoclonal antibodies have been used to analyze the distribution of SZP in human articular cartilage recovered from osteoarthritic patients at the time of joint replacement surgery.

METHODS:

Human articular cartilage was recovered from patients with osteoarthritis at the time of hip or knee replacement surgery. Samples were collected from patients after their informed consent and with Rush institutional review board approval. For comparison, normal looking human articular cartilage was collected from organ donors through collaboration with the Regional Organ Bank of Illinois within 24 h of death with IRB approval. Cartilage tissues were fixed in 4% formaldehyde in PBS for 30 minutes at room temperature. Tissues were mounted in OCT embedding compound and 10-um-thick frozen sections were cut. Sections were dried on gelatin-coated slides. Sections were incubated with 5 mg/ml hyalurondase, 1% BSA in TBS buffer for 30 minutes at 37°C and then blocked with normal horse serum for 30 minutes at room temperature. Specimens were stained with MOUSE monoclonal anti-human SZP antibodies. An anti-chicken type X collagen monoclonal antibody, which does not bind to human collagen X, was used as a negative control and an anti-type II collagen antibody (H6B3) was used as a positive control. The primary antibodies were detected with a Pierce Immunopure ABC Alkaline Phosphatase Mouse IgG staining kit according to the manufacturer's protocol. A 1-step NBT-BCIP substrate was incubated on the slides for 7 minutes to detect the alkaline phosphatase activity of the ABC complex.

RESULTS AND DISCUSSION:

Cartilage tissues were recovered from twelve osteoarthritic patients undergoing joint replacement surgery. Most of these tissues were from femoral heads removed during hip arthroplasties. A few of the specimens were from knee replacement surgeries. Cartilage tissues were recovered from regions adjacent to full thickness lesions as well as more "normal-looking" areas of cartilage further removed from lesion areas. For comparison, human cartilage specimens also were collected from ankle joints of organ donors. These donors have no known history of arthritis or other skeletal disorder. The specimens were collected from the talar dome of ankle joints which had a macroscopic assessment score of 0 on the Collin's scale (0 = normal; 4 = heavy damage over most of surface) and therefore represent "normal", undamaged cartilage.

In the normal looking cartilage of organ donors, the anti-SZP antibody strongly stained the surface of the cartilage. This acellular region appears to be the lamina splendens described by other investigators and was consistently stained in almost all specimens. The most superficial cells are flattened and sometimes stain for SZP. The staining of these superficial cells is variable. Sometimes the cells show a weak intracellular signal; other cells display an extensive pericellular signal. In normal looking cartilage it is rare to find a chondrocyte in the middle or deep zone which stains positive with the anti-SZP antibodies.

In osteoarthritic cartilage several different staining patterns were observed using the anti-SZP antibodies. The most common pattern showed a thin band of reactivity at the surface of the cartilage. Sometimes this band would be less than that seen in normal cartilage. In some areas this band appeared as if parts of this matrix had been ripped away. In this common pattern, usually the chondrocytes in the superficial layer did not stain with the anti-SZP antibodies. A different, less common pattern showed antibody reactivity for SZP evident throughout the cartilage matrix and extending from the top of the cartilage surface to a boundary front running through the middle zone of the cartilage. Individual chondrocyte reactivity was less evident because the entire matrix behind the front was positive. Such a pattern might be expected if much of the proteoglycan was lost from the cartilage matrix in a zone extending from the top of the cartilage to the front deeper in the cartilage. This would make the matrix more permeable and possibly allow the penetration of SZP from synovial fluid into the cartilage matrix until it reaches a region of cartilage matrix containing higher concentrations of proteoglycans so the large SZP molecule can no longer penetrate. Other osteoarthritic specimens showed regions of altered SZP distribution compared to normal looking cartilage. In some cases chondrocytes in clusters would show isolated reactivity for SZP even though most of the matrix surrounding the cells did not contain SZP. In other areas SZP was detected throughout the matrix although SZP producing chondrocytes were not evident. This pattern suggested SZP might locally diffuse from the synovial fluid into the cartilage in regions of local matrix damage.

In order to test the ability of SZP to diffuse into the cartilage, a cartilage surface was cut and the tissue incubated in synovial fluid for several hours. After this time the tissue was stained with the antibody and little if any penetration of SZP into the cartilage was evident. However if slices of human articular cartilage are maintained in serum-free organ culture containing insulin, transferrin and selenium, then the SZP accumulated in the medium. Immunostaining of these tissues maintained for over a month in organ culture showed SZP at the original cartilage surface, absent from most of the cartilage matrix within the tissue piece, but was able to bind to the deep cut surface of the tissue. This demonstrates that SZP is able to bind to macromolecules on the surface of the deep zone cartilage matrix, but did not penetrate into the cartilage matrix. Thus the penetration of SZP into osteoarthritic cartilage may indicate regions of cartilage matrix damage.

REFERENCES:


ACKNOWLEDGEMENTS: Supported by NIH (2PO-AR39239) and a collaborative research agreement with Glaxo-Wellcome Corporation. OA tissue was provided by Dr. Richard Berger, Department of Orthopedic Surgery and ROBI tissue collected by Dr. Arkady Margulis.

**University of Illinois, Chicago, IL

49th Annual Meeting of the Orthopaedic Research Society Poster #0774

Fig. 1 Immunohistochemical stain for SZP in cartilage lesion.