SUPERFICIAL ZONE PROTEIN (SZP): DEGRADATION BY CATHEPSINS AND REGULATION OF SYNTHESIS IN HUMAN ARTICULAR CHONDROCYTES.

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INTRODUCTION

SZP and lubricin are large proteoglycans secreted in to the synovial fluid by superficial zone chondrocytes and synovial fibroblasts, respectively [1,2]. Both proteins are encoded by the megakaryocyte stimulating factor (MSF)/proteoglycan-4 (PRG4) gene, but appear to differ as a result of differential exon usage and O-linked glycosylation [3]. Together, these proteins are primarily responsible for joint lubrication, and loss of lubricin in synovial fluids has been correlated with increased frictional coefficient in an animal knee injury model and in arthritic joints [4]. The factors responsible for lubricin/SZP synthesis and degradation are incompletely known but it is possible that the normal balance is disturbed in OA as a result of reduced synthesis and/or increased degradation, leading to a net loss. Previous studies have shown the ability of neutrophil elastase (NE), cathepsin B and MMP-7 to digest purified lubricin and SZP-like proteins in synovial fluid [4,5]. We have investigated (i) the ability of proteases to directly degrade SZP present in synovial fluid, and (ii) factors that regulate the production of SZP in monolayer cultures of human articular chondrocytes (HACs).

METHODS

Aliquots of bovine synovial fluid (BSF, 0.1µL equivalent) or purified human SZP were incubated with recombinant human cathepsin B (R&D Systems), cathepsin L (Sigma) and NE (Sigma) for 16 hours at 37°C in assay buffers comprising 0.1M sodium acetate, 5mM L-cysteine, 5mM EDTA and 1mM CHAPs (cathepsin B and L) or 0.1M Tris-HCl, 0.1M NaCl (NE), at both pH 5.5 and 7.0. Reactions were stopped by the addition of 1µM E-64 (cathepsin B and L) or 100µg/mL PMSF (NE). Degradation of SZP was determined by western blot analysis in which digests were separated by non-reducing SDS-PAGE on 3-8% Tris-acetate gels followed by transfer to nitrocellulose. Blots were probed with an anti-mouse monoclonal antibody (Ab) to human SZP [6].

Human articular chondrocytes obtained from OA knees and seeded into 6-well plates were cultured as monolayers in Hams F12 medium with 10% FBS. After 4-6 days in culture, medium was replenished and 10% OSM (10ng/mL) was added to some cultures for 48-72 hours and expression was determined by real-time PCR using primers/probe designed to exons 7 and 8 of human proteoglycan-4 (PRG4) (Applied Biosystems). SZP expression was determined from parallel cultures by a modified phenol/guanidine HCl procedure, described above. Real-time PCR was performed using RNA extracted for cathepsin B in OA pathology is the more likely [8].

RESULTS

Cathepsin B, cathepsin L and NE were able to degrade purified SZP. In addition, two bands recognized by the SZP mAb in bovine synovial fluid - a protein co-migrating with SZP and one of slightly higher MW – were also degraded by each protease (Figure 1). For each protease tested the extent of SZP degradation was dose-dependent over the 100 – 500nM concentration range of enzyme. Cathepsin B exhibited comparable degradation at both the acidic and neutral pH, suggesting that this activity is due to exopeptidase activity, since cathepsin B exopeptidase activity is largely absent at neutral pH [7]. Western blotting of HAC conditioned medium with the anti-SZP mAb identified a single band of ~350 kDa which was up-regulated in the presence of TGFβ, down-regulated by IL-1α and unaffected by OSM (Figure 2). SZP production by HACs was unaffected by IGF-1 (not shown). The inhibitory effect of IL-1α was also observed in combined treatment with TGFβ and OSM. To determine whether the inhibitory effect of IL-1α was due to increased protease-mediated SZP degradation, HACs were treated simultaneously with IL-1α and inhibitors of cysteine proteases (E-64 and cell permeable derivative EST) and cathepsin B (Ca-074 and its more cell permeable form Ca-074-Me). However, none of the protease inhibitors reversed the IL-1α mediated suppression, suggesting that IL-1α modulation of SZP levels is not cathepsin-mediated. The effects of TGFβ and IL-1α were conducted, at least in part, at the transcriptional level since real-time PCR revealed PRG4-derived transcripts to be stimulated ~3-fold by TGFβ and inhibited to around 35% of the control level by IL-1α (data not shown).

DISCUSSION

These results confirm and extend previous reports by demonstrating that SZP is a potential substrate for degradation by cathepsin L, in addition to cathepsin B and NE. Also, SZP degradation may occur at both acidic and neutral pH. Since cathepsin B appears to be the major active cysteine protease in OA cartilage rather than cathepsin L, a role for cathepsin B in OA pathology is the more likely [8]. The regulation of SZP synthesis in HACs by IL-1α and TGFβ suggest that these cytokines may contribute to disease progression and prevention through the modulation of mechanical wear and hence cartilage damage. Our findings additionally suggest that inhibition of SZP production by IL-1α does not appear to be mediated by cysteine proteases, but rather through direct suppression of transcription.

REFERENCES

1. Schumacher BL et al. (1994) Arch. Biochem. Biophys. 311: 144-152

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