TSG-6 EXPRESSION IS UPREGULATED IN THE MURINE STR/OR T MODEL OF OSTEOARTHRITIS

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Introduction Tumour necrosis factor-stimulated gene-6 (TSG-6) is a secreted glycoprotein of ~35 kDa that is induced in vitro in several cell types by proinflammatory cytokines and growth factors and in pathological conditions such as RA and OA (1,2). TSG-6 interacts with hyaluronan (HA), chondroitin-6-sulphate and the G1-domain of aggrecan via its Link module (3). The function of TSG-6 in vivo has not been established, but it is likely to be involved in cartilage remodelling, for example during inflammation, as its ligands are all components of extracellular matrix. We have suggested previously that TSG-6 may have a role in destabilising complexes of HA and aggrecan, that provide cartilage with its load-bearing properties, thus taking part in tissue homeostasis, repair and contributing to matrix disruption in disease (2,4).

TSG-6 immunoreactivity has been detected in cartilage and synovium of RA and OA patients, whereas is is absent in the joints of individuals with no known history of arthritis (2). In the present study we tested the hypothesis that there is a spatial and temporal change in the expression of TSG-6 during the development of degenerative joint disease. The expression of TSG-6 mRNA and protein was examined in the knee joints of STR/ort mouse which develops a natural form of OA in the medial tibial articular cartilage. OA-like lesions in the STR/ort mice begin to appear in older (>12 weeks) animals and can vary in the severity of cartilage damage (5).

Methods Serial cryostat sections (10µm) were cut through the knee joints of STR/ort male mice (6, 12-, 18-, 30- and 35-weeks of age) and age matched CBA mice that acted as control animals. Sense and antisense oligonucleotide probes were 3' and 5'-labelled with digoxigenin-dUTP and sections were subjected to in situ hybridization for TSG-6 mRNA. Prior to hybridization the sections were fixed, digested with proteinase K and acetylated. Hybridization overnight at 42°C was followed by detection of mRNA for TSG-6 with an anti-digoxigenin antibody labeled with alkaline phosphatase, visualized with BCIP/NBT. Control sections were treated with RNase or with hybridization solution alone. TSG-6 was immunolocalized using a rabbit anti-mouse polyclonal antisera raised against the C-terminal 15 amino acids of the mouse protein (521-535). Immunodetection was with the Vectastain ABC Elite kit and colour was developed using diaminobenzidine.

Results TSG-6 mRNA was expressed by the chondrocytes in the lateral and the medial tibial plateau of the STR/ort mice (Figure 1a) at a higher level than in the CBA controls. Sections treated with RNase or with TSG-6 sense probe gave no signal. The medial tibial cartilage showed stronger expression when it was compared with the lateral cartilage from the same knee joint. In STR/ort mice, chondrocytes adjacent to major OA lesions strongly expressed TSG-6 mRNA, compared to cells further from the lesion site (Figure 1b). TSG-6 protein was also localized in the lateral and medial tibial cartilage of STR/ort mice. In contrast, protein could not be detected in control CBA mice at any of the ages studied. In young STR/ort mice (6 weeks) where OA lesions had not yet developed, expression of the protein was confined mainly to the pericellular matrix surrounding the chondrocytes (results not shown). In older STR/ort mice (>18 weeks) that exhibited mild OA lesions, strong protein staining was noted in the interterritorial matrix of the superficial and mid-zones closest to the lesion site (Figure 2a). There was only weak expression of protein in the pericellular matrix of cartilage from joints with severe OA lesions (Figure 2b). Control sections that were treated with TSG-6 antibody preincubated with immunizing peptide, gave a very low signal, confirming the specificity of the antiserum. Reduced levels of alcian blue staining also coincided with the location of TSG-6 expression.

Discussion The pattern of TSG-6 expression has not been described before in mouse cartilage. The function of TSG-6 in cartilage is not clear, although it has been demonstrated to interact with HA and other matrix components (3,4). In this study, we have detected an increased expression of the protein in the extracellular matrix surrounding OA-like lesions in STR/ort mice. Previously it has been shown that TNFα, IL-1α, IL-1β and TGFβ1 are upregulated at sites of cartilage damage in STR/ort mice (5) and these factors are known to induce TSG-6 expression in cartilage (1). Regions of high TSG-6 immunoreactivity showed a decrease in alcian blue staining suggesting that TSG-6 may be involved in aggrecan turnover. Interestingly, there was a higher level of TSG-6 expression in minor OA lesions compared to more severely damaged cartilage. This may be a consequence of loss of the protein from the cartilage following the breakdown of the collagen network and the subsequent release of extracellular matrix components or, alternatively, due to cell death leading to reduced protein expression. It might, however, indicate that TSG-6 expression is maximal at early stages of lesion development.


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