AGGRECAN DEGRADATION IN HUMAN ARTICULAR CARTILAGE EXPLANTS IS MEDIATED BY BOTH ADAMTS-4 AND ADAMTS-5

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INTRODUCTION

Proteoglycan loss associated with cartilage destruction in osteoarthritis is thought to occur primarily through the action of aggrecanases, which are members of the ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) proteinase family. ADAMTS-4 (aggrecanase-1) and ADAMTS-5 (aggrecanase-2) in particular have been considered to be likely mediators of this process because among the ADAMTSs studied, they have the highest specific activity for aggrecan cleavage in vitro, both proteins are expressed in human osteoarthtritic cartilgage, and expression is colocalized in areas of aggrecan depletion [1, 2, unpublished data]. However recent published studies have shown that cartilage from ADAMTS-5, but not ADAMTS-4, knockout mice is significantly protected from degradation *in vitro* and *in vivo* [3, 4], raising questions about the relative contributions of these enzymes in human joint disease. To evaluate the respective roles of these enzymes in aggrecan breakdown in human cartilage, we employed a siRNA approach to evaluate the effects of specifically inhibiting each enzyme's expression in normal and osteoarthritic articular explants.

METHODS

Cells and Tissues: Human normal cartilage explants and chondrocytes were harvested from knees of 16 donors raging in age from 40 to 85 years. Osteoarthritic cartilage explants were taken from four patients that underwent knee replacement surgery ranging in age from 61 to 73 years.

Design and transfection of siRNA: siRNAs were designed via the "siRNA Founder" online algorithm (Ambion), and synthesized by Ambion or Dharmacon. Primary chondrocytes and cartilage explants were transfected with siRNAs using OligoFectamine reagent. At 24h (cells) or 48h (explants) post-transfection, cells or normal cartilage explants were treated with or without TNF-α and Oncostatin M. Conditioned media were collected for analysis after 24h (cells) or 48h (explants) additional incubation. Cytokine treatment was omitted in studies using OA cartilage explants.

RNA expression: Total RNA was isolated from chondrocytes and cartilage explants, and quality was assessed using an Agilent Bioanalyzer 2100. mRNA levels for each gene were determined by quantitative real-time RT-PCR (Taqman).

Cartilage degradation: Proteoglycan released in conditional media was measured by DMMB dye binding. Aggrecanase-generated aggrecan neoepitopes were detected by Western blot analysis using antibodies directed against the ARGS and AGEG cleavage sites.

Statistical Analysis: GAG release is expressed as the mean ± standard deviation of the results obtained from separate studies using at least four human donors. Significance was determined by comparing control and target siRNA group means using a randomized complete block analysis of variance to exclude donor variability with the assay as the blocking factor and the treatment group as the main effect. A two-sided p value <0.05 was considered significant.

RESULTS

Transfection of ADAMTS-4 and ADAMTS-5 siRNAs into monolayer cultured human primary chondrocytes specifically decreased the mRNA levels of each gene by 65% and 80%, respectively. Similarly, each siRNA decreased its target mRNA expression by at least 60% in cytokine-treated human normal cartilage explants. Cytokine-induced aggrecan degradation measured by GAG release and neoepitope formation was significantly decreased in normal cartilage by suppressing the expression of ADAMTS-4 or ADAMTS-5 individually, or in combination (Fig. 1). Reduction in aggrecan degradation was also observed following siRNA-mediated knockdown of either gene in osteoarthritic cartilages in the absence of added cytokine (Fig. 2). In contrast, transfection with a siRNA targeting ADAMTS-1 did not inhibit proteoglycan loss or aggrecan cleavage in either normal or osteoarthritic cartilage.

DISCUSSION

These results indicate that both ADAMTS-4 and ADAMTS-5 contribute to aggrecan loss in cytokine-stimulated normal human cartilage, and to ongoing aggrecan loss in osteoarthritic cartilage explants. Therefore, in contrast to the apparent dominant role of ADAMTS-5 in aggrecan degradation in mice suggested by mouse knockout studies, our studies suggest that both proteinases contribute to the structural damage that accompanies osteoarthritis in man.

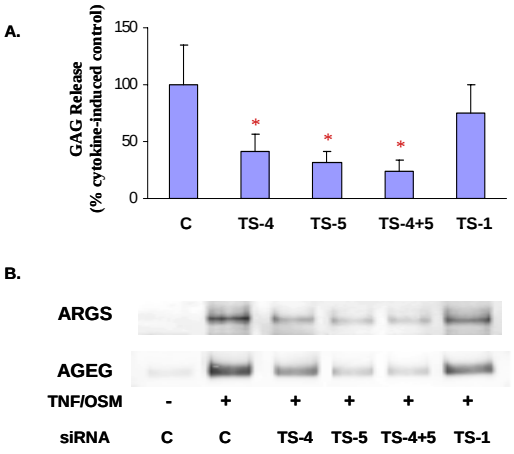

Fig. 1. Effect of transfection with control (c) or ADAMTS-targeted siRNAs on aggrecan breakdown in cytokine-stimulated normal cartilage explants. **(A)** Glycosaminoglycan in media 48 hr after treatment with 100 ng/ml TNFα + 100 ng/ml oncostatin M. *, $p < 0.005$ **(B)** Western analysis of neoepitopes generated by aggrecan cleavage.

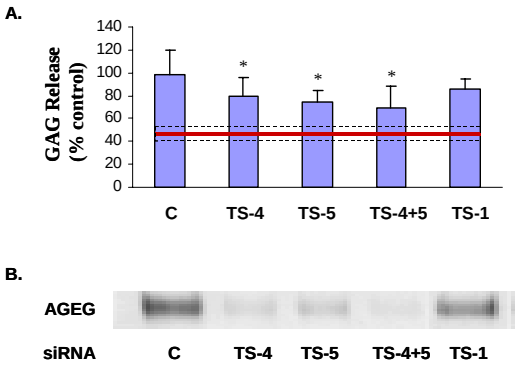

Fig. 2. Effect of transfection with control (c) or ADAMTS-targeted siRNAs on aggrecan breakdown in osteoarthritic cartilage explants. **(A)** Glycosaminoglycan in media. For comparison, the median and range of values obtained in separate studies using untreated normal cartilages are indicated by horizontal solid and dashed lines. *, $p < 0.05$ **(B)** Western analysis of neoepitopes generated by aggrecan cleavage.

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