

Chondrocyte secreted CRTAC1: an extracellular matrix molecule of human articular cartilage facilitates cell adhesion.

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INTRODUCTION: Cartilage acidic protein 1 (CRTAC1) is a novel human marker which allowed discrimination of human chondrocytes from osteoblasts and mesenchymal stem cells in culture. It was so far studied only on the RNA-level [1-3]. Based on sequence analyses the following protein domains were predicted: a signal peptide for secretion, four FG-GAP repeat domains, one RGD integrin binding motif, and an EGF-like Calcium-binding domain. Two splice variants (CRTAC1-A and CRTAC1-B) resulting from alternate last exon usage were detected in human. The aim of this study was to characterize the expression and localization of CRTAC1 protein and to test our hypothesis that CRTAC1 represents a so far unrecognized extracellular matrix molecule of cartilage.

METHODS: Two different polyclonal CRTAC1 antisera were generated by immunising rabbits with two distinct CRTAC1 specific peptide epitopes. These antisera were used for Western blot analysis and for immunohistochemical staining of cartilage-bone-sections. Human chondrocytes were obtained by enzymatic digestion of cartilage slices, cultured in monolayer and cellular lysates as well as medium supernatants were subjected to Western blotting. Functional cell adhesion experiments were performed with a Multiple Substrate Array (MSA™) containing either different matrix molecules or different quantities of type II collagen. Adhesion assays were performed with SaOS cell lines stably transfected with CRTAC1-A, CRTAC1-B or the control vector. In addition CRTAC1 conditioned medium compared to CRTAC1 free medium was analyzed on its ability to influence cell adhesion on different substrates.

RESULTS: Western blot analyses showed CRTAC1 reactive bands in supernatants of cultured chondrocytes indicating an effective secretion of the molecule. In addition, polyclonal CRTAC1 antisera stained the extracellular matrix of deep zone cartilage close to the subchondral bone, a region where

calcified cartilage matrix is located. Interestingly, consistent with this localization, some consensus domains of the molecule (repeat 3 and 4 of the FG-GAP domains and the EGF-like domain) were predicted to bind calcium, and the acidic nature of the molecule predisposes it for divalent cation binding. Of five putative O-glycosylation motifs in the last exon of splice variant CRTAC1-A, the most C-terminal one was modified according to exposure of serial C-terminal deletion mutants to the O-glycosylation inhibitor Benzyl- α -GalNAc. A functional assay on a Multiple Substrate Array showed that CRTAC1 supported cell adhesion of recombinant cells to several different matrix compounds. Its capacity to enhance cell-matrix interaction to type II collagen, the most prominent extracellular matrix compound of articular cartilage, was significant from 12.6 to 270 μ g/ml of spotted substrate. In addition CRTAC1 conditioned medium enhanced adhesion of human cells to extracellular matrix molecules like fibronectin, vitronectin and type VI collagen.

DISCUSSION & CONCLUSIONS: In summary, human CRTAC1-A represents a chondrocyte-secreted new extracellular matrix molecule of deep zone articular cartilage that is glycosylated at Thr-626 with function in cell-matrix interaction.

REFERENCES: ¹E.Steck, et al. (2001) *Biochem. J.* **353**: 169-174. ²K.Benz, et al. (2002) *BBRC* **293**: 284-292. ³A.Winter, et al. (2003) *Arthritis Rheum.* **48**: 418-429

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