A 384-well format screening of the compounds that inhibit collagen-protein interactions

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Objective: Collagen is a multifunctional protein that exhibits diverse biological activities. These functions are elicited by interactions between dozens of collagen-binding proteins (CBPs) and the collagen triple-helix. Since interactions of CBPs with collagen are often related to pathophysiological events in human body, some CBPs are currently regarded as targets for drug development. In this paper, we developed a high-throughput turbidmetric assay system to obtain inhibitors of collagen-CBP interactions.

Methods and Results: Our assay system is based on the finding that CBPs retard spontaneous collagen fibril formation in vitro, and the fibril formation is restored in the presence of compounds that disrupt the collagen-protein interaction. In this paper we show results of 384-well format screening of a set of test compounds against five recombinantly expressed CBPs, such as heat-shock protein 47, pigment epithelium derived factor, von Willebrand factor, glycoprotein VI and bacterial collagenase.

Conclusions: Using the system, the inhibitory activity of a set of test compounds were effectively evaluated for the five target CBPs in the same assay platform. Moreover, the use of the common assay platform will also bring us information about the specificity in the inhibitory action of a compound. The rapid screening system is a powerful tool for obtaining inhibitors for disease-related CBPs.

APC-induced MMP Activation in Human Diseased Chondrocytes Requires EPCR and Thrombomodulin

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Objectives: Activated protein C (APC) is derived from its inactive precursor Protein C (PC) through binding to endothelial protein C receptor (EPCR). We have previously shown that in cytokine-stimulated ovine cartilage, APC leads to collagenolytic MMP activation and cartilage degradation. This study investigates if APC can activate MMPs in human articular chondrocytes and the mechanisms/pathways whereby APC has its effects.

Methods: Chondrocytes were isolated from knee joints of normal young (6-12 month old) sheep, and patients undergoing joint replacement surgery. Cell monolayers were cultured serum free ± IL-1 and APC for 3 days. MMP-2, -9 and -13 activity was measured using gelatin zymography and fluorogenic substrate assays, and expression of enzymes and matrix components analysed using real-time RT-PCR. Activation of human MMP-2, -9 and -13 proenzymes by APC was examined in vitro.

Results: APC was unable to directly activate recombinant human proMMP-2, -9 or -13 in solution in vitro. In contrast, APC led to the activation of MMP-2, -9 and -13 in IL-1-stimulated cultures of normal ovine chondrocytes. APC also activated MMPs in IL-1-stimulated but not control human chondrocyte cultures. Interestingly, this activation only occurred in half of the OA-human patients, even though they all synthesised pro-MMPs that could be activated by APMA. Patients in which MMP-activation by APC was observed were distinguished by upregulation of EPCR, thrombomodulin and MMP-9 mRNA by IL-1 + APC.

Conclusions: These results suggest that APC is a physiologically relevant activator of chondrocyte MMPs implicated in cartilage breakdown in arthritis in humans. The differentiation of human OA patients into two sub-populations suggests that MMP activation by APC requires chondrocyte EPCR and TM and could be important in disease progression and as a therapeutic target in OA.