427i Identification of Shear Stress-Responsive Elements of the Cyclooxygenase-2 Gene in Human Chondrocytes

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Cyclooxygenase-2 (COX-2) is a pivotal proinflammatory enzyme that catalyzes the rate-limiting step in the synthesis of prostaglandins, which play a key role in the erosion of cartilage. Overexpression of the COX-2 protein in articular cartilage, an earmark of arthritis, is induced by mechanical-loading derived fluid shear stress in chondroctyes. Using cDNA microarray technology coupled with bioinformatics tools, we have recently documented that high levels of fluid shear (20 dyn/cm^2) induce COX-2 expression in chondrocytic cells via a mitogen-activated protein kinase (MAPK) c-jun N-terminal kinase 2 (JNK2)/c-jun-dependent pathway. Different regulatory elements present in the 5'-flanking region of the COX-2 gene have been demonstrated to regulate transcription in various cell types, however, COX-2 promoter analysis in shear-activated chondrocytes had yet to be performed. Hence, the goal of this study was to identify the cis- and trans-acting controls of shear-induced COX-2 expression in human chondrocytic cells. To map the binding sites of the COX-2 promoter that respond to shear, a series of promoter reporter constructs are used in which deletions or site-specific mutations have been introduced. Supershift gel assays are performed to identify the cognate transcription factors involved in COX-2 shear induction. Their functional role is further elucidated via functional knock-out experiments. Determination of key regulatory elements of the COX-2 promoter may provide insights into developing novel therapeutic strategies for targeting molecular pathways relevant to arthritis.