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HYALURONAN SUPPRESSES P38 MITOGEN-ACTIVATED PROTEIN KINASE IN OSTEOARTHRITIC CHONDROCYTES STIMULATED WITH COOH-TERMINAL HEPARIN-BINDING FIBRONECTIN FRAGMENT

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Purpose: Increased fibronectin fragments are thought to be involved in cartilage destruction in osteoarthritis (OA) through their catabolic activities. Hyaluronan of high molecular weight (HA) is now widely used for treatment of knee OA by intraarticular injection. Although there is evidence that HA suppresses fibronectin fragment action, the inhibitory mechanism is not entirely clear. While some fibronectin fragments can activate p38 mitogen-activated protein kinase in articular chondrocytes, HA effects on fibronectin fragment-activated p38 remain to be clarified. This study was aimed to examine the inhibitory effect of HA on nitric oxide (NO) production through p38 activation by COOH-terminal heparin-binding fibronectin fragment (HBFN-f) in OA chondrocytes.
Methods: OA chondrocytes in monolayer or cartilage explants were cultured with HBFN-f. Secreted levels of NO in conditioned media were determined. Activation of p38 and induction of inducible form of NO synthase (iNOS) were evaluated by immunoblot analysis. Cultures were pretreated with 2700 kDa HA to evaluate the inhibitory effect on HBFN-f action.
Results: HBFN-f activated p38, leading to NO production in association with iNOS up-regulation. The specific inhibitor of p38 confirmed the requirement of p38 for NO induction by HBFN-f. Pretreatment with HA resulted in significant suppression of p38 activation by HBFN-f. HA also inhibited HBFN-f-stimulated NO production with down-regulation of iNOS.
Conclusions: The present study clearly demonstrated that HBFN-f activated p38 in OA chondrocytes, while HA inhibited such activation. When HA is therapeutically introduced into OA joints, therefore, HA could suppress the catabolic actions of fibronectin fragments like HBFN-f as a potent p38 inhibitor.

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CHARACTERIZATION OF THE REG RECEPTOR AND ITS SIGNALING PATHWAY IN OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is the most common form of arthritis, affecting one in ten people. The disease whose aetiology is still unknown, is characterized by the progressive degeneration of the cartilage, and also affects bone and muscle tissue. Besides the articular degeneration, OA is also associated to inflammation and local chronic pains. Our studies have shown that the over-expression of the REG/Extl3 receptor enhances the action of cytokine TNFα in the activation of NFκB. This data suggests possible implication of the REG receptor in certain inflammatory mechanisms, including osteoarthritis, since the expression of this receptor is strongly increased in articular chondrocytes of OA patients. The first goal of this project is to verify whether the activation of NF-κB by pro-inflammatory cytokines such as TNFα in osteoarthritis, is mediated through the direct interaction between TNFRI receptor and the REG/Extl3 receptor. Secondly, we would like to characterize the signaling pathway of the candidate members of the Reg family that could modulate REG/Extl3 receptor function, and identify feasible interactions with TNFRI and/or other molecules implicated in TNFα signal transduction.
Methods: HEK293 and C28/I2 cells were transfected with REG receptor expression plasmids, and treated with TNFα alone, or in combination with increasing concentration of purified recombinant Reg I. We have studied the effect of REG/Extl3 receptor on the activation of NF-κB pathway by performing Luciferase assays with the pNF-κB-LUC construct. In parallel, different constructs of the Reg receptor have been tested in the similar manner to identify the important activation domain (s). Interactions between TNFR1 and REG/Extl3 were also characterized by immunoprecipitation.
Results: The activation of the NF-κB pathway by TNFα is 5 times higher in the presence of the EXTL3 receptor than in control cells. In addition, this effect seems generalized because it was detected with other pro-inflammatory cytokines. The data obtained with the receptor constructs showed that the transmembrane region located at the N-terminal is essential for the receptor activation. We have identified the TRAF binding domain in REG receptor, which is conserved in all known co-receptors of TNFRI. This suggests that the Reg/Extl3-TNFRI interaction takes place via the TRAF2 protein.
Conclusions: EXTL3 seems to play an important role in the inflammatory response induced by TNFα and other cytokines. Its characterization will allow us to identify possibly new therapeutic targets for OA.

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INTEGRIN LINKED KINASE-MEDIATED RAS ACTIVATION IS REQUIRED FOR MECHANOTRANSDUCTION-INDUCED PROLIFERATION AND DIFFERENTIATION IN ARTICULAR CHONDROCYTES

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Purpose: Mechanical forces are important regulators of cartilage function and structure, acting as potent exogenous regenerative stimuli on cells. Here we show that integrin linked kinase (ILK) mediated Ras activation plays a critical role in chondrocyte proliferation and differentiation. Furthermore, these signals activate multiple pathways to induce regulate chondrocytes functions.
Methods: Articular chondrocytes (ACs) obtained from the cartilage of shoulders and knees of Sprague Dowley rats (12-14 wk, females) were cultured in Ham's F-12 containing 10% FCS, 2 mM glutamine, 100 U/ml penicillin and 10 μg/ml Streptomycin. The cells were loaded on 3-D PCL scaffolds, grown for 5 days and subsequently subjected to dynamic compressive forces (15% DCF) at 0.25 Hz for various time intervals (5, 15, 30 60 min). Proteins were then extracted and signaling molecules analyzed for activation by Western blot analysis. In some cases expression of genes/transcription factors was suppressed by transient transfection of siRNA, confirming the gene silencing, and consequences of gene deletion assessed by pathway analysis.
Results: ACs activated by DTF exhibited a rapid activation of ILK and Ras in response to DCF. Deletion of ILK expression by siRNA or mutant geen insertion, inhibited Ras activation suggesting that ILK activation upstream of RAS is essential for AC activation. Ras activation induced activation of c-Raf (Ser338), Mitogen activated kinase kinase MEK1/2, and ERK1/2 activation directly as well as phospho-inositol-3-kinase (PI-3K) mediated phosphorylation of PAK1. Since we observed that PI-3K is activated during AC activation by DCF, these observations led to examination of Akt activation. We observed that in addition to activation of Ras DTF also activates Akt by phosphorylating Ser473 residue. Inhibition of Ras activity by its specific inhibitor GGT12133 also inhibited ERK1/2 activation at Thr 202/Tyr204. ERK1/2 phosphorylation