I-27 TARGETING NF-KAPPAB SIGNALING IN OA: IS IT ONLY ABOUT INFLAMMATION?

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Purpose: To provide evidence in support of the hypothesis that selectively impeding OA chondrocyte differentiation towards a hypertrophic-like state (by blocking NF-kappaB independent functions of IKKalpha) could represent a rational strategy for ameliorating the course of OA disease. Without argument OA is a complex often considered untreatable disease that develops with ageing due to a variety of genetic and environmental phenomena. Abnormal, progressive changes occur in the delicate balance between anabolic and catabolic processes of chondrocyte homeostasis in OA, thereby tipping the balance in favor of deregulated catabolic activity, excessive ECM remodeling and thus cartilage degradation. This excessive degree of cartilage destruction has a strong inflammatory component, as is the case for other debilitating, ageing associated diseases; but it remains unclear whether an inflammatory state actually initiates or exacerbates OA phenotype. Importantly OA pathology is also characterized by cycles of attempts at faulty ECM repair and enhanced angiogenesis in conjunction with chondrocyte hypertrophy leading to abnormal endochondral ossification that produces underlying bone thickening and the appearance of bony outgrowths. The latter features of OA disease are suggestive of specific perturbations in cellular homeostasis characterized by faulty, inappropriate chondrocyte differentiation. Along this line of reasoning selectively impeding adult articular chondrocyte differentiation towards a hypertrophic-like condition in OA could represent a logical strategy for stabilizing chondrocyte ECM and maintaining their normal homeostasis in general to avoid the onset of disease phenotype. In this regard experimental evidence will be presented that IKKalpha plays a surprisingly NF-kappaB independent, important role in modulating the onset of ECM remodeling in conjunction with the conversion of chondrocytes to a hypertrophic-like status and as such could represent a novel OA target.
Methods: Stable retroviral transduction of targeted interfering RNAs into OA and normal chondrocytes, in vitro chondrocyte differentiation, immunohistochemistry, immunoblotting, Elisa, real time PCR.
Results: Ablation of either IKKbeta or IKKalpha expression blocked ECM remodeling and chondrocyte differentiation by NF-kappaB dependent and independent mechanisms respectively. Importantly IKKalpha knockdown (KD) profoundly blocked chondrocyte differentiation prior to the onset of ECM remodeling. Moreover IKKalpha compromised articular chondrocytes maintained a highly organized ECM in conjunction with enhanced cell viability. IKKalpha KD suppressed MMP13 activity but not its expression. Interestingly we also find that IKKalpha KD and MMP13 KD chondrocytes share a number of common features.
Conclusions: The NF-kappaB signaling pathway and a significant portion of its downstream target genes are well known to orchestrate cellular stress-like responses. Moreover NF-kappaB deregulation strongly contributes to the poor prognosis of diseases with a strong inflammatory component, thus also making it a preferred OA target. However anti-NF-kappaB based therapeutics can be a double edged sword due to potentially deleterious consequences for cell survival and other targeted anti-inflammatory modalities (including anti-MMP or anti-Cox or anti-Il-1beta based therapeutics) can cause unwanted, collateral side affects. Our new findings provide evidence that IKKalpha, in a kinase and NF-kappaB independent pathway, could represent a novel OA disease target. Future experiments will in part be devoted to establishing a functional role for IKKalpha in an in vivo OA model, wherein IKKalpha ablation is induced in murine chondrocytes in vivo during the course of experimentally induced OA disease.

I-28 INFLAMMATION IN OA AS A TREATMENT TARGET

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Purpose: Osteoarthritis (OA) is a multifactorial disease in which joint failure results from an intricate interplay among the pathologic processes taking place in cartilage, synovial membrane (SM) and subchondral bone. The specific contribute of each of these tissues in determining the joint damage is matter of debate. SM is generally believed to play a minor role in OA pathogenesis and to be involved following the release of cartilage debris that would induce the SM reaction and inflammation. Inflamed SM secrete an array of proinflammatory molecules which implement the cartilage damage by promoting the activation of MMP (metalloproteinase) cascade leading to matrix breakdown.
We focused on the possible early involvement of the SM in OA pathophysiology and on the role of some inflammatory mediators in causing cartilage damage.
Methods: We induced right knee OA in nine goats by resecting the anterior cruciate ligament (ACL). Histology of SM, cartilage and subchondral bone was performed and the expression of Il-1 and Il-4 was assessed by immunohistochemistry and flowcytometry. Our experimental model has shown that cartilage and SM were firstly and simultaneously involved in OA (at 1 month) whilst morphometric analysis of subchondral bone detected significant differences between OA and control knee only after 6 months. Furthermore, the expression of Il-1 was already increased in SM at 1 month and, interestingly, the intracellular levels of Il-4 were significantly increased in OA cultured osteoblasts not before 3 months.
Results: Among the proinflammatory mediators produced by the SM, substance P (SP) is an intriguing molecule with pleiotropic functions. SP is a neuropeptide released from the fibers C terminal nerve endings and plays a pivotal role in controlling nociception, vascular tone and inflammation. SP has been found increased in synovitis and is also directly produced by synoviocytes. We demonstrated that chondrocytes synthesize SP and its expression increased in human OA chondrocytes both at mRNA (RT-PCR) and protein (flocytometry) levels. Furthermore, the cell-membrane bound peptidases (CD10 and CD26), which hydrolyze the neuropeptides, were found decreased on OA human chondrocyte surface. To further explore the role of SP in cartilage metabolism and OA pathogenesis, we studied its effect on proteoglycans (PG) remodeling and aggrecanase-5 (ADAMTS-5) and MMP-13 synthesis. Preliminary data suggest the SP (10^{-8} M) increases the breakdown of PG from both OA and normal cartilage explants, as indicated by reduced safranin staining, and enhances the release of ADAMTS-5 and MMP-13 (western blot). Preincubation with spantide II (a selective antagonist of SP) prevents these effects. Nevertheless, TIMP-3, a specific inhibitor of ADAMTS-5, does not seem to be regulated by SP. Hence, SP, beside its proinflammatory functions, exerts a direct effect on matrix cartilage remodeling and its increase in OA may represent a further mechanism of cartilage damage.
Conclusions: The relevance of inflammation in OA pathophysiology is worthy of further studies since its role in OA is not limited to appearance of pain but directly takes part to joint damage and its targeting, since the early stage of disease, with selective modulators can be a promising strategy to cure OA.

I-29 DISCOVERY AND VALIDATION OF AN IMMUNOAFFINITY LC-MS/MS ASSAY FOR THE QUANTIFICATION OF THE COLLAGEN TYPE II NEOEPITOPE PEPTIDE BIOMARKER

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Purpose: To validate an assay for the quantification of type II collagen neoepitope (TIINE) peptides for its biomarker applications. Application of a biomarker in scientific decision making requires its validation for the given purpose and therefore, should include two distinct activities: technical validation of the assay procedure and biological/pharmacological validation for its fit-for-purpose use. This work describes recent discovery and analytical validation of the immunoaffinity LC-MS/MS assay for the quantification of the TIINE 45-mer peptide in human urine to demonstrate that the assay is specific, sensitive and reliable (precise and accurate) for measuring type II collagen breakdown in clinical settings.
Methods: Quantitation of TIINE-containing 45-mer peptides with 5-hydroxyproline (HyP) was performed by two-dimensional LC-MS/MS, utilizing an HP1100 high-performance liquid chromatography (HPLC) system, a CTC Analytics HTS PAL autosampler, switching valve, and interfaced to an API 4000 triple quadrupole mass spectrometer. A 45-mer peptide containing 5-Hyp that was deuterated (d5) at the C-terminal glutamine residue was used as an internal standard. The standard curve of the 45-mer TIINE peptide was prepared in aqueous solution with protein present (10 μg/mL of BSA), whereas quality control samples were made in urine matrix. Methods used for validation were based on utilizing the authentic peptide spiked into urine samples and evaluated for sensitivity, selectivity, linearity, accuracy and precision as well as carry-over, long and short-term stability. Urine samples from the second morning void were collected without preservatives, aliquoted for separate