NOVEL BIOMARKERS OF KNEE OSTEOARTHRITIS IDENTIFIED USING METABOLIC PROFILING

G. Zhai¹, R. Wang-Sattler², D. Hart¹, T. Ilig², T.D. Spector¹
¹King’s Coll. London, London, United Kingdom; ²Inst. of Epidemiology, Neuherberg, Germany

Purpose: There is a pressing need to develop reliable molecular biomarkers that can inform on the process of joint destruction in osteoarthritis (OA). Such biomarkers could aid in drug development by identifying fast progressors and early response to therapy. Recent advances in metabolomics (the quantitative analysis of all metabolites present within a biological sample) has opened new avenues for biomarker identification. The aim of the study, therefore, was to identify serum metabolic biomarkers for OA using a metabolomics approach.

Methods: 123 knee OA cases and 299 controls were selected from the TwinsUK cohort. Knee OA was defined as either radiographic, self-reported OA, or total knee joint replacement due to primary OA. All the subjects were unrelated Caucasian females. Their frozen serum samples were retrieved and assessed for targeted metabolite profiling using API4000 Q TRAP LC/MS/MS System (Applied Biosystems, Darmstadt, Germany) equipped with Schimadzu Prominence LC20AD pump and SIL-20AC auto sampler with AbsoluteIDQTM Kit (Biocrates life sciences AG, Austria).

Results: A total of 163 serum metabolites were assessed and their concentrations were obtained. The mean of the coefficient of variation (CV) for the 163 metabolites was 0.07±0.05 and 90% of the metabolites had a CV of less than 0.10. The ratios of all pair metabolite concentrations were calculated and tested for significance level after conservative Bonferroni correction. These ratios associated with knee OA with p<0.01. Overall 14 ratios were significantly associated with knee OA and have potential clinical use as biomarkers.

Conclusions: This is the first study reporting the identification of novel OA biomarkers using a metabonomics approach. The results suggest that amino acid and acylcarnitine metabolic pathways involving valine and fumaryl-L-carnitine are implicated in the development of OA and have potential clinical use as biomarkers.

IDENTIFICATION OF A NOVEL HTRA1-SUSCEPTIBLE CLEAVAGE SITE IN HUMAN AGGREGAN: EVIDENCE FOR THE INVOLVEMENT OF HTRA1 IN AGGREGAN PROTEOLYSIS IN VIVO

A. Chamberland, E. Wang, A.R. Jones, E.A. Morris, C.R. Flannery, Z. Yang
Wyeth Res., Cambridge, MA

Purpose: Htra1 (also called PRSS11) is a member of the High Temperature Requirement family of serine proteases, defined by a characteristic trypsin-like serine protease domain and one or more C-terminal PDZ domains. HtrA1 expression levels are up-regulated approximately 7-fold in osteoarthritics (OA) and HtrA1 has been shown to degrade critical components of cartilage extracellular matrix (ECM) in vitro. Here, we investigated the effect of HtrA1 overexpression on cartilage matrix formation by using a chondrocyte alginate culture. In addition, we identified a novel Htra1-susceptible cleavage site within the interglobular domain (IGD) of aggrecan and have generated neoepitope antibodies to specifically detect aggrecan degradation products. We examined the physiological relevance of aggrecan cleavage by Htra1 in OA by determining if these neoepitopes are present in cartilage extracts.

Methods: Wildtype HtrA1 and an inactive Htra1 mutant (Htra1-ASM) were overexpressed in 3-D alginate cultures of human primary chondrocytes. Total sulfated glycosaminoglycan (sGAG) was measured by DMMB assay. Purified human aggrecan or recombinant aggrecan proteins comprised of either the G1-IGD-G2 or IGD alone were incubated with recombinant Htra1 (aa 157-480) or an inactive Htra1 mutant (aa 157-480, ASM). Cleavage products were analyzed by N-terminal sequencing. Polyclonal neoepitope antibodies, which specifically recognize the cryptic termini of aggrecan fragments (but not intact aggrecan), were generated and characterized by Western blotting. Conditioned media from human cartilage explants treated with Htra1 and human cartilage protein extracts from human donors were subjected to Western blot analysis using one of the neoepitope antibodies. Immunohistochemistry was used to determine the localization of Htra1-mediate aggrecan cleavage in OA cartilage.

Results: Overexpression of wild-type Htra1, but not the inactive mutant, caused a marked reduction of total proteoglycan content in chondrocyte-seeded alginate cultures. Incubation of recombinant aggrecan proteins with Htra1 resulted in distinct cleavage of these substrates. N-terminal sequencing identified the Htra1-specific cleavage site as VQVT−357 TWPD within the aggrecan IGD. Western blot analysis of conditioned media from cartilage explants revealed that a neoepitope-specific cleavage product was released when the explants were treated with Htra1, with or without the inclusion of a peptide agonist (CPII), but not when treated with ASM or ADAMTS-4. To determine whether Htra1 cleavage of aggrecan occurs physiologically in OA cartilage, Western blot analysis was performed on human cartilage protein extracts from 7 patients with OA and 7 age-matched donors. Htra1-generated aggrecan fragments were significantly more abundant in osteoarthritic cartilage compared to cartilage from healthy joints. Immunohistochemistry demonstrated the Htra1-mediated cleavage of aggrecan occurs mainly in the surface layer of cartilage where matrix degradation typically initiates in OA.

Conclusions: Htra1 has been shown to be upregulated in OAcartilage and may play a role in disease progression by breaking down extracellular matrix proteins. Here, we show Htra1 is capable of digesting aggrecan and have identified a novel Htra1-susceptible cleavage site. Using a newly generated neoepitope antibody, we show that Htra1-generated aggrecan fragment is more abundant in cartilage from human OA patients relative to normal donors, suggesting that Htra1-mediated digestion of aggrecan is a physiological relevant event that could contribute to OA disease progression.