Conclusions: ESE-1b functions to regulate the expression of matrix proteinases and IL-1β in articular cartilage. Thus, this transcription factor might be potentially involved in slowing down disease progression of chronic arthritides by suppressing proteinases and IL-1β expression.

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IDENTIFICATION OF POST-TRANSCRIPTIONALLY REGULATED GENES FOLLOWING EXPOSURE OF HUMAN ARTICULAR CHONDROCYTES TO HYPEROSMOTIC CONDITIONS

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Purpose: A number of physiochemical stimuli are thought to be important for regulation of chondrocyte function. One of the most widely studied is extracellular osmolarity which is thought to be altered as water is expelled from articular cartilage during loading. A number of studies have demonstrated that chondrocyte extracellular matrix (ECM) synthesis can be regulated by changes in the osmotic environment. We previously demonstrated that human articular chondrocytes (HAC) grown in hyperosmotic conditions increase the expression of the transcription factor SOX9, a key regulator of cartilage ECM genes, by stabilizing its mRNA. We were interested in whether other genes were controlled in a similar manner and so conducted a microarray screen to examine genes regulated by hyperosmolarity. Then, using *in silico* and *in vitro* approaches, we identified genes which were regulated post transcriptionally.
Methods: Human articular cartilage was obtained with informed consent from patients undergoing knee replacement operations for osteoarthritis. HAC were isolated by collagenase digestion, plated at 100,000 cells/cm^2 and used in experiments within 24 hours. Cells from four donors were used to perform microarray experiments and a further two for mRNA decay studies. For microarray analysis the cells were initially grown in serum free media adjusted to 380mOsm by addition of NaCl for 2 hours. The media was then replaced with more 380mOsm media (controls) or media adjusted to 550mOsm (hyperosmotic) and the cells were cultured for a further 5 hours. Total RNA was then extracted from the HAC cultures using Tri Reagent and sent to The Genome Centre, Queen Mary University London where the samples were processed and used to probe an Illumina Human Ref8 v.3.0 array. Genes increased by hyperosmolarity by an average of 2-fold across all four donors were subjected to further analysis. The presence of potential AU-rich elements (AREs) in the 3'UTR of regulated genes was determined by searching the AU-rich element database (ARED - http://brp.kfshrc.edu.sa/ARED/). Real time PCR analysis was performed to examine mRNA decay in actinomycin-D chase experiments which were conducted on HAC under control or hyperosmotic conditions.
Results: Principal components analysis of microarray data revealed that the major variation was caused by the difference in osmolarity between cultures. 88 genes were upregulated >2-fold and 51 genes were downregulated >2 fold by hyperosmotic conditions. We focused on upregulated genes in order to find candidates that may have undergone mRNA stabilization. Interrogation of the ARED database showed an overrepresentation of ARE's in the genes strongly upregulated by hyperosmolarity. Overall, 32% of hyperosmotically regulated genes contained predicted ARE's compared to a genome wide value of less than 7%. The largest subset of ARE's belonged to the ARED defined cluster type 5. RNA decay analysis of a number of these genes showed that they demonstrated short mRNA half lives (min 1 hour max 7.4 hours, average 3.2 hours) in HAC under control conditions. Furthermore in 9 out of 15 cases, hyperosmotic culture conditions stabilized the mRNA of these genes.
Conclusions: Recent microRNA research has underlined the importance of post transcriptional control to gene regulation. Building on previous work on post transcriptional control of SOX9 mRNA we have now described similar regulation of a diverse set of genes. By combining microarray analysis with *in silico* interrogation of the ARED database we have been able to rapidly identify many genes which are post transcriptionally regulated in HAC following exposure to hyperosmotic conditions. These genes have a broad range of functions but all harbor ARE's in their 3'UTRs indicating that common molecular mechanisms may be acting on these elements. It is not clear whether these responses are limited to hyperosmotic stimulation and further analysis of the stimuli and mechanisms involved is warranted. An understanding of post transcriptional gene control by chondrocytes will improve our appreciation how chondrocytes maintain cartilage homeostasis and could result in identification of therapeutic targets for treating joint diseases.

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EVIDENCE FOR INCREASED TYPE I COLLAGEN BEING AN EARLY FEATURE OF CARTILAGE DISEASE IN ANTEROMEDIAL OSTEOARTHRITIS OF THE KNEE

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Purpose: Antero-medial gonarthrosis (AMG) is a distinct phenotype of knee osteoarthritis (OA) that displays a constant and repeatable spatial pattern of disease.
There is evidence from the literature supported by our own work for type I collagen immunohistochemical staining in chondrocyte clusters and their territorial matrix at the surface of damaged cartilage regions.
The aim was to quantitatively assess regional differences in type I and type II collagen in cartilage from knees with AMG and aged, non-osteoarthritic controls
Methods: Ten medial tibial joint resection specimens obtained from patients undergoing partial knee replacement were examined (mean age = 67 years). Full thickness cartilage was harvested from defined zones: N (macroscopically preserved cartilage) and T1, T2, and T3 (partial thickness cartilage damage).
Sixteen equivalent control specimens were harvested from patients undergoing above knee amputation for peripheral vascular disease (mean age = 76 years). Only knees with no macroscopic evidence of osteoarthritis were included. Control specimens were grouped according to histological grade (modified Mankin score): 8 low-grade (score 3, "early OA group"). Cartilage was harvested from equivalent zones. All samples were prepared using optimised protocols for immunoassay for collagen type I and type II, and quantitative real-time polymerase chain reaction (qrt-PCR) for the genes COL1A1 and COL2A1.
The immunoassay used specific antibodies that have been validated and published in the literature. The collagen content in micrograms is expressed as a percentage of sample dry weight. GAPDH was used as the endogenous reference gene for qrt-PCR. Non-parametric tests were used for statistical analysis of the results (Prism 5, Graphpad Software Inc.): the Kruskal-Wallis test (post-hoc Dunn's test) for the immunoassay; the Wilcoxon signed ranked test for qrt-PCR. Statistical significance was set at p=0.05.
Results: *Immunoassay:* Type II collagen content (~50% of dry weight) was not significantly different between cartilage zones in AMG, low-grade or high-grade control specimens (p=0.375). There was no significant different between AMG and control specimens. Type I collagen content showed a significant regional difference in AMG (p=0.001) and high-grade control specimens (p=0.015). The median type I collagen content was ~1% of dry weight in the damaged zones (T1, T2 & T3) and ~4.5% of dry weight in the preserved cartilage (N) for both AMG and high-grade controls. There was

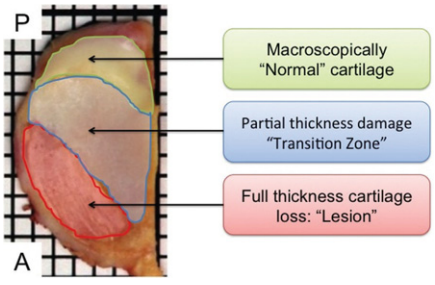

Figure 1

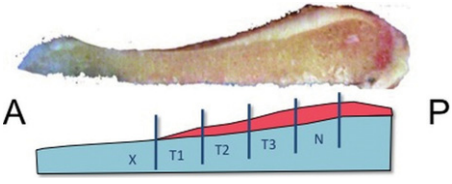

Figure 2

no such regional difference in type I collagen content in low-grade control specimens (p=0.181).
Real-time PCR: COL2A1 expression was not significantly different in regions of damaged and preserved cartilage in AMG (p=0.156), low-grade control (p=0.625) or high-grade control specimens (p=0.813). COL1A1 expression in AMG specimens was significantly greater in the preserved cartilage than damaged cartilage (p=0.031). However, the regional difference in type I collagen expression was not seen in low-grade (p=0.625) or high-grade controls (p=0.219).
Conclusions: Type I collagen is abundant in fibro-cartilage but only found in very low levels in mature hyaline cartilage and is not usually found in articular cartilage. Macroscopically normal cartilage in AMG exhibits increased COL1A1 gene expression and a subsequent increase in type I collagen matrix content. Type I collagen is also increased in the equivalent region of knees with early histological features of OA
These findings suggest that these cartilage matrix and chondrocyte phenotype changes may be an early feature of OA change in AMG.

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A ROLE FOR TNF-LIKE WEAK INDUCER OF APOPTOSIS (TWEAK) DERIVED FROM SYNOVIAL TISSUES AND CARTILAGE IN THE PATHOGENESIS OF OSTEOARTHRITIS

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Purpose: Previous studies have suggested that TWEAK may have an important role in stimulating bone and cartilage destruction in various arthritidies. The aim of this study was to investigate TWEAK and TWEAK receptor (Fn14) expression in the cartilage and synovial tissues from osteoarthritic and normal (healthy) individuals. Protein expression of TWEAK and TWEAK receptors (Fn14) was determined in cartilage from samples of various grades of osteoarthritis (OA) and synovial tissue from patients with OA and normal controls. TWEAK and Fn14 gene expression was studied in various grades of OA cartilage. In addition, levels of soluble TWEAK present in OA synovial fluid were measured.
Methods: Synovial tissues from total of 10 OA and 10 normal subjects were studied for TWEAK and Fn14 using immunohistochemistry. Osteoarthritic cartilage was obtained from 21 patients undergoing primary hip replacement and stained for TWEAK and Fn14 expression. Staining was assessed using a standard semi-quantitative assessment carried out by two independent observers. In addition, mRNA levels of TWEAK and Fn14 were determined by real time RT PCR in the various grades of cartilage tissue. TWEAK levels were measured in 15 OA synovial fluid samples using a commercial ELISA kit.
Results: Significantly higher TWEAK (Figure 1A and B) and Fn14 was detected in OA synovial tissue compared to the normal synovial tissue ($p<0.05$) and expression was predominantly in the synovial lining.
In OA cartilage, the majority (18/21) all grades of samples expressed TWEAK protein at low levels, whereas all samples (OA and normal) expressed high levels of Fn14 (Figure 1C and D). TWEAK and Fn14 mRNA was more abundant in OA patients (Figure 2) with grade 2 cartilage damage compared to those without any cartilage damage ($p<0.05$). TWEAK was present in all OA synovial fluids tested at a mean concentration of 713±134 pg/ml.

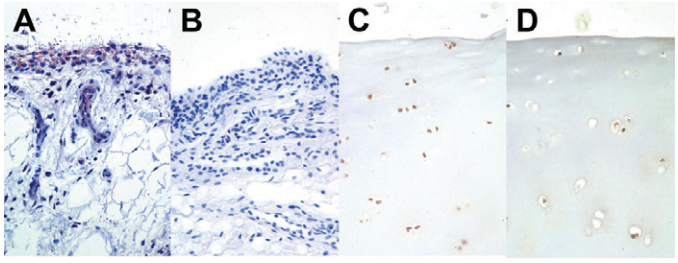

Figure 1. Synovial tissue expression of TWEAK (red stain) in OA and normal (B) tissues. Cartilage tissue expression of Fn14 (red stain) in grade 2 (C) and grade 0 (D) tissues.

Conclusions: Previous studies have shown that TWEAK can induce metalloproteinase production in vitro by human chondrocytes and in murine intervertebral disc tissues. As yet no studies have directly investigated the presence of TWEAK and its receptor (FN14) in human OA tissues. The high expression of Fn14 by chondrocytes in OA cartilage indicates that these cells are able to respond to TWEAK. In OA tissues TWEAK was expressed by chondrocytes in cartilage and highly expressed in the adjacent synovial tissues. This is consistent with TWEAK protein in the OA synovial fluid. Overall the study indicates that TWEAK released from the synovial tissue and chondrocytes in OA has a role in cartilage degradation.

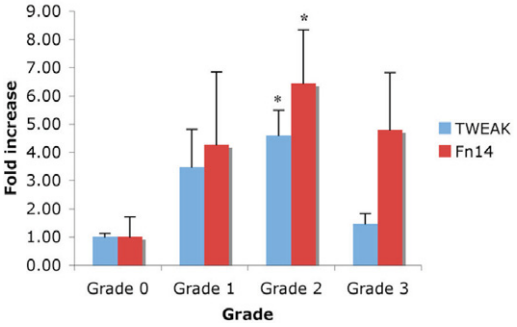

Figure 2. TWEAK and Fn14 mRNA in various grades of OA cartilage.

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ELUCIDATION OF THE HUMAN CHONDROCYTE GLYCOME AND OF ALTERING GLYCANS UNDER CYTOKINE TREATMENT

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Purpose: Recent evidence has indicated a relationship between osteoarthritis and altered glycosylation of cartilage and chondrocytes. Although the glycobiology of cells is known to affect adhesion, receptor activity or apoptosis, little information exists on the N- or O-glycome of human chondrocytes and on their significance for the functionality of cartilage tissue. In part, this fact may result from the methodological challenges encountered with low glycosyltransferase transcription levels and linkage-type specific glycoprotein glycan analysis. This study aimed to elucidate the glycome of cultured human chondrocytes using a combination of RT-qPCR and structural glycan analysis (LC-ESI-MS) and to define alterations of cellular glycans upon treatment of chondrocytes with pro-inflammatory cytokines.
Methods: Primary chondrocytes were isolated from human OA cartilage (n=8). Primary cells, SW1353 and C-28/I2 chondrocytes were cultured in DMEM with 10% FCS or 1% ITS. 10 ng/ml IL-1β or 40 ng/ml TNF-α were added as a pro-inflammatory stimulus. SYBR green based RT-qPCR assays for 19 glycosyltransferases and 5 galectins were established using GAPDH as reference. N- and O-glycan isolation was performed by PNGase (N-glycans) or sodium hydroxide (O-Glycan) digestion prior to structural identification by LC-ESI-MS using Porous Graphitized Carbon as stationary phase.
Results: About 100 different N- and O-glycans were detected and quantified in human chondrocytes in the presence or absence of FCS. Major N-glycans were found as sialylated diantennary structures, oligomannosidic or hybrid-type glycans. The isomers of diantennary and oligomannosidic N-glycans were fully assigned. Some structures were sulfated, contained GalNAc or Lewis fucose residues, whereas tri and tetraantennary glycans were present only to a minor extent. O-glycosylation mainly constituted of core-1 and core-2 structures bearing one or two α2,3 linked sialic acids.
Primary chondrocytes predominantly expressed α2,6-specific sialyltransferases (SiaT) and α2,6-linked sialic acid residues in glycoprotein N-glycans. In contrast, the preponderance of α2,3-linked sialyl residues and reduced levels of α2,6-specific SiaT were associated with the altered chondrocyte phenotype of C-28/I2 and SW1353 cells. Importantly, we found that both IL-1β and TNF-α increased overall sialylation of N- and O-glycans and induced a shift towards α2,3-linked sialic acid residues in primary chondrocyte glycoproteins. These results were supported by RT-qPCR showing increased expression of α2,3 SiaT in treated cells. Moreover, we found that both cytokines induced a considerable shift from oligomannosidic glycans towards complex-type N-glycans, whereas core α1,6-fucosylation was found to be reduced particularly by TNF-α.