In the PDT experiment on human RA synovial biopsies, the groups incubated with 28 H1–700DX and exposed to light showed apparent cell death in the synovial tissue as evident by the positive staining of both the gh2AX and caspase 3 markers (figure J and K). Staining of these markers co-localised with areas of high FAP staining (figure L). This was not the case in the control samples that were not exposed to either 28 H1–700DX and/or light (figure A – I). All biopsies did show FAP staining indicating that the cell death was only achieved when the biopsies were exposed to both the antibody and the light.

Conclusions: We have demonstrated fibroblast-specific cell death by targeted PDT using 700DX-conjugated 28 H1. Furthermore, we demonstrated that PDT also induces cell death of FAP-positive cells in synovial tissue from RA patients, suggesting FAP-targeted PDT as a promising new tool in treating RA.

Disclosure of Interest: None declared


**METABOLIC ANALYSIS OF THE SECRETOME OF ARTICULAR CARTILAGE AND CHONDROCYTES IN RESPONSE TO PRO-INFLAMMATORY CYTOKINES**

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**Background:** Chondrocytes rely primarily on glycolysis to meet their energy requirements, but can support cell survival and matrix synthesis during periods of starvation. This ‘spare respiratory capacity’ requires optimal mitochondrial function. Impaired mitochondrial function is implicated in osteoarthritis (OA). Metabolic adaptation is evident in early-stage OA, however cartilage from late-stage disease does not seem to have this flexibility. A deeper understanding of these complex metabolic pathways may identify new markers of disease stage, and support therapeutic strategies for treating OA.

**Objectives:** Metabolomics has the potential to reveal pathological pathways and identify novel biomarkers. The aim was to identify metabolic processes involved in early stage disease by analysis of metabolites and metabolic function in pro-inflammatory models of cartilage degradation.

**Methods:** Mass spectrometric analysis of murine articular cartilage was obtained from extensor tendons of C57/BL6 wildtype mice and chondrocytes from equine cartilage explants. Metabolic pathways and markers involved in OA were identified using the Seahorse XFp and XFe24 analysers. Cells were treated with species-specific 10 ng/ml IL-1α, 10 ng/ml tumour necrosis factor-α (TNF-α). Secretome metabolite levels were measured using AbsoluteEQ p180 targeted metabolomics kit (Bioscience) with Waters Xevo TQ-S mass spectrometer coupled to an Acquity UPLC system. PCA and OPLS-DA were performed using SIMCA-P v12.0 software. Metabolic function of primary equine (n=9) and bovine chondrocytes (n=3) was determined using Seahorse XFp and XFe24 analysers. Cells were treated with species-specific 10 ng/ml IL-1β and/or 10 ng/ml TNF-α for 18 hour, and metabolically challenged with the Mito Stress Test. Metabolite levels, and oxygen consumption rates, were normalised to total cell protein, and values analysed by ANOVA with Tukey’s multiple comparison post-tests.

**Results:** Cytokine treatment decreased proline, ornithine and α-aminoadipic acid (p<0.0001) in explant secretome. Citrulline increased with cytokine treatment (p<0.0001) and glutamate, present in DMEM (Gibco) with or without 10 ng/ml interleukin-1α (IL-1α) and 10 ng/ml tumour necrosis factor-α (TNF-α). Secretome metabolite levels were measured using AbsoluteEQ p180 targeted metabolomics kit (Bioscience), with Waters Xevo TQ-S mass spectrometer coupled to an Acquity UPLC system. PCA and OPLS-DA were performed using SIMCA-P v12.0 software. Metabolic function of primary equine (n=9) and bovine chondrocytes (n=3) was determined using Seahorse XFp and XFe24 analysers. Cells were treated with species-specific 10 ng/ml IL-1β and/or 10 ng/ml TNF-α for 18 hour, and metabolically challenged with the Mito Stress Test. Metabolite levels, and oxygen consumption rates, were normalised to total cell protein, and values analysed by ANOVA with Tukey’s multiple comparison post-tests.

**Conclusions:** Metabolites which decreased with cytokine treatment are all downstream of glutamate. With elevated glutamate, this suggests that cytokines inhibit glutamate uptake and metabolism. Elevated citrulline in cell and explant models may be attributed to disruption of the urea cycle via induction of nitric oxide synthase. IL-1β alone negated spare respiratory capacity, and chondrocytes remained glycolytic. In conclusion, cytokines disrupt glutamate and citrulline metabolism, normally tightly regulated mitochondrial pathways, and IL-1β alone is responsible for the metabolic switch. These metabolic pathways could provide markers of early-stage inflammatory disease.

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REFERENCE:


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CD14+CD16+ MONOCYTE SUBPOPULATION IS DOMINANT IN THE INFLAMMATION OF OSTEOARTHRITIS SYNOVIAL FLUID

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Background: In osteoarthritis (OA), the activation of inflammation response involving the interaction of cartilage and synovial hyperplasia may contribute to disease progression. However, inflammatory cells in OA synovial fluid (OASF) have been rarely studied.

Objectives: To investigate the phenotype of CD14+ cells and the secretion of proinflammatory cytokines by these cells

Methods: Immunohistochemistry staining in OA synovium was performed using anti-CD14, anti-CD16 and anti-CD56 antibody. The OASF was obtained through arthrocentesis. Mononuclear cells from OASFs were stained with anti-CD3, anti-CD4, anti-CD14, anti-CD16, anti-TLR4 or anti-TLR2 and analysed using flow cytometry. CD14 +CD16+ and CD14+CD16+ mononuclear cells in OASF were selected with magnetic microbeads. In the supernatant of these cells culture, the concentration of IL-1β, IL-6, IL-8, TNFα, MIP-1α, were measured by Luminex.

Results: In OA synovium, CD14+CD16+ was stained, but CD56 was not expressed. In OASF, there was a substantial number of CD14+ cells (36.6%±25.2%), CD3+ cells (37.4%±12.9%), with a rarity of CD90+ cells (1.7%±1.3%). The proportion of CD14+ cells was increased significantly in recurrent synovial fluid, compared with the proportion in initial synovial fluid. Among CD14+ cells in OASF, CD14+CD16+ monocyte subgroup (21.2%±21.8%) was more abundant than CD14+CD16− monocyte subgroup (10.9%±10.0%). TLR4 and TLR2 expressions were higher in CD14+CD16+ cells than in CD14+CD16− cells. The concentration of IL-8 and MIP-3 was more increased in the supernatant of CD14+CD16+ cells than in that of CD14+CD16− cells.

Conclusions: In OASF, the proportion of CD14+ cells was increased in recurrent synovial effusion. Compared to CD14+CD16− monocyte subpopulation, CD14+CD16+ monocyte subpopulation release more cytokine such as IL-8 and MMP-3, and had higher expressions of TLR4 and TLR2.

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THE LONG NONCODING RNA (LncRNA) HOTTIP IS A MASTER REGULATOR OF CELL CYCLE IN HAND SYNOVIAL FIBROBLASTS IN ARTHRITIS

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Background: Rheumatoid arthritis (RA) and other types of inflammatory arthritis follow a characteristic anatomical pattern of joint involvement. We have recently shown that local synovial stromal cells, specifically synovial fibroblasts, exhibit joint-specific transcriptomes and functions. In particular, hand SF exhibited prominent proliferative and chemoattractant activities. Density of stroma and leukocyte infiltration were increased in hand synovium.

Objectives: To explore the role of hand/foot-specific IncRNA HOTTIP in shaping the transcriptions of hand synovial fibroblasts in arthritis.

Methods: We studied transcriptomes and epigenomes of hand, shoulder and knee SF from patients with RA or osteoarthritis and from knees of non-arthritis subjects using RNA-sequencing, Illumina HiSeq 2000 (n=21), histone Chip-sequencing (Illumina HiSeq 2500, n=7) and Infrum HumanMethylation450 BeadChip (n=12). qPCR was used to confirm RNA-sequencing data in a larger cohort of SF from different joints. We silenced the IncRNA HOTTIP in hand SF using LNA GapmeRs, followed by RNA-sequencing, qPCR, protein-protein interaction analysis of RNA-sequencing data (STRING), and in vitro assays for proliferation (Brdu assay) and apoptosis (Annexin V/PI staining).

Results: Genome-wide DNA methylation patterns and histone marks at actively transcribed DNA regions (H3K27ac) and enhancers (H3K4me1) defined joint-specific origin of SF. SF from hands and feet specifically expressed the IncRNA HOTTIP. This distal-specific HOTTIP expression coincided with the enrichment of H3K4me3 and H3K27ac and a decrease in repressive marks (H3K27me3, DNA methylation) at the HOTTIP promoter in hand SF. In contrast, the HOTTIP promoter displayed scarce activating, but abundant repressive epigenetic markers in shoulder and knee SF. Silencing of HOTTIP in hand SF altered the expression of 447 protein-coding genes (log ratio >2, FDR<0.05). These genes were strongly enriched in the mitotic cell cycle protein interaction network (n=48 genes, p=4.3x10−7). Several of the enriched mitotic cell cycle genes, including NCAIPG, CENPO, ZWILCH and BUB1 were confirmed as downregulated by HOTTIP silencing in a larger cohort of hand SF (n=8). The basal expression of 36 out of the 48 enriched cell cycle genes correlated with the basal HOTTIP expression in hand SF (n=6, RNA-sequencing R=0.86). We further measured these correlations in a larger cohort of hand SF (n=21) for a subset of the 36 genes using qPCR. Among the measured genes, TADA3 and C0D32 were confirmed to correlate with HOTTIP expression in hand SF (R=0.5, p<0.05). Silencing of HOTTIP for 24 hour, 48 hour and 72 hour decreased the incorporation of Brdu into DNA of

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CHONDROCYTES UNDER PATHOLOGICAL CONDITIONS PREFERENTIALLY DEGENERATE SELECTED tRNAs TO INHIBIT PROTEIN SYNTHESIS

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Background: Oxidative and ER stress play an important role in the pathogenesis of osteoarthritis (OA). These stress conditions can induce the cleavage of transfer RNA (tRNA) via angiogenin. This cleavage at the anticodon loop of the tRNA produces tRNA half fragments (tiRNAs) that may be involved in the downregulation of protein synthesis.

Objectives: To identify specific tRNA to tiRNA isotype fragments that occur during oxidative or ER stress in human OA chondrocytes

Methods: Primary human articular chondrocytes were enzymatically isolated for 72 hour and then treated with tert-butyl hydroperoxide (TBHP) over a 12 hour time course or with Interleukin-1β (IL-1β) over a 24 hour time course. RNA was extracted for RNA expression analysis (qRT-PCR). Protein was harvested for western blot analysis. A tRF and tRNA PCR Array that profiles 55 known tRNAs was used to perform an unbiased dissection of the formation of tRNAs in OA chondrocytes. Total RNA was adapted using a First-Strand cDNA Synthesis Kit designed to create cDNA libraries from small RNAs for qPCR detection to identify ten novel tRNAs associated with cell stress in OA chondrocytes.

Results: Using qRT-PCR RNase gene expression was measured. Of these RNAs 250 μM TBHP treatment at 12 hour induced KIAA0430 (p<0.05), RNase30 (p<0.001) and ZC3H12A (p<0.0001) but not angiogenin or SN012, 10 ng/ml IL1β treatment at 6 hour induced SN012 (p<0.0001), RNase 7 (p<0.01), ZC3H12A (p<0.01) but not angiogenin or KIAA0430. Chondrocytes undergoing oxidative or ER stress produced tRNA halves in a time dependent manner, tRNA formation was highest at 6 hour of IL1β treatment and after 12 hour of TBHP treatment. tRNA fragments of RNAs specific for arginine, glutamic acid, glycine, histidine, lysine and valine were increased after TBHP or IL1β treatment. Ten tRNAs that were induced by IL1β and TBHP were selected for further study. Primer sets for these tRNA and the parent tRNAs were designed and screened across a wider time course, and with higher patient numbers.

Conclusions: The expression of several RNAs was increased in a time dependent manner in stressed OA chondrocytes and may be involved in the cleavage of tRNA fragments. tRNA halves were grouped together based on their parent tRNA amino acid target. We hypothesised that an increase in fold change in these grouped RNAs may result in less production of proteins that contain a high percentage of that specific amino acid. The changes in amino acid distribution may account for decreases seen in amino acid rich proteins such as Type II collagen (a glycin rich protein) in stressed chondrocytes. By designing tRNA and parent tRNA primers we will discover if tRNA information effects total levels of parent RNAs. The outcomes from this research will provide us with an increased understanding of tRNA to tRNA formation in human chondrocytes and may allow us to identify therapeutic targets for the treatment of OA and provide insights into novel stress signalling pathways.

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