REFERENCES:


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CD14+CD16+ MONOCYTE SUBPOPULATION IS DOMINANT IN THE INFLAMMATION OF OSTEARTHROIS ACARTILAGE AND SYNOVIAL FLUID

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-CD68 antibody. The OASF was obtained through arthrocentesis. Mononuclear cells from OASFs were stained with anti-CD3, anti-CD4, anti-CD14, anti-CD16, anti-CD4, anti-CD14, TrlR4 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, TNF-α, MMP-1, -3 was measured by Luminex.

Results: In OA synovium, CD14+CD16+ was stained, but CD68 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 ratio 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 subpopulation (21.2%±21.8%) was more abundant than CD14+CD16− monocyte subpopulation (10.9%±10.0%). TrlR4 and Tlr2 expressions were higher in CD14+CD16+ cells than in CD14+CD16− cells. The concentration of IL-8 and MMP-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 released more cytokines such as IL-8 and MMP-3, and had higher expressions of Tlr4 and Tlr2.

Disclosure of Interest: None declared


SAT0065 CHONDROCYTES UNDER PATHOLOGICAL CONDITIONS PREFERENTIALLY DEGRADE 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) into tiRNAs. This cleavage at the anticodon loop of the tRNA produces 5-tiRNA and 3-tiRNA halves known as tiRNAs.

Objectives: We aim 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 from OA cartilage. Cells were cultured in vitro for 72 hours and then treated with tert-butyl hydroperoxide (TBHP) over a 12 hour time course or with Interleukin-1β (IL-1β) over 24 hour time course. RNA was collected for tRNA expression analysis (qRT-PCR). Protein was harvested for western blot analysis. A TRF and tiRNA PCR Array that profiles 55 known tiRNAs was used to perform an unbiased detection of the formation of tiRNAs 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 tiRNAs associated with cell stress in OA chondrocytes.

Results: Using qRT-PCR, the mRNA expression was measured of these tiRNAs. The mRNA expression of these tiRNAs was enhanced in the presence of TBHP or IL-1β. These results suggest that tiRNAs are involved in the stress signalling pathways.

Disclosure of Interest: None declared


SAT0066 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 chemoattractive 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 transcriptome 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-arthritic subjects using RNA-sequencing, Illumina HiSeq 2500 (n=21), histone Chip-sequencing (Illumina HiSeq 2500, n=7) and Infinium 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 marks 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=3.3x10^{-7}). Several of the enriched mitotic cell cycle genes, including NCPAG, CENPO, TWILCH 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 revealed that HOTTIP silencing resulted in a larger cohort of hand SF (n=21) for a subset of the 36 genes using qPCR. Among the measured genes, TADA 3 and CDC27 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