THU0013

DOWNREGULATION OF MICRORNA MAY CONTRIBUTE TO ACTIVATION OF THE INTERFERON SIGNALLING PATHWAY IN THE IDIOPATHIC INFLAMMATORY MYOPATHIES

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Background: The idiopathic inflammatory myopathies (IIM) are a group of rare autoimmune conditions characterised by weakness and inflammation of skeletal muscle. MicroRNA are short single stranded RNA which regulate gene expression by binding to particular target mRNA and suppressing translation or inducing degradation. MicroRNA are known to play a role in muscle homeostasis and immune regulation and therefore may be involved in IIM pathology.

Objectives: To profile both microRNA and mRNA in whole blood samples from polymyositis (PM), dermatomyositis (DM) and inclusion body myositis (IBM) patients compared to non-myositis controls using next generation RNA sequencing.

Methods: Whole blood samples in tempus tubes were collected as part of the myositis research tissue bank held at the University of Manchester. Total RNA was extracted from 7 PM, 7 DM, 5 IBM and 5 non-myositis control samples and both microRNA and mRNA profiles were determined by sequencing on the Illumina HiSeq 4000.

Results: Analysis of microRNA expression identified 19 microRNAs as significantly differentially expressed (DE) ($p < 0.05$) for PM, 11 for DM and 27 for IBM, compared to controls. Analysis of myositis specific autoantibody anti-Jo1 positive cases (four PM and one DM) vs controls identified 18 DE microRNAs, 12 of which were not identified for any of the clinical subgroups.

Gene expression analysis identified 129 significantly DE genes for PM (false discovery rate < 0.05), 53 for DM, 24 for IBM and 691 for anti-Jo1 compared to controls. Ingenuity pathway analysis (IPA) of RNAseq data identified the most significant canonical pathway as 'Interferon signalling' which was predicted to be upregulated in PM, DM and anti-Jo1 subgroups. The anti-Jo1 subgroup had a higher number of DE genes and a higher activation score in the interferon signalling pathway compared to PM or DM. The IPA tool microRNA target filter was used to match DE microRNA with corresponding DE mRNA predicted or previously observed to be targets. When mRNA targets were filtered for involvement in interferon signalling, two downregulated microRNA targeted two upregulated mRNA in PM and five downregulated microRNA targeted five upregulated mRNA in the anti-Jo1 group as shown in figure 1. No microRNAs targeting mRNA involved in the interferon signalling pathway were identified for DM or IBM.

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Abstract THU0013 – Figure 1. Relationship between differentially expressed microRNA in anti-Jo1 samples versus controls and mRNA targets involved in the interferon signalling pathway. Green colouring indicates downregulation; red colouring indicates upregulation and darker shades indicate greater fold change.

Conclusions: To our knowledge this is the first mRNA and microRNA profiling study in whole blood from myositis patients using RNA sequencing. Grouping by anti-Jo1 positivity identified different microRNA and increased predicted activation of interferon signalling compared to the clinical subgroups analysed. Our results suggest that increased activation of the interferon pathway in anti-Jo1 positive patients may be due to downregulation of microRNAs which target genes in this pathway.

Disclosure of Interest: None declared

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THU0014

GENETIC SCREENING FOR IDENTIFYING HUMAN GENES REGULATING FIBROSIS IN SYSTEMIC SCLEROSIS

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Background: Systemic sclerosis (SSc) is a prototypic multi-systemic fibrotic disease. Fibrosis involves exacerbated collagen production. A lack of effective therapies has partly been due to incomplete understanding of the mechanisms of fibrosis and inadequate subsetting of this heterogeneous disease to allow targeted treatment. Identification of a collagen-synthesis gene signature in SSc will enhance our ability to molecularly subset SSc patients. We reasoned that there exists several unidentified genes regulating collagen synthesis, whose dysregulation play key roles in fibrosis underlying SSc.

Objectives: To discover novel genes/pathways involved in elevated collagen synthesis

Methods: We conducted a pioneering forward-genetics approach using genome-wide ribonucleic acid interference (RNAi) –screening. We performed RNAi-screening in dermal fibroblasts from three patients with early diffuse cutaneous systemic sclerosis (onset from the first non-Raynaud's manifestation of less than 3 years). Dermal fibroblasts were immortalised using ectopic expression of human telomerase. The assay used a collagen promoter driver reporter system for identifying genes regulating collagen transcription.

Results: The genetic screen identified 187 genes whose silencing altered the activation of collagen promoter activity. This included 82% and 18% positive and negative regulators, respectively. A system-level view of genes and signalling pathways regulating collagen synthesis was generated using bioinformatics analysis.

Conclusions: We anticipate that this catalogue of collagen expression regulating genes will help to unravel the dysregulated genetic regulatory modules of fibrosis and may aid in the diagnostics of different clinical subsets of SSc.

Disclosure of Interest: None declared

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THU0015

OESTROGEN DEPENDENT REGULATION OF MICRO-RNA IN RHEUMATOID ARTHRITIS

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Background: Oestrogen has ameliorating effects on rheumatoid arthritis (RA). Oestrogen receptor (ER) signalling affects micro-RNA (miR) expression and processing in breast cancer, while its effect on miR profile in RA have never been studied.

Objectives: To study the effect of the oestrogen replacement therapy and oestrogen receptor signalling on the miR transcription and bioprocessing in RA patients.

Methods: The expression of the key miR processing enzymes Dicer, Drosha and DGCR8 was measured in the leukocytes of the peripheral blood of 145 female RA patients (age 53 years, disease duration 9.8 years) by RT-PCR and analysed with respect to the levels of ER α , used as surrogate marker of active ER α signalling. Total RNA was prepared from the serum samples of 46 postmenopausal female RA patients who received treatment with oestradiol (E2), noretisterone acetate, vitamin D₃ and calcium (n=22, mean E2 levels 229.4 $\pm$ 143.2 pg/ml) or vitamin D₃ and calcium supplementation only (n=24, mean E2 levels 30.8 $\pm$ 8.7 pg/ml). Serum was obtained after 12 and 24 months of treatment and was stored at -70°C until the time of analysis. MicroRNA transcription was performed by 3D-Gene microarray measuring >2560 miRs (TATAA Biocenter, Gothenburg).

Results: A higher ER α expression in RA female patients was associated with increased transcription of the major miR processing proteins Dicer ($p=0.0057$), Drosha ($p=0.019$) and DGCR8 ($p=0.0095$). This activated transcription of miR biomachinery could indicate a higher demand and a facilitated miR maturation. The E2-treatment significantly changed the profile, but not the levels, of miRs in serum, where only 50% of the miR transcripts were present both in E2-treated and control samples. Also the profile of miRs related to RA and of those regulating translation of ER α and Dicer, Drosha, and DGCR8 proteins was affected. Extensive bioinformatic analysis of the miR-targeting genes affected during E2-treatment, revealed a reduction of gene clusters aiding autoimmune pathology and the RA-associated molecular processes including DNA replication ($p=2.6 \times 10^{-4}$), peptidyl-serine phosphorylation ($p=2 \times 10^{-4}$), protein localization to nucleus and nuclear import ($p=5 \times 10^{-4}$).

Conclusions: Activation of ER α significantly enhances the miR processing, and affects the profile of miR transcription in female RA patients. The change in miR profile during E2-treatment could contribute to a significantly change in the miR landscape and disposition of intracellular processes in RA.

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THU0016

TRANSMITOCHONDRIAL CYBRIDS SHOW THAT OXPHOS VIA, BUT NO GLYCOLYSIS VIA, IS INVOLVED IN THE ATP REDUCTION OF OA HUMAN CHONDROCYTES

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Background: Mitochondrial dysfunction is well documented in OA and has the capacity to alter chondrocyte function and viability, contributing to cartilage degeneration. It is important to evaluate the influence of mitochondria in the pathogenesis of OA using an *in vitro* model to explain the functional consequences of this association and help us to identify potential diagnostic biomarkers and/or therapeutic targets. Transmitochondrial cybrids are a useful cellular model to study the mitochondrial biology and function implications in the cellular behaviour, since they carry different mitochondrial variants with the same nuclear background, therefore, excluding the variations because of nuclear genome.

Objectives: The aim of this work is test mitochondrial activity in the OA chondrocytes using transmitochondrial cybrids with mtDNA from healthy donors (without OA) and from patients with OA.

Methods: Cybrids were developed using 143B. TK⁺ Rho-0 cell line (nuclear donor) and platelets (mitochondrial donors) from healthy and OA donors. Human articular chondrocytes were obtained from patients with hip replacement. The mtDNA copy number was measured by real-time PCR method. The ROS production was evaluated using flow cytometry. The metabolic status was evaluated by glucose consumption and glucose oxidation. The glycolytic activity was measured after addition of glucose, oligomycin and 2-dioxylglucose using Seahorse XFp (ECAR). The OXPHOS function was evaluated by Seahorse XFp (OCR) after addition of oligomycin, FCCP and Rotenone/Antimycin. Appropriate statistical analyses were performed with GraphPad Prism v6.

Results: The analysis of mtDNA copy number showed that the OA have higher levels than N in cybrids and human chondrocytes. The analysis of ROS production showed that OA had higher levels than N in both types of cells. The metabolic status analysing glucose consumption, glucose oxidation and total glucose cellular uptake reflected higher values in OA cybrids than N cybrids. But the analysis of glycolysis data showed lower values in OA than N cybrids. The analysis of ATP obtained through glycolysis did not show any difference between cybrids. The analysis of OXPHOS function showed that OA had lower basal respiration and maximal respiratory capacity than N in both types of cells. The ATP obtained via OXPHOS was lower in OA than in N.

Conclusions: The analysis of OXPHOS function supports the participation of mitochondria in cybrids and human chondrocytes metabolism. Both types of cells use the mitochondria to obtain ATP and OXPHOS via, but no glycolysis, is involved in the reduction of ATP synthesis by OA cells. All these data support that N cybrids and chondrocytes use mitochondria with more efficiency.

Disclosure of Interest: None declared

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THU0017

DNA METHYLATION OF REGULATORY SITES OF HAND OSTEOARTHRITIS SUSCEPTIBILITY GENES IN FINNISH WOMEN

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Background: Despite the hard effort in OA genetic studies only a small part of the estimated effect has been found so far and thus the focus has been changing from genetic to epigenetic studies. The most widely studied epigenetic control mechanism is DNA methylation. There are only few studies on hand OA concerning DNA methylation but their results are promising.

Objectives: Our aim was to study methylation of the regulatory sites of our previously found hand OA susceptibility genes and to replicate some previously reported methylation sites associated with hand OA, in our Finnish women hand OA material.

Methods: The study design was cross-sectional. Bilateral hand radiographs of 542 occupationally active Finnish female dentists and teachers aged 45 to 63 and

classified them according to the presence of OA by using reference images. Radiographic hand OA at least mild, in at least three joints (ROA2_3), and radiographic findings summary score (OASUM) were used to define hand OA status. The methylation percentages were determined by bi-sulfite converted DNA pyrosequencing with commercial CpG Assays. Statistical analyses were performed using hierarchical multiple linear regression.

Results: Of the studied methylation sites the COL2A1_1 methylation site was associated with ROA2_3 ($p=0.04$). Also ALDH1A2_01 methylation site was associated with OASUM ($p=0.02$). When the data was stratified by occupation the association was only significant, and stronger, in teachers but not in dentists (COL2A1_1 $p=0.02$ vs. $p=0.36$ for ROA2_3, and ALDH1A2_1 $p=0.01$ vs. $p=0.36$ for OASUM, respectively). The studied methylation sites in TGFB1, RRP9 and TRAPP5 genes had methylation percentages under the detection limit and they were excluded from the analysis.

Conclusions: Our results lend further support to COL2A1 and ALDH1A2 being hand OA susceptibility genes at the epigenetic level.

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THU0018

MIRNAS-146A AND -499 GENE EXPRESSION AND THEIR POLYMORPHISMS AS DIAGNOSTIC MARKERS FOR RHEUMATOID ARTHRITIS

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Objectives: To investigate the expression of miRNAs-146a and -499 and their polymorphisms in Egyptian patients with RA and to evaluate their relationship to clinical manifestations and disease activity.

Methods: Fifty-two RA patients and 56 matched controls were studied. Disease activity score-28 was assessed. MicroRNAs expression and polymorphisms were assayed by polymerase chain reaction (PCR).

Results: Patients mean age was 39.5 ± 10.8 years and the disease duration of 7 ± 5.1 years. The DAS28 was 3.1 ± 1.7 ; 23 were in remission, 5 had mild disease activity, 18 moderate and 6 severe. There was a 15.5 ± 27.2 fold increase in miRNA-146a and 3.3 ± 6.1 in miR-499. The fold change of miRNA-146a was significantly higher in those without joint deformities ($n=18$) (28.1 ± 42.6) compared to those with (8.8 ± 8.4 ; $p=0.01$). Both miRNA-146a and -499 fold change were significantly decreased in those with a positive ANA ($n=7$) (2.5 ± 2.1 and 0.07 ± 0.08) compared to those with a negative test (18.1 ± 29.6 and 3.8 ± 6.5 ; $p=0.002$ and $p=0.001$ respectively). The fold changes tended to be higher in those in remission compared to active patients. However, miRNA-499 in those with severe disease activity tended to be higher. There was no significant correlation of the fold change of the miRNAs with the clinical manifestations or medications received. Only ANA positivity significantly inversely correlated with the fold increase in miRNA-146a ($r=-0.42$, $p=0.003$). The fold change in miRNA-146a significantly correlated with miRNA-499 ($r=0.56$, $p<0.0001$).

Conclusions: Both miRNAs-146a and -499 are highly expressed in RA patients and can be considered as diagnostic markers. Increased expression of miRNA-146a expression is protective in those with negative ANA and both in those without joint deformities.

Disclosure of Interest: None declared

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THU0019

ARE MICRORNAS A MOLECULAR CLOCK? THE NEWCASTLE THOUSAND FAMILIES COHORT STUDY

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Background: No single biomarker has been identified for monitoring ageing trajectory. To date, biological clocks are based on DNA methylation, telomere length, p16^{ink4a} expression and microsatellite mutations¹. Body ageing is a complex phenomenon, including a progressive, pro-inflammatory state, termed 'inflammaging'. MicroRNAs have been linked with cellular senescence and inflammaging. MicroRNAs are short, non-coding sequences of RNA regulating post-transcriptional gene expression with impressive stability and ubiquitous presence, making them ideal candidate biomarkers. Previous studies of circulating microRNAs in ageing were small-scale² or compared individuals of different ages, which is methodologically less robust³. MicroRNA biomarkers can bring greater understanding to