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THU0009 IN VITRO STUDIES USING CYBRIDS, SHOW THAT MTDNA FROM HEALTHY AND OA PATIENTS HAVE DIFFERENT MITOCHONDRIAL ACTIVITY

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Background: Mitochondrial dysfunction is well documented in OA and has the capacity to promote abnormalities in chondrocyte function and viability contributing to cartilage degeneration. Previous studies have showed the mtDNA haplogroup J are associated with incidence and progression of OA. Cybrids are optimal cellular models to study the real 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 to test the real role of mtDNA in cellular activity, using cybrids with mtDNA from healthy donors (without OA) and from patients with knee OA.
Methods: Cybrids were developed using 143B.TK$^-$ Rho-0 cell line (nuclear donor) and platelets (mitochondrial donors) from healthy (without AO-N-) and knee OA donors. The OXPHOS function was evaluated by SeaHorse XFp after addition of oligomycin, FCCP and Rotenone/Antimycin A. The metabolic status was evaluated by glucose consumption and lactic acid production. The glycolytic activity was measure after addition of glucose, oligomycin and 2-dioxyglucose using Seahorse XFp. Appropriate statistical analyses were performed with GraphPad Prism v6.
Results: OA cybrids had lower basal respiration (92.07±39.9 and 155.5±54.08, $p<0.0005$), and maximal respiratory capacity (114.7±50.1 and 160.6±44.7, $p<0.05$) than N. The analysis of ATP production was lower in OA than in N cybrids (66.69±28.69 versus 101.5±42.04 $p\leq0.05$). The % spare respiratory capacity value for the N was significantly lower than in OA cybrids (107±16.21 versus 124.7±5.97, $p\leq0.0005$). Cybrids carrying the mtDNA from OA patients showed higher glucose consumption than N cybrids (43.77±8.87 mg/ml and 31.91±13.69 mg/ml; $p<0.05$) however in the lactic acid production did not exit differences. The glycolytic activity was evaluated and showed that OA cybrids had lower glycolysis (71.05±4.83 versus 85.43±11.18, $p<0.05$) but higher glycolytic reserve than N cybrids (56.60±7.5 versus 39.73±19.13, $p\leq0.05$).
Conclusions: Cybrids have different metabolic behaviour, being N more efficient using glucose via glycolysis. We found differences statistical significate in the parameters that describe the mitochondrial respiration capacity, in this line OA cybrids had lower mitochondrial respiration and produce less ATP than the cybrids obtained from healthy patients. These results showed that the mitochondria obtained from healthy and OA donors had a different behaviour. These data also offer a real rationale for why mitochondria alterations play an important role in the incidence of OA.
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THU0010 ALTERED MIRNAS PROFILES IN PLASMA-DERIVED EXOSOME OF PATIENTS WITH ANKYLOSING SPONDYLITIS BY SMALL RNA-SEQ ANALYSIS

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Background: Ankylosing spondylitis (AS) is a chronic inflammatory disease, which is difficult to diagnose in the early stages. Increasing evidences have shown that MicroRNAs (miRNAs) may serve as novel biomarkers for AS. Exosome can function as vehicles to deliver miRNAs in body fluids including saliva and plasma. However, the relationship between exosome-delivered miRNAs and AS has yet to be determined.
Objectives: The aim of this study is to detect the altered miRNAs profiles of plasma-derived exosome in AS patients by small RNA-Seq Analysis.
Methods: Ribo^{TM} kit was used to isolate exosome. Small RNA Sample Pre Kit was used to build libraries in 3 AS patients and 3 healthy volunteers (HV), following by IlluminaHiSeq platform sequencing and bioinformatics analysis. Quantitative reverse-transcription PCR (qRT-PCR) was used to confirm the expression of the highly-expressed miRNA in another 10 AS patients and 10 HV, and receiver-operator characteristic (ROC) curve was used to evaluate the diagnostic value of miRNAs.
Results: Small RNA-Seq analysis showed that the Q30 value of HV and AS patients were higher than 95% (Fig.1-A). The amount of miRNA in HV and AS patients were (509.667±77.501) and (632.000±43.555). 80 up-regulated and 19 down-regulated exosomal miRNAs were identified in AS patients, compared with HV ($|\log2Ratio|>1$, $P<0.05$) (Fig.1-B-C). The target genes of the 34 highly-expressed miRNAs from the 99 differently-expressed miRNAs were 7869, and the main function of these target genes are involved in the regulation of endocytosis and protein modification process analyzed by GO and KEGG. The qRT-PCR results indicated that the expression level of miRNA21–5P and miRNA423–5P in AS patients were (2.940±1.572) and (2.520±1.401) times higher than that of HV (Fig.1-D-E). ROC curve analysis showed that miRNA21–5P and miRNA423–5P had significant diagnostic value for AS with the AUC of 0.890 (CI95%: 0.723–1.057) and 0.835 (CI95%: 0.621–1.039) respectively (Fig.1-F).

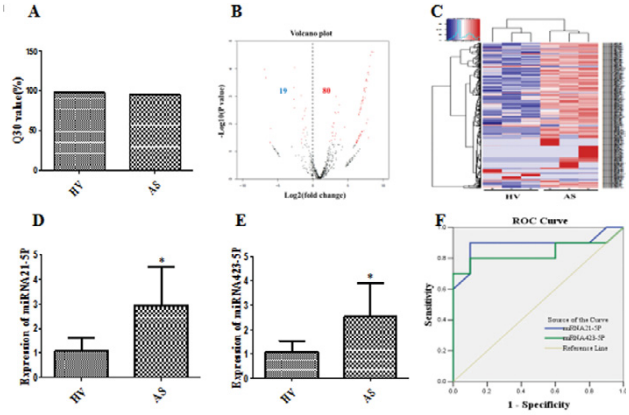

Fig.1 Altered miRNAs profiles in plasma-derived exosome of AS by small RNA-Seq Analysis
A: The Q30 value of each group. B-C: The differentially-expressed miRNAs. D-E: qRT-PCR was used to confirm the level of miRNA21-5P and miRNA423-5P. F: ROC cure was used to evaluate the diagnostic value of miRNA21-5P and miRNA423-5P. *$P<0.05$ vs HV.

Conclusions: The miRNAs profiles in plasma-derived exosome of AS patients are significant different from HV. miRNA21–5P and miRNA423–5P are higher expressed in AS patients. Thus, plasma-derived exosomal miRNAs might be reliable biomarkers to identify AS.
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THU0011 IMMUNE SIGNAL 2 CHECKPOINT MOLECULE EXPRESSION IN RHEUMATOID ARTHRITIS DISEASE PROGRESSION

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Background: Deep profiling of synovial tissue samples from rheumatoid arthritis (RA) patients may reveal the molecular underpinnings of phases of RA progression and provide new therapeutic targets to intervene earlier in disease pathogenesis.
Objectives: We sought to identify the molecular pathways expressed in different stages of disease (from seropositive subjects without clinically apparent synovitis to those with established disease) in synovial tissue compared to non-RA controls.
Methods: Transcriptomics profiling was performed on RNA isolated from synovial tissue biopsies. Normal synovium was collected from subjects with knee pain and without diagnosis of OA or RA (n=28). Arthralgia tissue was collected from ACPA-positive subjects without synovitis (n=10). Early RA tissue was collected from patients recently diagnosed (<1 year) with RA (n=57). Established RA tissue was collected from ACPA-positive subjects with >1 year of disease duration (n=95). Protein expression was confirmed on infiltrating immune cells from synovial biopsy cell suspensions by flow cytometry in separate RA subjects.
Results: Several pathways previously identified as important for RA pathogenesis (e.g., lymphocyte activation, osteoclast differentiation, NF-kappa B signaling) were enriched in differentially expressed genes in disease synovial biopsies compared to normal tissue samples. Interestingly, several genes known to function in T cell activation as signal 2 co-stimulatory or co-inhibitory molecules were differentially expressed, even in arthralgia and early RA subjects. 66 of 81 known co-stimulatory or co-inhibitory genes profiled were differentially expressed (FDR <5% and absolute fold-change >2) in disease samples from at least one cohort. The genes encoding co-stimulatory proteins that were increased compared to normal included *CD28*, *CD40LG*, *CD40* and *ICOS*. Interestingly, some of the genes encoding co-inhibitory proteins were increased (*PDCD1*/PD-1, *CD274*/PD-L1, *HAVCR2*/TIM3, *TIGIT, BTLA*), whereas others showed decreased expression (*C10orf54*/VISTA and *LAG3*) compared to normal controls. We focused on *CD28* expression, which is elevated in "pre-RA" arthralgia samples, proposing that anti-CD28 therapeutics could be candidates for RA disease prevention. By flow cytometry we demonstrated that a majority of CD4+ (>90%) and CD8+ (>60%) T

cells from RA synovial biopsy cell suspensions (n=4) showed surface expression of CD28.
Conclusions: We have generated a unique dataset from different phases of RA progression. Our results provide guidance for the selection of co-signaling molecules as therapeutic targets as well as for preventing the progression to RA. In particular, *CD28* expression was elevated in synovial tissue biopsies before the development of RA and it was also expressed on the majority of synovial tissue infiltrating CD4+ and CD8+ T cells from RA patients. These observations provide rationale to target CD28 for both RA treatment and disease interception.
Disclosure of Interest: A. Walsh Shareholder of: Johnson & Johnson, Employee of: Johnson & Johnson, M. Canavan: None declared, Y. Guo Shareholder of: Johnson & Johnson, Employee of: Johnson & Johnson, T. McGarry: None declared, X. Yin Shareholder of: Johnson & Johnson, Employee of: Johnson & Johnson, M. Wechalekar Grant/research support from: Johnson & Johnson, M. Smith Consultant for: Johnson & Johnson, S. Proudman: None declared, C. Orr: None declared, S. Kelly: None declared, C. Pitzalis Grant/research support from: Johnson & Johnson, Consultant for: Abbott, Astellas, MedImmune, BMS, Celgene, Grunenthal, GSK, MSD, Pfizer, Sanofi, Roche, UCB, D. Veale: None declared, U. Fearon: None declared, S. Nagpal Shareholder of: Johnson & Johnson, Employee of: Johnson & Johnson
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THU0012 HLA-DBR1 ALLELES PROFILE IN PATIENTS WITH RHEUMATOID ARTHRITIS: RELATION TO DISEASE SUSCEPTIBILITY AND SEVERITY

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Background: Rheumatoid arthritis (RA) is a complex, chronic autoimmune disease (AID) that affects approximately 0.5%–1% of the population worldwide. RA is considered to be complex diseases of unknown etiology, where the environmental, immunological and genetic factors contributed to susceptibility and severity of the disease. Since the first evidence suggesting the involvement of human leukocyte antigens (HLAs) in RA was reported in 1969, the HLA region have been at the center of genetic studies of RA.
Objectives: We undertook this study to elucidate the association between HLA-DRB1 alleles profile and susceptibility to and severity of RA in a Polish population.
Methods: We analyzed the data on a total of 472 RA patients in whom HLA-DRB1 allele genotyping had been performed. DNA sequences for exon 2 of the DRB1 locus were typed using polymerase chain reaction-sequence-based typing (PCR-SBT).
Results: Identification of the variants potentially associated with risk and protection was carried out by comparison to the DKMS Polish Bone Marrow Donor Registry (41306 alleles). A significant increase in the frequency of the HLA-DRB1*15:01 ($p=0.001$), DRB1*04 ($p<0.0001$), DRB1*01:01 ($p<0.0001$),DRB1*10:01 ($p=0.003$) and *09:01 ($p<0.0001$) were identified in patients with RA and showed strong association with the disease susceptibility. Furthermore, the HLA-DRB1*07:01 allele was found to be protective ($p<0.0001$) in our RA patients. In the RA patients with HLA-DRB1*52:01 allele the mean value of PLT ($p=0.03$) and organ symptoms ($p=0.02$) were more frequent than in RA patients without this allele. In RA patients with DRB1*01:01, DRB*04 as well as DRB1*15:01 alleles the number of tender joints ($p=0.03$), diabetes ($p=0.03$) and renal failure) $p=0.03$) were observed less frequently compared with RA patients without these alleles. We also observed that in RA patients with DRB1*10:01 the VAS score was higher than in RA patients without this allele.
No association was detect between the HLA-DRB1*07:01 and DRB1*09:01 and disease activity and laboratory parameters among RA patients.
Conclusions: Current finding indicated that HLA-DRB1*15:01 DRB1*04, DRB1*01:01 and DRB1*10:01 alleles may be associated with susceptibility to as well as severity of RA.
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THU0013 TOWARDS THE DEFINITION OF A RISK MODEL PROFILE OF PERICARDITIS IN SYSTEMIC LUPUS ERYTHEMATOSUS: A GENETIC STUDY

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Background: We have recently demonstrated that polymorphisms (SNPs) in TRAF3IP2 gene are associated with susceptibility for systemic lupus erythematosus (SLE) and can predispose to the development of pericarditis (1). Moreover, we found that other genes previously associated with susceptibility to SLE (MIR1279, STAT4, PTPN2) seem to be involved in the development of pericarditis (2). At this purpose, we built a genetic risk model for the development of pericarditis in SLE (3).
Objectives: We aimed to expand the knowledge on the genetic risk of pericarditis in SLE by studying the role of rs2205960 (TNFSF4) and rs2233945 (ATG16L1) SNPs, previously associated with SLE susceptibility, improving our genetic risk model.
Methods: We recruited SLE patients (diagnosed according to 1997 revised ACR criteria) and healthy subjects served as controls. Study protocol included complete physical examination and blood drawing. The clinical and laboratory data were collected in a standardized, computerized, electronically-filled form (3). Clinical and laboratory features were assessed with a dichotomous score (present=1; absent=0). SNPs genotyping was performed by allelic discrimination assay. A case/control association study and a genotype/phenotype correlation analysis were performed and a risk profile model for pericarditis in SLE was built.
Results: Three-hundred fifteen SLE patients [285 F (90.5%), 30 M (9.5%), mean age 43.11±11.28 years, mean age at onset 32.19±11.84 years] and 278 healthy controls were enrolled. Pericarditis was present in 56 (17.8%) SLE patients. Deviations from Hardy–Weinberg equilibrium for the studied SNPs were not observed. The variant alleles of the rs2205960 (TNFSF4, P=0.013, OR=2.14) and of the rs2233945 (ATG16L1 P=0.009, OR=2.32) were significantly associated with susceptibility to pericarditis. A risk profile model for pericarditis considering the risk alleles of TRAF3IP2, MIR1279, STAT4, PTPN2, TNFSF4 and ATG16L1 showed that patients with more than 5 risk alleles have a significantly higher risk to develop pericarditis ($P<0.001$, OR=8.01). Anti-Sm antibodies were the only laboratory parameter associated with the development of pericarditis. Thus, a multivariate analysis by binary regression analysis, considering as dependent variable the presence or absence of pericarditis and as independent variables all the studied SNPs associated with pericarditis, was performed. In a stepwise approach including anti-Sm, TRAF3IP2, MIR1279, STAT4, PTPN2, TNFSF4 and ATG16L1 SNPs, the model explains about 25% (R^2 Cox &Snell) of the variability involved in the susceptibility to pericarditis.
Conclusions: We describe for the first time the contribution of TNFSF4 and ATG16L1 SNPs in pericarditis development in SLE patients. We improved our genetic risk profile model to better and earlier identify SLE patients more susceptible to develop this complication.
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THU0014 NO ASSOCIATION OF SNP RS3761847 WITH THE EXPRESSION OF THE TRAF1-C5 LOCUS AND INVASION OF RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS

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Background: Polymorphisms in the *TRAF1-C5* locus have been associated with susceptibility to rheumatoid arthritis (RA). A single nucleotide polymorphism (SNP) rs3761847, located in the first intron of the *TRAF1* gene, has been identified as an RA risk locus in a genome-wide association study (GWAS); the relationship of rs3761847 with joint damage are unclear. One of the key mechanisms of joint destruction is the degradation of and invasion of cartilage by RA synovial fibroblasts (RASF).
Objectives: To determine whether the expression of *TRAF1-C5* genes and the invasion of RASF are associated with the genotype at rs3761847.
Methods: The genotype at rs3761847 was determined using TaqMan Allelic Discrimination Assay. Invasion assays were performed using Matrigel-coated invasion chambers. Peripheral blood mononuclear cells (PBMC) were obtained by density gradient centrifugation. Neutrophils were isolated from the red blood cell layer by erythrocyte lysis; $CD14^+$ cells were separated using magnetic cell sorting. Macrophages were differentiated from plastic-adhered monocytes in M-CSF (50ng/ml) for 8 days. Cells were stimulated with lipopolysaccharide (LPS, 100ng/ml) plus interferon γ (IFNγ, 20ng/ml), interleukin 4 (IL-4, 20ng/ml) or tumour necrosis factor α (TNF-α, 10ng/ml). Gene expression was measured by SYBR Green real-time PCR using HPRT1 as a housekeeping gene.
Results: rs3761847 was in Harvey-Weinberg equilibrium in RASF (n=54, p=0.98) with genotype frequencies of 0.42 AA, 0.45 AG and 0.13 GG. When grouped by genotype, the expression of TRAF1 (total), TRAF1 transcript variant 2 and complement C5 mRNA showed no significant difference (p=0.929, p=0.583, p=0.980 respectively). This was also the case for invasion of RASF (n=43, p=0.548; mean cell count 10.4 AA, 8.3 AG and 11.1 GG). There was no correlation of genotype with common RA disease markers (CRP p=0.718, ESR p=0.179, RF titre p=0.466, ACPA titre p=0.712). In contrast, analyzing TRAF1 expression in different types of blood cells, significantly higher levels of TRAF1 mRNA were detected in monocytes carrying the AA genotype compared with the GG genotype at rs3761847, both in unstimulated (2.26-fold) as well as LPS+IFNγ-activated cells (2.04-fold; $p<0.05$, n=3 each). No significant association of TRAF1 expression or transcript variant utilization with rs3761847 was observed in the other blood-derived cell types studied, i.e. PBMC, macrophages and neutrophils,