MIRNAS CONTRIBUTE TO DYSREGULATED ROS METABOLISM OF IMMUNE CELLS IN THE INFLAMMED JOINT

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Background: In the last years miRNAs have emerged as critical regulators of innate and adaptive immune responses and an altered expression or function is associated with several inflammatory and autoimmune diseases. Therefore miRNAs are also believed to promote inflammatory processes within the inflamed joint of juvenile idiopathic arthritis (JIA) patients. It is furthermore known that oxidative stress is associated with JIA. Free radicals are implicated in joint damage and play an important role as secondary messengers in immunological responses. How and if miRNAs contribute to dysregulated reactive oxygen species (ROS) metabolism in JIA remains to be elucidated.

Objectives: We aimed to identify miRNAs and miRNA regulated pathways, which contribute to dysregulated immune cell responses within the inflamed joint.

Methods: miRNA profiling was performed on peripheral blood mononuclear cells (PBMCs) from healthy children, PBMCs from 9 JIA patients and synovial fluid mononuclear cells (SFMCs) from the same JIA patients. Subsequently, GO and pathway enrichment analyses were performed on predicted target genes. Upregulation of miRNAs was confirmed in vitro after incubation with synovial fluid by qRT-PCR. Mitochondrial integrity, cellular ROS and Nrf2 protein expression were measured by flow cytometry.

Results: Transcriptome analysis of JIA SFMCs compared to HC PBMCs revealed strongly enhanced expression of miR23a and miR23a, miR7a, miR146a, and miR155, which are involved in oxidative stress responses. In addition, expression of those could be induced in healthy control PBMCs by synovial fluid ex vivo. ROS level in synovial fluid T cells were enhanced, while expression of Nrf2, the main regulator of anti-oxidative responses and a target of miR7a, remained low. Furthermore mitochondrial cyclophilin, which regulates ROS escape from mitochondria and is suppressed by miR23a, was downregulated in SFMCs as well.

Conclusion: SFMCs within the inflamed joint reveal a distinct miRNA expression profile. Especially miRNAs that are involved in regulation of ROS metabolism are upregulated. In line with this, expression of Nrf2 and mitochondrial cyclophilin, which are important regulators of cellular ROS metabolism are reduced while production of ROS is enhanced. We suggest that higher abundance of miRNAs, that are involved in oxidative stress pathways, contribute to redox dysregulations within the inflamed joint and thereby contribute to inflammatory processes.

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OLIGOARTICULAR JUVENILE IDIOPATHIC ARTHRITIS DOES NOT SHOW SIGNIFICANT CYTOKINE EXHUSATION IN SPITE OF INCREASED EXPRESSION OF CO-INHIBITORY RECEPTORS

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Background: Oligoarticular Juvenile idiopathic arthritis (O-JIA) is a common inflammatory joint disease in children, driven by continuous local T-cell activation. [1] T cell activation is counter-balanced via signals generated by co-inhibitory receptors (co-IRs) such CTLA-4, PD-1, LAG-3, and TIM-3.[2]

Objectives: Here we identify the role of co-IRs in the pathogenesis of O-JIA.

Methods: Pairend synovial fluid (SF) and plasma, PBMCs and SFMCs, were obtained from O-JIA patients together with clinical data (n=14). Plasma from healthy controls (HC, n=14); paired SF and plasma from 5 non-arthrititics juvenile orthopedic patients (OC, n=5) served as controls. Soluble levels of co-IRs were measured by ELISA and their cellular expression by flow cytometry. Spontaneously differentiated fibroblast like synoviocytes (FLS) from SFMCs were co-cultured with autologous PBMCs/SFMCs and used as an ex vivo disease model. Functional effects of co-IRs were evaluated via blocking them with checkpoint inhibitors in these ex vivo disease models.

Results: In O-JIA patients, increased levels of sPD-1, sLAG-3 and sTIM-3, but not sCTLA-4, were present in the SF compared with plasma. (Figure 1A) There was a close correlation between sPD-1 levels in the plasma and SF (r=0.65, p=0.029). In plasma, sTIM-3 levels correlated with sPD-1 levels (r=0.84, p<0.001) and sLAG-3 levels (r=0.68, p=0.021). In the SF, there was a significant correlation between sLAG-3 levels and sPD-1 levels (r=0.54, p=0.047). None of the soluble co-IR levels correlated with disease activity scores. Plasma and SF levels of sLAG-3 and sTIM-3 were higher in O-JIA patients when compared with HC and OC. (Figure 1A) On the CD3+CD4+CD45RO+ T cells, the surface expression of PD-1 (p=0.02), LAG-3 (p<0.001), TIM-3 (p<0.001), but not CTLA-4 (p=0.48), were higher in the SFMC compared with PBMC.

MHC class II expression was induced on FLS when these were co-cultured with autologous PBMCs and SFMCs, together with an increased Monocyte Chemoattractant Protein-1 (MCP-1) production. (Figure 1B) Only addition of neutralizing anti-LAG3 antibodies significantly increased the MCP-1 production in PBMC mononucleares (p<0.01) and FLS/SFMC co-cultures (p<0.01). PBMCs and SFMCs produced significantly higher levels of IFN-γ after CD3/CD28 activation both in mononucleares and co-cultures (p<0.001), but they were not affected from addition of any of the checkpoint inhibitor.

Conclusion: This is the first report studying the effects of different co-IRs in O-JIA. Both the soluble levels and the surface expressions of the co-IRs were higher at the site of inflammation in O-JIA. SFMCs and PBMCs of O-JIA patients are not exhausted, based on their ability to respond to CD3/CD28 activation. This is opposite to what has been shown in adult inflammatory arthritis.[3] Co-cultures of autologous FLSs and PBMCs/SFMCs may serve as an ex vivo arthritis model to perform functional analysis. LAG-3 stands out among the co-IRs and might play a role in O-JIA pathogenesis and a potential therapeutic option for O-JIA.

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BROADENING OUR UNDERSTANDING OF THE GENETICS OF JUVENILE IDIOPATHIC ARTHRITIS: INTERROGATION OF THREE DIMENSIONAL CHROMATIN STRUCTURES WITHIN JIA-ASSOCIATED RISK LOCI

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Background: Our group has shown that, like most complex traits, the risk loci for juvenile idiopathic arthritis (JIA) identified on genome-wide association studies (GWAS) and genetic fine mapping studies are highly enriched for enhancers. Enhancers are regulatory elements that fine-tune gene expression to specific

physiologic circumstances. Enhancers do not always regulate the nearest gene, and may regulate more than one gene. Enhancers typically regulate genes within the same chromatin loop, or topologically associated domain (TAD).

Objectives: To gain a better understanding of the genetics of JIA by examining the broader chromatin architecture that encompasses the known risk haplotypes.

Methods: We used publicly available chromatin conformation HiC data and the online JuiceBox software suite to query known JIA haplotypes for evidence of physical interactions between putative enhancers within the haplotypes and immunologically relevant genes. We specifically queried 20 haplotypes in which H3K4me1/H3K27ac marks were prominent within both lymphoid and myeloid cells. We queried data from GM12787 (B cell), K562 (lymphoblast), and THP-1 (monocyte/macrophage) cells, as well as human cord blood T cells. To identify TADs associated with specific enhancers, we used a 5KB resolution (which allowed us to visualize chromatin loops as peaks), setting the cursor at the center of each putative enhancer. We also identified the genes within the identified chromatin loop domains and used gene ontology (GO) analyses to identify functional associations among genes within the TADs incorporating the JIA risk loci.

Results: We identified at least one chromatin loop structure in all 20 of the JIA risk haplotypes for each of the 4 cell lines we queried. These loops were not cell type specific. That is, almost identical loops structures could be seen in each of the cell lines at each of the loci, suggesting that these enhancers regulate a broad range of common leukocyte functions. The TADs incorporating the JIA haplotypes invariably included genes of immunologic interest. For example, the TAD incorporating the IL2RA haplotype including IL15R (the alpha chain of the IL15 receptor) and PKCQ, a protein kinase C-family enzyme important in both T and B cell activation. The most complex locus was C5orf56 which encompassed 23 genes (incuding IL4R, IL15R, C5orf56) and 3 major loops and sub-loops. Genes within the TADs were highly enriched for multiple GO terms for processes involved in leukocyte activation (e.g., MAP kinase signaling cascade), JAK-STAT responses, chemotaxis, and cytokine-mediated signaling pathways.

Conclusion: These 20 JIA-associated risk loci are situated within complex chromatin regions that show similar features in both lymphoid and myeloid cells. HiC data demonstrate direct physical contacts between putative enhancers within the risk loci and multiple genes of immunologic interest. We hypothesize that at least some of the genes within the haplotype-associated TADs are the long sought “target genes” of JIA-associated genetic variants. This work emphasizes the importance of broadening our focus beyond the “nearest gene” to the GWAS-identified tag SNPs.

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A NOVEL RELA TRUNCATION IN A 3-GENERATION FAMILY WITH BEHCET’S DISEASE ALTERS THE APOPPTOTIC RESPONSE TO INFLAMMATORY STIMULANTS

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Background: Behcet’s disease (BD) is a heterogeneous multifactorial auto-inflammatory condition characterised by recurrent episodes of oral and genital ulceration, uveitis and skin lesions, with less frequent involvement of the gastrointestinal tract, large vessel bloods and central nervous system. The NF-κB pathway is a ‘master-regulator’ of immune and inflammatory signaling, with the ability to control the expression of key inflammatory genes and genes associated with apoptosis and proliferation.

Objectives: To identify the pathobiology associated with a novel genetic mutation identified in a 3-generation family with Behcet’s-like mucocutaneous ulceration syndrome, primarily involving childhood-onset chronic oral and genital ulcerations.

Methods: The novel RELA mutation was identified using whole exome sequencing. Immunoblot of peripheral blood mononuclear cell (PBMCs) lysates from affected family members was used to determine if the predicted truncated protein was expressed. PBMCs were stimulated with TNF; NFκB phosphorylation was measured relative to unstimulated cells form affected and unaffected family members. HEK293T cells transplanted with plasmids encoding either wild-type or the novel RELA-mutant and overexpression confirmed via immunoblot. An in vitro model of the RELA truncation was used to observe the effect of the RELA truncation on response to TNF stimulation. Apoptosis protein arrays, western blots, and ELISA assays were used to investigate the effect of TNF on wild-type RELA compared to the mutant protein. Mouse Embryonic Fibroblasts (MEFs) isolated from RELA-/- mice, which do not express endogenous RELA, were transfected with plasmids encoding either wild-type or the novel RELA-mutant. Wild Type MEF cells (with endogenous RELA) were used as a control. Cells were stimulated with LPS (2 μg/ml) or no treatment control for 12 hours.

Results: A heterogeneous cysteine deletion at position 1459 in RELA was detected in all affected individuals as previously reported. This mutation results in a frameshift His487ThrfsTer7, has increased pro-apoptotic proteins (BAD, cleaved caspase 3 and SMAC) whereas anti-apoptotic proteins (BCL2, CLASP1N) were decreased compared to cells transfected with wildtype RELA. Cells transfected with RELAHis487ThrfsTer7 were less sensitive to TNF stimulation compared to wild-type controls, as measured by induction of TNF-sensitive proteins.

Conclusion: This study gives novel information on both the genetic basis and biological mechanisms of BD in individual families. Familial mutations that induce haptinsufficency of RELA have recently been associated with BD. However, the His487ThrfsTer7 results in protein truncation rather than haptinsufficiency. Our study reports several recently published studies that loss-of-function mutations in the NF-κB pathway are linked with the development of familial early-onset BD-like syndromes. We propose that RELAHis487ThrfsTer7 causes altered NFκB signaling resulting in reduced mucosal cell recovery and the observed Behcet-like mucocutaneous ulceration syndrome.

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OP0154-HPR-TARGETS FOR REDUCING PREMATURE MORTALITY IN OLDER ADULTS WITH OSTEOARTHRITIS: RESULTS FROM A NOVEL PATH ANALYSIS WITHIN A COX PROPORTIONAL HAZARDS MODEL

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Background: There is increasing evidence that it is the impact of osteoarthritis rather than the osteoarthritis itself that explains an excess risk of mortality. This indicates that potentially modifiable targets may reduce mortality for those with osteoarthritis. Mediation analysis can be used to investigate pathways, although this has rarely been undertaken using survival analysis due to the challenge of accounting for time. The study uses a novel approach to examine mediation using path analysis with Cox proportional hazard modelling (survival analysis).

Objectives: The objectives of this study were to identify potential mechanisms of the impact of osteoarthritis on mortality and examine the role of modifiable targets (anxiety, depression, insomnia and walking frequency) for health professionals in primary care.

Methods: A population-based prospective cohort study was conducted using data from the North Staffordshire Osteoarthritis Project (NorSiOP), in which primary care medical record data was linked to self-report information collected by questionnaire in adults aged 50 years and over (n=8066). Individuals were defined as having osteoarthritis if they had consulted general practice for osteoarthritis, identified by Read codes in the primary care medical record, and indicated moderate to severe pain interference in daily life in the Medical Outcomes Short Form 36 at baseline (2002). A Cox proportional hazards analysis was performed to determine the total effect (TE) of osteoarthritis on mortality, both with adjustment for confounding variables (age, sex, education, occupation, smoking status, ischaemic heart disease, chronic obstructive pulmonary disease, non-steroidal anti-inflammatory drugs, obesity, cognitive impairment). Within the Cox proportional hazard analysis was used to decompose the TE to assess the indirect (IE) and direct effects (DE) for each of four potential mediators (anxiety, depression, insomnia, walking frequency; all measured by questionnaire) with adjustment for confounders. Results are expressed as adjusted hazard ratios (aHR); bootstrap resampling was used to generate 95% confidence intervals (95% CIs).

Results: Mean age of participants was 65.2 (SD 9.8) years and 51.6% were female. 2396 (29.7%) had osteoarthritis. Participants were followed up over 10 years. Mean age of participants was 65.2 (SD 9.8) years and 51.6% were female. 2396 (29.7%) had osteoarthritis. Participants were followed up over 10 years. Osteoarthritis was significantly associated with mortality (aHR 1.14; 1.00, 1.28). The relationship between osteoarthritis and mortality was mediated by walking frequency, depression and insomnia (anxiety did not mediate the relationship (IE HR 1.00; 0.98, 1.02)). The strongest mediator was walking frequency (TE 1.14; 1.00, 1.29; DE 1.04; 0.91, 1.18; IE 1.08; 1.06, 1.11), followed by depression (IE 1.06; 1.03, 1.08) and insomnia (IE 1.01; 1.00, 1.03).

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