GENOTYPIC EFFECTS OF ANKYLOSING SPONDYLITIS ASSOCIATED IL7R POLYMORPHISMS ARE MEDITATED THROUGH MONOCYTES IN INFAMMATION

M. H. Al-Mosawi1, E. Laut1, S. Danielli2, N. Yager1, J. de Wit1, E. Mahe1, L. Rizvi1, J. Knight1, B. Fairall1,*, P. Bowes1, 1Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences; 2Wellcome Trust Centre for Human Genetics; 3Weatherall Institute of Molecular Medicine; 4Department of Oncology, University of Oxford, Oxford, UK

Background: Interleukin 7 (IL-7) plays a key role in T cell survival and proliferation. Both cell-surface expressed and soluble forms of the IL-7 receptor (sIL7R) are recognised. sIL7R has been shown to prolong IL-7 activity in inflammation.

Methods: Monocyte cell surface IL7R expression was measured by flow cytometry in the presence or absence of LPS or TNF in a cohort of volunteers recruited from the Oxford biobank. Soluble IL7R was quantified by ELISA in purified monocyte cultures stimulated with LPS. RNA sequencing was performed for 8 paired samples of control and recombinant IL-7 exposed stimulated monocytes.

Results: Surface IL7R expression was induced after 24 hours of LPS stimulation both in isolated monocytes (n=84, p=8.3e-19) and CD14+ cells in whole PBMC cultures (n=103, p=3.9e-31). We find the key genetic regulator of this response to be rs6897932, previously associated with AS predisposition, both in isolated monocytes (n=85, p=9e-7) and CD14+ cells in whole PBMC cultures (n=103, p=9.4e-5). There was no genetic effect seen in unstimulated monocytes. Notably IL7R positivity of CD4+, CD8+ and CD56+cells measured within the PBMC culture both before or after LPS stimulation showed no association with rs6897932. The addition of anti-TNF (Infliximab) abrogated the genotypic effect. In a second independent cohort, genotype-specific surface IL7R induction was also observed after stimulation with recombinant human TNF (n=62, p=0.0007).

Conclusions: Monocytes upregulate IL7R expression and soluble IL7R secretion after LPS treatment in a functional, genotype- and TNF-alpha-dependent manner. SpA synovial monocytes express IL7R suggesting preactivation. These data draw attention to an unappreciated key myeloid role for AS risk variants at IL7R.

Disclosure of Interest: None declared

REFERENCE:

OP0288

TRANSCRIPTIONAL PERTURBATION OF RA-RISK ENHANCER BY CRISPR-DEADCAS9 REGULATES LONG RANGE GENE TARGETS

K. Duffus1, M. Imran2,3, T. Katopodi4, H. Ray-Jones1, G. Orozco1, A. Adamson1, S. Eyre1. 1Arthritis Research UK Centre for Genetics and Genomics, Centre for Musculoskeletal Research, Manchester Academic Health Science Centre; 2University of Manchester, Manchester; 3Wellcome Trust Sanger Institute, Cambridge, UK

Background: Findings from genome wide association studies in complex diseases indicate over 90% of genetic variants associated with risk of developing disease are found outside protein coding regions, suggesting regulation of gene expression is key to disease susceptibility. For rheumatoid arthritis (RA) it has been demonstrated that risk variants are found in gene regulation regions, and are significantly enriched in T-cell specific enhancers. In addition, a significant proportion of associated variants lay some distance from the nearest gene and enhancers may not necessarily regulate the closest gene, effectively ‘skipping’ genes. Using chromatin conformation technology (HiC) we have demonstrated that an enhancer region intrinsic of the COG6 gene, containing variants associated with RA, make robust physical contact with the promoter of FOXO1, almost 1 Mb away on the linear chromosome. COG6 is not an obvious candidate risk gene for RA, whilst FOXO1 is involved in T-cell development and shown to be over expressed in RA synovium. The challenge now is to provide empirical evidence that the enhancer found within COG6 does regulate FOXO1 expression, and how an RA risk genetic background affects this regulation.
Objectives: Use CRISPR-Cas9 to perturb the COG6 intrinsic enhancer region and measure the downstream effect on the expression of FOXO1.

Methods: We utilised a modified form of the Cas9 enzyme, dead Cas9 (dCas9), that can precisely target DNA, but will not induce a cut. Using the dCas9 attached to either enhancers (p300) or repressors (KRAB) of expression we investigated how perturbation of the enhancer intrinsic of COG6 changed the expression of FOXO1.

We designed 3 guides across the COG6 enhancer, and transduced a cell line (HEK293) using a lentiviral dCas9 CRISPR system, with either dCas9-KRAB or dCas9-p300 and each of the three guides. We cultured the cells until 70%–80% confluent, GFP sorted the cells and then extracted RNA. A quantitative PCR was performed (QuantStudio) for both COG6 and FOXO1 gene transcript expression and normalised to housekeeping genes.

Results: Up to 90% of HEK cells were transduced with the dCas9 enzyme and guide, and these were sorted by FACS using GFP to sort the top 60%. The 3 guides gave consistently increased levels of FOXO1 expression with the dCas9-p300, compared to both control and dCas9-KRAB (p<0.02). This was particular evident for guide 3, with a 40% increase (p300) and 10% decrease (KRAB) of FOXO1 expression observed. Expression of COG6 was also perturbed, but in a less consistent manner, with both increase and decrease expression for KRAB and p300.

Conclusions: Over 90% of HEK cells were transduced with the dCas9 enzyme and guide, and these were sorted by FACS using GFP to sort the top 60%. The 3 guides gave consistently increased levels of FOXO1 expression with the dCas9-p300, compared to both control and dCas9-KRAB (p<0.02). This was particular evident for guide 3, with a 40% increase (p300) and 10% decrease (KRAB) of FOXO1 expression observed. Expression of COG6 was also perturbed, but in a less consistent manner, with both increase and decrease expression for KRAB and p300.


Disclosure of Interest: None declared


---

OP0289

INTEGRATION OF CHROMATIN CONFORMATION, TRANSCRIPTOME AND GENOME-WIDE LANDSCAPE OF BRD2 AND BRD4 BINDING MOTIFS IDENTIFIES MECHANISMS OF BET INHIBITOR ACTION IN RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS

S. Nagpal1, V. Krishna1, X. Yin1, D. Pocalyko2, A. Walsh3, K. Bachman4, I. Anderson5, L. Madakamutil1, S. Nagpal1, V. Krishna1, X. Yin1, D. Pocalyko2, A. Walsh3, K. Bachman4, I. Anderson5, L. Madakamutil1

1Immunology; 2Discovery Sciences, Janssen Research, Spring House, USA

Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterised by infiltration of immune cells into the synovium and hyperplasia of the synovial lining, resulting in the formation of pannus that degrades cartilage and bone. Fibroblast-like synoviocytes (FLS) are the main cells of the rheumatoid synovium and possess phenotypic and molecular characteristics of transformed cells. JQ1, an inhibitor of the bromodomain and extra terminal domain (BET) family that includes BRD2, BRD3, BRD4 and BRD1, has shown efficacy in vitro on RA-FLS proliferation and in vivo in a murine model of arthritis.

Objectives: We sought to elucidate the mechanism of action of BET proteins in FLS biology and determine the potential therapeutic utility of targeting BRD2/BRD4 for RA disease treatment and interception.

Methods: To understand the mechanism of JQ1 action, we subjected JQ1-treated RA-FLS to transcriptional profiling by RNA-Seq and determined BRD2 and BRD4 cistromes by identifying global BRD2/BRD4 chromatin binding sites by ChIP-Seq. In addition, Assay for Transposase Accessible Chromatin by high throughput Sequencing (ATAC-Seq) was employed to identify open and closed regions of chromatin in JQ1-treated RA-FLS.

Results: We demonstrate that the active isomer of JQ1 but not its inactive isomer inhibits IL-1β-induced RA-FLS activation and proliferation. Through an integrated analysis of RNA-Seq, ATAC-Seq to profile changes in chromatin accessibility and ChIP-Seq of BRD2/4 and Pol2 proteins, we found that JQ1 inhibited multiple key inflammatory pathways, and altered the genome wide occupancy of crucial transcription factors involved in inflammatory signalling. Specifically, JQ1 treatment resulted in reduced occupancy of both BRD2 and BRD4 in approximately 2000 regions genome-wide, and a loss of Pol2 occupancy in approximately 600 genomic regions. Collectively we found that 105 genes had altered occupancy in all three proteins (BRD2, BRD4 and Pol2) and were also differentially expressed. Most prominently, JQ1 resulted in down regulation of IL6, IL8, p38 MAP kinase and HMGB1/TLR4 signalling pathways. In addition, we have identified BRD2/BRD4 super-enhancer genes and demonstrate that JQ1 altered BRD2/BRD4 occupancy in the IL6 and IL8 super-enhancer regions, and significantly down-regulated IL6, IL8, TLR4 and IL1β expression.

Conclusions: Our results suggest pleiotropic effects of JQ1 on pathways that have been individually targeted and shown to be efficacious for the treatment of RA. These studies provide a strong rationale for targeting of BRD2/BRD4 in RA.

Disclosure of Interest: S. Nagpal Shareholder of: Johnson and Johnson, Grant/research support from: Johnson and Johnson, Employee of: Johnson and John-son, V. Krishna Shareholder of: Johnson and Johnson, Grant/research support from: Johnson and Johnson, Employee of: Johnson and Johnson, X. Yin Shareholder of: Johnson and Johnson, Grant/research support from: Johnson and Johnson, Employee of: Johnson and Johnson, D. Pocalyko Shareholder of: Johnson and John-son, Grant/research support from: Johnson and Johnson, Employee of: Johnson and Johnson, A. Walsh Shareholder of: Johnson and Johnson, Grant/research support from: Johnson and Johnson, Employee of: Johnson and Johnson, K. Bachman Shareholder of: Johnson and Johnson, Grant/research support from: Johnson and Johnson, Employee of: Johnson and Johnson, I. Anderson Shareholder of: Johnson and Johnson, Grant/research support from: Johnson and Johnson, Employee of: Johnson and Johnson, L. Madakamutil Shareholder of: Johnson and Johnson, Grant/research support from: Johnson and Johnson, Employee of: Johnson and Johnson, V. Krishna Shareholder of: Johnson and Johnson, Grant/research support from: Johnson and Johnson, Employee of: Johnson and Johnson, A. Walsh Shareholder of: Johnson and Johnson, Grant/research support from: Johnson and Johnson, Employee of: Johnson and Johnson, D. Pocalyko Shareholder of: Johnson and Johnson, Grant/research support from: Johnson and Johnson, Employee of: Johnson and Johnson, A. Walsh Shareholder of: Johnson and Johnson, Grant/research support from: Johnson and Johnson, Employee of: Johnson and Johnson, I. Anderson Shareholder of: Johnson and Johnson, Grant/research support from: Johnson and Johnson, Employee of: Johnson and Johnson, L. Madakamutil Shareholder of: Johnson and Johnson, Grant/research support from: Johnson and Johnson, Employee of: Johnson and Johnson


FRIDAY, 15 JUNE 2018

PARE abstract session

OP0290-PARE

KEEP AN EYE OUT FOR INVISIBLE PAINS – A DANISH CAMPAIGN

C. R. Ziegler, Gigtforeningen, Gentofte, Denmark

Background: How does it feel to live with a RMD and have chronic pain? How do you explain pain caused by RMD to your friends and family? How do you make others understand how pain affects your daily life? The communication department of the Danish Rheumatism Association needed to convey living with invisible pain, because it is of such importance to people with RMDs. Based on interviews with the users (people with RMDs) we found the best way of making others understand was to create a film, which illustrated living with invisible pain.

Objectives: We wanted to increase the general understanding of RMDs and living with invisible pain in society. The secondary objective was to brand awareness of the Danish Rheumatism Association and to generate leads for further processing, e.g. signing up for membership.

Methods: Based on a few interviews with users and several posts from Social Media we focused on making invisible pain visible. By choosing the media of a film we wanted to explain pain caused by RMD to your friends and family? How do you explain pain caused by RMD to your friends and family? How do you make others understand how pain affects your daily life? The communication department of the Danish Rheumatism Association needed to convey living with invisible pain, because it is of such importance to people with RMDs. Based on interviews with the users (people with RMDs) we found the best way of making others understand was to create a film, which illustrated living with invisible pain.

Results: We launched the film on World Arthritis Day 2017. In 3½ weeks the film reached 1 million users and more than 1000 comments on Facebook, and the number of followers went up by 2000. The total impact of the film will be presented on September 30, 2023 by guest. Protected by copyright.