OP0191

EPIGENETIC PROFILING OF SYNOVIAL FIBROBLASTS REVEALS STRUCTURAL DNA DYNAMICS AT DISEASE IMPLICATED CHROMOSOME REGIONS

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Background: One of the more striking findings from genome-wide association studies (GWAS) is how the majority of disease associated genetic variants are found within gene regulatory regions, known as enhancers. It is now well established that these enhancers regulate target genes through physical interactions. We, and others, have shown that these interactions can vary between cell types and act over long distances. Therefore, if we are to fully translate GWAS findings, we need link disease associated enhancers to their target genes, in the relevant cell types. So far we only have a limited picture in rheumatoid arthritis (RA) relevant cell types, since synovial fibroblasts (SF) have been omitted from this type of chromatin interaction analysis.

Objectives: Investigate dynamic Hi-C interactions in unstimulated and stimulated SF, mapping RA enhancers to their target genes, and correlating with gene expression.

Methods: We cultured SF from synovial tissues of RA patients. After stimulation of the SF (n=6) with 10 ng/ml TNF for 24h, Hi-C and RNA libraries were generated and sequenced on the Illumina HiSeq 4000. We called dynamic active and inactive regions of the genome (A/B compartments), mapped on RA associated enhancers, linked these to target genes and correlated the interactions with dynamic expression.

Results: We found a region on the chromosome 6q23, intergenic between *OLIG3* and *TNFAIP3*, with an enhancer containing SNPs associated with RA to be dynamically linked to the *TNFAIP3* gene through DNA activity, interactions and corresponding gene expression.

As shown in figure 1a, the genomic region containing the RA associated variants (red square) only resides in an open, active region of DNA (black bar, compartment A) upon TNF-stimulation of the SF. This region then makes a strong interaction with the promoter of *TNFAIP3* (figure 1a) in stimulated cells, which corresponds to a more than 100-fold increase in *TNFAIP3* gene expression (figure 1b).

Conclusion: Using independent data, we have indicated how the RA region on 6q23 could influence the expression of *TNFAIP3*, under stimulatory conditions in SF cells. This supports previous work, where this particular 6q23 enhancer is preferentially active in RA synovium, compared to osteoarthritis, reflecting the different stimulatory conditions in each disease (Ai *et al.* 2018). We have successfully linked an RA associated enhancer to its target gene in SF, indicating how the enhancer works over a large distance to interact with and regulate the *TNFAIP3* gene. Furthermore we show that this enhancer containing RA associated SNPs is only active upon stimulation with TNF in SF.

REFERENCE:

[1] Ai R, et al. Nat Commun. 2018 May 15;9(1):1921

Figure 1a

Figure 1b

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DISSECTING THE LONG-RANGE GENE REGULATION OF RHEUMATOID ARTHRITIS RISK ENHANCERS AT THE 5Q11 LOCUS USING THE COMPLEMENTARY APPROACHES OF CRISPRa AND CRISPRi

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Background: Large-scale genome wide association studies (GWAS) in rheumatoid arthritis (RA) have revealed that the majority of associated risk variants lie in non-coding regions of the genome. For RA it has been demonstrated that risk variants are significantly enriched in T cell specific enhancers. One such susceptibility locus known as 5q11 is the third strongest association with RA. At this locus the lead risk variants lie in two distinct active enhancer elements intronic to the *ANKRD55* gene. Previous work using chromatin conformation has shown these enhancers make physical contact with the promoter of *IL6ST*, 150 kb away on the linear chromosome¹. Whilst *ANKRD55* is a gene of unknown function, *IL6ST* is part of the IL6R complex which regulates the IL6 signalling pathway. Tocilizumab is already an approved drug for RA, which works against the IL6R. CRISPR is an ideal tool to dissect this enhancer-gene relationship to provide us with empirical evidence that the intronic enhancer within *ANKRD55* regulates the expression of *IL6ST*. Understanding how RA risk genetic background affects gene regulation will give us a better understanding of the underlying biology of the disease and can ultimately be used to help repurpose or discover novel drug therapies.

Objectives: To use CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) to perturb the *ANKRD55* intronic enhancer and measure the downstream effect on the expression of *IL31RA*, *IL6ST*, *ANKRD55*.

Methods: We used dead (dCas9) a modified CRISPR Cas9 enzyme that is catalytically inactive, that precisely targets DNA, but does not cut. Instead, effector molecules are fused to the dCas9 (VP64 for activation and KRAB for repression) to alter the transcription of the targeted genes. Two lead functional risk SNPs at the 5q11 risk locus rs10065637 and rs7731626 ($R^2 = 0.4472$) lie in two distinct enhancer elements separated by 5.5kb. We designed 4 guides across each enhancer, and transduced a T cell line (Jurkat) using lentiviral delivery; first dCas9-KRAB and dCas9-VP64 and subsequently the pool of guides for each enhancer alongside controls. We cultured the cells until confluent and then sorted the top 60% expression of BFP-GFP (KRAB) or MCherry-GFP (VP64). RNA was extracted and then a qPCR was performed (Quantstudio) for *IL31RA*, *IL6ST* and *ANKRD55*.

Results: In the CRISPRa Jurkat cells the guides targeted to both SNP containing enhancers significantly increased the expression of *ANKRD55*: rs7731626 ($p < 0.0001$) and rs6859219 ($p = 0.0003$) respectively. They also increased the expression of *IL6ST* rs7731626 ($p = 0.0023$) and rs6859219 ($p = 0.01$). In CRISPRi Jurkat cells the guides targeted to both SNP containing enhancers significantly decreased the expression of *ANKRD55*: rs7731626 ($p < 0.001$) and rs6859219 ($p = 0.0071$). When guides were simultaneously used for both enhancers rs6859219+rs7731626 there was also a significant decrease in expression of *ANKRD55*. ($p < 0.0001$). Only the pool guides against both enhancers significantly decreased the expression of *IL6ST* ($p < 0.0001$). In both systems no significant effect was seen on the expression of *IL31RA* (Figure 1).

Figure 1