High resolution genetic mapping has identified a novel disease target for rheumatoid arthritis

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Nucleome THERAPEUTICS

Introduction

Genetic support increases the probability of a drug's success, with identification of the causal gene further improving success rates[1,2].

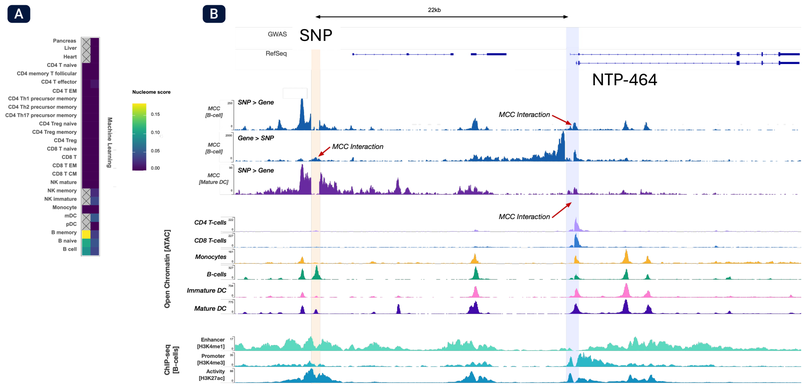
90% of autoimmune disease-related SNPs are in non-coding regions of the genome, making their functional validation and linkage to causal genes difficult[3].

Nucleome's Machine Learning (ML) algorithm has prioritized a SNP as being a strong loss-of-function variant that predisposes carriers to the development of autoimmunity, including rheumatoid arthritis.

The aim of this study was to functionally validate this target genetically and with a tool agonist antibody *in vitro* and *in vivo*.

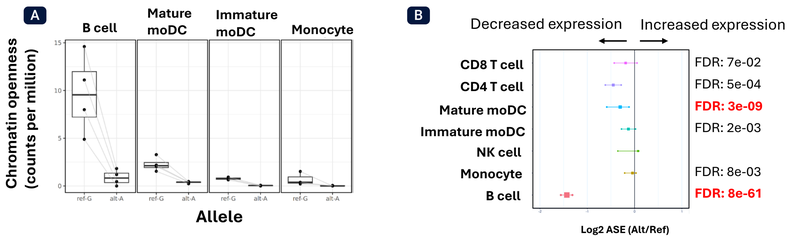
[1]Nelson, Net Genet, 2015; [2]Nelson, Net Genet, 2024; [3]Edwards Am J Hum Genet, 2013

1 NTP-464 was selected by Nucleome's machine-learning and 3D genome technology



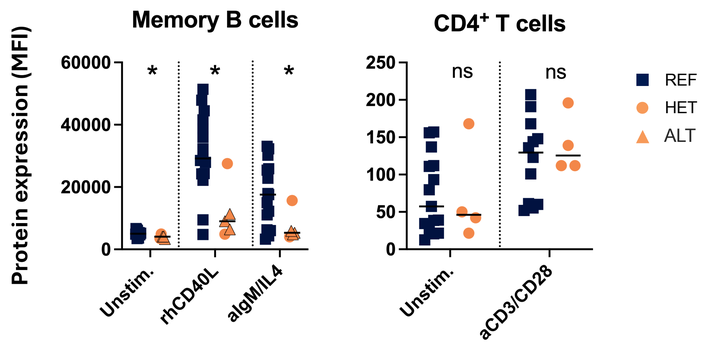
(A) Nucleome's ML algorithm predicted this RA-associated SNP would have a profound effect on chromatin in B cells. (B) Multi-omics analyses including 3D genome, ATAC-seq and ChIP-seq, demonstrated the disease-associated allele of this variant destroys a B cell-specific enhancer-promoter link.

2 NTP-464 variant closes chromatin and decreases RNA expression in B cells



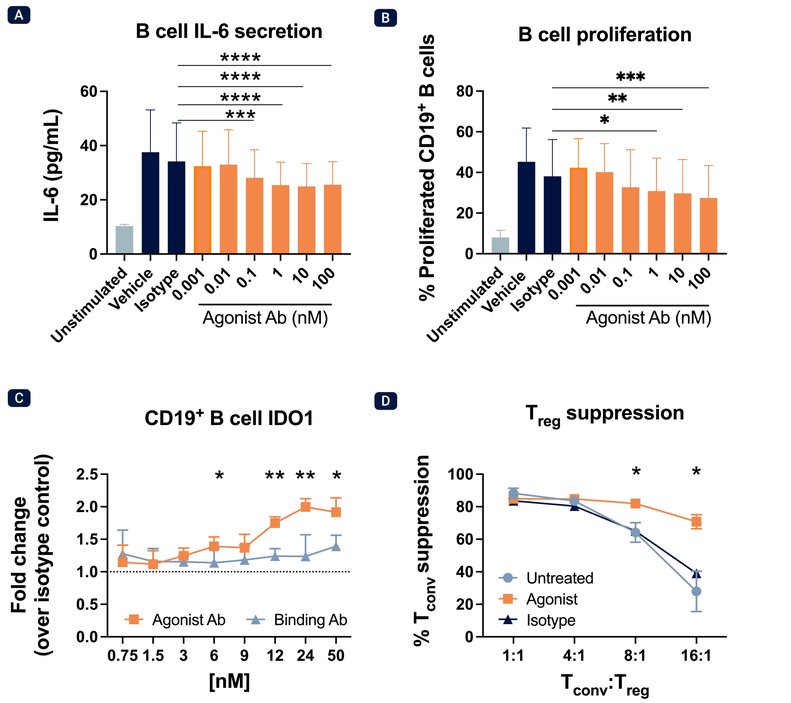
Allelic skewing of the NTP-464 gene was observed in B cells from heterozygous donors as measured by (A) ATAC-seq and (B) RNA-seq.

3 NTP-464 variant impacts protein expression in B cells not T cells



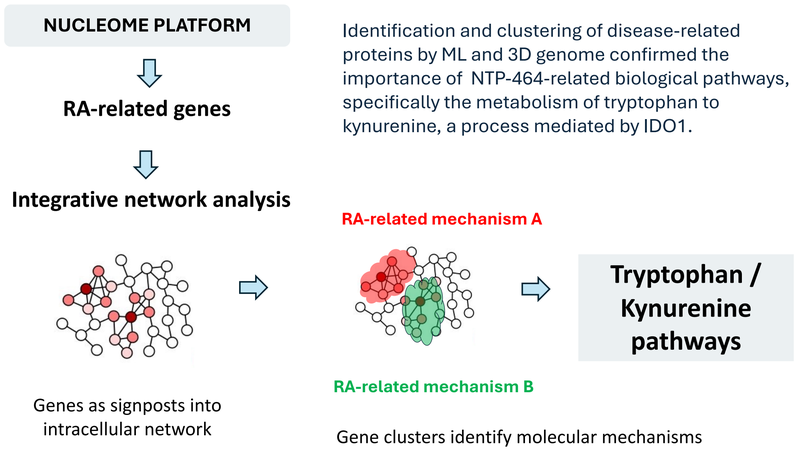
NTP-464 protein expression was measured by flow cytometry in PBMCs with cell-specific stimuli in n=15 donors homozygous for the reference allele and n=5 donors bearing the disease-associated allele. Statistical significance measured by Mann Whitney test. * = p<0.05, ns = non-significant.

4 NTP-464 agonism promotes anti-inflammatory activities *in vitro*



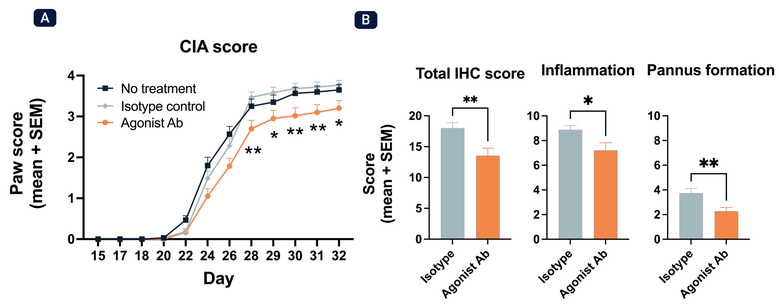
Effect of antibody on (A) IL-6 release following 24h treatment, (B) proliferation at day 5, (C) intracellular IDO1 measured by flow cytometry after 48h and (D) Treg suppression of autologous conventional T cells proliferation after 5 days. Statistical significance by 2way ANOVA or one sample t-test of null hypothesis. * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001.

5 Moving beyond single loci: mechanism identification converges with NTP-464 related biology



Identification and clustering of disease-related proteins by ML and 3D genome confirmed the importance of NTP-464-related biological pathways, specifically the metabolism of tryptophan to kynurenine, a process mediated by IDO1.

6 NTP-464 agonism reduces disease severity in a murine model of collagen-induced arthritis



NTP-464 agonism reduces collagen-induced arthritis severity by (A) clinical score and (B) immunohistochemistry compared to isotype controls. Statistical significance by 2way ANOVA or unpaired t-test, * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001.

Conclusions

Nucleome's technology mapped a disease-associated SNP in the Dark Genome to its target gene and correctly proposed a therapeutic agonist modality.

The disease-associated allele of this variant destroys an enhancer to promoter link, affecting protein expression.

Agonism of the target had anti-inflammatory effects in vitro and in vivo.

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