Case Study

Insights on Differential Gene Expression and Treatment Response in Rheumatoid Arthritis

Problem: Most patients do not respond to TNFi therapy

Tumor necrosis factor inhibitors (TNFi) are prescribed to suppress inflammation in a variety of autoimmune diseases. For rheumatoid arthritis (RA), TNFi is the first biologic prescribed to 90% of patients; however, 58-73% do not respond in a clinically meaningful way to this expensive first line of defense[1].

Solution: Predicting response by IFN expression profiling

In 2015, researchers at the University of Liverpool and Institute of Ageing and Chronic Disease used RNA sequencing to stratify RA patients according to their interferon (IFN) expression patterns. Both disease severity and TNFi treatment response were seen to correlate with IFN expression clustering[2].

Patients that showed higher expression levels of IFN response genes prior to treatment responded better to TNFi therapy.

Wright *et al.* isolated total RNA from neutrophils, enriched for mRNA, and sequenced on an Illumina HiSeq 2000 instrument. Data analysis software was used to identify genes that were differentially expressed in RA patients versus healthy controls and to predict which signaling pathways were likely to be driving the differential expression. Genes belonging to the IFN signaling pathway differed significantly between RA patients and healthy controls, likely influenced by their upstream regulators in RA patients.

A clustering analysis of 178 IFN genes that were differentially regulated in RA versus controls revealed that RA patients sub-cluster into two distinct populations largely defined by 59 IFN genes.

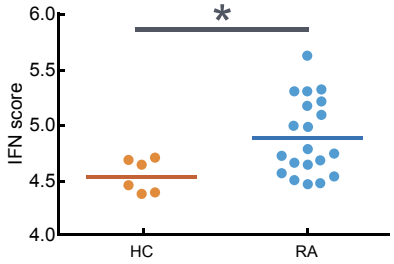

***Figure 1**. The IFN score for patients with rheumatoid arthritis (RA) vs. healthy controls (HC) as determined by Wright* et al[2]*. *p-value < 0.05.*

Disease severity was measured by the metric DAS28 (a disease activity score derived from examining 28 joints). High IFN scores prior to treatment correlated with an improved change in DAS28 12 weeks after treatment with TNFi. Statistical analyses revealed that IFN score could actually be used to predict whether patients would have good, moderate, or no response to TNFi with an area under the curve (AUC) of 0.76.

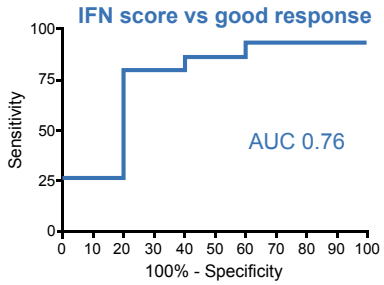

***Figure 2**. ROC curve relating IFN score to predictions of good response to TNFi therapy based on the European League Against Rheumatism's "good response" criteria as demonstrated by Wright* et al[2].

Context: This work adds to a growing body of evidence for the power of IFN stratification

Expression of a smaller panel of IFN response genes had been previously shown to increase for some patients following treatment with the TNFi infliximab[3]. Patients with an induced IFN response had a poor clinical outcome. Thus, IFN signatures can potentially help predict patient outcomes prior to treatment and monitor patient response following treatment.

TNFi is often the first biologic prescribed for patients that do not respond to immunosuppressant small molecule drugs like methotrexate. Patients who do not respond to TNFi are often moved on to another biologic in a costly trial and error process.

Increased IFN activation was previously associated with a subset of patients for four additional rheumatic diseases[6]—systemic lupus erythematosus (SLE), dermatomyositis (DM), polymyositis (PM), and systemic scleroderma (SSc)—suggesting that these conditions may also benefit from profiling of IFN-responsive RNA signatures.

Research opportunities for applying the HTG EdgeSeq Immune Response Panel

The vast majority of the genes used by Wright *et al.* to calculate IFN score are included in the HTG EdgeSeq Immune Response Panel (*listed below*). Using the semi-automated HTG EdgeSeq Immune Response Panel and associated HTG EdgeSeq Reveal software can thus accelerate expression stratification studies, providing an easy-to-use platform to enhance your ability to gain insight into interferon-based treatment responses in autoimmune diseases.

***Table 1**. Genes identified by Wright* et al. *to be associated with differential stratification in rheumatoid arthritis that are included in the HTG EdgeSeq Immune Response Panel.*

ADAR	CD274	HBD	IFIT1	IRF2	MMP9	TICAM1	TIMP1
AHR	CD36	HERC5	IFIT2	IRF3	MX1	RARRES3	TLR5
AIM2	CDC37	HERC6	IFIT3	IRF4	MX2	RNASEL	TLR9
ALOX15	CEBPA	HLA-A	IFITM1	IRF5	MYC	RSAD2	TNFAIP6
ALOX5AP	CISH	HLA-C	IFITM2	IRF7	MYD88	S100A9	TRIM22
ARG1	CMPK2	HLA-DQB1	IFITM3	IRF9	NCR1	SERPINA1	TYK2
BCL2L1	CTSD	HLA-DRA	IFNAR1	IRS2	NOD2	SERPING1	UBE2L6
BCL3	EGR1	HLA-E	IFNAR2	ISG15	OAS1	SOCS1	USP18
BST2	FADD	HLA-F	IL12RB1	ISG20	OAS2	SOCS3	XAF1
CASP1	FBP1	HSP90AB1	IL1RL1	ITGA4	OAS3	SP100	ZBP1
CASP5	FCER1A	HSPB1	IL1RN	JAK1	OASL	SPI1	ZFP36
CCL3L1	FCER1G	IFI16	IL27	JAK3	PF4	SREBF1	
CCL3L3	FCGR1A	IFI30	IL32	KLF4	PRF1	STAT1	
CCL5	FCGR1B	IFI35	IL4R	LCN2	PSMB8	STAT2	
CCND3	FKBP5	IFI44	IL8	LGALS3BP	PTPN1	TAP1	
CCR2	GBP1	IFI44L	IP6K2	LTB4R	PTPN11	TBX21	
CCR3	GBP4	IFI6	IRAK2	LY6E	PTPN6	TGFB1	
CD14	GBP5	IFIH1	IRF1	MAPK14	RAC2	THBS1	

References

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