

TNF-mediated gene expression in HUVEC

Abstract

Gene expression in TNF treated Human Umbilical Vein Endothelial Cells (HUVEC) was compared to that in untreated HUVEC using the Affymetrix® GeneChip® Human Genome U133A array. Using filtering criteria of a 1.5 or greater fold-change in expression and a false discovery rate of less than 5%, 624 out of over 19,000 transcripts examined were differentially expressed. The expression of 335 transcripts was increased by TNF treatment, while 289 showed decreased expression. The Gene Ontology and KEGG pathway terms associated with these genes were examined to identify biological themes associated with each set of genes. These analyses identified distinct biological themes associated with each group of genes. The ontology term analysis showed that TNF treatment regulates the expression of genes involved in the immune response, cell cycle, apoptosis and cell adhesion in HUVEC. The analysis of KEGG pathway terms identified a significant over representation of genes involved in the Jak-Stat signaling pathway and cytokine-cytokine receptor interactions among the up-regulated genes and an over representation of cell cycle genes among the down-regulated genes.

Introduction

In this study the GeneSifter® microarray data analysis system was used to identify TNF-mediated changes in gene expression in HUVEC (Human Umbilical Vein Endothelial Cells). The analysis process used can be broken down into two discrete tasks: identification of differentially expressed genes and the determination of the biological significance of both individual genes and groups of genes. GeneSifter uses Gene Ontology (GO) Reports and z-scores to summarize the biological processes associated with a gene list. Z-scores can then be used to identify GO terms that are significantly over- or under-represented in a gene list. This study outlines the use of these methods to identify biological themes associated with genes regulated by TNF in HUVEC.

The Data

Gene expression profiles were measured in HUVEC stimulated for 5 hours with TNF and in untreated HUVEC using the Affymetrix® GeneChip® Human Genome U133A array. Four biological replicates were prepared for each condition. The CEL files for all samples were downloaded from GEO (GSE2639) and loaded into GeneSifter. Expression measurements were derived using GC-RMA.

Identification of differentially expressed genes

The Affymetrix® GeneChip® Human Genome U133A array contains probe sets representing over 20,000 transcripts. Using filtering criteria of a 1.5 or greater fold-change in expression, and a p-value of < 0.05 from a unpaired, two sample t-test produced a list of 764 genes that were differentially expressed. Correction for multiple testing was then performed using the method of Benjamini and Hochberg (Reiner, et al., 2003) to derive a false discovery rate estimate from the raw p-values. A false discovery rate of 5% was used as a cutoff for statistical significance giving a final list of 624 differentially expressed genes. 335 of these genes showed increased expression following TNF stimulation, while 289 showed decreased expression. The parameters used for this filtering are summarized in figure 1.

Biological significance

The biological process ontologies and KEGG pathway terms associated with the differentially expressed genes were examined using a z-score report. The z-score report identifies ontologies or pathway terms that are significantly over-represented in a gene list (Doniger, et al., 2003). The most significantly enriched biological process ontology identified for the up-regulated genes was 'immune response'

A

Signal	GC-RMA
Fold-change	1.5
Statistics	t-test
Correction	Benjamini and Hochberg
Confidence	5% False Discovery Rate

B

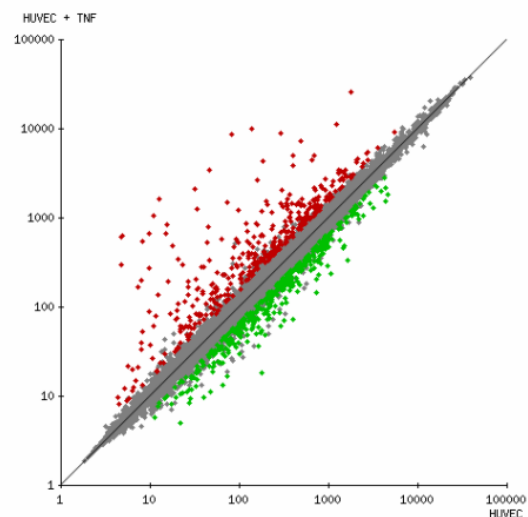


Figure 1: A) Parameters used to identify differentially expressed genes using GeneSifter. B) Scatterplot showing all genes. Red and green points indicate significantly up-regulated and down-regulated genes, respectively.

A

Ontology	List	Up	Down	Array	z-up	z-down
immune response	60	56	4	644	11.5	-2.45
chemotaxis	17	17	0	102	9.92	-1.41
inflammatory response	22	21	1	172	8.93	-1.27
negative regulation of apoptosis	14	13	1	97	7.48	-0.62
antigen processing, endogenous antigen via MHC class I	4	4	0	12	7.29	-0.48
positive regulation of I-kappaB kinase/NF-kappaB cascade	11	10	1	69	6.91	-0.27
cytoplasmic sequestering of transcription factor	4	3	1	8	6.75	2.21
signal transduction	108	75	33	1990	5.23	-0.85
regulation of cell proliferation	22	16	6	240	4.71	0.7
positive regulation of cell proliferation	11	8	3	107	3.69	0.7
protein kinase cascade	15	9	6	146	3.24	1.99
cell adhesion	25	19	6	466	2.76	-0.98
negative regulation of cell proliferation	10	7	3	126	2.54	0.41

B

Ontology	List	Up	Down	Array	z-up	z-down
DNA replication	12	0	12	122	-1.7	6.49
S phase of mitotic cell cycle	12	0	12	124	-1.7	6.41
cell cycle	41	13	28	609	-0.2	5.08
DNA dependent DNA replication	6	0	6	61	-1.2	4.57
cytokinesis	6	0	6	92	-1.5	3.28
DNA repair	8	1	7	164	-1.4	2.26

C

KEGG Pathway	List	Up	Down	Array	z-up	z-down
Cytokine-cytokine receptor interaction	33	32	1	233	10.1	-1.68
Apoptosis	16	13	3	91	6.46	1.04
Jak-STAT signaling pathway	15	13	2	142	4.45	-0.4
Cell cycle	12	1	11	93	-1.1	7.27
Toll-like receptor signaling pathway	11	11	0	94	5.07	-1.35

Table 1: Z-score reports identified distinct biological themes associated with genes regulated by TNF in HUVEC. A) Selected biological process ontologies for up-regulated genes. B) Selected biological process ontologies for down-regulated genes. C) KEGG pathway terms.

with a z-score of 11.5. Other more specific terms associated with the immune response such as “inflammatory response”, “antigen processing” and “chemotaxis” were also identified. A group of ontologies associated with the cell cycle were over-represented among the down-regulated genes. See

table 1 for a list of selected ontology terms identified by the z-score report. Z-score analysis of the KEGG pathway terms found that genes involved in cytokine-cytokine receptor interactions, the Jak-Stat signaling pathway, the cell cycle, apoptosis and the toll-like receptor signaling pathway were regulated by TNF stimulation (Table 1 and figure 2). See supplemental material to view a comprehensive list of ontologies and pathway terms identified as being regulated by TNF in HUVEC.

Summary

624 differentially expressed genes were identified as being regulated by TNF in this analysis. Analysis of the Gene Ontology and KEGG pathway terms associated with these genes showed enrichment of gene families associated with immune response, signal transduction, apoptosis and cell adhesions among the 624 genes.

Supplemental Material

Raw data is available from the Gene Expression Omnibus (GSE2639).

Data can be viewed and analyzed using the GeneSifter Data Center (www.genesifter.net/web/DC).

References

Reiner, et al. 2003. Identifying differentially expressed genes using false discovery rate controlling procedures. *Bioinformatics* 19(3):368-375
 Doniger, et al. 2003. MAPPFinder: using Gene Ontology and GenMAPP to create a global gene-expression profile from microarray data. *Genome Biology* 4:

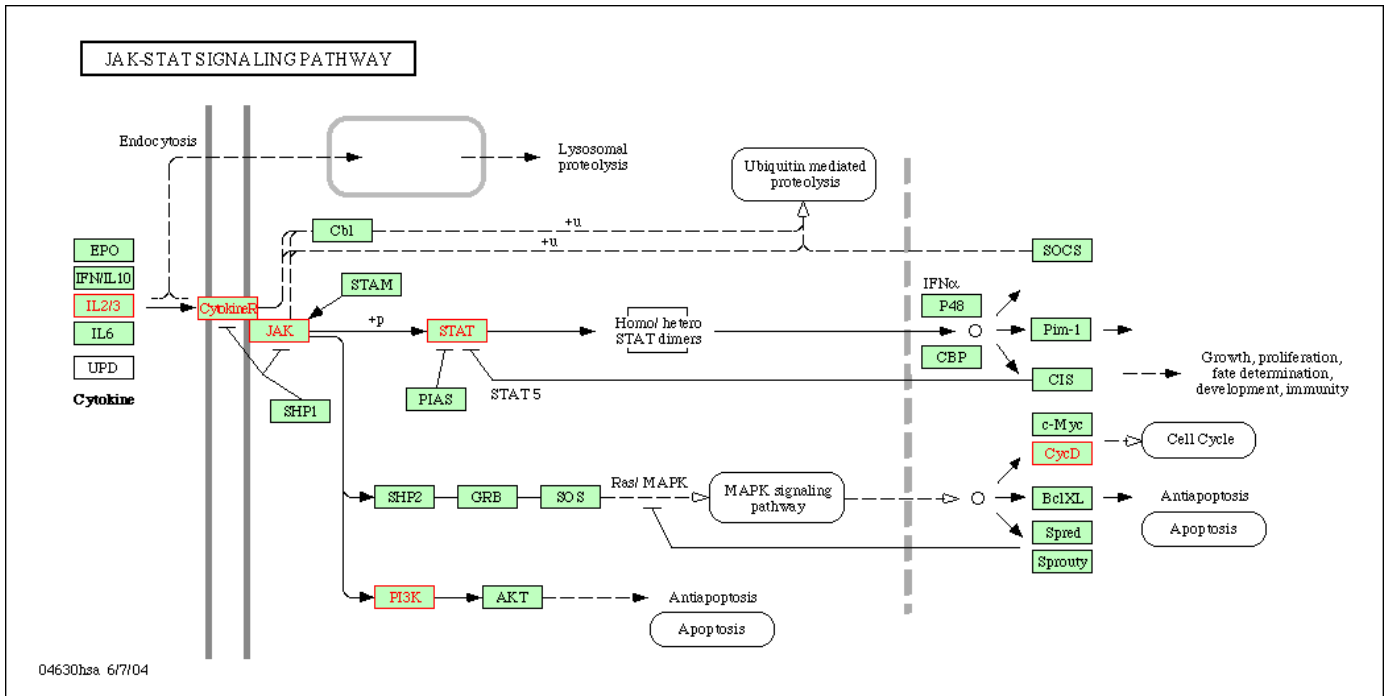


Figure 2: KEGG pathway diagram showing genes involved in the Jak-Stat Signaling Pathway regulated by TNF in HUVEC. The genes that were regulated are highlighted in red.