

Microarray analysis of CD40-mediated gene expression in Ramos cells

Abstract

Gene expression in Ramos cells stimulated with anti-CD40 was compared to that in untreated cells using the Affymetrix® GeneChip® Human Genome U95A array. Using filtering criteria of a 1.5 or greater fold-change in expression, all present calls in at least one of the groups and a false discovery rate of less than 5%, 598 out of over 10,000 transcripts examined were differentially expressed. The expression of 117 transcripts was increased by anti-CD40 treatment, while 481 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. The ontology term analysis showed that anti-CD40 treatment regulates the expression of genes involved in the immune response, transcription, cell cycle, apoptosis and cell-matrix adhesion in Ramos cells. The analysis of KEGG pathway terms identified a significant over representation of genes involved in the MAPK signaling pathway, apoptosis 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 CD40-mediated changes in gene expression in the Burkitt lymphoma cell line Ramos. 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 CD40 in Ramos cells.

The Data

Gene expression profiles were measured in Ramos cells stimulated for 24 hours with anti-CD40 and in untreated Ramos cells using the Affymetrix® GeneChip® Human Genome U95A array. Six biological replicates were prepared for each condition. Data from the CHP file for all samples were downloaded from GEO (GSE2350) and loaded into GeneSifter.

Identification of differentially expressed genes

The Affymetrix® GeneChip® Human Genome U95A array contains probe sets representing over 10,000 transcripts. Data for each sample was normalized to the median intensity for that sample prior to identification of differentially expressed genes. Using filtering criteria of a 1.5 or greater fold-change in expression, all present calls in at least one of the groups and a false discovery rate (Reiner, et al., 2003) of less than 5% from an unpaired, two sample t-test produced a list of 598 differentially expressed genes. 117 of these genes showed increased expression following CD40 stimulation of Ramos cells, while 481 showed decreased expression. The parameters used for this filtering are summarized in figure 1. See supplemental material to view the full list of differentially expressed genes.

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 included 'immune

A

Fold-change	1.5
Detection Filter	Present Only
Statistics	t-test
Correction	Benjamini and Hochberg
Confidence	5% False Discovery Rate

B

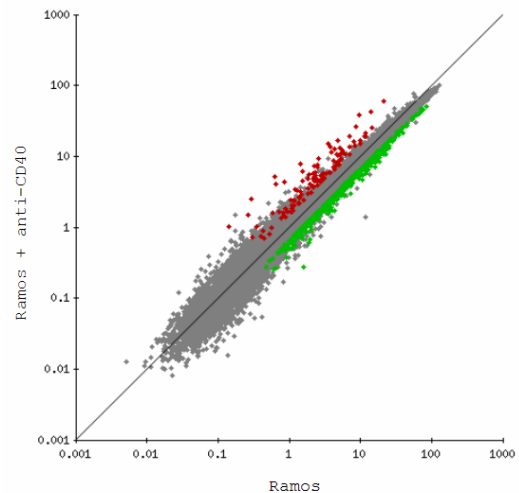


Figure 1: A) Parameters used to identify differentially expressed genes using GeneSifter. B) Scatterplot showing all genes. Red and green points indicate significantly up-regulated and down-regulated genes, respectively.

A

Ontology	List	Up	Down	Array	z-up	z-down
negative regulation of apoptosis	11	8	3	77	8.47	-0.34
positive regulation of I-kappaB kinase/NF-kappaB cascade	6	4	2	53	4.9	-0.32
immune response	33	15	18	536	4.49	-1.54
protein complex assembly	10	4	6	89	3.41	0.91
antimicrobial humoral response	7	3	4	71	2.81	0.37
protein amino acid dephosphorylation	10	3	7	87	2.37	1.48

B

Ontology	List	Up	Down	Array	z-up	z-down
RNA interference	2	0	2	2	-0.1	6.36
M phase of mitotic cell cycle	16	0	16	90	-1	5.89
regulation of transcription	93	15	78	1042	1.67	4.59
mitotic cell cycle	24	0	24	221	-1.5	4.38
ubiquitin-dependent protein catabolism	10	0	10	70	-0.8	3.8
steroid hormone receptor signaling pathway	3	0	3	13	-0.4	3.13
nuclear mRNA splicing, via spliceosome	12	1	11	109	-0.1	2.67
cell-matrix adhesion	6	0	6	47	-0.7	2.61
cell cycle checkpoint	4	0	4	26	-0.5	2.57

C

KEGG Pathway	List	Up	Down	Array	z-up	z-down
MAPK signaling pathway	18	8	10	197	3.46	0.63
Cytokine-cytokine receptor interaction	13	7	6	199	2.78	-0.88
Cell cycle	12	0	12	90	-1.1	4.39
Apoptosis	10	7	3	83	5.71	-0.28
Jak-STAT signaling pathway	9	6	3	127	3.4	-1.07
Pyrimidine metabolism	8	1	7	62	0.18	2.81
Toll-like receptor signaling pathway	7	5	2	85	3.7	-0.87

Table 1: Z-score reports identified distinct biological themes associated with genes regulated by CD40 in Ramos. A) Selected biological process ontologies for up-regulated genes. B) Selected biological process ontologies for down-regulated genes. C) KEGG pathway terms.

response' and 'negative regulation of apoptosis'. Among the down-regulated genes, terms associated with the cell cycle, cell-matrix adhesion and protein catabolism were over-represented. 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 the MAPK signaling pathway, cytokine-cytokine receptor interactions, the Jak-Stat signaling pathway, the cell cycle, apoptosis and the toll-like receptor signaling pathway were regulated by CD40 stimulation (table 1 and figure 2). See supplemental material to view a comprehensive list of ontologies and pathway terms identified as being regulated by CD40 in Ramos cells

Summary

598 genes were identified as being regulated by CD40 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, cell cycle, apoptosis and cell-matrix adhesion among the 598 genes.

Supplemental Material

Raw data is available from the Gene Expression Omnibus (GSE2350).

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:

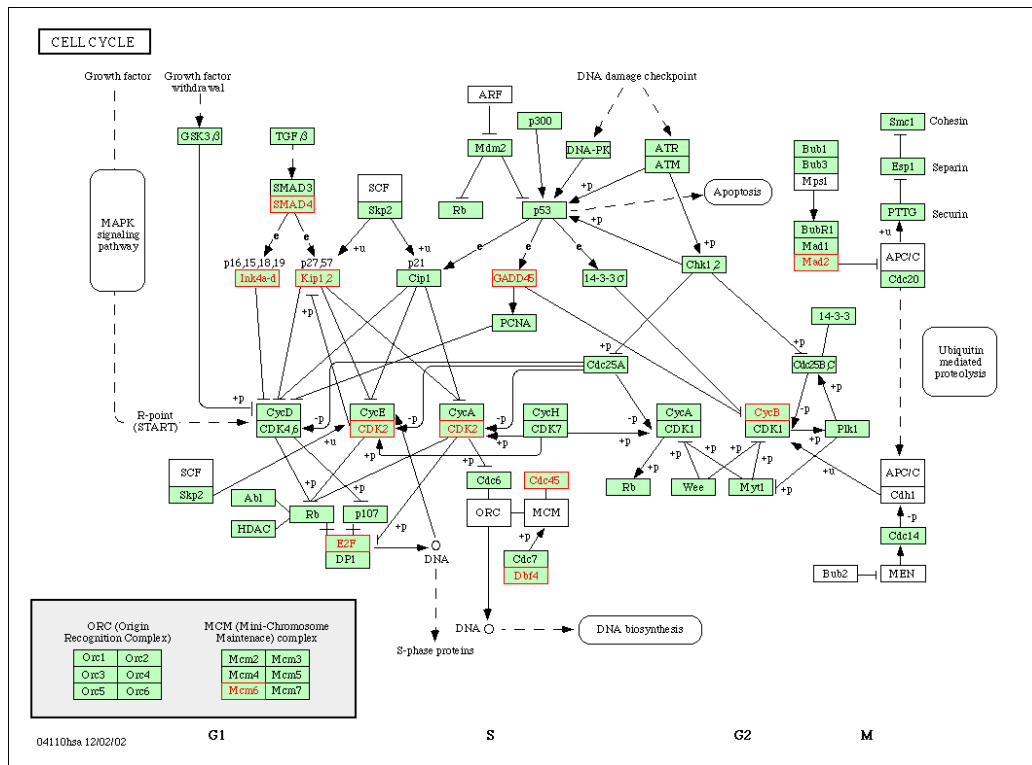


Figure 2: KEGG pathway diagram showing genes (highlighted in red) involved in the Cell cycle that are regulated by CD40 in Ramos cells..