Differentially Expressed Genes in Chronic Lymphocytic Leukaemia (CLL) Cells

A project to identify potential therapeutic targets to treat CLL

Introduction

- This project makes use of RNA-seq data in order to identify which genes are differentially expressed in human cells from patients with Chronic Lymphocytic Leukaemia, as opposed to non-cancerous cells.
- Chronic Lymphocytic Leukaemia (CLL) is a type of blood cancer in which the bone marrow begins to produce too many lymphocytes.
- While the exact cause is not fully understood, it is believed to occur due to certain genetic mutations that can happen over the course of one’s life. It is most prevalent in older adults.
- CLL is currently incurable. Precision-based treatments include CAR T cell therapy, BTK, PI3K and BCL2 inhibitors, among others.

Overview

- *What is RNA-seq analysis?*

RNA-seq analysis is the statistical analysis of data generated from RNA sequencing. It is used to detect the abundance of RNA transcripts in a biological sample.

- *Why is it used?*

RNA-seq has many purposes. This particular project will explore its usefulness in detecting genes with expression levels that are statistically significantly higher in one condition (e.g. a disease) as opposed to another (e.g. the absence of the disease).

- *What is limma?*

limma is an acronym for ‘Linear Models for Microarray and Omics Data’. It is an R package used to analyse differential expression for omics data, such as RNA-seq data.

- *How does it work?*

limma fits linear models to data to analyse experiments and check for statistically significant differential expression. It uses empirical Bayesian methods to produce stable results.

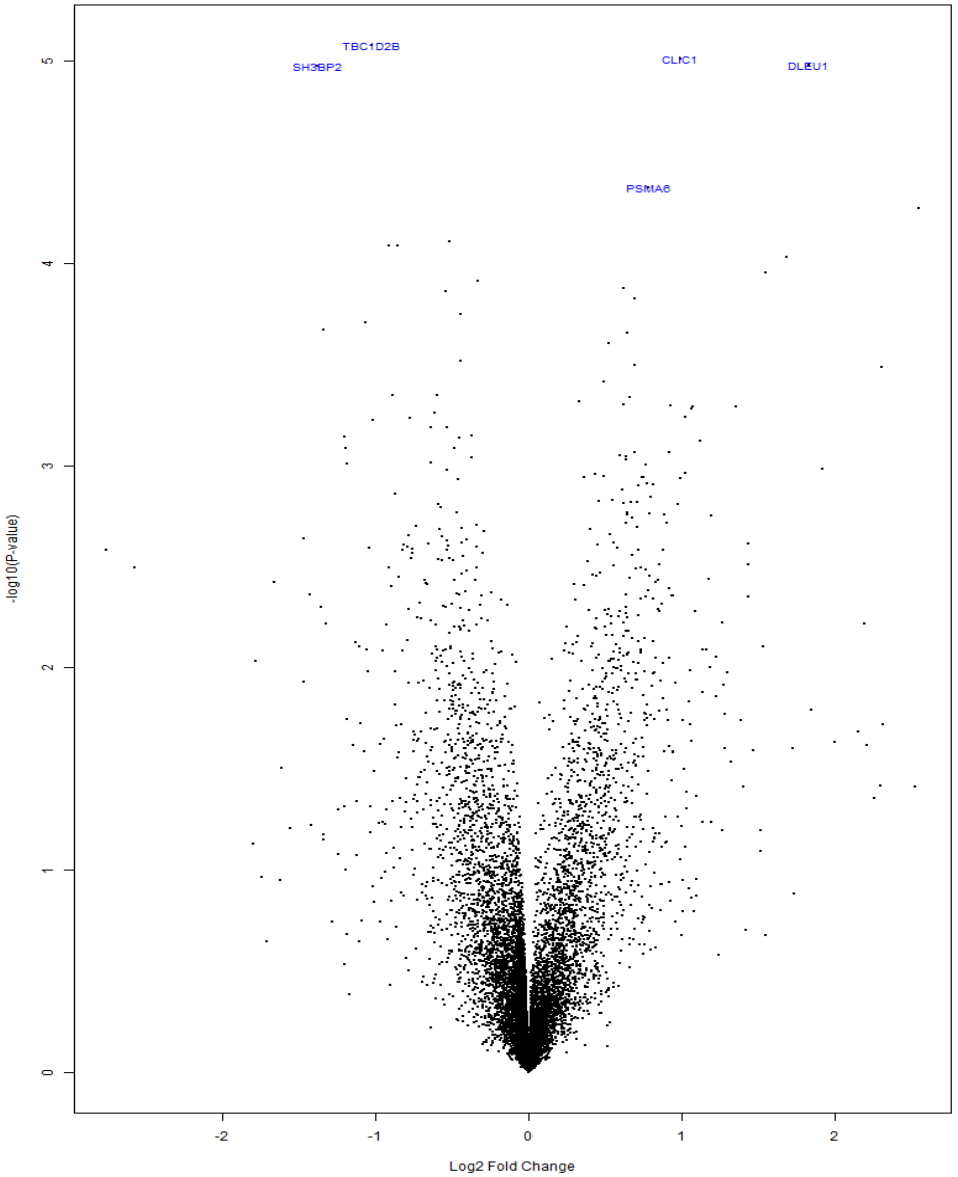
Data Availability and Methods

- The data was obtained from a study of patients with CLL and was made available through the ‘Differential Expression Analysis with *limma* in R’ course, offered on the DataCamp platform.

- The data was analysed in R with the `limma` package. Post analysis, the gene ontologies and KEGG pathways of the most significant genes were identified with the `GO.db` and `Biobase` packages.

Results

The following plot displays the top 5 most significantly enriched genes:



The following table shows the most significantly enriched KEGG pathways:

		Pathway	N	Up	Down	P.Up	P.Down
2	hsa04650	Natural killer cell mediated cytotoxicity	100	0	1	1	0.0220488237472998
3	hsa00780	Biotin metabolism	2	0	0	1	1
4	hsa01320	Sulfur cycle	2	0	0	1	1
5	hsa03271	Virion - Rotavirus	2	0	0	1	1
6	hsa00750	Vitamin B6 metabolism	2	0	0	1	1
7	hsa00524	Neomycin, kanamycin and gentamicin biosynthesis	3	0	0	1	1
8	hsa00440	Phosphonate and phosphinate metabolism	3	0	0	1	1
9	hsa00290	Valine, leucine and isoleucine biosynthesis	3	0	0	1	1
10	hsa00470	D-Amino acid metabolism	4	0	0	1	1
11	hsa00400	Phenylalanine, tyrosine and tryptophan biosynthesis	4	0	0	1	1
12	hsa04122	Sulfur relay system	4	0	0	1	1
13	hsa03267	Virion - Adenovirus	4	0	0	1	1
14	hsa03260	Virion - Human immunodeficiency virus	4	0	0	1	1
15	hsa03264	Virion - Flavivirus and Alphavirus	5	0	0	1	1
16	hsa00232	Caffeine metabolism	6	0	0	1	1
17	hsa00130	Ubiquinone and other terpenoid-quinone biosynthesis	6	0	0	1	1
18	hsa00740	Riboflavin metabolism	7	0	0	1	1
19	hsa00920	Sulfur metabolism	7	0	0	1	1
20	hsa00730	Thiamine metabolism	7	0	0	1	1
21	hsa03266	Virion - Herpesvirus	7	0	0	1	1

Discussion

- The results indicate that pathways involved in natural killer cell mediated cytotoxicity are significantly downregulated.
- Moreover, querying the Entrez IDs of the top 5 most significantly enriched genes using the NCBI Gene database revealed that these genes may all be implicated in natural killer cell functioning and the progression of CLL or other cancer types.
- SH3BP2 encodes a protein that positively regulates transcriptional activity in natural killer cells, while DLEU1 is thought to be a tumour-suppressor gene and is often deleted in CLL patients.
- CLIC1, encoding a chloride intracellular channel, is essential to maintain cell membrane potential and volume, among other things. It should be noted that this protein is ubiquitous in the bone marrow, a vital site for the maturation and development of natural killer cells. Therefore, disruptions in the activity of this gene could affect natural killer cell production, with downstream effects on NK cell mediated cytotoxicity.
- Studies have implicated PSMA6 and TBC1D2B in certain lung cancers. Therefore, it is probable that these genes may also play a role in the progression of CLL.

Conclusions and Caveats

- The results of the analysis, in combination with other research findings, appear to provide evidence that the five described genes may play a direct or indirect role in the progression of Chronic Lymphocytic Leukaemia.
- This analysis appears to suggest that these genes may be explored as therapeutic targets to develop new precision-based treatments for this disease.
- However, further research and experimentation is needed in order to attain conclusive evidence of the therapeutic potential of these genes.

Acknowledgements

- The RNA-seq analysis with the `limma` library was built off of the code provided as part of the course ‘Differential Expression Analysis with *limma* in R’.

- Further information was obtained from the official documentation for the package, available on the Bioconductor website.

References

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- National Cancer Institute

- Cancer Research UK

- NCBI Database

- The Bioconductor Project