

RNA expression differences in mantle cell lymphoma cells with and without ABT-199 resistance

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Zhao et al original paper - Aims

Main aim was to look at the development of ATB-199 (BCL-2 targeting drug) resistance in mantle cell lymphoma cells.

They used HBL-2 cell line and produced DTPs by exposing to ABT-199 and cloning cells that managed to survive.

These cells are the parent and derived cells that we used in our RNA-seq analysis

Zhao et al
original
paper -
Background


Drug resistance limits the effectiveness of medications

Increasing evidence –
Epigenetic and transcriptional reprogramming.

DTP and DETP facilitate drug resistance.




BCL-2

- Part of the BCL2 protein family
 - Transcribed by the BCL2 gene – 18q21
 - Major role – apoptic inhibitor
 - Combinatorial dysregulation, e.g. MYC – development of lymphomas
 - For example – Mantle Cell lymphoma, MCL
- 



ABT-199

- A selective inhibitor of the BCL2 protein
 - Considered a great improvement on its predecessors
 - Promising in the treatment for MCL
 - However – many patients develop resistance
 - The mechanism of resistance underexplored.
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Significance

Drug resistance is major problem

Reduce drug resistance which could lead to more successful cancer treatments.

Develop new biomarkers and treatments

What they did :

Modelled response to MCL-derived lines (HBL-2)

Cultured three independent ABT-199 resistant lines

Performed RNA-seq analysis

Parental – amplification of chromosome 18q21

Derived – Deletions.

High Throughput data analysis project



- Conducted group data analysis project using high-throughput data analysis
- Attempted to define the differences between transcriptomes of the parental (HBL-2) lines and the derived resistant line.

Aims

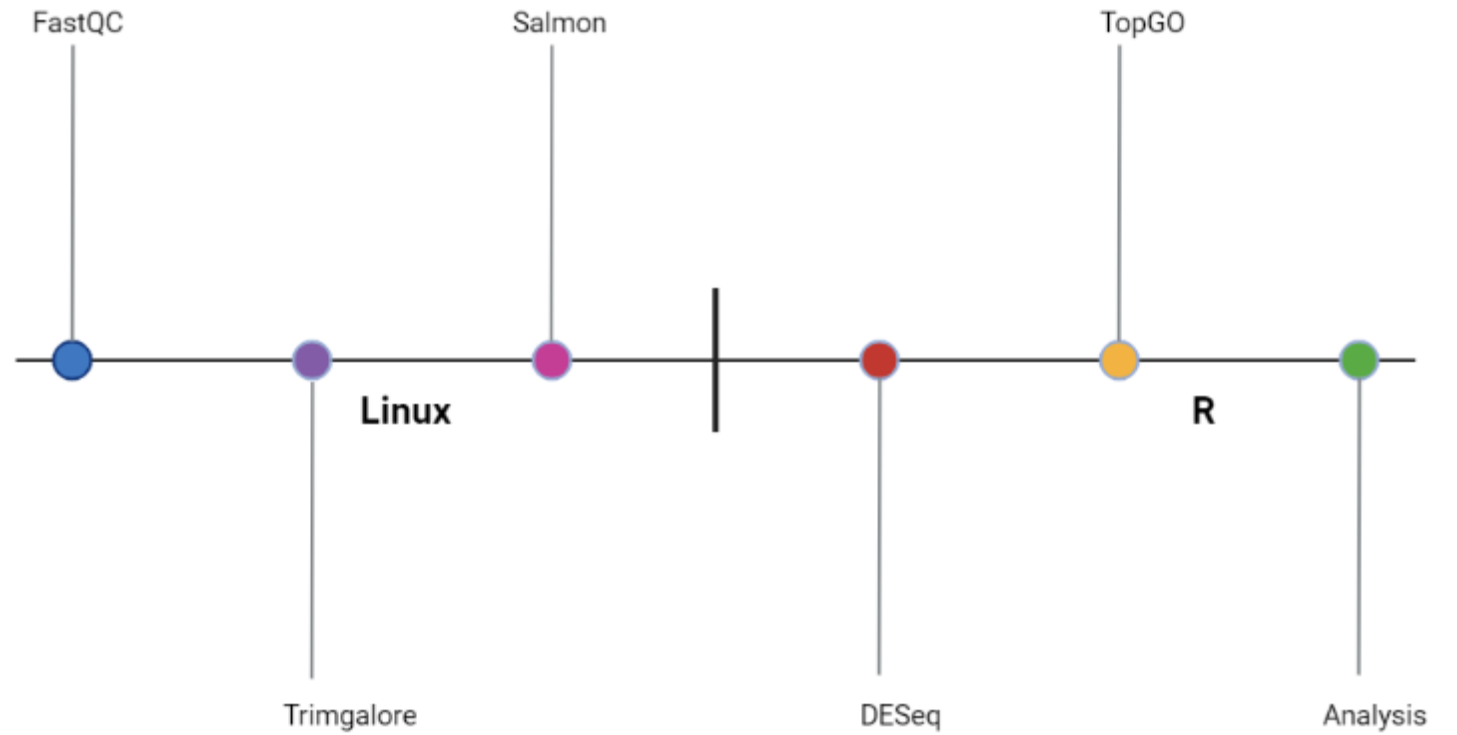


PREPARE AND CHECK THE
QUALITY OF THE RNA SEQ
DATA



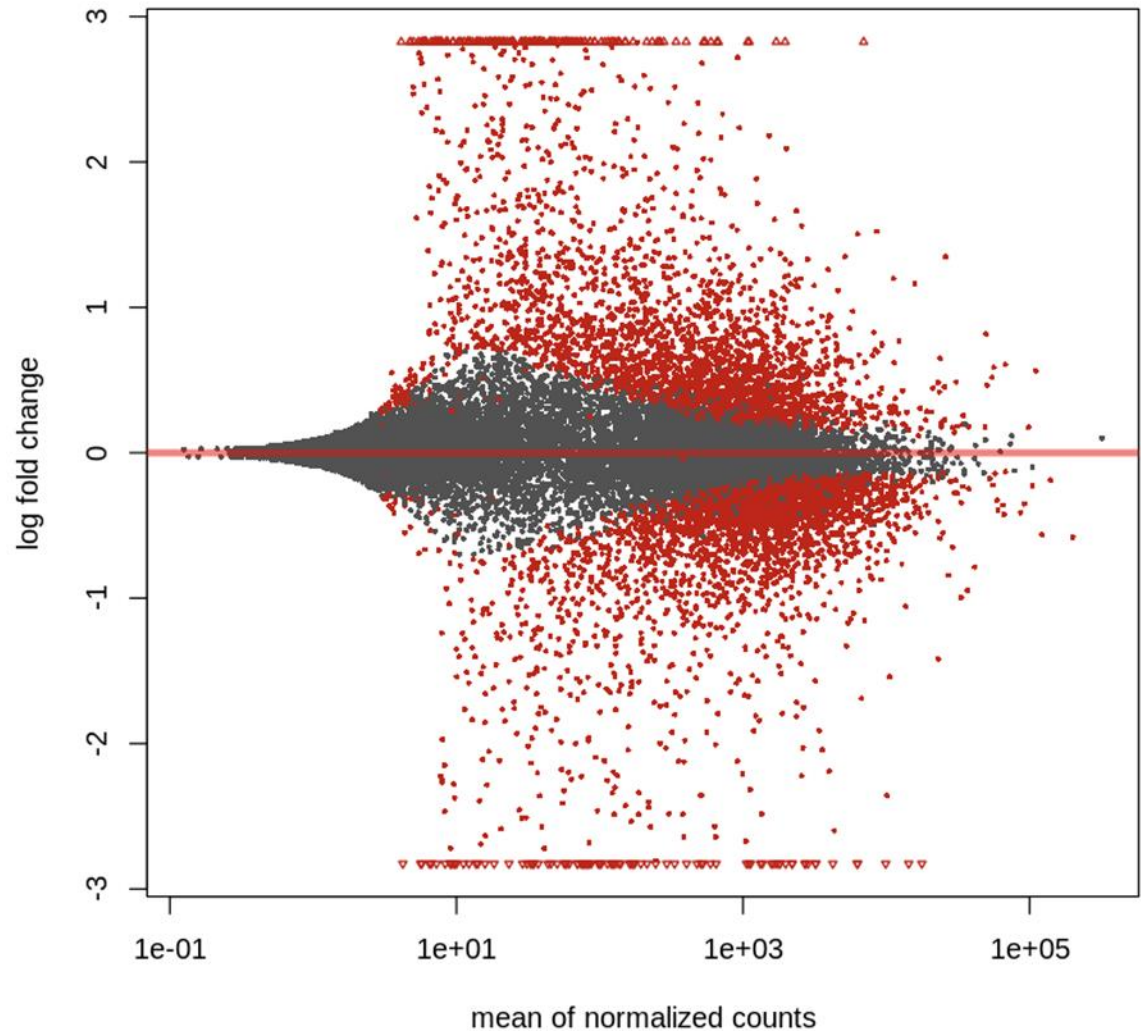
COMPARE EXPRESSION OF
PARENTAL VS DERIVED
LINES

Methods



Results DESeq

- What is it a measure of
- Can see a significant difference in the expression
 - If there was no difference the points would centre more towards the zero line
- Overexpressed in derived above zero, overexpressed in parent (under expressed in derived).
- Red points represent p-values < 0.1 – significant



Top 10 differentially expressed genes

- Top 10 genes according the p value
- Many of these genes are centred around the 18q21 location - BCL2 is included within these genes
 - In agreement with Zhao et al.

Ensembl ID	Gene Name	POS	log2FoldChange	Adjusted P-value
ENSG00000074695	LMAN1	18q21.32	-11.24423	0
ENSG00000091164	TXNL1	18q21.31	-3.579859	0
ENSG00000119541	VPS4B	18q21.33	-6.589036	0
ENSG00000134046	MBD2	18q21.2	-3.928278	0
ENSG00000163683	SMIM14	4p14	4.07336	0
ENSG00000171791	BCL2	18q21.33	-2.358777	0
ENSG00000196735	HLA-DQA1	6p21.32	-3.325505	8.03E-256
ENSG00000211685	IGLC7	22q11.22	8.06943	9.16E-252
ENSG00000103522	IL21R	16p12.1	-2.937142	9.04E-246
ENSG00000026508	CD44	11p13	-2.603008	1.76E-229

LMAN1: membrane mannose
specific lectin

TXNL1: thioredoxin-like protein
– previously identified in cancer

VPS4B: Vacuolar protein sorting
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MBD2: Methyl-CpG binding
domain protein 2

Genes on
18q21

Significant genes in other chromosome locations

- SMIM14
- HLA-DQA1
- IL21R
- CD44



Ensembl numbers for genes on the paper

Gene name	Ensembl	Chromosome location	P-value	Log2fold change
PMAIP1 (NOXA)	ENSG00000141682	18q21.32	1.68E-142	-10.92254
TCF4	ENSG00000196628	18q21.2	1.18E-79	-3.263973
FOXC1	ENSG00000054598	6p25.32	2.75E-43	2.43224
IRF5	ENSG00000128604	7q32.1	2.17E-74	1.67013
IKZF1	ENSG00000185811	7p12.2	0.904251804	0.17465
BCL2	ENSG00000171791	18q21.33	0	-2.358777

TopGO testing

Are the most significant genes enriched for any particular GO term annotations?

Classic Fisher Test	Kolomogorov-Smirnov
Compares expected number of significant genes at random to the observed number of significant genes – arrives at a probability	Compares the distribution of gene p-values expected at random to the observed distribution of the gene p-values to arrive at a probability

Top 10 GO Terms-rank by elimKS

GO.ID	Term	Annotated	Significant	Expected	Rank in classicFisher	classicFisher	classicKS	elimKS
GO:0002479	antigen processing and presentation of e...	68	36	19.01	140	1.20E-05	4.00E-07	4.00E-07
GO:0060333	interferon-gamma-mediated signaling path...	77	41	21.53	120	2.40E-06	7.70E-07	7.70E-07
GO:0034975	protein folding in endoplasmic reticulum	11	10	3.08	155	2.40E-05	4.60E-06	4.60E-06
GO:0043312	neutrophil degranulation	386	151	107.92	102	9.60E-07	1.30E-05	1.30E-05
GO:0002250	adaptive immune response	343	148	95.9	36	7.10E-10	2.40E-11	1.80E-05
GO:0050852	T cell receptor signaling pathway	167	70	46.69	191	6.70E-05	7.20E-06	3.00E-05
GO:0035116	embryonic hindlimb morphogenesis	14	10	3.91	326	9.00E-04	3.40E-05	3.40E-05
GO:0050900	leukocyte migration	295	121	82.48	98	7.10E-07	1.70E-07	4.50E-05
GO:0019221	cytokine-mediated signaling pathway	592	237	165.52	26	5.40E-11	2.70E-10	5.90E-05
GO:0007568	aging	238	98	66.54	132	6.50E-06	7.80E-05	7.80E-05

Top 10 GO terms-rank by-Classic fisher

GO.ID	Term	Annotated	Significant	Expected	classicFisher	classicKs	elimKS
GO:0002376	immune system process	2235	817	624.88	4.40E-23	2.50E-20	0.25091
GO:0006955	immune response	1550	595	433.36	8.10E-22	2.00E-20	0.0381
GO:0045321	leukocyte activation	988	393	276.23	3.60E-17	3.00E-16	0.50554
GO:0002252	immune effector process	938	375	262.25	8.30E-17	4.40E-16	0.28316
GO:0001775	cell activation	1080	419	301.96	4.00E-16	7.20E-15	0.16099
GO:0006952	defense response	1175	448	328.52	1.10E-15	9.70E-14	0.5205
GO:0070887	cellular response to chemical stimulus	2310	795	645.85	2.00E-14	1.30E-12	0.00069
GO:0045087	innate immune response	705	288	197.11	2.00E-14	2.20E-12	0.00088
GO:0002682	regulation of immune system process	1207	452	337.46	2.60E-14	7.60E-14	0.30868
GO:0006950	response to stress	3026	1005	846.04	9.50E-14	3.40E-11	0.14977

Top 10 go terms ranked by KS test

GO.ID	Term	Annotated	Significant	Expected	Rank in classicFisher	classicFisher	classicKs	elimKS
GO:0006955	immune response	1550	595	433.36	2	8.10E-22	2.00E-20	0.0381
GO:0002376	immune system process	2235	817	624.88	1	4.40E-23	2.50E-20	0.25091
GO:0045321	leukocyte activation	988	393	276.23	3	3.60E-17	3.00E-16	0.50554
GO:0002252	immune effector process	938	375	262.25	4	8.30E-17	4.40E-16	0.28316
GO:0001775	cell activation	1080	419	301.96	5	4.00E-16	7.20E-15	0.16099
GO:0002682	regulation of immune system process	1207	452	337.46	9	2.60E-14	7.60E-14	0.30868
GO:0006952	defense response	1175	448	328.52	6	1.10E-15	9.70E-14	0.5205
GO:0002684	positive regulation of immune system pro...	847	330	236.81	14	4.40E-13	1.90E-13	0.1778
GO:0050776	regulation of immune response	823	323	230.1	12	2.70E-13	9.50E-13	0.00351
GO:0070887	cellular response to chemical stimulus	2310	795	645.85	7	2.00E-14	1.30E-12	0.00069

Biological Implications

- Used Fisher test results
 - Top ranking – “Immune system response” and “immune response”
- Shows that the most significant genes cause a dysregulation to the body’s response to disease
 - <http://amigo.geneontology.org/> - searching by GO terms to find associations to BCL2



Conclusions

- Our results show good agreement with ZHAO et al.
 - Detect the loss of the 18q21 Amplicon
 - Proven lack of expression at this point from analysis of DESeq result
- TopGO analysis shows significant differential expression in relevant biological processes.
- There is a clear difference between expression in Parent vs Derived