RNA-SEQ OF FV-INDUCED ERYTHROLEUKEMIA CELLS SUGGESTS A ROLE OF WASP IN APOPTOSIS

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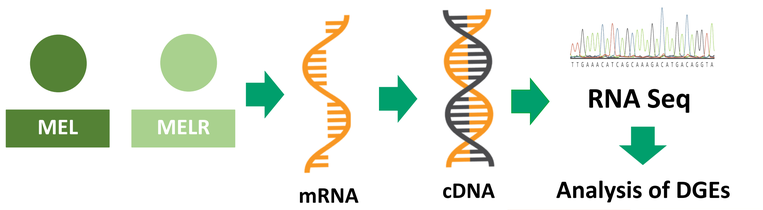
INTRODUCTION

In previous studies, *RNA-seq* was used to compare the transcriptome of virus-induced murine erythroleukemia cell line (MEL), a useful model to evaluate tumor cell reprogramming with chemical inducers, and a derived cell line with induced resistance to differentiation (MEL-R).



Among the differentially expressed genes (DGEs), a group up-regulated in MEL corresponded to proteins related to the actin cytoskeleton organization, in which Was and Btk stood out because they are responsible for severe hematologic diseases.

The role of Was has been extensively studied as one of the key players in actin cytoskeleton regulation in the lymphoid lineage, but its role in the myeloid lineage context remains poorly understood. The combination of novel roles of Was in transcription, apoptosis and chromatin remodeling, its importance in lymphoid differentiation and its relation to the Wiskott-Aldrich Syndrome, make Was a very interesting gene to be studied.

In this study, we obtained the cell line MEL Was$^{-/-}$ derived from MEL-DS19, by using the CRISPR/Cas9 strategy. MEL Was$^{-/-}$ is a useful *in vitro* model to study the molecular functions of Was and Btk in the erythroid linage and to further investigate the resistance to chemical inductors, through transcriptome analysis.

GOAL OF THE RESEARCH

1. Analyze the molecular function of Was in progenitors of the erythoid linage and its role in reprogramming celular differentiation in erythroleukemia cells.

RESULTS

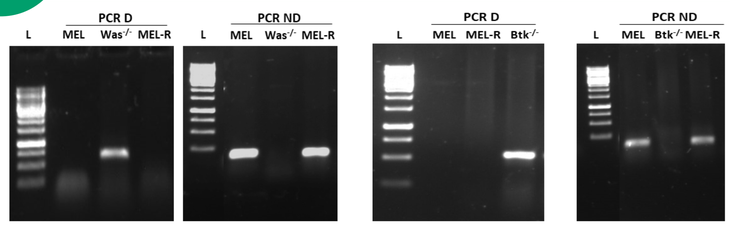
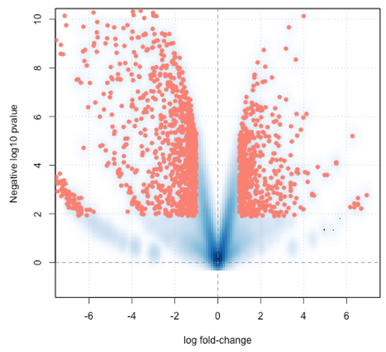


Fig. 1 Validation of deletion of Was and Btk genes. Electrophoresis of agarose gels of the PCR products done with the no deletion (ND) and deletion primers (D)n MEL/Was$^{-/-}$, clon 73 **(A)** and in MEL/Btk$^{-/-}$, clon 41 **(B)**.



Cutoff
- Adjusted p-value < 0.05
- FC equal or greater to 2

Fig. 2. Differential expressed genes are shown in pink, while not DEGs are shown in blue.

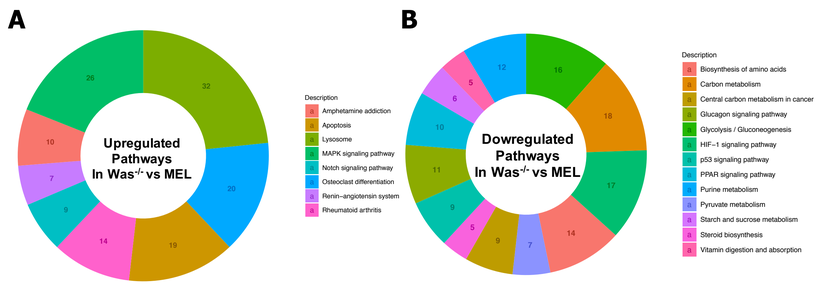


Fig. 3. Functional annotation of DGEs according to related pathways from KEGG database. **A)** Upregulated DGEs fin MEL/Was$^{-/-}$, with respect to MEL **B)** Downregulated DGEs in MEL/Was$^{-/-}$, with respect to MEL.

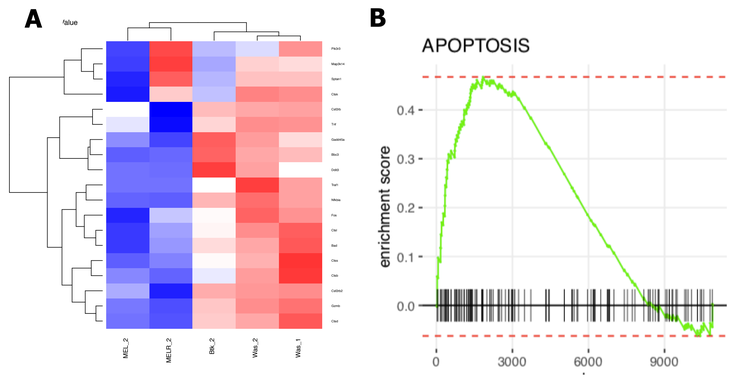


Fig. 3. Functional annotation of genes. A) Genes found to be related to Apoptosis are more downregulated in MEL-R and MEL (using KEGG) **B)** Enrichment of Apoptosis related genes in Was with respect to MEL via GSEA

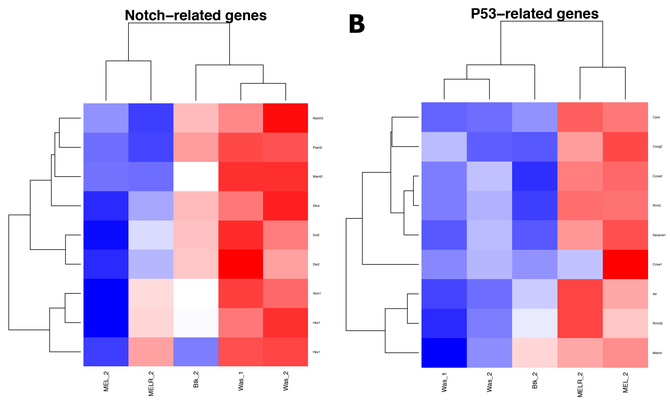


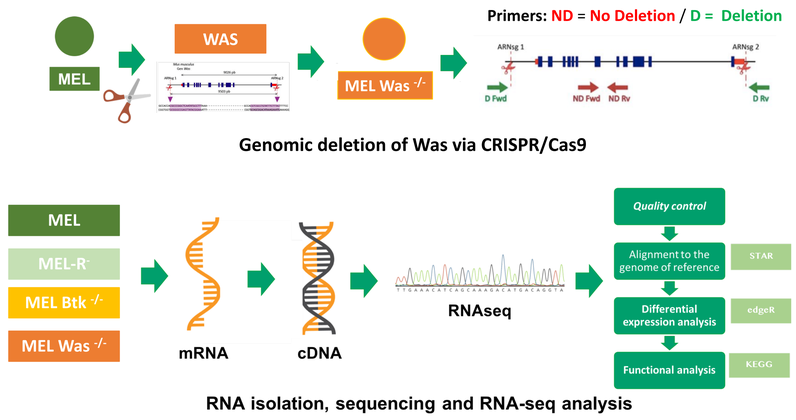
Fig. 4. Functional anotation of genes. A) Genes found to be related to Notch signaling are more upregulated in MEL Was$^{-/-}$ (using KEGG) **B)** Genes related to p53 in turn are downregulated in MEL Was$^{-/-}$ samples.

CONCLUSION

1. A total of 1933 genes were reported as DEGs (with an adjusted p-value lower than 0.05 and a fold change greater or equal to two), of which 1119 were upregulated in MEL Was$^{-/-}$ with respect to MEL and 814 were downregulated in MEL Was$^{-/-}$ when compared to MEL.
2. It has been demonstrated that a group of DGEs is related to apoptosis and Notch signaling pathway, both well known players in cell fate, proliferation and survival.
3. On the other hand, p53 related genes were seen to be downregulated in MEL Was$^{-/-}$ samples, suggesting that apoptosis might not be activated by p53 downstream-related genes in the absence of Was.

Taken together, these results identified differentially expressed genes belonging to the above pathways, which can serve as excellent candidates for experimental studies to confirm their role in reprogramming of erythroid progenitors with chemical inducers.

PROCEDURES



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