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Regulation of Autophagy and Oxidative Phosphorylation Is Predominantly Altered at the Transcription Level in Leukemic Stem Cells (LSCs) in Relapsed AML

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INTRODUCTION

Acute myeloid leukemia (AML) is a highly aggressive malignancy of uncontrolled myeloid progenitor cell proliferation and has a dismal survival rate. Despite the availability of standard and targeted therapeutic regimens, the rate of relapse is high in patients when leukemic stem cells (LSCs) are present. LSCs can self-renew and reinitiate leukemia in patients at any time point. Altered gene expression patterns in LSCs at diagnosis and relapse might help elucidate the factors responsible for relapse in AML.

AIM

- Transcriptomic Profiling of LSCs in treatment naïve and relapse AML
- To identify and characterize various factors responsible for tumorigenesis and chemoresistance in AML

METHOD

- **Sorting of LSCs (predominantly residing in CD34+ CD38- fractions) from bone marrow aspirate (BMA) samples based on the presence of aberrant markers using BD FACSAria™ III.**
- **Transcriptomic profiling of LSCs was done using paired diagnostic and relapse samples (n=18) collected from the patients.**
- **The differentially expressed genes identified by DESeq2 were used for pathway enrichment analysis via ShinyGO and Cytoscape.**
- **Additionally, we employed the rMATS tool to identify altered splicing patterns between relapse and diagnostic LSCs, along with rMAPS to pinpoint significant RNA-binding proteins instrumental in regulating alternative splicing events.**

RESULTS

- The most significantly altered pathways were those involved in **autophagy** and **oxidative phosphorylation processes.**
- The identified genes ULK1, ATG13, GABARAPL1, ATP5F1D, UQCRC2, and UQCR10 were significantly downregulated at relapse in AML LSCs,
- This downregulation highlights the altered metabolic profiles at relapse compared to diagnosis.
- We also identified substantial splicing aberrations in genes that play pivotal roles in autophagy.

Fig.1 Gating strategy for sorting LSC enriched fractions from BMA collected from AML patients

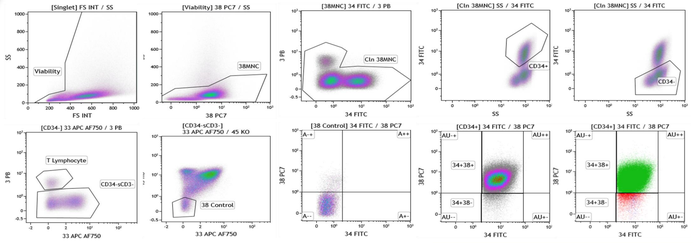


Fig.2 Genemania network for genes involved in Autophagy (a) and Oxidative Phosphorylation (b)

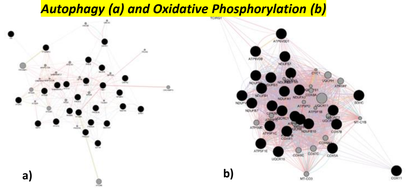


Fig.3 Top 10 Hub genes involved in Autophagy (a) and Oxidative Phosphorylation (b)

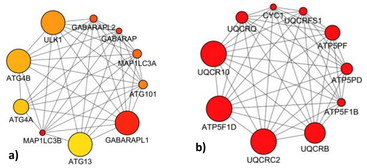
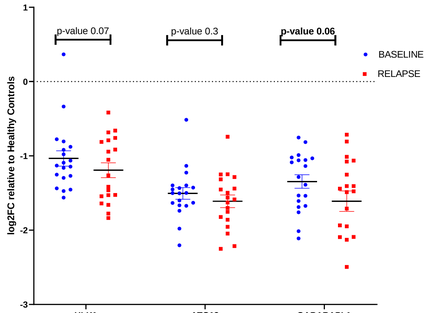


Fig.4 qPCR validation of ULK1, ATG13 AND GABARAPL1 showing downregulation at relapse in 20 paired AML samples



CONCLUSIONS

- Autophagy and oxidative phosphorylation pathways are significantly altered in AML patients at relapse.
- These alterations occur at levels of transcription and its regulation during splicing.
- The future scope is identifying RNA-binding proteins that regulate these processes. This will help decipher the regulatory networks to better understand LSC biology and unveil novel therapeutic targets to improve patient survival outcomes.

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