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The role of BCLAF1 and its impact on Acute Myeloid Leukemia (AML)

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INTRODUCTION

Acute Myeloid Leukemia is the most frequent acute leukemia in adult patients. Despite new medical advances, AML remains an incurable disease with a 5-year overall survival rate of about 20%, highlighting the need for a better understanding of leukemogenesis in order to develop novel therapies. Recent studies suggest that dysregulated RNA binding proteins (RBPs) may act as genetic vulnerabilities in AML, thereby constituting potential therapeutic targets. In fact, aberrant expression of RBPs occurs ubiquitously in cancer, and some particular dependencies for tumor progression have already been identified. However, despite this considerable progress, the promise of targeting RBPs therapeutically is still limited by a lack of knowledge about the precise mechanisms behind its dependencies in cancer.

We have recently performed a genome-wide CRISPR dropout screen using primary mouse leukaemia cells and found that several RBPs are indeed essential for the maintenance of the leukemic state (Barbieri *et al*, Nature 2017). One of them, BCLAF1 (BCL2-associated transcription factor 1), contains a domain rich in alternating arginine and serine residues (RS domain), a feature that is frequently found in proteins involved in mRNA splicing, and is a component of nuclear speckles, which are enriched in proteins that facilitate mRNA processing. Thus, it seems apparent that BCLAF1, which we found to be required for AML cell growth, may be particularly involved in mRNA splicing.

OBJECTIVES:

The main aim of this work is to study the role of BCLAF1 and its impact on AML biology.

METHODS:

AML patient data was obtained from The Cancer Genome Atlas (TCGA). Patients were classified into quartiles based on BCLAF1 expression levels, and differential gene expression analysis was performed using DESeq2 package. Up- and down-regulated genes were subjected to a Gene Ontology (GO) enrichment analysis.

In order to generate stable cell clones with doxycycline inducible reduced levels of BCLAF1, MOLM13 AML cells were infected with the corresponding shRNA expressing constructs. Total RNA was extracted using Qiagen Rneasy kit, and PolyA+ mRNA was isolated using oligo(d)T magnet beads. cDNA libraries were generated using Oxford Nanopore technologies Direct cDNA Sequencing kit following manufacturer instructions. Nanopore sequencing resulted in ONT reads that were mapped to human reference genome hg38 using IsoQuant. The resulting TSV and GTF files were used for transcript identification and quantification analysis with R packages IsoformSwitch Analyzer and DESeq2.

RESULTS:

In order to shed some light into the role of BCLAF1 in AML biology, we first performed differential gene expression analyses using AML patient RNA sequencing data publicly available at TCGA. To do so, we classified patients into quartiles based on BCLAF1 expression levels and compared the upper and lower one. Interestingly, GO enrichment analysis indicated that patients with low BCLAF1 levels display reduced expression levels of several genes involved in mRNA splicing, suggesting that BCLAF1 may participate in alternative splicing.

To test this hypothesis, we generated an AML cell line with doxycycline inducible reduced levels of BCLAF1 and performed mRNA splicing analysis using NANOPORE long-read sequencing. We observed several intron retention/exclusion, appearance/disappearance of non-mediated decay (NMD) isoforms and changes at the 3'UTR events. In particular, we found that BCLAF1 depletion led to changes in isoform usage of more 100 genes. Notably, among them we identified different target genes that are involved in mRNA splicing themselves.

CONCLUSIONS:

BCLAF1 was originally identified as a death-inducing transcriptional repressor. However, its exact molecular functions still need to be properly defined. Our results are consistent with more recent reports suggesting that BCLAF1 plays a role during alternative mRNA splicing. Interestingly, it has been reported that AML cells carrying splicing factor mutations are particularly vulnerable to genetic or pharmacologic modulation of splicing, thereby providing a therapeutic window of opportunity. Thus, understanding BCLAF1 function and its impact on AML biology has potential for clinical relevance.