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ORAL ABSTRACTS

603.LYMPHOID ONCOGENESIS: BASIC

Regulation of Alternative Splicing in B-Cell ALL By DYRK1A

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DYRK1A, located in the Down syndrome critical region of chromosome 21, is a serine and threonine kinase that controls multiple cellular processes including apoptosis, cell cycle, transcription and signal transduction. We previously demonstrated that DYRK1A is required for maturation of B cells and is a therapeutic target in B-cell ALL, including both DS and non-DS subtypes. Key substrates within the B-cell lineage include D-type cyclins, FOXO1, and STAT3. Given that several studies have reported that DYRK1A phosphorylates various splicing factors and participates in splicing of Tau in neuronal cells, we investigated the contribution of DYRK1A to splicing in malignant B cells.

We first treated B-ALL cell lines with two DYRK1A inhibitors, EHT 1610 and GNF 2133 and performed RNA-sequencing to detect changes in alternative splicing (AS). GO analysis of AS events showed RNA binding proteins among top differentially spliced genes. Of note, mis-splicing of *RBM39* was one of the top differentially AS events associated with DYRK1A inhibition; this event involves inclusion of a "poison" exon that contains a premature termination codon and leads to a transcript predicted to be degraded by nonsense-mediated mRNA decay (NMD). We confirmed NMD by knocking down an essential NMD factor, UPF1, which stabilized the NMD isoform. The NMD isoform was also detected in B-ALL patient samples treated with DYRK1A inhibitors and in NALM6 cells in which DYRK1A was inducibly degraded.

RBM39 has been reported to be a therapeutic target in AML, where cells with splicing factor mutations are more sensitive to loss of RBM39 than cells lacking splicing factor mutations. To determine whether RBM39 is a therapeutic target in B-ALL, we silenced *RMB39* with shRNAs *in vitro* and *in vivo*. Genetic knockdown of *RBM39* impeded B-ALL cell growth both *in vitro* and in xenograft models. Furthermore, inducible silencing of *RBM39* hampered B-ALL progression *in vivo* evidenced by a significant increase in survival of mice engrafted with human B-ALL cells. We also found that indisulam and its analogs, which lead to DCAF15-mediated degradation of RBM39, suppressed the growth of B-ALL cells *in vitro* and *in vivo*. Importantly, the anti-tumor effect of RBM39 disruption extended beyond DS-ALL to Ph-like B-ALL, which continues to be an area of unmet medical need.

We next analyzed the contributions of splicing factors that are known DYRK1A substrates to AS of RBM39. Knockdown of SRSF1 and SRSF2 led to increased inclusion of the poison exon even in the absence of DYRK1A inhibition, indicating that they normally function to exclude the exon. By contrast, knockdown of SF3B1 led to a reduction of the NMD isoform in the presence of EHT1610. Surprisingly, phosphorylation of SF3B1 by DYRK1A was not essential for the change in RBM39 splicing, as cells engineered to harbor two SF3B1 T434A phospho-deficient mutant alleles, did not show aberrant splicing. These results indicate that SF3B1, SRSF1, and SRSF2 are not the key substrates that mediate the effect of DYRK1A on alternative splicing. Next, given that DYRK1A is known to phosphorylate the C-terminal domain (CTD) of RNA polymerase II (Pol II) on Ser2 and Ser5, we investigated the contribution of Pol II phosphorylation to RBM39 splicing. IP-MS revealed an interaction between RNA Pol II and SF3B1, consistent with previous reports of co-transcriptional splicing. We found that inhibition of DYRK1A disrupted the colocalization of SF3B1 and RNA Pol II as well as with *RBM39* RNA. However, DYRK1A inhibition had a transient effect on Ser2 and Ser5 phosphorylation, suggesting that cells have alternative pathways for Ser2/5 phosphorylation. Combining DYRK1A inhibition with inhibition or loss of CDK9, which also phosphorylates the CTD of RNA Pol II, led to a

persistent decrease in RNA Pol II phosphorylation, marked increased NMD isoform and a profound reduction in RBM39 protein. Finally, we found that combining the CDK9 inhibitor dinaciclib with EHT 1610 synergistically inhibited growth of B-ALL patient cells *in vitro* and *in vivo* shown by significantly improved survival. Ectopic expression of RBM39 partially rescued the growth inhibition upon dinaciclib and EHT 1610 treatment suggesting downregulation of RBM39 is the key event in drug targeting.
Together, our results reveal that DYRK1A controls RNA splicing by regulating the SF3B1-RNA Pol II interaction and that RBM39 is a therapeutic target in B-ALL.

Disclosures Crispino: *SAB of Alethiomics:* Other: Member; *Cellarity:* Consultancy.

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