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ORAL ABSTRACTS

636.MYELODYSPLASTIC SYNDROMES-BASIC AND TRANSLATIONAL

Aberrant Splicing of *MBD1* Reshapes the Epigenome to Drive Convergent Myeloerythroid Defects in MDS

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Splicing defects are a characteristic feature of myelodysplastic syndromes (MDS) and typically associate with specific recurrent splicing factor mutations. However, a subset of transcripts exhibit abnormal splicing regardless of mutational background, occurring even in the absence of splicing-related mutations. These shared splicing events likely include common underlying drivers of MDS hematopoietic defects, yet the functions of the resulting transcripts remain unknown. We identified a long isoform of Methyl-CpG-Binding Domain 1 (*MBD1*) as the product of one such mutation-independent splicing event. To understand whether altered *MBD1* splicing contributes to hematopoietic dysfunction, we overexpressed isoforms of *MBD1* in cord blood CD34+ cells and found that the MDS-associated full-length isoform (MBD1-L), containing MBD1's 3rd CXXC domain, impaired erythroid differentiation by stalling cell cycling and promoting apoptosis. In contrast, the MBD1-ΔCXXC3 isoform (MBD1-S), preferentially produced in healthy cells, did not induce these defects. Recapitulating these findings, the MBD1-L isoform uniquely impaired reconstitution capacity *in vivo*, particularly in the erythroid and myeloid lineages, and in addition produced an enrichment of the MDS transcriptomic signature on RNA-seq profiling.

The unique CXXC3 domain of MBD1-L specifically binds non-methylated CpGs, and exhibits greater target affinity than the shared MBD domain, which is responsible for mCpG binding. Given MBD1's key role in heterochromatin maintenance at mCpG regions, we hypothesized that the unique function of MBD1-L in MDS may be attributable to the refocusing of MBD1-mediated epigenetic repression from canonical, methylated DNA sites to unmethylated sites. Isoform-specific CUT&RUN and multi-omics profiling in cord blood CD34+ cells revealed that the inclusion of the CXXC3 exon triggers a striking redistribution of MBD1 from gene bodies and intergenic regions to hypomethylated promoter CpG islands, resulting in widespread suppression of promoter chromatin accessibility and downregulation of cell-cycle-related transcripts. Characterization of the MBD1 interactome by rapid immunoprecipitation mass spectrometry showed that the MBD1-L isoform preferentially associates with the SETDB1:ATF7IP H3K9 methylator complex, supporting active heterochromatin establishment at MBD1-bound promoters. Among the direct targets uniquely repressed by MBD1-L is *BCOR*, a recurrent LOF gene in MDS whose loss perturbs hematopoietic differentiation and promotes self-renewal. Downregulation of *BCOR* by MBD1-L led to the derepression of *BCOR*-controlled genes, contributing to an enriched stem cell-associated transcriptomic signature resembling *BCOR* LOF.

To investigate whether reversal of MBD1-L splicing can restore hematopoietic output in diseased cells, we delivered lipid-encapsulated splice-switching antisense oligonucleotides targeting the CXXC3 exon into primary human MDS cells, and observed an increase in differentiation in vitro. In addition, depletion of MBD1-L in the MDSL cell line using isoform-specific shRNAs increased cell proliferation, confirming that targeted reduction of MBD1-L inverted the quiescent, differentiation-impaired phenotype imposed by its overexpression.

Our results demonstrate that *MBD1* isoforms act on separate compartments of genomic sequence to carry out divergent functions in hematopoietic cells, and that disease-associated overproduction of MBD1-L compromises hematopoietic output and lineage differentiation. These findings provide the first evidence that mutation-independent splicing changes can drive hematopoietic dysfunction in MDS.

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