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Enhanced Alternative Splicing Induced by Missense Mutations in U2AF1 Splicing Factor

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Myelodysplastic syndromes (MDS) are heterogeneous disorders of the bone marrow marked by ineffective hematopoiesis, and progress to highly chemotherapy-resistant secondary Acute Myeloid Leukemia (sAML) in up to 30% of patients. Identifying predictive biological markers that denote those higher risk cases of MDS that will transition into sAML is imperative to develop more effective therapies for the disease. Last year, we identified novel missense mutations in \textit{U2AF1} using whole genome sequencing in patients with MDS. \textit{U2AF1} is a subunit of the U2 small nuclear riboprotein auxiliary factor recruited to the spliceosome complex that mediates pre-mRNA splicing. The mutations occur at a highly conserved site in codon 34 [changing serine to phenylalanine (S34F) or tyrosine (S34Y); 1 patient with S34F had an additional missense mutation on the same allele (Q157R)] in \textasciitilde9% of patients with MDS. Pre-mRNA splicing is an essential, highly regulated posttranscriptional process in which noncoding intronic sequences are removed from pre-mRNA and exonic sequences are spliced together, resulting in the production of specific protein isoforms. Alternative splicing allows a single gene to express many different protein isoforms through the implementation of alternative 5’ and 3’ splice sites, the retention of introns, or the aberrant retention or skipping of exons. Pre-mRNA splicing is a ubiquitous eukaryotic cellular process, and modifications or dysfunction in this pathway could lead to expanded genetic diversity in cancer cells and disease. We hypothesize that \textit{U2AF1} missense mutations alter pre-mRNA splicing in MDS as a potential mechanism for MDS pathogenesis. We utilized \textit{GH1} and \textit{FMR1} minigene assays (well characterized systems to measure alternative splicing) and reported that S34F-mutated \textit{U2AF1} promoted enhanced exon skipping in \textit{GH1} and cryptic splice site usage in \textit{FMR1}, resulting in enhanced alternative splicing. We extended this analysis to include additional \textit{U2AF1} mutations not previously investigated and new mutations discovered by other groups—S34Y, S34F/Q157R, Q157R, and Q157P. We now show that S34F-mutated \textit{U2AF1} displayed the most profound alternative splicing phenotype for both \textit{GH1} and \textit{FMR1}, with modestly enhanced alternative splicing by S34Y-mutated \textit{U2AF1}. Conversely, Q157R and Q157P-mutated \textit{U2AF1} both significantly enhanced splicing of the canonical isoforms of \textit{GH1} and \textit{FMR1}. The S34F/Q157R allele displayed an intermediate splicing phenotype. With S34F-mutated \textit{U2AF1} displaying the most profound alternative splicing phenotype, we are currently performing whole transcriptome analysis to identify additional genes that have enhanced alternative splicing in the presence of S34F-mutated \textit{U2AF1} to further elucidate its role in the pathogenesis of MDS. Understanding the molecular consequences of mutations in splicing machinery may lead to improve therapies for patients with MDS.