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Title: INVESTIGATING THE ROLE OF RNA HELICASES IN SPLICING CHANGES CAUSED BY CANCER ASSOCIATED SF3B1 MUTATIONS

Abstract

The spliceosome is a large, dynamic ribonucleoprotein (RNP) complex responsible for the accurate and flexible processing of pre-mRNA to mRNA. It accomplishes this with five small nuclear RNPs (snRNPs) and numerous other proteins including NTP-dependent RNA helicases. SF3B1, the largest subunit of the SF3B complex, is tasked with 3' splice-site (3'ss) positioning and coordination of multiple factors leading to splicing catalysis. SF3B1 is frequently mutated in a multitude of cancers and pre-malignancies. Mutant SF3B1 alters the splicing of many transcripts, including alternative 3'ss choice and detained introns (DIs). However, the vast majority of splicing appears to be unaffected. SF3B1 interacts with RNA helicases that play a role in 3' ss choice: Prp5/DDX46 and Sub2/DDX39B. Recently, a third helicase-Prp43/DHX15-was also implicated in this step of splicing. Additionally, loss of SUGP1, a coactivator of DEAH-box helicases, phenocopies SF3B1 mutations at alternative 3' ss. Finally, expression of a dominant-negative mutant of another helicase, Prp2/DHX16, causes increased prevalence of DIs, and DHX16 is itself mutated in cancers. We hypothesize that SF3B1 mutants alter its interactions with multiple helicases during the splicing cycle, and that different subsets of introns exhibit unique dependencies on those helicases and other splicing factors, accounting for the resulting splicing patterns. We are targeting these helicases with auxin-inducible degrons. We will characterize changes in splicing upon target protein depletion, and then compare the splicing changes resulting from the depletion of the helicases to the splicing changes resulting from SF3B1 mutations to gain mechanistic insight into how these factors interact.