CRISPR Screen Identifies TOP3B as a Key Regulator in RNA Splicing

Shar-yin N. Huang
Laboratory of Molecular Pharmacology, Developmental Therapeutics Branch, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD 20892, USA

Abstract
Among the six human topoisomerases, topoisomerase IIIb (TOP3B) is uniquely capable of catalyzing topological conversions of RNA in addition to DNA. Although TOP3B orthologs exist in all domains of life, their cellular functions remain undefined. To characterize the molecular functions of TOP3B, we performed a genome-wide CRISPR-Cas9 loss-of-function screen in human wild-type and TOP3B knockout cells. Genetic interaction analysis identified extensive functional connections between TOP3B and RNA-processing pathways, with the spliceosome emerging as a prominent vulnerability in HAP1 TOP3B knockout cells. Consistent with these findings, affinity purification coupled with mass spectrometry showed TOP3B physically associates with spliceosome components. Transcriptomic analyses of multiple human TOP3B deficient cancer cell lines revealed widespread changes in alternative splicing patterns, suggesting that loss of TOP3B leads to dysregulation of pre-mRNA splicing, with the most pronounced effects in long transcripts or long exons/introns. Our study establishes TOP3B as an important regulator in pre-mRNA splicing.