Comprehensive Bulk and Single Cell Transcriptomic Characterization of *SF3B1* Mutation Reveals Its Pleiotropic Effects in Chronic Lymphocytic Leukemia

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INTRODUCTION

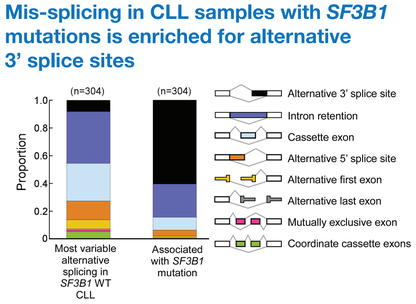
Mutations in the core spliceosome component *SF3B1* have been associated with adverse clinical outcome in chronic lymphocytic leukemia (CLL), but how this mutation contributes to the cancer phenotype remains poorly understood. We undertook a transcriptomic characterization of primary human CLL cells in relation to *SF3B1* mutation status using both total and poly-A selected RNA to comprehensively identify affected transcripts and pathways. Significantly altered spliced events associated with *SF3B1* mutation were enriched for events affecting the 3' splice site. We observe similar patterns of significant alternative splicing at the single cell level for CLL cells harboring *SF3B1* HEAT repeat mutations compared to CLL cells without *SF3B1* mutation within the same patients. Presence of *SF3B1* mutation was coordinated with changes in expression and splicing in genes across diverse cancer- and CLL-associated pathways, including DNA damage response, apoptosis, cell cycle, metabolism, protein synthesis, telomere maintenance, and Notch activation. Overexpression of full-length mutated *SF3B1* in cell lines resulted in modulation of the DNA damage and Notch signaling pathways, with activity mediated through altered splice forms of DVL2. Mutation in *SF3B1* is thus an efficient mechanism by which numerous complex changes in CLL biology are generated that can contribute to disease progression.

SUMMARY

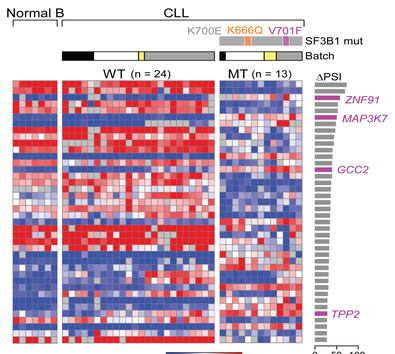
- *SF3B1* mutation causes alternative splicing
- *SF3B1* mutation impacts gene expression changes that affect a wide array of pathways, including Notch activation
- Novel microfluidic approach detects mutation, expression, and alternative splicing in the same single cells to reveal relevance of *SF3B1* mutation within the HEAT repeat mutational hotspot
- Altered DVL2 splice variant is associated with *SF3B1* mutation in CLL and increases Notch activation
- *SF3B1* mutation results in multiple alterations in transcript sequence or expression to impact CLL in a concerted fashion across CLL pathways

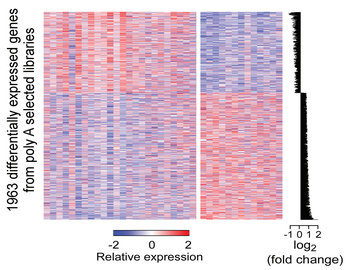
BULK APPROACH

Mis-splicing in CLL samples with *SF3B1* mutations is enriched for alternative 3' splice sites

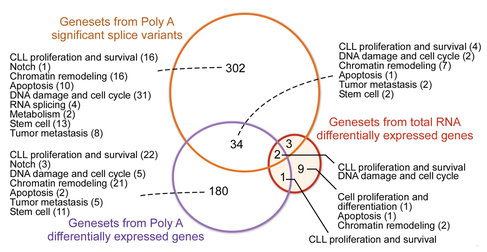


Using bulk total and poly-A selected RNA-seq, we find that splice events significantly associated with mutant *SF3B1* in CLL show evidence of enrichment at 3' alternative splicing. We identify numerous differentially altered splice events and differentially expressed genes. Gene set enrichment analysis identifies diverse cancer- and CLL-associated pathways affected by both splice variants and gene expression, including Notch.

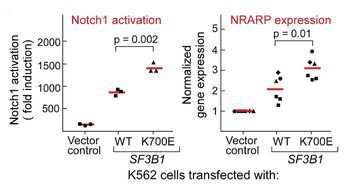




SF3B1 mutation impacts multiple cellular pathways including Notch



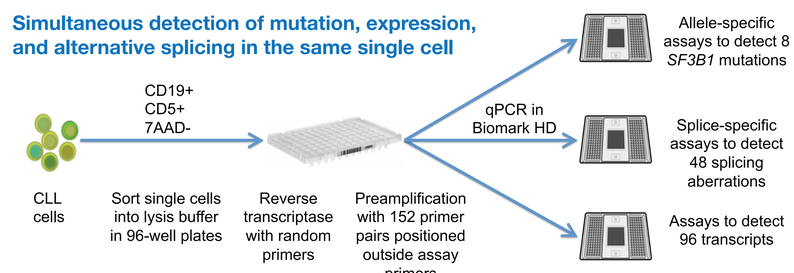
We confirm expression of SF3B1K700E induces elevated Notch pathway activation in K562 cells as indicated by Notch1 and Notch pathway target NRARP expression.

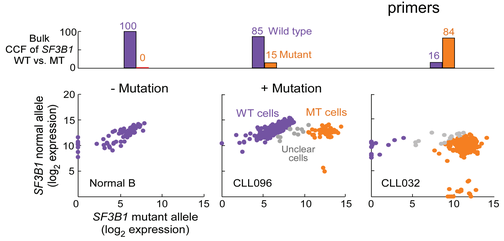


Pathway	293T	U20S	HeLa	K562	HG3	JeKo-1	MEC2
RNA splicing	√	√	√	√	√	√	√
WNT pathway	√	√			√	√	
Cell cycle				√	√	√	
Apoptosis				√	√	√	
DNA damage response	√	√	√	√			√
Notch signaling	√	√		√		√	√

SINGLE CELL APPROACH

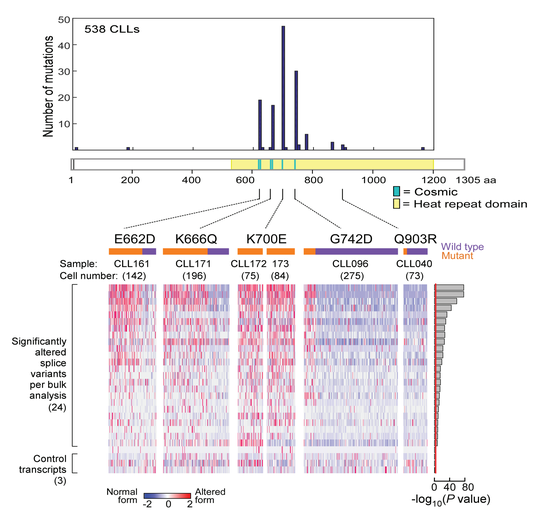
Simultaneous detection of mutation, expression, and alternative splicing in the same single cell





We adapted a novel and sensitive microfluidic approach that uses multiplexed targeted amplification of RNA on the Fluidigm Biomark platform to simultaneously detect somatic mutation status, gene expression, and alternative splicing within the same single cell.

single CLL cells with *SF3B1* mutations demonstrate altered splicing

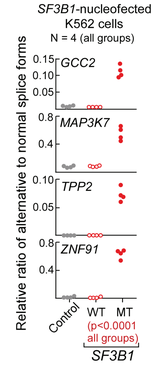


To explore the extent to which *SF3B1* mutations other than K700E share a similar spectrum of altered splicing as that generated by the K700E mutation, we examined single cells from 6 patient samples across 5 different *SF3B1* mutations. Of the 4 *SF3B1* mutations that lie within the HEAT repeat mutational hotspot of *SF3B1* exhibited similar patterns of alternative splicing to bulk while 1 mutation outside did not. Thus, our results suggest that not all *SF3B1* HEAT repeats are equally critical for proper splicing function.

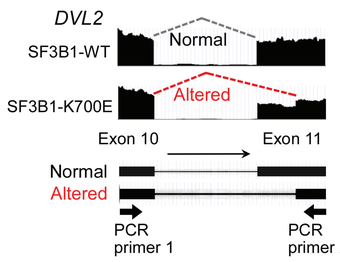
FUNCTIONAL VALIDATION

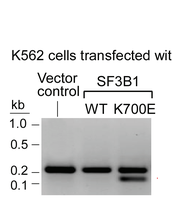
SF3B1 mutation causes alternative splicing

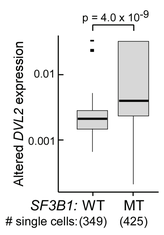
We validate that expression of mutant *SF3B1* causes alternative splicing by cloning wild-type and K700E-mutated *SF3B1* into expression constructs. Introduction of wild-type and mutated *SF3B1* expression constructs into a various cell types consistently resulted in upregulation of splice variants associated with *SF3B1* mutation and increased 3' alternative splicing. Using this construct, we assessed the effects of *SF3B1* mutation on CLL cellular pathways were in 293T, HeLa, U2OS, K562, HG3, JeKo-1 and MEC2 cells.

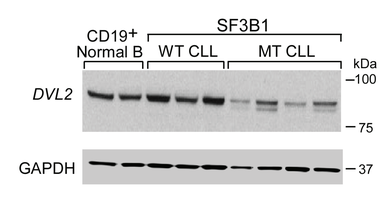


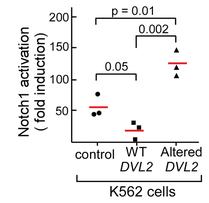
SF3B1 mutation affects Notch signaling through a splice variant of *DVL2*

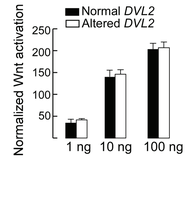












Focusing on splicing events in genes involved in Notch signaling, we identified an altered splicing event in DVL2. We confirm association of the DVL2 splice variant K562 cells overexpressing mutated *SF3B1* and in primary CLL single cells. We identified the protein product of altered DVL2 by western blot in a series of *SF3B1* primary CLL samples with *SF3B1* mutation but not in those lacking the mutation. Enforced expression of altered DVL2 into K562 cells in the presence of Notch 1-expressing plasmid increased Notch activation, while wild-type DVL2 was repressive.