Abnormal Splicing Patterns and Novel Intron Retentions Affect Genes Regulating Splicing, NFKB, ERK, and TP53 in Waldenstrom Macroglobulinemia

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Waldenstrom’s Macroglobulinemia (WM) is characterized by activating mutations in MYD88 and CXCR4 in 93.95% and 30-40% of patient’s, respectively. Functional analysis of these mutations has revealed downstream activation of NF-kB, through BTK/IRAK as well as AKT/ERK activation downstream of HCK and CXCR4. Transcriptome analysis using next generation sequencing demonstrated strong upregulation of BCL2 and overall dysregulation of the BCL-2 family of genes.

Isomorphic based evaluation of splicing assumes that the data consists of a mixture of known isoforms, which may not be a reasonable assumption for a cancer transcriptome wherein mutations in splicing and transcription factor dysregulation may cause unique combinations of splicing events not observed in healthy donors (HD). To explore alternative splicing in Waldenstrom’s Macroglobulinemia (WM), we developed a MISO (Kat et al, Nat Meth 2010) based protocol to analyze next generation RNA sequencing data from 77 WM patients, 16 HD plasma cell (PC) samples, 9 HD CD19+CD27- peripheral blood B-cells (PB) and 9 HD CD19+CD27+ memory B-cells (MB). Fifty-five patients harbored MYD88 mutations, 20 of whom also carried activating mutations in CXCR4. Twenty-two patients were wild-type (WT) for both genes, and was specifically enriched to ensure adequate numbers for comparative study. To identify dysregulated splicing events, the memory B-cell cohort was merged and down-sampled for use as common comparator control for the WM and other HD samples. Estimating the variance in the control group is critical for statistical analysis so a jackknife permutation analysis where control samples pulled out and compared to the remaining samples was performed. This analysis was built into an automated pipeline based on two-pass STAR aligned bam files and corresponding tools to aggregate and analyze the data were developed using R. Novel Intron retentions were investigated by identifying intronic regions not overlapping known exons. The number or reads mapping to these regions relative their flanking exons was calculated and compared.

Filtering against events observed in the control permutation analysis and other healthy donor samples allowed for the identification of individual splicing events associated with WM. Splicing events were present in genes that regulated splicing itself (SF1, PTBP1), as well as pathways critical to WM growth and survival including NF-kB (ZFAND5, LYN, PKCδ), ERK/2 (CTBP1, VPS25, CC2M), and P53 (PML, EJFG1). PCR was successfully used to validate many of these splicing events. Preliminary intron retention analysis identified 4/17 (2%) of patients had intrinsic retentions on CD79B that were caused by intrinsic somatic mutations near the intron exon boundary. CD79B is a critical component of the B-cell receptor pathway and coding mutations have been observed in 7-15% of WM patients (Hunter et al, Blood 2013; Poulan et al, Am J Hematol, 2013).

The retentions identified in this study represent a novel form of CD79B mutation that has not been previously reported. Additional analysis and functional characterization of these events is ongoing. These studies aim to identify novel biomarkers and therapeutic targets for WM while developing analytical tools applicable to other malignancies.

Summary

- MISO based permutation analysis can be used to identify significant splicing events that are missed by gold standard isoform quantification algorithms.
- Alternative splicing patterns distinguish WM samples from healthy donors, WM samples based on MYD88 mutation status, and by stage of B-cell differentiation.
- Alternative splicing events in WM have been validated and affect clinically relevant pathways including NF-kB, ERK/2 and TP53. Functional characterization of these events is ongoing and may reveal novel therapeutic targets in WM.
- Recurrent intron retentions caused by splice site mutations are found CD79B in WM and may be present in related lymphomas where the same mutations have been observed. Transcriptional and functional characterization of these retentions are ongoing.