Whole Genome and Transcriptome Analysis of Waldenström's Macroglobulinemia Cell Lines, Including the Novel BCWM.2 Cell Line, Show Preservation of Many Important Genomic Features of Primary Disease, and Identify a Non-L265P Mutation in MYD88

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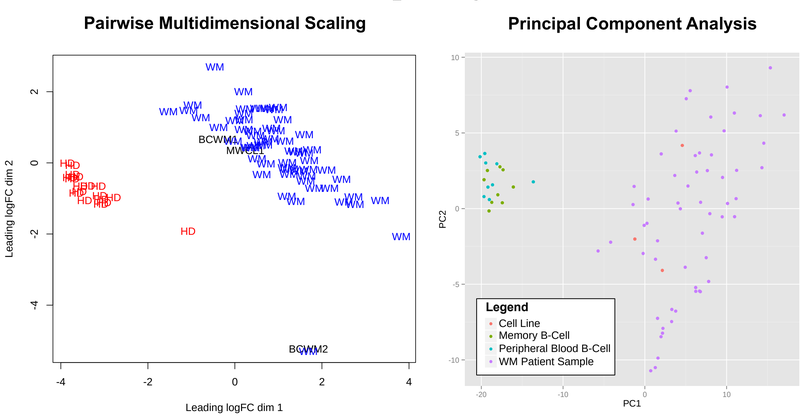
Background

WM cell lines represent a vital tool for discovery of mechanisms responsible for WM pathogenesis, cell signaling, development of targeted therapeutics, and evaluation of mechanisms responsible for drug resistance. To date, few WM cell lines exist and there has been no comprehensive genomic and transcriptomic profiling of WM cell lines using next generation sequencing.

Methods

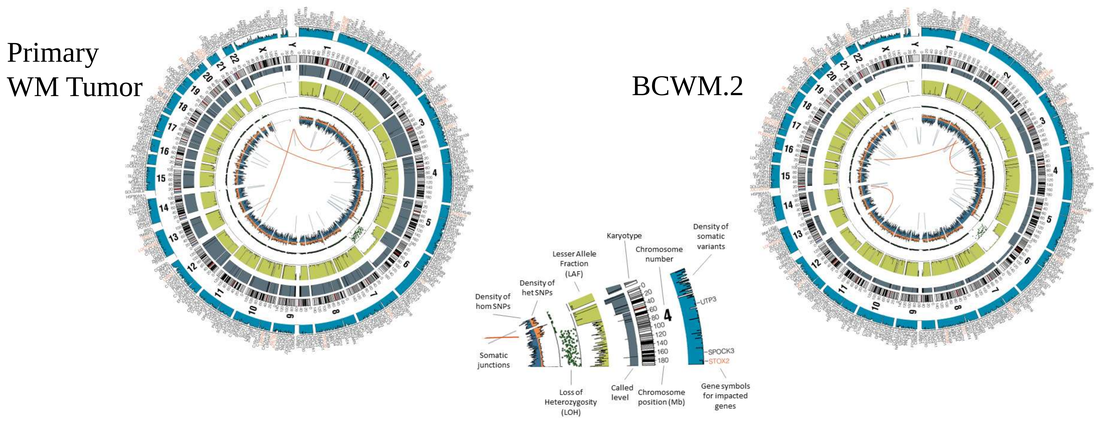
We performed whole genome (WGS) and transcriptome sequencing (WTS) on three established (BCWM.1, MWCL-1, RPCI-WM1), and one novel (BCWM.2) cell line established from continuous culture of $CD19^+$ selected bone marrow cells from a WM patient with an immunophenotype consistent with primary WM disease ($CD5^-$, $CD10^-$, $CD19^+$, $CD20^+$, $sIgM^+$, λ^+, $CD11c^-$, $CD38^+$, and $CD138^+$). Samples from primary tumor and germline from the founder of BCWM.2, along with tumor samples from 57 WM patients, as well as memory ($CD19^+CD27^+$) and non-memory ($CD19^+CD27^-$) B-cells from 9 healthy donors were also sequenced. WGS was performed using Complete Genomics platform, and WTS using Illumina HiSeq. Reads were aligned to KnownGene HG19/GRCh37 reference using STAR. Read counts per gene were obtained using featureCounts from Rsubread, and analyzed using voom from the edgeR/limma Bioconductor packages in R. Differential isoform expression was assessed using the Cufflinks software suite.

RNASeq Analysis

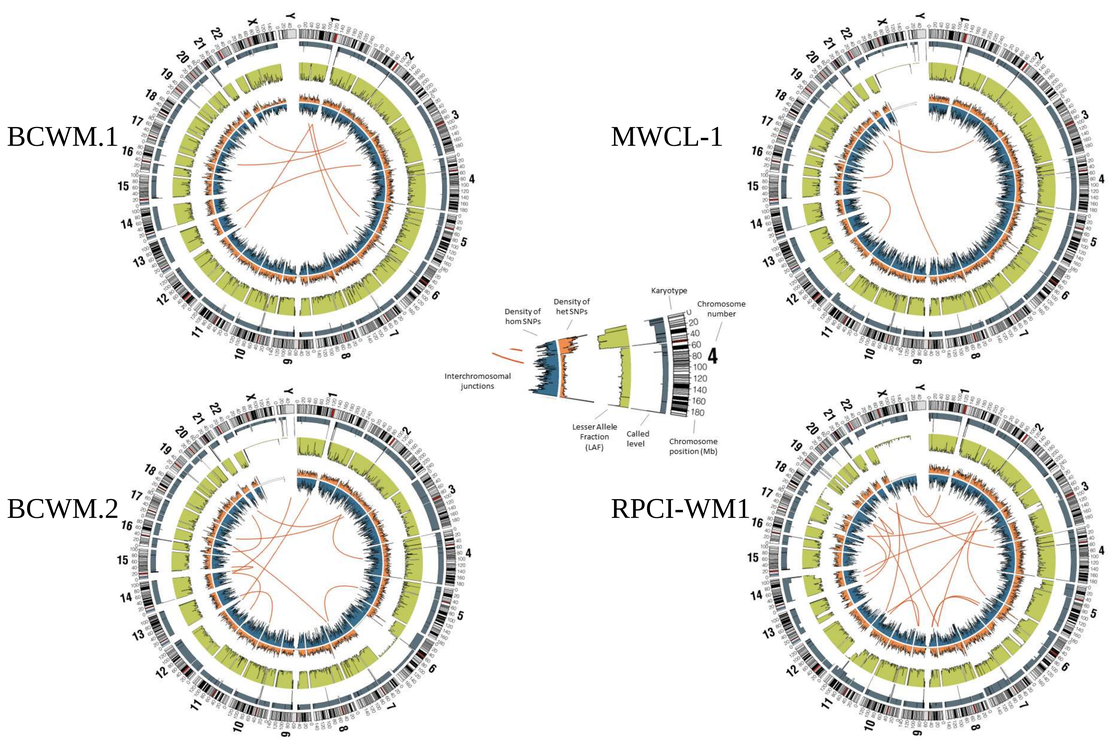


Disclosures: No relevant conflicts of interest to declare.

Somatic Events in BCWM.2 and Parent Primary WM BM Sample Compared with CD19 Depleted Peripheral Blood Germline Controls



Comparison of Waldenström's Cell Lines by Whole Genome Sequencing



Results

WGS revealed no cytogenetic level amplifications or deletions in BCWM.1, whereas MWCL-1 harbored monoallelic deletions in 17p and 17q, and amplifications in 11q. RPCI-WM1 showed deletions in 3p, 6q, 9p, 13q, 18q, 19q and X, with amplifications in 5p, 6p, 7q, 11q, 14q, 18q, 21q, and acquired uniparental disomies on 17p, 18q, and X. BCWM.2 matched its parent tumor profile with trisomy 3 and 12, as well as deletion of the entire arm of 6q and corresponding amplification of 6p. All 4 cell lines were wild type for CXCR4 and all but BCWM.2 harbored the MYD88 L265P mutation. Like its founding clone, BCWM.2 carried an activating heterozygous MYD88 G/A S243N mutation (Nature 470(7332):115-9) previously described in ABC DLBCL. In addition, a novel I318N mutation in LYN that was predicted to be activating was found in BCWM.2, but not in the founder primary tumor or germline samples. While WTS analysis of RPCI-WM1 is pending, principal component analysis using the top 500 high variance genes showed that BCWM.1, BCWM.2 and MWCL-1 cells clustered with primary WM tumor cells, and were distinct from memory and non-memory B-cell samples from healthy donors. Likewise, multidimensional scaling of pairwise similarity clustered BCWM.2 directly with its corresponding primary sample. BCWM.1, BCWM.2, and MWCL-1 cells showed a high degree of recapitulation with primary MYD88 mutated CXCR4 wild-type WM cells including IL17RB, CABLES1, WNT5A, GPER, or WNK2 over-expression. As a group, WM cell lines were found to over-express many cancer associated genes, including MUC13, MMP7, LMO3, IL4I1, and several SRC family kinases.

Conclusions:

This study provides the first ever comprehensive genomic and transcriptomic profiling of WM cell lines by next generation sequencing including the novel BCWM.1 cell line. The findings show preservation of many important genomic features of primary WM disease, and identified the existence of a non-L265P activating MYD88 mutation, as well as dysregulation of many cancer associated genes in WM. The findings provide an important new resource for the study of WM disease