Comprehensive Integration of Whole Genome, Transcriptome and Methylation Profiling Reveals Novel Gene Dysregulation Including IL15, SOCS6 and CARD11 Associated with MYD88 and CXCR4 Genotype Status in WM

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Background

Waldenstrom's Macroglobulinemia/IgM lymphoplasmacytic lymphoma is a B-cell lymphoma defined by highly recurring mutations in *MYD88* (95-97%) and *CXCR4* (30-40%) by whole genome (WGS), PCR and Sanger sequencing (*Treon et al NEJM 2012, Hunter et al Blood 2013, Xu et al, BJH 2016*). Using next generation RNA sequencing studies (RNASeq) of on biopsy samples previously analyzed using WGS, we subsequently observed that *MYD88* and *CXCR4* mutation status were the primary determinants of differential gene expression in WM (*Hunter et al, Blood 2016*). We have now integrated enhanced reduced representation bisulfite sequencing (ERRBS) data into the existing WGS and RNASeq data to present of more complete picture of genomic regulation in WM.

Methods

CD19+ selected bone marrow samples from 54 patients with WM, and CD19- peripheral blood mononuclear samples were used for tumor and germline controls, respectively. WGS and RNASeq were conducted as previously described (Hunter et al, Blood 2014; 2016) and methylation profiling was conducted using ERRBS at the Weil Cornell Medical Epigenomics Core and analyzed using Bismark. Results were filtered for methylation sites supported by at least 10 reads across all of the samples. Differential methylation analysis was conducted at the individual site level and aggregated promoter level using an established edgeR protocol. Differential methylation data was then compared with Salmon derived gene and isoform expression data which was available for 49/54 (91%) of the samples.

Disclosures:
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Treon: *Pharmacyclics: Consultancy, Other: Travel, Accommodations, Expenses, Research Funding; BMS: Research Funding; Johnson & Johnson: Consultancy; Janssen: Consultancy, Other: Travel, Accommodations, Expenses.*

Results

Clinical characteristics of patients were as follows: Median age 60 (range 40-87 years); BM involvement 43% (range 4-95%); serum IgM 3590 (range 416-8320 ug/dl). Most patients (67%) were untreated. WGS data revealed 33 (61%), 18 (33%), and 3 (6%) were *MYD88^{Mutant}/CXCR4$^{Wild\text{-}Type}$*, *MYD88^{Mutant}/CXCR4^{Mutant}* and *MYD88$^{Wild\text{-}Type}$/CXCR4$^{Wild\text{-}Type}$*, respectively. As was observed in our RNASeq analysis, pairwise multidimensional scaling of the methylation status of the top 2,000 high variance promoters revealed segregation of the MYD88^{Mutant}/CXCR4$^{Wild\text{-}Type}$ from both *MYD88^{Mutant}/CXCR4^{Mutant}* and *MYD88$^{Wild\text{-}Type}$/CXCR4$^{Wild\text{-}Type}$* (Figure 1A). Log fold change in methylation in differentially methylated promoters from MYD88^{Mutant}/CXCR4^{Mutant} and MYD88$^{Wild\text{-}Type}$/CXCR4$^{Wild\text{-}Type}$ samples relative to *MYD88^{Mutant}/CXCR4$^{Wild\text{-}Type}$* were negatively correlated with log-fold change in gene expression (Rho = -0.463, p <0.0001; Figure 1B). Promoter level analysis revealed 556 differentially methylated promoters in *MYD88^{Mutant}/CXCR4^{Mutant}* of which 440 (78%) had increased levels of methylation relative to *MYD88^{Mutant}/CXCR4$^{Wild\text{-}Type}$* samples. Affected genes included *IL15*, *GNAO1*, *HIF1A*, *SOCS6*, *PIK3R5*, *IRF8* and *CD38*. For MYD88$^{Wild\text{-}Type}$/CXCR4$^{Wild\text{-}Type}$ samples, 126 promoters were significantly differentially methylated with only 28 (22%) demonstrating increased methylation relative to MYD88^{Mutant}/CXCR4$^{Wild\text{-}Type}$ samples. Affected promoters included *NRIP1*, *FNBP1L*, and *PTK2*. Single site analysis revealed 127 and 1 significantly differentially methylated CpG loci for MYD88^{Mutant}/CXCR4^{Mutant} and MYD88$^{Wild\text{-}Type}$/CXCR4$^{Wild\text{-}Type}$, respectively. This analysis revealed a large intronic CpG island in *CARD11* that was demethylated only in *MYD88^{Mutant}/CXCR4$^{Wild\text{-}Type}$* patients and a methylated site in the *PPP1CC* promoter found in *MYD88$^{Wild\text{-}Type}$/CXCR4$^{Wild\text{-}Type}$*.

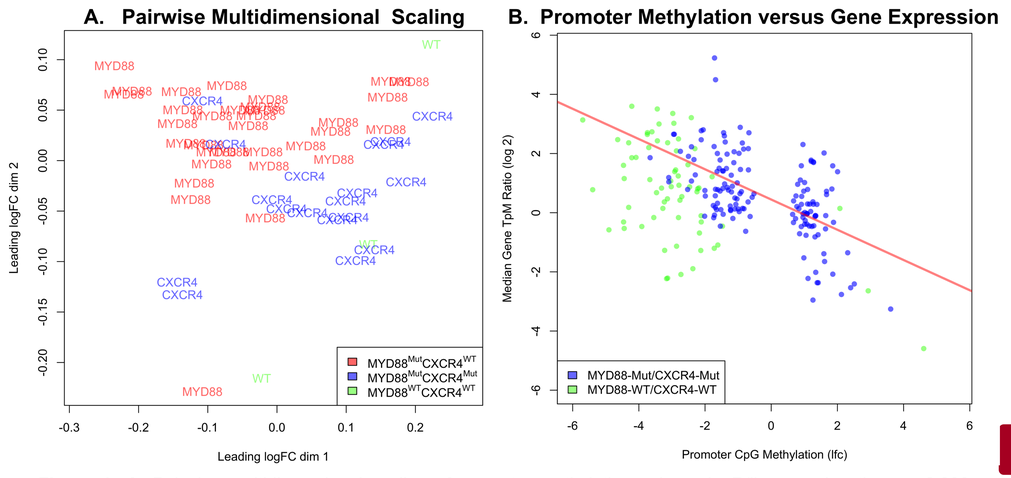
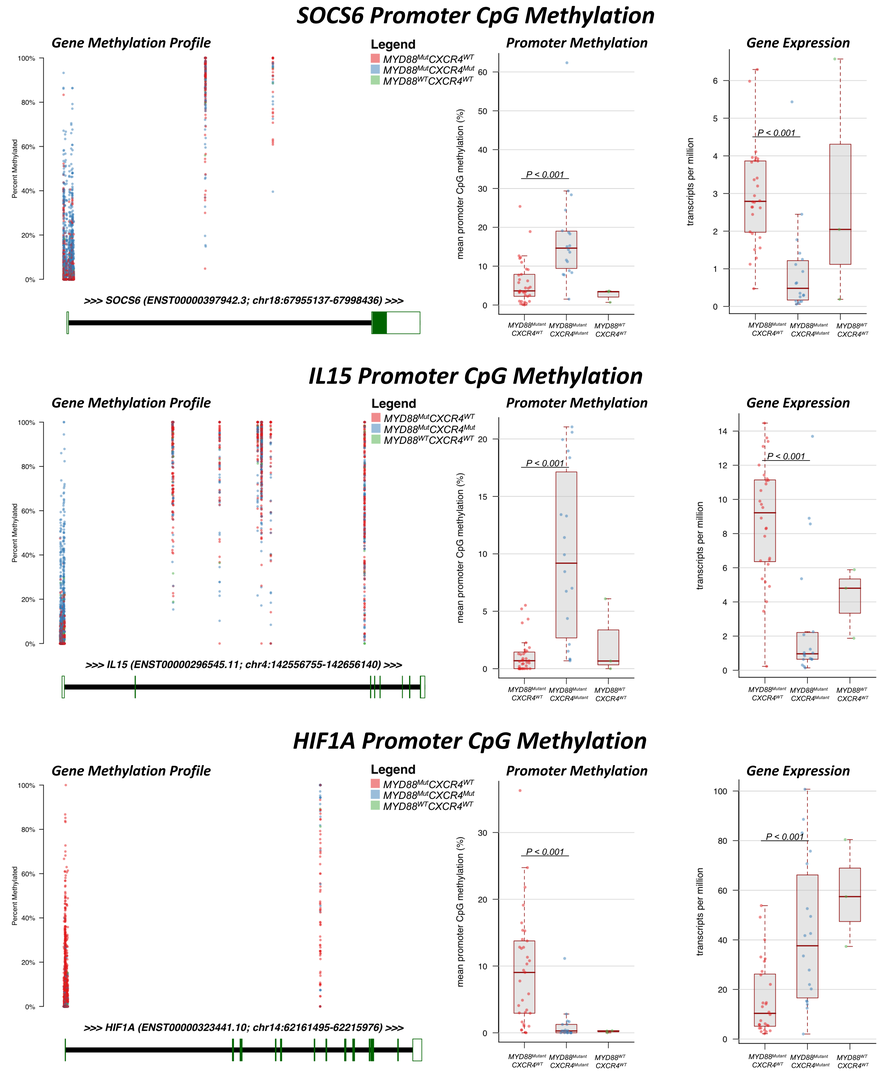


Figure 1: A. Pairwise multidimensional scaling of promoter methylation using edgeR/limma using the top 2,000 promoters with the highest variance in methylation shows segregation based on sample genotype. **B.** A strong correlation between the log fold change (lfc) of differentially methylated promoters and gene expression relative to MYD88^{Mutant}CXCR4$^{Wild\text{-}Type}$ (rho = -0.463, p <0.0001) was observed. This analysis was restricted to polyadenylated genes with a mean transcripts per million (TpM) of at least 0.1.

Differential CpG Methylation by Genotype



Conclusions

The studies provide the first comprehensive insights into epigenomic regulation of WM and show that *MYD88* and *CXCR4* mutation status drives methylation and confers a distinct transcriptomic profile that includes genes such as *IL15*, *SOCS6*, *CARD11*, *HIF1A*, and *PIK3R5* with important pro-survival and immune regulatory roles.