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 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.

Results

Clinical characteristics of patients were as follows: Median age 60 (range 40-97 years); BM involvement 43% (range 4-95%); serum IgM 3590 (range 416-8320 ug/d). Most patients (67%) were untreated. WGS data revealed 33 (61%), 18 (33%), and 3 (6%) were MYD88Mutant/CXCR4Wild-Type, MYD88Wild/Mutant/CXCR4Mutant and MYD88Wild-Type/CXCR4Wild-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 MYD88Mutant/CXCR4Wild-Type from both MYD88Wild/Mutant and MYD88Wild-Type/CXCR4Wild-Type (Figure 1A). Log fold change in methylation in differentially methylated promoters from MYD88Mutant/CXCR4Mutant and MYD88Wild-Type/CXCR4Wild-Type samples relative to MYD88Wild/Mutant/CXCR4Wild-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 MYD88Mutant/CXCR4Mutant of which 440 (78%) had increased levels of methylation relative to MYD88Wild/Mutant/CXCR4Wild-Type samples. Affected genes included IL15, GNAO1, HIF1A, SOCS5, PIK3R5, IRF8 and CD38. For MYD88Wild-Type/CXCR4Wild-Type samples, 126 promoters were significantly differentially methylated with only 28 (22%) demonstrating increased methylation relative to MYD88Wild-Type samples. Affected promoters included NRP1, FNBP1L, and PTK2. Single site analysis revealed 127 and 1 significantly differentially methylated CpG loci for MYD88Mutant/CXCR4Mutant and MYD88Wild-Type/CXCR4Wild-Type, respectively. This analysis revealed a large intronic CpG island in CARD11 that was demethylated only in MYD88Wild-Type/CXCR4Wild-Type patients and a methylated site in the PPP1CC promoter found in MYD88Wild-Type/CXCR4Wild-Type.

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, SOCS5, CARD11, HIF1A, and PIK3R5 with important pro-survival and immune regulatory roles.