Multi-omics analysis reveals a unique epigenetic signature in *MYD88* wild-type Waldenström macroglobulinemia

MYD88 L265P mutations (MUT) are found in approximately 90% of patients with Waldenström macroglobulinemia (WM) and lead to the activation of pro-survival signaling.[1,2] Prior studies have demonstrated that patients with *MYD88* wild-type (WT) WM have inferior responses to conventional therapies and a higher likelihood of histological transformation to an aggressive lymphoma.[3] The importance of the role of non-coding genomics in lymphomagenesis is being increasingly appreciated, but the epigenetic landscape of *MYD88*^{WT} WM remains largely underexplored.[4] Previously, our group showed how DNA methylation and non-coding RNA may alter the control of critical genes which may be responsible for the progression of IgM-monoclonal gammopathy to WM disease.[5-7] Given the demonstrated importance of these non-coding epigenetic regulators, the aim of this study was to utilize a multi-omics approach to assess the role of microRNA (miRNA) and DNA methylation in driving *MYD88*^{WT} WM biology through comparison with *MYD88*^{MUT} WM patients and healthy controls.

Bone marrow samples were prospectively collected from 20 subjects (3 with *MYD88*^{WT}/*CXCR4*^{WT} WM, 12 with *MYD88*^{MUT} WM [3 had CXCR4-mutated disease], and 5 healthy controls). WM was defined as ≥10% bone marrow involvement by lymphoplasmacytic lymphoma and serum IgM monoclonal protein of any size. Positive selection for CD19^{+} and/or CD138^{+} B cells was performed on samples utilizing STEMCELL CD19^{+} and CD138^{+} selection kits (17854, 17877). Clonal sorting was not performed. Analyses were conducted as per prior reports and focused on comparisons between *MYD88*^{WT} WM and normal controls and between *MYD88*^{WT} WM and *MYD88*^{MUT} WM patients.[5-7] Briefly, RNA differential expression for miRNA and mRNA was determined using a $\log_2$ fold change (FC) >0.5 or <-0.5 and false discovery rate (FDR) <0.05. Genome-wide DNA methylation analysis was performed, and CpG methylation ratios were segmented into 200 bp regions. Differentially methylated regions (DMR) were determined using a Q-value <0.05 and an absolute methylation difference ≥10%. The absolute methylation difference of ≥10% was established after investigation of different threshold values, as it allowed sufficient identification of DMR while effectively reducing background noise. Next, RNA-sequencing data from matched samples were integrated to the methylome and analyzed to identify differentially expressed genes ($\log_2$FC >0.5 or <-0.5 FDR <0.05) with corresponding promoter hypo- or hypermethylated DMR. For miRNA analysis, miRNA-mRNA target analysis was performed, and differentially expressed miRNA-mRNA pairs with a correlated biological expression (i.e., either upregulated miRNA experimentally predicted to regulate downregulated mRNA or *vice versa*) were selected. Ingenuity Pathway Analysis was used to determine potentially epigenetically regulated pathways using a corrected P<0.05. This study received approval from the Mayo Clinic Institutional Review Board.

The patients' characteristics for the whole cohort and stratified by *MYD88* mutation status are included in Table 1. Baseline characteristics were comparable between *MYD88*^{MUT} and *MYD88*^{WT} WM patients. All *MYD88*^{WT} WM patients were *CXCR4*^{WT}, and three patients from the *MYD88*^{MUT} WM cohort were *CXCR4*^{MUT}. The characteristics of the healthy control samples were not available because of institutional policy.

Comparing *MYD88*^{WT} WM samples to controls, a greater number of hypermethylated DMR were observed across genomic regions (hyper- *vs*. hypomethylated): promoter (29,129 *vs*. 6,300), 3' untranslated region (UTR) (2,068 *vs*. 1,551), 5'UTR (10,169 *vs*. 735), intron (30,344 *vs*. 24,511), exon (22,224 *vs*. 6,855), CpG island (30,372 *vs*. 1,240) and CpG shore (14,477 *vs*. 5,872) (all P<0.0001) (Figure 1A). Next, mRNA analysis demonstrated that 352 differentially expressed genes had concordant up-/downregulation with promoter hypo-/hypermethylated DMR.

On the assessment of promoter-methylation-based pathways, comparing *MYD88*^{WT} WM to controls, multiple pathways involved in cell signaling, proliferation, gene regulation, cytokine and immune signaling, and metabolism were found to be differentially regulated (Figure 1B). Most pathways were observed to be negatively enriched, including several members of interleukin (IL) signaling (IL-2, IL-3, IL-7) and regulators of cell proliferation and signaling, including mTOR, PI3K/AKT, and p53. Underlying multiple pathways were promoter hypermethylation and mRNA downregulation of *AKT3*, *MAP3K1*, and *PIK3CA* genes.

Next, methylation studies comparing *MYD88*^{WT} to *MYD88*^{MUT} WM samples demonstrated a similar hypermethylated profile in *MYD88*^{WT} with a statistically greater number of hypermethylated DMR observed across genomic regions (hyper- *vs*. hypomethylated): promoter (24,829 *vs*. 2,544), 3'UTR (3,534 *vs*. 378), 5'UTR (6,111 *vs*. 605), intron (52,292 *vs*. 5,360), exon (21,066 *vs*. 2,187), CpG island (18,334 *vs*. 1,551) and CpG shore (16,351 *vs*. 1,957) (all P<0.0001) (Figure 1C). Assessing mRNA expression, 212 differentially expressed genes had concordant up-/downregulation with promoter hypo-/hypermethylated DMR.

Methylation-based pathway analysis comparing *MYD88*^{WT} to *MYD88*^{MUT} WM samples showed downregulation of cytokine signaling, including IL-8, IL-17, and regulators of intracellular signaling, including RAC (Figure 1D). Of note, we have previously demonstrated reduced levels of IL-8 in WM and, in the present analysis, we found hypermethylation with associated downregulation of downstream RAS superfamily genes, including *RHOC* and *RRAS2* (Figure 1E).[8] Additionally, underlying multiple downregulated cytokine pathways (IL-8, IL-4, IL-13) was promoter hypermethylation with associated mRNA downregulation of *BCL2*, an important regulator of apoptosis, and a current therapeutic target in WM.[9] In relation to RAC inactivation, we observed potential upstream inhibition of this pathway via promoter hypermethylation of the integrin genes *ITGAM* (integrin alpha M) and *ITGB1* (integrin beta 1), both found to be downregulated.[10]

On miRNA analysis, comparing *MYD88*^{WT} WM to control samples, we observed 192 differentially expressed transcripts (70 downregulated; 122 upregulated) experimentally targeting 6,255 mRNA (Figure 2A). After target analysis and filtering, pathway analysis demonstrated multiple intracellular signaling, and metabolism pathways were downregulated (Figure 2B). Here, we observed miRNA-based downregulation of the RAC signaling pathway, a pathway also downregulated in our methylation analysis. Underlying this pathway was upregulation of miR-98-3p (FC: 2.4; FDR <0.005) and let-7a-5p (FC: 1.1; FDR <0.005) targets of *PIK3C3* and *MAP3K1*, respectively, found to be downregulated. These targets are members of the PI3K/AKT and MAPK family, and prior reports have described the role of miR-98 in regulating tumor progression via targeting of these pathways.[11] Relating back to our methylation analysis, we observed similar downregulation of PI3K/AKT and MAPK signaling comparing *MYD88*^{WT} WM to controls (Figure 1B); thus, these findings indicate multiple epigenetic regulators may influence key intracellular signaling pathways that regulate cytokine and immune function.

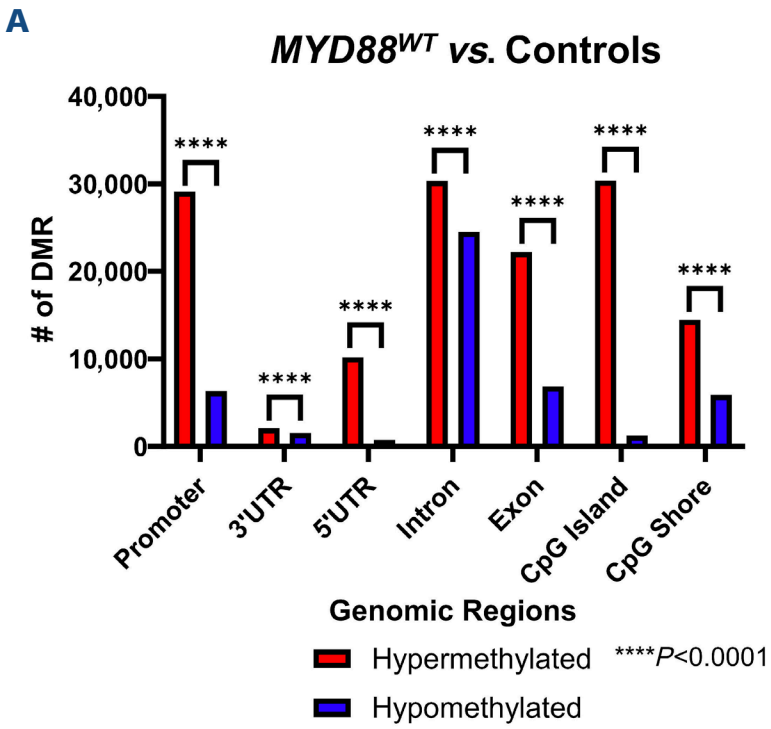
In comparing *MYD88*^{WT} to *MYD88*^{MUT} WM samples, our miRNA analysis identified 11 differentially expressed transcripts (all downregulated) experimentally targeting 3,011 differentially expressed mRNA (Figure 2C). Here, pathway analysis revealed upregulation of chromatin organization and histone modification signaling (Figure 2D). Underlying both pathways was the downregulation of miR-138-5p (FC: -9.8; FDR: 0.015), which targets *DNMT3A* and *DOT1L*, both of which were observed to be upregulated on mRNA analysis. Of relevance, miR-138-5p has been demonstrated to act as a tumor suppressor, and *DOT1L* is a histone methyltransferase involved in the regulation of mRNA transcription.[12,13] Furthermore, *DNMT3A* is a critical epigenetic regulator responsible for the addition of methyl groups at CpG sites; thus, upregulation of this gene corresponds with the CpG hypermethylation seen across genomic regions in *MYD88*^{WT} WM patients (Figure 2E).[14]

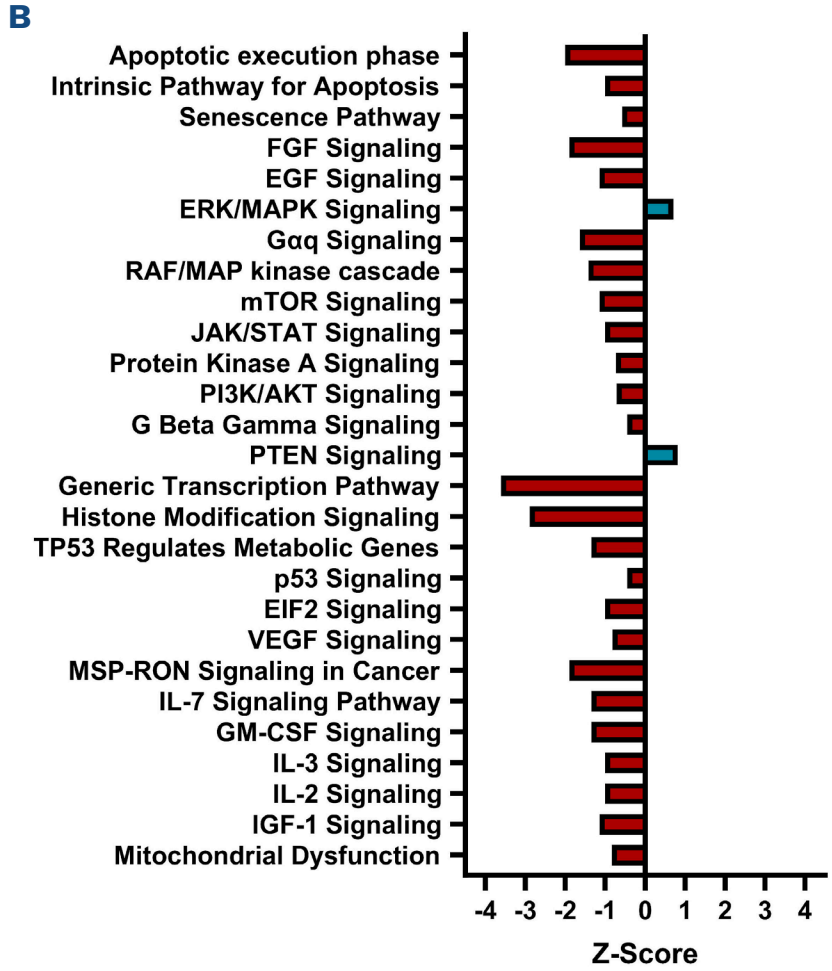
Overall, we identified for the first time a distinct epigenetic signature in *MYD88*^{WT} WM. An important limitation of this study is the smaller sample size of patients with *MYD88*^{WT} WM given the rarity of this diagnosis. To address this limitation, we sought to compare our findings with a previously published cohort of 18 patients with *MYD88*^{WT} disease.[15] In this prior report, utilizing gene set enrichment analysis comparing *MYD88*^{WT} to *MYD88*^{MUT} WM patients, significant enrichment for the upregulation of E2F, MYC, PI3K-AKT-MTOR, and G2M checkpoint signaling targets and the downregulation of inflammatory response genes and TNFA signaling via NF-κB was found.[15] Performing a similar mRNA-based gene set analysis comparing our *MYD88*^{WT} to *MYD88*^{MUT} WM samples, we observed a concordant pattern

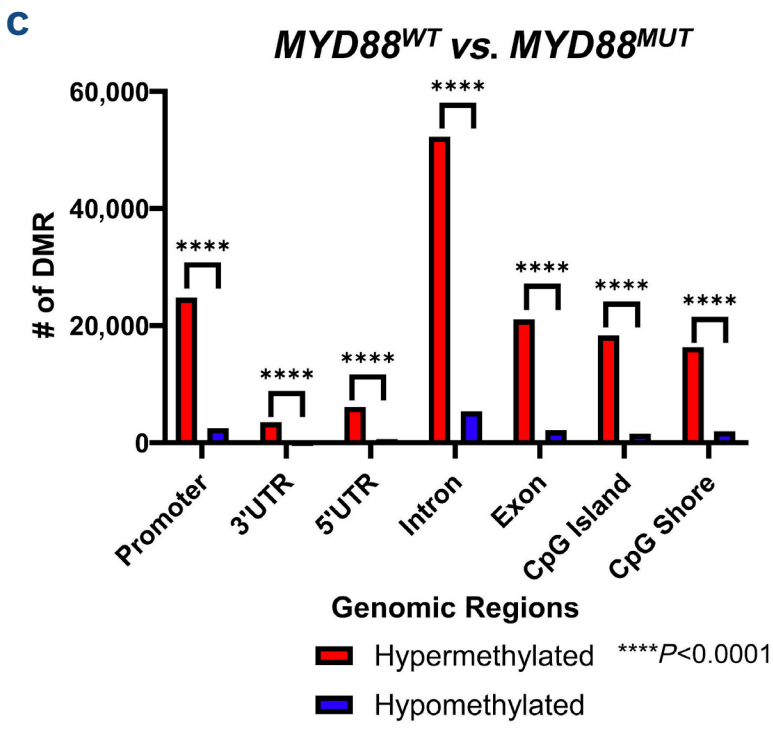
Table 1. Patients' characteristics of Waldenström macroglobulinema samples.

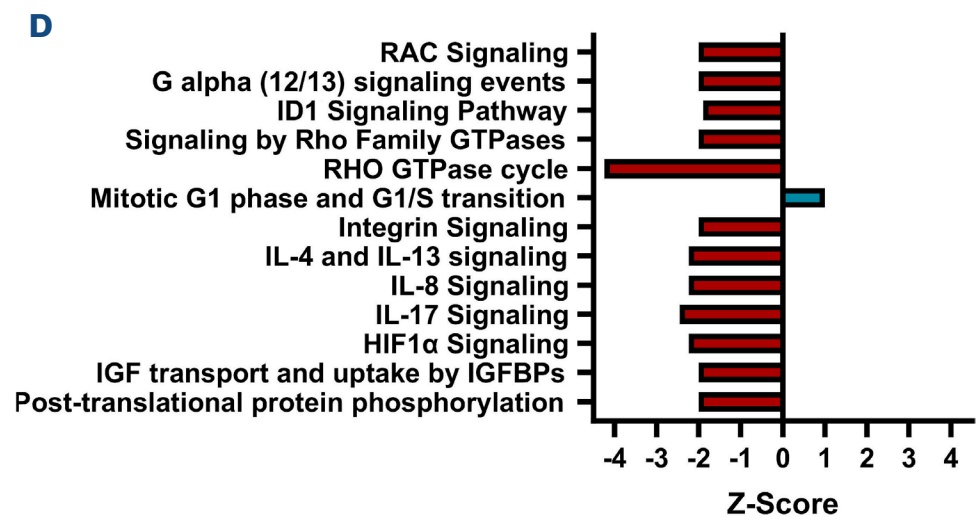
Parameter	All patients N=15	*MYD88*^{MUT} WM N=12	*MYD88*^{WT} WM N=3	*P*
Age at diagnosis, years, median (range)	60 (46-76)	61 (49-76)	48 (46-60)	0.07
Male sex, N (%)	9 (60)	7 (58)	2 (67)	0.79
Hemoglobin, g/dL, median (range)	12.2 (9.5-15.8)	12.0 (9.5-15.8)	12.7 (11.1-13.4)	0.72
Platelets, x10^9/L, median (range)	213 (96-512)	193 (96-512)	242 (213-348)	0.68
β_2 microglobulin, μg/mL, median (range)	3.01 (1.64-5.91)	3.07 (1.64-5.91)	3.04 (1.75-3.07)	0.35
IgM, mg/dL, median (range)	2,240 (173-10,000)	2,790 (173-10,000)	1,400 (604-3,400)	0.43
BM involvement, %, median (range)	50 (10-90)	50 (10-90)	20 (20-40)	0.15
Abnormal FLC ratio, N (%)	3 (23)	3 (30)	0	0.28
CXCR4-mutated, N (%)	3 (20)	3 (25)	0	0.33
Dx to LFU, months, median (range)	87.4 (29.4-142.3)	91.0 (29.4-142.3)	80.0 (47.6-92.2)	0.63

All characteristics at the time of sample collection. MUT: mutated; WM: Waldenström macroglobulinemia; WT: wild-type; N: number; IgM: immunoglobulin M; BM: bone marrow; Dx: diagnosis; LFU: last follow-up; FLC: free light chain; FLC data were available for 13 patients (WT: 3; MUT: 10).









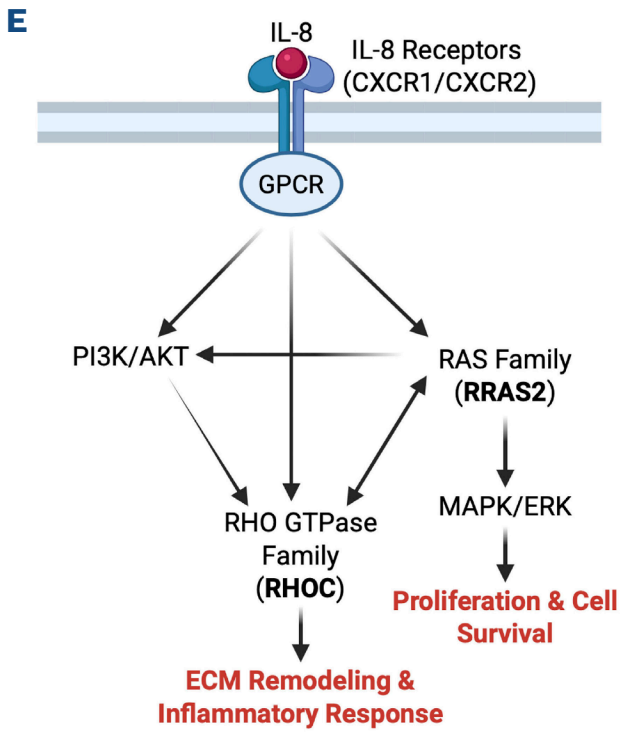


Figure 1. Genome-wide methylation analyses with promoter-methylation-based pathway integration. (A, B) Analysis evaluating differentially methylated regions (DMR) comparing patients with *MYD88*^{WT} Waldenström macroglobulinemia (WM) to controls (A) and methylation-based pathway analysis evaluating promoter methylation and mRNA expression (B). (C, D) DMR analysis comparing WM patients with *MYD88*^{WT} to those with *MYD88*^{MUT} (C) with corresponding pathway analysis (D). (E) IL-8 pathway analysis demonstrating downstream involvement of RHOC and RRAS2.

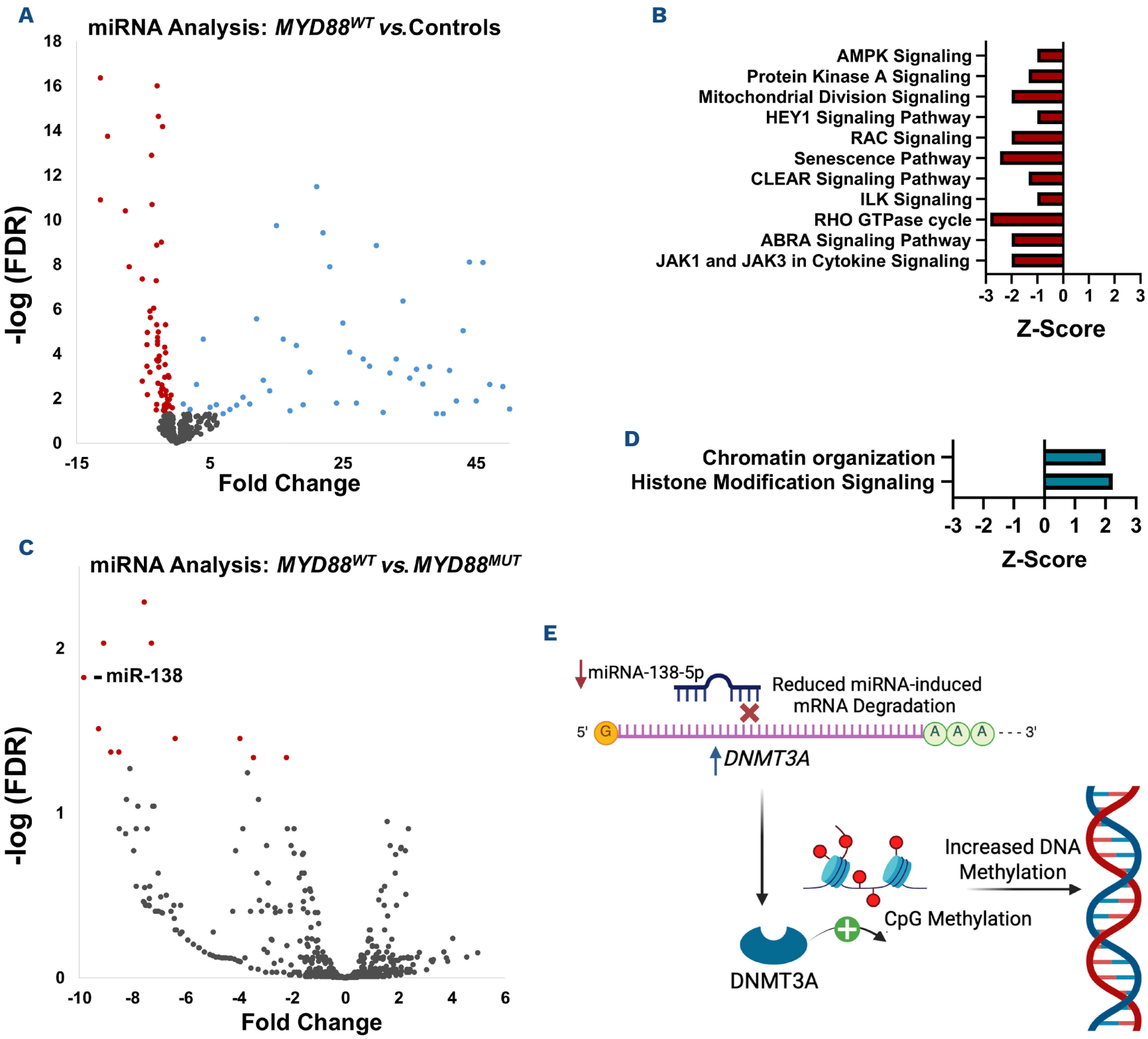


Figure 2. MicroRNA (miRNA) differential expression with miRNA-based pathway analysis. (A, B) Volcano-plot demonstrating differential expression miRNA between patients with *MYD88*^{WT} Waldenström macroglobulinemia (WM) and controls (A) and miRNA-based pathway analysis integrating mRNA differential expression (B). (C, D) miRNA differential expression comparing patients with *MYD88*^{WT} and *MYD88*^{MUT} WM (C) with miRNA-based pathway analysis (D). (E) A postulated mechanism whereby downregulation of miR-138-5p permits increased DNMT3A expression and consequent CpG hypermethylation. Volcano-plots demonstrate $\log_2$(fold change) data and false discovery rate (FDR).

(*Online Supplementary Table S1*) with positive enrichment of E2F, MYC, MTOR and G2M checkpoint signaling, and negative enrichment of TNFA signaling via NF-κB and inflammatory response signaling (all $P<0.05$). These consistent patterns offer external support for the observations presented in the current study.

A central feature of our analysis of *MYD88*^{WT} WM patients was the epigenetic inactivation of cytokine and intracellular pathways involved in immune regulation, including the IL-8 and the PI3K/AKT/mTOR pathways. Although constitutional activation of MYD88 serves a critical role in the pro-inflammatory signaling observed in *MYD88*^{MUT} WM patients, these findings suggest that both methylation and miRNA-based mechanisms may epigenetically regulate the reduced inflammatory signaling in *MYD88*^{WT} WM beyond only the mutation in *MYD88*.[1,2] In relation to treatment, given that BTK inhibitor efficacy relies on suppression of activated inflammatory pathways, these findings also suggest a potential non-coding mechanism for the reduced response to BTK inhibitors in patients with *MYD88*^{WT} WM. Furthermore, our observation of BCL2 promoter hypermethylation with mRNA downregulation

indicates that an additional epigenetic mechanism may confer resistance to venetoclax in *MYD88*^{WT} WM, which has been predominantly evaluated in patients with *MYD88*^{MUT} disease.[9]

Overall, the results of this study offer novel insights into the biology of *MYD88*^{WT} WM, suggest its epigenetic distinction from *MYD88*^{MUT} WM, and propose how DNA methylation and non-coding RNA may contribute to the unique clinical phenotype of patients with this disease. Some of the key epigenetic findings identified, such as the non-coding downregulation of cytokine and inflammatory signaling pathways, may play a role in the inferior outcomes and reduced response to conventional WM-directed therapies in patients with *MYD88*^{WT} WM. Larger studies are needed to investigate the epigenetic similarities between *MYD88*^{WT} WM and other B-cell lymphomas, including marginal zone lymphoma, which may help to improve biological subclassification. Furthermore, functional studies are needed to validate these findings at the proteomic level and explore the potential utility of epigenetic therapies for the treatment of *MYD88*^{WT} WM.

Authors

Karan Chohan,[1*] Jonas Paludo,[1*] Surendra Dasari,[2] Joseph P. Novak,[1] Jithma P. Abeykoon,[1] Saurabh Zanwar,[1] Zhi-Zhang Yang,[1] Shahrzad Jalali,[1] Vaishali Bhardwaj,[1] Jordan E. Krull,[1] Esteban Braggio,[3] Michelle K. Manske,[1] Craig B. Reeder,[4] Sikander Ailawadhi,[4] Asher Chanan-Khan,[4] Prashant Kapoor,[1] Robert A. Kyle,[1] Morie A. Gertz,[1] Anne J. Novak,[1] Patrizia Mondello[1] and Stephen M. Ansell[1]

[1]Division of Hematology, Mayo Clinic, Rochester, MN; [2]Department of Health Sciences Research, Mayo Clinic, Rochester, MN; [3]Division of Hematology and Medical Oncology, Mayo Clinic, Scottsdale, AZ and [4]Division of Hematology and Oncology, Mayo Clinic, Jacksonville, FL, USA

**KC and JP contributed equally as first authors.*

Correspondence:
S.M. ANSELL - ansell.stephen@mayo.edu

https://doi.org/10.3324/haematol.2025.289068

Received: August 28, 2025.
Accepted: November 24, 2025.
Early view: December 4, 2025.

Disclosures

SMA receives research funding from Bristol-Myers Squibb, Seattle Genetics, Affimed Therapeutics, Regeneron, Trillium Therapeutics, AI Therapeutics and ADC Therapeutics. The other authors have no conflicts of interest to disclose.

Contributions

KC, JP, SD and SMA designed this study, analyzed/interpreted the data, and wrote the manuscript. JPN, JPA, SZ, Z-ZY, SJ, VB, JEK, EB, MKM, CBR, SA, AC-K, PK, RAK, MAG, AJN and PM interpreted the data and assisted in writing the manuscript. All authors provided final approval of the manuscript and are accountable for all aspects of the work.

Funding

This study was supported by research funding from the BINK Foundation, and the International Waldenström Macroglobulinemia Foundation (IWMF) to SMA and the Mayo Clinic. PM is supported by the NCI K08CA279652, LRF Clinical Investigator Award (CDA 1020588), the Gerstner Family Career Development Award, the ASH Junior Faculty Scholar Award, the IWMF/LLS Strategic Research Roadmap Initiative, and the IWMF/LLS Enhanced Research Roadmap Initiative.

Data-sharing statement

RNA data are available at GEO under the accession numbers GSE232994 and GSE232995. Methylation data are available on Zenodo under the record number: 11206554. For additional data on the patients, please contact the corresponding author.

References

1. Treon SP, Xu L, Yang G, et al. MYD88 L265P somatic mutation in Waldenström's macroglobulinemia. N Engl J Med. 2012;367(9):826-833.
2. Varettoni M, Arcaini L, Zibellini S, et al. Prevalence and clinical significance of the MYD88 (L265P) somatic mutation in Waldenström's macroglobulinemia and related lymphoid neoplasms. Blood. 2013;121(13):2522-2528.
3. Zanwar S, Abeykoon JP, Durot E, et al. Impact of MYD88(L265P) mutation status on histological transformation of Waldenström macroglobulinemia. Am J Hematol. 2020;95(3):274-281.
4. Yang H, Green MR. Harnessing lymphoma epigenetics to improve therapies. Blood. 2020;136(21):2386-2391.
5. Chohan K, Paludo J, Dasari S, et al. MicroRNA and long non-coding RNA analysis in IgM-monoclonal gammopathies reveals epigenetic influence on cellular functions and oncogenesis. Haematologica. 2024;109(5):1570-1575.
6. Chohan K, Paludo J, Dasari S, et al. Multiomics analysis of IgM monoclonal gammopathies reveals epigenetic influence on oncogenesis via DNA methylation. Blood. 2024;144(12):1284-1289.
7. Mondello P, Paludo J, Novak JP, et al. Molecular clusters and tumor-immune drivers of IgM monoclonal gammopathies. Clin Cancer Res. 2023;29(5):957-970.
8. Elsawa SF, Novak AJ, Ziesmer SC, et al. Comprehensive analysis of tumor microenvironment cytokines in Waldenström macroglobulinemia identifies CCL5 as a novel modulator of IL-6 activity. Blood. 2011;118(20):5540-5549.
9. Castillo JJ, Allan JN, Siddiqi T, et al. Venetoclax in previously treated Waldenström macroglobulinemia. J Clin Oncol.

2022;40(1):63-71.
10. Lawson CD, Burridge K. The on-off relationship of Rho and Rac during integrin-mediated adhesion and cell migration. Small GTPases. 2014;5:e27958.
11. Akhlaghipour I, Moghbeli M. MicroRNA-98 as a novel diagnostic marker and therapeutic target in cancer patients. Discov Oncol. 2024;15(1):385.
12. Yeh M, Wang Y-Y, Yoo JY, et al. MicroRNA-138 suppresses glioblastoma proliferation through downregulation of CD44. Sci Rep. 2021;11(1):9219.
13. Wong M, Polly P, Liu T. The histone methyltransferase DOT1L: regulatory functions and a cancer therapy target. Am J Cancer Res. 2015;5(9):2823-2837.
14. Yang L, Rau R, Goodell MA. DNMT3A in haematological malignancies. Nat Rev Cancer. 2015;15(3):152-165.
15. Hunter ZR, Xu L, Tsakmaklis N, et al. Insights into the genomic landscape of MYD88 wild-type Waldenström macroglobulinemia. Blood Adv. 2018;2(21):2937-2946.