MLL1 inhibition reduces IgM levels in Waldenström macroglobulinemia

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Abstract

Waldenström macroglobulinemia (WM) is a B cell lymphoma characterized by the overproduction of a monoclonal IgM antibody, a leading pathogenic feature of the disease. Current therapies are based on our knowledge at the signaling and genetic scale, but recent research has identified epigenetic dysregulation, one of the important dynamics in the biology of this disease. In this study, we found that Mixed-lineage leukemia 1 (MLL1) histone methyltransferase and its chromatin tethering partner Menin are upregulated in WM patients. *KMT2A* knockdown using short hairpin RNA (shRNA) and inhibition of MLL1 function using the menin-MLL1 inhibitor (MI-2) in WM cells resulted in a significant reduction in IgM levels without significantly impacting WM cell growth and survival. Further analysis identified MLL1 binding at multiple sites in the 5' Em enhancer of the immunoglobulin heavy (IGH) chain. We found increased histone 3 lysine 4 trimethylation (H3K4me3) enrichment at multiple MLL1 binding sites upon LPS stimulation, a known inducer of IgM. Finally, we found that disruption of Menin-MLL1 complex using the MI-2 inhibitor in tumor-bearing mice significantly reduced human IgM levels in mice sera. Taken together, these results identify MLL1 as a regulator of IgM and define MLL1 as a new therapeutic target for WM.

Results

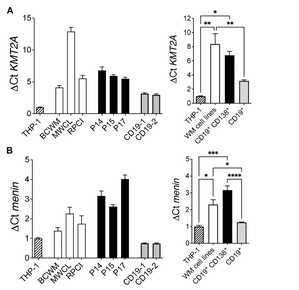


Figure 1: *KMT2A* and *Menin* are overexpressed in WM patients and cell lines. Expression of A) *KMT2A* and B) *MEN1* in WM cells lines (BCWM.1, MWCL-1 and RPCI-WM1), CD19$^+$ CD138$^+$ cells from WM patients (P14, P15 and P17), normal CD19$^+$ cells from PBMCs were compared to expression in THP-1 cells. Right panels represent the average expression of *KMT2A* and *MEN1*. Bars represent ΔCt relative to THP-1 +/- SEM.

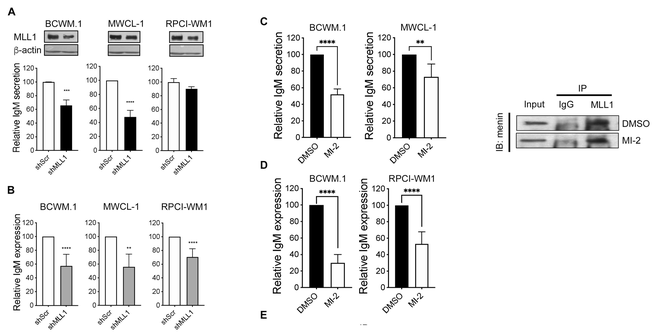


Figure 2: MLL1 depletion and disruption of menin-MLL1 complex reduce Cμ expression and secretion in WM. A) WM cells (4 x 10^6) were transfected with shMLL1 or scrambled control (shScr) for 48 hr followed by quantification of IgM levels in the culture supernatant by ELISA. An additional set of cells were transfected and lysed in RPIA buffer followed by western blot to confirm MLL1 knockdown (blots shown above each graph). B) WM cells were transfected with shMLL1 or shScr for 48 hr. Cells were harvested and RNA was purified and used to determine Cμ expression by qRT-PCR. C) WM cells (2 x 10^6) were treated with 5 mM MLL1 inhibitor MI-2 or DMSO control for 72 hr followed by ELISA to quantify IgM levels or D) qRT-PCR to determine Cμ expression. These experiments were repeated at least 3 times with similar results and the bars represent the average of 3 independent experiments performed in triplicate +/- SEM. E) Immunoprecipitation assay was performed to assess inhibition of Menin-MLL1 interaction following treatment with 5 mM MI-2. BCWM.1 cells were lysed and MLL1 protein was immunoprecipitated using aMLL1 antibody. IgG pull-down was used as negative control. Menin protein was detected by western blot using a Menin antibody.

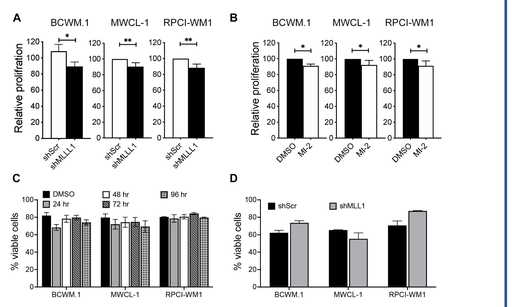
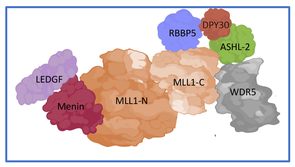


Figure 3: MLL1 inhibition does not affect WM cell viability. A) WM cells (2 x 10^6) were transfected with shMLL1 or shScr. Cells were resuspended in 0.6 ml and 100 ml were plated/well in 96-well plates. After 3 days of culture, cell proliferation was assessed using XTT assay. B) WM cells (25 x 10^3 cells/well) were plated in 96-well plates and treated with either 5 mM MI-2 or DMSO control. After 3 days, cell proliferation was assessed using XTT. C) WM cells (0.25 x 106 cells/ml) were cultured in triplicated wells in 24-well plates and treated with 5 mM MI-2 or DMSO control. Cell viability was assessed daily using trypan blue exclusion. D) WM cells (4 x 10^6) were transfected with shMLL1 or shScr for 72 hr followed by examination of cell viability by trypan blue exclusion. Bars represent the average of at least 3 independent experiments performed in triplicate +/- SEM.



MLL1 histone methyltransferase (HMT) complex.

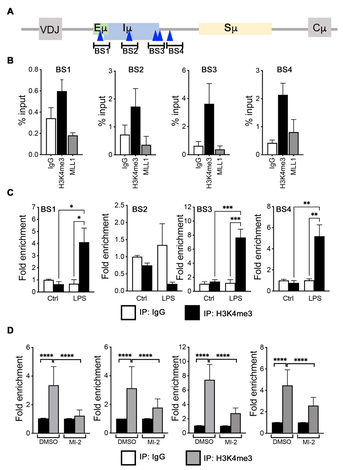


Figure 4: H3K4me3 deposition near IGH Eμ enhancer and Im region. A) Schematic of IGH region. Variable region (VDJ) and constant region (Cm) are shown in gray. Blue triangles represent five candidate MLL1 binding sites. The Eμ enhancer (green), Iμ region (blue) and switch (Sm) region are indicated. Black brackets indicate primers used for ChIP-qPCR to detect binding to MLL1 binding sites (BS1, BS2, BS3 and BS4). B) BCWM.1 cells (10 x 10^6) were fixed and lysed as indicated in materials and methods. Lysates were used in ChIP assay followed by qPCR to determine basal MLL1 or it's mark H3K4me3 deposition in the IGH region in WM cells. C) CD19+ cells were purified from PBMCs followed by stimulation with LPS for 3 hr to activate B cells. Cells were then used in ChIP-qPCR to determine H3K4me3 deposition in the IGH region. D) BCWM.1 cells were treated with 5 mM MI-2 or DMSO control and cells were used to determine H3K4me3 deposition in IGH region by ChIP-qPCR. These experiments were performed at least 3 times.

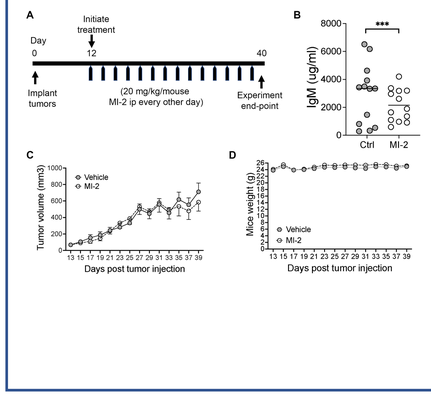


Figure 5: MLL1 inhibition reduces IgM secretion in tumor-bearing mice. A) Hariless SCID mice were implanted with 10 x 10^6 BCWM.1 cells. Groups of mice were treated with either 5 mM MI-2 or vehicle control. B) Human IgM levels were quantified in mice sera at experiment end-point using ELISA. C) Tumor volume in MI-2 and Vehicle treated mice. D) Mice weight was determined in MI-2 and vehicle treated mice.

Summary

- *KMT2A* and *MEN1* expression are elevated in WM patients and WM cell lines and promotes disease biology.
- Inhibition of menin-MLL1 reduces IgM expression and secretion *in vitro* and *in vivo*.

Acknowledgement

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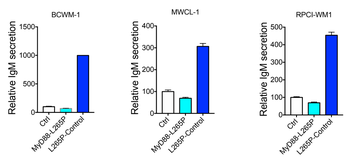
We thank Dr. Steven Treon and Dr. Asher Chanan-Khan for providing the BCWM.1 and RPCI-WM1 cells and Dr. Anne Novak for providing the MyD88 expression constructs.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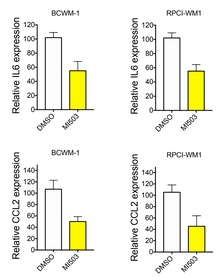
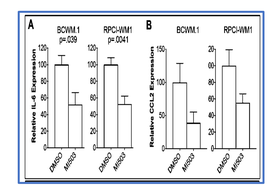
MWCL-1
RPCI-WM1
Relative IgM secretion
Ctrl
MyD88-L265P
L265P-Control


BCWM-1
RPCI-WM1
Relative IL6 expression
Relative CCL2 expression
DMSO
MI503


A
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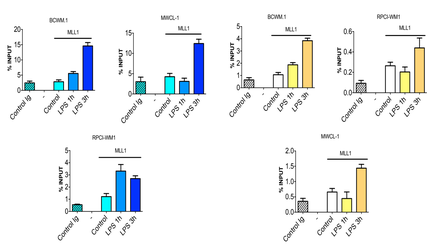
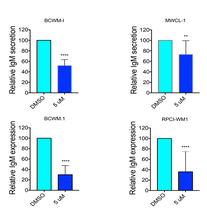
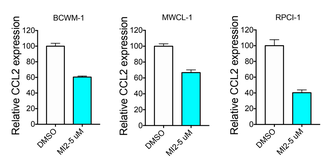
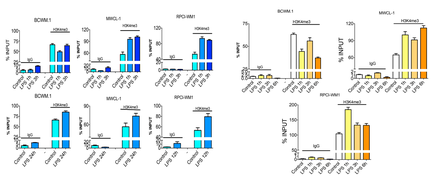
RPCI-WM1
p= 039
p= 0041
Relative IL-6 Expression
B
Relative CCL2 Expression
DMSO
MI503


BCWM.1
MWCL-1
RPCI-WM1
MLL1
% INPUT
Control Ig
Control
LPS 1h
LPS 3h

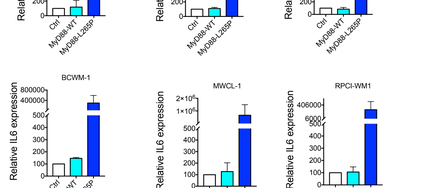

BCWM-I
MWCL-1
BCWM.1
RPCI-WM1
Relative IgM secretion
Relative IgM expression
DMSO
5 uM

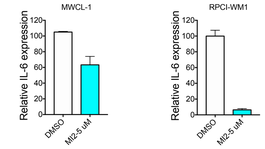

BCWM-1
MWCL-1
RPCI-1
Relative CCL2 expression
DMSO
MI2-5 uM

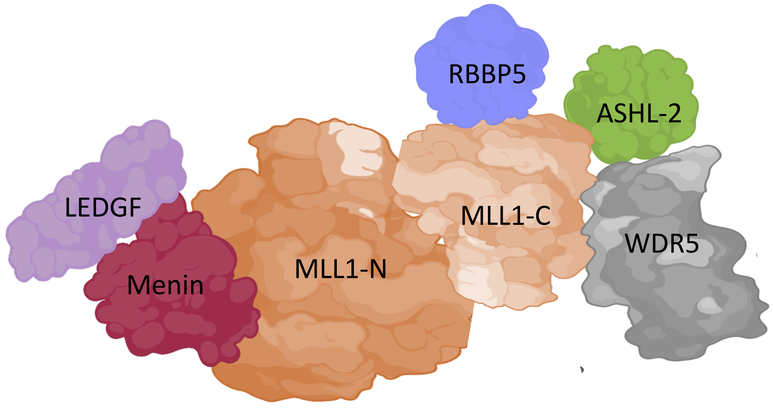

BCWM.1
MWCL-1
RPCI-WM1
H3K4me2
H3K4me3
% INPUT
IgG
Control
LPS 1h
LPS 3h

BCWM.1
MWCL-1
H3K4me3
% INPUT
IgG
Control
LPS 1h
LPS 3h

RPCI-WM1
H3K4me3
% INPUT
IgG
Control
LPS 1h
LPS 3h


BCWM-1
MWCL-1
RPCI-WM1
Relative IgM expression
Relative IL6 expression
Ctrl
MyD88-WT
MyD88-L265P


MWCL-1
RPCI-WM1
Relative IL-6 expression
DMSO
MI2-5 uM


RBBP5
ASHL-2
LEDGF
MLL1-C
WDR5
MLL1-N
Menin