MLL1 modulates IgM and inflammatory cytokines in Waldenström macroglobulinemia

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Abstract

Waldenström macroglobulinemia (WM) is a low-grade B-cell lymphoma associated with the accumulation of lymphoplasmacytic cells in the bone marrow (BM) and a high level of monoclonal IgM protein in the serum. Genome-wide studies revealed genomic alternations in WM, with the most prevalent being a point mutation in the myeloid differentiation primary response gene 88 (MyD88) resulting in an amino acid change (L265P) that is present in over 90% of WM patients. MyD88 is an effector of Toll-like receptor (TLR) signaling pathway that mediates inflammatory cytokine expression and secretion upon activation. We have identified a change in the chromatin landscape in response to TLR stimulation in WM cells. We found increased levels of trimethylation of histone 3 lysine 4 (H3K4me3) at the promoters of the inflammatory cytokines IL-6 and CCL2 by chromatin immunoprecipitation (ChIP) assay followed by qPCR. This modification is catalyzed by six related homologs of the yeast histone methyltransferase (HMT) family. In addition, we have identified the Mixed-lineage leukemia 1 (MLL1) as the enzyme bound to these promoters in response to TLR-MyD88 stimulation. Analysis of WM cell lines and primary WM patient cells showed that MLL1 and its binding partner, menin, are expressed at significantly higher levels in CD19+CD138+ cells from WM patients compared to CD19+ cells from peripheral blood . We found an increase in H3K4me3 deposition on IL-6 and CCL2 promoters during early (1-3 hr) and late (12-24 hr) kinetics following TLR-MyD88 stimulation with LPS. This also coincided with increased deposition of MLL1 on these cytokine promoters by ChIP-qPCR. Disruption of menin-MLL1 using the MI-2 or MI-503 menin-MLL1 inhibitors significantly reduced IL-6 and CCL2 expression in WM cell lines. Finally, MLL1 knockdown using RNAi significantly reduces IgM expression in BCWM.1, MWCL and RPCI-WM1 and secretion and treatment of WM cell lines with the menin-MLL1 inhibitor significantly reduced IgM expression and secretion. Taken together, these results identify a novel role for menin-MLL1 in regulating inflammatory cytokines and IgM secretion in WM and provide the rationale for targeting these molecules in WM patients.

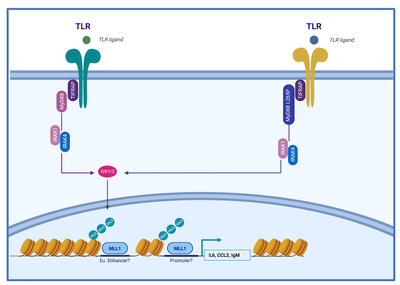

Fig. 1: TLR-MyD88L265P-ERK1/2-MLL1 axis mediated regulation of inflammation and tumor growth in WM.

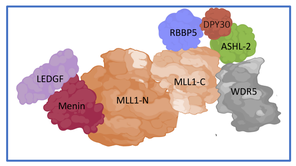

Fig. 2: MLL1 histone methyltransferase (HMT) complex.

Results

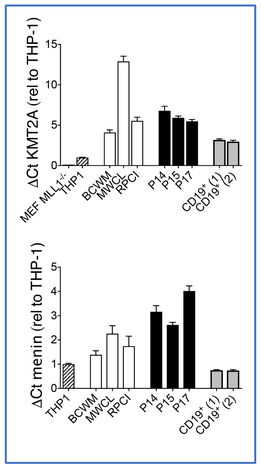

Fig. 3: KMT2A and menin expression in WM cells lines and CD19+ CD138+ cells from WM patients.

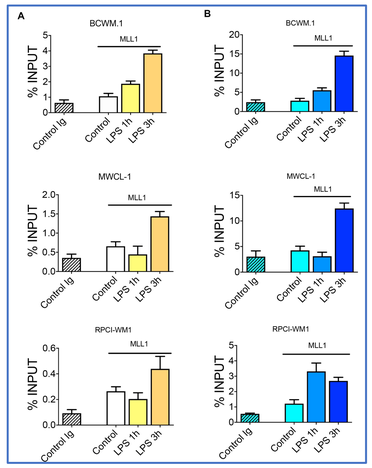

Fig. 4: TLR-MyD88L265P stimulation induces MLL1 deposition on cytokine promoters. WM cells were stimulated with LPS followed by ChIP-qPCR to determine MLL1 binding to (A) IL-6 and (B) CCL2 promoter regions.

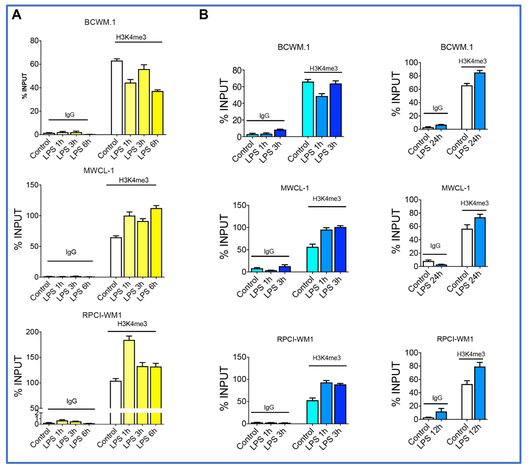

Fig. 5: TLR-MyD88L265P stimulation increases H3K4me3 deposition on cytokine promoters. WM cells were stimulated with LPS and ChIP-qPCR was performed at the indicated times to determine H3K4me3 deposition on (A) IL-6 and (B) CCL2 promoters.

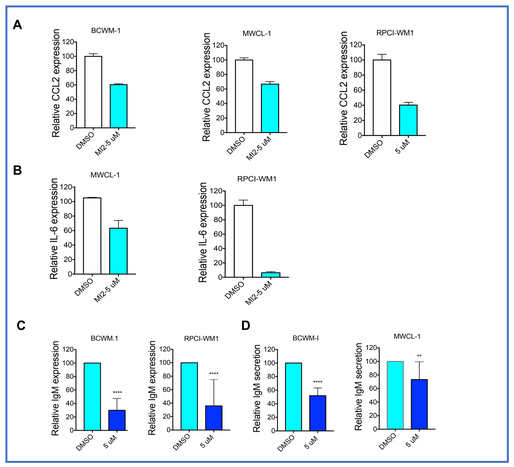

Fig. 6: Disruption of menin-MLL1 reduces IgM and cytokine expression in WM. WM cells were treated with menin-MLL1 inhibitor MI-2 followed by qPCR to determine (A) CCL2, (B) IL-6, (C) IgM expression and (D) IgM secretion.

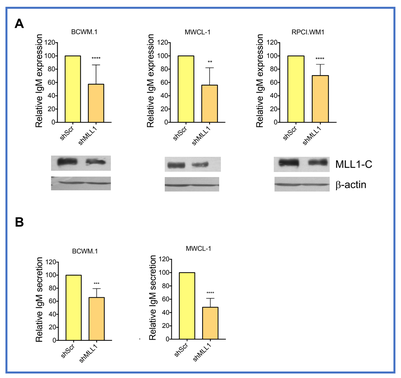

Fig. 7: MLL1 knockdown reduces IgM expression and secretion in WM. WM cells were transfected with shRNA targeting KMT2A gene or scrambled control followed by (A) qPCR to determine IgM expression and western blot to determine KMT2A/MLL1 expression; and (B) IgM secretion.

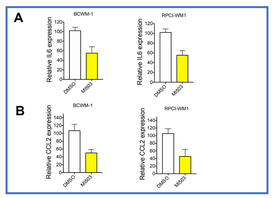

Fig.8: Disruption of menin-MLL1 reduces cytokine expression in WM. WM cells were treated with menin-MLL1 inhibitor Mi503 followed by qPCR to determine (A) IL-6 and (B) CCL2 expression.

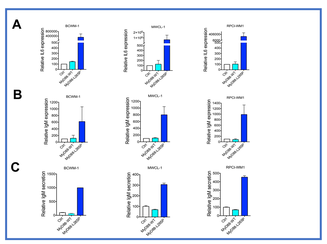

Fig. 9: MyD88-L265P induces IL-6 and IgM expression and secretion in WM. WM cells were transfected with WT MyD88, MyD88-L265P or empty vector followed by qPCR and ELISA to determine (A) IL-6 expression; (B) IgM expression and (C) IgM secretion.

Summary

- KMT2A and Menin expression are elevated in WM patient cells and WM cell lines and promotes disease biology.
- MLL1 is an effector of TLR-MyD88-L265P-induced gene expression in WM.
- Inhibition of menin-MLL1 reduces IL-6, CCL2 and IgM expression.
- MyD88-L265P (but not WT MyD88) induces increased expression of IL-6 and IgM in WM cells.

Acknowledgement

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