Targeting Myddosome Self-assembly in Waldenström's Macroglobulinemia

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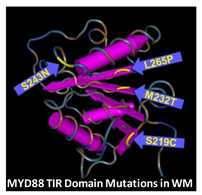
Background

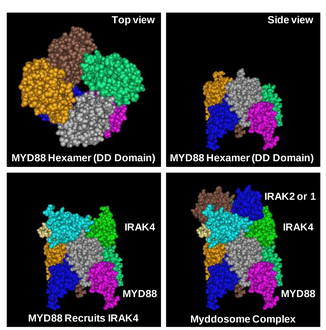
MYD88 mutations are present in over 95% of patients with Waldenstrom's Macroglobulinemia (WM) (*N Engl J Med,* 373:584-86), and promote Myddosome self-assembly that triggers NF-κB dependent survival through BTK and IRAK1/IRAK4 (*Blood,* 122(7):1222-32). While current therapeutic strategies are aimed at blocking these downstream kinases, peptidomimetics that interfere with Myddosome self-assembly may offer a more targeted approach for blocking aberrant MYD88 signaling.



MYD88 TIR Domain Mutations in WM

MYD88 mutations are present in over 95% (91% is L265P mutation) of patients with Waldenstrom's Macroglobulinemia (WM) (*N Engl J Med,* 373:584-86). All of these mutations are located on the TIR domain of MYD88 protein.

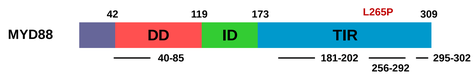
MYD88 mutations cause the TIR domain conformation change and initiate MYD88 homodimerization followed by hexamerization and recruit IRAK4 tetramer, then IRAK2 or IRAK1 tetramer to form a signaling complex – Myddosome. Both MYD88 TIR domain and DD domain are responsible for Myddosome self-assembly.



Methods

We expressed mini-peptides of MYD88 Toll/Interleukin-1 Receptor (TIR) or Death Domain (DD) sequences (indicated on figure below) in GFP fusion protein by lentiviral transduction in mutated MYD88 WM and wild-type MYD88 control cells with an inducible vector. Phospho-flow analysis was used to evaluate changes in pBTK, pIRAK1/IRAK4, and pNF-kB, and determined cell growth and survival by AlamarBlue® Assay, Annexin V staining.

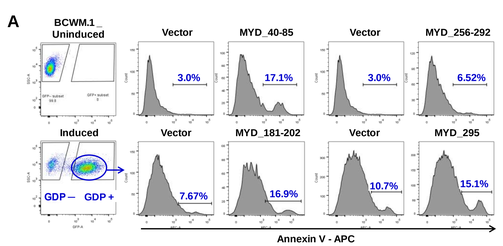
MYD88 domain structure and mini-peptides

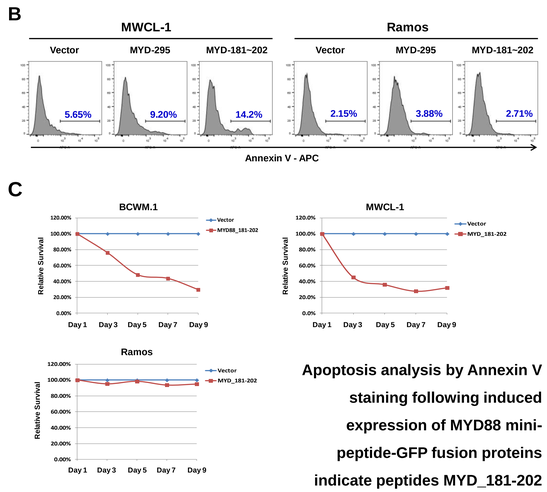


DD: death domain
ID: intermediate domain
TIR: TIR domain

Results

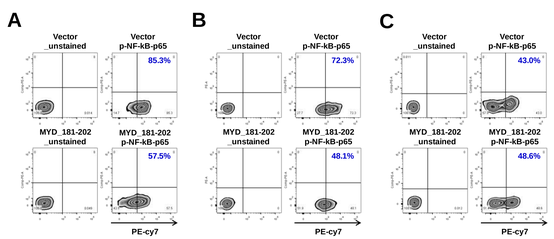
Transduction of TIR or DD mini-peptides in mutated MYD88 WM cells but not wild-type MYD88 control cells reduced tumor cell growth and survival.





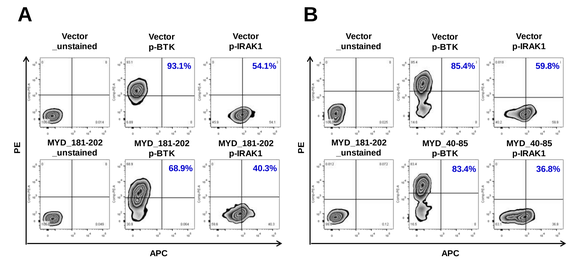
Apoptosis analysis by Annexin V staining following induced expression of MYD88 mini-peptide-GFP fusion proteins indicate peptides MYD_181-202 and MYD_40-85 induced more pronounced cell death than MYD_256-292 and MYD_295-302 in BCWM.1 cells (A). Similarly, a significant apoptosis was induced by mini-peptide MYD_181-202 in MWCL-1 cells but not in MYD88 wild type lymphoma cell line, Ramos (B). The AlamarBlue® cell viability assay confirmed a sustained cell death was induced by mini-peptide MYD_181-202 in MYD88 L265P expressing WM cell lines but not in wild type MYD88 expressing Ramos cells (C).

Transduction of MYD88 mini-peptides in mutated MYD88 WM cells but not wild-type MYD88 control cells reduced NFKB activation.



A, BCWM.1 (L265P+); B, MWCL-1 (L265P+); C, Ramos cells (MYD88-WT)

MYD88 TIR domain interfering mini-peptides impacted BTK but not IRAK1/IRAK4 activation, whereas MYD88 DD domain interfering mini-peptides showed an opposite effect.



PhosFlow analysis indicates MYD88 TIR domain mini-peptide MYD_181-202 reduced BTK phosphorylation more robustly than IRAK1 phosphorylation, while the DD domain mini-peptide MYD_40-85 reduced IRAK1 phosphorylation more significantly than BTK phosphorylation indicating a pathway preferential interruption by these two peptides.

Conclusion

The findings demonstrate differences in BTK versus IRAK1/IRAK4 directed NF-kB signaling in response to Myddosome self-assembly in MYD88 mutated WM cells. The feasibility of directly targeting MYD88 homodimerization to block aberrant MYD88 signaling was also recognized, and suitable peptide sequences for the development of peptidomimetics that interfere with Myddosome self-assembly and signaling were identified. The findings provide a framework for direct targeting of the Myddosome in MYD88 mutated WM disease.