Mutated MYD88 activates the BCR component Syk and provides a rationale therapeutic target in Waldenstrom's Macroglobulinemia

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Background

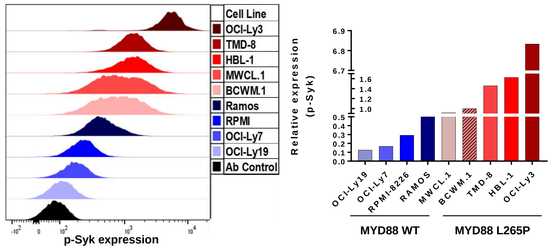
Mutations in the Toll receptor (TLR) pathway are highly prevalent in Waldenstrom's Macroglobulinemia (WM) and ABC DLBCL, wherein mutated MYD88 triggers NF-kB pro-survival signaling through BTK/IRAK. Activation of B-cell receptor (BCR) signaling can also be triggered by activating mutations in CD79A/B in ABC DLBCL, though are rare in WM (5-8%). Despite these findings, there is evidence for BCR activation in WM (Argyropoulos et al, Leukemia 2016). We hypothesized that crosstalk between TLR and BCR might account for aberrant BCR signaling in WM and ABC DLBCL.

Methods

Phospho-flow analysis of MYD88, Syk (pY525-pY526) and other BCR signaling components was performed in MYD88 mutated WM cells compared to wild-type MYD88 expressing control cells. Knockdown or overexpression of MYD88 by lentiviral transduction in MYD88 mutated BCWM.1 cells. Western blot and phospho-flow studies were used to detect protein expression and phosphorylation in WM cells. Co-Immunoprecipitation assay was used to detect the involvement of Syk in the Myddosome complex. Cell survival following treatment was assessed by Annexin V staining or *CellTiter-Glo*® Luminescent Cell Viability Assays.

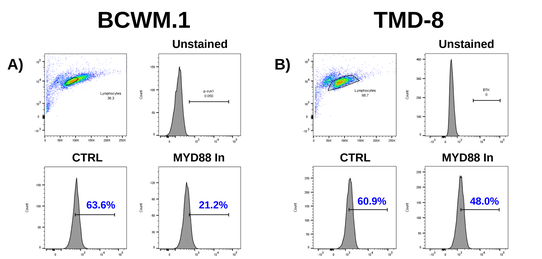
Results

MYD88 mutated WM and ABC-DLBCL cell lines express higher levels of phospho-Syk.



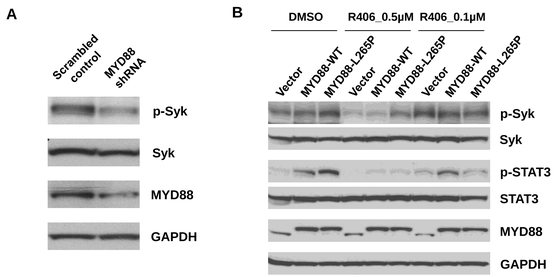
Phospho-Flow study shows much higher levels of phospho-Syk in MYD88 mutated WM (BCWM.1, MWCL-1) and ABC-DLBCL (TMD-8, HBL1, and OCI-Ly3) cell lines compared to MYD88 WT cell lines (OCI-Ly7, OCI-Ly19, RPMI-8226, RAMOS).

MYD88 inhibitor reduces the phosphorylation of Syk in MYD88 mutated WM and ABC-DLBCL cells.



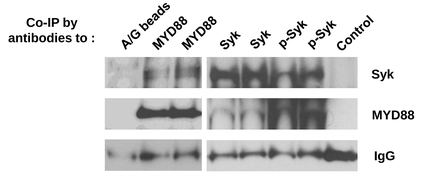
Phospho-flow study shows reduction of phospho-Syk in MYD88 mutated BCWM.1 (WM) and TMD-8 ABC-DLBCL cells following treatment with a MYD88 inhibitor peptide compared to a control peptide.

Knockdown of MYD88 reduces phospho-Syk while the over-expression of MYD88 L265P enhances phospho-Syk expression in MYD88 mutated BCWM.1 WM cells.



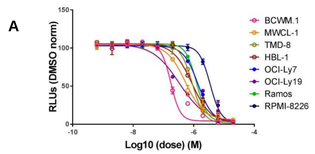
The knockdown of MYD88 in MYD88 mutated WM cells reduced the phosphorylation of Syk (A). The transduction of MYD88 L265P increases Syk phosphorylation compared to vector only or MYD88 WT in MYD88 mutated WM cell line (BCWM.1) as well as increases the phosphorylation of its downstream STAT3 (B). While this increased phosphorylation of STAT3 through Syk activation by the overexpression of mutated MYD88 were reduced by a Syk inhibitor, R406 in WM cell line, BCWM.1.

Syk is involved in the Myddosome complex in MYD88 mutated WM cells



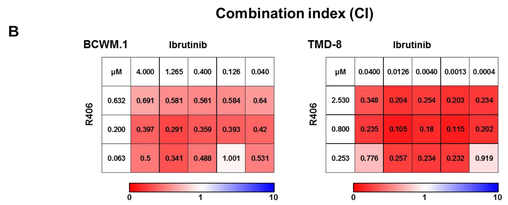
Co-Immunoprecipitation assay using BCWM.1 cells by antibodies to MYD88, Syk or phospho-Syk show binding between Syk and MYD88, with greater binding of MYD88 to phospho-Syk compared to total Syk, supporting the involvement of activated Syk in the Myddosome complex in MYD88 mutated WM cells.

The Syk inhibitor R406 shows a cytotoxic effect against MYD88 mutated WM and ABC-DLBCL cells and the combination of R406 with ibrutinib demonstrates synergistic tumor cell killing.



EC_{50} for Syk inhibitor

Cell lines	EC_{50} (μM) for R406
BCWM.1	0.247
MWCL-1	0.529
TMD8	0.901
HBL-1	0.865
OCI-Ly7	1.383
OCI-Ly19	0.329
Ramos	1.237
RPMI-8226	3.467



The Syk inhibitor R406 shows tumor cell killing at sub-micromolar EC50 dosing against MYD88 mutated WM and ABC-DLBCL cells (A), The combination of R406 and ibrutinib shows synergistic killing in MYD88 mutated WM and ABC-DLBCL cell lines. The combination index (CI) <1.0 indicates a synergistic effect. The combined doses for R406 and Ibrutinib are shown (μM) (B).

Conclusion

Our findings demonstrate that in addition to activation of the TLR pathway, mutated MYD88 activates the BCR component Syk. These findings provide the rationale for combined therapeutics targeting the TLR and BCR pathways in MYD88 mutated WM and possibly other mutated MYD88 driven B-cell malignancies including ABC DLBCL.