How do MYD88 mutations impact WM cell growth and survival?

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Activating mutations leading to production of constitutive growth and survival signals is one of the major elements that transform normal cells into cancer cells. By whole genome sequencing, we discovered highly recurring MYD88 L265P (*MYD88^{L265P}*) mutation in >90% of WM patients. There are also other rare MYD88 mutations in WM, including M232T, S243N. These MYD88 mutations, all affect Toll/Il-1 Receptor (TIR) domain of MYD88 and were demonstrated to be activating mutations in WM and ABC-DLBCL.[Treon et al, N Engl J Med., 2012; 2015; Ngo et al, Nature, 2011] MYD88 is an adapter protein that plays a central role in the innate and adaptive immune response. The initiation of the growth and survival signaling by the mutated MYD88 start with the TIR domain conformation change and followed by the MYD88 homodimerization and Myddosome assembly through recruiting IRAKs and many other signaling molecules. **(1). In addition to interleukin-1 receptor-associated kinases (IRAKs), mutated MYD88 activates Bruton tyrosine kinase (BTK).**[Yang et al, Blood, 2013] The lentiviral knockdown of MYD88 or use of a MYD88 inhibitor decreased the cell survival and reduced the phosphorylation of BTK and its downstream NF-kB gatekeeper IkBa in MYD88^{L265P} expressing WM cells. While, increased BTK phosphorylation was observed in WM cells transduced to overexpress *MYD88^{L265P}* vs wild-type MYD88 (*MYD88^{WT}*). Coimmunoprecipitation (co-IP) studies identified BTK complexed to MYD88 in L265P-expressing WM cells, with preferential binding of MYD88 to phosphorylated BTK (p-BTK). The results establish BTK as a downstream target of MYD88^{L265P} signaling. **(2). Mutated MYD88 causes aberrant expression and hyperactivation of Hematopoietic cell kinase (HCK).**[Yang et al, Blood, 2016; Liu et al, ASH, 2017] HCK was found highly expressed and activated in MYD88-mutated primary WM cells, WM, and ABC-DLBCL cell lines, but was absent or expressed at low levels in healthy donor B cells, as well as *MYD88^{WT}* cell lines. The overexpression of the MYD88^{L265P} protein itself markedly induced HCK transcription and activation across multiple B-cell lines. This aberrant up-regulation of HCK expression was found to be driven by PAX5 and the mutated MYD88 enhanced signaling of transcriptional factors, AP1, NF-kB and STAT3. Knockdown of HCK reduced cell survival and attenuated BTK, PI3K/AKT, and MAPK/ERK survival signaling in MYD88 mutated WM and ABC-DLBCL cells; conversely, the overexpression of HCK did the opposite. HCK inhibitor, A419259 blocked HCK activation and strongly induced apoptosis in MYD88 mutated WM and ABC DLBCL cells compared to *MYD88^{WT}* cells. The findings indicated that HCK represents a novel target for therapeutic development in WM, and possibly other diseases driven by mutated MYD88. **(3). Disruption of**

Myddosome assembly blocks mutated MYD88 signaling.[Liu et al, BJH, 2016] Since the homodimerization of MYD88 initiates the Myddosome assembly and downstream signaling, we tried to disrupt MYD88 homodimerization by overexpression of MYD88 mini-peptides in MYD88 mutated WM cells. The expression of TIR domain targeting MYD88$^{181-202}$ mini-peptide blocked growth and survival of BCWM.1 and MWCL-1 cells, but had no impact on *MYD88^{WT}* Ramos cells. Moreover, pro-survival signaling (p-BTK, p-IRAK1, and p-NFkB-p65) was abrogated in *MYD88^{L265P}* but not *MYD88^{WT}* cells transduced with MYD88$^{181-202}$. The death domain (DD) targeting MYD88$^{40-85}$ mini-peptide was also contributed to sustained growth and survival inhibition, as well as reduced p-IRAK1 and p-NFkB-p65 activation in BCWM.1 cells. The findings suggest that interference of Myddosome assembly within select regions of the TIR and DD domains can impact growth and survival signaling of *MYD88^{L265P}* mutated WM cells. **(4). Mutated MYD88 cross-talk to BCR signaling.**[Munshi et al, ASH, 2017] In addition to triggering IRAKs, BTK and HCK signals in supporting WM cell growth and survival, we recently found that mutated MYD88 also cross-talk to BCR signaling through the activation of SYK. High levels of p-SYK were observed in MYD88 mutated WM and ABC DLBCL cell lines vs. *MYD88^{WT}* cell lines, and in primary MYD88 mutated WM cells compared to healthy donor peripheral blood B-cells. MYD88 knockdown or using MYD88 inhibitory peptide robustly reduced p-SYK levels in MYD88 mutated WM cell lines and primary WM tumor cells; while, the reduction of p-SYK was modest in ABC DLBCL cell lines with activating CD79B mutations. The overexpression of MYD88^{L265P} but not MYD88^{WT} augmented p-SYK levels in both MYD88 mutated and *MYD88^{WT}* cell lines. SYK, particularly p-SYK was found complexed with MYD88 in BCWM.1 cells by co-IP. Knockdown of SYK greatly reduce the cell survival in MYD88 mutated WM and ABC-DLBCL cells and showed an association of downstream p-STAT3 and p-AKT signaling on MYD88 triggered p-SYK. SYK inhibitors, R406 and Entospletinib (GS-9973) produced higher cytotoxicity in MYD88 mutated B-cell lines. Importantly, the combination of ibrutinib with either R406 or Entospletinib produced synergistic tumor cell killing in MYD88 mutated WM and ABC DLBCL cell lines and more robust tumor cell apoptosis in primary MYD88 mutated WM cells. Our studies illustrated multiple signaling pathways driven by mutated MYD88 in supporting WM cell growth and survival, and provided novel targets for drug development or clinical applications in WM.