What drives mutated MYD88 pro-survival signaling in WM?

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MYD88 L265P (*MYD88*^{L265P}) is the most recurrent somatic mutation in Waldenström's macroglobulinemia (WM), and takes about 96% of all MYD88 mutations in WM patients. There are also other rare MYD88 mutations in WM, including M232T, S243N, all affect Toll/Il-1 Receptor (TIR) domain of MYD88. [Treon et al, N Engl J Med., 2012; 2015] Like L265P, M232T and S243N were demonstrated to be oncogenic mutations and shown high levels of NF-κB transactivation in ABC-DLBCL. [Ngo VN, Nature, 2011] The initiation of the pro-survival signaling by mutated MYD88 start with the TIR domain conformation change by these mutations followed by the homodimerization of MYD88 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] Coimmunoprecipitation studies identified BTK complexed to MYD88 in L265P-expressing WM cells, with preferential binding of MYD88 to phosphorylated BTK (pBTK). Increased pBTK was observed in WM cells transduced to overexpress *MYD88*^{L265P} vs wild-type MYD88 (*MYD88*^{WT}). In addition, lentiviral knockdown or use of a MYD88 inhibitor decreased the phosphorylation BTK and NF-κB gatekeeper IκBα and resulted in reduced survival in MYD88 L265P-expressing WM cells. The results establish BTK as a downstream target of MYD88 L265P signaling. **(2). Disruption of Myddosome assembly blocks BTK, IRAKs and NF-kB activation by mutated MYD88.** [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 (pBTK, pIRAK1, and pNFkB-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 pIRAK1 and pNFkB-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. **(3). Mutated MYD88 causes aberrant expression and hyperactive of HCK in supporting tumor cell growth and survival.** [Yang et al, Blood, 2016] Recently, we observed that mutated MYD88 WM and ABC DLBCL cell lines, as well as primary WM cells show enhanced HCK transcription and activation, and that HCK is activated by IL6. This aberrant up-regulation of HCK expression was found to be driven by the mutated MYD88 enhanced signaling of transcriptional factors, AP1, NF-kB and STAT3. Over-expression of mutated MYD88 triggers HCK and IL6 transcription, while knockdown of HCK reduced survival and attenuated BTK, PI3K/AKT, and MAPK/ERK signaling in mutated MYD88 WM cells. Ibrutinib and the more potent HCK inhibitor A419259 blocked HCK activation and induced apoptosis in mutated MYD88 WM and ABC DLBCL cells. Docking and pull-down studies confirmed that HCK was a target of ibrutinib. Ibrutinib and A419259 also blocked ATP binding to HCK, while transduction of mutated MYD88 expressing WM cells with a mutated HCK gatekeeper greatly increased the EC_{50} for ibrutinib and A419259. The findings support that HCK expression and activation is triggered by mutated MYD88, supports growth and survival of mutated MYD88 WM and ABC DLBCL cells, and is a direct target of ibrutinib. HCK represents a novel target for therapeutic development in WM, and possibly other diseases driven by mutated MYD88.