HCK transcription is regulated by AP1, NF-kB and STAT3 transcription factors in MYD88 mutated WM and ABC-DLBCL cells.

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Hematopoietic cell kinase (HCK) is a member of the SRC family of tyrosine kinases (SFKs). HCK transcription is aberrantly upregulated in Waldenström's Macroglobulinemia (WM) and Activated B-cell (ABC) subtype Diffuse Large B-cell Lymphoma (DLBCL) in response to activating mutations in MYD88 (Yang et al, Blood 2016). To clarify the mechanism responsible for the aberrant upregulation of HCK transcription inMYD88 mutated cells, we analyzed the promoter sequence of HCK using PROMO and identified consensus binding sites for transcription factors (AP1, NF-kB, STAT3, and IRF1) that are regulated by mutated MYD88 (Ngo et al, Nature 2011; Treon et al, NEJM 2012; Yang et al, Blood 2013; Juilland et al, Blood 2016; Yang et al, Blood 2016). We performed Chromatin Immuno-precipitation (ChIP) assays using ChIP grade antibodies to JunB, c-Jun, NF-kB-p65, STAT3 and IRF1 in MYD88 mutated WM (BCWM.1, MWCL-1) and ABC DLBCL (TMD-8, HBL-1, OCI-Ly3) cells that highly express HCK transcripts, as well as wild type MYD88 expressing GCB DLBCL (OCI-Ly7, OCI-Ly19) cells that show low HCK transcription. Following ChIP, a HCK promoter specific quantitative PCR assay was used to detect HCK promoter sequences. These studies showed that JunB, NF-kB-p65 and STAT3 bound more robustly to the HCK promoter in MYD88 mutated WM and ABC-DLBCL cells versus MYD88 wild type GCB DLBCL cell lines, while c-Jun bound more abundantly to the HCK promoter sequence in all DLBCL cell lines, regardless of MYD88 mutation status. In contrast c-Jun binding was low in MYD88 mutated WM cells. IRF1 binding to the HCK promoter was similar in all cell lines, regardless of the MYD88 mutation status. To further investigate HCK regulation, we developed an HCK promoter driven luciferase reporter vector (WT) with mutated AP-1 binding (AP1-mu-1~6), NF-kB binding (NF-kB-mu-1~5), and STAT3 binding (STAT3-mu) sites and investigated their impact on HCK promoter activity in MYD88 mutated BCWM.1 cells. We observed that mutation of AP1-mu-1,4,5,6; NF-kB-mu-1,4,5, as well as STAT3-mu binding sites greatly reduced HCK promoter activity, thereby supporting a role for AP-1, NF-kB and STAT3 transcription factors in HCK gene expression in MYD88 mutated cells. To further clarify the importance of these transcription factors in aberrant HCK

gene expression in MYD88 mutated cells, we treated BCWM.1, MWCL-1, TMD-8 and HBL-1 cells with the AP-1 inhibitor SR 11302; NF-kB inhibitor QNZ; and the STAT3 inhibitor STA-21. Treatment of cells for 2 hours with SR 11302, QNZ, and STA-21 at sub-EC_{50} concentrations resulted in decreased HCK expression in MYD88 mutated all cell lines. Lastly, we investigated the contribution of BCR signaling to HCK transcription. BCWM.1, MWCL-1, TMD-8, and HBL-1 cells were treated with the Syk kinase inhibitor R406, and HCK transcription levels were then assessed. Differences in HCK expression were observed between MYD88 mutated WM and ABC DLBCL cells following R406, supporting a contributing role for BCR signaling in ABC DLBCL but not WM cells to HCK expression. Our data provide critical new insights into HCK regulation, and a framework for targeting pro-survival HCK signaling in WM and ABC DLBCL cells dependent on activating MYD88 mutations.