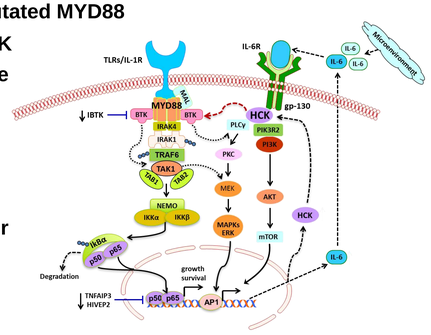
Aberrant HCK Survival Signaling By Mutated MYD88 Requires PAX5 in Waldenström's Macroglobulinemia and ABC-DLBCL Cells.

Xia Liu, Jiaji Chen, Manit Munshi, Lian Xu, Nicholas Tsakmaklis, Maria Demos, Amanda Kofides, Gloria Chan, Zachary Hunter, Christopher Patterson, Robert Manning, Philip Brodsky, Joshua Gustine, Patricia Severns, Toni Dubeau, Jorge Castillo, Steven Treon, and Guang Yang

Bing Center for Waldenström's Macroglobulinemia, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA USA

Background

Activating mutations in MYD88 are present in 95% and 40% of patients with WM and ABC-DLBCL, respectively. MYD88 mutations are known to trigger growth and survival of WM and ABC-DLBCL cells through BTK/IRAK1/IRAK4 directed NF-kB signaling (Ngo et al, Nature 2011; Yang et al, Blood 2013). Recently, we identified that mutated MYD88 can trigger the SRC family member HCK, a kinase that is downregulated in later stages of B-cell ontogeny (Yang et al, Blood 2016). HCK promotes malignant cell survival through activation of BTK, as well as AKT and ERK1/2, whereas knockdown of HCK leads to decreased survival in WM and ABC-DLBCL cells. The regulatory mechanisms that promote mutated MYD88 directed HCK signaling are unknown, and may permit therapeutic advances for MYD88 mutated diseases.



Pro-Survival Signaling Driven by Mutated MYD88 in Waldenström's Macroglobulinemia

Methods

To clarify whether HCK expression is regulated by TLRs/MYD88 or BCR signaling, we performed activation studies using triggers of TLR9 (ODN-2006) and TLR4 (LPS-EP) signaling, and BCR (anti-IgM/IgG) signaling in MYD88 wild-type or mutated B-cell lymphoma cells. To better understand the mechanism for the aberrant upregulation of HCK transcription in MYD88 mutated B-cell lymphoma cells, we performed promoter-binding

Transcription factor (TF) profiling in BCWM.1 cells, and performed Chromatin Immuno-precipitation (ChIP) assays 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. Lentiviral knockdown of PAX5 was conducted in MYD88 mutated WM and ABC-DLBCL cell lines, BCWM.1 and TMD8 respectively. We also developed an HCK promoter driven luciferase reporter vector (WT) with mutated AP-1 binding (AP1-mut-1~6), NF-kB binding (NFkB-mut-1~5), and STAT3 binding (STAT3-mut) sites and investigated their impact on HCK promoter activity in MYD88 mutated BCWM.1 and TMD-8 cells. To further clarify the importance of these transcription factors in aberrant HCK gene expression in MYD88 mutated cells, we treated wild type HCK promoter vector carrying BCWM.1 and TMD-8 cells with AP-1, NF-kB or STAT3 inhibitors at sub-EC_{50} concentrations and detected HCK promoter activities or HCK mRNA levels .

Results

Stimulation of TLRs/MYD88 signaling increases HCK transcription in wild type MYD88 B-cell lymphoma cells

Stimulation of TLRs/MYD88 signaling but not BCR signaling increased HCK transcription, particularly in wild-type MYD88 expressing B-cell lymphoma cells thereby demonstrating the importance of TLR/MYD88 signaling in driving HCK expression (Figure 1.).

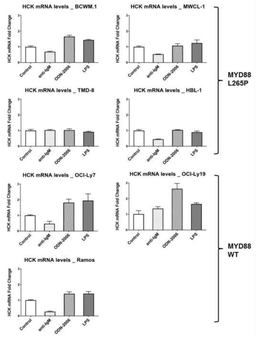


Figure 1. HCK mRNA levels following the stimulation of BCR or TLRs in B-cell lymphoma cell lines.

HCK promoter binding transcription factor profiling indicates PAX5 and mutated MYD88 directed signaling regulate HCK transcription in MYD88 mutated cells.

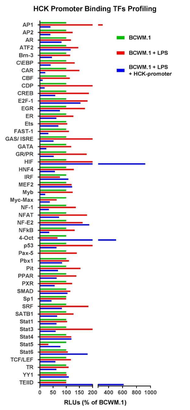


Figure 2. HCK promoter binding transcription factor profiling

The promoter-binding TFs profiling using nuclear extracts of BCWM.1 cells, BCWM.1 cells following LPS stimulation for TLR/MYD88 activation, with/without HCK promoter sequences. Among the TFs, PAX5 signal was reduced the most by the addition of HCK promoter into BCWM.1 cell nuclear extracts. The signals of many other TFs that can be regulated by NF-kB (AP1, C\EBP, Myb, NF-kB), MAPK (AP1, C\EBP, CREB, HNF4, NF-1) and STAT3 signaling were also reduced (Figure 2.). These results indicate that PAX5 and mutated MYD88 directed TFs including NF-kB, AP-1 and STAT3 bind to endogenous HCK promoter sequence.

PAX5 activates HCK transcription in MYD88 mutated WM and ABC-DLBCL cells.

Knockdown of PAX5 reduced HCK mRNA level in BCWM.1 and TMD-8 cells indicating an activating function of PAX5 in the regulation of HCK expression (Figure 3.).

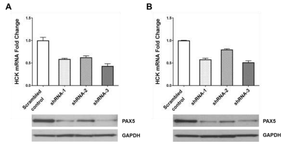


Figure 3. Knockdown of PAX5 reduces HCK transcription in MYD88 mutated WM cell line, BCWM.1 and ABC-DLBCL cell line TMD-8 cells.

ChIP study indicates transcription factors AP1, NF-kB and STAT3 directly bind to HCK promoter.

ChIP results indicate NFkB-p65, JunB and STAT3 bound more robustly to the HCK promoter in MYD88 mutated WM (BCWM.1, MWCL-1) and ABC-DLBCL (TMD-8, HBL-1, OCI-Ly3) cells versus MYD88 wild type GCB-DLBCL (OCI-Ly7, OCI-Ly19) cell lines, while c-Jun bound more abundantly to the HCK promoter sequence in all DLBCL cell lines, regardless of MYD88 mutation status. (Figure 4.).

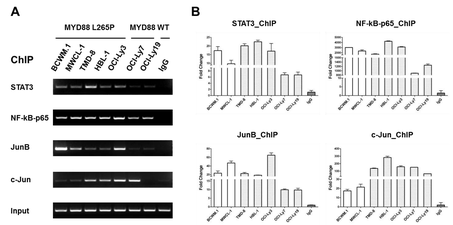


Figure 4. Chromatin immuno-precipitation (ChIP) revealed that AP1, NF-kB and STAT3 transcription factors bind to HCK promoter sequence.

Deletion of TF consensus binding site on HCK promoter reduces HCK promoter activity.

The deletion of AP-1, NF-kB and STAT3 binding sites on HCK promoter reduced HCK promoter activity, thereby supporting a role for AP-1, NF-kB and STAT3 in HCK gene expression in MYD88 mutated cells. (Figure 5.) Treatment of BCWM.1 and TMD-8 cells with inhibitors of AP-1, NF-kB and STAT3 decreased HCK promoter activities by luciferase reporter assay, as well as endogenous HCK mRNA transcription levels in MYD88 mutated cells.

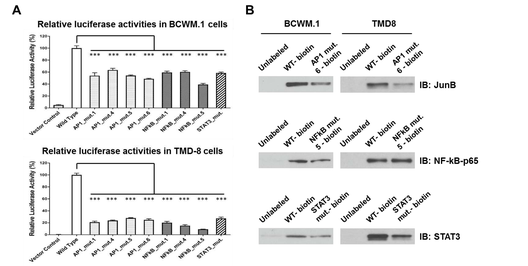


Figure 5. HCK promoter activities and corresponding TF binding are reduced by the deletion of TF binding sites for AP1, NF-kB and STAT3.

Conclusion

Our data indicate PAX5 along with mutated MYD88 trigger transcription factors NF-kB, AP-1 and STAT3 are necessary to trigger aberrant HCK expression. The findings provide a framework for efforts directed at blocking MYD88 triggered HCK survival signaling in MYD88 mutated B-cell lymphomas.