

HCK Transcription is Regulated by AP1, NF-kB and STAT3 Transcription Factors in MYD88 Mutated WM and ABC-DLBCL Cells.



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Background

Hematopoietic cell kinase (HCK) is a member of the SRC family of tyrosine kinases (SFKs). Our recent studies demonstrate 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. HCK is also a direct target of Ibrutinib and contributes to its efficacy in WM and ABC-DLBCL (Yang et al, Blood 2016). HCK transcription and activation is triggered by mutated MYD88, and is an important determinant of pro-survival signaling.

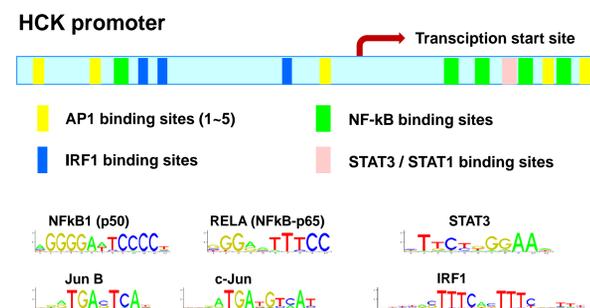
Methods

To clarify the mechanism responsible for the aberrant upregulation of HCK transcription in MYD88 mutated cells, we analyzed the promoter sequence of HCK using PROMO software and 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, an HCK promoter sequence specific quantitative PCR assay was used to detect HCK promoter sequences.

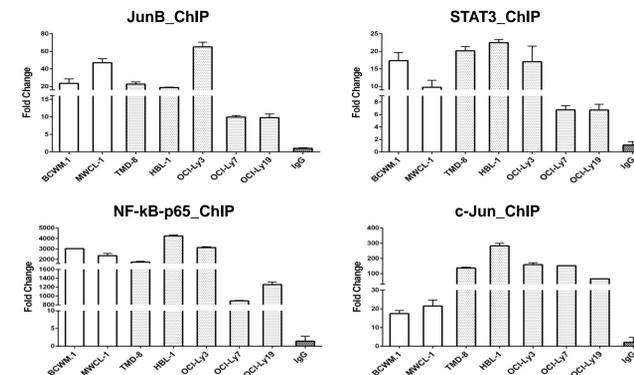
We also developed an HCK promoter driven luciferase reporter vector (WT) with mutated AP-1 binding (AP1-mut-1~6), NF-kB binding (NFkB-mut-1~5), and STAT3 binding (STAT3-mut) sites and investigated their impact on HCK promoter activity in MYD88 mutated BCWM.1 and TMD-8 cells. Biotin labeled HCK promoter wild type or AP1-mut-6, NFkB-mut-5 and STAT3-mut sequences were produced to pull-down transcription factors from nuclear extract of BCWM.1 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 inhibitor SR 11302; NF-kB inhibitor QNZ; and the STAT3 inhibitor STA-21 at sub-EC₅₀ concentrations.

Results

Consensus transcription factors AP1, NF-kB, STAT3 binding sequences identified on HCK promoter region.

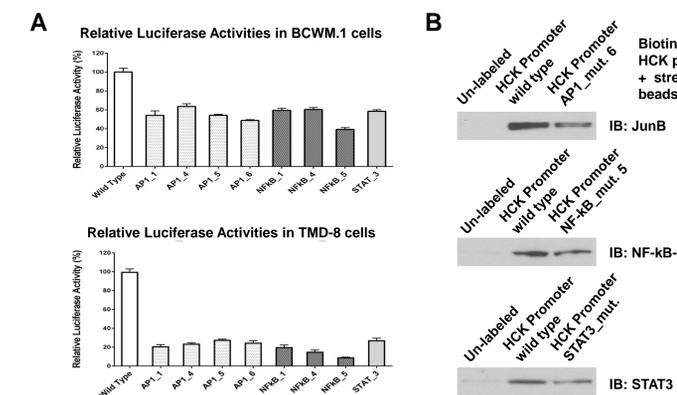


Chromatin Immunoprecipitation (ChIP) Study Reveals HCK Transcription Is Regulated by AP1, STAT3 and NFkB Transcription Factors.



Following the Chromatin IP, HCK promoter sequence specific quantitative PCR assays were used to detect HCK promoter sequences. The results indicate JunB, NFkB-p65 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. IRF1 binding to the HCK promoter was similar in all cell lines, regardless of the MYD88 mutation status (data not shown).

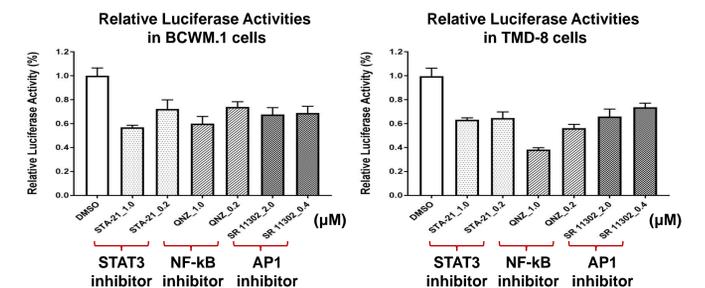
HCK Promoter Activities Reduced by Transcription Factor Binding Site Mutations in MYD88 Mutated Cells.



To further understand HCK regulation, we developed an HCK promoter driven luciferase reporter vector (wild type) and its mutants with mutated AP-1 binding sites (1~6), NF-kB binding sites (1~5) and STAT3 binding site. Using these vectors, we looked at their impact on HCK promoter activity in MYD88 mutated cells and found that mutation of AP1-mut-1,4,5,6; NF-kB-mut-1,4,5, as well as STAT3-mut binding sites greatly reduced HCK promoter activity (A).

In addition, we also performed pull-down assays using biotin labeled wild type or mutant HCK promoter sequences and probed with corresponding transcription factor antibodies, and found the reduced binding of JunB, NF-kB-p65 and STAT3 with corresponding binding site mutations. These data support the importance for AP-1, NF-kB and STAT3 transcription factors in HCK gene expression in MYD88 mutated cells (B).

HCK Promoter Activities Reduced by Inhibitors Specific to Transcription Factors in MYD88 Mutated Cells



To further clarify the importance of these transcription factors in aberrant HCK gene expression in MYD88 mutated cells, we treated HCK promoter driven luciferase wild type vector transduced BCWM.1 and TMD-8 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 these inhibitors at below one fifth EC₅₀ concentrations, resulted in decreased HCK promoter activities in these two MYD88 mutated cell lines.

Conclusion

Chromatin Immuno-precipitation (ChIP) and mutational analysis on HCK promoter region indicate HCK transcription is regulated by AP1, NF-kB and STAT3 transcription factors in MYD88 mutated WM and ABC-DLBCL cells. 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.