Aberrant Expression of Spliced WNK2 is An Early Event in MYD88 Mutated WM that Activates ERK1/2 and Supports Tumor Growth

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BACKGROUND

Waldenström's Macroglobulinemia (WM) patients carry MYD88 ($MYD88^{MUT}$) and CXCR4 ($CXCR4^{MUT}$) mutations in 95-97% and 30-40% of cases, respectively.[1,2] The mechanisms of MYD88-driven lymphomagenesis remain to be clarified. Our previous transcriptome analysis highlighted WNK2 as a highly dysregulated gene in $MYD88^{MUT}$ WM.[3] WNK2 is a serine/threonine protein kinase that negatively regulates ERK1/2 activation in a kinase-dependent manner and is a known tumor suppressor in certain solid cancers, where it is primarily silenced by promoter methylation.[4-9] ERK1/2 is a critical pro-survival signaling in WM; its activation is accompanied by the release of inflammatory cytokines and can mediate ibrutinib resistance.[10] We previously demonstrated WNK2 transcriptional and protein upregulation in $CXCR4^{WT}$ and silencing in $CXCR4^{MUT}$ WM and identified several mechanisms of WNK2 transcriptional dysregulation including i) the preferential expression of short isoforms lacking the kinase domain; ii) the recurrence of an identical, aberrant, exon skipping event in all gene isoforms; and iii) aberrant methylation and chromatin accessibility in $CXCR4^{WT}$ vs $CXCR4^{MUT}$ WM. We therefore hypothesized that spliced WNK2 may contribute to $MYD88^{MUT}$ WM oncogenesis.[11]

METHODS

We investigated WNK2 regulation in 249 untreated WM patients by combined transcriptome and PacBio IsoSeq analysis in a subgroup of 11 WM and 4 healthy donors (HD). Lentiviral transduction was used to overexpress and functionally characterize WNK2 isoforms in $MYD88^{MUT}$ BCWM1 and MWCL1 WM and control lymphoma cell lines.

RESULTS

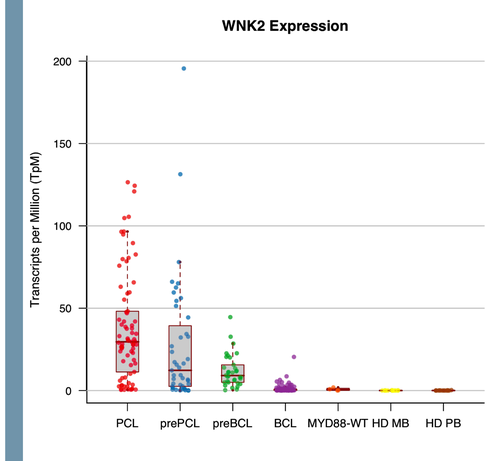

Figure 1. Transcriptome analysis of 249 untreated WM samples based on a novel subtype and early/late evolutionary staging classification for WM[12] revealed that WNK2 is aberrantly upregulated from an early stage of WM and is either silenced or further upregulated with disease progression in the B-cell like (BCL) or Plasma cell like (PCL) WM subgroups, respectively. Pre-BCL and pre-PCL are earlier disease stages that respectively evolve into BCL and PCL over time. $CXCR4^{MUT}$ were most prevalent in the pre-BCL vs pre-PCL (52.6% vs 7.4%) and in the BCL vs PCL (78.4% vs 8.7%, p<0.001) groups. HD MB: healthy donor memory B cells; HD PB: healthy donor peripheral B cells.

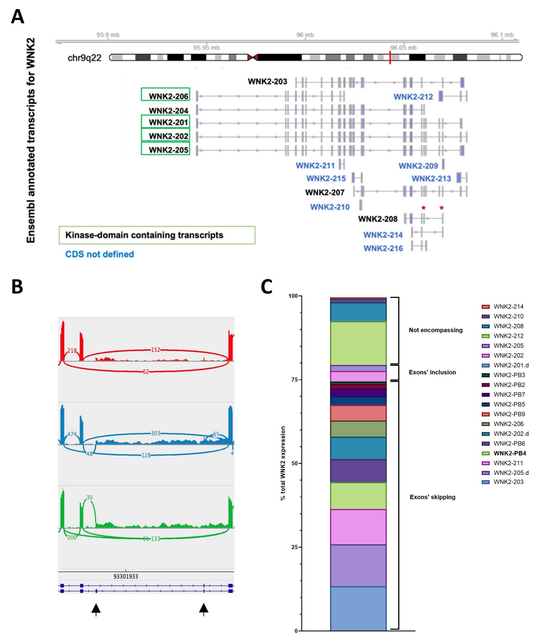

Figure 2. **WNK2 isoforms**. **A)** Ensembl annotated isoforms for the WNK2 gene. The location of the 2 aberrantly spliced exons is highlighted (red stars). **B)** Raw read coverage shows the newly discovered RNA splicing aberration consisting in the preferential skipping of 2 exons (highlighted) in 3 representative patients of our cohort of 249 WM. Layout of the WNK2 transcript from genome browser is shown as reference. **C)** WNK2 isoform analysis, including the Ensembl/Gencode and PacBio newly discovered isoforms (WNK2-PB2 to -PB9), was conducted in 29 WM high expressors of WNK2. WNK2-PB4 was among the most expressed short gene isoforms. The expression of isoforms carrying the newly identified RNA splicing aberration accounts for ~75% of total WNK2 expression in WM.

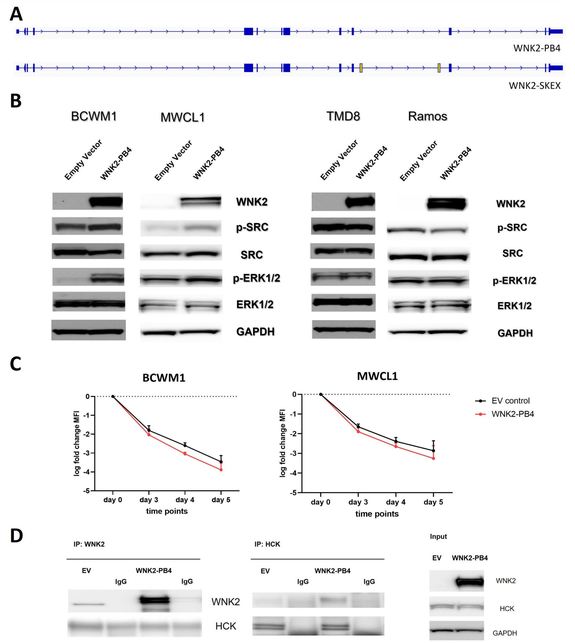

Figure 3. **Stable WNK2 cell lines**. **A)** WNK2 was overexpressed in $MYD88^{MUT}$ (BCWM1, MWCL1, TMD8) and $MYD88^{WT}$ (Ramos) cell lines by lentiviral transduction. The artificial WNK2-SKEX construct contains the 2 exons skipped in WNK2-PB4 (highlighted in yellow). **B)** $BCWM1^{WNK2\text{-}PB4}$ and $MWCL1^{WNK2\text{-}PB4}$ cells showed increased phosphorylation of ERK1/2 and SRC family members, including HCK, compared to the control lines. Increased activation of ERK1/2 was also observed in WM cell lines overexpressing WNK2-SKEX (not shown). **C)** $BCWM1^{WNK2\text{-}PB4}$ and $MWCL1^{WNK2\text{-}PB4}$ cells showed greater proliferation compared to the empty vector control by CellTrace Violet. **D)** By pull-down with both WNK2 and HCK antibodies, WNK2 is shown in complex with the SRC family member HCK in BCWM1.

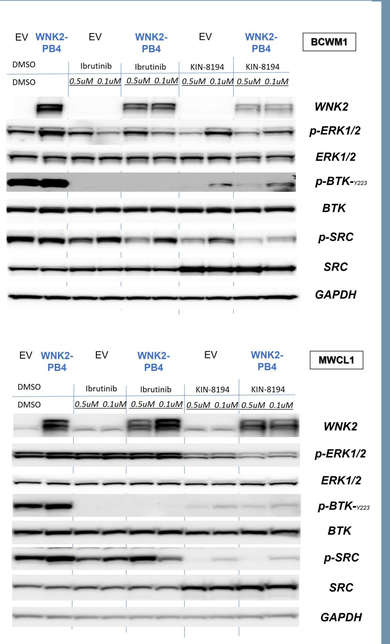

Figure 4. **Ibrutinib and KIN-8194 (dual HCK/BTK inhibitor) treatment of stable WM cell lines overexpressing WNK2.** Upon treatment with the highly potent inhibitor KIN-8194, we observed dose-dependent decreases in phosphorylation levels of SRC family members (including HCK), BTK, and ERK and total levels of WNK2 in $BCWM1^{WNK2\text{-}PB4}$ (**A**) and $MWCL1^{WNK2\text{-}PB4}$ (**B**) *versus* vector control transduced cells.

CONCLUSIONS

Taken together, our findings show a high selection pressure to aberrantly regulate WNK2 expression at an early stage of $MYD88^{MUT}$ WM with different evolution in $CXCR4^{WT}$ vs $CXCR4^{MUT}$ WM, thus suggesting a role for aberrantly spliced WNK2 in the oncogenesis of $MYD88^{MUT}$ WM. Overexpression of the aberrantly spliced WNK2-PB4 isoform in stable WM cell lines provided a proliferation advantage and led to ERK1/2 activation, which may be induced by the WNK2/HCK interaction.

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ACKNOWLEDGEMENTS

MLG is an IWMF Robert Kyle award Fellow, ZRH is an ASH Scholar. The authors gratefully acknowledge the generous support of Peter Bing M.D., International Waldenström's Macroglobulinemia Foundation, Leukemia and Lymphoma Society (Grant: 6673-24), NIH SPORE in Multiple Myeloma (Grant: 2P50CA100707-16A1), the Orszag and Kitchen Fund for WM Research, the Kerry Robertson Fund for WM Research, the Siegel Family Fund for WM, the WM Foundation of Canada, the WMR Fund, and the WM patients who provided samples for these studies.

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