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POSTER ABSTRACTS

651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

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. 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 (Guerrera ML et al, Haematologica 2018). WNK2 is a serine/threonine protein kinase that negatively regulates ERK1/2 activation in a kinase-dependent manner; is a known tumor suppressor in certain solid cancers, where it is primarily silenced by promoter methylation. ERK1/2 is a pro-survival signal in WM; its activation is accompanied by the release of inflammatory cytokines and can mediate ibrutinib resistance. 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.
Methods: We investigated WNK2 regulation in 253 untreated WM patients by combined transcriptome and PacBio IsoSeq analysis in a subgroup of 12 WM and 3 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: We identified that tumors from patients with MYD88 ^{MUT}CXCR4 ^{WT} WM preferentially expressed the short Ensembl WNK2-207 transcript, whose annotation is incomplete. Via PacBio analysis, we identified 8 previously undocumented short isoforms of WNK2 mapping to the original WNK2-207 annotation, all of which lack the kinase domain and bear the same mRNA skipping of two exons near the 3' end. RNA-Seq analysis of 253 WM patents confirmed the IsoSeq predictions and showed that the novel PacBio isoform WNK2-PB4 was among the most expressed transcripts in WM. We next sought to functionally characterize WNK2-PB4 overexpression in MYD88 mutated lymphoma cells. We successfully cloned WNK2-PB4 from patient CD19+ BM cells and obtained an artificial construct of WNK2-PB4 that included the 2 skipped exons, WNK2-SKEX, through the Genewiz gene synthesis service. Stable cell lines overexpressing either WNK2-PB4 or WNK2-SKEX or the empty vector were successfully generated by lentiviral transduction. Overexpression of WNK2-PB4 showed increased phosphorylation of ERK1/2 and SRC family members in the BCWM1 and MWCL1 cells; in contrast, no activation of those targets was seen in MYD88 ^{MUT} DLBCL TMD8 and MYD88 ^{WT} Burkitt Ramos cell lines (**Figure 1**). Increased activation of ERK1/2 was also observed in WM cell lines overexpressing WNK2-SKEX. Study of the downstream signaling of ERK1/2 by WB revealed increased phosphorylation of p90RSK in BCWM1 $^{WNK2\text{-}PB4}$ cells. BCWM1 and MWCL1 overexpressing WNK2-PB4 showed greater proliferation compared to the empty vector control by CellTrace Violet, while no differences in apoptosis were noticed. By co-immunoprecipitation, we observed WNK2 complexed with HCK. Upon treatment with the highly potent dual HCK/BTK inhibitor KIN-8194, we observed dose-dependent decreases in phosphorylation levels of HCK, BTK, and ERK and total levels of WNK2 in WNK2-PB4 overexpressing BCWM.1 and MWCL-1 cells versus vector control transduced cells. Transcriptome analysis of WM samples based on a novel subtype and early/late evolutionary staging classification for WM (Hunter et al, ASH 2022) revealed that WNK2 was aberrantly upregulated from an early stage of WM and was either silenced or further upregulated with disease progression in the B-cell like or Plasma cell like WM subgroups, respectively.

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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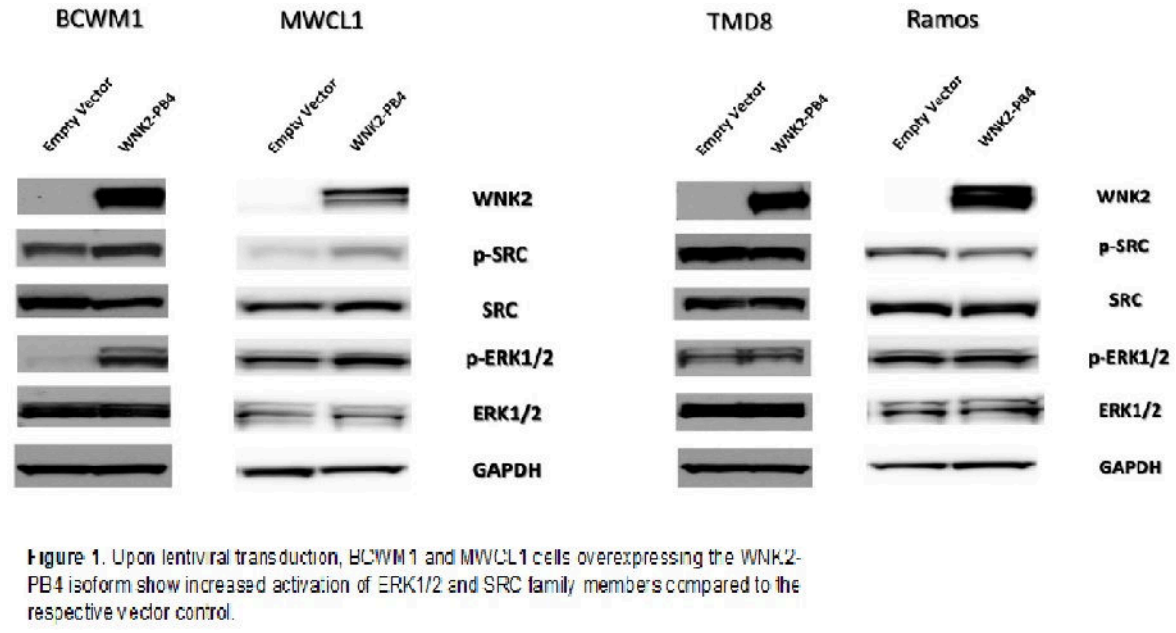

Figure 1. Upon lentiviral transduction, BCWM1 and MWCL1 cells overexpressing the WNK2-PB4 isoform show increased activation of ERK1/2 and SRC family members compared to the respective vector control.

Figure 1

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