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Aberrant Expression of Spliced WNK2 Is an Early event in MYD88-Mutated WM that Activates ERK 1/2 and supports Tumor Growth

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Introduction: Although MYD88 mutations ($MYD88^{MUT}$) are present in 95-97% of WM patients, they are insufficient for lymphomagenesis. The serine/threonine protein kinase WNK2 emerged as a putative second "hit" candidate in our previous studies. WNK2 is a known tumor suppressor and negatively regulates ERK1/2 activation in a kinase-dependent manner. We investigated WNK2 regulation in WM patients by combined transcriptome, PacBio IsoSeq and methylome, and modeled WNK2 function by lentiviral transduction in WM cell lines.

Main points: Unlike healthy B cells, WNK2 was aberrantly upregulated in 60% of WM, starting from early-stage disease. Aberrant methylation differentially regulated gene expression between WM vs HD, and $CXCR4^{MUT}$ vs $CXCR4^{WT}$ WM. The WNK2 isoforms grouped into three distinct families: the canonical full-length (C-K), the novel aberrantly spliced (S-K), and the short non-kinase domain (S-NK) family. S-NK variants were the most prevalent in WM, while S-K were cancer-specific. Stable WM lines overexpressing the dominant S-NK isoform in WM showed increased phosphorylation of ERK1/2 and SRC family members. By RNA-Seq, they showed enrichment of the neutrophil chemotaxis and RNA splicing pathways *vs* vector controls, confirmed by targets' validation. Treatment of primary cells with WNK463, a commercially available potent and selective oral WNK kinase inhibitor, induced a dose-dependent B cell death.

Conclusions: Our findings showed aberrant, methylation-mediated WNK2 transcription in $MYD88^{MUT}$ WM patients that differentially aligns with CXCR4 mutational status. We identified novel and cancer-specific isoforms of WNK2, highly expressed in WM, of which the dominant S-NK isoform promotes inflammation and myeloid chemotaxis, suggesting a novel oncogenic function for WNK2. Our findings provide a critical framework for the selective therapeutic targeting of cancer-specific WNK2 isoforms.