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ORAL ABSTRACTS

802.CHEMICAL BIOLOGY AND EXPERIMENTAL THERAPEUTICS

The Atypical Protein Kinase WNK1 Controls Leukemia Progression in TAL/LMO T-Cell Acute Lymphoblastic Leukemia

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Genomic and proteomic approaches have been crucial in identifying genetic mutations and protein expression profiles underlying leukemia cell growth and survival, especially in heterogeneous diseases like T-cell acute lymphoblastic leukemia (T-ALL). T-ALL is mainly driven by "poorly druggable" transcription factors along with "fairly druggable" secondary events. However, only a few kinases, such as JAK2 and LCK, have shown dependency for successfully matched therapies, limiting treatment options for T-ALL patients.

To identify druggable active pathways in T-ALL, we profiled 31 phosphorylated kinases in T-ALL cell lines, clinical T-ALL blasts, and T cells, and showed that WNK1 exhibited the most significant difference between T-ALL and T cells ($p < 0.0001$). WNK (with no lysine = K) proteins (WNK1-4) are atypical serine-threonine kinases in which the catalytic lysine, necessary for ATP binding, is replaced by cysteine and situated in subdomain I instead of II. WNK proteins regulate ion flux transport across cellular membranes and facilitate the release/recycling of carrier vesicles; however, their role in leukemia remains uncharacterized.

From the analysis of different transcriptome datasets, we demonstrated that WNK1 is the most highly expressed WNK isoform in T-ALL samples, T-ALL cell lines, and patient-derived leukemia xenografts (PDLX) ($p < 0.0001$). WNK1 and p-WNK1 are predominately expressed in lymphoblasts compared to normal thymus or lymph nodes, indicating the involvement of disease-causing events that lead to the activation of this pathway. To correlate phosphorylation levels with protein and mRNA expression, we intersected the FORALL database, which encompasses ALL's proteomic, transcriptomic, and pharmacoproteomic profiling with the functional Cancer DepMap dataset. *WNK1* emerged as a T-ALL lineage transcriptional dependency, with a high correlation between WNK1 protein and mRNA expression (Pearson r= 0.64, p=0.00123, n=22), providing additional support for targeting WNK1 in T-ALL.

The next question is whether this level of *WNK1* expression can identify a specific T-ALL subgroup. From two independent T-ALL datasets, we found that *WNK1* is overexpressed in the *TAL/ LMO*-related (TAL-R) subgroups ($p < 0.01$), where PTEN/AKT mutations are notably abundant. An outlier analysis of WNK1 expression revealed a correlation with 6q14-q16 deletions (Fisher's exact test = 0.0268), a privileged abnormality in the *TAL/LMO* subgroup, suggesting a potential area for clinical exploitation in poor-prognosis T-ALL.

To understand the contribution of *WNK*1 in leukemogenesis, we performed a network-based analysis (NetBID2) from RNA-seq data of T-ALL cells lacking *WNK1* expression or treated with WNK463, an orally bioavailable pan-WNKs inhibitor. Results showed that modulation of WNK1 enriched signatures associated with the G2M checkpoint and mitotic spindle control ($p < 0.0001$). Consistently targeting *WNK1* with shRNA or sgRNA-CRISPR Cas9 in TAL-R cell lines promoted polyploidy, resulting in cell cycle arrest and inhibition of cell proliferation *in vitro*. Similarly, treatment with WNK463 led to morphometric changes, such as incomplete cell division or chromosome segregation through mitotic spindles and abscission defects.

Due to the potential translational significance of our findings, we established a PDLX TAL-R model by serially transplanting *SIL-TAL1* $^{+}$ leukemia cells into NOD/SCID mice for *in vivo* drug treatment. Mice (n=10) were exposed to WNK463 at 1.5 mg/Kg/day for 26 days. At the control group endpoint, we sacrificed four mice per group for efficacy studies to reveal a decrease of leukemic cells infiltration in the bone marrow and spleen (p=0.0286), an effect pharmacodynamically associated

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with a reduction in the WNK1 phosphorylation levels. The remaining mice were observed for survival analysis. Treated mice showed a significant survival advantage (p=0.0009, log-rank Mantel-Cox test) compared to vehicle control. No relevant drug-related toxicity was recorded in the treatment group.
In summary, our work demonstrates the significant contribution of WNK1 kinase in the leukemia progression in *TAL/ LMO* T-ALL cases. The lack of sequence similarity in the ATP binding domain compared to other kinases makes the identification of leukemia's dependence on atypical WNK1 kinase particularly promising for the development of selective inhibitors as a potential treatment for T-ALL.

Disclosures No relevant conflicts of interest to declare.

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