(PB2541) ONCOGENIC ROLE AND DRUG SENSITIVITY OF A NEW IKZF1 MUTATION IN B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

Topic: 01. Acute lymphoblastic leukemia - Biology & translational research

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Background

The *IKZF1* gene encoded IKAROS protein, a zinc finger transcription factor, plays a crucial role in the differentiation and development of lymphocytes. Deletion /mutation of the *IKZF1* gene, as a hallmark of high-risk B-ALL, leads to IKAROS loss of function and is associated with poor prognosis in high-risk B-cell acute lymphoblastic leukemia (B-ALL). We have identified a novel *IKZF1* mutation (c.643T>C: p.S215P) in a patient who exhibited treatment resistance and a poor prognosis. The underlying molecular mechanism of this mutation and drug sensitivity remains to be further elucidated.

Aims

To investigate the oncogenic role of the new *IKZF1* mutation (c.643T>C: p.S215P) and the sensitivity of CK2 inhibitor CX-4945 and HDAC inhibitor Chidamide to B-ALL with the mutation.

Methods

Nalm6 cells with vector only, $IKZF1^{WT}$ and $IKZF1^{S215P}$ were established by lentiviral transduction. CCK-8 cell proliferation assay, Annexin V apoptosis assay, and colony formation assay were performed in the Nalm6-vector only, $IKZF1^{WT}$ and $IKZF1^{S215P}$ cells for 48 hours. The synergistic effect was determined by the combination indices (CIs) with CalcuSyn software. Human leukemia cell-derived xenograft (CDX) mouse models were established by using Nalm6-vector only, $IKZF1^{WT}$ and $IKZF1^{S215P}$ cells.

Results

Lentiviral IKZF1 wild type ($IKZF1^{WT}$), $IKZF1^{S215P}$ mutant were successfully transduced into Nalm6 cells with comparable expression levels (**Fig.1A**). The cell proliferation, apoptosis, and colony-forming assay showed that $IKZF1^{S215P}$ significantly promotes cell proliferation ($P<0.05$, **Fig.1B**), inhibits apoptosis ($P<0.001$, **Fig.1C**), and suppresses colony-forming ($P<0.01$, **Fig.1D, 1E**). In vivo CDX experiments demonstrated that $IKZF1^{WT}$ reduced the spleen size and weight, as well as % $mCD45^{-}hCD19^{+}$ cells in both the spleen and bone marrow; while the $IKZF1^{S215P}$ mutant increased spleen size (**Fig.1F**) and weight ($P<0.001$, **Fig.1G**), along with an elevated % $mCD45^{-}hCD19^{+}$ cells in the spleen ($P<0.001$, **Fig.1H**) and bone marrow ($P<0.001$, **Fig.1I**). We found that the combination of CX-4945 and Chidamide also has a synergistic effect on the proliferation ($P<0.001$, **Fig.1J**) and apoptosis ($P<0.001$, **Fig.1K**) in $IKZF1^{S215P}$ mutant cells. Moreover, the expression of TCL1A, a crucial coactivator of AKT signaling, is significantly up-regulated in $IKZF1^{S215P}$ mutant cells compared with vector-only control ($P<0.001$, **Fig.1L**). CX-4945 or Chidamide down-regulates *TCL1A* expression in $IKZF1^{S215P}$ mutant cells, and the reduction is more significant in the combination of CX-4945 and Chidamide compared with either single drug alone ($P<0.001$, **Fig.1M**).

Summary/Conclusion

Our results indicate that the IKZF1^{S215P} mutation promotes oncogenesis in B-ALL. The combination of CX-4945 with Chidamide demonstrates potent synergistic efficacy by suppressing TCL1A expression in IKZF1^{S215P} mutant cells. This study supports the combination therapy as a promising strategy for high-risk IKZF1-mutated B-ALL.

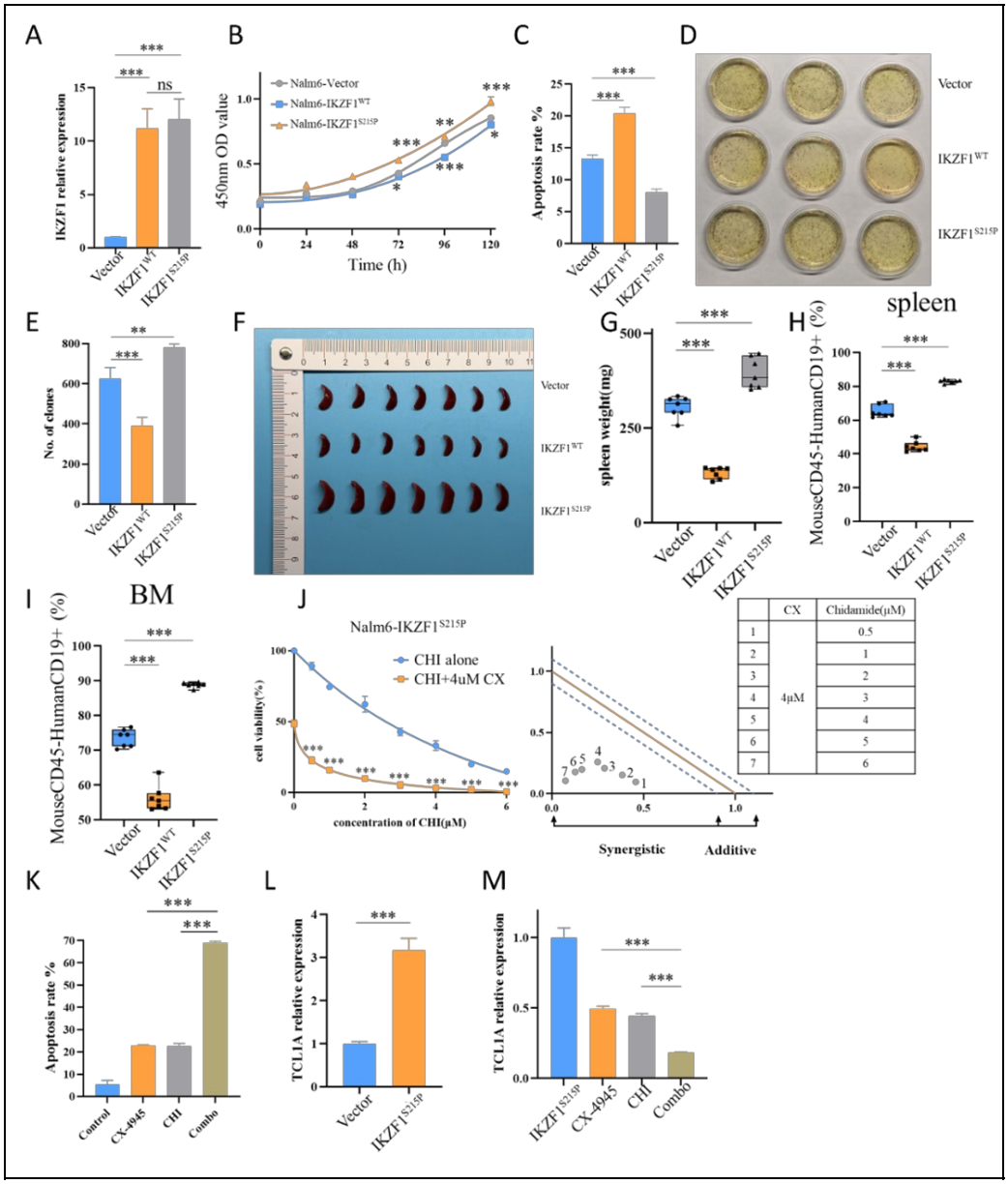

Fig. 1 Novel Oncogenic IKZF1S215P Mutation is Sensitive to CX-4945 and Chidamide in B-ALL (A) Expression level of IKZF1WT and IKZF1S215P in Nalm6 cells by qPCR. Cells were stably expressed with lentiviral IKZF1WT, IKZF1S215P, and vector only. (B-E) Effect of IKZF1WT and IKZF1S215P on cell proliferation (B), apoptosis (C) and colony-forming (D-E) in the Nalm6 cells compared with that of vector only. (F-G) Spleen image (F) and spleen weight (G) of the mice with IKZF1WT, IKZF1S215P, and vector only cells. (H-I) The percentage of mCD45-hCD19+ cells in the spleen (H) and bone marrow (I) from IKZF1WT, IKZF1S215P, and vector only in Nalm6 cells of mice. (J) Effect and synergistic analysis of the combination of CX-4945 and Chidamide vs single drug on cell proliferation in IKZF1S215P cells. (K) The statistic histogram of apoptosis in IKZF1S215P mutant cells that were treated with CX-4945, Chidamide alone, and two drugs combination for 48h. (L) The expression of TCL1A in IKZF1S215P and vector only in Nalm6 cells by qPCR. (M) The expression of TCL1A in IKZF1S215P cells treated with CX-4945 alone, Chidamide alone, and two drugs combination for 48 h detected by RT-qPCR.