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POSTER ABSTRACTS

605.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

Genome-Wide CRISPR Screen Identifies ZNF451 Regulating Sensitivity to Topoisomerase 2 Inhibitors in Peripheral T-Cell Lymphoma Cells

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Introduction: Despite the recent therapeutic developments for peripheral T-cell lymphomas (PTCLs), their prognosis remains dismal. Topoisomerase 2 inhibitors (TOP2i), which increase the amount of TOP2-DNA covalent complex and block DNA double-strand break repair, have long been used as key drugs in the treatment of PTCLs. The molecular mechanisms underlying sensitivity to TOP2i remain to be fully elucidated.

Methods: To elucidate the cell-intrinsic genes affecting TOP2i sensitivity in PTCL cells, we performed whole-genome loss-of-function CRISPR screenings in adult T-cell leukemia/lymphoma (ATLL) cell lines. ST1 and KK1 cells were transduced with the Brunello CRISPR knockout pooled library targeting 19,114 genes (4 sgRNAs/gene) followed by puromycin selection. The transduced cells were then treated with etoposide (ETP), doxorubicin (DOX) or DMSO control for 8 days. The MAGeCK algorithm was used to rank the sgRNAs responsible for TOP2i sensitivity. Among the top 500 enriched genes, we further selected the those with at least 2 sgRNAs with a log2 fold change (TOP2i/DMSO) < -0.5 or >0.5 in both ST1 and KK1 cells as genes whose knockout conferred TOP2i sensitivity or resistance, respectively.

Results: Using the whole genome loss-of-function CRISPR screen, we identified 3 genes and 2 genes whose knockout conferred ETP sensitivity and resistance, respectively. TOP2A, a known ETP target, was ranked as a top gene for ETP resistance, confirming the reliability of our screenings (Fig. 1). In the same way we also extracted 3 genes whose knockout conferred DOX sensitivity. Interestingly, zinc finger protein 451 (ZNF451) was identified as an overlapping gene whose knockout conferred sensitivity in both screens for ETP and DOX. ZNF451 is an E3 SUMO ligase and was previously reported to be an endogenous DNA repair factor for TOP2i-induced DNA double-strand breaks (Schellenberg et al. *Science* 2017). The role of ZNF451 in PTCL cells has not been previously reported, prompting us to focus on ZNF451 in further analyses. For a confirmatory experiments, we transduced sgRNAs targeting ZNF451 (sgZNF451) in ATLL cell lines. To see the knockout efficiencies of the sgZNF451, we performed immunoblot analysis by using anti-ZNF451 antibody and found three bands with different levels of reduction. To ensure whether the bands detected by the anti-ZNF451 antibody represented endogenous ZNF451 protein, we engineered the ATLL cell line with an HA-tag at the 5′ UTR of the endogenous ZNF451 gene locus by CRISPR-mediated knock-in. Immunoblot analysis of sgZNF451-transduced engineered cells expressing HA-tag-ZNF451 by using anti-HA antibody showed almost the same pattern of the bands as shown in the blot by anti-ZNF451 antibody. Thus, we confirmed that sgZNF451 worked efficiently and concluded that post-transcriptional modification affected the stability of ZNF451 protein in ATLL cells. Next, we investigated the role of ZNF451 on cell viability. While sgZNF451-transduced ATLL cells did not differ from control sgRNA-transduced cells in terms of cellular proliferation under normal culture conditions, sgZNF451-transduced ATLL cells showed increased sensitivity to both ETP and DOX compared to control cells with statistical significance, confirming our screening results (Fig. 2). Importantly, sgZNF451-mediated sensitization to ETP and DOX was found not only in ATLL cells but also in other PTCL cell lines including ALK-positive and ALK-negative anaplastic large cell lymphoma (ALCL). Mechanistically, apoptosis and G2/M cell cycle arrest were induced at significantly higher frequencies by ETP treatment in ZNF451 knocked out cells compared to control cells. Given the role of ZNF451 in TOP2i sensitivity in PTCL cells, we investigated whether ZNF451 expression levels stratify the prognosis of PTCL patients treated with CHOP-like regimens. To test this, FFPE samples from 45 PTCL-NOS patients were used for Q-PCR analysis of ZNF451 mRNA but we did not find any correlation between the ZNF451 expression and prognosis.

Conclusions: We performed unbiased genome-wide CRISPR/Cas9 library screens and identified ZNF451 as an important regulator of sensitivity to TOP2i in PTCL cells. Further studies are needed to determine whether measuring ZNF451 protein levels can be used as a biomarker in PTCL patients who are treated with TOP2i.

Disclosures Goto: *Novartis:* Honoraria; *Bristol-Myers Squibb:* Research Funding; *SymBio:* Research Funding; *Chugai:* Honoraria; *Kyowa Kirin:* Honoraria, Research Funding. **Teshima:** *SHIONOGI:* Research Funding; *Astellas:* Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding; *Priothera SAS:* Research Funding; *ONO:* Research Funding; *LUCA Science:* Research Funding; *Otsuka:* Research Funding; *Meiji Seika Pharma:* Membership on an entity's Board of Directors or advisory committees; *Janssen:* Honoraria, Membership on an entity's Board of Directors or advisory committees; *Celgene:* Honoraria, Membership on an entity's Board of Directors or advisory committees; *Merck Sharp & Dohme:* Honoraria; *DAIICHI SANKYO:* Membership on an entity's Board of Directors or advisory committees; *AstraZeneca:* Membership on an entity's Board of Directors or advisory committees; *Roche Diagnostics:* Membership on an entity's Board of Directors or advisory committees; *Takeda:* Membership on an entity's Board of Directors or advisory committees; *Sanofi:* Membership on an entity's Board of Directors or advisory committees; *Chugai:* Honoraria, Research Funding; *Asahi Kasei Pharma:* Membership on an entity's Board of Directors or advisory committees, Research Funding; *Kyowa Kirin:* Honoraria, Research Funding; *NIPPON SHINYAKU:* Honoraria, Research Funding; *Fuji Pharma:* Research Funding; *Eisai:* Research Funding; *Sumitomo Pharma:* Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding; *AbbVie:* Honoraria; *Novartis:* Honoraria, Membership on an entity's Board of Directors or advisory committees; *Bristol-Myers Squibb:* Honoraria. **Nakagawa:** *Astrazeneca:* Honoraria; *Mundipharma:* Honoraria; *Meiji Seika pharma:* Honoraria; *AbbVie Inc.:* Research Funding; *Takeda Pharmaceutical Company Limited:* Honoraria, Research Funding.

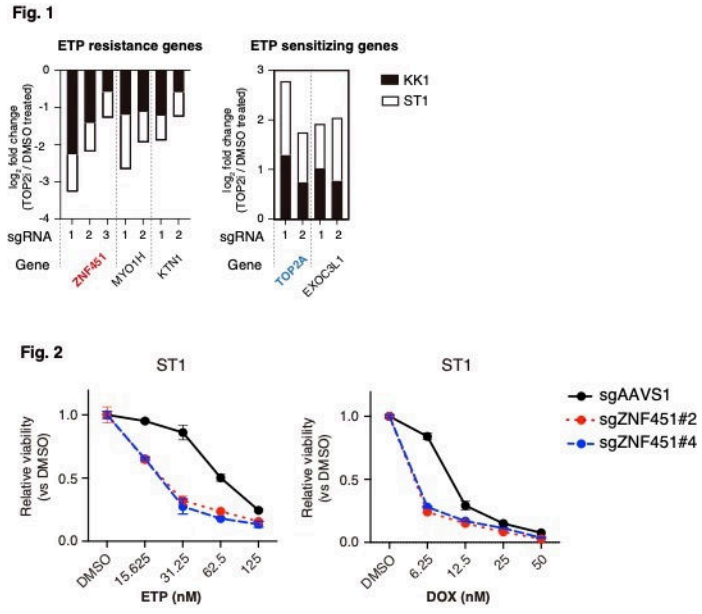

Figure 1

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