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604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

Exploring the Mechanisms of Venetoclax Resistance Via Drug Screening and Genome-Wide CRISPR Screening

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Introduction: Combining Venetoclax, a selective BCL-2 inhibitor, with hypomethylating agents has revolutionized frontline acute myeloid leukemia (AML) treatment. Despite its success, the occurrence of resistance remains a significant concern, limiting the range of available treatment alternatives. Little is known about the mechanism by which the cells escape the treatment. Recent studies have observed *MCL1* upregulation in AML cells to promote resistance, and the rationale to use an MCL1 inhibitor became attractive. Although MCL1 inhibitors work exceptionally well *in vitro* their clinical trials were unsuccessful due to their toxicity. Here, we aim to identify other genes responsible for acquiring resistance and tackle the problem through screening for clinically approved drugs to rapidly benefit the patients with our study.
Methods: First, we generated a model of the venetoclax resistance from 3 AML cell lines: MOLM-13, HL-60 and MV4-11. Resistance was achieved through chronic administration of the compound, inhibiting cell viability to 80-90% in multiple rounds by gradually increasing concentrations until the cells developed resistance. We have then characterized these Venetoclax-resistant (VeR) cells using quantitative RT-PCR and RNA sequencing to reveal the mechanisms behind their resistance. VeR cell lines were subjected to drug screening with a library of 859 FDA and EMA-approved compounds with various clinical indications. Cell viability in response to the drug library was assessed after 72h by Cell-Titer Glo and evaluated against the wild-type counterparts. Top-performing compounds were validated in dose-response curves, and synergy scores were identified from combinational treatments. Simultaneously, we performed genome-wide CRISPR/Cas9 knockout screening using Brunello CRISPR knockout library on both MOLM-13 wild-type (WT) and VeR cell lines to identify genes that maintain the resistance to venetoclax and genes that may potentially synergize with targeting BCL-2.
Results: Results from the CRISPR screen pointed out multiple genes whose loss of function may contribute to venetoclax resistance in MOLM-13 cells, among which *BAX, NOXA, ELAVL1* and *TP53* ranked as top hits. On the contrary, we also identified several genes whose loss of function sensitized the cells to venetoclax (e.g., *MCL1, OPA1, CDK2, MCAT*), suggesting them to be promising therapeutic targets. Among these hits, *CDK2* correlates with our drug screening data indicating Flavopiridol as an effective compound in treating VeR cell lines. Moreover, we confirmed the elevated *MCL1* gene expression for the MOLM-13 VeR cell line through RNAseq (and qPCR) and detected an altered expression of genes mainly in metabolic pathways and PI3K-Akt signaling pathway via differential gene expression analysis. Our drug screening results revealed overall high efficacy of DNA-damaging agents, proteasome and HDAC inhibitors for both WT and VeR cell lines. We also included an MCL1 inhibitor as a positive control in our drug library and confirmed a synergistic effect when combined with venetoclax.
Conclusion: Integrating a range of screening approaches may lead to discovering previously unknown therapeutic targets for venetoclax-resistant AML. Our screens confirmed previously described involvement of the apoptotic pathway genes in venetoclax resistance as well as identified some novel promising targets. Our ongoing efforts involve thorough validations of the identified hits from CRISPR and drug screening to enhance the reliability and translatability of the results.
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Disclosures No relevant conflicts of interest to declare.

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