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604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

Functional Interrogation of Clinically Associated Venetoclax-Sensitive Genes Identifies an Essential Role of Transcription Factor ZNF740 in Therapeutic Response

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Venetoclax, a targeted inhibitor of BCL-2, has shown therapeutic benefits in acute myeloid leukemia (AML). However, the emergence of drug resistance remains a significant clinical challenge, emphasizing the need for deeper understanding of its mechanisms. While comprehensive transcriptome analyses have identified many genes associated with venetoclax responsiveness, it remains crucial to distinguish between genes that play a direct role and those that are simply associated.

In this study, we focused on genes strongly linked to a positive venetoclax response in primary AML samples using the BeatAML2.0 dataset. We identified ~1,400 top genes whose expression inversely correlated with venetoclax AUC. We theorized that if any of these genes were essential for venetoclax sensitivity, their absence would lead to resistance. To explore their significance, we developed a focused CRISPR knockout library of these ~1,400 genes, with each gene represented by 7 guide RNAs, and an additional 250 guide RNAs as negative controls. We then performed CRISPR screens on AML cell lines, using either DMSO or venetoclax as treatments. Interestingly, the majority of the gene knockouts did not lead to resistance, suggesting that these genes likely have an associative rather than a direct impact on venetoclax sensitivity.

However, our screen did identify several genes that, when depleted, consistently resulted in resistance across different AML cell models. One of the top hits was the zinc finger transcription factor ZNF740, whose expression is strongly correlated with venetoclax AUC ($R = -0.52$, $p = 2.71e-26$). For verification, we used CRISPR approach to knock out ZNF740 in Cas9-expressing OCI-AML2 and MOLM-13 cell lines. We then performed IC_{50} and competitive proliferation assays under venetoclax treatment. We found that AML cells without ZNF740 exhibited a markedly higher IC_{50} and demonstrated enhanced survival under venetoclax exposure. Conversely, enforced overexpression of ZNF740 rendered hypersensitivity to venetoclax. Intriguingly, our transcriptomic study showed that ZNF740 deletion upregulated monocyte differentiation signature genes, a change previously linked to venetoclax resistance.

Collectively, our findings indicate that ZNF740 serves as a newly identified, clinically relevant transcriptional modulator of venetoclax responsiveness. We are now investigating the molecular targets of ZNF740, particularly its role in guiding AML lineage decisions and its impact on venetoclax response. Gaining insight into ZNF740's function and mechanism could pave the way for innovative strategies to counteract venetoclax resistance observed in clinical settings.

Disclosures No relevant conflicts of interest to declare.

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