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CORRESPONDENCE

CRISPR screening and cell line IC50 data reveal novel key genes for trametinib resistance

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Dear Editor,

In addition to advancing the development of clinical gene-editing therapeutics, CRISPR/Cas9 has significantly accelerated the identification of new genes associated with specific phenotypes through its use in genomic screens. High-throughput arrayed and pooled lentiviral-based CRISPR/Cas9 screens have been employed in various contexts, including the identification of essential genes, genes involved in cancer metastasis and tumor growth, and genes related to viral response. Additionally, this technology has been successfully utilized to identify drug targets [1] and mechanisms of drug resistance [2]. Moreover, databases such as the Cancer Therapeutics Response Portal (CTRP) and the Genomics of Drug Sensitivity in Cancer (GDSC) provide conventional approaches for drug-resistance gene discovery data. These data are valuable resources in the field of cancer research, particularly for discovering genes associated with drug resistance, by providing extensive datasets and tools for correlating genetic features with drug sensitivity and resistance.

Here, we integrated CRISPR screening data for resistance to methotrexate, trametinib, and vemurafenib from iCSDB [3], a database of CRISPR screens, to identify key genes for resistance to these drugs and validate the drug sensitivity association of these genes with CTRP and GDSC data. These genes were positively selected in the CRISPR screen experiments for corresponding drug resistance. In CRISPR screen experiments, if certain genes are "positively selected" for corresponding drug resistance, it means that knockouts of these genes enable the cells to have higher tolerance or resistance to the drug. Specifically, CRISPR screen experiments typically involve systematically knocking out or editing a large number of genes to observe how the loss of these genes affects cell survival under specific conditions. When cells are treated with a certain drug, the cells that survive usually have resistance to the drug. By analyzing the genomes of the surviving cells, researchers can determine which gene knockouts or mutations led to this resistance. These genes are described as being "positively selected" under these conditions because their mutations are beneficial for cell survival. Results showed that six gene–drug pairs remain in the candidate list. Five of these are key genes for trametinib, and one is a key gene for vemurafenib, with only trametinib having CRISPR screening evidence of gene-positive selection on at least two cell lines (Fig. 1A). Hence, we believe that the five key genes for trametinib resistance are reliable.

In the next round of analysis, we validated the gene candidates from the CRISPR screen experiments. Given that the CRISPR screen experiments were conducted in one single-cell line, it is essential to use data from much larger range of cell lines to demonstrate the general role of these genes in drug resistance. The CTRP and GDSC provided IC50 and expression data of a number of cell lines of different cancer types; with these data, an IC50 prediction model by gene expression profile can be trained, which subsequently applied in different cancer type bulk sequencing data from TCGA [4]. With this approach, we predicted the IC50 for individual samples from TCGA and calculated the correlation between predicted IC50 and the expression of candidate genes in a few cancer types that relevant to trametinib (Fig. 1B). In addition to melanoma, trametinib is widely used for non-small cell lung cancer (NSCLC). Based on the DRESIS database, melanoma (ICD-11: 2C30) and pancreatic cancer (ICD-11: 2C10) have clinically reported

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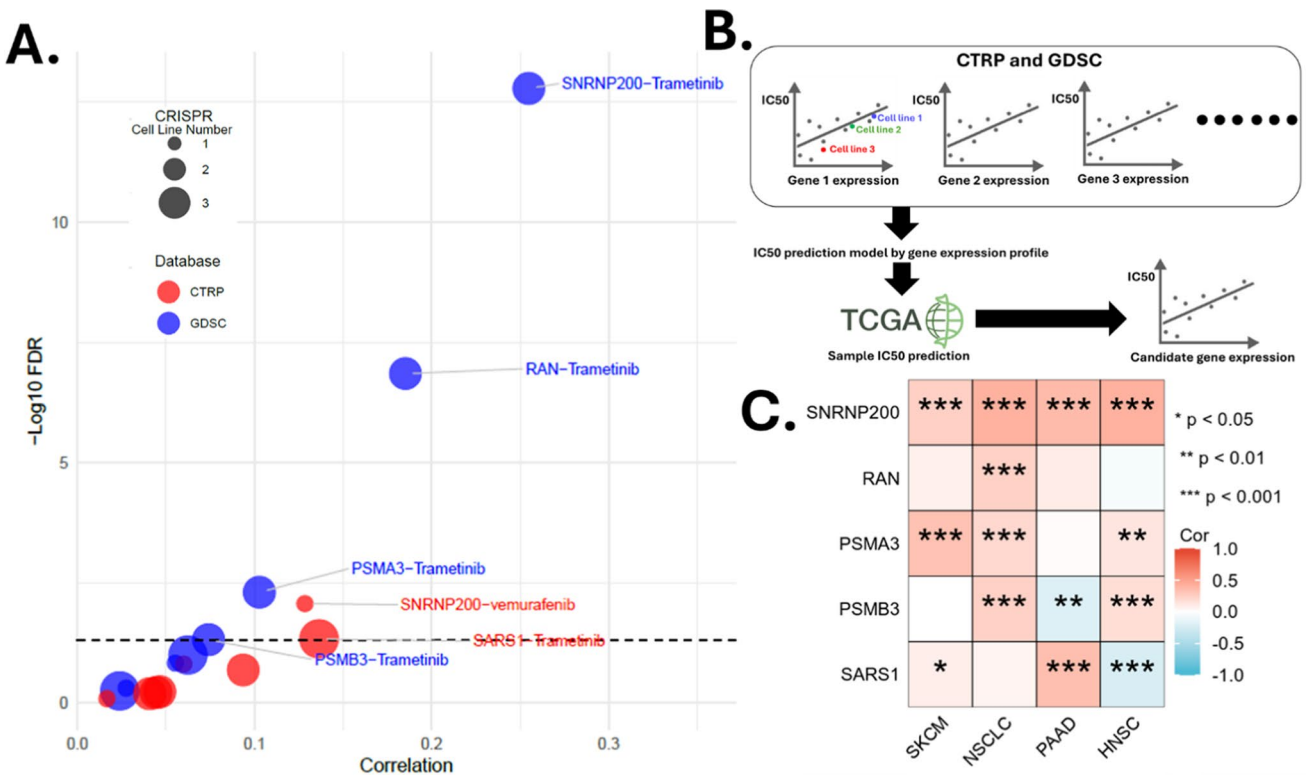

Fig. 1 **A** Volcano plot of expression/IC50 correlation of CRISPR screening obtained gene–drug pairs. **B** Schematic diagram for validating the correlation of drug-resistant gene candidates using data from CTRP/GDSC and TCGA. **C** Heatmap of correlation between gene level and predicted IC50 in selected cancer types

resistance to trametinib, and head and neck cancer (ICD-11: 2D42) has resistance information discovered by cell line testing. Therefore, we focused our analysis on these four cancer types. Results showed that SNRNP200 exhibited a remarkable positive correlation in all four cancer types (SKCM, NSCLC, PAAD, and HNSC). RAN showed a significant correlation only in NSCLC. PSMA3 demonstrated a significant positive correlation in SKCM, NSCLC, and HNSC, but not in PAAD. PSMB3 displayed a significant positive correlation in NSCLC and HNSC, but not in SKCM; in PAAD, the correlation trend was negative and inconsistent with CRISPR screening and GDSC data IC50 correlation analysis. SARS1 showed a slight positive correlation in SKCM and PAAD, but in HNSC, the correlation trend was negative and inconsistent with CRISPR screening and GDSC data IC50 correlation analysis (Fig. 1C).

Therefore, after these analyses, we concluded that for trametinib resistance:

- SNRNP200 is key in SKCM, NSCLC, PAAD, and HNSC.
- RAN is key in NSCLC.
- PSMA3 is key in SKCM, NSCLC, and HNSC.
- PSMB3 is key in NSCLC and HNSC.
- SARS1 is key in SKCM and PAAD.

In summary, this correspondence integrated CRISPR screening data and cell line IC50 data to identify novel genes, including SNRNP200, RAN, PSMA3, PSMB3, and SARS1, associated with trametinib resistance, which has not been previously reported. This data-driven analysis enriches our understanding of trametinib resistance mechanisms; however, further experimental validation is crucial to conclusively confirm these findings.

Author contributions HL write the paper, and PW reversed the paper.

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Data availability No datasets were generated or analyzed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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