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Research Article
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Targeting *WTAP* sensitizes hepatocellular carcinoma to sorafenib by inhibiting the ERK signaling pathway

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Abstract: Hepatocellular carcinoma (HCC) often requires targeted therapy and immunotherapy due to frequent delayed diagnosis. Sorafenib, the first targeted drug applied to treat HCC, has demonstrated a remarkable therapeutic effect in the clinic. However, its clinical application has been limited by drug resistance and the insufficient understanding of the relevant mechanism. Wilms' tumor 1-associated protein (WTAP), associated with tumor progression, remains unstated in sorafenib resistance. In this study, *WTAP* expression patterns in HCC were systematically characterized through integrative analysis of The Cancer Genome Atlas (TCGA) datasets and spatial transcriptomic profiling. To delineate the potential mechanisms of *WTAP*-mediated sorafenib resistance in HCC, multimodal approaches integrating gene set enrichment analysis (GSEA), predictions from the "oncoPredict" package in vitro experiments, molecular docking simulations, and western blot validation were applied. To further investigate the role of *WTAP* in drug resistance, hydrodynamic tail vein injection (HTVi) mouse models and immunohistochemistry were utilized. Significant *WTAP* upregulation was identified in HCC tissues, showing strong associations with tumor progression and adverse clinical outcomes. The knockdown of *WTAP* sensitized HCC cells to sorafenib in vitro. GSEA, molecular docking analysis, and western blot analysis demonstrated that *WTAP* induces the activation of the extracellular signal-regulated kinase (ERK) signaling pathway, a critical link in chemoresistance mechanisms. In the HTVi HCC model, the combination of *WTAP* knockdown with sorafenib markedly suppressed tumor progression and boosted survival rates. These findings highlight that *WTAP* positively regulates the ERK pathway in HCC, promoting sorafenib resistance; therefore, targeting *WTAP* may represent a novel strategy to potentiate sorafenib responsiveness in HCC.

Key words: Wilms' tumor 1-associated protein (WTAP); Sorafenib resistance; Extracellular signal-regulated kinase (ERK) pathway; Hepatocellular carcinoma (HCC)

1 Introduction

Hepatocellular carcinoma (HCC) persists as a critical oncologic challenge, ranking third globally in terms of cancer-related mortality, with a 5-year survival probability below 23% (Global Burden of Disease 2019 Cancer Collaboration, 2022; Vogel et al., 2022; Siegel et al., 2024). The delayed diagnosis of HCC often severely impacts treatment outcomes and patient prognosis. Current treatment paradigms for advanced HCC primarily revolve around interventional therapy, targeted therapy, and immunotherapy (Keating, 2017). Sorafenib is a first-generation drug for targeted therapies for advanced HCC, and its efficacy has been confirmed in clinical trials (Galle et al., 2018). However, durable therapeutic responses are limited in most HCC

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patients due to the early onset of sorafenib resistance, significantly compromising treatment efficacy (Xia et al., 2020). Sorafenib has also been associated with some adverse reactions, such as diarrhea, fatigue, palmoplantar keratoderma, and hyperkeratosis (Zhang DD et al., 2024). If the sensitivity of HCC to sorafenib could be improved and its effective dose could be reduced, the incidence and degree of adverse reactions could also be alleviated. Given the suboptimal response rates to current therapies, elucidating the resistance mechanisms in targeted therapeutic approaches is critical for optimizing therapeutic strategies and clinical outcomes in HCC management.

Wilms' tumor 1-associated protein (WTAP), a nuclear speckle-localized protein, exhibits functional co-localization with splicing factors. Recent research has highlighted its pivotal role in promoting tumor progression. Specifically, WTAP has been identified as a key molecule that promotes breast, lung, colon, and especially liver cancer (Chen et al., 2019; Li et al., 2024; Zhu et al., 2024; Zhou et al., 2025). Our previous studies have shown that the promoting effect of WTAP in liver cancer is mediated through the cell cycle (Chen et al., 2019). However, we did not clarify whether WTAP inhibition could be used in combination with existing drugs, particularly sorafenib, to improve treatment outcomes in liver cancer. Thus, we hope to further explore whether the therapeutic synergy of combined WTAP inhibition and sorafenib can improve drug sensitivity in targeted therapy for liver cancer and explore its potential mechanisms of action.

This study delves into the potential of WTAP to potentiate sorafenib sensitivity in HCC cells, revealing a promising therapeutic strategy to improve clinical survival outcomes.

2 Results

2.1 Elevated expression and unfavorable prognostic association of *WTAP* in HCC

In this study, to clarify the biological function of *WTAP*, its transcriptional profile in clinical HCC specimens obtained from The Cancer Genome Atlas (TCGA) database was first analyzed. The findings revealed a significant upregulation of *WTAP* expression in the tumor tissues of liver cancer patients (Fig. 1a). Consistently, when paired normal and tumor tissues adjacent to HCC (n=50) were compared, a higher expression of *WTAP* was detected in the cancerous tissues (Fig. 1b). Spatial transcriptomics data visually confirmed the significant upregulation of *WTAP* transcript levels in HCC tissues from liver cancer patients (Fig. 1c). Immunohistochemical profiling of HCC specimens archived in the Human Protein Atlas (HPA) revealed significantly elevated WTAP protein expression compared with that in non-neoplastic hepatic tissues (Fig. 1d). The *WTAP* expression levels showed a statistically significant association with HCC clinical stage (P< 0.05; Fig. 1e), suggesting that patients with more advanced clinical stages typically exhibit higher *WTAP* transcript levels. Furthermore, Kaplan-Meier survival analysis indicated that higher *WTAP* messenger RNA (mRNA) expression is predictive of a poorer overall survival rate (P<0.001; Fig. 1f).

2.2 Diminished susceptibility of HCC cells to sorafenib therapy by *WTAP* in vitro

In order to investigate the role of *WTAP* in targeted therapy for HCC, we leveraged data from the Gene Expression Omnibus (GEO) dataset (GSE199092) and observed that the treatment with sorafenib (7.5 μmol/L for 3 d) led to an upregulation of *WTAP* mRNA expression in the HepAD38 wild-type (WT) cell line (Fig. 2a), suggesting a potential link between *WTAP* and resistance to HCC targeted therapy. Subsequent GSEA of *WTAP* single-gene differential expression data from TCGA database revealed significant enrichment of the "primary bile acid biosynthesis" and "drug metabolism cytochrome P450 pathways" (Fig. 2b). The literature has reported that alterations in the metabolic flux of the tumor microenvironment, particularly lipid metabolism, can contribute to increased cellular resistance to drugs (Cao, 2019; Tang et al., 2020). Cytochrome P450, a membrane-bound protein, plays a pivotal role in drug detoxification and cellular metabolism (Zhao et al., 2021) and can serve as a target for anticancer treatments and a biomarker within tumor cells (Paolini et al., 2017; Wang et al., 2020). Therefore, we hypothesized that *WTAP* is associated with resistance to targeted therapies in HCC. By utilizing the R package "oncoPredict" to predict the sensitivity of HCC to sorafenib treatment, high expression levels of *WTAP* were found to be correlated with increased resistance to HCC-targeted therapies, while lower *WTAP* levels were found to enhance the sensitivity of HCC to

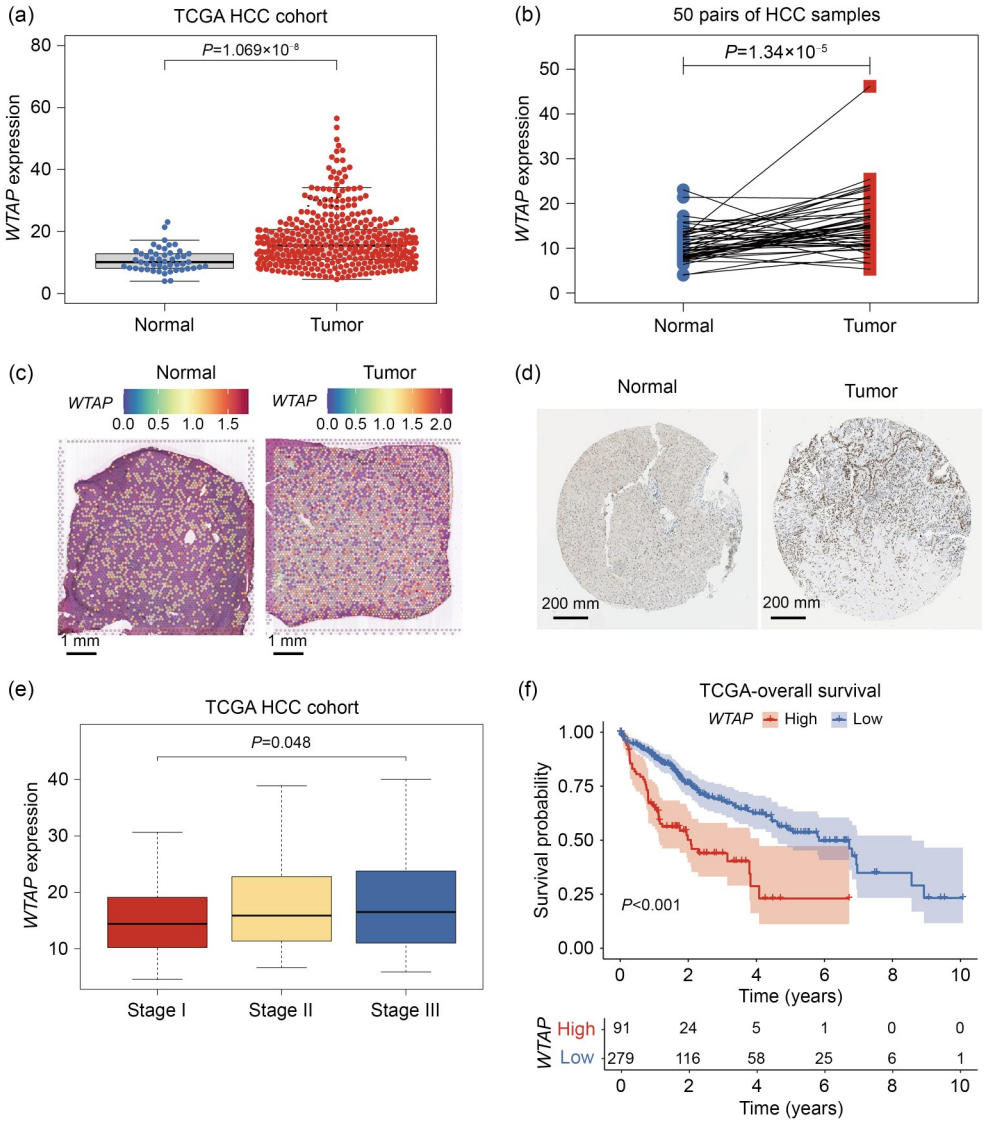

Fig. 1 Association of upregulation of Wilms' tumor 1-associated protein (*WTAP*) in hepatocellular carcinoma (HCC) with adverse outcomes. (a) The Cancer Genome Atlas (TCGA) database was utilized to analyze the messenger RNA (mRNA) expression of *WTAP* in HCC. (b) *WTAP* mRNA expression in 50 pairs of HCC tissues in the TCGA cohort. (c) Spatial feature plots of the signatures of *WTAP* based on spatial hematoxylin and eosin (HE) staining of HCC-N (normal) and HCC-T (tumor) tissue sections. (d) Representative immunohistochemistry (IHC) images of WTAP in normal and HCC tissues were obtained from the Human Protein Atlas (HPA) database. (e) The analysis of TCGA database showed significant differences in *WTAP* mRNA expression among different cancer stages and tumor grades ($P<0.05$). (f) Kaplan-Meier survival analysis demonstrated a significantly higher survival rate in the low-*WTAP* group compared with the high-*WTAP* group ($P<0.001$).

sorafenib treatment (Fig. 2c). *WTAP*-knockdown Huh7 cells were generated using two small interfering RNAs (siRNAs; siWTAP1 and siWTAP2), and both *WTAP* interference RNAs decreased WTAP protein expression (Fig. 2d). Furthermore, *WTAP* knockdown ameliorated the resistance of Huh7 cells to sorafenib compared with siRNA control (siControl) combined with sorafenib (Fig. 2e). Collectively, these data suggest that the inhibition of *WTAP* could enhance sorafenib sensitivity in HCC.

2.3 Targeting *WTAP* with siWTAP for potentiating sorafenib efficacy in HCC treatment in vivo

In order to elucidate the in vivo efficacy of siRNA-targeting *WTAP* in combination with sorafenib in a murine model of HCC, an HCC model was initially

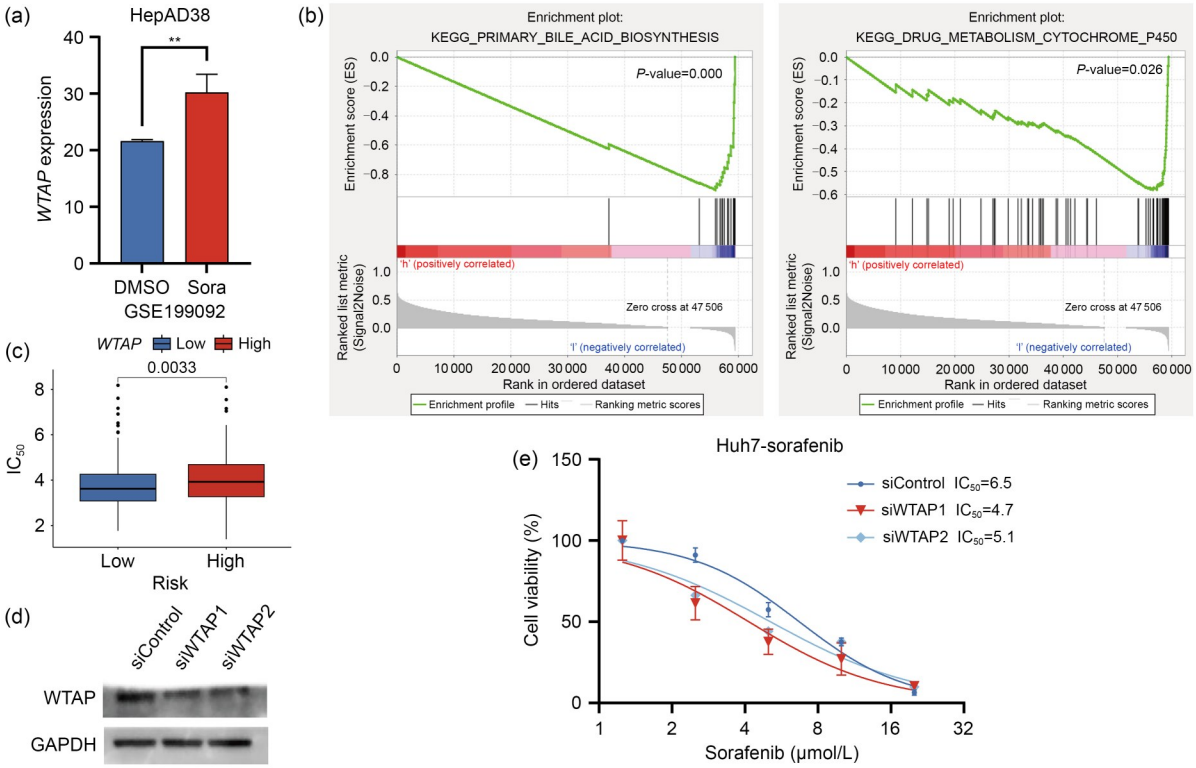

Fig. 2 Resistance of hepatocellular carcinoma (HCC) cells to sorafenib following targeting Wilms' tumor 1-associated protein (*WTAP*) in vitro. (a) The expression of *WTAP* messenger RNA (mRNA) in HepAD38 wild-type (WT) cells treated with sorafenib (7.5 μmol/L, 3 d) based on Gene Expression Omnibus (GEO) dataset (GSE199092). DMSO: dimethyl sulfoxide; Sora: sorafenib. (b) Gene set enrichment analysis (GSEA) revealed significant enrichment of the PRIMARY_BILE_ACID_BIOSYNTHESIS and DRUG_METABOLISM_CYTOCHROME_P450 pathways in HCC samples stratified by low-*WTAP* and high-*WTAP* expression levels, as determined by RNA sequencing (RNA-seq) data from The Cancer Genome Atlas (TCGA) database. KEGG: Kyoto Encyclopedia of Genes and Genomes. (c) The differential expression groups of *WTAP* were used to predict sensitivity to sorafenib treatment. IC_{50}: half maximal inhibitory concentration. (d) Western blot analysis was performed to determine the transfection efficiency in Huh7 cells. GAPDH: glyceraldehyde-3-phosphate dehydrogenase. (e) A dose–response curve was generated to evaluate the effect of *WTAP* knockdown on sorafenib-treated HCC cells. The data are expressed as mean±standard error of the mean (SEM), n=3. ** P<0.01.

established using protein kinase B (AKT)/Ras plasmids. One month post-inoculation, the mice were administered sorafenib at a dosage of 12.5 mg/kg daily via oral gavage and siWTAP at 1 mg/kg via tail vein injection every 3–4 d for a total of five injections (Fig. 3a). The experimental groups received corn oil, siControl, combined sorafenib (12.5 mg/kg) with siControl, or combined sorafenib (12.5 mg/kg) with siWTAP (Fig. 3b). Notably, compared with the control group, sorafenib alone inhibited tumor growth and improved the health status of the mice, while the combination of sorafenib with siWTAP had a more significant tumor inhibition effect and could effectively prolong the survival time of the mice (Fig. 3c). Data on the body weight of the siRNA-treated mice indicated that the depletion of *WTAP* in hepatocytes was effective (Fig. 3d). Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) apoptosis marker and Ki67 staining confirmed that siWTAP enhanced the drug sensitivity to sorafenib. TUNEL apoptosis marker staining showed that the apoptosis of liver cancer cells in mice was increased after treatment with sorafenib, and its degree was significantly increased after treatment with siWTAP and sorafenib. Ki67 staining revealed that the proliferation of liver cancer cells in mice was significantly inhibited after treatment with siWTAP and sorafenib, compared with that in the mice treated with sorafenib alone. siWTAP enhanced the sensitivity of HCC cells to sorafenib (Fig. 3e). Collectively, our findings suggest that targeting *WTAP* may serve as a potential sensitizer for molecular targeted therapy in HCC.

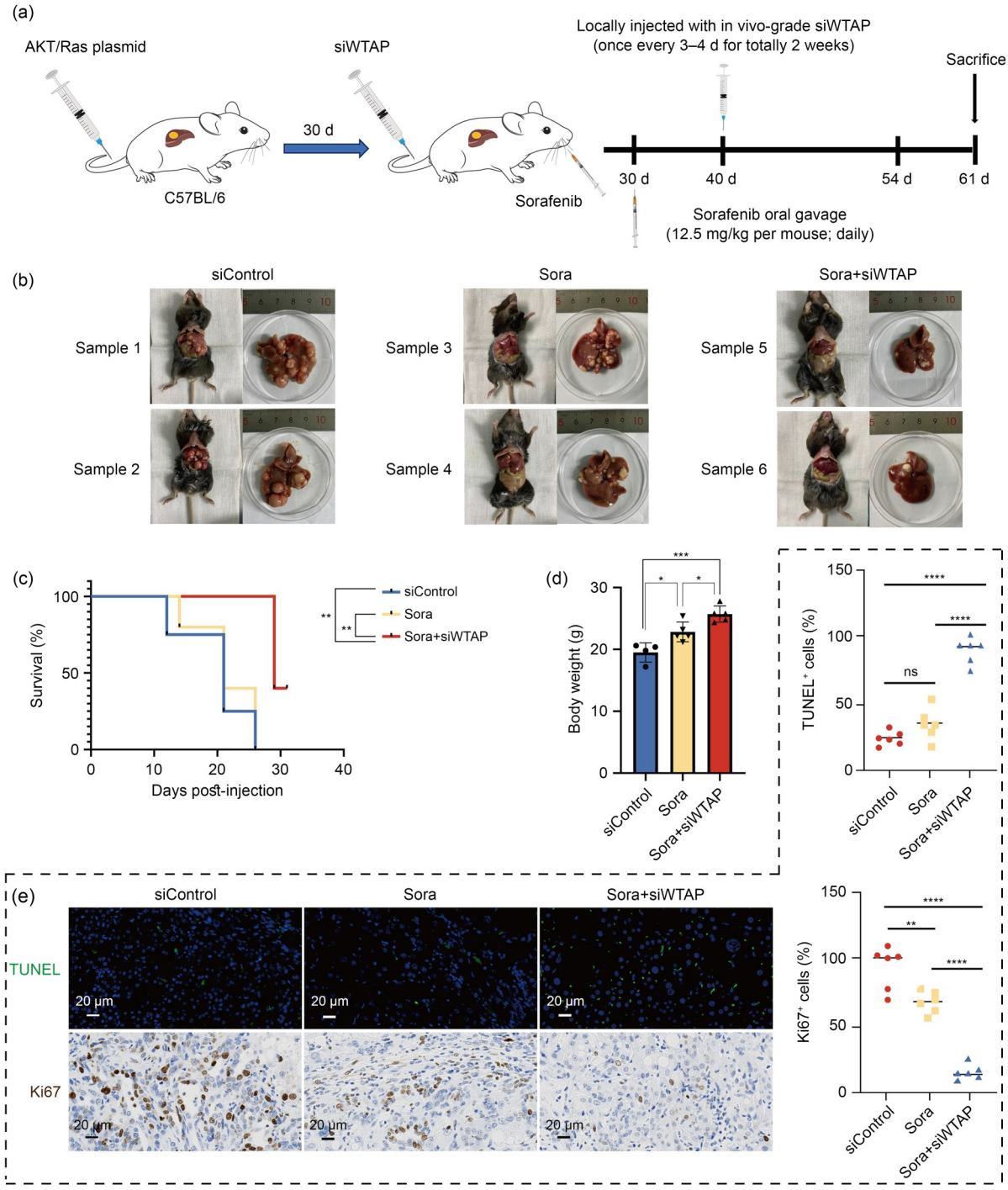

Fig. 3 Increased sensitivity of hepatocellular carcinoma (HCC) to sorafenib by Wilms' tumor 1-associated protein (*WTAP*) knockdown in vivo. (a) Schematic representation of siWTAP treatment in combination with sorafenib (12.5 mg/kg) in protein kinase B (AKT)/Ras HCC mouse models. (b) Orthotopic and macroscopic appearance of livers extracted from AKT/Ras mice 30 d post-treatment with siControl, combined sorafenib (12.5 mg/kg) with siControl, or combined sorafenib (12.5 mg/kg) with siWTAP. (c) Kaplan-Meier estimation of survival over time in AKT/Ras mice (siControl, *n*=4; sorafenib, *n*=5; sorafenib+siWTAP, *n*=5) analyzed by log-rank test (Mantel-Cox). (d) Quantification of body weight at the end of the single or combination treatment. (e) Representative images and quantitative analyses of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) fluorescence staining and Ki67 immunohistochemistry (IHC) staining in AKT/Ras mice treated with either monotherapy or combination therapy. The data are expressed as mean±standard error of the mean (SEM), *n*=3. * *P*<0.05, ** *P*<0.01, *** *P*<0.001, **** *P*<0.0001; ns: not significant. Sora: sorafenib.

2.4 *WTAP* regulation of sorafenib resistance in HCC through the ERK signaling pathway

Drug resistance in targeted therapy most commonly develops by the reactivation of the extracellular signal-regulated kinase (ERK) cascade, which allows tumors to acquire resistance to the drug through a series of signal transduction upregulation mechanisms (Johannessen et al., 2013; Lu et al., 2017; Sugiura et al., 2021). To investigate whether *WTAP* modulates the sensitivity of HCC to targeted therapies through the ERK signaling pathway, we conducted a GSEA with *WTAP* as a single gene to identify downstream targets regulated by the *WTAP* epigenetic axis in HCC. The analysis revealed a significant positive correlation between *WTAP* expression and the ERK (mitogen-activated protein kinase (MAPK)) pathway (P<0.001; Fig. 4a). By visualizing the three-dimensional (3D) molecular structures of WTAP and MAPK from the AlphaFold database using PyMOL software, multiple potential binding sites could be predicted between WTAP and ERK, suggesting a possible interaction between the two proteins (Fig. 4b). Further analysis using the scatter plot correlation tool from the Gene Expression Profiling Interactive Analysis (GEPIA) database (https://gepia.cancer-pku.cn/index.html) confirmed a strong correlation between *WTAP* and ERK (MAPK), with an R value of 0.62 (Fig. 4c). Western blot experiments in Huh7 cells after *WTAP* knockdown showed that reducing *WTAP* levels decreased the phosphorylation levels of the ERK pathway, thereby exerting a positive regulatory effect (Fig. 4d). Building upon the spatial hematoxylin and eosin (HE) staining results of *WTAP* in the HCC-N (normal) and HCC-T (tumor) specimens (Fig. 1c), we performed parallel spatial mapping of ERK (MAPK) while maintaining consistency. A joint evaluation of both datasets identified positively correlated expression patterns between *WTAP* and ERK (MAPK) in the spatial transcriptomics analysis (Fig. 4e). Phosphorylated ERK (p-ERK) staining revealed that, in mice treated with sorafenib or the combination of sorafenib with siWTAP, ERK activation in mouse liver cancer cells was significantly reduced compared with that in mice treated with sorafenib alone. These results are consistent with the spatial transcriptome data (Fig. 4f). Lastly, Kaplan-Meier analysis evaluating the co-expression patterns of *WTAP* and MAPK demonstrated reduced overall survival in HCC patients exhibiting dual elevated expression compared with other cohorts (P=0.011), most notably versus those displaying low co-expression levels of *WTAP* and MAPK (Fig. 4g). These data advance our understanding of the molecular mechanisms of *WTAP* in HCC-targeted therapy and may offer potential targets for optimizing therapeutic regimens.

3 Discussion

This investigation aimed to tackle the current clinical challenges posed by HCC, a globally prevalent malignancy with roughly 700 000 new cases detected annually (Vogel et al., 2022). According to the current scientific consensus, the development of liver cancer is recognized as a multi-step progression mediated by diverse genetic predispositions and environmental exposures (Dhar et al., 2018), in which the dynamic interaction between these factors and cross-talk among signaling pathways collectively drive HCC progression and foster its characteristic heterogeneity (Marquardt and Thorgeirsson, 2014). As the first U.S. Food and Drug Administration (FDA)-approved molecular targeted drug, sorafenib has been shown to have beneficial clinical effects (Cheng et al., 2009), exerting its antitumor effects by inhibiting the Raf/MAPK signaling pathway (Zhu et al., 2017). However, liver cancer often develops resistance to sorafenib during continuous drug administration, necessitating the need for new therapies (Chen et al., 2015). Our research was driven by the need to understand the mechanism of resistance to targeted therapies, which represents a significant barrier to improving treatment efficacy and patient prognosis.

There is already a volume of research addressing potential mechanisms of resistance to targeted therapies in liver cancer. For instance, methylation modifications were shown to play a crucial role in sorafenib resistance in HCC (Shi et al., 2024; Fu et al., 2025), with the methylated circRNA-SORE (a circular RNA upregulated in sorafenib-resistant HCC cells) maintaining sorafenib resistance in liver cancer by regulating β-catenin signaling (Xu et al., 2020b). Zhang XY et al. (2024) elucidated the YTH domain-containing family protein 1 (YTHDF1)–N^6-methyladenosine (m^6A)–neurogenic locus notch homolog protein 1 (NOTCH1) axis in the regulation of resistance to sorafenib and lenvatinib. m^6A RNA methylation-mediated hepatocyte

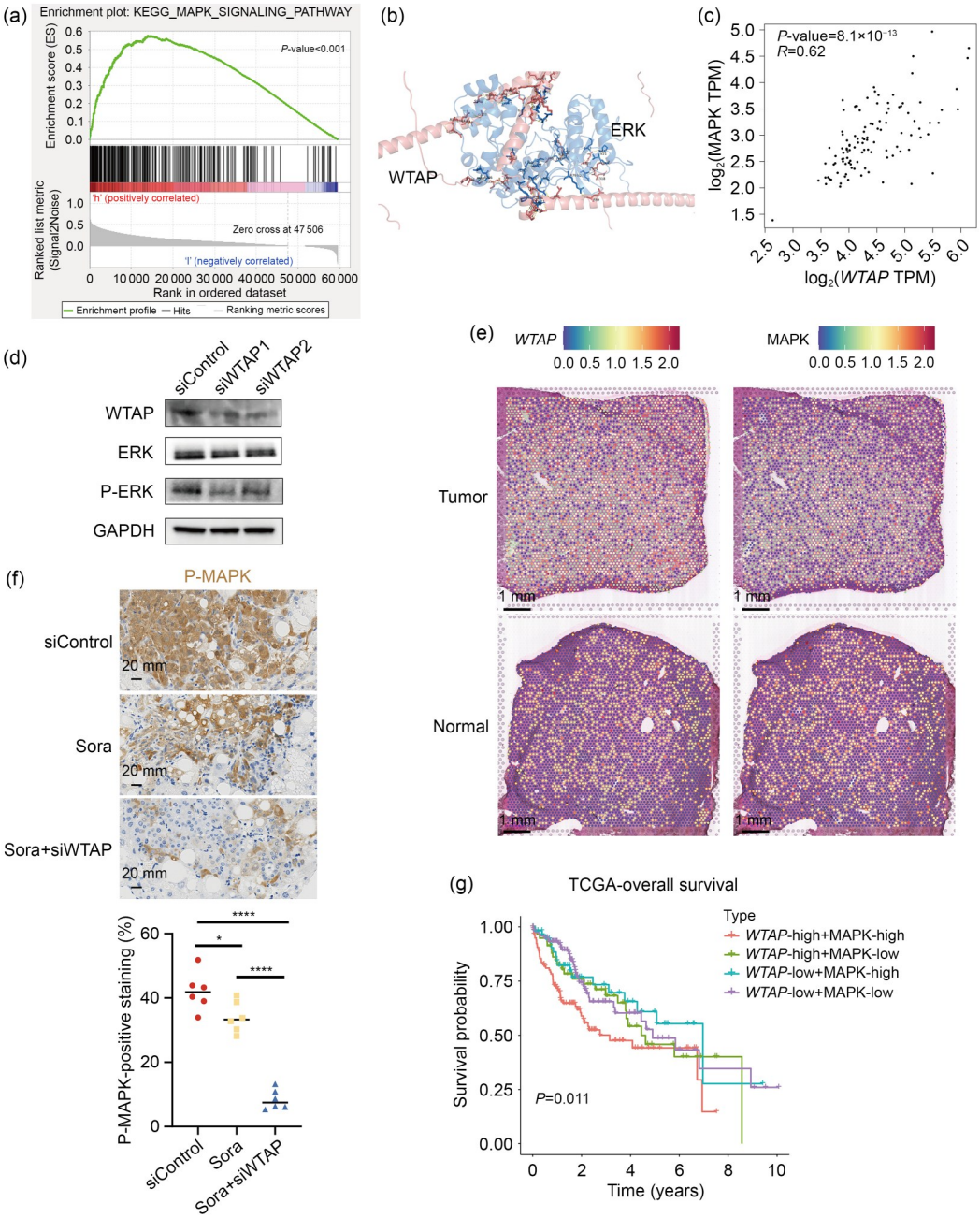

Fig. 4 Wilms' tumor 1-associated protein (*WTAP*) regulation of the extracellular signal-regulated kinase (ERK) pathway in hepatocellular carcinoma (HCC). (a) The gene set enrichment analysis (GSEA) results reveal a significant positive correlation between *WTAP* expression and the ERK (mitogen-activated protein kinase (MAPK)) pathway. KEGG: Kyoto Encyclopedia of Genes and Genomes. (b) Docking analysis demonstrates the interaction between WTAP and ERK. (c) Gene expression profiling interactive analysis (GEPIA) (https://gepia.cancer-pku.cn/index.html) confirms the correlation between *WTAP* and ERK (MAPK). TPM: transcripts per million. (d) Protein levels of WTAP and ERK pathway markers (ERK and phosphorylated ERK (p-ERK)) upon *WTAP* knockdown by western blotting in Huh7 cells. GAPDH: glyceraldehyde-3-phosphate dehydrogenase. (e) Spatial hematoxylin and eosin (HE) staining and spatial feature plots of the signatures of *WTAP* (the same as Fig. 1c) and ERK (MAPK) in HCC-N (normal) and HCC-T (tumor) tissue sections. (f) Representative images and quantitative analysis of phosphorylated MAPK (p-MAPK) immunohistochemistry (IHC) staining in protein kinase B (AKT)/Ras mice treated with either monotherapy or combination therapy. Sora: sorafenib. The data are expressed as mean±standard error of the mean (SEM), n=3. * P<0.05, **** P<0.0001. (g) Overall survival analysis of HCC patients stratified by ERK (MAPK) expression status in the *WTAP*-high and *WTAP*-low groups according to The Cancer Genome Atlas (TCGA) data.

nuclear factor 3γ (HNF3γ) is another potential resistance mechanism (Zhou et al., 2020). Additionally, heat shock protein 90β (HSP90β) regulates STIP1 homology and U box-containing protein 1 (STUB1)-induced YTHDF2 ubiquitination, which is involved in the resistance mechanism of HCC to sorafenib (Liao et al., 2023). Xu et al. (2020a) reported that circRNA-SORE mediates sorafenib resistance by stabilizing Y-box-binding protein 1 (YBX1). While WTAP as a binding partner of Wilms' tumor 1 (WT1) is well-known for its methylation functions (Liu et al., 2014), previous findings from our team indicated that it enhances the proliferation of liver cancer cells by silencing ETS proto-oncogene 1 (ETS1) (Chen et al., 2019). However, whether *WTAP* can improve the efficacy of existing targeted drugs in liver cancer treatment has not been investigated.

The findings of this study revealed that *WTAP* plays a critical role in HCC. The analysis of TCGA dataset demonstrated elevated *WTAP* expression in liver cancer tumor tissues, suggesting a correlation between high *WTAP* levels and poor prognosis. The significant association between *WTAP* expression and advanced clinical tumor stage, along with Kaplan-Meier survival analysis, further confirmed that *WTAP* is associated with poor prognosis. These results are consistent with our prior investigation (Chen et al., 2019). In vitro studies further clarified the role of *WTAP* in drug resistance, demonstrating that *WTAP* knockdown sensitizes HCC cells to sorafenib, a standard targeted therapy drug for HCC. This finding is particularly significant, as the high expression of *WTAP* is associated with increased resistance to targeted therapy, as predicted by the R package "oncoPredict" (Maeser et al., 2021). Co-treatment with siWTAP and sorafenib demonstrated synergistic activity in promoting chemosensitivity and triggering apoptotic cell death in HCC cells, underscoring the therapeutic potential of *WTAP* for overcoming drug resistance.

In vivo studies corroborated our in vitro findings, demonstrating that the combination of siWTAP with sorafenib significantly inhibited tumor growth and improved the health status of mice with HCC compared with sorafenib alone. This suggests that targeting *WTAP* could potentially enhance the efficacy of sorafenib, providing a novel strategy for HCC treatment.

Furthermore, our research uncovered the molecular mechanisms by which *WTAP* may regulate drug resistance. The ERK (MAPK) pathway encompasses multiple signaling cascade frameworks that rely on phosphorylation events for activation, with various interacting pathways involved. These activated kinases participate in controlling cellular growth, differentiation, proliferation, apoptosis, and migration, and are significant players in tumorigenesis (Santarpia et al., 2012; Braicu et al., 2019; Sugiura et al., 2021). ERK is a well-known key molecule in cell signal transduction, and is involved in the development of drug resistance (Zhang et al., 2009). Our data revealed a strong positive association between *WTAP* levels and ERK/MAPK signaling activity. Molecular docking analysis further supported the interaction between *WTAP* and MAPK, and protein immunoblot analysis confirmed the downregulation of ERK phosphorylation after *WTAP* knockdown.

While our study provides valuable insights into the role of *WTAP* in HCC, there are limitations that warrant further investigations. First, our analysis was focused on a single gene, *WTAP*, and did not explore the broader landscape of genes involved in HCC progression and drug resistance. Second, this study primarily addressed post-transcriptional regulation and failed to delve into the potential transcriptional control of *WTAP*. Third, while our in vivo results suggest a synergistic effect of *WTAP* knockdown with sorafenib, the long-term effects and potential side effects of this combination therapy in a clinical setting have yet to be determined. Finally, the specific mechanisms by which *WTAP* interacts with the ERK pathway require further elucidation, as does the exploration of other potential signaling pathways that may be influenced by *WTAP*.

Targeting *WTAP* to overcome sorafenib resistance by siRNA knockdown strategy has certain limitations and technical challenges. The knockdown efficiency and specificity of siWTAP may be affected by various factors, including sequence composition, chemical modification patterns, and delivery methods (Hu et al., 2020). All these factors make differences in efficacy and off-target effects. At the same time, due to the crucial role of *WTAP* in the RNA methylation process, its systematic knockdown may disrupt normal cellular functions. To overcome these challenges, our future research will adopt an integrated optimization approach, combining the use of established bioinformatics tools for computational prediction of off-target effects, as well as a rational sequence design considering thermodynamic parameters and secondary structure. Moreover, we may attempt to construct liver-specific delivery

systems, such as *N*-acetylgalactosamine (GalNAc) linkage (Prakash et al., 2016), to enhance tissue-specific targeting and to minimize systemic effects. These optimization strategies will be systematically evaluated through well-designed animal experiments to rigorously test their therapeutic effects and biological safety, thereby promoting the translational application of this therapy to target *WTAP*.

4 Conclusions

Our study provides compelling evidence for the role of *WTAP* in HCC progression and drug resistance. By targeting *WTAP*, we may be able to sensitize HCC cells to sorafenib, offering a promising avenue for improving patient outcomes. Future research should focus on validating these findings in larger cohorts and exploring the potential of *WTAP* as a therapeutic target in HCC.

Materials and methods

Detailed methods are provided in the electronic supplementary materials of this paper.

Data availability statement

To acquire the TCGA-liver hepatocellular carcinoma (LIHC) dataset, we utilized the TCGA data portal website (https://gdac.broadinstitute.org). The publicly available RNA sequencing (RNA-seq) dataset employed in this study was retrieved from the GEO database (https://www.ncbi.nlm.nih.gov/geo), specifically the dataset GSE199092. Additionally, transcriptome sequencing data from four samples, including HCC and normal tissues, were obtained from the study by Wu et al. (2021). Additional research data created in this work are available from the corresponding authors upon reasonable request.

Acknowledgments

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Author contributions

Yu WU and Guomin JU contributed equally to designing the study, developing methods, analyzing the data, and writing the first draft. Xueyu ZHOU was responsible for data management. Jian WU and Chuanhui PENG contributed to manuscript revision and obtained funding. Chuanhui PENG and Shusen ZHENG participated in the research investigations and project management. The finalized manuscript underwent rigorous review and unanimous approval by all the authors, who additionally confirmed full dataset accessibility while accepting responsibility for its precision and secure management.

Compliance with ethics guidelines

Yu WU, Guomin JU, Xueyu ZHOU, Jian WU, Shusen ZHENG, and Chuanhui PENG declare that they have no conflicts of interest.

All animal procedures strictly adhered to ethical standards and were formally authorized by the Institutional Animal Ethics Committee of The First Affiliated Hospital, Zhejiang University School of Medicine (Ethics Approval No. 2025069).

Declaration on the use of generative AI tools

During the preparation of this work, the authors used DeepSeek in order to improve language and readability and check for grammatical errors. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

References

Braicu C, Buse M, Busuioc C, et al., 2019. A comprehensive review on MAPK: a promising therapeutic target in cancer. *Cancers*, 11(10):1618. https://doi.org/10.3390/cancers11101618

Cao YH, 2019. Adipocyte and lipid metabolism in cancer drug resistance. *J Clin Invest*, 129(8):3006-3017. https://doi.org/10.1172/JCI127201

Chen J, Jin RN, Zhao J, et al., 2015. Potential molecular, cellular and microenvironmental mechanism of sorafenib resistance in hepatocellular carcinoma. *Cancer Lett*, 367(1):1-11. https://doi.org/10.1016/j.canlet.2015.06.019

Chen YH, Peng CH, Chen JR, et al., 2019. WTAP facilitates progression of hepatocellular carcinoma via m6A-HuR-dependent epigenetic silencing of ETS1. *Mol Cancer*, 18:127. https://doi.org/10.1186/s12943-019-1053-8

Cheng AL, Kang YK, Chen ZD, et al., 2009. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol*, 10(1):25-34. https://doi.org/10.1016/S1470-2045(08)70285-7

Dhar D, Antonucci L, Nakagawa H, et al., 2018. Liver cancer initiation requires p53 inhibition by CD44-enhanced growth factor signaling. *Cancer Cell*, 33(6):1061-1077.e6. https://doi.org/10.1016/j.ccell.2018.05.003

Fu CL, Zhao ZW, Zhang QN, 2025. The crosstalk between cellular survival pressures and N6-methyladenosine modification in hepatocellular carcinoma. *Hepatob Pancreat Dis Int*, 24(1):67-75. https://doi.org/10.1016/j.hbpd.2024.08.004

Galle PR, Forner A, Llovet JM, et al., 2018. EASL Clinical Practice Guidelines: management of hepatocellular carcinoma.

J Hepatol, 69(1):182-236. https://doi.org/10.1016/j.jhep.2018.03.019

Global Burden of Disease 2019 Cancer Collaboration, 2022. Cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life years for 29 cancer groups from 2010 to 2019: a systematic analysis for the global burden of disease study 2019. *JAMA Oncol*, 8(3):420-444. https://doi.org/10.1001/jamaoncol.2021.6987

Hu B, Zhong LP, Weng YH, et al., 2020. Therapeutic siRNA: state of the art. *Sig Transduct Target Ther*, 5:101. https://doi.org/10.1038/s41392-020-0207-x

Johannessen CM, Johnson LA, Piccioni F, et al., 2013. A melanocyte lineage program confers resistance to MAP kinase pathway inhibition. *Nature*, 504(7478):138-142. https://doi.org/10.1038/nature12688

Keating GM, 2017. Sorafenib: a review in hepatocellular carcinoma. *Targ Oncol*, 12(2):243-253. https://doi.org/10.1007/s11523-017-0484-7

Li TJ, Huang YQ, Cui SE, et al., 2024. RNA methylation patterns of tumor microenvironment cells regulate prognosis and immunotherapeutic responsiveness in patients with triple-negative breast cancer. *Sci Rep*, 14:26075. https://doi.org/10.1038/s41598-024-77941-2

Liao YN, Liu Y, Yu CF, et al., 2023. HSP90β impedes STUB1-induced ubiquitination of YTHDF2 to drive sorafenib resistance in hepatocellular carcinoma. *Adv Sci*, 10(27): 2302025. https://doi.org/10.1002/advs.202302025

Liu JZ, Yue YN, Han DL, et al., 2014. A METTL3-METTL14 complex mediates mammalian nuclear RNA N^6-adenosine methylation. *Nat Chem Biol*, 10(2):93-95. https://doi.org/10.1038/nchembio.1432

Lu HZ, Liu SJ, Zhang G, et al., 2017. PAK signalling drives acquired drug resistance to MAPK inhibitors in *BRAF*-mutant melanomas. *Nature*, 550(7674):133-136. https://doi.org/10.1038/nature24040

Maeser D, Gruener RF, Huang RS, et al., 2021. oncoPredict: an R package for predicting *in vivo* or cancer patient drug response and biomarkers from cell line screening data. *Brief Bioinform*, 22(6):bbab260. https://doi.org/10.1093/bib/bbab260

Marquardt JU, Thorgeirsson SS, 2014. SnapShot: hepatocellular carcinoma. *Cancer Cell*, 25(4):550-550.e1. https://doi.org/10.1016/j.ccr.2014.04.002

Paolini M, Poul L, Darmon A, et al., 2017. A new opportunity for nanomedicines: micellar cytochrome P450 inhibitors to improve drug efficacy in a cancer therapy model. *Nanomed Nanotechnol Biol Med*, 13(5):1715-1723. https://doi.org/10.1016/j.nano.2017.03.006

Prakash TP, Yu JH, Migawa MT, et al., 2016. Comprehensive structure-activity relationship of triantennary *N*-acetylgalactosamine conjugated antisense oligonucleotides for targeted delivery to hepatocytes. *J Med Chem*, 59(6): 2718-2733. https://doi.org/10.1021/acs.jmedchem.5b01948

Santarpia L, Lippman SM, El-Naggar AK, 2012. Targeting the MAPK-RAS-RAF signaling pathway in cancer therapy. *Expert Opin Ther Targ*, 16(1):103-119. https://doi.org/10.1517/14728222.2011.645805

Shi YX, Li K, Yuan YC, et al., 2024. Comprehensive analysis of m6A modification in immune infiltration, metabolism and drug resistance in hepatocellular carcinoma. *Cancer Cell Int*, 24:138. https://doi.org/10.1186/s12935-024-03307-3

Siegel RL, Giaquinto AN, Jemal A, 2024. Cancer statistics, 2024. *CA Cancer J Clin*, 74(1):12-49. https://doi.org/10.3322/caac.21820

Sugiura R, Satoh R, Takasaki T, 2021. ERK: a double-edged sword in cancer. ERK-dependent apoptosis as a potential therapeutic strategy for cancer. *Cells*, 10(10):2509. https://doi.org/10.3390/cells10102509

Tang WW, Chen ZY, Zhang WL, et al., 2020. The mechanisms of sorafenib resistance in hepatocellular carcinoma: theoretical basis and therapeutic aspects. *Sig Transduct Target Ther*, 5:87. https://doi.org/10.1038/s41392-020-0187-x

Vogel A, Meyer T, Sapisochin G, et al., 2022. Hepatocellular carcinoma. *Lancet*, 400(10360):1345-1362. https://doi.org/10.1016/S0140-6736(22)01200-4

Wang Q, Sun MC, Li D, et al., 2020. Cytochrome P450 enzyme-mediated auto-enhanced photodynamic cancer therapy of co-nanoassembly between clopidogrel and photosensitizer. *Theranostics*, 10(12):5550-5564. https://doi.org/10.7150/thno.42633

Wu R, Guo WB, Qiu XY, et al., 2021. Comprehensive analysis of spatial architecture in primary liver cancer. *Sci Adv*, 7(51): eabg3750. https://doi.org/10.1126/sciadv.abg3750

Xia SJ, Pan Y, Liang YL, et al., 2020. The microenvironmental and metabolic aspects of sorafenib resistance in hepatocellular carcinoma. *EBioMedicine*, 51:102610. https://doi.org/10.1016/j.ebiom.2019.102610

Xu JJ, Ji L, Liang YL, et al., 2020a. CircRNA-SORE mediates sorafenib resistance in hepatocellular carcinoma by stabilizing YBX1. *Sig Transduct Target Ther*, 5:298. https://doi.org/10.1038/s41392-020-00375-5

Xu JJ, Wan Z, Tang MY, et al., 2020b. N^6-methyladenosine-modified circRNA-SORE sustains sorafenib resistance in hepatocellular carcinoma by regulating β-catenin signaling. *Mol Cancer*, 19:163. https://doi.org/10.1186/s12943-020-01281-8

Zhang DD, Cai Y, Sun YX, et al., 2024. A real-world pharmacovigilance study of Sorafenib based on the FDA Adverse Event Reporting System. *Front Pharmacol*, 15:1442765. https://doi.org/10.3389/fphar.2024.1442765

Zhang XY, Su TH, Wu YF, et al., 2024. N^6-methyladenosine reader YTHDF1 promotes stemness and therapeutic resistance in hepatocellular carcinoma by enhancing NOTCH1 expression. *Cancer Res*, 84(6):827-840. https://doi.org/10.1158/0008-5472.CAN-23-1916

Zhang Z, Zhou XY, Shen HJ, et al., 2009. Phosphorylated ERK is a potential predictor of sensitivity to sorafenib when treating hepatocellular carcinoma: evidence from an *in vitro* study. *BMC Med*, 7:41. https://doi.org/10.1186/1741-7015-7-41

Zhao MZ, Ma JS, Li M, et al., 2021. Cytochrome P450 enzymes and drug metabolism in humans. *Int J Mol Sci*, 22(23): 12808. https://doi.org/10.3390/ijms222312808

Zhou CF, Wang M, Du XM, et al., 2025. WTAP/IGF2BP3 mediated m6A modification of SOD2 mRNA aggravates the tumourigenesis of colorectal cancer. *J Biochem Mol Toxicol*, 39(1):e70117. https://doi.org/10.1002/jbt.70117

Zhou TF, Li SC, Xiang DM, et al., 2020. m6A RNA methylation-mediated HNF3γ reduction renders hepatocellular carcinoma dedifferentiation and sorafenib resistance. *Sig Transduct Target Ther*, 5:296. https://doi.org/10.1038/s41392-020-00299-0

Zhu XX, Chen XY, Zhao LT, et al., 2024. WTAP-mediated m^6A modification of circSMOC1 accelerates the tumorigenesis of non-small cell lung cancer by regulating miR-612/CCL28 axis. *J Cell Mol Med*, 28(23):e70207. https://doi.org/10.1111/jcmm.70207

Zhu YJ, Zheng B, Wang HY, et al., 2017. New knowledge of the mechanisms of sorafenib resistance in liver cancer. *Acta Pharmacol Sin*, 38(5):614-622. https://doi.org/10.1038/aps.2017.5

Supplementary information

Materials and methods