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Targeting WTAP for sensitized sorafenib therapy in hepatocellular carcinoma by activating the ERK signaling pathway

Yu WU[1,2,3,4,*], Guomin JU[1,2,3,4,*], Xueyu ZHOU[5], Jian WU[1,2,3,4], Shusen ZHENG[1,2,3,4]✉, Chuanhui PENG[1,2,3,4]✉

[1]*Division of Hepatobiliary and Pancreatic Surgery, Department of Surgery, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, China*
[2]*NHC Key Laboratory of Combined Multi-organ Transplantation, Key Laboratory of Organ Transplantation, Zhejiang Province, Hangzhou 310003, China*
[3]*Key Laboratory of The Diagnosis and Treatment of Organ Transplantation, Research Unit of Collaborative Diagnosis and Treatment For Hepatobiliary and Pancreatic Cancer, Chinese Academy of Medical Sciences (2019RU019), Hangzhou 310003, China*
[4]*State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, Hangzhou 310003, China*
[5]*Department of Thyroid Surgery, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, China*

Abstract: Hepatocellular carcinoma (HCC) often requires targeted therapy and immunotherapy due to frequent delayed diagnosis. Sorafenib, as the first targeted drug applied to HCC, has demonstrated a remarkable therapeutic effect in clinical treatment. However, its clinical implication 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 paper, 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 and western blot analysis demonstrated that WTAP induces the activation of 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)

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1 Introduction

Hepatocellular carcinoma (HCC) persists as a critical oncologic challenge, ranking third globally in cancer-related mortality with a five-year survival probability below 23% (Global Burden of Disease 2019

✉ Chuanhui PENG, peng_chuanhui@zju.edu.cn
Shusen ZHENG, shusenzheng@zju.edu.cn
*The two authors contribute equally to this work
Chuanhui PENG, https://orcid.org/0000-0002-0800-1417

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Cancer Collaboration et al., 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, whose efficacy has been confirmed in clinical trials (Galle et al., 2018). However, durable therapeutic responses are limited in most HCC 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 diarrhoea, fatigue, palmoplantar keratoderma, and hyperkeratosis (D. Zhang et al., 2024). If the sensitivity of sorafenib to HCC could be improved and its effective dose could be reduced, this would mean that the incidence and degree of adverse reactions could also be alleviated. Obviously, 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 promotive 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 enhance clinical survival outcomes.

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2 Materials and methods

2.1 Cell culture

The Huh7 human HCC cell line (Shanghai Institutes for Biological Sciences, CAS) was maintained in Minimum Essential Media (MEM, Gibco) containing 10% fetal bovine serum (FBS, Gibco), 1% penicillin-streptomycin, and incubated at 37 °C with 5% CO_2 (Thermo Scientific, USA).

2.2 Animal experiments

All animal experiments received ethical approval from the Institutional Animal Care and Use Committee (IACUC) at the First Affiliated Hospital of Zhejiang University School of Medicine. C57BL/6J mice (Gem-Pharmatech Co., Ltd., Nanjing, China) were housed in a specific pathogen-free (SPF) facility with regulated humidity, temperature, and 12-h light/dark cycles, with free access to food and water. All procedures complied with the institutional ethical guidelines.

In order to evaluate the role of siWTAP in AKT/Ras-driven HCC and its therapeutic potential, 8-week-old C57BL/6J mice (n=8) were randomized into three groups. Hydrodynamic tail vein injections were administered using a plasmid cocktail containing 5 μg pT3-EF1α-myr-AKT, 5 μg NRasV12/pT2-CAGGS, and 0.8 μg pCMV/SB dissolved in 2 mL saline. Four weeks post-AKT/Ras induction, mice received daily oral sorafenib (12.5 mg/kg) for 10 d, followed by intravenous siWTAP or scramble siRNA (1.5 mg/kg) every 3–4 d for two weeks.

2.3 RNA interference

WTAP-targeting small interfering RNAs (siRNAs) and negative control (siNC) were synthesized commercially (GenePharma, Shanghai, China). Transient transfections were carried out using the jetPRIME Polyplus kit (France) following standard protocols. Cells were collected for immunoblot analysis 48 hours post-transfection. All siRNA sequences are listed in Table S1.

2.4 Cell counting Kit-8 (CCK-8) assay

Cell proliferation was quantified via CCK-8 assay (Dojindo Laboratories), wherein Huh7 cells plated at

2×10^3 cells/well in 96-well plates and incubated for 24 h under standard conditions. Cells were then exposed to sorafenib at gradient concentrations ranging from 1.25 to 20 μmol/L for 96 hours. A volume of 10 μL CCK-8 reagent was added to each well, followed by 1-hour incubation. Absorbance at 450 nm was measured, and 50% inhibitory concentration (IC_{50}) were calculated.

2.5 Western blot analysis

Protein lysates were extracted using ice-cold RIPA lysis buffer (Biyuntian) containing protease/phosphatase inhibitors (Thermo Scientific), quantified via DC Protein Assay (Thermo Scientific), and resolved electrophoretically on 4%–12% SDS-PAGE gels (GenScript). After transferring to PVDF membranes (Millipore), the membranes were incubated overnight at 4 °C with primary antibodies, followed by HRP-conjugated secondary antibody detection and chemiluminescent visualization (Bio-Rad). All Antibodies are listed in Table S2.

2.6 Immunohistochemistry (IHC) and TUNEL assay

Formalin-fixed, paraffin-embedded tissue blocks were sectioned into 5-μm-thick slices for histological analysis. After deparaffinization and antigen retrieval, slides were incubated with primary antibodies and visualized using the Real Envision Detection System (DAKO). Apoptosis was assessed via the TUNEL Kit (Abcam). A quantitative assessment of positively stained regions or cell counts was conducted through systematic sampling of six randomized fields per histological section. The subsequent statistical evaluation was performed using ImageJ software equipped with the IHC Analyzer module.

2.7 Gene Set Enrichment Analysis (GSEA)

WTAP-associated pathways were analyzed using TCGA-LIHC data via GSEA software (https://www.gsea-msigdb.org). Hallmark and KEGG_C2 gene sets were evaluated to compute enrichment scores (ES), normalized ES (NES), and significance (*P*-value).

2.8 Molecular docking

The three-dimensional structural coordinates of WTAP (UniProt ID: Q15007) and MAPK1 (UniProt ID: P28482) were obtained from the AlphaFold Database (https://alphafold.ebi.ac.uk/). The docking results of the proteins with their targets were visualized using PyMOL software (https://pymol.org/2). In the visualization, WTAP is represented in red, MAPK1 in blue, and the docking interface in yellow.

2.9 Exploration of the Model in Chemotherapy Response

Sorafenib IC50 values were calculated using the R package "oncoPredict". The IC_{50} value signifies the concentration of a substance required to inhibit a specific biological or metabolic process by 50%. To ascertain differences between groups, the Wilcoxon signed-rank test was utilized.

2.10 Statistical analysis

Statistical analyses were performed with GraphPad Prism 9.5.1 and R programming language (version 4.4.1). All experimental data were presented as mean values with standard error of the mean (SEM). For comparisons between two experimental groups, unpaired Student's t-test was applied, while one-way analysis of variance (ANOVA) was utilized for multi-group analyses. The statistical significance thresholds were defined as follows: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

3 Results

3.1 WTAP exhibits elevated expression in hepatocellular carcinoma and is associated with unfavorable prognosis

In this study, to clarify the biological function of WTAP, its transcriptional profile in clinical HCC specimens obtained from 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 comparing paired normal and tumor tissues adjacent to hepatocellular carcinoma (n=50), 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 within HCC tissues in liver cancer patients (Fig. 1c). The immunohisto-

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chemical profiling of HCC specimens archived in the Human Protein Atlas (HPA) revealed significantly elevated WTAP protein expression compared to non-neoplastic hepatic tissues (Fig. 1d). The WTAP expression levels showed a statistically significant association with HCC clinical staging ($P < 0.05$) (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 mRNA expression is predictive of a poorer overall survival rate ($P<0.001$) (Fig. 1f).

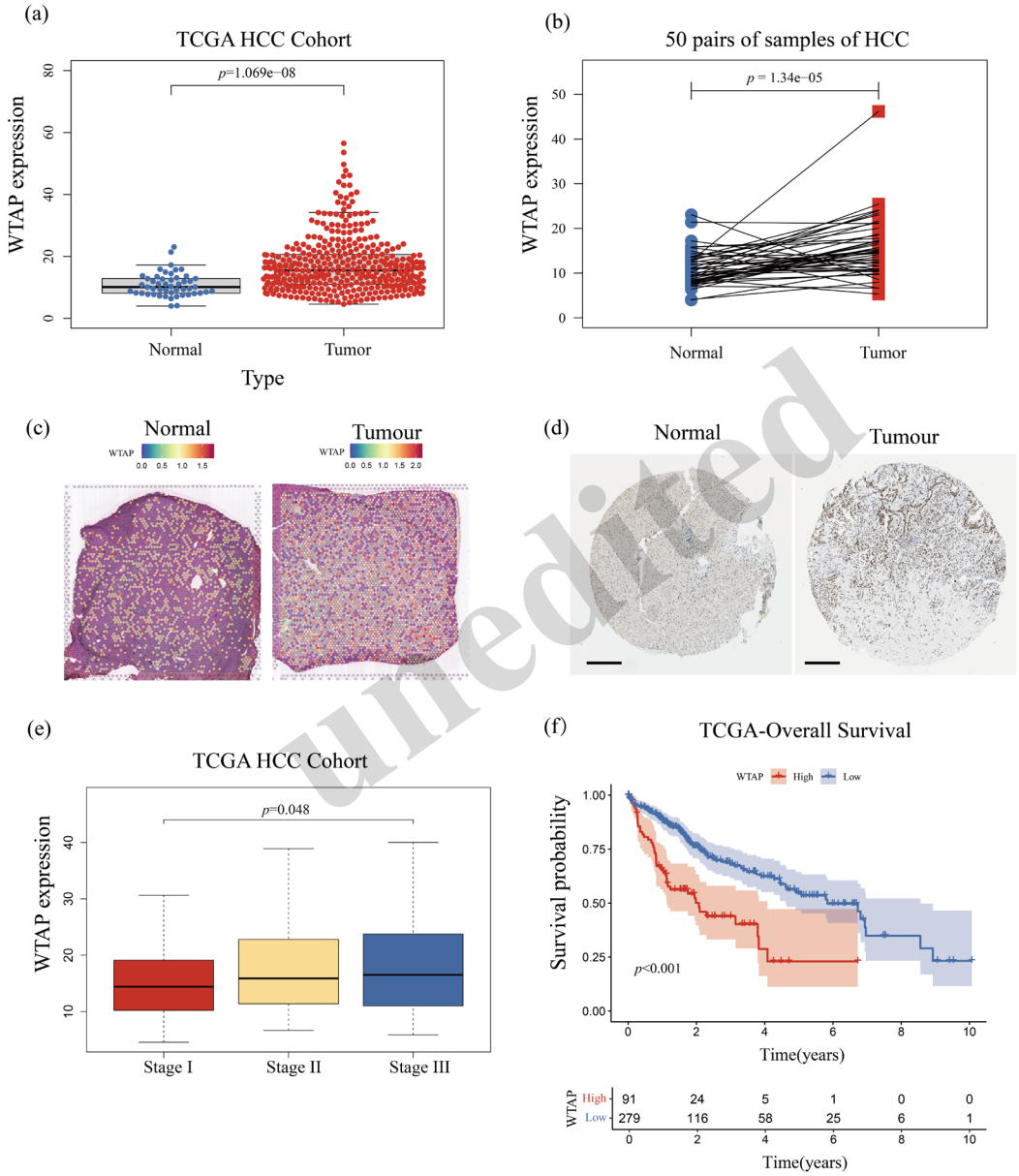

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Fig. 1 The up-regulation of Wilms' tumor 1-associated protein (WTAP) in Hepatocellular carcinoma (HCC) is associated with adverse outcomes. (a) The Cancer Genome Atlas (TCGA) database was utilized to analyze the mRNA expression of WTAP in HCC. (b) WTAP mRNA expression of 50 pairs of HCC tissues in the TCGA cohort. (c)Spatial feature plots of the signatures of WTAP base on spatial HE staining in HCC- 3N and HCC- 3T tissue sections. (d) Representative immunohistochemistry (IHC) images of WTAP in normal tissue and HCC were provided by the Human Protein Atlas (HPA) database. (e) The analysis of TCGA databases 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 to the high-WTAP group ($P<0.001$). Scale bars, 200 mm.

3.2 WTAP diminishes the susceptibility of HCC cells to sorafenib therapy in *vitro*.

In order to investigate the role of WTAP in targeted therapy for HCC, we leveraged data from the GEO dataset (GSE199092) and observed that treatment with Sorafenib (7.5 μM for 3 days) led to an upregulation of WTAP mRNA expression in the HepAD38WT cell line (Fig. 2a), suggesting a potential link between WTAP and resistance to HCC targeted therapy. Subsequent GSEA on WTAP single-gene differential expression data from the TCGA database revealed significant enrichment of the 'primary bile acid biosynthesis' and 'drug metabolism cytochrome P450 pathways' (Fig. 2b). Literature has reported that alterations in the metabolic flux of the tumor microenvironment, particularly lipid metabolism, can contribute to increased cellular resistance to drugs (Tang et al., 2020; Cao, 2019). 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 (Wang et al., 2020; Paolini et al., 2017). Therefore, we hypothesized that WTAP is associated with resistance to targeted therapies in HCC. 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 sorafenib treatment (Fig. 2c). WTAP knockdown Huh7 cells were generated using two short interfering RNAs (siWTAP-1, siWTAP-2), and both WTAP interferences RNAs decreased WTAP protein expression (Fig. 2d). Furthermore, WTAP knockdown ameliorated the resistance of Huh7 cells to sorafenib compared with siRNA control combined with sorafenib (Fig. 2e). Collectively, these data suggest that the inhibition of WTAP could enhance Sorafenib sensitivity in HCC.

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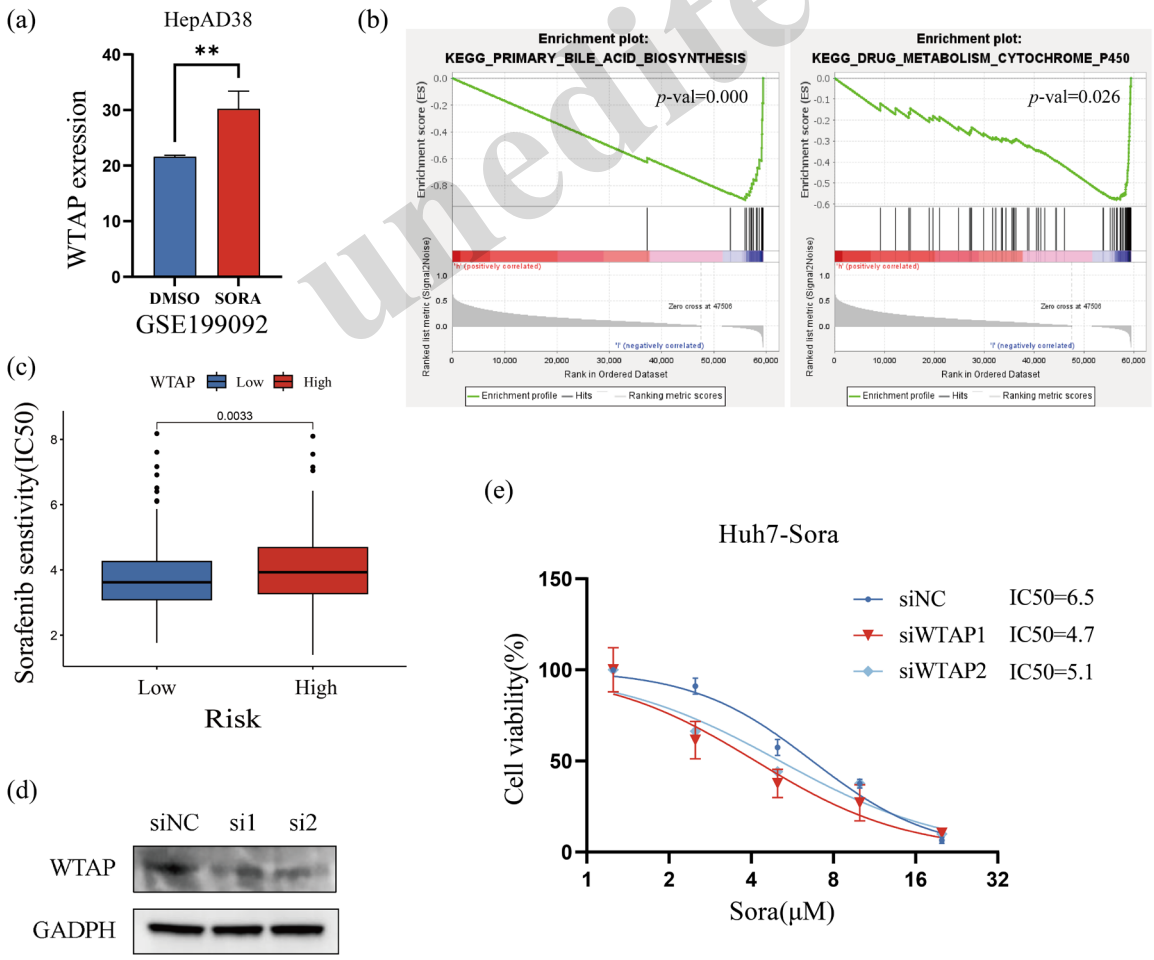

Fig. 2 WTAP targeted sorafenib resistance of HCC cells in *vitro*. (a) The expression of WTAP mRNA in HepAD38WT treated with sorafenib (7.5 μM, 3days) based on GEO datasets (GSE199092). (b) Gene set enrichment analysis (GSEA) analysis revealed significant enrichment of the PRIMARY_BILE_ACID_BIOSYNTHESIS and DRUG_METABOLISM_CYTOCHROME_P450 pathways in HCC samples stratified by low-WTAP and high-WTAP expression levels, as determined by RNA-seq data from the TCGA database. (c) The differential expression groups of WTAP were used to predict sensitivity to sorafenib treatment. (d) Western blot analysis was performed to determine the transfection efficiency in Huh7 cells. (e) A dose-response curve was generated to evaluate the effect of WTAP knockdown on sorafenib-treated HCC cells. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

3.3 Targeting WTAP with siWTAP can potentiate the therapeutic efficacy of sorafenib in the treatment of HCC 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 established using AKT/Ras plasmids. One month post-inoculation, 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 days for a total of five injections (Fig. 3a). The experimental groups received corn oil, siControl, and combined sorafenib (12.5 mg/kg) with siControl or siWTAP (Fig. 3b). Notably, compared to the control group, sorafenib alone inhibited tumor growth and improved the health status of mice, while the combination of sorafenib with siWTAP had a more significant tumor inhibition effect and could effectively prolong the survival time of mice (Fig. 3c). Data on the body weight of siRNA-treated mice indicated that the depletion of WTAP in hepatocytes was effective (Fig. 3d). 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. In Ki67 staining, the proliferation of liver cancer in mice was significantly inhibited after treatment with siWTAP and sorafenib, compared with that in mice treated with sorafenib alone. SiWTAP enhanced the sensitivity of hepatocellular carcinoma cells to sorafenib (Fig. 3e). Collectively, our findings suggest that targeting WTAP may serve as a potential sensitizer for molecular targeted therapy in HCC.

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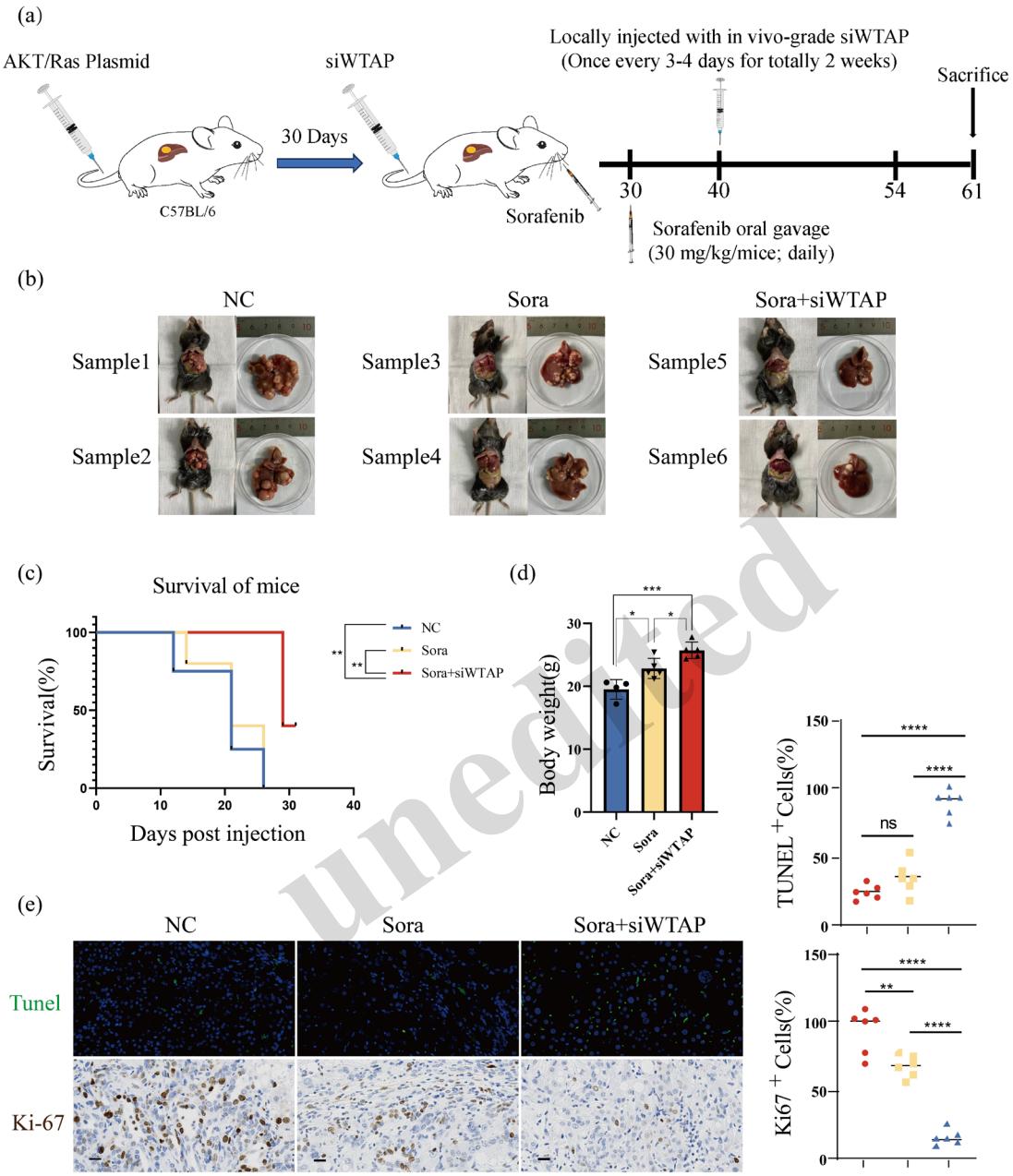

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Fig. 3 The sensitivity of HCC to sorafenib is increased by WTAP knockdown in *vitro*. (a) Schematic representation of si-WTAP treatment in combination with sorafenib (30 mg/kg) in AKT/Ras HCC mouse models. (b) Orthotopic and macroscopic appearance of livers extracted from AKT/Ras mice 30 days post-treatment with sorafenib, siWTAP, or combination therapy. (c) Kaplan-Meier estimation of survival over time in AKT/Ras mice (NC, n=4; sorafenib, n=5; sorafenib + siWTAP, n=5) analyzed by log-rank test (Mantel-Cox). (d) Quantification of liver weight, body weight, and liver weight/body weight at the end of single or combination treatment. (e) Representative images and quantitative analysis of TUNEL fluorescence staining and Ki-67 IHC staining in AKT/Ras mice treated with either monotherapy or combination therapy. Scale bars, 20 μm.*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

3.4 WTAP may regulate the mechanism of sorafenib resistance in HCC through the ERK signaling pathway.

Drug resistance in targeted therapy most commonly develops by the reactivation of the ERK cascade, which makes the tumor 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 molecular structures of WTAP and MAPK1 from the AlphaFold database using PyMOL software, multiple potential binding sites could be predicted between WTAP and MAPK1, suggesting a possible interaction between the two proteins (Fig. 4b). Further analysis using the scatter plot correlation tool from the GEPIA database (http://gepia.cancer-pku.cn/index.html) confirmed a strong correlation between WTAP and ERK (MAPK1), with an R value of 0.65 (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 HE staining results of WTAP in HCC-3N and HCC-3T specimens (Fig. 1c), we performed parallel spatial mapping of ERK (MAPK1) while maintaining consistency. A joint evaluation of both datasets identified positively correlated expression patterns between WTAP and ERK (MAPK1) in the spatial transcriptomics analysis (Fig. 4e). In p-ERK staining, after the treatment of mice with siWTAP and sorafenib alone, ERK activation in mouse liver cancer cells was significantly reduced compared with that of 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 MAPK1 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 MAPK1 (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.

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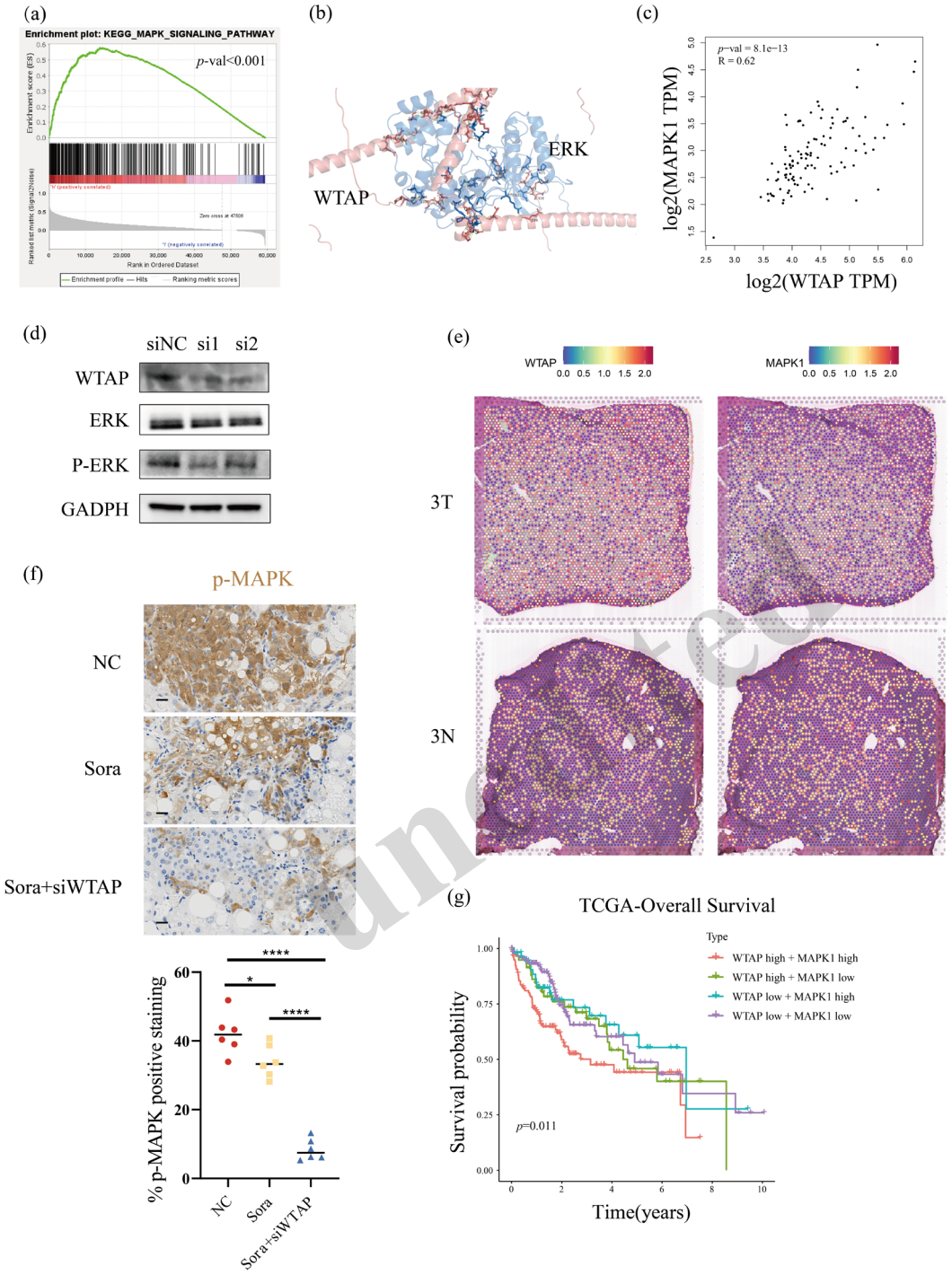

Fig. 4 WTAP regulates the ERK pathway in HCC. (a) The GSEA results reveal a significant positive correlation between WTAP expression and the ERK (MAPK) pathway. (b) Docking analysis demonstrates the interaction between WTAP and ERK. (c) GEPIA (http://gepia.cancer-pku.cn/index.html) analysis confirms the presence of a correlation between WTAP and ERK (MAPK1). (d) Protein levels of WTAP (Same as Fig. 1C) and ERK pathway markers (ERK, P-ERK) upon WTAP knockdown by western blot in Huh7 cells. (e) Spatial HE staining and spatial feature plots of the signatures of WTAP and ERK (MAPK1) in HCC-3N and HCC-3T tissue sections (from left to right). (f) Representative images and quantitative analysis of p-MAPK IHC staining in AKT/Ras mice treated with either monotherapy or combination therapy. Scale bars, 20 mm. (g) Overall survival analysis for HCC patients by ERK (MAPK1) expression status in the WTAP-high and WTAP-low groups according to TCGA data. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

4 Discussion

This investigation aimed to tackle the current clinical challenges posed by hepatocellular carcinoma (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 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 mechanisms of resistance to targeted therapies, which represent a significant barrier to improving treatment efficacy and patient prognosis.

There is already a volume of research addressing potential mechanisms of resistance to targeted therapy 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., 2024), with methylated circRNA SORE maintaining sorafenib resistance in liver cancer by regulating β-catenin signaling (Xu et al., 2020b). Zhang et al. elucidated the YTHDF1-m6A-NOTCH1 axis in the regulation of resistance to sorafenib and lenvatinib (X. Zhang et al., 2024). M6A RNA methylation-mediated HNF3γ is another potential resistance mechanism (Zhou et al., 2020). Additionally, HSP90β regulates STUB1-induced YTHDF2 ubiquitination, which is involved in the resistance mechanism of HCC to sorafenib (Liao et al., 2023). Xu et al. reported that circRNA-SORE mediates sorafenib resistance by stabilizing YBX1 (Xu et al., 2020a). While WTAP as a binding partner of WT1 is well-known for its methylation modification functions (Liu et al., 2014), previous findings from our team indicated that it enhances the proliferation of liver cancer cells by silencing ERS1 (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 staging, along with Kaplan-Meier survival analysis, further confirmed that WTAP is associated with poor prognosis. These results are consistent with our prior investigations (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". 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 to 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 (Sugiura et al., 2021; Santarpia et al., 2012; Braicu et al., 2019). ERK is a well-known key molecule in cell signaling transduction, 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.

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Molecular docking analysis further supported the interaction between WTAP and MAPK1, 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. Firstly, 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. Secondly, this study primarily addressed post-transcriptional regulation and failed to delve into the potential transcriptional control of WTAP. Thirdly, 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 are yet to be determined. Lastly, 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 si-WTAP 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.

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5 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 improved 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.

Data availability statement

To acquire the TCGA-LIHC dataset, we utilized TCGA data portal website (http://gdac.broadinstitute.org/). The publicly available 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 tumors and normal tissues, were obtained from the study by Wu et al (Wu et al., 2021). Additional research data created in this work is available from the corresponding authors upon reasonable request.

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Author contributions

Yu Wu and Guomin Ju contributed equally to designing the study, developing methods, analyzing 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 research investigations and project management. The finalized manuscript underwent rigorous review and unanimous approval by all authors, who additionally confirmed full dataset accessibility while accepting responsibility for its precision and secure management.

Compliance with ethics guidelines

The authors Yu Wu, Guomin Ju, Xueyu Zhou, Jian Wu, Shusen Zheng, and Chuanhui Peng affirm no financial or competing interests in this work.

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 ID: 2025069).

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Supplementary information

Table S1. Target sequences of siRNAs

Gene	Sequences (5'-3')
Si WTAP#1	GCUUUGGAGGGCAAGUACATT
Si WTAP#2	GGUUCGAUUGAGUGAAACATT

Table S2. Antibodies

Antigens	Host	Source	Identifier
GAPDH	Mouse	Proteintech	CAT#60004-1-Ig
WTAP(WB)	Mouse	Proteintech	CAT#60188-1-Ig
MAPK	Rabbit	Cell Signaling Technology	CAT#4695
P-MAPK	Rabbit	Cell Signaling Technology	CAT#4370

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