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ORIGINAL RESEARCH

ARQ-197 enhances the antitumor effect of sorafenib in hepatocellular carcinoma cells via decelerating its intracellular clearance

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Background: Hepatocellular carcinoma (HCC) is one of the heaviest malignant burdens in China. Molecular targeting agent, sorafenib, is the main therapeutic option for antitumor therapy of advanced HCC, but it is currently too expensive for the public and its therapeutic effect does not satisfy initial expectation. Therefore, it is important to develop more effective molecular targeted therapeutic strategies for advanced HCC.

Materials and methods: The antitumor effects of sorafenib or ARQ-197, an antagonist of c-MET (tyrosine-protein kinase Met or hepatocyte growth factor receptor), were examined by MTT or in murine tumor model. The effect of ARQ-197 on epithelial–mesenchymal transition (EMT) or multidrug resistance (MDR) was examined by quantitative real-time PCR for the expression of related genes. The clearance of sorafenib in HCC cells was detected by liquid chromatography-mass spectrometry/mass spectrometry.

Results: ARQ-197 treatment enhanced the sensitivity of HCC cells to sorafenib. Mechanistic studies indicated that ARQ-197 inhibited the expression of EMT- and MDR-related genes. Moreover, ARQ-197 treatment decelerated the clearance of sorafenib in cultured HCC cells and subcutaneous HCC tumors in nude mice.

Conclusion: In the present work, our data suggested that ARQ-197 decelerated the clearance of sorafenib in HCC cells and enhanced the antitumor effect of sorafenib.

Keywords: advanced hepatocellular carcinoma, molecular targeted agents, ARQ-197 and sorafenib, drug clearance, epithelial–mesenchymal transition, multidrug resistance

Introduction

In China, viral hepatitis (infection by hepatitis B or hepatitis C) has a high morbidity and >80 million people suffer from hepatitis-related chronic liver diseases.[1–5] Despite the progress made in the current prevention approaches of hepatitis, patients suffering from chronic liver diseases have a sky-high chance of developing into hepatocellular carcinoma (HCC).[6–8] Unfortunately, due to the poor clinical diagnosis technology, most patients with HCC are not diagnosed until the disease develops into advanced stage, when radical treatments such as surgical resection and liver transplantation are not useful.[6–8] Currently, molecular targeted agent sorafenib is the first-class anticancer drug used for advanced HCC treatment.[9,10] Although global multicenter clinical trials, such as the Oriental trial and the SHARP trial,[11,12] have demonstrated that sorafenib treatment could prolong patient survival, there are many shortcomings of the clinical application of sorafenib: 1) only a low proportion of patients are sensitive to sorafenib and can be benefited from the treatment;[11,12] 2) for patients who are initially sensitive to sorafenib, drug resistance may occur as treatment progresses;[13] and 3) sorafenib is

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expensive, which puts a huge financial burden on patients. Therefore, it is of great clinical significance to develop new and more effective treatment strategies to improve the antitumor effect of sorafenib.

Receptor tyrosine kinase c-MET was initially discovered in human osteosarcoma, and hepatocyte growth factor (HGF) is the only known ligand of c-MET.[14,15] Recently, multiple evidences have shown that elevated level of c-MET is found in 20%–40% cases of HCC and it is associated with high risk of metastasis and poor prognosis.[16–18] HGF regulates hepatic regeneration and hepatocyte apoptosis in normal tissues and promotes the metastasis of HCC cells.[19–21] On HCC cells, HGF binds and activates c-MET, further inducing the transduction of c-MET downstream pathways, such as Raf/MEK/ERK and AKT/ETS-1 signaling pathways, and leads to the proliferation, migration, and invasion of HCC cells.[22–24] Thus, based on the roles of c-MET in many such features of tumor cells, we hypothesized that it may participate in multidrug resistance and the clearance of antitumor agents. In this study, we used ARQ-197 (also named Tivantinib), a highly selective and non-ATP competitive inhibitor of c-MET for HCC cell treatment, and found that it inhibited the activation of c-MET, promoted apoptosis, and inhibited proliferation and metastasis of HCC cells.[25–30] Our results revealed that ARQ-197 decelerated the clearance of sorafenib in cultured HCC cells or subcutaneous HCC tumors in nude mice, and the combination of ARQ-197 and sorafenib could upregulate the antitumor effect of sorafenib on HCC cells.

Materials and methods

Cell line and reagents

L-02 (a nontumor hepatic cell line) HCC cell lines, HepG2 (an HCC cells line), LM-3 (a highly aggressive HCC cell line), MHCC97-H cells (a highly aggressive HCC cell line), Hu7 (an HCC cell line), BEL-7402 (an HCC cell line), MHCC97-L (a lowly aggressive HCC cell line), and SMMC-7721 (an HCC cell line), which were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, People's Republic of China) or the National Infrastructure of Cell Line Resources, Chinese Academy of Medical Sciences (Beijing, People's Republic of China), the culture collection centers of the Chinese government, have been described in our previous publication and were maintained under conditions described in our previous work.[31–34] ARQ-197 (catalog no. S2753) or sorafenib (catalog no. S7397) was purchased from Selleck Corporation (Houston, TX, USA). Dimethyl sulfoxide (DMSO) was purchased from Sigma Aldrich Corporation (St Louis, MO, USA). The usage of cell lines was permitted by the Ethics Committee, the Fifth Medical Center of the PLA General Hospital.

Quantitative polymerase chain reaction

Total RNA of HCC cells was extracted by using a PARISTM Kit (Thermo Fisher Scientific, Waltham, MA, USA) and reverse transcribed by Multiscribe™ Reverse Transcriptase (Thermo Fisher Scientific) agent. Quantitative real-time PCR (qPCR) was performed following the methods described in references.[35,36] The level of β-actin mRNA was measured as a loading control. Primers used in qPCR experiments are listed in Table 1.

MTT assay

ARQ-197 or sorafenib was dissolved in DMSO. Then, the drugs were diluted with DMEM (Thermo Fisher Scientific). The concentrations of ARQ-197 or sorafenib used are shown in Table 2. HCC cells were treated with indicated concentration of drugs and MTT assay was performed. Inhibition rates and IC_{50} values were calculated following the methods described in our previous studies.[37]

Gene reporter luciferase assay

Luciferase reporters EBS-Luc (ETS-1 binding site luciferase reporter), direct repeat 3 (DR3)-Luc (PXR binding site DR3 luciferase reporter), and everted repeat 6 (ER6)-Luc (PXR binding site ER6 luciferase reporter) have been described in our previous work.[38,39] HCC cells, which were treated with indicated concentrations of drugs, were harvested to analyze luciferase or β-galactosidase activities following the methods described in references.[40,41]

Subcutaneous tumor

All methods or protocols of the animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of the Fifth Medical Center of the PLA General Hospital. All animal studies were performed in accordance with the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines. Nude mice (T-cell deficient) mice aged 4–6 weeks were purchased from Si-Bei-Fu Biotechnology Corporation (Beijing, People's Republic of China). For subcutaneous tumor models,[42–44] MHCC97-H cells were injected into nude mice (1×10^6 cells per animal).

Pharmacokinetic experiments

The sequences and effects of PXR siRNA and ETS-1 siRNA are described in our previous work.[38,45] The pharmacokinetic

Table 1 Primers used in quantitative real-time PCR experiments

Targets	Forward/reverse	Sequences of primer (5′–3′)
E-cadherin	Forward sequence	GCCTCCTGAAAAGAGAGTGGAAG
	Reverse sequence	TGGCAGTGTCTCTCCAAATCCG
N-cadherin	Forward sequence	CCTCCAGAGTTTACTGCCATGAC
	Reverse sequence	GTAGGATCTCCGCCACTGATTC
Vimentin	Forward sequence	AGGCAAAGCAGGAGTCCACTGA
	Reverse sequence	ATCTGGCGTTCCAGGGACTCAT
MMP-3	Forward sequence	CACTCACAGACCTGACTCGGTT
	Reverse sequence	AAGCAGGATCACAGTTGGCTGG
MMP-9	Forward sequence	GCCACTACTGTGCCTTTGAGTC
	Reverse sequence	CCCTCAGAGAATCGCCAGTACT
TIMP-1	Forward sequence	GGAGAGTGTCTGCGGATACTTC
	Reverse sequence	GCAGGTAGTGATGTGCAAGAGTC
MDR-1	Forward sequence	GCTGTCAAGGAAGCCAATGCCT
	Reverse sequence	TGCAATGGCGATCCTCTGCTTC
CYP3A4	Forward sequence	CCGAGTGGATTTCCTTCAGCTG
	Reverse sequence	TGCTCGTGGTTTCATAGCCAGC
UGT1A9	Forward sequence	GCTCTAAAAGCAGTCATCAATGAC
	Reverse sequence	GTGCCTCATCACAAACTCCACC
β-actin	Forward sequence	CACCATTGGCAATGAGCGGTTC
	Reverse sequence	AGGTCTTTGCGGATGTCCACGT

experiments were performed according to the methods provided by Shao et al[46] and Wu et al.[47] For cell-based experiments, HCC cells were treated with 1 μmol/L sorafenib for 12 hours. Then the cells were harvested at indicated time points. For subcutaneous tumor experiments, HCC cells were injected into nude mice to form subcutaneous tumors. Sorafenib solution was injected into subcutaneous tumors and tissue samples were harvested at indicated time points. The collected samples were extracted by acetonitrile, and the amount of sorafenib sustained in samples was identified by liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS) following the methods provided by Feng et al[47] and Shao et al.[46]

In vivo antitumor effect of sorafenib

In vivo antitumor effect of sorafenib was examined in a subcutaneous tumor model following the protocols described by Jia et al[32] and An et al.[48] HCC cells were injected into nude mice (1×10^6 cells per animal) and 3–4 days after injection, mice received sorafenib (2 mg/kg) treatment. Mice were orally administered sorafenib every 2 days. After 21 days of treatment (ten times treatment), mice were harvested and the volumes or weights of tumor tissues were examined. Inhibition rates of sorafenib on tumor volumes or tumor weight were calculated following the methods described by Jia et al[32] and An et al.[48]

Table 2 Concentrations of agents used in cell survival experiments

Agents	Concentrations (μmol/L)						
ARQ-197	0.01	0.03	0.1	0.3	1	3	10
Sorafenib	0.01	0.03	0.1	0.3	1	3	10

Statistical analysis

All statistical significance analyses were performed using SPSS 16.0 statistical software (IBM Corporation, Armonk, NY, USA). The IC_{50} values or half-life time ($t_{1/2}$) values were calculated by Origin software (Origin 6.1; OriginLab Corporation, Northampton, MA, USA). Statistical significance was analyzed by Bonferroni correction with two-way ANOVA, and paired samples were tested by paired-sample *t*-test (SPSS 16.0 statistical software; SPSS Inc., Chicago, IL, USA).

Results

c-MET is highly expressed in aggressive HCC cell lines

First, in order to investigate the role of c-MET in HCC cells, we measured the expression of c-MET in both non-malignant and HCC cell lines using qPCR. As shown in Figure 1, the expression level of c-MET in nontumor L-02 cell line was significantly lower than that in different HCC cell lines. In highly aggressive cell lines MHCC97-H and LM-3, the expression level of c-MET was significantly higher than that in less-aggressive MHCC97-L cells.

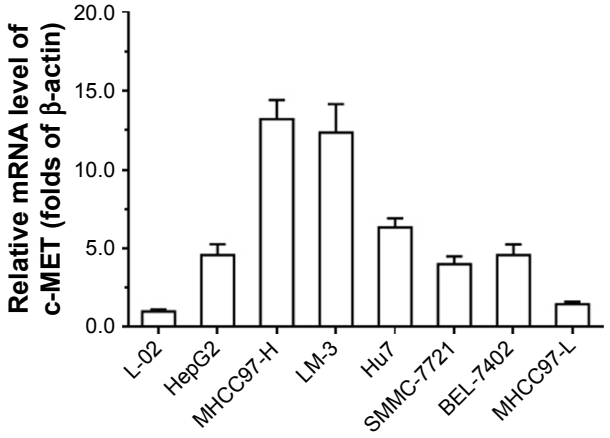


Figure 1 The mRNA level of c-MET in hepatic cells.
Notes: Hepatic cells – L-02, HepG2, LM-3, MHCC97-H, Hu7, BEL-7402, SMMC-7721, or MHCC97-L – were harvested for qPCR experiments.
Abbreviation: qPCR, quantitative real-time PCR.

The in vitro antitumor effect of ARQ-197 on HCC cells

Next, the in vitro antitumor effect of ARQ-197 on HCC cells was examined by MTT assay. As shown in Figure 2, ARQ-197 inhibited the survival of MHCC97-H (Figure 2A), LM-3 (Figure 2B), and HepG2 (Figure 2C) cells in a dose-dependent manner. Interestingly, we observed the effect of ARQ-197 was stronger in cells with higher endogenous c-MET expression level. The effective concentration (IC_{25}), half-inhibitory effective concentration (IC_{50}), and maximum action concentration (IC_{max}) of ARQ-197 on each cell line are summarized in Table 3.

ARQ-197 enhances the in vitro antitumor effect of sorafenib on HCC cells

To examine whether ARQ-197 enhances the antitumor effect of sorafenib on HCC cells, we performed combined treatment and MTT assay. As shown in Figure 3, ARQ-197 treatment at IC_{25} concentration enhanced the antitumor effect of sorafenib on HCC cells MHCC97-H (Figure 3A), LM-3 (Figure 3B), and HepG2 (Figure 3C). Moreover, ARQ-197 treatment decreased the IC_{50} value of sorafenib on HCC cells (Table 4). Therefore, ARQ-197 enhances the in vitro antitumor effect of sorafenib on HCC cells.

ARQ-197 inhibits the expression of epithelial–mesenchymal transition (EMT) and multidrug resistance (MDR) related genes

To examine the working mechanism of ARQ-197, the effect of ARQ-197 on the expression of genes related to EMT and

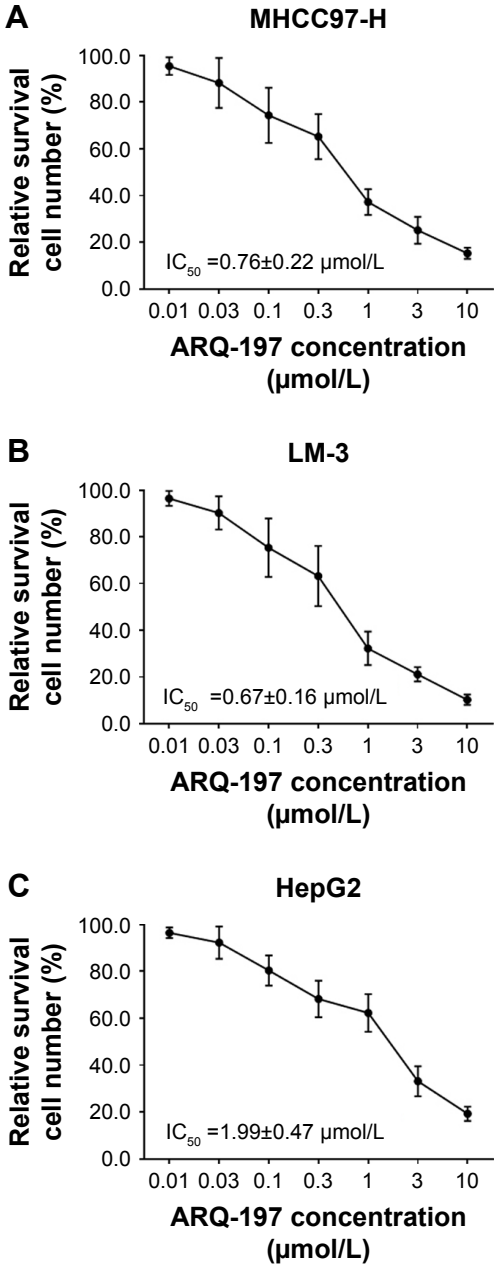


Figure 2 ARQ-197 inhibits the survival of HCC cells in a dose-dependent manner.
Notes: HCC cells MHCC97-H (**A**), LM-3 (**B**), and HepG2 (**C**), which were treated with indicated concentrations of ARQ-197, were analyzed by MTT experiments. Inhibition rate of ARQ-197 on HCC cells was calculated at OD 490 nm.
Abbreviation: HCC, hepatocellular carcinoma.

Table 3 ARQ-197 inhibits hepatic cells' survival in a dose-dependent manner

Cell lines	IC_{25} values	IC_{50} values	IC_{max} values
	Concentrations (μmol/L)		
L-02	3.44±0.24	7.59±0.78	–
MHCC97-H	0.09±0.01	0.76±0.22	3.56±0.65
LM-3	0.08±0.02	0.67±0.16	4.62±0.80
HepG2	0.23±0.07	1.99±0.47	9.55±0.57
MHCC97-L	1.29±0.52	4.63±0.93	–

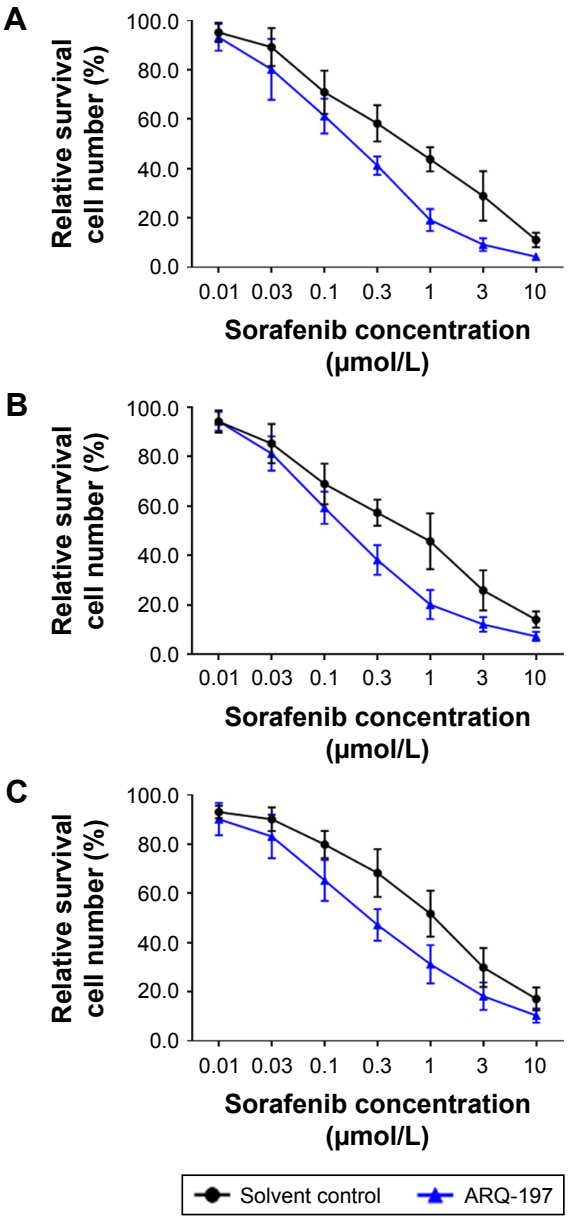


Figure 3 ARQ-197 enhances the antitumor effect of sorafenib or inhibits the survival of HCC cells in a dose-dependent manner.

Notes: The HCC cells MHCC97-H (**A**), LM-3 (**B**), and HepG2 (**C**), which were pretreated with IC_{25} concentration of ARQ-197, were treated with indicated concentrations of sorafenib. Next, the cells were analyzed by MTT experiments. Inhibition rates of sorafenib on HCC cells were calculated at OD 490 nm.

Abbreviation: HCC, hepatocellular carcinoma.

Table 4 ARQ-197 enhances the sensitivity of HCC cells to sorafenib

Cell lines	Solvent control	ARQ-197
	IC_{50} values of sorafenib (μmol/L)	
MHCC97-H	1.39±0.46	0.24±0.05
LM-3	1.29±0.25	0.22±0.07
HepG2	1.68±0.42	0.53±0.17
MHCC97-L	2.73±0.40	0.97±0.39

Abbreviation: HCC, hepatocellular carcinoma.

MDR was examined by qPCR. It is well known that EMT is an important process that participates in drug resistance. As shown in Figure 4, treatment of MHCC87-H cells with ARQ-197 at IC_{25} concentration enhanced the expression of E-cadherin (Figure 4A), an indicator of epithelial formation, and decreased the expression of N-cadherin (Figure 4B) and vimentin (Figure 4C), two indicators of mesenchymal formation. This result suggests that ARQ-197 treatment inhibited the EMT process of MHCC97-H cells. Next, the effect of ARQ-197 on PXR-induced drug resistance was examined. As shown in Figure 4, ARQ-197 treatment at IC_{25} concentration decreased the expression of drug resistance-related genes: *Cyp3a4* (Figure 4D), *Mdr-1* (Figure 4E), and *Ugt1a9* (Figure 4F). Similar results were obtained from Western blotting (Figure 5). Therefore, ARQ-197 inhibited the expression of genes related to MDR in MHCC97-H cells.

ARQ-197 inhibits the transcription factor activities of ETS-1 and PXR

Moreover, previous work from our lab had revealed that, HGF/c-MET signaling pathway promoted sorafenib resistance by enhancing PXR downstream drug resistance-related genes' expression via interaction between PXR and ETS-1.[45] To further examine the effect of ARQ-197, luciferase experiments were performed. We transfected ETS-1 responsible gene reporter plasmid EBS-Luc or PXR responsible gene reporter plasmids DR3-Luc or ER6-Luc, respectively, and then administered ARQ-197. As shown in Figure 6, ARQ-197 treatment inhibited the luciferase activities of EBS-Luc reporter (Figure 6A), DR3-Luc (Figure 6B), and ER6-Luc (Figure 6C) in a dose-dependent manner. These results indicate that ARQ-197 inhibits the expression of genes related to EMT or MDR by decreasing the transcription factor activities of ETS-1 and PXR.

ARQ-197 decelerates the clearance of sorafenib in HCC cells

The clearance of sorafenib in HCC cells was examined by LC-MS/MS. As shown in Figure 7, ARQ-197 treatment decelerated the clearance of sorafenib in MHCC97-H cells. The half-life time ($t_{1/2}$) of sorafenib in MHCC97-H cells increased from 9.08±0.43 to 13.60±0.65 hours. Moreover, ARQ-197 also decelerated the clearance of sorafenib in subcutaneous MHCC97-H tumors. The half-life time ($t_{1/2}$) of sorafenib in tumors increased from 19.49±0.79 to 30.33±0.98 hours. Table 5 shows the half-life of sorafenib in HCC cells with ARQ-197 or solvent control treatment. Moreover, to reveal the specificity of ARQ-197, PXR siRNA

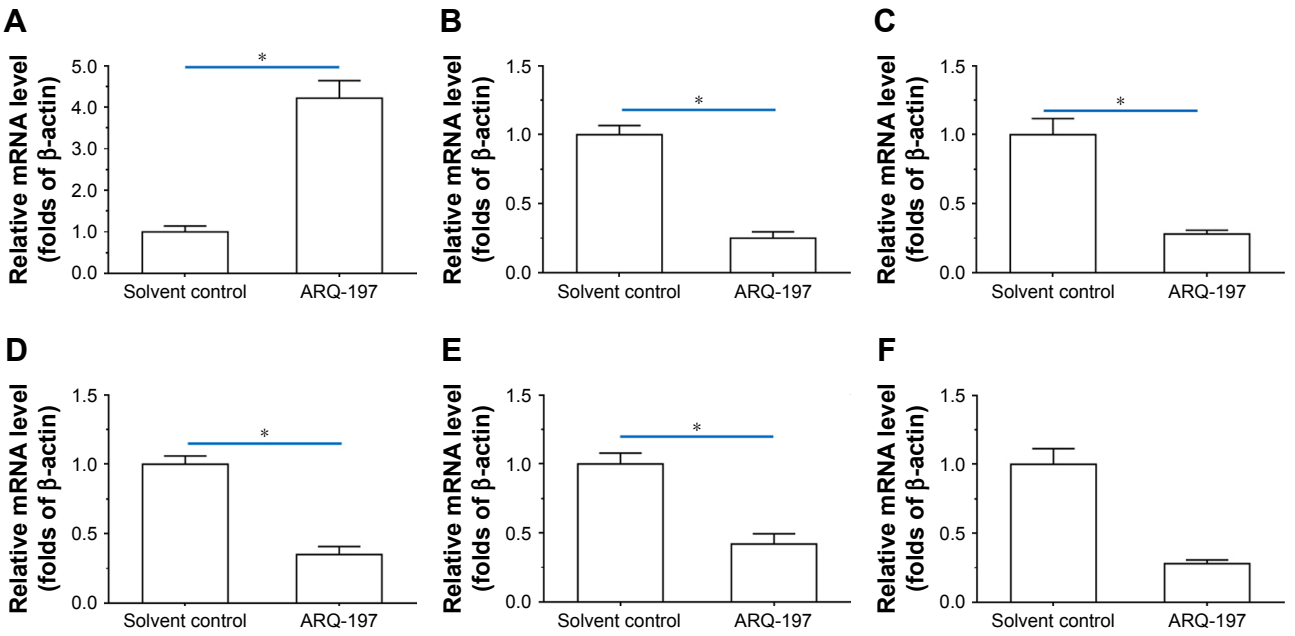


Figure 4 ARQ-197 inhibits EMT- or MDR-related genes' expression in MHCC97-H cells.
Notes: MHCC97-H cells, which were treated with IC_{25} concentration of ARQ-197, were harvested for qPCR experiments. The mRNA level of EMT-related genes, E-cadherin (**A**), N-cadherin (**B**), vimentin (**C**), or MDR-related genes, CYP3A4 (**D**), MDR-1 (**E**), UTG1A9 (**F**), was examined by qPCR. *$P<0.05$.
Abbreviations: EMT, epithelial–mesenchymal transition; MDR, multidrug resistance; qPCR, quantitative real-time PCR.

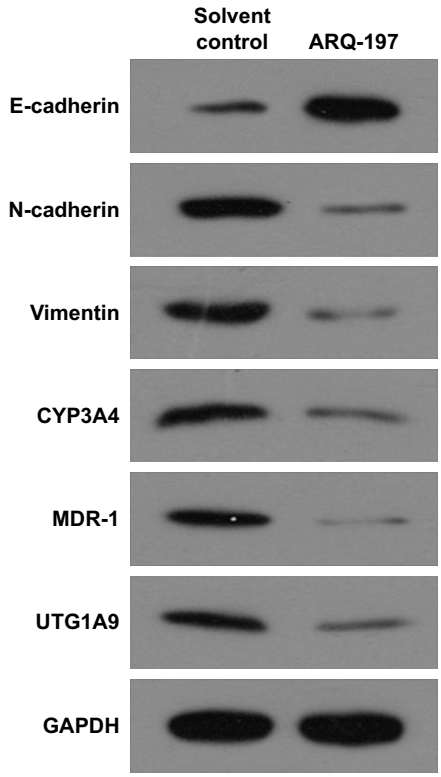


Figure 5 ARQ-197 inhibits the protein level of EMT- or MDR-related genes' expression in MHCC97-H cells.
Notes: MHCC97-H cells, which were treated with IC_{25} concentration of ARQ-197, were harvested for Western blot experiments. The protein levels of EMT-related genes, E-cadherin, N-cadherin, vimentin, or MDR-related genes, CYP3A4, MDR-1, UTG1A9, were examined by their antibodies.
Abbreviations: EMT, epithelial–mesenchymal transition; MDR, multidrug resistance.

or ETS-1 siRNA was used. As shown in Table 6, knockdown of ETS-1 or PXR's expression decelerated the clearance of sorafenib in MHCC97-H or LM-3 cells. ARQ-197 did not affect the half-life values of sorafenib in MHCC97-H or LM-3 cells in the presence of ETS-1 siRNA or PXR siRNA (Table 6). These data suggest that c-MET functions in a PXR/ETS-1-dependent manner.

ARQ-197 enhances the in vivo antitumor effect of sorafenib on HCC tumor model

To examine the in vivo antitumor effect of ARQ-197, we administered ARQ-197 to nude mice HCC models. As shown in Figure 8, treatment with 3 mg/kg ARQ-197 or 2 mg/kg sorafenib or together inhibited the subcutaneous growth of MHCC97-H tumors compared to the vehicle controls. ARQ-197 treatment significantly enhanced the antitumor effect of sorafenib, compared to sorafenib alone (Figure 8). Therefore, as expected, ARQ-197 treatment enhanced the antitumor effect of sorafenib on HCC tumor growth.

Discussion

At present, despite the fast progress made in the research of advanced HCC, patients' prognosis or clinical outcome is still poor.[49–51] Various local therapeutic strategies including radiofrequency ablation (RFA) and transcatheter arterial chemoembolization (TACE) can delay the disease progression and

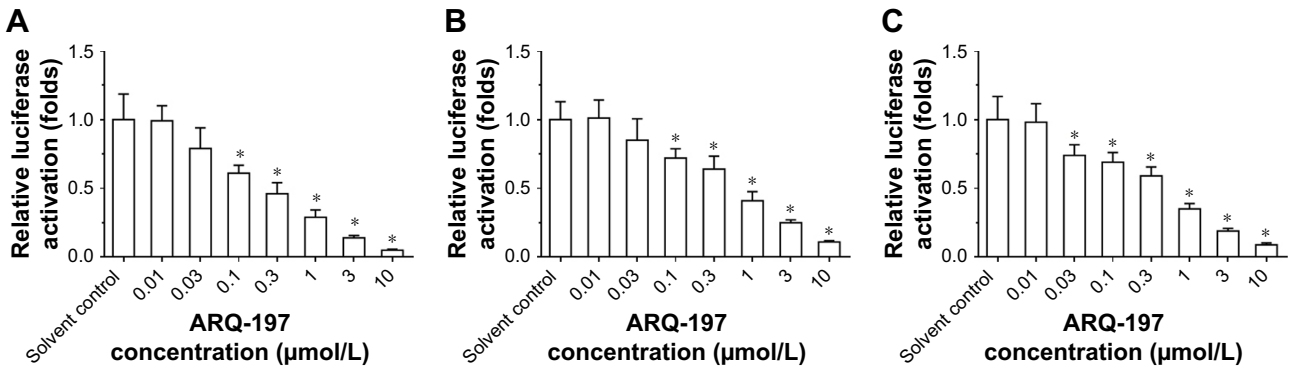


Figure 6 ARQ-197 decreases the transcription factor activation of ETS-1 and PXR in MHCC97-H cells.
Notes: MHCC97-H cells which were transfected with luciferase reporters of (**A**) ETS-1 (EBS-Luc) or luciferase reporters of PXR (DR3-Luc [**B**] or ER6-Luc [**C**]) were treated with indicated concentration of ARQ-197. *$P<0.05$.
Abbreviations: DR3, direct repeat 3; ER6, everted repeat 6.

reduce the tumor burden of advanced HCC, but the recurrence of the malignancy after treatment has made treating options even narrower.[52–55] These situations make chemotherapies important for advanced HCC treatment. Advanced HCC is insensitive to traditional cytotoxic chemotherapies and is featured to have MDR.[56,57] Therefore, development of more effective molecular targeted therapy has always been the top priority of research. In the present work, we demonstrated that ARQ-197 treatment enhanced the sensitivity of HCC cells to sorafenib. Currently, combination therapies are mainly considered as combinations of different treatment strategies, such as TACE + RFA, TACE + sorafenib, or RFA + sorafenib.[58–61] On the other hand, there are many reports of combined cytotoxic chemotherapy drugs and molecular targeted drugs or therapeutic antibodies.[62–65] Although there are very few reports on molecular targeted agents' combination, this type of combination per se is a promising strategy for cancer treatment. For example, vemurafenib + cobimetinib, dabrafenib + trametinib, and encorafenib + binimetinib have been proved to be effective in melanoma treatment.[66] Our results show that the combination of ARQ-197 and sorafenib helped to achieve better therapeutic effect.

Sorafenib resistance may be achieved through multiple molecular mechanisms. EMT process and HGF/c-MET pathway are foremost molecular mechanisms of antitumor drug resistance, which significantly promotes the antitumor effect of drugs on cancer cells.[67–77] Jiao et al showed that HGF treatment induced gefitinib resistance in human lung cancer cells and miR-1-3 p or miR-206 sensitizes cells to gefitinib by inhibition of c-Met and EMT process.[78] In the present work, we found that ARQ-197 inhibited the EMT process of HCC cells and enhanced the sensitivity of HCC cells to sorafenib.

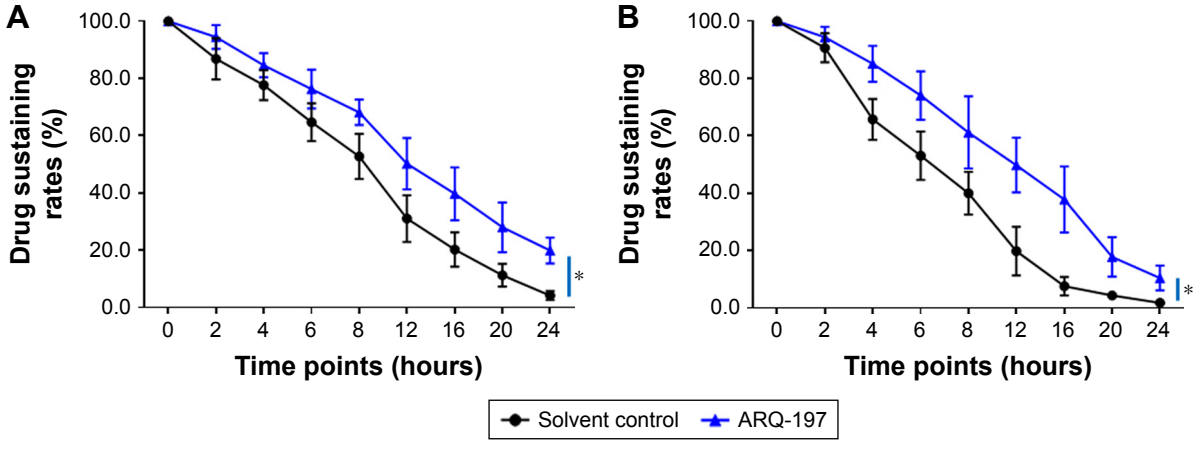


Figure 7 ARQ-197 decelerates the clearance of sorafenib in MHCC97-H cells.
Notes: (**A**) MHCC97-H cells, which were treated with IC_{25} concentration sorafenib for 12 hours, were harvested at indicated time points. (**B**) Sorafenib solution was injected into subcutaneous tumor formed by MHCC97-H cells, and tumor tissues were harvested at indicated time points. Samples were analyzed by LC-MS/MS. Drugs clearance curve was calculated based on the sustaining of sorafenib in cells or tumors. *$P<0.05$.
Abbreviation: LC-MS/MS, liquid chromatography–mass spectrometry/mass spectrometry.

Table 5 ARQ-197 decelerated the clearance of sorafenib in HCC cells

Cell lines	Models	Solvent control	ARQ-197
		Half-life of sorafenib ($t_{1/2}$, hours)	
MHCC97-H	Cultured cells	9.08±0.43	13.60±0.65
	Subcutaneous tumor	19.49±0.79	30.33±0.98
LM-3	Cultured cells	9.93±0.57	14.42±0.67
	Subcutaneous tumor	18.80±0.72	39.10±0.92
HepG2	Cultured cells	9.62±0.82	12.46±0.62
	Subcutaneous tumor	16.56±0.44	29.72±0.91
MHCC97-L	Cultured cells	10.21±0.51	12.59±0.18
	Subcutaneous tumor	18.09±0.22	24.36±0.55

Abbreviation: HCC, hepatocellular carcinoma.

Table 6 ARQ-197 decelerated the clearance of sorafenib in HCC cells in the presence of PXR or ETS-1

Cell lines	Models	Solvent control	ARQ-197	ETS-1 siRNA	PXR siRNA	ETS-1 siRNA + ARQ-197	PXR siRNA + siRNA
		Half-life of sorafenib ($t_{1/2}$, hours)					
MHCC97-H	Cultured cells	9.08±0.43	13.60±0.65	13.85±0.84	17.69±0.91	13.53±0.23	17.62±0.88
	Subcutaneous tumor	19.49±0.79	30.33±0.98	30.76±1.06	42.07±2.79	30.96±0.46	40.13±0.83
LM-3	Cultured cells	9.93±0.57	14.42±0.67	15.61±0.46	18.00±0.25	15.22±0.18	18.44±0.67
	Subcutaneous tumor	18.80±0.72	39.10±0.92	39.45±0.74	52.88±0.95	38.26±0.60	50.29±3.57

Abbreviation: HCC, hepatocellular carcinoma.

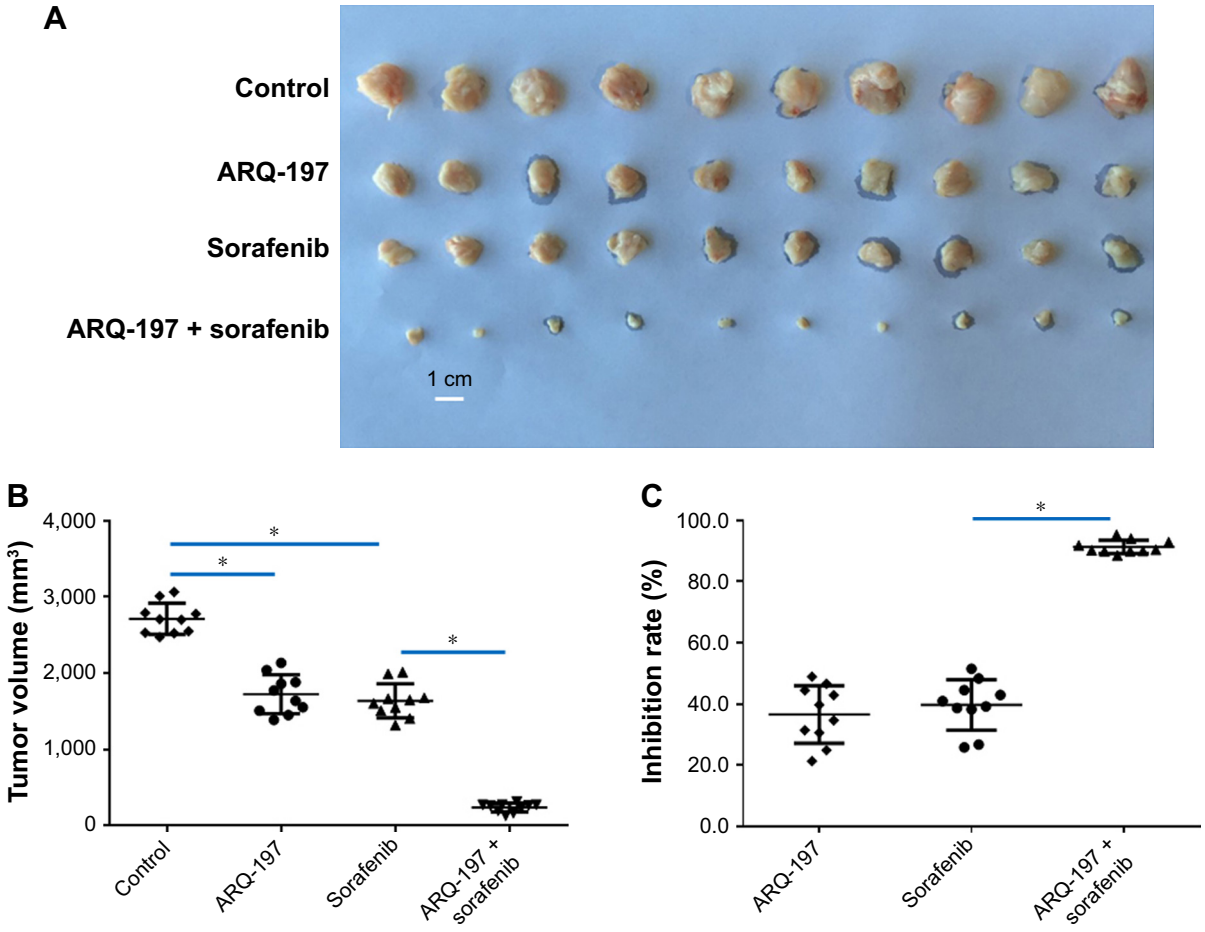


Figure 8 (*Continued*)

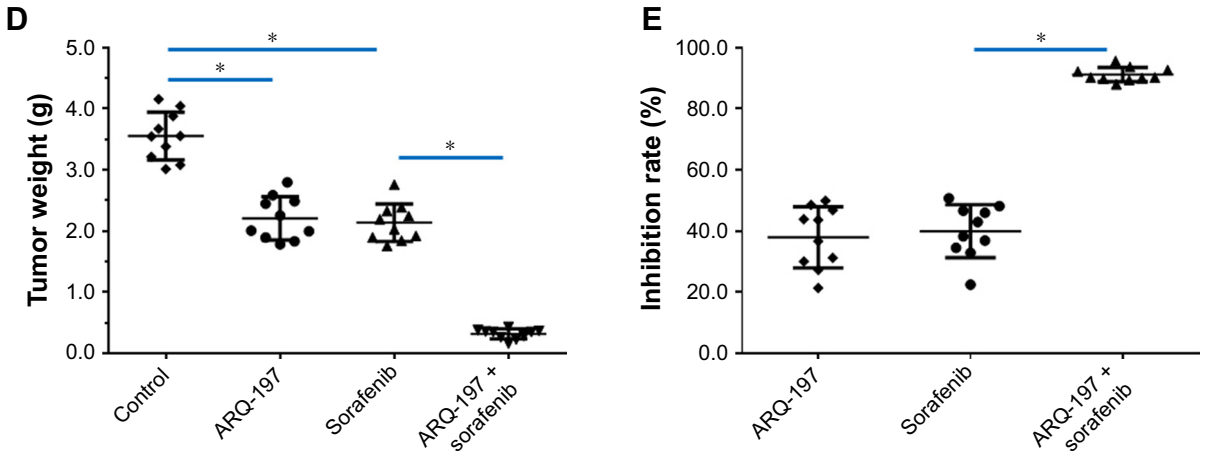


Figure 8 ARQ-197 enhances the antitumor effect of sorafenib on the subcutaneous growth of MHCC97-H cells in nude mice.
Notes: MHCC97-H cells were seeded into nude mice to form subcutaneous tumors. Nude mice received oral administration of solvent control, ARQ-197, sorafenib, or ARQ-197 + sorafenib. Tumors were harvested and tumor volumes or tumor weights were noted. Results are shown as photographs of subcutaneous tumors (**A**), tumor volumes (**B**), inhibition rates of agents on MHCC97-H cells' subcutaneous growth calculated from tumor volumes (**C**), or tumor weights (**D**), orinhibition rates of agents on MHCC97-H cells' subcutaneous growth calculated from tumor weights (**E**). *$P<0.05$.

Moreover, ARQ-197 also inhibited the activation of PXR, which is the key regulator for exogenous drug metabolism and detoxification in cells.[79,80] Previous studies have also shown that sorafenib can act as a ligand/agonist for PXR during treatment, promoting the expression of PXR downstream drug resistance–related genes by inducing PXR's transcription factor activity, ultimately resulting in sorafenib resistance.[47,81] In HCC cells, the transcription factor ETS-1, an important effector of HGF/c-MET signaling pathway, could function as a co-regulator of PXR and enhance sorafenib resistance. In the present work, we found that ARQ-197 inhibited the activation of PXR as the mechanistic explanation for the decelerated clearance of sorafenib in HCC cells. ARQ-197 decreased the expression of *Cyp3a4*, *Mdr-1*, and *Utg1a9*, the downstream genes of PXR, in HCC cells. MDR-1 is the most important Phase II drug-metabolizing enzyme,[82] CYP3A4 is a Phase I drug-resistance enzyme, and UTG1A9 is a Phase III drug-resistance enzyme mediating sorafenib metabolism.[83–85] It is well known that binding of HGF to c-MET induced the activation of multiple downstream metabolic pathways, including the mTOR pathway.[86] Our results extended our knowledge about HGF/c-MET in drug metabolism. Sorafenib is the first-class molecular targeted drug for advanced HCC approved by the US Food and Drug Administration. In addition to sorafenib, regorafenib and lenvatinib have recently been approved for marketing.[87,88] Future exploration of the combination of ARQ-197 with new molecular targeted drugs such as regorafenib and lenvatinib is of great significance.

Conclusion

We report that ARQ-197 enhanced the sensitivity of HCC cells to sorafenib by decelerating the clearance of sorafenib in cultured HCC cells or subcutaneous HCC tumors in nude mice. Also, our data indicate that the combination of ARQ-197 and sorafenib can upregulate the antitumor effect of sorafenib on HCC cells.

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Author contributions

All authors made substantial contributions to the design and conception, acquisition, analysis, and interpretation of data. Authors took part in either drafting or revising the manuscript. At the same time, authors gave final approval of the version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Disclosure

The authors report no conflicts of interest in this work.

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