# The Study of the Influence of Unfolded Protein Response and Autophagy on Sorafenib-Induced Tumor Inhibition of Hepatocellular Carcinoma

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**Abstract**

and adhesion ability of HCC cells were tested by MTT assay and fow cytometry, respectively. The invasion and migration abilities of cells were detected by Trans well assay. Moreover, we established HCC orthotopic transplantation tumor model of nude mice. The activation of UPR can inhibit the sorafenib-induced apoptosis of HCC cells. Inhibiting autophagy signi fcantly enhanced the sorafenib-induced apoptosis of HCC cells. Activating the UPR can enhance the sorafenib resistance of HCC cells. On the contrary, inhibiting autophagy causes opposite e fects.

**Keywords:** Hepatocellular carcinoma; Sorafenib; Unfolded protein response; Autophagy

## Introduction

Liver cancer is one of the most common malignancies worldwide. In China, the incidence of liver cancer ranks h among all malignant tumors and the fatality rate ranks second. Statistics show that about half of the newly diagnosed cases of liver cancer worldwide come from China [1]. According to the relevant prediction of the World Health Organization (WHO), the number of deaths due to liver cancer can reach millions in 2030 [2,3]. Hepatocellular carcinoma (HCC) is the major histological subtype of liver cancer, which is imperceptible at the early stage of the disease, develops rapidly, tends to recur and metastasize, is aggressive and has a poor prognosis, thus the overall survival rate of patients is low [4]. With the continuous development of medical technology, the diagnosis and treatment of liver cancer have been continuously improved [5,6]. For early stage patients, rst-line treatment includes hepatectomy, ablation and liver transplantation, and the survival rate a er treatment can reach 50%. For patients with advanced disease, other therapeutic strategies such as trans catheter arterial chemoembolization (TACE) are used [7]. However, to date, radical treatment of HCC remains a troublesome problem [6]. With the advent of molecular targeted therapies, researchers have invested more and more attention in the study of the mechanism of tumor progression as an attempt to nd better, safe, and e ective tumor therapeutic targets [8,9].

In 2006, sorafenib was approved by the Food and Drug Administration (FDA) for the treatment of advanced renal cell carcinoma, and in 2007 for the treatment of advanced HCC, which is currently the only molecular targeted drug for advanced HCC [10, 11]. Sorafenib is a small molecule compounds as well as an oral multiple kinase inhibitors that works mainly by inhibiting the proliferation of tumor cells, inhibiting angiogenesis, and promoting the apoptosis of tumor cells. Sorafenib can inhibit the proliferation of tumor cells by inhibiting the kinase activity of Raf1, B-Raf and Ras/Raf/MEK/ERK signaling pathways; it can inhibit tumor angiogenesis by inhibiting hepatic cytokine receptor (c-kit), Fms-like tyrosine kinase 3 ( t3) and vascular endothelial growth factor receptor (VEGFR2, VEGFR3); sorafenib can also induce apoptosis by reducing the phosphorylation level of eIF4E and the expression level of Mcl-1 in tumor cells [12]. Although it has been widely used in the treatment of HCC and is also of great signi cance to improve the survival time of patients, most patients su er from recurrence or even death of HCC due to the development of drug resistance [13-16]. erefore, in-depth study of

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and sorafenib. Cells in each group were treated for 12 h followed by relevant assays.

## MTT assay

Cells in logarithmic growth phase were adjusted to 5 × 104/mL, and were seeded (100 μL in each well) in a 96-well plate and cultured in a cell incubator at 5% $CO_2$ and 37 for 12 h. After the cells adhered, the culture medium in each well was discarded, and 100 μL of sorafenib at different experimental concentrations was added, respectively. The control group was added with an equal volume of DMEM culture medium (containing 10% fetal bovine serum), with 6 duplicate wells in each group. Only DMEM culture medium was added to the zero adjustment group without cells. After culture for 24 h, 48 h and 72 h, 20 μL MTT solutions was added to each well, the culture was continued for 4 h, after that, the liquid in the wells was discarded, 150 μL DMSO solution was added to each well, and the plate was shaken on a shaker at low speed for 10 min. The absorbance value was detected at the wavelength of 490 nm of microplate reader. Cell survival rate was calculated by the formula: [(OD value of experimental group - OD value of zeroing group)/(OD value of control group - OD value of zeroing group)] × 100%.

## Cell adhesion assay

96-well culture plates were first coated with 20 μL of Matrigel gel (2 μg/50 μL) per well, incubated overnight at 37 in a 5% $CO_2$ incubator, and incubated with 2% bovine serum albumin for 1 h until use. They were divided into 6 groups of cells as mentioned before, with 6 duplicate wells in each group; the above cells were seeded in the treated 96-well plate at 5 × 104 cells/well, respectively, incubated for 120 min and then the non-adhered cells in the wells were discarded. After rinsing twice with PBS solution, 20 μL of 5 g/L MTT solution was added, cultured for 4 h and then 150 μL of DMSO was successively added, and the absorbance value was detected at a wavelength of 490 nm on a microplate reader. Calculation of adhesion rate in each group = (mean OD value of treatment group/mean OD value of control group

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### e e ects of UPR activation or autophagy suppression on sorafenib-induced change of HCC tumor growth *in vivo*

For verifying the above results in vivo, a total of 30 nude mice were inoculated with 100% tumor survival rate and tumor formation rate. It

targeted drugs, maintain the genomic stability of tumor cells to prevent immunogenic death, and reduce the recognition ability of the immune system to tumor cells [29, 30]. Inhibition of autophagy can therefore disrupt the energy supply balance of tumor cells, inhibit the metabolic function of mitochondria and produce ROS, and perturb the intracellular nucleotide repertoire, which causes tumor cell damage [31]. Indeed, we found that, the inhibition of autophagy by treatment of 3-MA could reduce the malignant phenotypes of HCC cells and aggravate the sorafenib-induced tumor suppression.

In summary, activation of UPR can enhance the drug resistance of HCC cells and weaken the lethality of sorafenib; inhibition of autophagy can enhance the effect of sorafenib and improve the sensitivity of HCC cells to sorafenib. Therefore, we can get inspiration that the application of UPR blockers and autophagy inhibitors can enhance the therapeutic efficiency of sorafenib in HCC cells.

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None.

## Conflict of Interest