

Capillarisenol C, a novel bisphenol from *Artemisia capillaris*, induces autophagic death in hepatocellular carcinoma through endoplasmic reticulum stress

Xiaofang Wei

Gansu University of Chinese Medicine

Jie Yao

Shenzhen People's Hospital, Jinan University, Southern University of Science and Technology)

Wanying Shen

Gansu University of Chinese Medicine

Qiujie Xie

Shenzhen People's Hospital, Jinan University, Southern University of Science and Technology)

Yangfang Li

Shenzhen People's Hospital, Jinan University, Southern University of Science and Technology)

Lanlan Ge (✉ gelanlan4710@163.com)

Shenzhen People's Hospital, Jinan University, Southern University of Science and Technology)

Xiaobin Zeng

Shenzhen People's Hospital, Jinan University, Southern University of Science and Technology)

Xudong Tang

Gansu University of Chinese Medicine

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Abstract

In our previous work, we isolated a novel bisphenol named capillarisenol C (Cap C) from *Artemisia capillaris*. Preliminary studies indicated potential anti-hepatocellular carcinoma (HCC) activity. However, its mechanism remains unclear. In this study, we aimed to investigate the function and molecular mechanism of the anti-HCC activity of Cap C. First, We evaluated the inhibitory effect of Cap C on the viability of HepG2 and Huh7 cells using CCK8 assays. The results showed that Cap C sharply reduced the viability of HepG2 and Huh7 cells in a time- and concentration-dependent manner, however, lenvatinib (clinical drugs for the treatment of HCC) had no obvious growth inhibitory effect on HepG2 and Huh7 cells at the corresponding concentration. By calculation, the half maximal inhibitory concentrations (IC_{50}) of Cap C were 8.58 and 4.96 μ M for HepG2 and Huh7 cells at 48 h. Then, we investigated its autophagic effects on liver cancer cells using immunofluorescence staining and CRISPR/Cas9 assays. To study the mechanism of Cap C, we used quantitative PCR and western blotting. We found that Cap C effectively inhibited the proliferation of HepG2 cells and increased MAP1LC3-II expression. Moreover, Cap C-induced cell death was attenuated by autophagy-related gene ATG7 knockdown. Mechanistic studies showed that Cap C significantly promoted the expression of endoplasmic reticulum (ER) stress-related proteins. Our results suggest that Cap C may lead to autophagic HCC cell death by inducing ER stress.

Introduction

Hepatocellular carcinoma (HCC) is a common malignant liver tumor, ranking sixth among the most common neoplasms and fourth among the leading causes of cancer-related death worldwide [1]. The etiology of HCC is complex and multifaceted, and the early symptoms are not easily noticeable [2, 3]. Despite advancements in early detection technologies and clinical treatments, improving the survival rate of HCC patients remains a formidable challenge [4]. Sorafenib and lenvatinib, two first-line drugs for advanced HCC, cannot prolong survival significantly [5]. It is thus imperative to find effective drugs with few side effects to improve the prognosis and survival of HCC patients.

In recent years, autophagy has been shown to be an important regulator of cancer occurrence and development [6]. Autophagy is the process whereby lysosomes in eukaryotic cells participate in the degradation of long-lived proteins, damaged organelles, and invasive microorganisms to maintain intracellular homeostasis. It is a core molecular pathway that helps to maintain the balance between cells and cell environment *in vivo* [7, 8]. Autophagy can be stimulated by nutritional stress, redox stress, or endoplasmic reticulum (ER) stress [9]. The entire process is mediated by autophagy-related proteins (ATGs) [10] and can be divided into four steps: initiation, elongation, termination, and ribosome recycling [11]. Autophagy was initially considered a cytoprotective process initiated under conditions of starvation. However, excessive autophagy activation induces cell death [12, 13]. Numerous natural compounds, including kaempferol [14] and flavokawain B [15], have been reported to induce autophagic cancer cell death.

Disorders of the autophagy pathway are closely related to HCC [16]. On the one hand, autophagy plays a suppressive role in the initiation of HCC. Mice with heterozygous disruption of BECLIN1, which is the key autophagy-related gene, tended to initiate the spontaneous formation of HCC. On the other hand, autophagy has been believed to promoting the progression of HCC. Other studies showed that over-expression of LC3- was positively correlated with malignant progression and predictive of poor prognosis in HCC [17]. These observations imply that autophagy may be a potential target for HCC treatment.

Various conditions, including the accumulation of misfolded proteins, depletion of Ca^{2+} stores in the ER, and increased accumulation of certain lipids, can disrupt normal ER function and lead to ER stress. The activated ER stress is responded to restore endoplasmic reticulum and cellular homeostasis. There are three transmembrane ER stress protein sensors: endoplasmic reticulum to nucleus signaling 1 (ERN1/IRE1), eukaryotic translation initiation factor 2 alpha kinase 3 (eIF2a/PERK), and activating transcription factor 6 (ATF6) [18]. The eIF2a/PERK pathway is critical for autophagy induction after ER stress confirming the link between autophagy and the unfolded protein response (UPR). Among them, ATF4 and ATF4-induced transcription factor C/EBP homologous protein (CHOP) can regulate the transcription of more than a dozen ATG genes. IRE1 is also involved in the activation of autophagy [19]. Thus, activation of the UPR induces autophagy.

In recent years, natural products used to treat various diseases with few side effects and low toxicity have become increasingly popular [20, 21]. For example, in traditional Chinese medicine, abundant evidence supports the therapeutic value of the herbal medicine Yincheng for HCC, although the underlying mechanism remains to be investigated [22]. In our previous work, we isolated a novel bisphenol named capillarisin C (Cap C) from *Artemisia capillaris*. Preliminary studies indicated potential anti-HCC activity. However, its mechanism remains unclear. In this study, we further investigated the anti-HCC activity of Cap C to reveal its molecular mechanism.

Materials And Methods

Antibodies and reagents

LC3A/B (2741), PERK (5683), AMPK α (5832), ULK1 (8054S), phospho-ULK1 (Ser555; 5869), and phospho-AMPK α (Thr172; 50081), autophagy antibody sampler kit (4445), AKT (4691), phospho-AKT (Ser473; 4046), PI3K (4249S), anti-mouse IgG (7076S), and anti-rabbit IgG (7074S) were purchased from Cell Signaling Technology (Wuhan, China). CHOP (15204) and IRE1 (27528) were obtained from Proteintech Group (Wuhan, China). ATG5 (A19677), phospho-eIF2 α -S51 (AP0692), β -actin (AC004), Cell Counting Kit-8 (CCK8, 1018) were purchased from ABclon. Secondary antibody Alexa Fluor 594 goat anti-rabbit (A11037; Invitrogen), p62 (T55546; Abmart), chloroquine diphosphate salt (CQ; C6628; Sigma), 4-phenylbutyric acid (4-PBA; C6831; APExBIO), GF-transfect-mat (210423; GenePharma), dulbecco's modified eagle medium high glucose (DMEM High Glucose; C3113-0500; VivaCell), fetal bovine serum

(FBS; 10091148; Gibco), 0.25% trypsin-EDTA (25200-072; Gibco), Penicillin Streptomycin Solution (100×) (C0222; Beyotime) and DAPI (C0065; Solarbio) were purchased from indicated commercial sources.

Preparation method of Cap C

Cap C was isolated from the dried aerial parts of *A. capillaris* Thunb, which were gathered in Ganzhou, Jiangxi Province, China, in August 2019. A voucher specimen (No. 20190805) has been deposited in the Center Lab of Longhua Branch, Shenzhen People's Hospital, Second Clinical Medical College of Jinan University, Shenzhen, China. And the preparation method of Cap C has been patented China (application no. CN202111108858.6). High-performance liquid chromatography (HPLC) analysis of Cap C was performed using an Essentia LC-16 HPLC system (Shimadzu, Kyoto, Japan) equipped with a diode-array detector using a YMC-Pack ODS-A column (4.6 mm× 250 mm, 5 μm) at 30 °C and a flow rate of 1 ml/min. The gradient elution program of the mobile phases consisted of 0.1% trifluoroacetic acid/water (phase A) and methanol (phase B) as follows: 0–30 min, 10–100% B; 30–35 min, 100% B. The injection volume of the test sample solution was set at 10 μl each time, and the UV spectra were recorded at 280 nm for fingerprinting. Its purity was higher than 98% (Figure 1)

Cell cultures

Human hepatocellular carcinoma HepG2 and Huh7 cell lines were purchased from the American Type Culture Collection (ATCC). LX-2 was purchased from the Cell Bank of the Chinese Academy of Sciences. All cells were grown in DMEM containing 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin in an incubator with humidified 5% CO₂ at 37°C.

Cell Counting Kit-8 (CCK8) assay

CCK8 assays were conducted to estimate the cell survival rate. Cells in the logarithmic growth phase were collected and uniformly seeded into 96-well plates (5×10^3 cells/well). After incubation for 24 hours, the cells were treated with 0, 1, 2.5, 5, 8, 10, and 13 μM Cap C (five sub-holes per concentration), followed by incubation for 24 or 48 h. Subsequently, 10% CCK8 was added to each well, followed by incubation for 2–3 h in the dark. A microplate reader (Multiskan FC; Thermo) was then used to measure the absorbance at 450 nm.

Western blotting

HepG2 cells were seeded into a 12-well plate (2×10^5 cells/well). After incubation for 24 h, the cells were treated with 0, 1, 2.5, 4, 5, and 6.5 μM Cap C for 48 h. Whole-cell lysates were then collected in SDS lysis buffer (0.5 M Tris-HCl [PH 6.8], 2% SDS, 10% glycerol, and 0.1% bromophenol blue) and boiled at 95°C for 15 min. Subsequently, the lysates were subjected to 10% SDS-PAGE and transferred to PVDF membranes (IPVH00010; Amersham Life Sciences). The blots were blocked in a blocking buffer (5% skimmed milk in TBST) at room temperature for 30 min and then incubated with primary antibodies (LC3, p62, ATG5, ATG12, ATG16L1, ULK1, p-ULK1, AMPKα, p-AMPKα, AKT, p-AKT, PI3K, PERK, IRE1, p-eIF2α, and β-actin) at

room temperature for 1 h. The bands were then visualized using horseradish peroxidase–conjugated secondary antibody (1:5000) and enhanced chemiluminescence (ECL, Pierce Biotech, Rockford, IL, USA). In all experiments, β -actin was used as a loading control. The experiments were conducted in triplicate.

Quantitative real-time PCR

Total RNA was extracted using an RNA extraction kit (RN07; Aidlab) according to the manufacturer's instructions. Approximately 1 μ g of total RNA was used for reverse transcription using a first strand cDNA synthesis kit (R211-02; Vazyme). RT-qPCR was performed using a ChamQ Universal SYBR qPCR Master Mix (Q711-02-AA; Vazyme). PCR primers were custom-synthesized by Guangzhou IGE Technology (Guangzhou, China), and the information of primers were shown in the Table 1. The amount of mRNA was assayed using quantitative PCR. The amount of each sample was normalized using β -actin. The assays were performed at least three times. The data obtained from one representative experiment were expressed as means $\pm$ standard deviations.

Immunofluorescence assay

Glass coverslips were added to a 12-well plate, and then HepG2 cells were seeded into the plate (1×10^5 cells/well). After incubation for 24 h, the cells were treated with 0 and 4 μ M Cap C for 48 h. The cells were fixed in 4% paraformaldehyde for 20 min and then incubated with PBS containing 1% BSA and 0.1% Triton X-100 for 10 min. They were then incubated with the corresponding antibodies (1:200 dilution in 5% BSA) for 1 h and re-incubated with Alexa Fluor 594–conjugated secondary antibody in the dark at room temperature for 1 h. Finally, they were incubated with 0.5 μ g/ml DAPI in the dark for 10 min for staining. Slides were examined under a Leica TCS SP8 confocal microscope (Leica, Germany).

Transient transfection

For transient transfection, HepG2 cells were seeded into a 12-well plate (1×10^5 cells/well). Plasmids were transfected into the cells using GF-transfect-mat (210423; GenePharma) following the manufacturer's protocol. An immunofluorescence assay was performed after transfection for 24 h.

Lentivirus and retrovirus infections

For ATG7 stable knockdown cell lines, gRNAs targeting human ATG7 were designed by a website (<http://crispr.mit.edu>). Sequences (human ATG7: 5'-CACCGGCCCGTTGCTGCCAGCTAT-3') were selected and cloned into a lentiCRISPR v2 vector. Plasmids were then transfected into HEK293T cells together with psPAX2 and pMD2.G using calcium phosphate transfection. Two days after transfection, the medium was passed through a 0.45 μ m pore filter and mixed with polybrene (H9268; Sigma-Aldrich, St. Louis, MO, USA). The medium containing lentiviruses was transferred to HepG2 cells. The HEK293T cells were further cultured in a fresh medium for 24 h. After 8 h of infection, the medium was changed. The next day, the infection procedure was repeated. After lentivirus infection, cells were treated with 2 μ g/ml puromycin (P9620; Sigma-Aldrich, St. Louis, MO, USA) for 48 h. After two cell culture generations, follow-up

experiments were conducted. Stably overexpressing mRFP-GFP-LC3 cell lines were generated as follows: lentiviral plasmid mRFP-GFP-LC3 was transfected into HEK293T cells together with psPAX2 and pMD2.G using calcium phosphate transfection. The remaining steps were the same as those for the construction of the ATG7 knockdown cell lines.

Statistical analysis

The experimental data is expressed as mean $\pm$ standard deviation (SD), and the data is compared using two-sample t-test, $p < 0.05$ is considered statistically significant, and software such as Graph pad, Adobe Photoshop and ImageJ are used for graphing.

Results

Cap C reduces the viability of hepatocellular carcinoma cells

Our previous work indicated that Cap C potentially exerted anti-HCC activity. In this study, we evaluated the *in vitro* cytotoxic potency of Cap C against two HCC cell lines—HepG2 and Huh7—using CCK8 assays. The results showed that Cap C sharply reduced the viability of HepG2 and Huh7 cells in a time- and concentration-dependent manner (Figure 2A, 2B, 2D, and 2E), however, lenvatinib (clinical drugs for the treatment of HCC) had no obvious growth inhibitory effect on HepG2 and Huh7 cells at the corresponding concentration. By calculation, the half maximal inhibitory concentrations (IC_{50}) of Cap C were 8.58 and 4.96 μ M for HepG2 and Huh7 cells at 48 h, respectively. In contrast, Cap C had a relatively weak cytotoxic effect on the normal human liver cell line LX-2, with an IC_{50} of 16.36 μ M at 48h (Figure 2C and 2F). These results indicate that Cap C has a strong anti-proliferative effect on HCC cells and is less toxic to normal cells.

Cap C regulates autophagy in HepG2 cells

Studies have shown that bisphenol compounds can induce autophagic cancer cell death. For example, bisphenol A has been reported to induce autophagic ovarian granulosa cell death by activating the AMPK/mTOR/ULK1 signaling pathway [23]. Thus, we investigated whether Cap C, a new bisphenol compound, might also induce autophagic HCC cell death. Since autophagy is mediated by autophagy-related proteins, we first examined the effect of Cap C on autophagy-related proteins. We treated HepG2 cells with Cap C (0, 1, 2.5, 4, 5, and 6.5 μ M) for 48 h. Western blotting indicated that Cap C increased the expression of LC3-II in HepG2 cells in a dose-dependent manner (Figure 3A and 3B), as well as the expression of ATG12 (Figure 3A). Moreover, Cap C increased the mRNA levels of LC3 and p62 (Figure 3C). To further investigate autophagy regulation by Cap C, we performed immunoblotting assays. The results showed that the number of red (LC3) puncta increased in the Cap C-treated cells (Figure 3D). Moreover, Cap C treatment increased the number of green (p62) puncta in HepG2 cells transiently transfected with GFP-p62 (Figure 3E). These results suggest that Cap C can regulate autophagy in HepG2 cells.

Cap C induces autophagic HCC cell death

To further investigate whether Cap C mediated autophagy, we used HepG2 cells stably transfected with the tandem fluorescent-tagged LC3B (monomeric red fluorescent protein [mRFP]-GFP-LC3B) plasmid. GFP and RFP showed different pH sensitivities: GFP was quenched in acid, whereas RFP was stable [24]. As shown in Figure 4A, the number of yellow puncta increased in HepG2 cells treated with CQ, an autophagy inhibitor, indicating that autophagic flux was blocked. Cap C treatment also increased the number of red puncta, suggesting that autophagic flux was promoted. Then, we found that the autophagy inhibitor CQ attenuated the killing effect of Cap C in HepG2 cells (Figure 4B). Considering that ATG7 is a key ATG, we constructed an ATG7 knockdown HepG2 cell line using CRISPR/Cas9. ATG7 knockdown suppressed the Cap C-mediated increase in LC3-II expression (Figure 4D and 4E) and cell death (Figure 4C). These results suggest that Cap C could induce autophagic HepG2 cell death.

Cap C induces autophagy through the ER stress-dependent upregulation of PERK and IRE1

It has been shown that the PI3K/AKT/mTOR and AMPK/ULK1 signaling pathways are important for controlling autophagy [25, 26]. To determine whether these two signaling pathways played important roles in Cap C-induced autophagic HCC cell death, we examined the expression of proteins (AKT; p-AKT; PI3K; ULK1; p-ULK1; AMPKa; p-AMPKa) in the HepG2 cells treated with Cap C using western blotting. We found that with the increase of Cap C concentration did not significantly change the key proteins of the PI3K/AKT/mTOR and AMPK/ULK1 signaling pathways (Figure 5A, 5B, 5D, and 5E). Moreover, as accumulating evidence suggests that ER stress signals are closely related to autophagy [19], we examined whether Cap C induced ER stress in HepG2 cells. Western blotting revealed that Cap C treatment for 48 h led to a dose-dependent increase in the levels of PERK and IRE1 but not of p-eIF2 α in HepG2 cells (Figure 5C and 5F). Immunofluorescence assays confirmed the upregulation of PERK and IRE1 induced by Cap C treatment (Figure 5G and 5H). Furthermore, 4-PBA, an ER stress inhibitor, attenuated Cap C-induced HepG2 cell death (Figure 5I). These results indicate that Cap C may activate ER stress in HepG2 cells and induce autophagy through ER stress-dependent PERK and IRE1 upregulation.

Discussion

Cap C is a novel natural bisphenol isolated from *Artemisia capillaris*. In this study, we found that Cap C significantly inhibited HCC cell viability but had a negligible effect on normal hepatocytes. Moreover, Cap C treatment led to autophagic HepG2 cell death. The inhibition of autophagy blocked Cap C-induced a decrease in HepG2 cell viability. Furthermore, Cap C upregulated the ER stress-related proteins PERK and IRE1, and the ER stress inhibitor 4-PBA attenuated Cap C-induced cell death. These findings suggest that ER stress may be a crucial mediator of Cap C-induced autophagic HepG2 cell death (Fig. 6).

Our results indicate that HCC cells are more sensitive to Cap C than normal liver cells. The activation of the ER stress pathway might contribute to HCC cell selectivity. Cancer cells usually exhibit higher levels of ER stress and unfolded protein response (UPR) induced by conditions in the tumor microenvironment such as hypoxia, acidosis, and nutrient deprivation [27]. Thus, UPR-related gene expression is elevated in

most HCC cells [28]. Moreover, it has been reported that elevated mRNA levels of BiP, ATF6, and spliced XBP1 show stronger correlations with well-differentiated HCC tissue than healthy tissue [29]. The use of drugs that exacerbate the basal ER stress of cancer cells can counteract the cytoprotective effect of the UPR, leading to proapoptotic arm (DDIT3) activation and cell death. Conversely, normal cells show lower levels of ER stress and greater resistance to drug-induced cytotoxicity [30].

Autophagy has been shown to play a dual role in HCC. Wu et al. reported that adrenaline treatment in a DEN-induced HCC mouse model resulted in a remarkable increase in liver injury, along with an increase in tumor number and size. Mechanistically, adrenaline inhibited autophagy activation by disrupting the Beclin1/VPS34/ATG14 complex [31]. This suggests that autophagy plays a suppressive role in the initiation of HCC. On the other hand, the upregulation of hypoxia-induced Egr-1 has been shown to induce autophagy in HCC cell lines, promoting migration, which suggests that autophagy promotes the progression of HCC [32]. However, excessive autophagy activation may also lead to autophagic cell death [33]. Both autophagy inducers and inhibitors have been used for HCC treatment [34]. The autophagy inducer sorafenib is a first-line drug used for the treatment of advanced HCC [35]. On the other hand, the suppression of autophagy by 3-MA and inactive ATG4B suppresses the proliferation of Huh7 cells [36]. Thus, the inhibition of autophagy can promote HCC cell death. However, autophagy inhibitors may have side effects, highlighting the significant risk of triggering neoplasticism within normal hepatocytes [34]. Thus, inducing autophagic cell death may be ideal for HCC therapy. Our research suggests that the novel natural component Cap C can induce autophagic HepG2 cell death, suggesting that it could be used as a drug for HCC.

Declarations

Conflicts of Interest

All the authors stated that there were not any actual or potential conflicts of interest.

Acknowledgements

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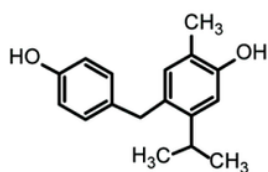
Table

Table 1 Information of primers

Gene name	Forward (5'-3')	Reverse (5'-3')
β -actin	CAGCACAATGAAGATCAAGA	GATCCACATCTGCTGGAAG
LC3B	CCAGATCCCTGCACCATG	CTGCTTCTCACCCTTGTATCG
p62	GGCACCTGTCTGAGGGCTT	TTTCCTGGTGGACCCATTTC
Beclin1	TCACCATCCAGGAAC TCACA	CCTGGCGAGGAGTTTCAATA

Figures

A



B

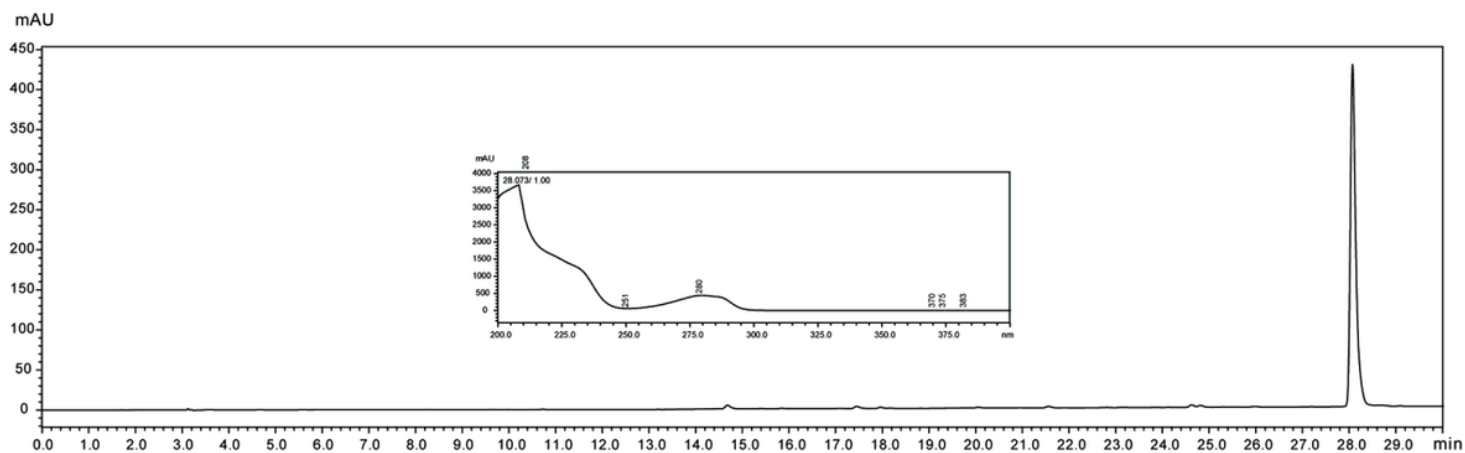


Figure 1

HPLC analysis of Cap C: **(A)** Chemical structure of Cap C. **(B)** HPLC analysis of Cap C. Cap C, capillarisenol C.

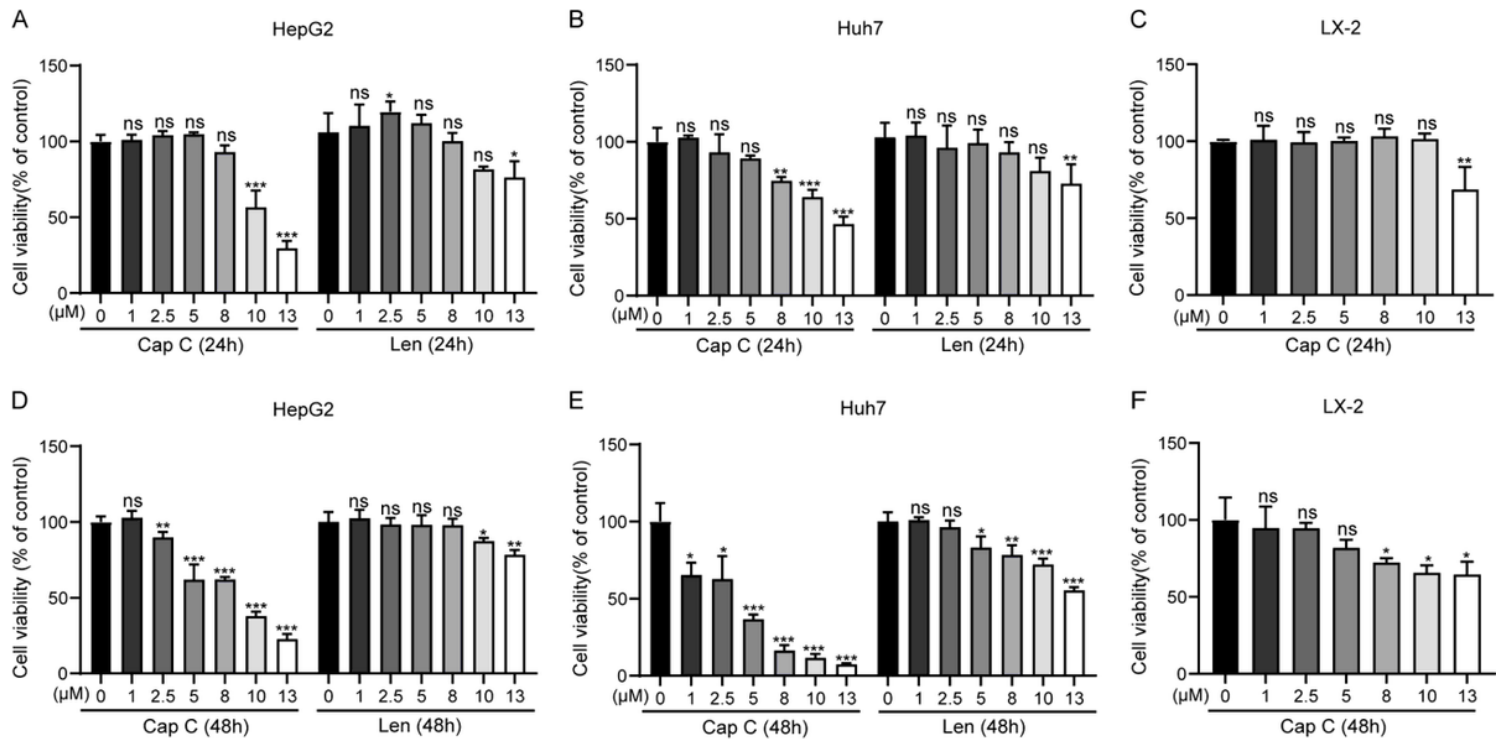


Figure 2

Cap C reduces the viability of hepatocellular carcinoma cells: **(A-C)** HepG2 **(A)**, Huh7 **(B)** and LX2 **(C)** cells were exposed to Cap C at the indicated doses for 24 h and dose-escalation effects of Cap C was assessed by CCK8 assay. **(D-F)** HepG2 **(D)**, Huh7 **(E)** and LX2 **(F)** cells were exposed to Cap C at the indicated doses for 48 h and dose-escalation effects of Cap C was assessed by CCK8 assay. Cap C, capillarisenol C; Len, lenvatinib. Results are mean $\pm$ SD for three experiments. * p -value ≤ 0.05 , ** p -value ≤ 0.01 , *** p -value ≤ 0.001 . (t-test).

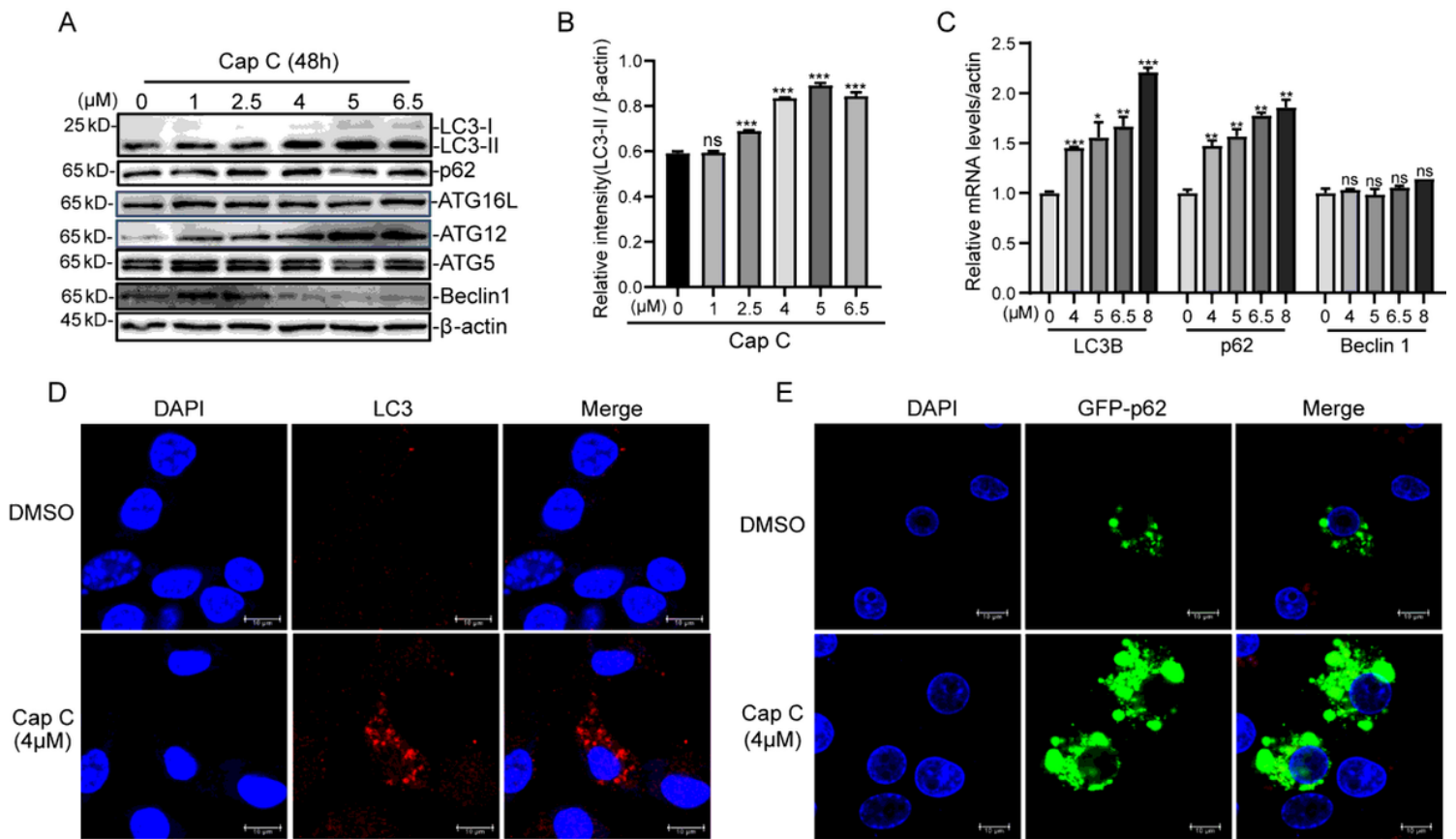


Figure 3

Cap C regulated autophagy in HepG2 cells: **(A)** HepG2 cells were exposed to the indicated dose of Cap C for 48 h and the expression levels of LC3 were detected by using western blot analysis. **(B)** The imageJ statistical analysis was performed simultaneously. **(C)** HepG2 cells were treated with 4 μM Cap C for 48 h, then qRT-PCR was performed. **(D)** HepG2 cells were exposed to the indicated dose of Cap C for 48 h and the distribution LC3B was examined by confocal microscopy. The scale bars represent 10 μm. **(E)** HepG2 cells transiently transfected with GFP-p62 plasmid were treated with indicated dose of Cap C for 48 h, then fixed and subjected to confocal microscopy. Scale bar: 10 μm. Cap C, capillarisenol C. Results are mean ± SD. for three experiments. * p -value ≤ 0.05, ** p -value ≤ 0.01, *** p -value ≤ 0.001. (t-test).

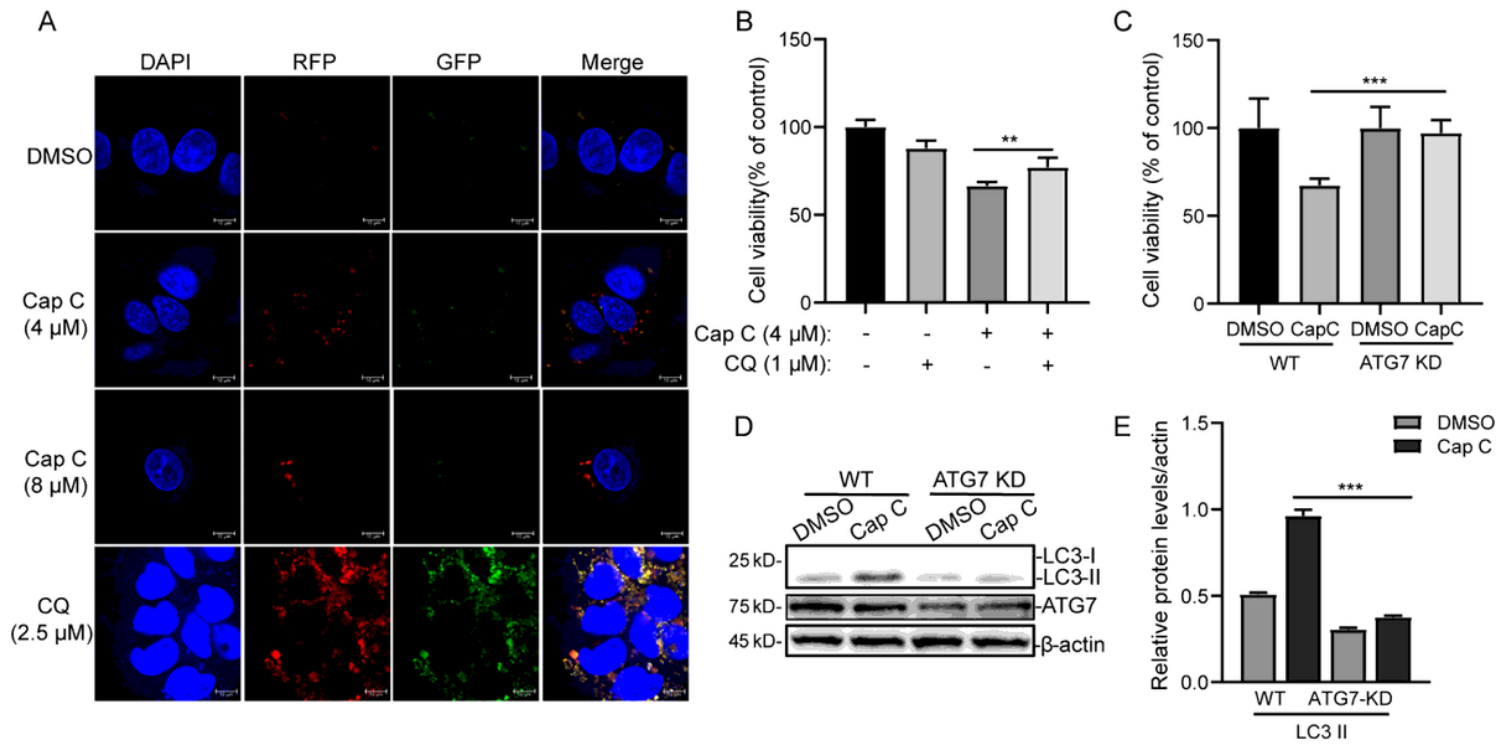


Figure 4

Cap C induces autophagic cell death in HCC cells: **(A)** HepG2 cells stably transfected with mRFP-GFP-LC3B plasmid were treated with indicated dose of Cap C or CQ for 48 h, then fixed and subjected to confocal microscopy. Scale bar: 10 μm. **(B)** Effect of autophagy inhibitor CQ on the growth of Cap C treated cells, which were treated with Cap C (4 μM) and CQ (1 μM) for 48 h; Cell viability was determined by CCK8. **(C)** WT, and ATG7 KD HepG2 cells were treated with 4 μM Cap C for 48 h, then cell viabilities were assessed by CCK8 assay. **(D)** ATG7 were stably knockdown in HepG2 cells using CRISPR/Cas9. Wild-type (WT), ATG7 knockdown (ATG7 KD) HepG2 cells were treated with 4 μM Cap C for 48 h and cell lysates were immunoblotted with indicated antibodies. **(E)** Gray scale analysis of LC3 II by imageJ. Cap C, capillarisenol C. Results are mean ± SD. for three experiments. ** p -value ≤ 0.01, *** p -value ≤ 0.001. (t-test).

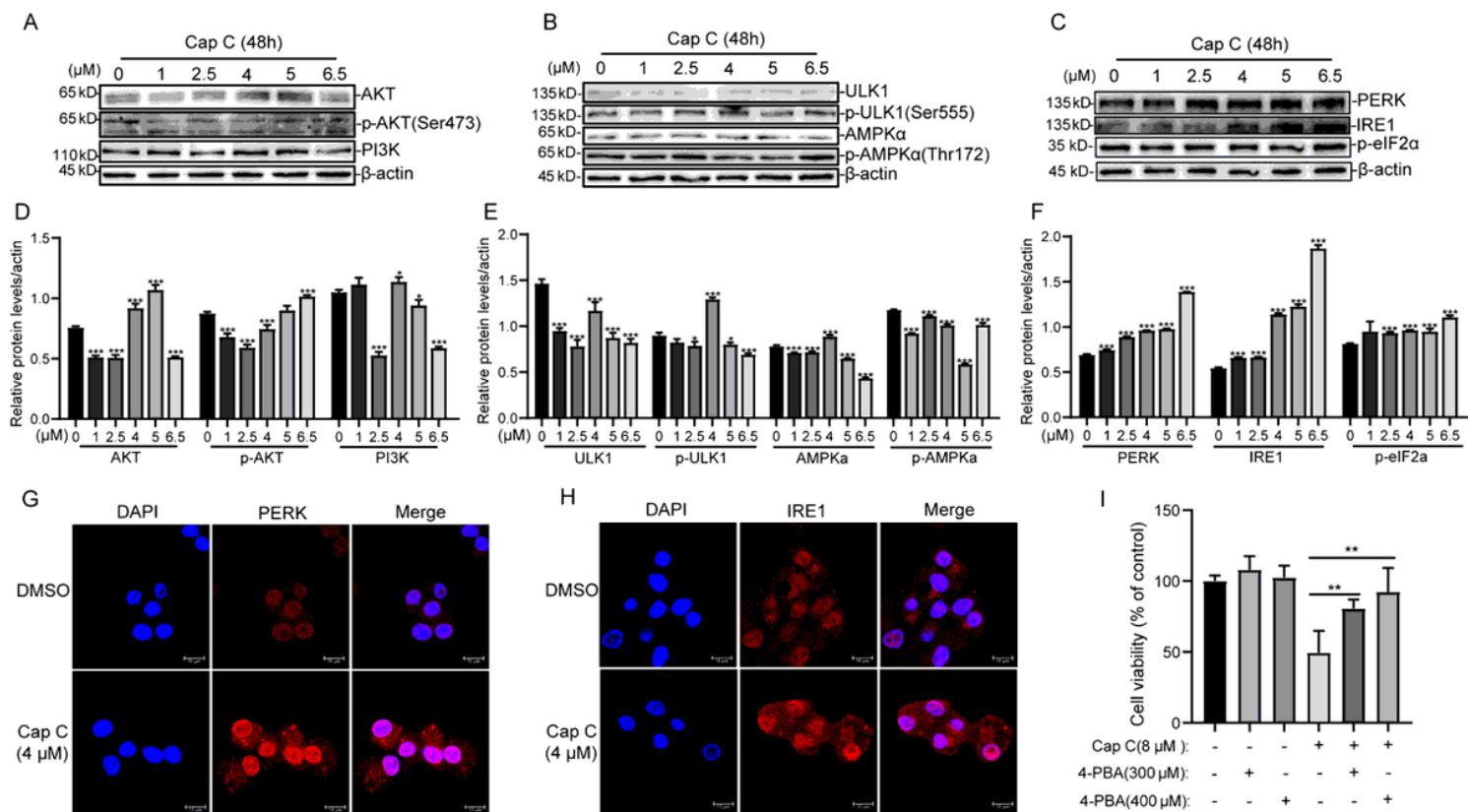


Figure 5

Cap C induces autophagy through ER stress-dependent upregulation of PERK and IRE1 in HepG2 cells: **(A-C)** HepG2 cells were treated with the indicated dose of Cap C for 48 h and cell lysates were immunoblotted with indicated antibodies. **(D-F)** The imageJ statistical analysis was performed simultaneously. **(G-H)** HepG2 cells were exposed to the indicated dose of Cap C for 48 h and the expression of PERK **(G)** and IRE1 **(H)** were examined by confocal microscopy. The scale bars represent 10 μm. **(I)** Effect of ER stress inhibitor 4-PBA on the growth of Cap C-treated cells, which were treated with Cap C (8 μM) and 4-PBA (300, 400 μM) for 48 h; Cell viability was determined by CCK8. Cap C, capillarisenol C. Results are mean ± SD for three experiments. * p -value ≤ 0.05, ** p -value ≤ 0.01, *** p -value ≤ 0.001. (t-test).

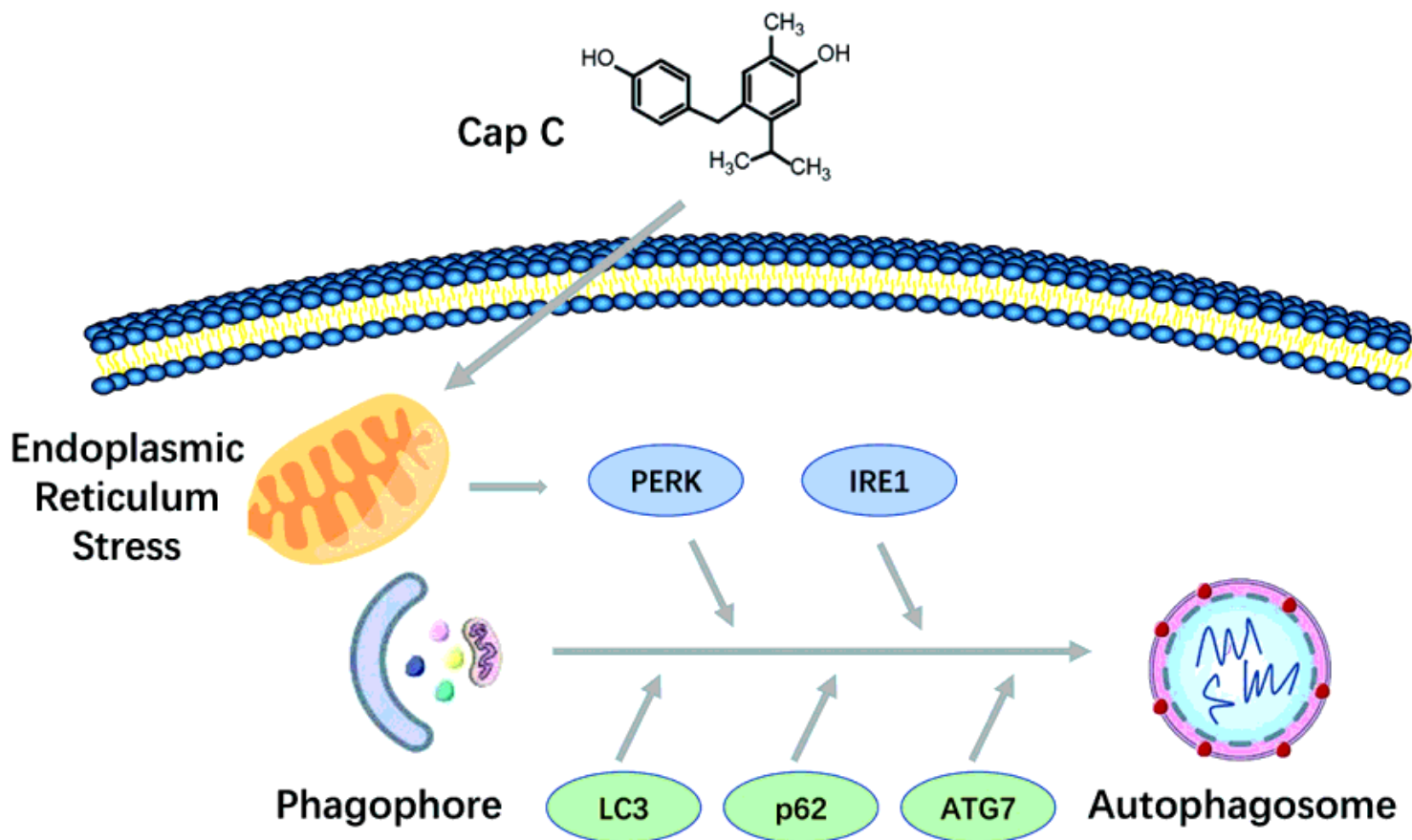


Figure 6

A hypothetical model for the Cap C induced autophagic death through endoplasmic reticulum in hepatocellular carcinoma. Cap C induces autophagy through the ER stress-dependent in HepG2 cells. Briefly, Cap C upregulates ER stress-related proteins PERK/IRE1 and autophagy related proteins. Cap C, capillarisenol C.

Supplementary Files

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- [Onlinefloatimage7.png](#)