

## Original Research Article

# Scoparone inhibits the growth of liver cancer cells by regulating MAPK signaling pathway

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### Abstract

**Purpose:** To investigate the possible effects of scoparone on the viability, apoptosis and motility of liver cancer cells, as well as the mechanism of action.

**Methods:** CCK-8 and colony formation assays were carried out to determine liver cancer cell viability, while flow cytometry (FCM) and Immunoblot assays were performed to evaluate cell apoptosis. Wound closure and Transwell assays were performed to determine the effect of scoparone on cell motility, while immunoblot assays were performed to assess the effect of the compound on MAPK pathway of liver cancer cells.

**Results:** Scoparone suppressed the viability of Hep3B2 and Huh-7 cells ( $p < 0.01$ ), as well as the apoptosis and motility of the cells ( $p < 0.01$ ). Furthermore, scoparone inhibited the growth of liver cancer cells via MAPK pathway.

**Conclusion:** Scoparone suppresses liver cancer cell growth via MAPK pathway, and is thus a promising drug for liver cancer treatment. However, in vivo studies are required to validate these findings.

**Keywords:** Scoparone, Liver cancer, Viability, Apoptosis, MAPK pathway

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## INTRODUCTION

Liver cancer is the most common cancer type in the world [1]. The main cause is HBV, HCV or alcohol [2]. Liver cancer has a rapid growth as well as deterioration rate, and the survival rate varies greatly among countries [3]. Patients diagnosed with liver cancer at the late stage have a poor prognosis and low survival rate [3]. Although there are many treatments available such as natural compounds, chemotherapy,

immunotherapy and combination therapy, the incidence and mortality rate remain high [4]. Recently, a targeted therapy for this cancer has been widely reported [5].

Scoparone, the main ingredient of the Chinese herb, artemisinin, has been used in Asia to treat neonatal jaundice and it possesses multiple biological attributes [6]. It provides protection against hyperbilirubinemia by activating the constitutive androgen receptor (CAR) [7]

improved hepatic inflammation and autophagy in mice with nonalcoholic steatohepatitis via the ROS/P38/Nrf2 pathway and in macrophages [8]; it alleviates the inflammation, apoptosis and fibrosis of non-alcoholic steatohepatitis by suppressing the TLR4/NF- $\kappa$ B pathway [9] inhibits the activity of the MAPK pathway, and the invasion of PDGF-BB-treated vascular smooth muscle cells, thereby reducing atherosclerotic vascular injury [10] also induced the expression of pluripotency transcription factors SOX2 and NANOG in dermal papilla cells [11]. It alleviated Ang II-induced pathological myocardial hypertrophy in mice by suppressing oxidative stress [12].

Scoparone has been shown to have antitumor effects. For example, scoparone suppressed the proliferation of melanoma cells [13], and inhibit pancreatic cancer through the PI3K/Akt signaling pathway [14]. Scoparone also showed anti-tumor activity against DU145 prostate cancer cells by suppressing STAT3 activity [15]. However, the possible effect of Scoparone in liver cancer has not been determined.

The aim of this study was to investigate the anticancer activity of scoparone and the mechanism of action.

## EXPERIMENTAL

### Antibodies and drugs

The following materials were used in this work. Anti-Bax (1: 500 dilution, ab32503, Abcam), anti-Bcl-2 (1: 500 dilution, ab32124, Abcam), anti-cleaved caspase3 (1: 500 dilution, ab32042, Abcam), anti-cleaved caspase9 (1: 1000 dilution, ab2324, Abcam), anti-P38 (1: 500 dilution, ab170099, Abcam), anti-p-P38 (1: 500 dilution, ab178867, Abcam), anti-ERK (1:500 dilution, ab32537, Abcam), anti-p-ERK (1: 500 dilution, ab131438, Abcam), anti-GAPDH (1: 2000 dilution, ab8245, abcam). Scoparone (HY-N0228) was purchased from MedChemExpress (USA).

### Cell culture

The two liver cancer cell lines, including Hep3B2 and Huh-7 were all purchased from ATCC. Both cells were maintained in DMEM, supplemented with 10 % of FBS and incubated at 37 °C and 5 % CO<sub>2</sub> incubator.

### Immunoblot assay

The samples were lysed with lysis buffer (RIPA, Beyotime, China), and then separated by 8 %

SDS-PAGE experiment. The total proteins were transferred onto PVDF membranes in a sequence (Millipore, USA). The PVDF membranes were blocked with 5 % dry milk in TBST buffer and antibodies. After washing, the membranes were treated with HRP secondary antibodies for 45 min. Each blot was then visualized using ECL kit (GE, SA).

### Cell viability assay

For CCK-8 assay, cells were plated on 96-well plates (1000 cell per well) and maintained in complete growth media for 24 h at 37 °C. The cells were exposed to CCK-8 reagent at 37 °C for 1.5 h. Relative cell viability was assessed spectrophotometrically in a microplate at 450 nm (Bio-Rad, USA) for 48 h.

For colony formation assays, the cells were plated into 6-well plates (1000 cell per well) and maintained in complete growth media for 14 days at 37 °C. Then cells were fixed with PFA for 20 min and stained with Crystal violet agent (0.1 %) for 20 min. Then cells were photographed.

### Cell apoptosis assay

The cells were washed with phosphate buffered saline (PBS). Subsequently, the cells were fixed with 70 % ethanol at -20 °C for 1 h, and then stained with propidium iodide (PI) and a FITC-labelled Annexin V agent at 4 °C for 10 min, and the apoptosis level was measured by a BD FACS caliber.

### Cell migration assay

For wound closure assays, the liver cancer cells were grown to 100 % confluency. Then scratches were made on them using a pipette tip, and washed with PBS buffer. Serum-free culture medium was added so as to induce wound healing. Then the pictures of cells were taken at 0 and 24 h time points.

For transwell assays, after transfection for 48 h, the cells were seeded onto the upper chamber in the culture medium without serum. Then, complete culture medium was added into the bottom to induce cell invasion. After 24 h, cells in the upper were removed, and the remaining cells were fixed, stained with crystal violet agent (0.1 %) and number was counted.

### Statistics

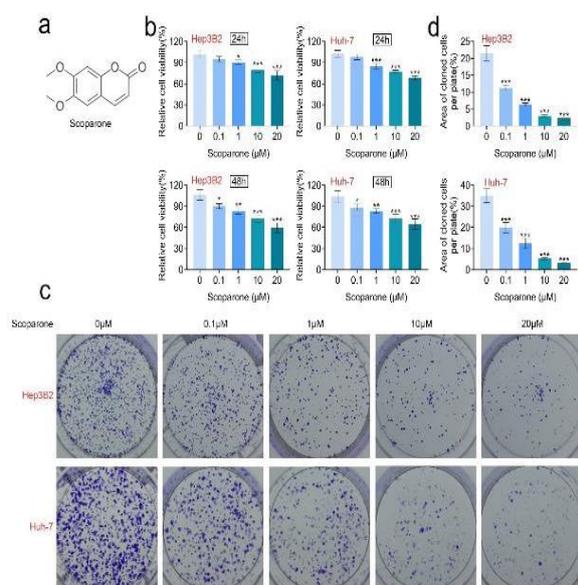
Data are presented as mean  $\pm$  SD. Statistical significance of the difference was evaluated

using Student's t test with the aid of SPSS software.  $P < 0.05$  was considered significant.

## RESULTS

### Scoparone inhibited proliferation of liver cancer cell lines

Since scoparone affected the progression of multiple types of tumors, its possible role was investigated in liver cancer. The molecular formula of scoparone is shown in Figure 1 A. Scoparone suppressed the viability of both types of liver cancer cells (Figure 1 B). Scoparone also decreased colony numbers of liver cancer cells (Figure 1 C and D). Therefore, scoparone suppressed liver cancer cell viability.

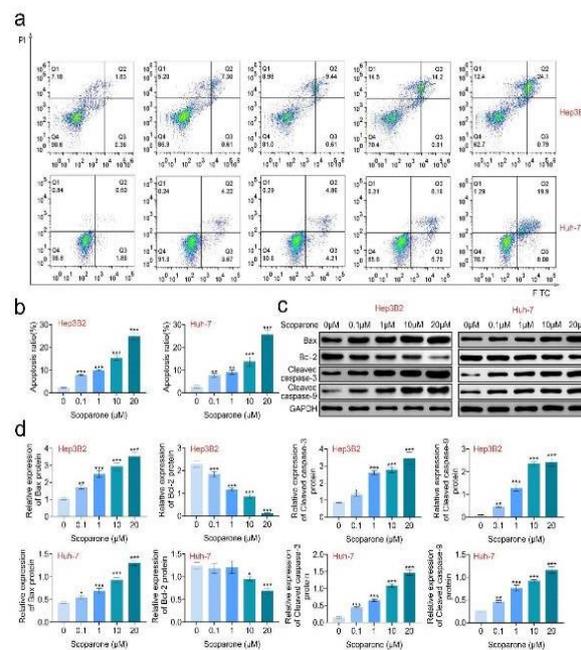


**Figure 1:** Scoparone inhibits proliferation of liver cancer cell lines. (A) Molecular formula of scoparone (B). CCK-8 assays showed the effects of scoparone (0, 0.1, 1, 10, 20  $\mu\text{M}$ ) on the viability of Hep3B2 and Huh-7 cells for 24 and 48 h. The absorbance value at 450 nm wavelength was measured (C and D). Colony formation assays showed the effects of scoparone on the colony formation of Hep3B2 and Huh-7 cells (C), and the colony numbers were measured (D). Data are presented as mean  $\pm$  SD. \* $P < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

### Scoparone stimulates apoptosis in liver cancer cell lines

Scoparone treatment significantly induced the apoptosis of both Hep3B2 and Huh-7 cells as shown in Figure 2 A and B. The increased expression levels of Bax, cleaved-caspase3, and cleaved-caspase9 in Hep3B2 and Huh-7 cells upon scoparone treatment are shown in Figure 2 C and D; however, the expression of BCL-2 significantly decreased (Figure 2 B). Scoparone,

therefore, stimulated the apoptosis of liver cancer cell lines.



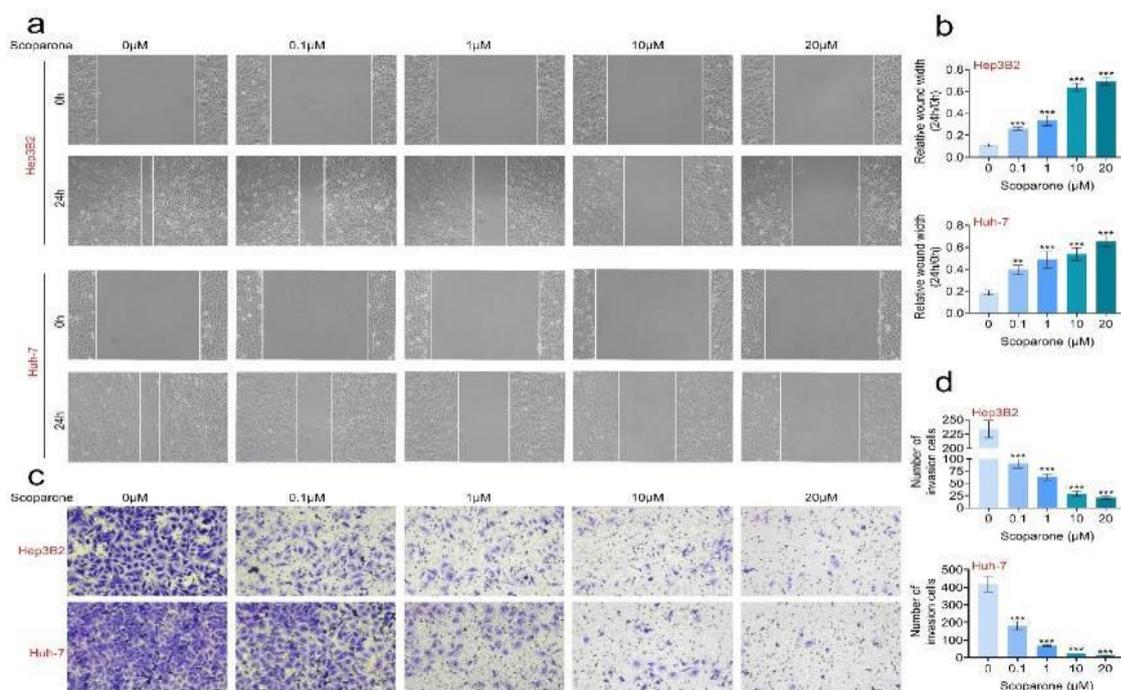
**Figure 2:** Scoparone stimulates apoptosis in liver cancer cell lines. (A). Effects of scoparone (0, 0.1, 1, 10, 20  $\mu\text{M}$ ) on apoptosis of Hep3B2 and Huh-7 cells (A), and the percentage of apoptosis cells are indicated (B). C and D: Effect of scoparone (0, 0.1, 1, 10, 20  $\mu\text{M}$ ) on the expression of apoptosis markers, including Bax, Bcl-2, cleaved-caspase 3, and cleaved-caspase 9 of Hep3B2 and Huh-7 cells (C), and their expression levels (D). Data are presented as mean  $\pm$  SD. \* $P < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

### Scoparone suppressed the migration of liver cancer cell lines

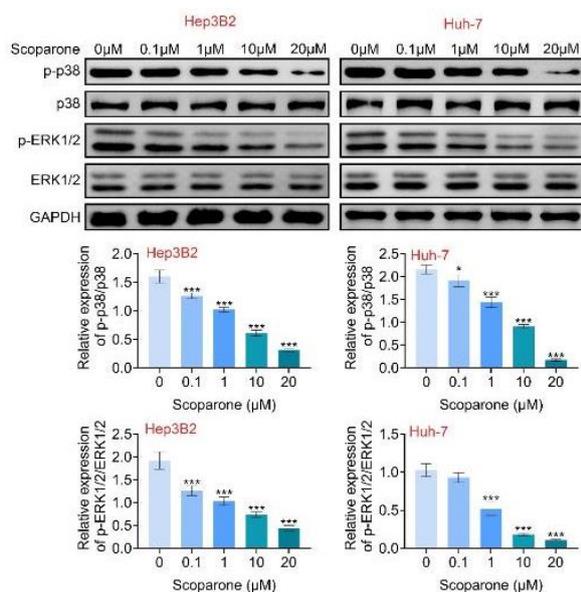
The effects of scoparone on the motility, migration and invasion of liver cancer cells were revealed through wound closure and transwell assays. The increased wound width caused by scoparone treatment occurred at different concentrations in Hep3B2 and Huh-7 cells (Figure 3 A and B). Furthermore, scoparone suppressed the invasion of Hep3B2 and Huh-7 cells, as evidenced by the decreased invasive cell numbers (Figure 3 C and D). Therefore, scoparone suppressed the migration of liver cancer cell lines.

### Scoparone mediated MAPK pathway in liver cancer cell lines

Scoparone significantly decreased the phosphorylation levels of p38 and ERK, in a dose-dependent manner (Figure 4). Scoparone suppressed MAPK pathway in liver cancer cell lines.



**Figure 3:** Scaparone suppressed the migration of liver cancer cell lines. (A, B). Wound closure assays showed the effects of scoparone (0, 0.1, 1, 10, 20 uM) on the migration of Hep3B2 and Huh-7 cells upon the indicated treatment (A), and the wound width was measured (B). (C, D): Transwell assays showed the effects of scoparone (0, 0.1, 1, 10, 20 uM) on the apoptosis of Hep3B2 and Huh-7 cells upon the indicated treatment (C), and the invasion cell numbers were measured (D). Data are presented as mean ± SD (\*\*\*)  $p < 0.001$



**Figure 4:** Scaparone mediated MAPK pathway in liver cancer cell lines. Effect of scoparone (0, 0.1, 1, 10 and 20 uM) on the expression of P38, pP38, ERK, and p-ERK of Hep3B2 and Huh-7 cells. Data are presented as mean ± SD; \* $p < 0.05$ , \*\*\* $p < 0.001$

## DISCUSSION

For malignant tumors occurring in the liver [3], the initial symptoms are not obvious, but late symptoms are mainly liver pain, fatigue,

emaciation, jaundice, ascites and other symptoms [16]. Clinically advanced cases of these patients have a low cure rate due to the spread of cancer cells, hence early diagnosis and treatment of liver cancer should be achieved [4]. To improve the survival rate of patients, it is still necessary to conduct in-depth research on the pathogenesis of liver cancer, to identify the key regulatory proteins and screen out effective therapeutic drugs [4].

In the present study, scoparone, could serve as a promising drug for liver cancer. Through series of *in vitro* assays, such as MTT, colony formation, FCM, Immunoblot, wound closure, and Transwell assays, the effects of scoparone were assessed. The anti-tumor effects of scoparone on various types of tumours have been widely reported [14]. Scaparone inhibited the progression of pancreatic cancer cells through via PI3K/Akt pathway [14]. It also exerted anti-tumor activity against prostate cancer cells by suppressing the activity of STAT3 [15]. These studies demonstrated scoparone may serve as a promising drug for tumor treatment.

Besides the effects on tumor progression, scoparone displays multiple biological activities. Scaparone inhibits ultraviolet radiation-induced lipid peroxidation [17]. In addition, scoparone suppressed the expressions of SOX2 and

NANOG in dermal papilla cells, and altered the development of stem cells [11]. It also has potentials for the treatment of several other diseases. For example, scoparone alleviates Ang II-induced pathological myocardial hypertrophy in mice by suppressing oxidative stress [12], as well as also mitigates hepatic fibrosis via suppressing of TLR-4/NF- $\kappa$ B pathway [6].

Scoparone has been predicted as a potential drug candidate for various types of liver diseases, such as acute liver injury, alcohol-induced hepatotoxicity, fulminant hepatitis, non-alcoholic fatty liver disease and fibrosis [18]. Scoparone ameliorates hepatic inflammation and autophagy in macrophages [8]. Interestingly, it suppressed the progression of liver cancer in the present work. However, the precise regulatory mechanism needs further study. In the present study, scoparone mediated MAPK pathway in liver cancer cell lines, and this pathway has been reported to mediate the viability, apoptosis, autophagy, and EMT of liver cancer cells [19]. Several proteins and drugs affect the progression and metastasis of liver cancer via this pathway. For example, PNO1 mediated the autophagy and apoptosis of liver cancer via this pathway [20]. EB2 also promoted liver cancer proliferation and metastasis via MAPK pathway by regulating microtubule dynamics [21]. Therefore, this pathway might also act as a therapeutic target for liver cancer therapy.

## CONCLUSION

Scoparone suppresses cell viability and stimulates apoptosis in liver cancer cell lines. It regulates MAPK pathway in liver cancer cells, and thus, may inhibit the proliferation of cancer cells and promote cell apoptosis via MAPK pathway. Therefore, scoparone is a potential therapeutic agent for the management of liver cancer.

## DECLARATIONS

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### Funding

None provided.

### Ethical approval

None provided.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Conflict of Interest

No conflict of interest associated with this work.

### Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Xitao Liu and Jie Huang designed the study and carried them out, supervised the data collection, analyzed the data, interpreted the data, prepare the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the manuscript.

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