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Nobiletin inhibits cell proliferation and invasion via suppression of UBE2C in hepatocellular carcinoma

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ABSTRACT: Nobiletin is a polymethoxylated flavone derived from Citrus. It is used in traditional Chinese medicine due to its antitumor activity against a variety of malignant tumors. However, the biological function of nobiletin in hepatocellular carcinoma (HCC) and its underlying mechanism is largely unknown. The experiments were performed by treatment of two HCC cell lines (SMMC-7721 and Huh-7) with nobiletin at various concentrations for 48 h. The results showed that nobiletin possessed cytotoxicity to both HCC cell lines, as determined by MTT assay, in a dose-dependent manner. Meanwhile, cell proliferation, migration, and invasion, as assessed by colony formation and Transwell assays, were inhibited in both cells after being treated with nobiletin. Western blotting was performed to measure the expression of ubiquitin-conjugating enzyme E2 C (UBE2C), E-cadherin, and N-cadherin. UBE2C expression was reduced in nobiletin-treated HCC cells, while its expression was increased in HCC tissues and cell lines, and positively correlated with a shorter survival time of patients with HCC. Mechanistically, nobiletin exhibited anti-proliferation and anti-aggressive effects on HCC cells via downregulation of UBE2C. Moreover, nobiletin administration restricted tumor growth in the HCC xenografts model conducted on nude mice. In conclusion, nobiletin suppressed proliferation, migration, and invasion of HCC cells by reducing UBE2C, suggesting its potential use as an effective therapeutic agent for the treatment of HCC.

KEYWORDS: nobiletin, UBE2C, hepatocellular carcinoma, proliferation, metastasis

INTRODUCTION

Liver cancer is the sixth most commonly diagnosed cancer and third most commonly caused cancer-related deaths globally, with $> 900,000$ new cases and $> 830,000$ new deaths in 2020 [1]. Hepatocellular carcinoma (HCC), which accounts for more than 80% of liver cancers, is among the top three causes of cancer-related deaths in 46 countries and among the top five in 90 countries [2]. Based on data from the global burden of disease 2019 study, the number of disease-adjusted life years from liver cancer increased from 11,278,630 (95% UI, 10,062,526–12,677,403) in 1990 to 12,528,422 (95% UI, 11,400,671–13,687,675) in 2019 [3]. In the past decades, there have been several methods for HCC treatment on clinical, such as surgical resection, hepatic artery embolization chemotherapy, radiofrequency ablation, and immunotherapy [4], but the prognosis of patients with HCC is still unsatisfactory with a 5-year overall survival rate of less than 20% [5]. The immune system disorders and organ damage caused by the above treatment strategies still plague the majority of patients [6]. Therefore, seeking a safe and effective therapeutic agent for HCC patients was a vital issue that needed to be solved urgently.

Nowadays, the therapeutic efficacy of traditional Chinese herbs (TCHs) for human diseases is receiving more and more attention, especially its proven use in cancer treatment [7]. Nobiletin is a common biologically active ingredient of TCH, which is a polymethoxylated flavone extracted from *Citrus nobilis* Lour, *Citrus aurantium* L, and *Citrus reticulata* Blanco [8]. Of note, nobiletin has been shown to have multiple biological activities [9] such as antitumor, detoxification, antioxidant, neuroprotective, hepatoprotective, anti-inflammatory, and anti-diabetic. Many studies have confirmed that nobiletin has antitumor activity [10] and enhanced the sensitivity of cancer cells to chemotherapy with the advantages of low toxicity, safe use, and non-addictive properties [11]. Currently, nobiletin possesses anti-proliferation and anti-metastasis activities in human malignancies [12], including solid tumors and hematological cancers. However, the inhibitory effect of nobiletin on the malignant phenotypes of HCC cells and its anticancer mechanism remains unclear.

The ubiquitin-conjugating enzyme E2C (UBE2C) is an important member of the ubiquitin-conjugating enzyme E2 family that is located on human chromosome 20q13.12 [13]. Previous studies have demonstrated that aberrantly expressed UBE2C has been shown to play an important role in tumorigenesis and facilitated poor outcomes in patients with tumors [14], and acted as a novel biomarker for cancer diagnosis [15]. Moreover, knockdown of UBE2C inhibited the malignant biological behavior of cancer cells, including HCC [16], esophageal squamous cell carcinoma [17], and pancreatic ductal adenocarcinoma [18]. The above studies suggest that UBE2C may be an effective target for

malignant tumors in early diagnosis and treatment, however, whether UBE2C acts as an effective therapeutic target for nobiletin in the treatment of HCC has not been confirmed.

The aim of the present study is to deeply understand the anti-tumor molecular mechanism of nobiletin and provide guidance for its effective application in the treatment of HCC. Firstly, we explored the cytotoxicity of nobiletin on HCC cells by detecting cell viability and proliferation, as well as its effects on the migration and invasion abilities of HCC cells were also evaluated. Moreover, we verified the effect of nobiletin on UBE2C expression in HCC cell lines, and further analyzed the expression of UBE2C in HCC tissues from the Gene Expression Profiling Interactive Analysis (GEPIA) database and its effect on the survival time of HCC patients. Functionally, we investigated the effect of UBE2C on the malignant biological behavior of HCC cells treated with or without nobiletin *in vitro*. Furthermore, we assessed the therapeutic effect of nobiletin on tumor growth in a xenograft nude model of HCC *in vivo*.

MATERIALS AND METHODS

Cell culture and treatment

Human HCC cell lines (SMMC-7721 and Huh-7) and human liver epithelial cells (THLE-3) were purchased from the Shanghai Institute for Biological Science, Chinese Academy of Science (Shanghai, China). All cell lines were cultivated in Dulbecco's modified eagle medium (DMEM) with an added 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin-streptomycin (Solarbio, China). Cells were cultured at 37 °C in an environment with 5% carbon dioxide. Subsequently, both SMMC-7721 and Huh-7 cells were treated with nobiletin (> 99% purity; MedChemExpress, USA) at different doses (0–100 μM and 0–50 μM, respectively) for 48 h.

Cell transfection

For cell transfection, lentiviral UBE2C shRNA (sh-UBE2C), UBE2C overexpression (oe-UBE2C) plasmids, and their respective negative controls were sourced from Genechem company (Shanghai, China). These vectors were introduced into SMMC-7721 and Huh-7 with the help of a Lipofectamine™ 3000 Transfection Reagent (Invitrogen, USA). After the 48 h cell transfection, Western blotting was performed to estimate the efficiency of the transfections in each group.

MTT assay

Cell viability was measured by MTT assay. The half-maximal inhibitory concentration (IC_{50}) value is a critical index of the dose-response curve. According to IC_{50} values, SMMC-7721 and Huh-7 cells (5×10^3 cells/well) were added into 96-well plates, treated with different concentrations (30 and 50 μM) of nobiletin, or transfected with oe-UBE2C or sh-UBE2C for 48 h. Next, a 20 μl MTT reagent (5 mg/ml; Solarbio) was added to each well. Following incubation for 4 h, the DMEM medium was replaced by 100 μl dimethyl sulfoxide (DMSO; Beyotime, China). The absorbance at 490 nm was detected with a microplate reader (Bio-Gene Technology, Guangzhou, China).

Colony formation assay

Long-term cell proliferation was detected by colony formation assay. In detail, approximately 1,000 SMMC-7721 and Huh-7 cells were transfected with corresponding plasmids or plus treated with nobiletin (30 and 50 μM) and added to 6-well plates. Dishes were taken out after being cultured for 14 days and then fixed with 4% paraformaldehyde for 40 min. The numbers of cell clones were counted after staining with crystal violet for 2 h.

Transwell assay

The migration and invasion abilities of SMMC-7721 and Huh-7 cells were evaluated using the Transwell system (Corning, USA) according to the manufacturer's instructions. In brief, cells were trypsinized, counted, and resuspended in serum-free media. Subsequently, both cell lines were cultured in serum-free DMEM without/with nobiletin (0, 30, and 50 μM) for 48 h at 37 °C or transfected HCC cells were cultured in serum-free DMEM with 40 μM nobiletin for 48 h at 37 °C with 5% CO_2 in an incubator. Both SMMC-7721 and Huh-7 cells (5×10^4) resuspended in serum-free media were seeded into the upper chamber. The lower chambers were filled with 600 μl media containing 10% FBS. After 24 h of incubation at 37 °C, cells on the bottom surface of the membrane were fixed and stained with 0.1% crystal violet dye. Subsequently, the cells on the upper surface of the inserts were removed by scraping with a cotton swab. For cell invasion, both HCC cells suspended in the serum-free medium were added into Matrigel (BD Biosciences, USA)-coated upper chambers. Cell migration and invasion were assessed by counting the number of penetrated cells in five random fields.

Western blotting

Both SMMC-7721 and Huh-7 cells were treated with nobiletin (0, 30, and 50 μM) for 48 h at 37 °C, then total protein was extracted from cells using RIPA lysis buffer (Beyotime) containing Complete Mini Protease Inhibitor Cocktail and Phosphostop (Roche, Germany). An equal amount of protein samples was separated on a 10% polyacrylamide gel, and then transferred onto a polyvinylidene fluoride membrane (Millipore, Germany). After blocking with 5% skim milk for 1 h at 37 °C, the membrane was incubated with indicated 1:1,000 primary antibodies (anti-UBE2C, AB-

clonal, China; anti-E-cadherin and anti-N-cadherin, Abcam, USA; anti-GAPDH, Proteintech, USA) at 4 °C overnight followed by incubation with a goat anti-rabbit/anti-mouse-peroxidase-conjugated second antibody (1:8,000, Abcam, UK). The signals of the membrane were visualized using an enhanced chemiluminescent detection reagent (Rockford, USA).

Tumor xenografts

A total of 10 BALB/c nude mice (male, 4-week-old) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China). Huh-7 cells (5×10^6 cells/mice) were inoculated into mice on the flanks via subcutaneous injection. After the tumor volume reached 100 mm^3, mice were randomly assigned to two groups ($n = 5$ mice/group), including the vehicle group and the nobiletin treatment (NOB) group. The mice in the nobiletin group were injected intraperitoneally with a dose of nobiletin at 40 mg/kg/day. Mice in the vehicle group were injected intraperitoneally with an equal volume of PBS. Body weight and tumor volume were detected every 3 days, and tumor volume was calculated as: length (mm) × width^2 (mm^2)/2. After cell injection for 4 weeks, mice were killed via cervical dislocation. Tumor samples were weighed and harvested to examine the expression of UBE2C. The protocol was approved by the Laboratory Animal Welfare and Ethics Committee of Zhejiang Chinese Medical University (Approval No. 202104-0155).

Statistical analysis

All experiments were repeated at least three times, and all data are shown as mean ± standard deviation (SD). The Student's *t*-test was utilized to evaluate the comparisons in two groups, and a one-way analysis of variance (ANOVA) followed by Tukey's test was used to assess the differences in multiple groups. The differences were considered statistically significant at $p < 0.05$.

RESULTS

Nobiletin inhibits the viability and proliferation abilities of HCC cells

To explore the cytotoxicity effect of nobiletin on HCC cells *in vitro*, both SMMC-7721 and Huh-7 cells were treated with nobiletin at different concentrations (0 to 100 μM) for 48 h. MTT assay showed that nobiletin reduced the viability of both cells in a dose-dependent manner (Fig. 1A,B), and IC_{50} of both cells was respectively 39.60 μM and 41.63 μM. In addition, the effect of nobiletin (0, 30, 50 μM) on the proliferation ability of both HCC cells was performed by *in vitro* clonogenic assay. As expected, treatment with nobiletin significantly suppressed the relative colony number of both HCC cells compared with the untreated group (0 μM nobiletin) (Fig. 1C–E). Taken together, nobiletin treatment inhibited cell viability and proliferation in both HCC cells.

Nobiletin suppresses the invasion and migration of HCC cells

To further assess whether nobiletin exhibited anti-migration and anti-invasion effects on HCC cells *in vitro*, both SMMC-7721 and Huh-7 cells were treated with nobiletin at the dose of 30 and 50 μM. As the incubation time with nobiletin increased, the viabilities of both cells decreased, and the inhibitory effect became more significant with the increase in treatment time ($p < 0.05$, Fig. S1). Based on these results, both HCC cells treated with 30 and 50 μM nobiletin for 48 hours were suitable for subsequent tests. As shown in Fig. 2A,B, the detection results of the Transwell assay showed that the number of migrated cells was reduced after treatment with nobiletin. Similarly, nobiletin treatment significantly downregulated the invasion capability of both HCC cells in comparison to the untreated group (Fig. 2C,D). Of note, 50 μM nobiletin showed a stronger inhibitory effect on the migration and invasion of HCC cells (Fig. 2A–D). Moreover, Western blotting showed that nobiletin significantly promoted the protein level of E-cadherin in both cells (Fig. 2E,F), but reduced the protein level of N-cadherin. The above results suggested that nobiletin possessed an anti-aggressive pharmacological effect on HCC cells.

UBE2C is the critical therapeutic target for nobiletin and is associated with HCC progression

Previous studies have confirmed that UBE2C was aberrantly highly expressed in many malignancies and contributed to the occurrence and progression of malignant tumors [19]. Importantly, GEPIA database analysis results showed that UBE2C expression was significantly upregulated in HCC tissues compared to the normal liver tissues (Fig. 3A). Kaplan-Meier analysis demonstrated that HCC patients with high UBE2C expression exhibited a shorter time of overall survival (Fig. 3B) and disease-free survival (Fig. 3C) than those patients with low UBE2C expression. Moreover, the protein level of UBE2C in HCC cell lines (Huh-7 and SMMC-7721) was higher than that in human liver epithelial cells (THLE-3) (Fig. 3D). Furthermore, we have examined the effect of nobiletin on the tumor-associated genes that were highly expressed in HCC, including MELK, CCNB2, MYBL2, KIF20A, UBE2C, XTP8, MCM2, and TOP2A. The results showed that nobiletin treatment significantly reduced the expression of UBE2C (Fig. S2). Importantly, treatment with 40 μM nobiletin notably reduced the expression of UBE2C protein in both HCC cells compared with the untreated group (Fig. 3E). Collectively, UBE2C was associated with HCC progression and decreased in HCC cells treated with nobiletin.

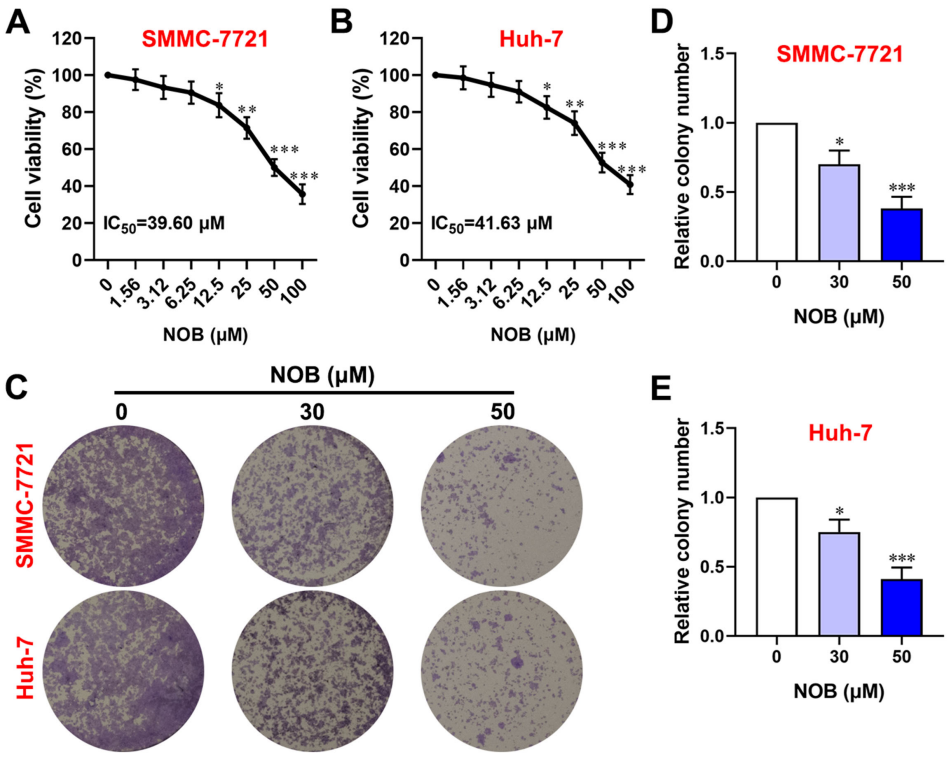

Fig. 1 Nobiletin inhibited the proliferation of HCC in a dose-dependent manner. A and B: Both SMMC-7721 and Huh-7 cells were treated with nobiletin at different concentrations for 48 h, MTT assay was performed to measure cell viability; C–E: Colony formation assay was used to examine the number of colonies in both cells treated with 0, 30, or 50 μM nobiletin for 48 h. Compared with the untreated group (0 μM nobiletin), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. NOB: Nobiletin.

Overexpression of UBE2C ameliorates the inhibitory effect of nobiletin on the malignant phenotype of HCC cells

To verify whether nobiletin showed antitumor effects on HCC progression via regulating UBE2C *in vitro*, we transfected with UBE2C overexpression (oe-UBE2C) plasmids or shRNA (sh-UBE2C) into both SMMC-7721 and Huh-7 cells. Western blotting confirmed that UBE2C was lowly expressed with the interference of UBE2C in both HCC cells (Fig. 4A,B), but its protein level was enhanced in the oe-UBE2C group in comparison with the Con group. MTT assay revealed that knockdown of UBE2C or nobiletin treatment significantly inhibited both HCC cell viability (Fig. 4C), while overexpression of UBE2C alone significantly promoted the proliferation of both SMMC-7721 and Huh-7 cells (Fig. 4D). Meanwhile, colony formation assay showed that UBE2C-silenced or nobiletin treatment reduced the relative number of clonogenic cells (Fig. 4E) compared with the Con group. However, overexpression of UBE2C significantly reversed the inhibitory effect of nobiletin on HCC cell proliferation. In addition, UBE2C-silenced or treatment of nobiletin notably suppressed the number of migrated (Fig. 4F) and invaded cells (Fig. 4G), whereas UBE2C-elevated facilitated the migration and invasion abilities of both HCC cells treated with nobiletin (Fig. 4F,G). Similarly, UBE2C overexpression alone promoted both HCC cell migration and invasion compared with the Con group (Fig. 4F,G). Furthermore, UBE2C overexpression alone enhanced the expression of N-cadherin protein and reduced E-cadherin protein expression (Fig. 4H,I), but nobiletin treatment reversed these changes. Taken together, nobiletin treatment inhibited the proliferation, migration, and invasion of HCC cells via suppression of UBE2C.

Nobiletin inhibits xenograft tumor growth of HCC *in vivo*

We investigated the therapeutic effect of nobiletin on the tumor growth of HCC by establishing a xenograft nude model. The results showed that administration of nobiletin at the dose of 40 mg/kg per day exhibited antitumor effects (Fig. 5A), as demonstrated in the reduction of tumor volume (Fig. 5B) and tumor weight (Fig. 5C). Moreover, the protein level of UBE2C was lower in the nobiletin group than that in the vehicle group (Fig. 5D). What's more, there was no significant

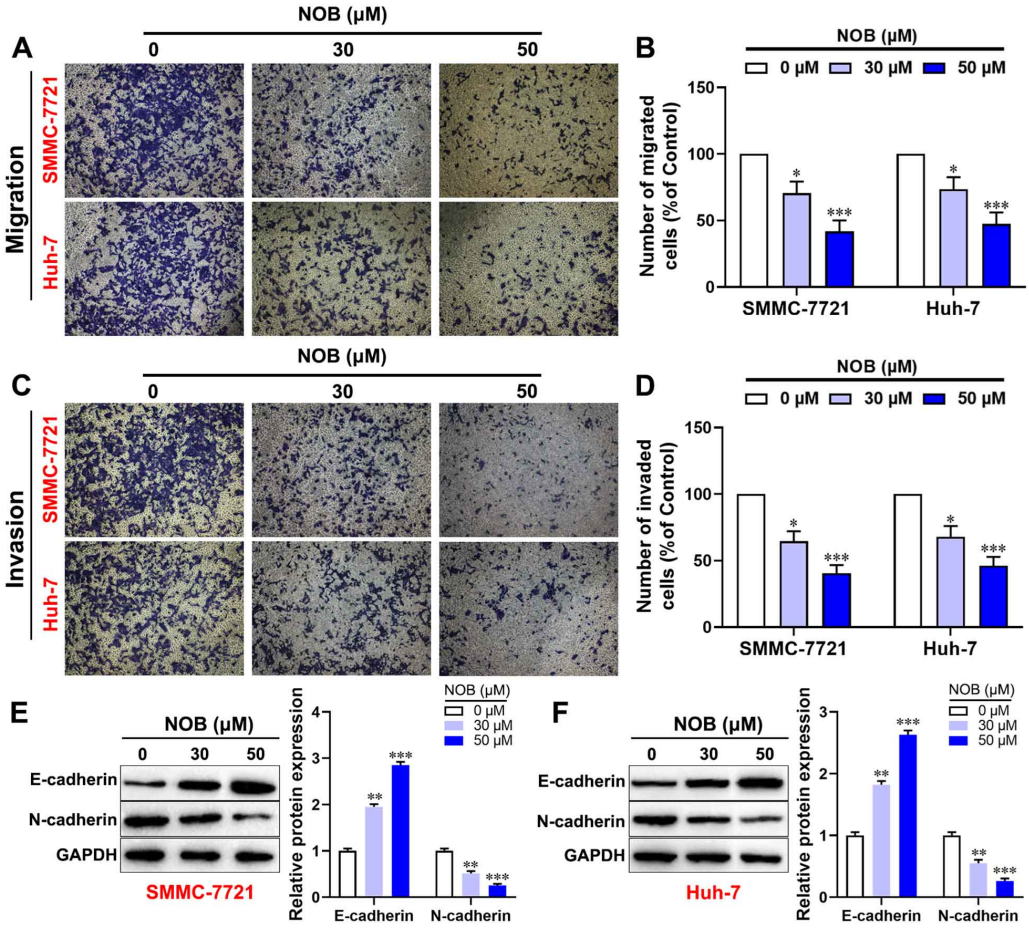

Fig. 2 Nobiletin suppressed the migration and invasion abilities of both SMMC-7721 and Huh-7 cells. A and B: The number of migratory cells was determined by Transwell assay; C and D: The number of invasive cells was examined by Transwell assay. E and F: The levels of E-cadherin and N-cadherin proteins were examined by Western blotting. Compared with the untreated group (0 μM nobiletin), * $p < 0.05$, *** $p < 0.001$. NOB: Nobiletin.

difference in body weight of nude mice with or without nobiletin administration (Fig. 5E). Collectively, nobiletin suppressed the tumor growth of HCC in the xenograft nude model.

DISCUSSION

HCC is a serious threat to human health and life with high morbidity and mortality [20]. At present, most of the commonly used antitumor chemotherapeutic drugs have many problems such as strong side effects, high prices, and poor targeting [21]. Most importantly, TCH cannot only improve various clinicopathological features of patients with malignant tumors, but also prolong the survival time of cancer patients [22]. Therefore, it is urgent to seek a safe, efficient, and low side effect TCH for HCC treatment. In the present study, our data showed that nobiletin treatment significantly inhibited HCC cell viability and proliferation in a dose-dependent manner, as well as suppressed the migration and invasion capacities of HCC cells. Similarly, ginkolic acid is a natural compound obtained from leaves and seed coats of *Ginkgo biloba* L., and it inhibits the proliferation and migration of HCC cells [23]. Moreover, nobiletin treatment reduced the expression of UBE2C protein in HCC cells, and high UBE2C expression showed a shorter survival time in HCC patients. Mechanistically, nobiletin exhibited antitumor effects on HCC via downregulation of UBE2C *in vitro* and *in vivo*.

Previous studies have confirmed that nobiletin acted as an ingredient of TCH and played an important role in effective cancer therapy [24]. Of note, nobiletin has been shown to act as an inducer of programmed cell death in cancer [10]. For example, nobiletin treatment inhibited migration, invasion, sphere formation, and angiogenesis of breast cancer cells [25].

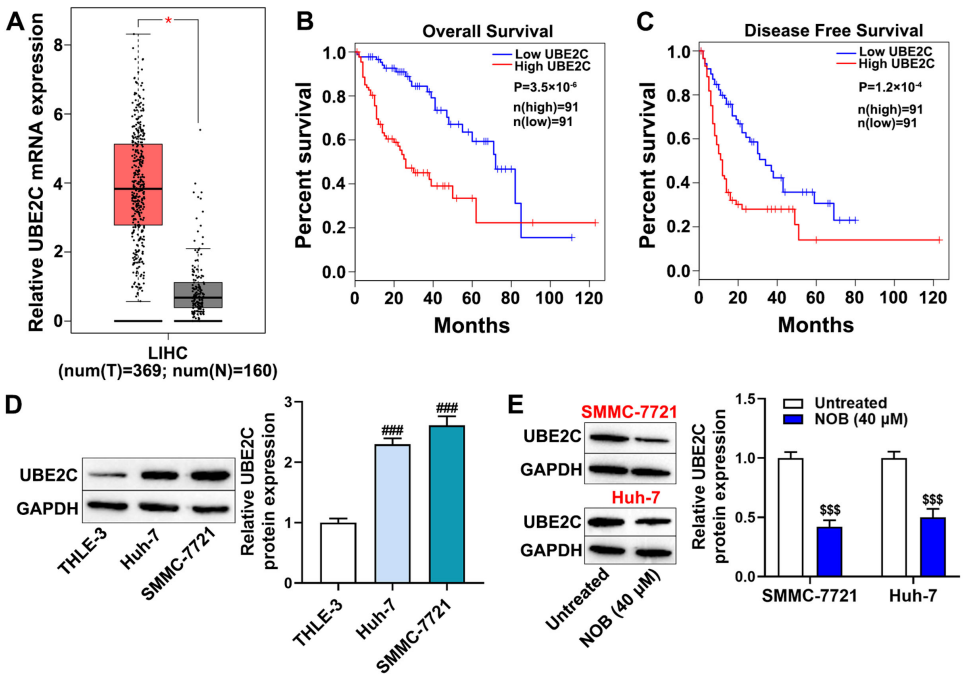

Fig. 3 UBE2C is the critical therapeutic target for nobiletin and is associated with HCC progression. A: The mRNA expression of UBE2C in HCC tissues and normal liver tissues was analyzed from the GEPIA database; B and C: Survival curves based on UBE2C expression level were constructed by Kaplan-Meier analysis; D: Western blotting was performed to examine the protein level of UBE2C in HCC cell lines and human liver epithelial cells (THLE-3); E: Both SMMC-7721 and Huh-7 cells were treated with 40 μM nobiletin for 48 h, the protein level of UBE2C was measured by Western blotting. Compared with the normal group, * $p < 0.05$; compared with the THLE-3 cells, $^{\#\#\#}$ $p < 0.001$; compared with the untreated group, $^{\$\$\$}$ $p < 0.001$. NOB: Nobiletin.

Functionally, nobiletin exhibited an antitumor effect on cancer cells via regulating tumor-related genes or several signaling pathways. For example, nobiletin inhibited cell proliferation by restricting protein kinase A/-cAMP response element binding protein-induced aerobic glycolysis in oral squamous cell carcinoma [26]. Wang et al [27] reported that nobiletin promoted the pyroptosis of breast cancer cells via regulation of the miR-200b/juxtaposed with another zinc finger gene 1 axis. In addition, increasing evidence has proved that nobiletin treatment contributed to enhancing the sensitivity of cancer cells to standard chemotherapy [28]. In the present study, our data showed that nobiletin suppressed HCC cell proliferation, migration, and invasion *in vitro*, and restricted tumor growth in a xenograft nude model of HCC *in vivo*. Similarly, the inhibitory effect of nobiletin on HCC *in vitro* and *in vivo* was also confirmed by the study of Ma et al [29] in 2014. However, the anticancer mechanism of nobiletin in HCC has been unknown yet.

Numerous studies have shown that UBE2C was overexpressed in many human tumors and served as a potential oncogene [30]. Upregulation of UBE2C facilitated the proliferation, migration, invasion, and epithelial-mesenchymal transition of cancer cells, as well as inhibited cancer cell apoptosis [31]. Yang et al [32] showed that DUB3 knockdown inhibited proliferation, migration, invasion, and angiogenesis of colorectal cancer cells, which functions in ubiquitination reversible process similarly to UBE2C. Additionally, UBE2C acted as a target gene of miRNA and was involved in cancer progression. For instance, miR-300 targets UBE2C to repress the malignant biological behavior of gastric cancer cells [33]. Chen et al [34] proved that miR-525-5p overexpression inhibited metastasis and overcame anoikis resistance by targeting UBE2C in cervical cancer cells. Most importantly, UBE2C acted as a potential oncogene in HCC by promoting cancer cell proliferation, invasion, and drug resistance [16], and served as a therapeutic target gene of HCC [35]. In this study, we confirmed that UBE2C was upregulated in HCC tissues and cell lines, and high UBE2C expression was positively associated with a shorter survival time in HCC patients. Moreover, nobiletin treatment could reduce UBE2C expression in HCC cells, and overexpression of UBE2C reversed the inhibitory effect of nobiletin on the malignant phenotype of HCC cells. Likewise, icaritin inhibited

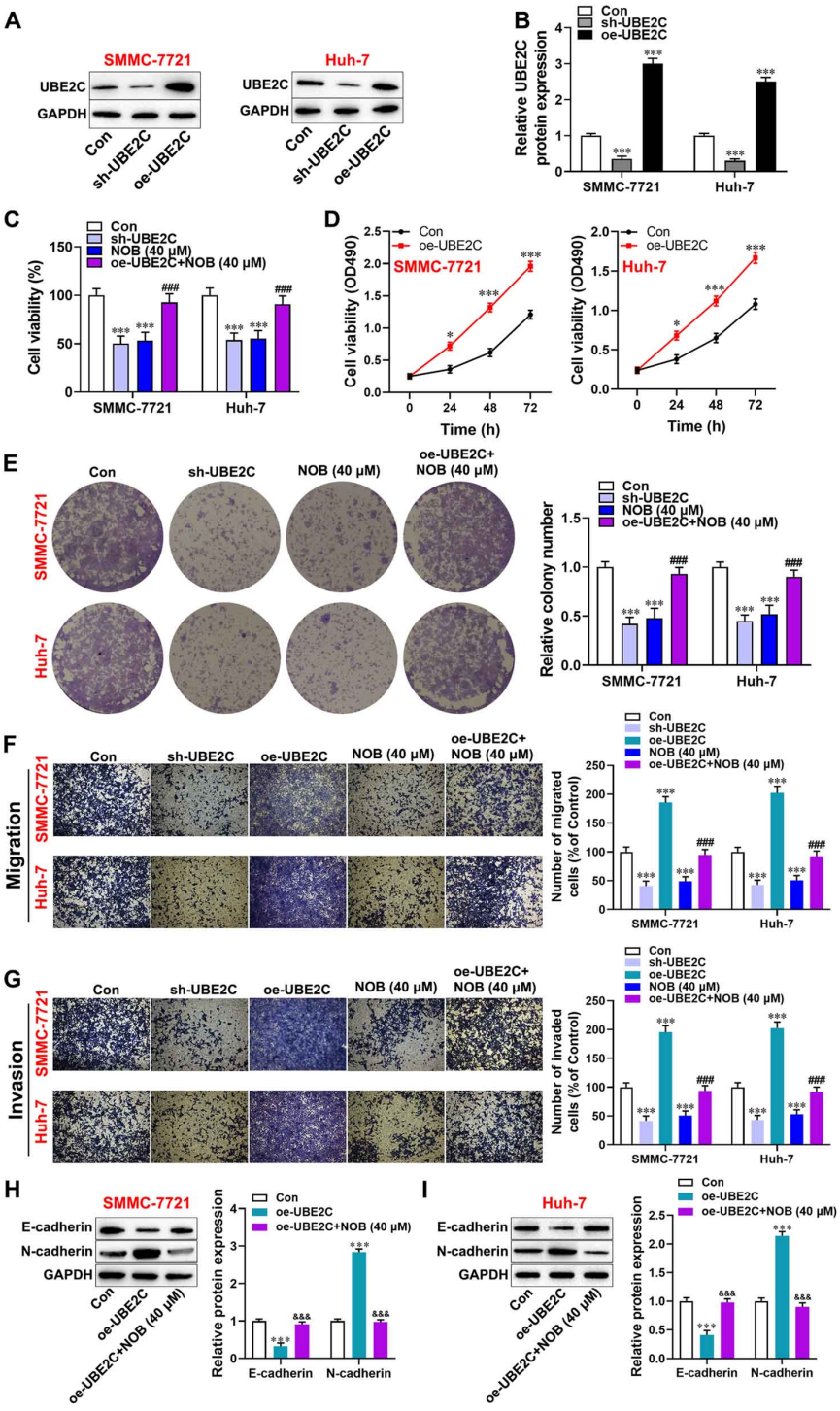

Fig. 4 Nobiletin inhibited the proliferation, migration, and invasion of HCC cells via reducing UBE2C expression *in vitro*. A and B: Both SMMC-7721 and Huh-7 cells were transfected with sh-UBE2C and oe-UBE2C, and Western blotting was used to examine the protein level of UBE2C; C and D: MTT assay was performed to assess cell viability; E: Colony formation assay was employed to examine cell proliferation; F and G: Transwell assay was utilized to measure the migration and invasion abilities of HCC cells; H and I: The expression of E-cadherin and N-cadherin protein was examined by Western blotting. Compared with the Con group, *** $p < 0.001$; compared with the NOB group, $^{\#\#\#}$ $p < 0.001$; compared with the oe-UBE2C, $^{\&\&\&}$ $p < 0.001$. NOB: Nobiletin.

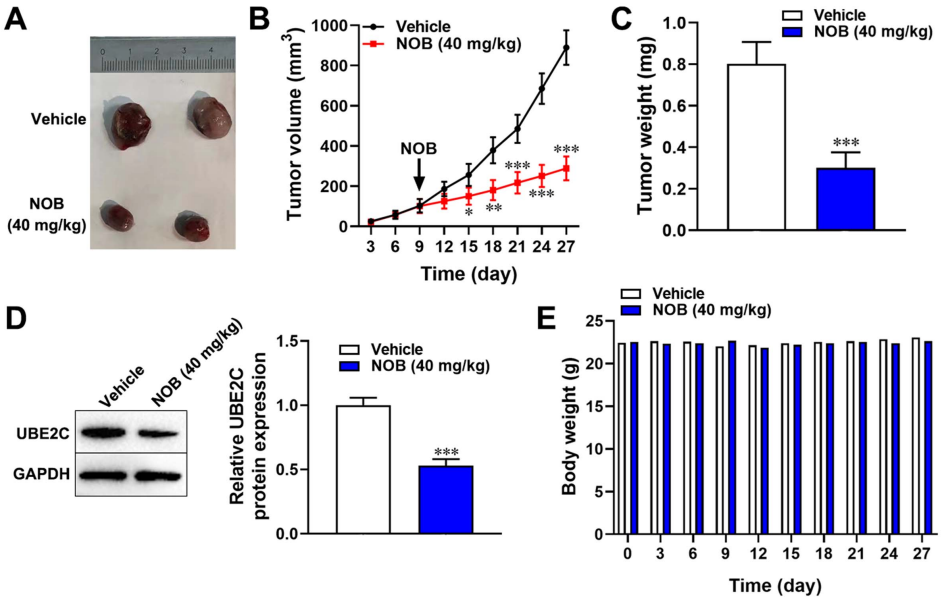

Fig. 5 Nobiletin administration inhibited tumor growth in xenograft nude model of HCC. A: Representative image of xenograft tumors in each group; B: Tumor volume was measured every three days; C: Tumor weight was examined; D: The protein level of UBE2C in tumor tissues was detected by Western blotting; E: Body weight was measured every three days. Compared with the vehicle group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. NOB: Nobiletin.

the proliferation and invasion of prostate cancer cells via reducing UBE2C expression, as well as hampered xenograft tumor growth *in vivo* [36]. Moreover, UBE2C affected the malignant biological behavior of cancer cells via the mediation of tumor-related signaling pathways, such as ERK [37], Akt/mTOR [38], and Wnt/β-catenin [39]. However, it is still unknown whether nobiletin exerts an antitumor effect on HCC by modulating the downstream signaling pathway of UBE2C. Certainly, these are the focus of our further research work.

CONCLUSION

In summary, nobiletin notably suppressed the proliferation, migration, and invasion abilities of HCC cells *in vitro*, and inhibited tumor growth in a xenograft mice model of HCC *in vivo*. Mechanistically, nobiletin possessed antitumor activity in HCC by downregulating oncogene UBE2C expression. Moreover, knockdown of UBE2C significantly inhibited the proliferation, migration, and invasion of HCC cells, and overexpression of UBE2C reversed the inhibitory effect of nobiletin on HCC. Our finding indicates that nobiletin might be a promising therapeutic agent for HCC treatment through inhibition of proliferation and aggressive ability.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2024.088.

REFERENCES

1. Brown ZJ, Tsilimigras DI, Ruff SM, Mohseni A, Kamel IR, Cloyd JM, Pawlik TM (2023) Management of hepatocellular carcinoma: A review. *JAMA Surg* **158**, 410–420.
2. Rumgay H, Ferlay J, de Martel C, Georges D, Ibrahim AS, Zheng R, Wei W, Lemmens VEPP, et al (2022) Global, regional and national burden of primary liver cancer by subtype. *Eur J Cancer* **161**, 108–118.
3. Choi S, Kim BK, Yon DK, Lee SW, Lee HG, Chang HH, Park S, Koyanagi A, et al (2023) Global burden of primary liver cancer and its association with underlying aetiologies, sociodemographic status, and sex differences from 1990–2019: A DALY-based analysis of the Global Burden of Disease 2019 study. *Clin Mol Hepatol* **29**, 433–452.
4. Vogel A, Meyer T, Sapisochin G, Salem R, Saborowski A (2022) Hepatocellular carcinoma. *Lancet* **400**, 1345–1362.
5. Liu S, Shao R, Bu X, Xu Y, Shi M (2021) Identification of the pyroptosis-related gene signature for overall survival prediction in patients with hepatocellular carcinoma. *Front Cell Dev Biol* **9**, 742994.
6. Xu S, Lai R, Zhao Q, Zhao P, Zhao R, Guo Z (2021) Correlation between immune-related adverse events and prognosis in hepatocellular carcinoma patients treated with immune checkpoint inhibitors. *Front Immunol* **12**, 794099.
7. Wang Y, Zhang Q, Chen Y, Liang CL, Liu H, Qiu F, Dai Z (2020) Antitumor effects of immunity-enhancing traditional Chinese medicine. *Biomed Pharmacother* **121**, 109570.
8. Yang J, Yang Y, Wang L, Jin Q, Pan M (2020) Nobiletin selectively inhibits oral cancer cell growth by promoting apoptosis and DNA damage *in vitro*. *Oral Surg Oral Med*

Oral Pathol Oral Radiol **130**, 419–427.

9. Singh B, Singh JP, Kaur A, Singh N (2020) Phenolic composition, antioxidant potential and health benefits of citrus peel. *Food Res Int* **132**, 109114.
10. Huang J, Chang Z, Lu Q, Chen X, Najafi M (2022) Nobiletin as an inducer of programmed cell death in cancer: A review. *Apoptosis* **27**, 297–310.
11. Feng S, Zhou H, Wu D, Zheng D, Qu B, Liu R, Zhang C, Li Z, et al (2020) Nobiletin and its derivatives overcome multidrug resistance (MDR) in cancer: total synthesis and discovery of potent MDR reversal agents. *Acta Pharm Sin B* **10**, 327–343.
12. Ashrafizadeh M, Zarrabi A, Saberifar S, Hashemi F, Hushmandi K, Hashemi F, Moghadam ER, Mohammadinejad R, et al (2020) Nobiletin in cancer therapy: how this plant derived-natural compound targets various oncogene and onco-suppressor pathways. *Biomedicines* **8**, 110.
13. Townsley FM, Aristarkhov A, Beck S, Hershko A, Ruderman JV (1997) Dominant-negative cyclin-selective ubiquitin carrier protein E2-C/UbcH10 blocks cells in metaphase. *Proc Natl Acad Sci USA* **94**, 2362–2367.
14. Wang Y, Shi F, Tao R, Wu J, Gu J, Yang R, Wu S (2021) The relationship between UBE2C and AGGF1 overexpression and tumor angiogenesis in non-small cell lung cancer. *Cancer Manag Res* **13**, 5919–5930.
15. Liu PF, Chen CF, Shu CW, Chang HM, Lee CH, Liou HH, Ger LP, Chen C-L, et al (2020) UBE2C is a potential biomarker for tumorigenesis and prognosis in tongue squamous cell carcinoma. *Diagnostics (Basel)* **10**, 674.
16. Xiong Y, Lu J, Fang Q, Lu Y, Xie C, Wu H, Yin Z (2019) UBE2C functions as a potential oncogene by enhancing cell proliferation, migration, invasion, and drug resistance in hepatocellular carcinoma cells. *Biosci Rep* **39**, BSR20182384.
17. Li R, Pang XF, Huang ZG, Yang LH, Peng ZG, Ma J, He RQ (2021) Overexpression of UBE2C in esophageal squamous cell carcinoma tissues and molecular analysis. *BMC Cancer* **21**, 996.
18. Wang X, Yin L, Yang L, Zheng Y, Liu S, Yang J, Cui H, Wang H (2019) Silencing ubiquitin-conjugating enzyme 2C inhibits proliferation and epithelial-mesenchymal transition in pancreatic ductal adenocarcinoma. *FEBS J* **286**, 4889–4909.
19. Xie C, Powell C, Yao M, Wu J, Dong Q (2014) Ubiquitin-conjugating enzyme E2C: a potential cancer biomarker. *Int J Biochem Cell Biol* **47**, 113–117.
20. Carr J, Cha DE, Shaltiel T, Zheng S, Siderides C, Golas B, Labow D, Magge D, et al (2022) African immigrants in New York City with hepatitis B-related hepatocellular carcinoma demonstrate high morbidity and mortality. *J Immigr Minor Health* **24**, 327–333.
21. Kumar B, Singh S, Skvortsova I, Kumar V (2017) Promising targets in anti-cancer drug development: recent updates. *Curr Med Chem* **24**, 4729–4752.
22. Wang K, Chen Q, Shao Y, Yin S, Liu C, Liu Y, Wang R, Wang T, et al (2021) Anticancer activities of TCM and their active components against tumor metastasis. *Biomed Pharmacother* **133**, 111044.
23. Chen JY, Wang R, Li Y, Li CH, Liu T, Xin Y, Li YL, Zhang D (2021) Ginkgolic acid inhibits proliferation and migration of human hepatocellular carcinoma cells by inducing G0/G1 cell cycle arrest. *ScienceAsia* **47**, 11–18.
24. Xiu LJ, Sun DZ, Jiao JP, Yan B, Qin ZF, Liu X, Wei PK, Yue X-Q (2015) Anticancer effects of traditional Chinese herbs with phlegm-eliminating properties - An overview. *J Ethnopharmacol* **172**, 155–161.
25. Sp N, Kang DY, Kim DH, Park JH, Lee HG, Kim HJ, Darvin P, Park Y-M, et al (2018) Nobiletin inhibits CD36-dependent tumor angiogenesis, migration, invasion, and sphere formation through the Cd36/Stat3/NF-κB signaling axis. *Nutrients* **10**, 772.
26. Lin CX, Tu CW, Ma YK, Ye PC, Shao X, Yang ZA, Fang YM (2020) Nobiletin inhibits cell growth through restraining aerobic glycolysis via PKA-CREB pathway in oral squamous cell carcinoma. *Food Sci Nutr* **8**, 3515–3524.
27. Wang JG, Jian WJ, Li Y, Zhang J (2021) Nobiletin promotes the pyroptosis of breast cancer via regulation of miR-200b/JAZF1 axis. *Kaohsiung J Med Sci* **37**, 572–582.
28. Feng SL, Tian Y, Huo S, Qu B, Liu RM, Xu P, Li YZ, Xie Y (2020) Nobiletin potentiates paclitaxel anticancer efficacy in A549/T xenograft model: Pharmacokinetic and pharmacological study. *Phytomedicine* **67**, 153141.
29. Ma X, Jin S, Zhang Y, Wan L, Zhao Y, Zhou L (2014) Inhibitory effects of nobiletin on hepatocellular carcinoma *in vitro* and *in vivo*. *Phytother Res* **28**, 560–567.
30. Dastsooz H, Cereda M, Donna D, Oliviero S (2019) A comprehensive bioinformatics analysis of UBE2C in cancers. *Int J Mol Sci* **20**, 2228.
31. Kim YJ, Lee G, Han J, Song K, Choi JS, Choi YL, Shin YK (2019) UBE2C overexpression aggravates patient outcome by promoting estrogen-dependent/independent cell proliferation in early hormone receptor-positive and HER2-negative breast cancer. *Front Oncol* **9**, 1574.
32. Yang DZ, Hui Y, Bai SK, Liang WJ, Hou JM, Jiang J (2022) DUB3 contributes to colorectal cancer cell migration and angiogenesis via NF-κB/HIF-1α. *ScienceAsia* **48**, 359–366.
33. Wang Y, Huang F, Liu M, Zhao Q (2021) UBE2C mRNA expression controlled by miR-300 and HuR determines its oncogenic role in gastric cancer. *Biochem Biophys Res Commun* **534**, 597–603.
34. Chen M, Liu LX (2020) MiR-525-5p repressed metastasis and anoikis resistance in cervical cancer via blocking UBE2C/ZEB1/2 signal axis. *Dig Dis Sci* **65**, 2442–2451.
35. Li C, Xu J (2020) Identification of potentially therapeutic target genes of hepatocellular carcinoma. *Int J Environ Res Public Health* **17**, 1053.
36. Hu J, Wu X, Yang C, Rashid K, Ma C, Hu M, Ding Q, Jiang H (2019) Anticancer effect of icaritin on prostate cancer via regulating miR-381-3p and its target gene UBE2C. *Cancer Med* **8**, 7833–7845.
37. Liu G, Zhao J, Pan B, Ma G, Liu L (2019) UBE2C overexpression in melanoma and its essential role in G2/M transition. *J Cancer* **10**, 2176–2184.
38. Lu ZN, Song J, Sun TH, Sun G (2021) UBE2C affects breast cancer proliferation through the AKT/mTOR signaling pathway. *Chin Med J (Engl)* **134**, 2465–2474.
39. Wang R, Song Y, Liu X, Wang Q, Wang Y, Li L, Kang C, Zhang Q (2017) UBE2C induces EMT through Wnt/β-catenin and PI3K/Akt signaling pathways by regulating phosphorylation levels of Aurora-A. *Int J Oncol* **50**, 1116–1126.

Appendix A. Supplementary data

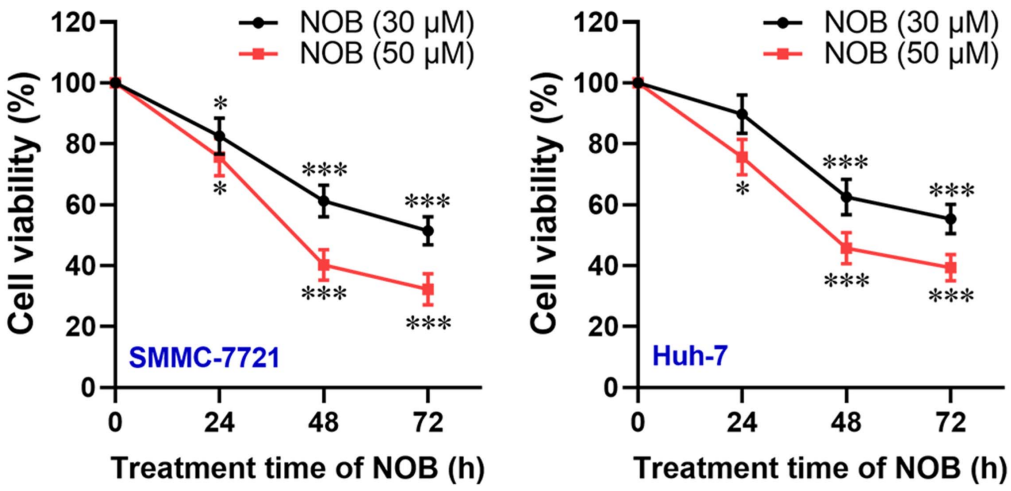

Fig. S1 The effect of nobiletin on the viability of HCC cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with 0 h.

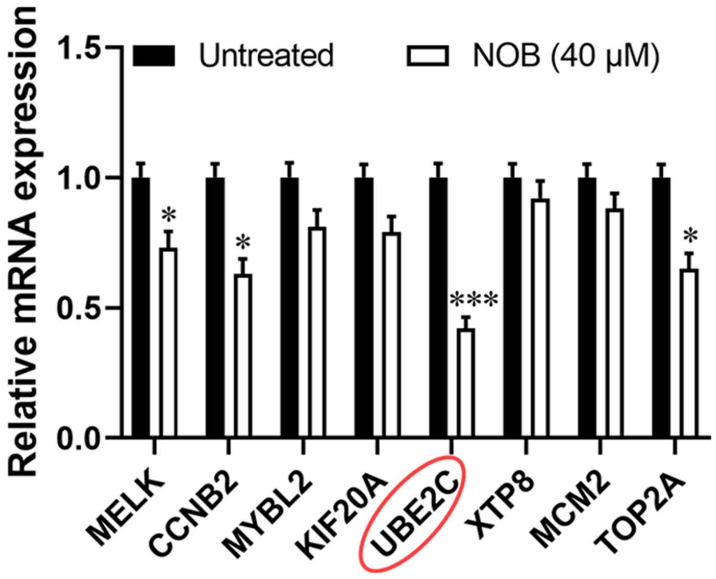

Fig. S2 The effect of nobiletin on tumor-associated genes related to HCC.