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Original Article

The Role of p-VEGFR2/p-AKT and HDAC1 in Apatinib-associated Inhibition of Gallbladder Cancer Cell Growth

Wei Huang[1], Yanmin Pang[2], Wenmei Zhao[1], Liang'e Xia[1], Luting Wang[3], Yingde Nong[1], Kai Xiao[4] and Yichong Ning[1*]

[1]*Chongzuo Key Laboratory of Biomedical Clinical Transformation, The People's Hospital of Chongzuo, Youjiang Medical University for Nationalities, Chongzuo, Guangxi, China;* [2]*Department of Hematology & Oncology, Affiliated Hospital of Youjiang Medical University for Nationalities, Baise, Guangxi, China;* [3]*Department of Hematology, Shenzhen Longhua District Central Hospital, Shenzhen, Guangdong, China;* [4]*Department of Neurosurgery, The Second Xiangya Hospital of Central South University, Changsha, Hunan, China*

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Abstract

Background and objectives: Apatinib has been shown to be efficacious in the treatment of gallbladder cancer. However, the underlying mechanisms remain unclear. This study aimed to explore pathways related to the antitumor effects of apatinib at the cellular level in gallbladder cancer.

Methods: NOZ and GBC-SD gallbladder cancer cells were treated with apatinib at concentrations of 0 μM, 10 μM, or 20 μM. The effect of apatinib on the proliferation of these cells was assessed using MTT and colony formation assays, and the effects of apatinib on cell cycle progression and DNA synthesis were evaluated using flow cytometry. Clinical cancer tissue samples, along with paired adjacent normal tissue samples, were obtained from 10 patients with gallbladder cancer. Immunohistochemistry, western blotting, and quantitative real-time polymerase chain reaction analyses were conducted to elucidate molecular changes induced by apatinib treatment.

Results: Treatment with 20 μM apatinib significantly inhibited the expression of phosphorylated (p)-vascular endothelial growth factor receptor 2 (VEGFR2), p-AKT, and histone deacetylase 1 (HDAC1). Additionally, apatinib treatment led to upregulated expression of p-cyclin-dependent kinase 1, p21, and Bax, and downregulated expression of cell division cycle 25B, B-cell lymphoma 2, Snail, and Slug. Apatinib decelerated DNA replication and induced cell cycle arrest at the G2/M phase, consequently suppressing the proliferation of gallbladder cancer cells.

Conclusions: Apatinib inhibits the proliferation of gallbladder cancer cells, and the mechanism involves VEGFR2/AKT, HDAC1, and downstream genes. These findings provide a basis for further investigation into the molecular mechanisms underlying the inhibitory effect of apatinib in gallbladder cancer.

Introduction

Gallbladder cancer, originating from the mucosal epithelial cells of the gallbladder, accounts for nearly 75% of biliary tract malignancies.[1] The standard chemotherapy regimen for gallbladder cancer is similar to that used to treat cholangiocarcinoma. Gemcitabine and cisplatin are used as first-line treatments, and FOLFOX is used as second-line therapy.[2] Most cases are already at an advanced stage at the time of initial diagnosis. Gallbladder cancer is characterized by rapid progression and strong invasiveness. Unfortunately, even patients who undergo surgical treatment have a high risk of recurrence and metastasis.[3] Strategies to improve the 5-year overall survival rate of individuals with advanced gallbladder cancer, as well as to improve their currently poor prognosis, are urgently needed.[4] Hence, the development of novel treatment regimens, such as immunotherapy combined with antiangiogenic agents, is essential for the effective treatment of gallbladder cancer. However, the

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***Correspondence to:** Yichong Ning, Chongzuo Key Laboratory of Biomedical Clinical Transformation, The People's Hospital of Chongzuo, Youjiang Medical University for Nationalities, 6 Longxiashan East Road, Jiangzhou District, Chongzuo, Guangxi 532200, China. ORCID: https://orcid.org/0000-0003-0223-4487. Tel: +86-15116470629, E-mail: ningyc@ymun.edu.cn
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efficacy and safety of such combination therapies have not been fully studied.[5] Currently, radical resection is considered the only potentially curative treatment for gallbladder cancer. With advances in the development of molecular inhibitors, more targeted agents are being used in clinical practice. However, their overall therapeutic effect in bile duct cancers has remained unsatisfactory, and their use has not improved patient survival.[6] Therefore, it is very important to develop more effective treatment strategies.

Apatinib, also known as YN968D1, is a small-molecule tyrosine kinase inhibitor. In gastric cancer and cholangiocarcinoma, apatinib is known to inhibit vascular endothelial growth factor-induced cell proliferation, epithelial–mesenchymal transition, and tumor microvessel density by inhibiting vascular endothelial growth factor receptor 2 (VEGFR2) activity.[7-10] Additional research has demonstrated that apatinib is a small-molecule antitumor agent with multi-targeted and anti-angiogenic properties that inhibits not only vascular endothelial growth factor receptor but also platelet-derived growth factor receptor, c-Kit, and c-Src activity.[11] At present, apatinib is mainly used in the treatment of advanced gastric cancer and gastroesophageal junction adenocarcinoma. Phase II/III clinical trials of apatinib are currently underway for liver cancer,[12] breast cancer,[13] non-small cell lung cancer,[14] and various other types of cancer.[15] In these clinical trials, apatinib has shown antitumor effects against a variety of advanced solid tumors, as well as clinical efficacy and safety in gallbladder cancer treatment.[16] Patients treated with apatinib achieved partial remission within 2 months, and the treatment duration was extended to nearly 1 year. Given these favorable outcomes, the present study aimed to better understand the role and molecular mechanisms of apatinib in gallbladder cancer.

Histone deacetylases (HDACs) not only remove acetyl groups from histones and mediate chromatin remodeling but also deacetylate certain transcription factors (such as p53).[17] Class I HDACs exhibit the strongest HDAC activity, and among them, HDAC1 plays a pivotal role in regulating cell cycle progression.[18] Notably, HDAC1 expression is increased in gallbladder cancer.[19] Among the many target genes of HDAC1 that affect tumor growth, *Snail* and *Slug* have been identified as representative downstream targets.[20] Interestingly, *Snail* and *Slug* are also p53 target genes.[21] Therefore, HDAC1 may regulate the expression of *Snail* and *Slug* either directly or indirectly through its interaction with p53.

Apatinib has demonstrated clinical effectiveness in the treatment of advanced gallbladder cancer, but its underlying mechanisms have yet to be fully elucidated. Therefore, the principal aim of this study was to explore the pathways involved in the antitumor effects of apatinib at the cellular level in gallbladder cancer.

Materials and methods

Clinical tissues

Clinical tissue samples were obtained from 10 patients with gallbladder cancer recruited at The People's Hospital of Chongzuo. The samples included gallbladder cancer tissues (n = 10) and paracancerous tissues (n = 10). This study was approved by the Ethics Committee of The People's Hospital of Chongzuo, Project ID: KS202308(06). All research methods adhered to the Declaration of Helsinki. All patients provided informed consent for the use of their tissues. The clinicopathological information for the gallbladder cancer patients included in this study is presented in Table 1.

Drug preparation

Apatinib was provided by Jiangsu Hengrui Pharmaceuticals Co., Ltd. Afuresertib (GSK2110183) and SC79 were obtained from Selleck Chemicals (Houston, TX, USA). Stock solutions were prepared by dissolving the drugs in dimethyl sulfoxide (DMSO). The storage concentrations of apatinib, afuresertib (AFU), and SC79 were 50 mM, 10 mM, and 10 mM, respectively.

Cell cultures

The GBC-SD and NOZ human gallbladder cancer cell lines, the HGBEC epithelial cell line, and the HGC-27 and SMMC-7721 cell lines were acquired from Cellcook (Guangzhou, China). The cells were cultured in RPMI 1640 medium (Gibco, Cat# 11875) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS; Gibco-BRL, Waltham, MA, USA) and 1% penicillin/streptomycin (Gibco-BRL). The cells were cultured at 37 °C in a humidified environment containing 5% CO_2. The cells were expanded to the fifth passage at a ratio of 1:2 and used in the experiments. For cryopreservation, the cells were suspended in freezing medium (10% DMSO, 20% FBS, and 70% RPMI 1640) and stored in liquid nitrogen.

Cell proliferation assay

Cells were seeded into 96-well plates at 5×10^3 cells per well. Each treatment group was cultured for 5 days. MTT solution (5 mg/mL MTT in phosphate-buffered saline, stored at −20 °C) was added to each well at a 1:10 dilution. After incubation at 37 °C for 4 h, the resulting crystals were dissolved in 100 µL DMSO. The absorbance of each well, which is positively correlated with relative viability, was measured at 490 nm using an enzyme-linked immunosorbent assay reader. All experiments were conducted in three parallel replicates.

Apatinib sensitivity

NOZ and GBC-SD cells were seeded in 96-well plates and treated with increasing concentrations of apatinib for 48 h. The MTT assay was used to evaluate cell viability, and the half-maximal inhibitory concentration (IC_{50}) of apatinib was calculated using the following formula: $\lg IC_{50} = Xm - I\,[P - (3 - Pm - Pn)/4]$.

Colony formation assay

NOZ and GBC-SD cells were seeded into 6-well plates at a density of 500 cells per well. The cells were cultured in 2 mL of RPMI 1640 medium supplemented with 10% FBS. Some cultures were treated with apatinib at various concentrations for 14 days. Control groups exposed only to culture medium were also included. After the treatment period, the cells were stained with 0.5% crystal violet in 4% paraformaldehyde for 5 min, and the number of colonies in each well was counted.

Lentiviral transduction

Lentiviruses were purchased from GeneChem (Shanghai, China). The cells were infected with lentiviral particles at a multiplicity of

Table 1. Clinicopathological information of gallbladder cancer patients who provided tissue samples for this study

No.	Gender	Age (years)	Differentiation status	Ki-67 expression
1	Male	63	Moderate	40%, +
2	Female	61	Well	20%, +
3	Male	61	Poor	70%, +
4	Female	77	Moderate/poor	50%, +
5	Female	66	Well	30%, +
6	Male	33	Well	20%, +
7	Male	46	Poor	60%, +
8	Male	64	Well/Moderate	30%, +
9	Female	71	Moderate/Poor	40%, +
10	Male	55	Moderate	60%, +

infection of 20 for 8 h and then cultured in fresh medium for 72 h. At 72 h post-infection, the cells were selected with puromycin (2.0 μg/mL) for an additional 72 h. The infection efficiency was greater than 90% based on Western blot analysis.[21]

Cell cycle analysis

A FACScalibur flow cytometer (BD FACSVia) was used to analyze cell cycle distribution after apatinib treatment, according to a previously described method.[22]

DNA synthesis analysis

In total, 2 × 10^6 cells were seeded into 6-well plates. To prepare 2× EdU labeling medium, 2 μL of EdU solution was added to 998 μL of complete culture medium. The 2× EdU labeling medium was prewarmed and then mixed with an equal volume of the original culture medium to obtain a 1× EdU solution. The final concentration of EdU was 10 μM, and 300 μL of EdU-containing medium was added to each well. The cells were incubated for 2 h, after which the medium was removed. The cells were washed twice with 1× phosphate-buffered saline for 5 min each time. Then, 150 μL of 4% paraformaldehyde was added to each well and incubated at room temperature for 30 min. Finally, the fixative was removed. Tetramethylrhodamine azide (excitation 541 nm, emission 567 nm) was detected by flow cytometry.

Western blot analysis

Western blot analysis was performed as previously described.[22] The following antibodies were used: CDK1 (Cell Signaling Technology (CST), 77055C), p-CDK1 (CST, 9111), CDC25B (CST, 9525), Bax (CST, 2772), p21 (Abclonal, A1483), p-VEGFR2 (Abclonal, AP0382), VEGFR2 (Abclonal, A5609), AKT (Abclonal, A11016), p-AKT (S473) (Abclonal, AP0140), HDAC1 (Abclonal, A18304), Slug (Abclonal, A1057), Snail (Abclonal, A5544), β-actin (ACTB, Abclonal, AC026), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Abclonal, A19056), BCL-2 (Wanleibio, WL01556), goat anti-rabbit IgG-HRP conjugate (GAR, Abclonal, AS014), and GAM (Abclonal, AS003). All antibodies were diluted 1:1,000 in antibody diluent (D608502, Sangon Biotech, Shanghai, China).

RNA extraction and quantitative reverse-transcription polymerase chain reaction (qRT-PCR)

Total RNA was isolated from cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). qRT-PCR was performed using PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, Foster City, CA, USA). Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. 18S rRNA was used as the internal control for messenger RNA (mRNA) expression. All assays were performed in triplicate. The following primers were purchased from Sangon Biotech:

HDAC1 (5'-GAATCCGCATGACTCATAAT-3', 5'-GCTGTGGTACTTGGTCATCT-3'); p21 (5'-TGTCCGTCAGAACCCATGC-3', 5'-AAAGTCGAAGTTCCATCGCTC-3'); BCL-2 (5'-GGTGGGGT-CATGTGTGTGG-3', 5'-CGGTTCAGGTACTCAGTCATCC-3'); Bax (5'-CCCGAGAGGTCTTTTTCCGAG-3', 5'-CCAGCCCAT-GATGGTTCTGAT-3'); and 18S (5'-CGGCGACGAC-CCATTCGAAC-3', 5'-GAATCGAACCCTGATTCCCCGTC-3').

Immunohistochemistry

Immunohistochemistry was performed using a previously described method.[22] The HDAC1 antibody was purchased from Abclonal (A18304). Tissue sections were incubated in citrate buffer at 92–98 °C for 10 min and then cooled to room temperature for antigen retrieval. Samples were incubated with the primary antibody (1:50) at 4 °C for 12 h, followed by incubation with the secondary antibody (1:200) at 30 °C for 30 min.

Statistical analysis

Double-blind procedure was implemented to ensure that both the researchers conducting the experiments and those analyzing the data were unaware of group assignments,All experiments were repeated three times. Statistical analyses were performed using SPSS 26.0. Prior to analysis of variance (ANOVA), normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test) were assessed to ensure assumptions were met. One-way ANOVA was used to assess differences among groups, followed by Tukey's post hoc test for multiple comparisons. Data are presented as mean ± standard deviation. Statistical significance was defined by the *P*-value.

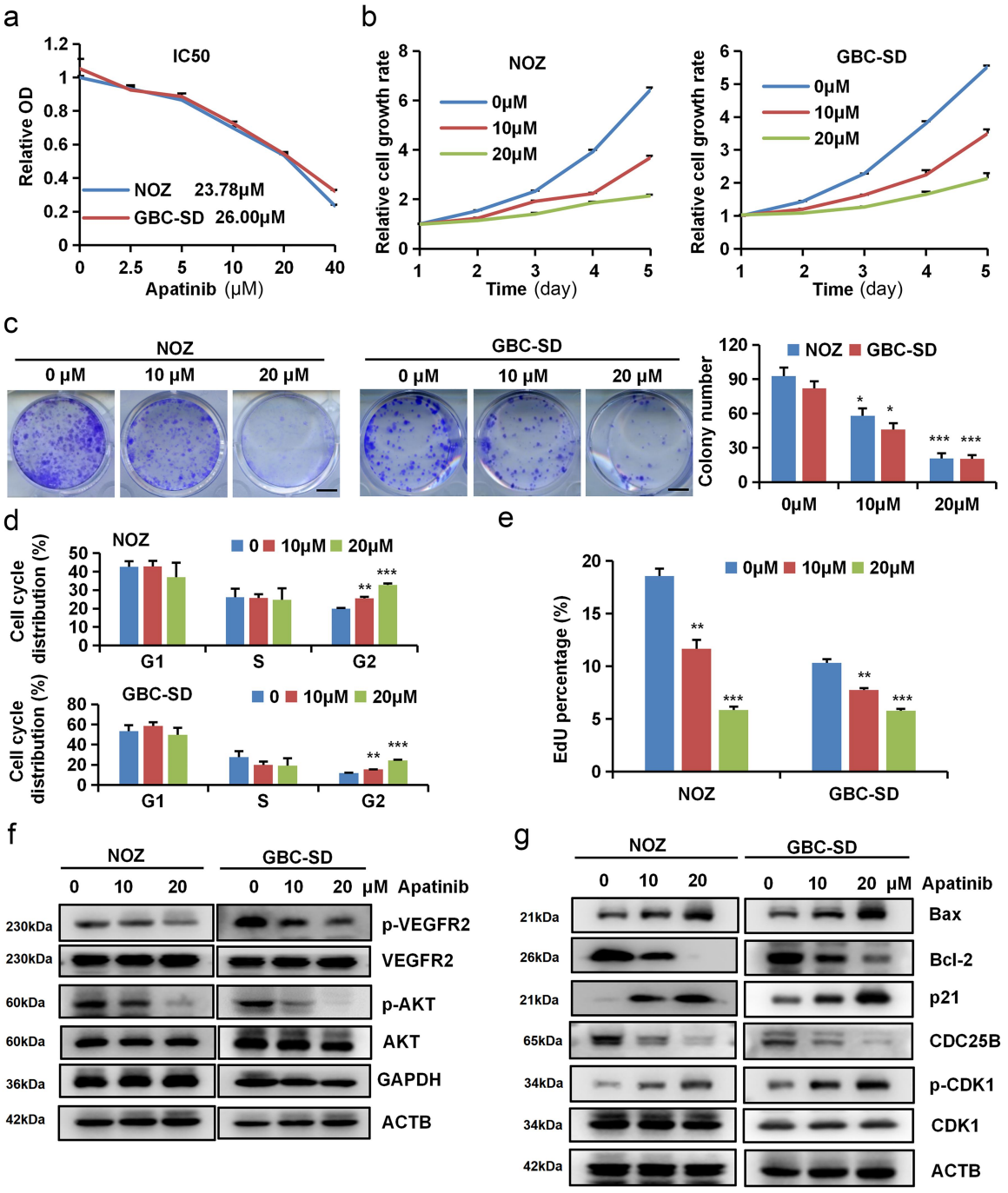

Fig. 1. Apatinib inhibits the proliferation of gallbladder cancer cells. (a) IC_{50} values of apatinib in NOZ and GBC-SD cells. (b) Cell viability based on MTT assay. (c) Representative images (left panel) and statistical analysis (right panel) of colony formation. Bar length: 1 cm. (d) Flow cytometric analysis of cell cycle distribution. (e) DNA synthesis represented by EdU staining (10 μM). (f–g) Western blot analysis of target protein expression. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. ACTB, β-actin; G1, first gap; G2, second gap; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IC_{50}, half maximal inhibitory concentration; OD, optical density; S, synthesis; WB, western blot.

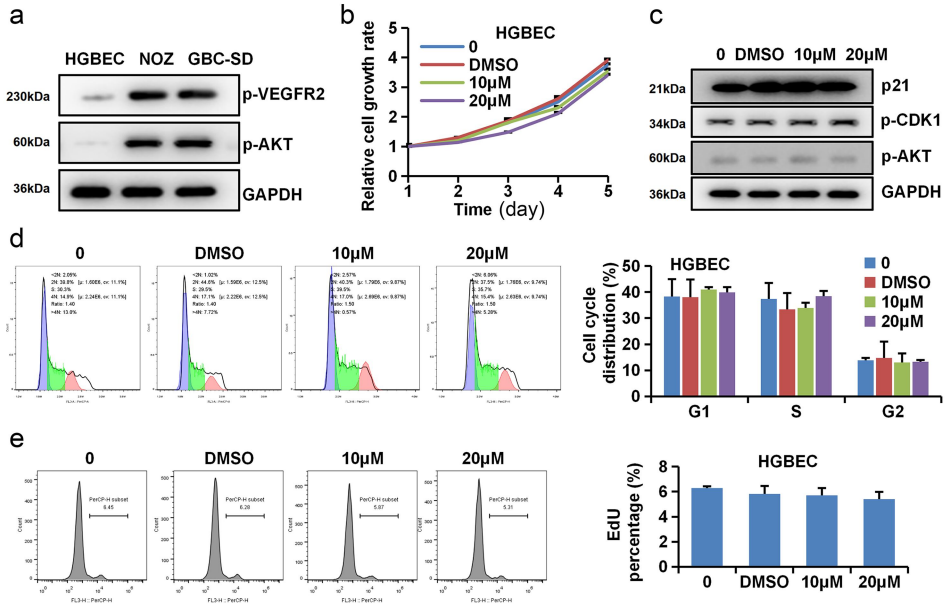

Fig. 2. Apatinib has limited effects on normal gallbladder epithelial HGBEC cells. (a) WB analysis showing p-AKT and p-VEGFR2 expression levels in HGBEC/NOZ/GBC-SD cells. (b) Cell viability was determined by MTT assay. (c) WB analysis of cell cycle checkpoint proteins. (d) Flow cytometry showing cell cycle distribution. (e) DNA synthesis was detected with EdU (10 μM). ACTB, β-actin; DMSO, dimethylsulfoxide; G1, first gap; G2, second gap; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; S, synthesis; WB, western blot.

Results

Apatinib inhibits the proliferation of gallbladder cancer cells

Tumor malignancy is characterized by uncontrolled cell proliferation, and treatment with apatinib has shown promise in improving progression-free survival in individuals with advanced or recurrent biliary tract cancer, including gallbladder cancer, who have undergone prior systemic treatment.[23] To investigate the mechanism underlying the effect of apatinib in gallbladder cancer, we used the poorly differentiated cancer cell lines GBC-SD and NOZ. In our experiment, the IC_{50} values for apatinib were 26.00 μM in GBC-SD cells and 23.78 μM in NOZ cells (Fig. 1a). Based on these IC_{50} values, we used concentrations of 10 μM and 20 μM apatinib in subsequent experiments to assess the drug's effects. MTT and colony formation assays demonstrated that apatinib significantly inhibited the proliferation of both GBC-SD and NOZ cells in a concentration-dependent manner (Fig. 1b and c).

Apoptosis is one of the main factors affecting cell proliferation, and it occurs when the cell cycle cannot progress past the checkpoint. Previous studies have reported that apatinib exerts antitumor effects in gastric cancer via VEGFR2–AKT signaling,[24] and thus we examined whether it also inhibits the activity of VEGFR2 and AKT in gallbladder cancer cells. Our results showed that the phosphorylation levels of VEGFR2 and AKT were decreased upon exposure of the cells to apatinib (Fig. 1f). Our findings in HGC-27/SMCC-7721/NOZ and GBC-SD cells treated with the same concentration of apatinib suggested that the inhibitory effect of apatinib on gallbladder cancer proliferation *in vitro* may be stronger than that on gastric cancer and liver cancer (Supplementary Fig. 1a and b). Importantly, apatinib did not significantly affect the viability of normal gallbladder epithelial HGBEC cells (Fig. 2a and b), indicating a degree of cancer cell selectivity. We also evaluated the inhibitory effects of the solvent DMSO on proliferation as well as the toxic effects of apatinib. The results showed that DMSO alone had no inhibitory effect on gallbladder cancer cells (Supplementary Fig. 1c and d).

Apatinib induces G2/M cell cycle arrest in gallbladder cancer cells

We found that apatinib inhibited the proliferation of gallbladder cancer cells. To further explore the mechanism of proliferation inhibition, we assessed its effects on cell cycle progression in these cells. Apatinib treatment induced G2/M phase arrest in both gallbladder cancer cell lines (Fig. 1d and Supplementary Fig. 1e), a finding distinct from its reported effects in some other cancers, suggesting context-specific mechanisms. It has been suggested that apatinib regulates other signaling pathways in gallbladder cancer. EdU incorporation assays revealed that apatinib significantly reduced the DNA synthesis rate in a concentration-dependent manner (Fig. 1e and Supplementary Fig. 1f). Western blot analysis of cell cycle regulatory proteins showed increased expression of p-CDK1, p21, and Bax, along with decreased expression of CDC25B and BCL-2 (Fig. 1g). These data suggest that apatinib inhibits gallbladder cancer cell proliferation by reducing DNA synthesis and inducing G2/M cell cycle arrest. These changes were not observed in HGBEC cells (Fig. 2c–e), indicating that apatinib's antiproliferative effect is not due to general cytotoxicity but rather to specific cell cycle modulation in cancer cells.

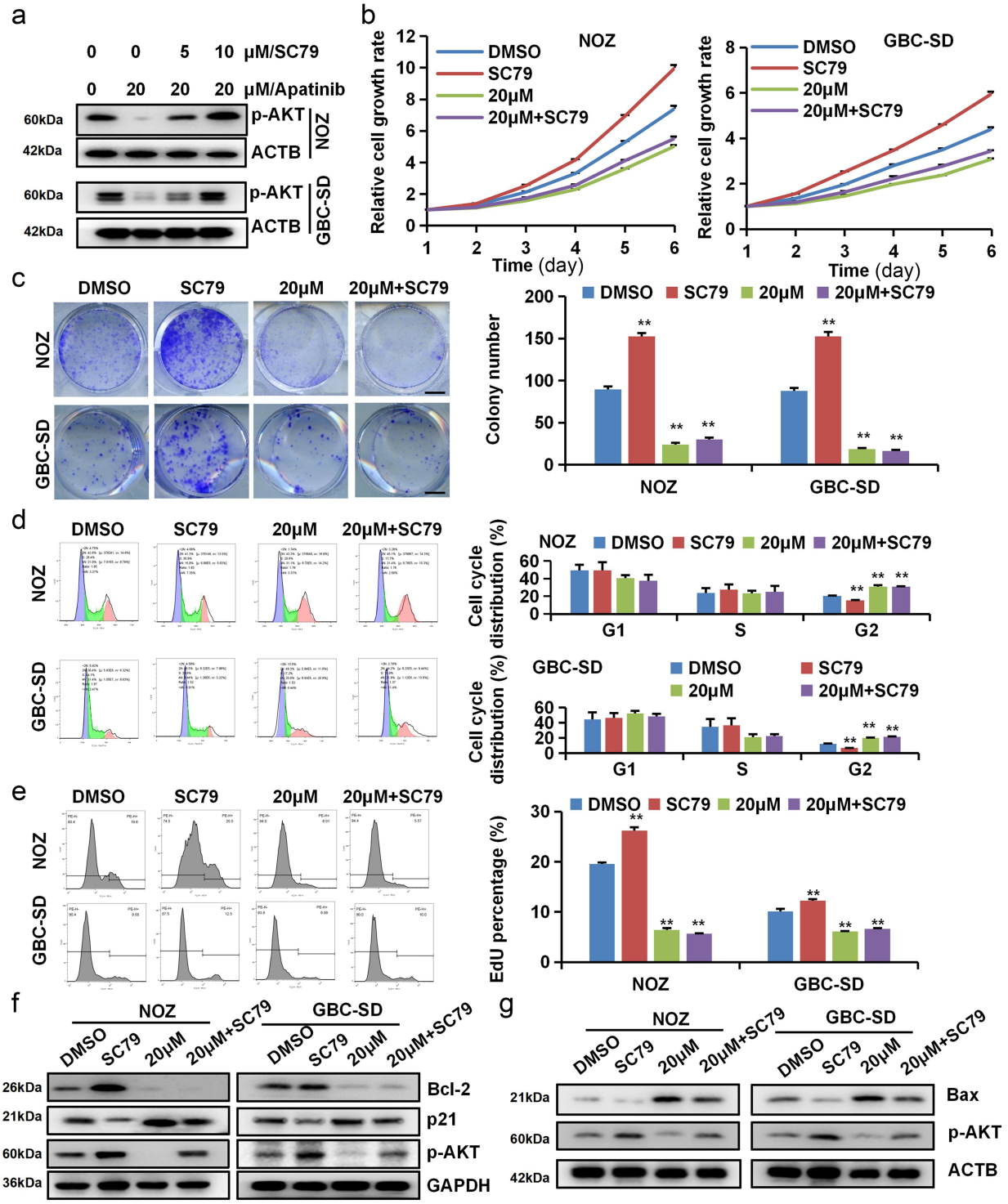

Fig. 3. Activation of p-AKT does not restore the inhibitory effect of apatinib on gallbladder cancer cells. (a) The optimal concentration of SC79 was analyzed using WB. (b–c) MTT and colony formation assays after treatment with apatinib (20 μM) or SC79 (10 μM) for 48 h. Bar length: 1 cm. (d) Flow cytometry showing cell cycle distribution. (e) DNA synthesis was detected by EdU. (f) WB analysis showing the expression levels of p-AKT, p21, BCL-2, and GAPDH. (g) WB analysis showing the expression levels of p-AKT, Bax, and ACTB. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. ACTB, β-actin; DMSO, dimethylsulfoxide; G1, first gap; G2, second gap; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; S, synthesis; WB, western blot.

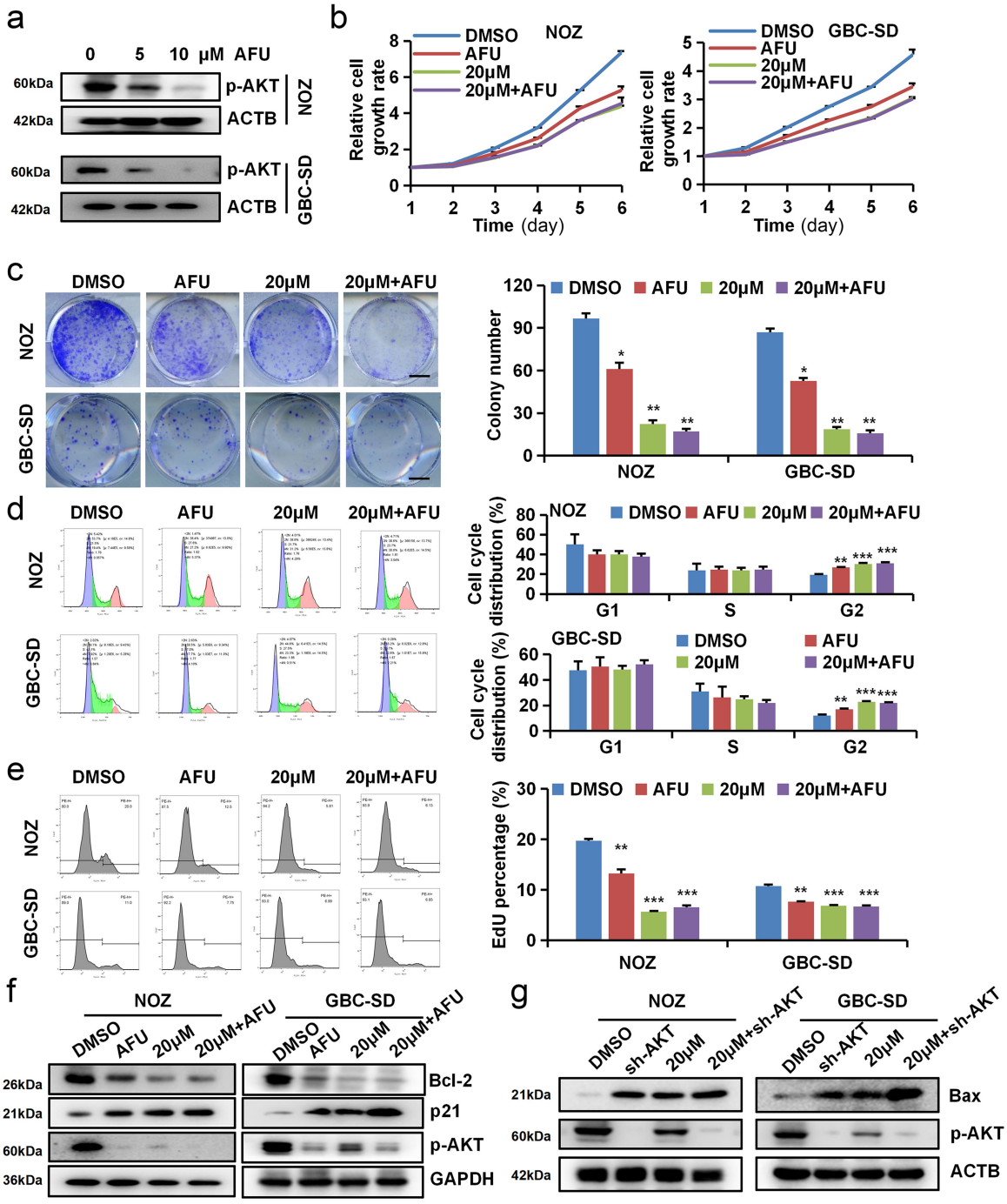

Fig. 4. p-AKT inhibition could not counteract the inhibitory effect of apatinib on gallbladder cancer cells. (a) The optimal concentration of AFU (10 μM) was analyzed by WB. (b–c) MTT and colony formation assays after treatment with apatinib (20 μM) or AFU (10 μM) for 48 h. Bar length: 1 cm. (d) Flow cytometry showing cell cycle distribution. (e) DNA synthesis was detected by EdU. (f) WB analysis showing the expression levels of p-AKT, p21, BCL-2, and GAPDH. (g) WB analysis showing the expression levels of p-AKT, Bax, and ACTB after interference with AKT. Compared with control: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. ACTB, β-actin; AFU, afuresertib; DMSO, dimethylsulfoxide; EdU, 5-ethynyl-2′-deoxyuridine; G1, first gap; G2, second gap; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; S, synthesis; WB, western blot.

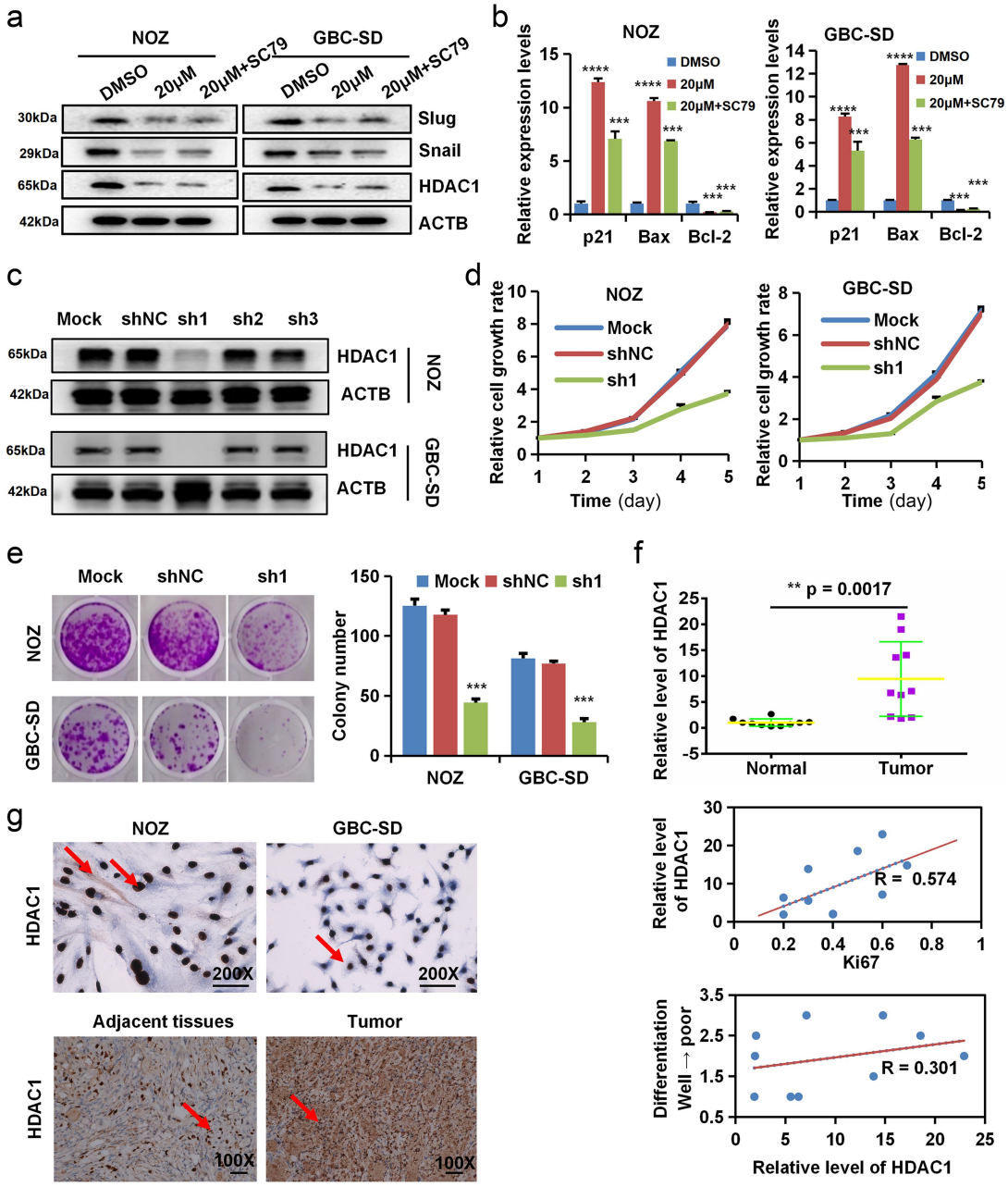

Fig. 5. Apatinib regulates downstream target genes through HDAC1. (a) WB analysis showing the expression levels of HDAC1 and tumor marker genes. (b) qRT-PCR showing transcriptional activity in NOZ and GBC-SD cells. (c) WB analysis of HDAC1 expression; sh1: shHDAC1-1, sh2: shHDAC1-2, sh3: shHDAC1-3. (d–e) MTT and colony formation assays after HDAC1 interference. (f) Detection of HDAC1 mRNA levels in clinical samples. Correlation analysis with Ki-67 and tumor differentiation degree. Well is defined as 1, and poor is defined as 3. (g) Immunochemical assay of cells and tissues with anti-human HDAC1 antibody. Bar length: 50 μm. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. ACTB, β-actin; DMSO, dimethylsulfoxide; HDAC1, histone deacetylase 1; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; WB, western blot.

AKT activation does not restore the inhibitory effect of apatinib in gallbladder cancer cells

To determine whether AKT inhibition mediates apatinib's effects, we treated the cells with the AKT activator SC79. Although SC79 restored p-AKT levels (Fig. 3a), it did not reverse apatinib's inhibitory effects on cell proliferation. These results showed that SC79 could not eliminate the impact of apatinib on gallbladder cancer cell proliferation (Fig. 3b–g). Hence, we concluded that apatinib plays an antitumor role in gallbladder cancer through molecular mechanisms other than the AKT signaling pathway.

p-AKT inhibition could not counteract apatinib's inhibitory effect on gallbladder cancer cells

To further understand the mechanism of apatinib's effects on gallbladder cancer cells, AFU, an AKT inhibitor, was used to treat gallbladder cancer cells. An optimal concentration of 10 μM AFU was determined (Fig. 4a) and used for subsequent combination experiments. NOZ and GBC-SD cells were categorized into DMSO, AFU, 20 μM apatinib, and 20 μM apatinib + AFU groups. Afterward, MTT, colony formation, cell cycle, and DNA synthesis assays were performed (Fig. 4b–e). AFU alone suppressed proliferation, but 20 μM apatinib alone exhibited stronger antitumor activity. Notably, combining AFU with apatinib did not enhance the effect beyond that of apatinib alone. The data indicated that the combination of apatinib and AFU did not have a better effect than 20 μM apatinib alone. The results also showed that apatinib not only inhibited AKT activity but also affected other genes. Finally, p-AKT, p21, and BCL-2 expression were detected by Western blot analysis (Fig. 4f). Although p-AKT was not completely inhibited by apatinib, downstream gene expression was significantly altered. Based on this, we used lentivirus to interfere with AKT expression, and Western blot analysis showed that p-AKT and Bax exhibited similar effects (Fig. 4g).

Apatinib regulates downstream target genes through HDAC1

Apatinib has a considerable impact on the rate of DNA synthesis. Numerous studies have reported that DNA synthesis rate is also closely associated with histone acetylation.[25] Furthermore, our Western blot analyses showed that treatment with 20 μM apatinib effectively reduced the protein expression levels of HDAC1, Snail, and Slug in gallbladder cancer cells, and the addition of SC79 could not restore their expression levels (Fig. 5a). Additionally, qRT-PCR was used to verify whether apatinib affects transcription in gallbladder cancer cells. Apatinib treatment resulted in reduced BCL-2 mRNA levels and increased Bax and p21 mRNA levels (Fig. 5b). To further study the role of HDAC1 in gallbladder cancer, we used lentivirus to knock down HDAC1 expression in NOZ and GBC-SD cells (Fig. 5c). MTT and colony formation assays indicated that HDAC1 knockdown inhibited gallbladder cancer cell proliferation (Fig. 5d and e). Consistent with a pro-tumorigenic role, HDAC1 mRNA levels were significantly higher in gallbladder cancer clinical specimens than in adjacent normal tissues. Furthermore, HDAC1 expression was positively correlated with both Ki-67 expression and tumor differentiation grade (Fig. 5f). We detected HDAC1 protein expression in NOZ and GBC-SD cells as well as clinical specimens by immunohistochemistry, which revealed high expression in gallbladder cancer cells and tissues (Fig. 5g). HDAC1 was mainly expressed in the nucleus of gallbladder cancer cells, with a small amount present in the cytoplasm. These findings highlight a crucial role for HDAC1, independent of the VEGFR2/AKT pathway, in mediating apatinib's therapeutic effects in gallbladder cancer by regulating downstream gene expression.

Discussion

Consistent with the established activity of apatinib in various advanced cancers, this study demonstrates that apatinib effectively inhibits gallbladder cancer cell proliferation *in vitro*.[26,27] The mechanisms of apatinib in gastric, liver, lung, and other cancers have been investigated previously. In particular, its mechanism of action in gastric cancer, especially in digestive tract tumors, has been thoroughly studied. Apatinib can suppress gastric cancer cell proliferation by inducing apoptosis and autophagy, which disrupts cell cycle progression and hinders cancer cell migration and invasion. In SGC7901 and MKN45 cells, the inhibitory effect of apatinib on gastric cancer cell proliferation is concentration dependent.[28] Although the clinical efficacy of apatinib for gallbladder cancer has been studied, and patients with advanced gallbladder cancer treated with apatinib have achieved longer progression-free survival, the safety and efficacy of apatinib in such cases remain incompletely understood.

Bax, p21, and BCL-2 are well-known target genes of p53,[21] and HDAC1 has been shown to regulate p53 acetylation in gallbladder cancer and to inhibit cancer cell proliferation.[29,30] The results of the present study revealed that apatinib exerts an antitumor effect via HDAC1; however, it remains to be elucidated whether HDAC1 directly or indirectly regulates downstream target genes through p53.

Limitations

This study was limited by the use of only two gallbladder cancer cell lines (NOZ and GBC-SD), and its findings may not be generalizable to other cell types or patient populations. The lack of *in vivo* validation restricts the translational relevance of the findings. Furthermore, the small number of clinical specimens collected (only 10 cases), and the fact that these patients did not receive apatinib treatment, precluded a more in-depth correlation analysis as well as integration of clinical therapeutic efficacy and prognostic outcomes. The study demonstrated only that HDAC1 expression is higher in gallbladder cancer tissues than in adjacent noncancerous tissues, and that HDAC1 expression is positively correlated with Ki-67 expression and tumor differentiation grade. Unfortunately, complete pathological staging data were not collected, precluding analysis of the correlation between HDAC1 expression and pathological stage.

Future directions

Considering the pivotal role of HDAC1, apatinib may exert its antitumor effects by modulating other molecular pathways via HDAC1, such as regulating ferroptosis and immune function. Further research is needed to bridge these gaps and achieve a more comprehensive understanding of the mechanism of action of apatinib in gallbladder cancer.

Conclusions

Apatinib inhibits the proliferation of gallbladder cancer cells, and the mechanism involves VEGFR2/AKT, HDAC1, and downstream genes. These findings provide insight into the molecular mechanisms underlying the inhibitory effects of apatinib on gallbladder cancer cell proliferation, and the reported data establish a theoretical foundation for the provide a basis for future mechanistic and *in vivo* studies.

Supporting information

Supplementary material for this article is available at https://doi.org/10.14218/ERHM.2025.00077.

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Conflict of interest

The authors declare that they have no conflicts of interest related to the contents of this article.

Ethical statement

This study was approved by the Ethics Committee of The People's Hospital of Chongzuo (Project ID: KS202308(06)) and conformed to the ethical guidelines of the Declaration of Helsinki (as revised in 2024). Written informed consent was obtained from each patient.

Author contributions

Study concept and design (WH, YcN), acquisition of data (YP, WZ), analysis and interpretation of data (WH, LW, YdN, YcN), drafting of the manuscript (YP, LW), critical revision of the manuscript for important intellectual content (KX, YcN), administrative, technical, or material support (WH), and study supervision (LX). All authors have made significant contributions to this study and have approved the final manuscript.

Data sharing statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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