

Baicalein suppresses the proliferation and invasiveness of colorectal cancer cells by inhibiting Snail-induced epithelial-mesenchymal transition

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Abstract. *Scutellaria baicalensis* (*S. baicalensis*) is a plant that is widely used for medicinal purposes. Baicalein, one of the primary bioactive compounds found in *S. baicalensis*, is thought to possess antitumor activity, although the specific mechanisms remain unclear. Therefore, the present study aimed to evaluate the ability of baicalein to disrupt the proliferation and metastatic potential of colorectal cancer (CRC) cells; a rapid and sensitive ultra-high performance liquid chromatography-tandem mass spectrometric method was employed for the identification of baicalein in an *S. baicalensis* aqueous extract and in rat plasma. To investigate the effects of baicalein, Cell Counting Kit-8 (CCK-8), western blotting, wound-healing and Transwell assays were performed. The data indicated that baicalein was absorbed into the blood and was able to effectively disrupt the proliferation, migration and invasion abilities of CRC cells in a dose- and time-dependent manner. Baicalein treatment was also revealed to decrease the expression of epithelial-mesenchymal transition (EMT)-promoting factors including vimentin, Twist1, and Snail, but to upregulate the expression of E-cadherin in CRC cells. The expression levels of cell cycle inhibitory proteins p53 and p21 also increased following baicalein treatment. In addition, Snail-induced vimentin and Twist1 upregulation, as well as E-cadherin downregulation, were reversed following treatment with baicalein. In conclusion, the results of the present study indicate that baicalein may suppress EMT, at least in part, by decreasing Snail activity.

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Introduction

Colorectal cancer (CRC) is the third most common cause of cancer-related deaths (1), and the incidence rates are rapidly increasing, with >1 million new cases and 694,000 mortalities each year worldwide (2). The current first-line treatment for CRC is a combination of radiotherapy and chemotherapy (3), where drugs such as cisplatin are frequently employed (4). However, such treatments have serious side effects and are often associated with drug resistance, resulting in the constant need to identify alternative treatment options with fewer side effects. This has led to increased interest in the use of natural products to treat CRC (5).

Epithelial-mesenchymal transition (EMT) is a key process in cancer metastasis, which is characterized by the decreased expression of cell-cell adhesion molecules such as E-cadherin, and the increased expression of mesenchymal proteins such as vimentin. During EMT, epithelial cells adopt a mesenchymal phenotype and exhibit increased migratory potential; this allows for increased invasiveness and resistance to apoptosis (6,7). Furthermore, EMT promotes cancer cell resistance to chemo- and radiotherapy (8). Snail is a member of the zinc-finger transcription factor family, and is reportedly one of the most important transcriptional regulators of EMT (9). Zheng *et al* (10), revealed that in CRC, Snail expression was significantly enhanced, which affected cancer progression. Additionally, Kwon *et al* (11) suggested that Snail may be a novel prognostic biomarker and therapeutic target in CRC. In fact; to an extent, the activation of Snail is considered to be the initiating factor for EMT in various malignant tumors.

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Abbreviations: UPLC-MS/MS, ultra-high performance liquid chromatography-tandem mass spectrometric; *S. baicalensis*, *Scutellaria baicalensis*; CCK-8, Cell Counting Kit-8; EMT, epithelial-mesenchymal transition; CRC, colorectal cancer; DMSO, dimethyl sulfoxide; OD, optical density; PBS, phosphate-buffered saline; FBS, fetal bovine serum

Key words: colorectal cancer, baicalein, Snail, epithelial-mesenchymal transition, p53, p21

Flavonoids are a diverse family of polyphenolic compounds derived from plant-based foods, including fruit, seeds, vegetables, herbs, tea and wine. Flavonoids have been extensively studied and their anticancer effects are well documented (12). Epidemiological studies have identified that the increased intake of dietary flavonoids is associated with a decreased risk of developing CRC (13). Previous studies also revealed that flavonoids suppressed the migration and invasion abilities (14), influenced cell cycle progression (15) and induced apoptosis in CRC cells (16-18). In Asia, *S. baicalensis*, whose constituents include numerous flavonoid compounds, is widely used for the treatment of hypertension (19), inflammation (20) and cancer (21), as well as bacterial and viral infectious diseases (22). Baicalein, which is currently one of the most representative flavonoid aglycones in *S. baicalensis*, has received considerable attention for its reported ability to suppress cellular proliferation and induce apoptosis (17,23-26). However, the specific mechanisms of these anti-metastatic properties remain unclear.

In the present study, baicalein was extracted and analyzed using ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), and its effect on cell proliferation, migration and invasion, and the expression of EMT markers was subsequently evaluated. Notably, Snail-induced EMT was partially blocked by baicalein treatment, which provides theoretical evidence for its use as a potential antitumor agent, and indicates a novel mechanism for its antitumor effects.

Materials and methods

Chemicals. High performance liquid chromatography (HPLC)-grade methanol and formic acid were obtained from Merck KGaA. Deionized water was prepared via Milli-Q water purification (EMD Millipore). Baicalein reference standards (cat. no. 18031608) were purchased from the Beijing Aoke Biological Technology Corporation, and stock solutions were prepared in dimethyl sulfoxide (DMSO) and stored at 4°C. Methyl p-hydroxybenzoate (internal standard; purity >98% by HPLC-UV) was obtained from Sigma-Aldrich (Merck KGaA).

UPLC/MS/MS instrument and conditions. In order to detect and analyze baicalein in rat plasma after oral administration of *S. baicalensis* extract, UPLC-MS/MS was conducted as previously described (27). An Acquity UPLC system (Waters Corporation) and an API 4000 Triple Quadrupole mass spectrometer (Shanghai AB SCIEX Analytical Instrument Trading Co.) equipped with an electrospray ionization (ESI) source were used as a part of this system, using Analyst 1.5 software (Applied Biosystems; Thermo Fisher Scientific, Inc.) for assay control. An Acquity UPLC HSS BEH C18 column (100 x 2.1 mm, 1.7 µm; Waters Corporation) maintained at 40°C was used for chromatographic separation, with a mobile phase of (A) 0.1% formic acid, and (B) methanol, with a gradient elution of 60-90% (A) for 0-6 min, 90% (A) for 6-7 min, 90-35% (A) for 7-10 min, and 35% (A) for 10-12 min. The flow rate of the mobile phase was 0.20 ml/min, with a 1-µl injection volume. The electrospray ionization source was performed in negative ionization mode with the following parameters: -4.5 kV ion spray voltage, 500°C turbo

spray temperature. For gas pressures, nebulizer, heater and curtain gas were set to 55, 50 and 25 psi, respectively, with a dwell time of 50 msec. Detection analysis was conducted in multiple reaction monitoring mode at the transition m/z [M-H]⁻ 269.2→195.0 for baicalein and 150.9→136.0 for IS, with collision energies of -35 eV and -19 eV, and cone voltages of -70 V and -56 V, respectively.

Preparation of *S. baicalensis* extract. To obtain an aqueous extraction of *S. baicalensis*, 100 g *S. baicalensis* roots were immersed in distilled water for 30 min with occasional stirring, and then boiled three times for 30 min each; plant:water ratios were maintained at 1:10. The three separate decoctions were combined and concentrated into a final volume of 100 ml to yield *S. baicalensis* extract, which was used for UPLC-MS/MS analysis. The raw materials were identified by Professor Minghua Qiu of Kunming Institute of Botany, Chinese Academy of Sciences (Kunming, China), where voucher specimens are retained.

Animal study. A total of six male Sprague-Dawley rats (weight, 250±20 g; age, 8 weeks) were provided by Kunming Medical University (Yunnan, China). The rats were housed under standard conditions (20±2°C with 60±5% humidity and 12-h light/dark cycles) with free access to food and water, and acclimated for 1 week. Animals were observed daily throughout the study. All rats were fasted, with free access to water, for 12 h prior to the experiment. The rats were then randomized into 2 groups (n=3 per group); the experimental group received *S. baicalensis* extract (4.5 g/kg) by intragastric administration once (28), and the control rats received distilled water only (10 ml/kg of body weight). At 45 min after oral administration, rats were then anesthetized using pentobarbital sodium at a dose of 50 mg/kg (i.p.), and blood samples (0.5 ml) were collected via retro-orbital bleeding. The anesthetic agent doses selected were based on existing literature (29). The sampling time-points were selected based on previous pharmacokinetic studies (27). Subsequently, the rats were sacrificed by cervical dislocation. Plasma was then separated from blood after centrifugation at 5,000 x g for 10 min at 4°C. The humane endpoint of this experiment was as follows: A marked reduction in food or water intake, labored breathing, inability to stand, and no response to external stimuli. No abnormal signs that signified the humane endpoints of the experiment were observed from any of the rats during the experiment. All animal procedures were approved and performed in compliance with the guidelines set by the Animal Care Committee of the First People's Hospital of Yunnan Province (30).

Sample preparation. A total of 10 µl IS (methyl p-hydroxybenzoate, 1 µg/ml in methanol) and 50 µl 0.2 M HCl were spiked into a 100-µl sample of rat plasma, mixed and allowed to rest for 10 min. Next, 800 µl ethyl acetate was added and mixed for 3 min, followed by centrifugation for 5 min at 5,000 x g (4°C). The supernatants were transferred to fresh tubes and evaporated using a nitrogen gas stream at room temperature. Any remaining residue was dissolved in 100 µl mobile phase, mixed for 1 min by vortexing and centrifuged at 15,000 x g for 5 min at 4°C. Finally, 1 µl supernatant was injected into the UPLC-MS/MS system for baicalein detection.

Cell culture. HT29 and DLD1 human colorectal cancer cell lines were obtained from Shanghai Cell Biological Institute of the Chinese Academy of Sciences, and cultured in RPMI-1640 media (Hyclone; GE Healthcare Life Sciences) containing 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin (Thermo Fisher Scientific, Inc.) at 37°C with 5% CO₂.

Cell Counting Kit-8 (CCK-8) assay. The CCK-8 assay (Beyotime Institute of Biotechnology) was used to assess the effects of baicalein on cancer cell viability. HT29 and DLD1 cells were seeded into a 96-well plate at a density of 2x10³ cells/well, and cultured until complete adherence. The cells were treated with baicalein at concentrations of 0, 20, 40, 60, 80, 100 and 120 μmol/l for 24, 48 and 72 h, using DMSO as a negative control. The media was then replaced with fresh media containing 10% CCK-8 solution. After a further 3 h of incubation, the optical density (OD) of each well was assessed using a spectrophotometer at a wavelength of 450 nm. The inhibition rates were calculated as follows: Inhibition rate = [1 - (OD drug treated - OD blank)/(OD control-OD blank)] x100%. The half inhibitory concentration (IC₅₀) for baicalein was determined using the Logit method (31), indicating the concentration of baicalein necessary to inhibit 50% cell proliferation at a given time-point.

Wound-healing assay. HT29 cells were plated in a 6-well plate at 1x10⁶ cells/well, and cultured to 90% confluency. A 10-μl sterile micropipette tip was used to create a wound across the monolayer, and the cells were washed twice with sterile phosphate-buffered saline (PBS) to remove debris. The cells were treated with 10, 20, and 30 μmol/l baicalein in RPMI-1640 media without FBS, and the control group was treated with 0.05% DMSO only. Cell migration was assessed using an inverted microscope (Zeiss Axio Vert.A1), original magnification, x10.

Transwell invasion assay. To assess the effects of baicalein on cancer cell invasiveness, a Transwell invasion assay was performed using 24-well Transwell chambers (pore size, 8 μm), pre-coated with Matrigel[®] for 1 h at 37°C. (BD Biosciences). Following treatment with 10, 20, and 30 μmol/l baicalein at 37°C for 24 h, HT29 cells were digested with 0.25% trypsin and resuspended in serum-free RPMI-1640 medium at a density of 1x10⁶/ml, and 100 μl cell suspension was added into the upper chambers. The lower chambers were filled with 500 μl medium supplemented with 10% FBS. Following incubation at 37°C for 24 h, the inserts were detached and non-invasive cells were gently removed with a cotton wool swab. Invaded cells were fixed with 4% paraformaldehyde for 20 min at room temperature and stained with 0.1% crystal violet for 15 min at room temperature. Stained cells were visualized using an inverted microscope (Zeiss Axio Scope.A1) and counted in 5 randomly selected fields (magnification, x10).

Plasmid transfection. pcDNA3.1-vector and pcDNA3.1-Snail plasmids were obtained from Shanghai GeneChem Co., Ltd., and verified by DNA sequencing. The pcDNA3.1-vector plasmids were used as the controls. HT29 and DLD1 cells (4x10⁵ cells/well) were seeded into 6-well plates with complete

medium and incubated at 37°C for 24 h prior to transfection. For transient transfections, cells were transfected with 2.5 μg plasmid using Lipofectamine[®] 3000 transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The cells were harvested 1-2 days after transfection for further investigation.

Western blot analysis. For western blotting, HT29 and DLD1 cells were plated at 3x10⁵ cells/well in 6-well plates, and treated with 10, 20, and 30 μmol/l baicalein or DMSO only for 24 or 48 h at 37°C. Cells were washed with PBS and lysed using ice-cold RIPA buffer (Beyotime Institute of Biotechnology) for 30 min to extract the total protein, which was quantified using the bicinchoninic acid assay method. The lysates were then denatured in loading buffer containing 4% SDS, and incubated at 95°C for 10 min. In total, 50 μg total protein per sample was separated by 10% SDS-PAGE gel and transferred to PVDF membranes, which were subsequently blocked for 2 h using 5% non-fat dry milk in TBS and 0.05% Tween 20. The membranes were incubated overnight at 4°C with primary antibodies targeted against: E-cadherin (cat. no. 14472; 1:1,000; Cell Signaling Technology, Inc.), vimentin (cat. no. 5741; 1:1,000; Cell Signaling Technology, Inc.), Snail (cat. no. 3879; 1:1,000; Cell Signaling Technology, Inc.), Twist1 (cat. no. 46702; 1:1,000; Cell Signaling Technology, Inc.), p53 (cat. no. 60283-2-Ig; 1:800; ProteinTech Group, Inc.), p21 (cat. no. 10355-1-AP; 1:800; ProteinTech Group, Inc.) and β-actin (cat. no. 4970; 1:10,000; Cell Signaling Technology, Inc.). Following primary incubation, the membranes were incubated with horseradish peroxidase-conjugated goat anti-mouse (cat. no. 6946; 1:8,000; Abcam) and anti-rabbit (cat. no. 6721; 1:6,000; Abcam) IgG (H+L) secondary antibodies for 2 h at room temperature. Protein bands were visualized in a dark-room using enhanced chemiluminescence reagents (New Cell & Molecular Biotech Co., Ltd.). Protein expression was quantified using ImageJ software (v.1.48; National Institutes of Health) with β-actin as the loading control.

Statistical analysis. All experiments were performed in triplicate. Data are presented as the mean ± standard deviation. Statistical analyses were performed using SPSS software (v.22.0; IBM Corp.). One-way ANOVA followed by Dunnett's post hoc test was used to compare the treatment and control groups. P<0.05 was considered to indicate a statistically significant difference. All graphs were generated using GraphPad Prism software (v.5.0; GraphPad Software, Inc.).

Results

Analysis of the absorption of baicalein from *S. baicalensis* extract. The *S. baicalensis* herb comprises a complex mixture of different phytochemicals, and contains >60 chemical components. Thus, the purpose of using the UPLC-MS/MS technique was to detect and identify baicalein in rat plasma following the oral administration of *S. baicalensis* extract. A representative chromatogram of baicalein is presented in Fig. 1.

Baicalein suppresses DLD1 and HT29 cell proliferation *in vitro*. The anti-proliferative activity of baicalein was assessed with a CCK-8 assay, using HT29 and DLD1 cells

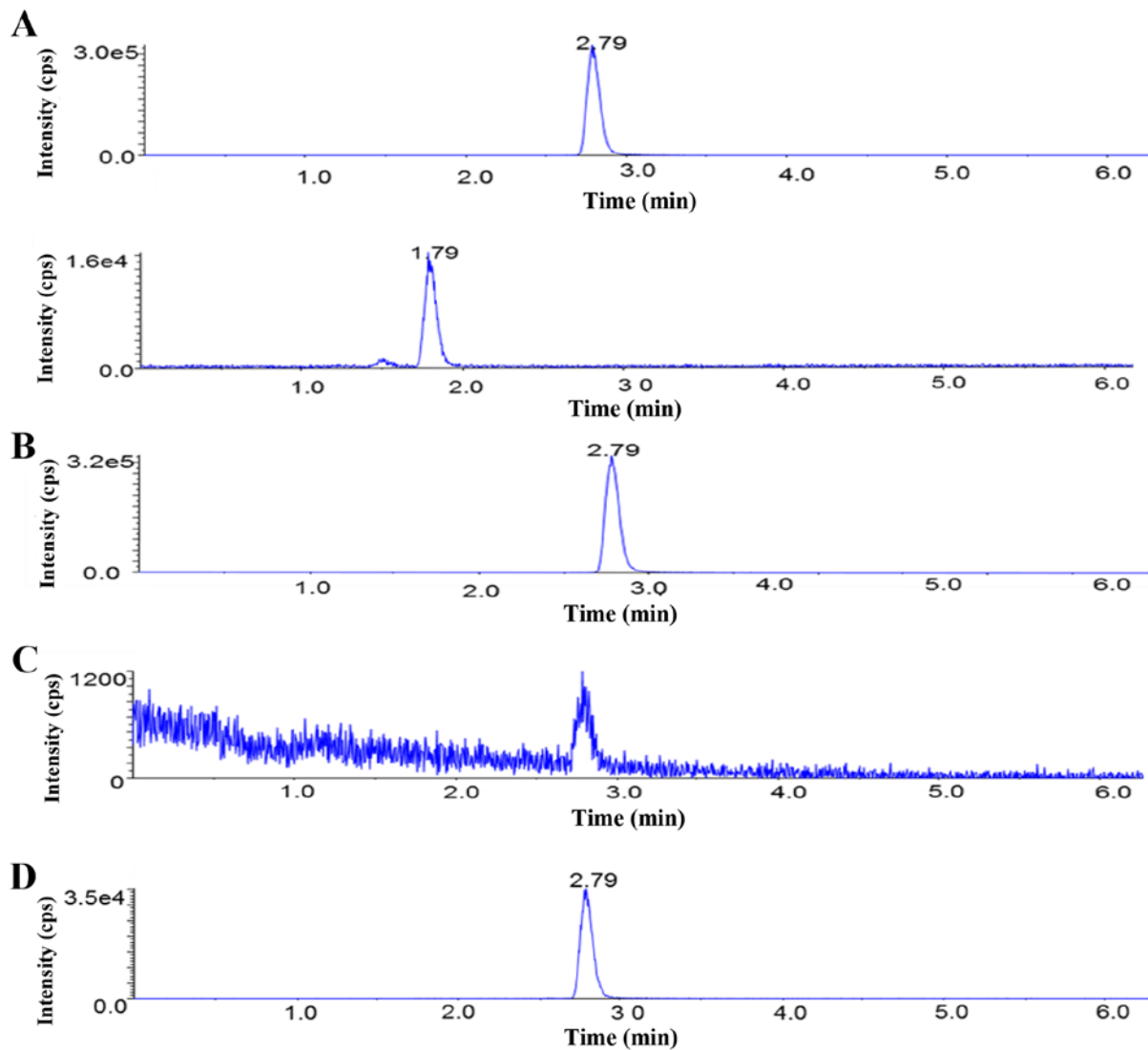


Figure 1. Ultra-high performance liquid chromatography-tandem mass spectrometric chromatogram of baicalein. (A) Baicalein and internal standard. (B) Crude *S. baicalensis* aqueous extract. (C) Blank rat plasma. (D) Rat plasma sample obtained 45 min after oral administration of crude *S. baicalensis* aqueous extract. *S. baicalensis*, *Scutellaria baicalensis*.

treated with 0-120 $\mu\text{mol/l}$ baicalein to identify the minimal non-lethal dose. Baicalein was revealed to inhibit the viability of HT29 and DLD1 cells in a dose- and time-dependent manner; in HT29 cells, the IC_{50} values at 24, 48 and 72 h were 49.77, 34.35 and 16.91 $\mu\text{mol/l}$, respectively; and in DLD1 cells, the IC_{50} values were 60.49, 34.70, and 18.75 $\mu\text{mol/l}$ at the same time-points, respectively (Fig. 2A). To avoid growth suppression, all subsequent experiments were conducted using baicalein concentrations $<34 \mu\text{mol/l}$.

p53 and p21 are reportedly involved in the baicalein-associated inhibition of CRC HCT116 cell proliferation (24). Therefore, the expression levels of p53 and p21 in HT29 and DLD1 cells were investigated, with or without baicalein treatment. As revealed in Fig. 2B and D, both p53 and p21 expression were significantly increased in baicalein-treated cells compared with the control cells, which was consistent with the aforementioned study (24).

Baicalein affects the mobility of HT29 cells in vitro. p53 is also important for the regulation of metastasis and E-cadherin

expression (32). Chang *et al* (33), revealed that p53 inhibits the invasiveness of CRC cells by regulating EMT. To further explore the effects of baicalein on CRC cells, the migration and invasion abilities of baicalein-treated HT29 cells were investigated. The wound-healing assay results demonstrated that baicalein inhibited the migratory ability of CRC cells in a dose-dependent manner (Fig. 3A). Following treatment with 10, 20 or 30 $\mu\text{mol/l}$ baicalein for 48 h, HT29 cell motility was inhibited by 49.65, 67.41 and 83.17%, respectively (Fig. 3B). In the Transwell assays, cells from the control group exhibited a higher invasive capacity than those that had been treated with baicalein, indicating that baicalein significantly inhibited the invasiveness of CRC cells in a dose-dependent manner (Fig. 3C and D). These findings indicated that baicalein may act as a suppressor of CRC cell migration and invasion.

Baicalein regulates the expression of EMT markers in HT29 and DLD1 cells. EMT is an important process which is characterized by the decreased expression of epithelial markers such as E-cadherin, and the concomitant increased

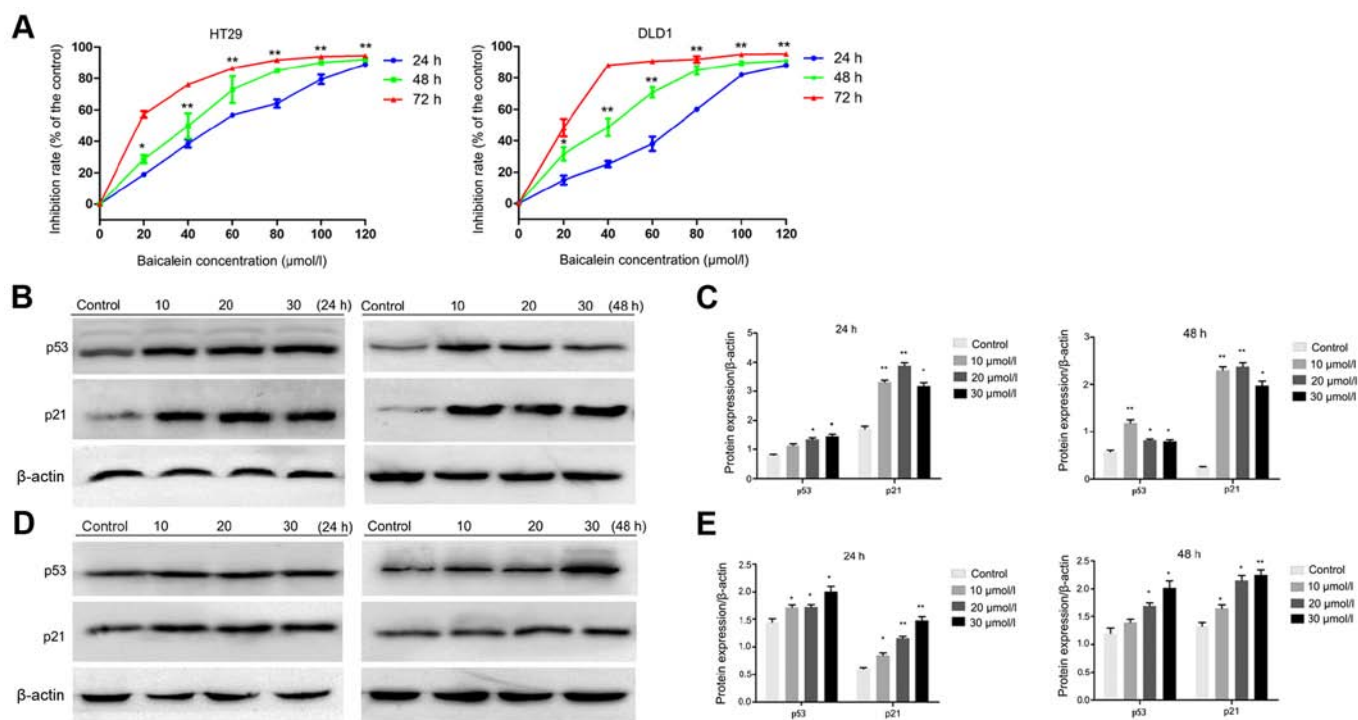


Figure 2. Baicalein suppresses cellular proliferation in CRC. (A) HT29 and DLD1 cells were treated with 0, 20, 40, 60, 80, 100 and 120 $\mu\text{mol/l}$ baicalein for 24, 48 and 72 h, and the CCK-8 assay was used to assess cellular proliferation. (B) Effects of baicalein on p53 and p21 expression in HT29 cells. Cells were treated with or without baicalein at the indicated concentrations for 24 or 48 h, and the protein expression levels of p53 and p21 were determined by western blotting. β -actin was used as the internal control. (C) p53 and p21 expression in HT29 cells was quantified using ImageJ software. (D) Effects of baicalein on p53 and p21 expression in DLD1 cells. Cells were treated with or without baicalein at the indicated concentrations for 24 or 48 h, and the protein expression levels of p53 and p21 were determined by western blotting. (E) p53 and p21 expression levels in DLD1 cells were calculated using ImageJ software. Results are expressed as the means \pm standard deviation of three separate experiments. * $P < 0.05$ and ** $P < 0.01$ vs. the control. CRC, colorectal cancer; CCK-8, Cell Counting Kit-8.

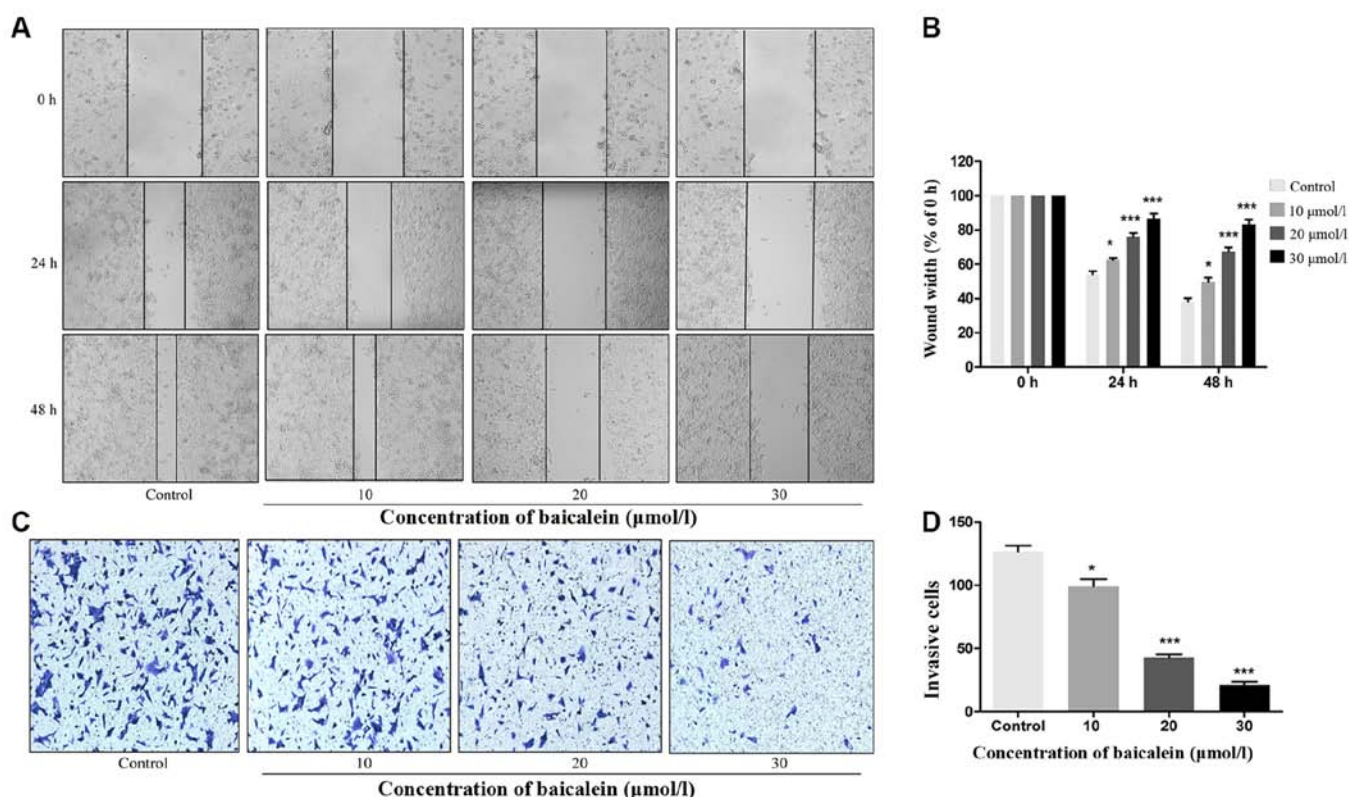


Figure 3. Baicalein inhibits cell migration and invasion in CRC. (A) Effects of baicalein on cell migration were detected using a wound-healing assay; original magnification, $\times 10$. (B) Wound width was measured to quantify the migratory potential in each group. (C) Effect of baicalein on cellular invasiveness was demonstrated by Transwell assays; original magnification, $\times 10$. (D) Invasive cells were measured to quantify the invasion potential in each group. A representative result of three independent experiments and statistical data are presented as the mean \pm standard deviation. * $P < 0.05$ and *** $P < 0.001$ vs. the control. CRC, colorectal cancer.

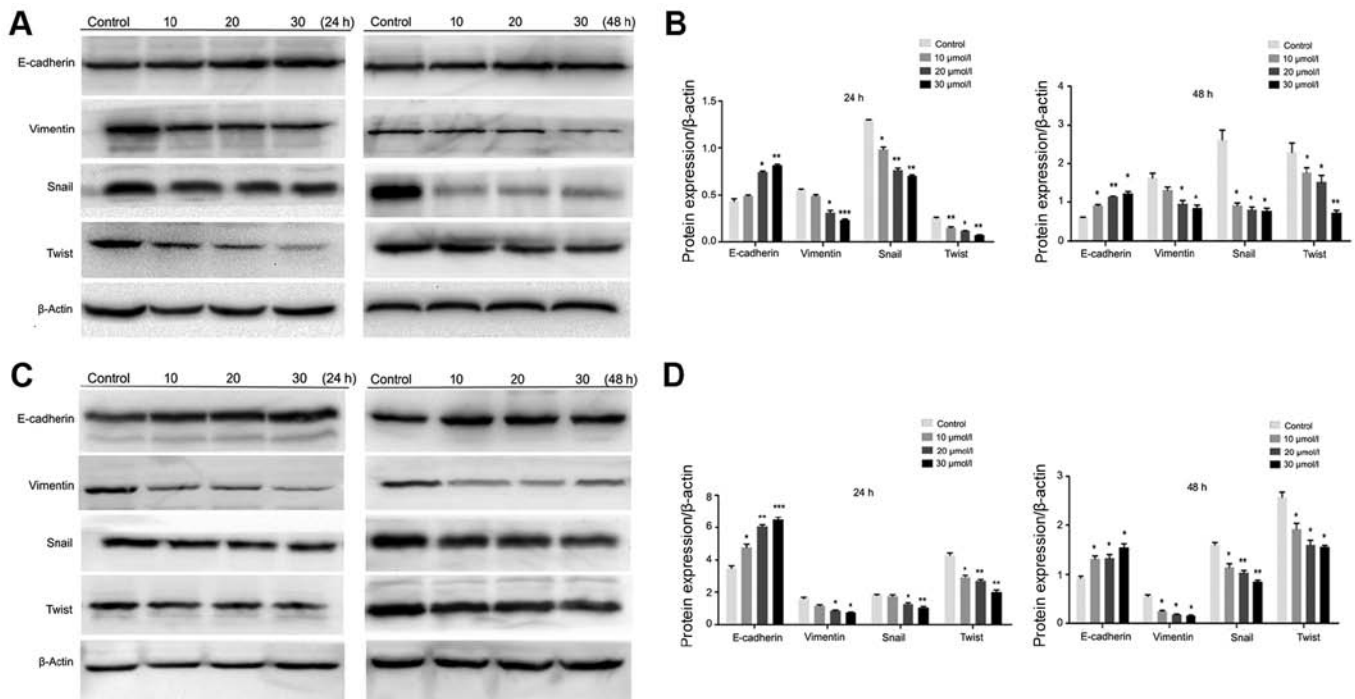


Figure 4. Baicalein regulates the expression of epithelial-mesenchymal transition-associated markers. (A) HT29 cells were treated with or without baicalein at the indicated concentrations for 24 or 48 h, and protein expression levels of E-cadherin, vimentin, Snail and Twist1 were determined by western blotting. (B) E-cadherin, vimentin, Snail and Twist1 expression levels in HT29 cells were quantified using ImageJ software. (C) DLD1 cells were treated with or without baicalein at the indicated concentrations for 24 or 48 h, and the protein levels of E-cadherin, vimentin, Snail and Twist1 were determined by western blotting. (D) E-cadherin, vimentin, Snail and Twist1 expression in DLD1 cells were calculated using ImageJ software. Results are presented as the means \pm standard deviation of three separate experiments. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. the control. β -actin was used as the internal control.

expression of mesenchymal markers such as vimentin, matrix metalloproteinase 9 and various transcription factors (34). In the present study, the effects of baicalein on the expression of EMT markers was assessed in HT29 and DLD1 cells, following a 24- or 48-h treatment with 0-30 $\mu\text{mol/l}$ baicalein. Compared with the control group, Snail, Twist1 and vimentin expression was decreased in baicalein-treated HT29 cells, while E-cadherin expression was increased at 24 and 48 h, respectively (Fig. 4A and B). Similar effects were observed in DLD1 cells (Fig. 4C and D). These results indicated that baicalein was able to impede EMT in CRC cells.

Baicalein inhibits Snail-induced EMT in CRC cells. Since Snail plays a key role in metastasis and baicalein suppresses the expression of Snail and Snail-associated target genes (35), it was hypothesized that the antitumor activity of baicalein was influenced by Snail. In order to confirm this hypothesis, CRC cells were transfected with a pcDNA3.1-Snail plasmid, and Snail overexpression was confirmed by western blotting (Fig. 5A). The overexpression of Snail was revealed to increase the migratory and invasive capacities of CRC cells, which were reversed by baicalein treatment (Fig. 5B and C). Snail is reported to be one of the most important EMT-associated transcription factors; therefore, the effects of baicalein on Snail-induced EMT were also evaluated. As revealed in Fig. 5D, transfection with Snail-pcDNA3.1 markedly increased the expression levels of Snail, vimentin and Twist1, while the level of E-cadherin expression was significantly decreased, compared with the control-transfected cells; these results were consistent with

the EMT expression profile. Notably, following baicalein treatment, Snail-induced vimentin and Twist1 upregulation, as well as E-cadherin downregulation were decreased both in HT29 and DLD1 cells, indicating that baicalein exhibits its suppressive effect partly through the inhibition of Snail-induced EMT.

Discussion

Plants possess a complex mixture of different phytochemicals; *S. baicalensis* contains >60 chemical components, of which baicalein is the primary contributor to its antitumor effects. In the present study, a UPLC-MS/MS technique was employed to detect and identify baicalein in rat plasma after the oral administration of *S. baicalensis* extract. The results demonstrate that baicalein is a major bioactive ingredient of *S. baicalensis*, which is absorbed into the blood via enterocytes. However, it should be noted that *S. baicalensis* is most often administered orally, and that flavonoids are usually unstable at a neutral pH (36). On the other hand, the flavonoid metabolite 2, 4, 6-trihydroxybenzoic acid was reported to mediate its effects through a CDK- and sodium-coupled monocarboxylate transporter 1-dependent pathway contributing to the prevention of CRC (37). Therefore, it is possible that flavonoids may be subjected to degradation by the intestinal microflora or its metabolites (38).

S. baicalensis is commonly used to treat cancer (39,40); baicalein is the primary active ingredient present within extracts of *S. baicalensis* (41,42). Baicalein treatment can inhibit cellular proliferation by blocking the cell cycle and

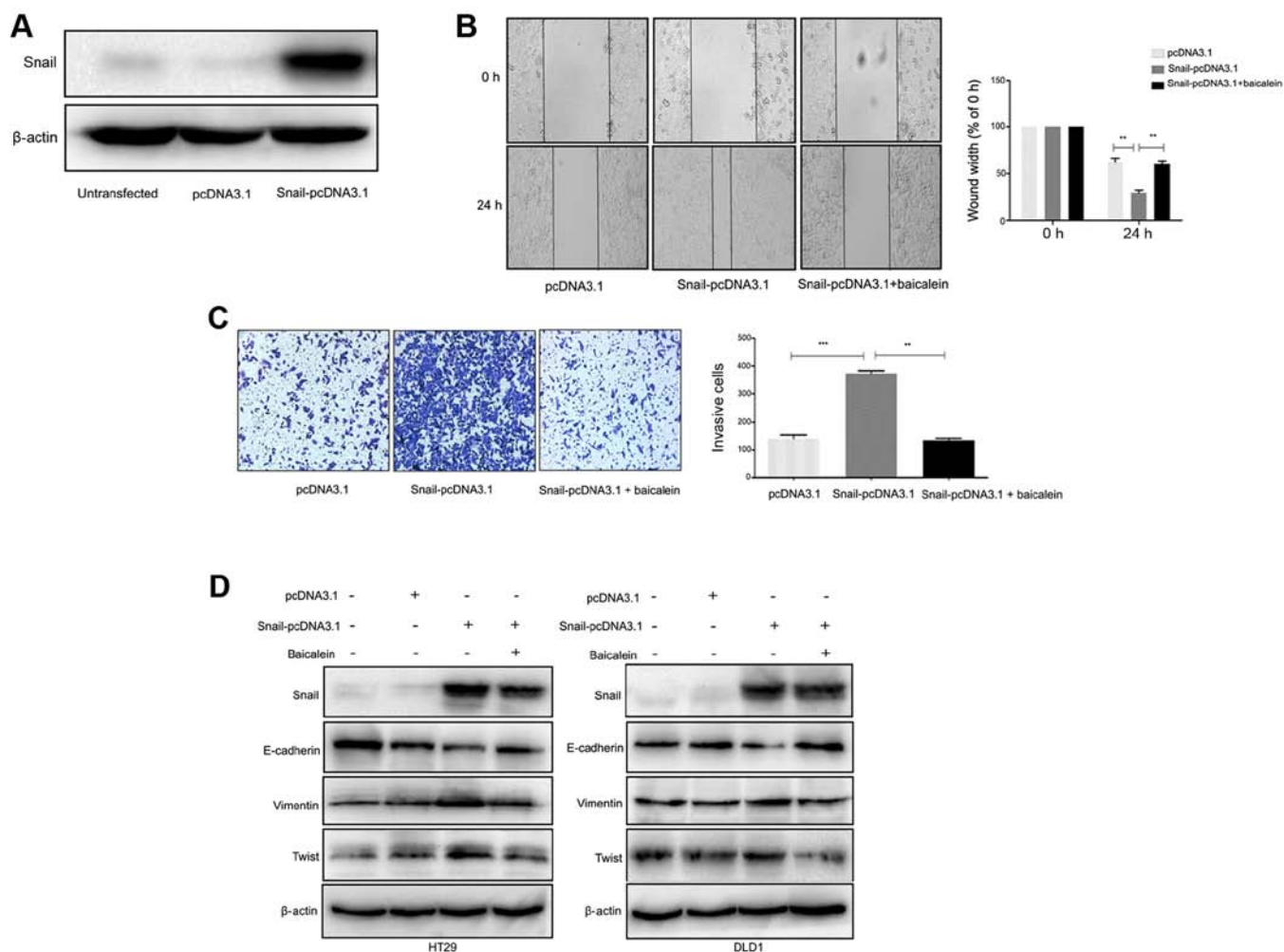


Figure 5. Overexpression of Snail reverses the inhibitory effects of baicalein on colorectal cancer cell migration and invasiveness. (A) HT29 cells were transfected with Snail-pcDNA3.1 or pcDNA3.1 and the overexpression of Snail was confirmed by western blotting. (B) Cell migration was evaluated via wound-healing assays. HT29 cells were transfected with Snail-pcDNA3.1 or pcDNA3.1. At 24 h post-transfection, the cells were treated with or without 30 $\mu\text{mol/l}$ baicalein for 24 h. Images of the wound closure were acquired; original magnification, $\times 10$; quantitative analyses are presented. Each experiment was independently repeated ≥ 3 times. (C) Cell invasiveness was evaluated using Transwell assays. HT29 cells were transfected with Snail-pcDNA3.1 or pcDNA3.1, and at 24 h post-transfection, were treated with or without 30 $\mu\text{mol/l}$ baicalein for a further 24 h. The invasive HT29 cells were observed under a microscope; original magnification, $\times 10$; quantitative analyses are presented. Each experiment was independently repeated ≥ 3 times. (D) Baicalein inhibits Snail-induced epithelial-mesenchymal transition; colorectal cancer cells were transfected with Snail-pcDNA3.1 or pcDNA3.1 for 24 h, and then treated with or without 30 $\mu\text{mol/l}$ baicalein for a further 48 h. Snail, E-cadherin, vimentin, Twist1 and β -actin expression levels were measured via western blotting. Images are representative of three experiments. Data are presented as the means \pm standard deviation of three independent experiments. ** $P < 0.01$ and *** $P < 0.001$ vs. the control.

inducing apoptosis and senescence, via the modulation of the mitogen-activated protein kinase 1 ERK-1/-2, and p53-p21 pathways (17,24). In the present study, baicalein exhibited its antitumor effects even at a low concentration (10 μM), at which p53 and p21 expression were significantly increased. Similar results were observed for E-cadherin, vimentin, Snail and Twist1 expression levels. It is well known that p53 induces apoptosis in response to a variety of cellular stimuli (43). Since the induction of apoptosis is a major mechanism of most chemotherapeutic agents, it was hypothesized that p53 may be involved in baicalein-mediated cellular proliferation. Notably, both p53 and p21 expression were increased in baicalein-treated cells in the present study.

Emerging evidence has revealed that EMT is responsible for the development of metastatic dissemination, a characteristic of the advanced clinical stages of CRC (44). Baicalein has been reported to inhibit EMT by regulating the Wnt/ β -catenin

signaling pathway (45). However, the effects of baicalein on Snail, a primary promoter of EMT, have not been previously reported, to the best of the authors' knowledge. In the present study, Snail-overexpression was revealed to significantly promote CRC cell invasiveness, which was partially reversed by baicalein treatment, resulting in the downregulation of E-cadherin and the upregulation of Snail and Twist1. Hence, to the best of the authors' knowledge, the present study is the first to report that baicalein suppresses EMT, partly through a decrease in Snail activity.

To conclude, the present study confirmed that baicalein is absorbed into the blood and can inhibit cellular proliferation, migration and invasiveness in CRC, potentially by regulating p53 and p21 expression, and disrupting EMT. These data suggest that baicalein, a primary component of *S. baicalensis*, exerts potent anticancer effects against human CRC cells, and is potentially an effective target drug for cancer therapy.

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Availability of data and materials

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

Authors' contributions

QG, WZ and QZ conceived and designed the experiments. WZ and QZ performed the experiments. YZ analyzed the data. QZ wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal procedures were approved and performed in compliance with the guidelines set by the Animal Care Committee of the First People's Hospital of Yunnan Province.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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