Original Article

Baicalein suppresses the proliferation of human cervical cancer cells via Notch 1/Hes signaling pathway

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ABSTRACT

Background: Baicalein is an active compound extracted from the roots of *Scutellaria baicalensis* georgi, which is widely and traditionally used in the anticancer therapy. Notch signaling pathway is usually abnormally activated in kinds of human cancers. The aim of the present study is to investigate the antitumor effects of baicalein in human cervical cancer and explore whether baicalein treatment affects notch signaling pathway in human cervical cancers.

Materials and Methods: Cervical cancer cells were treated with increasing concentrations of baicalein for 24, 48, and 72 h, respectively. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine cell viability of cervical cancer cells. The apoptosis rate was determined by FACS assay. Furthermore, the molecular mechanism was investigated. The expression levels of Notch 1, Notch 2, Notch 3, hairy enhancer of split-1 (Hes-1), and Hes-5 were determined by western blotting analysis.

Results: MTT assay results revealed that baicalein inhibited cell proliferation of HeLa cells and SiHa cells in a time- and dose-dependent manner. The data from FACS assay demonstrated that baicalein-induced cell apoptosis of cervical cancer cells at the final concentration of 100 μM for 24 h. Furthermore, baicalein treatment downregulated Notch 1/Hes-1, Hes-5 signaling pathway, and there was no obvious change on the expression of Notch 2 and Notch 3.

Conclusion: Baicalein inhibited the proliferation of human cervical cancer cells via Notch 1/Hes signaling Pathway. The study would provide some new clues in the clinical therapy of human cervical cancers.

KEY WORDS: Baicalein, cervical cancer, hairy enhancer of split-1, hairy enhancer of split-5, Notch 1

INTRODUCTION

Cervical cancer is one of the key public health issues in China.[1] Most cervical cancers are caused by human papillomavirus virus. Although the medical condition has been improved, the incidence and mortality of cervical cancer have been increasing in China.[2] Traditional Chinese medicine (TCM) has been widely used in cancer therapy, such as lung cancer, breast cancer, nasopharyngeal cancer, cervical cancer, and so on.[3-5] Baicalein is one of the major components in the roots of *Scutellaria baicalensis*, which is a kind of Chinese herbal medicine.[6] Recently, the antitumor effects of *Scutellariae* radix and its components baicalein are gradually reported in various human cancers. Gao *et al*. have found that baicalein inhibited the proliferation, migration, and invasiveness of breast cancer cells MDA-MB-231 by downregulating the expression of SATB1.[7] Baicalein and doxorubicin were simultaneously used for combined anticancer therapy to maximize the treatment effect and to overcome multidrug resistance in breast cancers.[8] Baicalein potently inhibited gastric cancer cell proliferation and colony formation by inducing apoptosis of gastric cancer cells through the mitochondrial pathway.[9] The therapeutic effects of baicalein on human bladder cancer were clarified by induction of apoptosis via reactive oxygen species -dependent activation of caspases in human bladder cancer.[10] Moreover, baicalein inhibited the cell viability of ovarian cancer cells, with less of an effect on normal cells, suggesting baicalein possessed potent potential for chemoprevention and treatment of ovarian cancers.[11]

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Baicalein inhibited the progression and migration of kinds of cancers involving several signaling pathway, including AKT signaling pathway,[12] Wnt/beta-catenin pathway,[13] AMPKalpha and MEK/ERK1/2 signaling pathways,[14] p38 signaling pathway,[15,16] caveolin-1/AKT/mTOR pathway,[17] and PTEN/AKT/HIF-1alpha signaling pathway.[18] Notch signaling pathway is highly conserved in evolution progression.[19] There were four different notch receptors in mammals, referred to as Notch 1, Notch 2, Notch 3, and Notch 4, which was the single-pass transmembrane receptor.[20] Notch signaling pathway played an important role in the regulation of embryonic development.[21,22] Aberrant gain or loss of notch signaling components would lead to multiple human diseases, especially the development and progression of various cancers.[23] Till now, it has not been investigated that whether baicalein treatment would affect the levels of notch signaling pathway in cervical cancer cells. Thus, in the present study, we used the different concentrations of baicalein to treat human cervical cancer cells and explored the molecular mechanism of baicalein on notch signaling pathway. It is helpful to give new clues in the clinical therapy for human cervical cancers and baicalein might be used as a new drug to treat cervical cancers.

MATERIALS AND METHODS

Cell lines and agents

Human cervical cancer cell lines (HeLa and SiHa) were cultured and kept in our laboratory. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml of penicillin, and 100 mg/ml streptomycin. The cervical cancer cells were kept in a humidified atmosphere of 5% CO_2 at 37°C. DMEM and FBS were obtained from Gibco BRL (Carlsbad, CA, USA). Penicillin and streptomycin were obtained from Sigma Chemical Co (St Louis, MO, USA). Baicalein (≥98.0% purity, 465119) was purchased from Sigma–Aldrich Biotechnology (St. Louis, MO, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) agent (Cat. No. 88417) was purchased from Sigma Inc. (Sigma, Saint Louis, MO, USA). All other chemicals were analytical grade.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

Cell viability was determined by colorimetric MTT assay. Briefly, human cervical cancer cells (3×10^4 cells/well in 100 μl of medium) were plated into 96-well plate. The cells were then treated with baicalein at the final concentration of 0.1, 1.0, 10, 50, and 100 μM, respectively. The cells were treated with baicalein for 24, 48, and 72 h, respectively. Next, 10 μl of MTT (5 mg/mL) in PBS solution was added to each well, and then, the plate was further incubated for 4 h. The medium was removed and 100 μl of dimethyl sulfoxide was added to each well and mixed thoroughly to dissolve the formed crystal formazan. The data were measured at 490 nm using an enzyme-linked immunosorbent assay reader (RT600, Guangdong, China).

FACS assay

The apoptosis rate was determined by Annexin V-FITC/propidium iodide (PI) double staining assay. Briefly, human cervical cancer cells (3×10^6) were collected and washed twice with PBS buffer. Then, the cells were suspended in 500 μL of binding buffer (adding 5 μL of Annexin V-FITC and 10 μL of PI) and incubated in the dark for 10 min at 4°C. The samples were analyzed on a flow cytometry (Millipore Corporation, Billerica, MA, USA). The experiment was repeated twice.

Western blotting analysis

The expression levels of Notch 1, Notch 2, Notch 3, hairy enhance of split-1 (Hes-1), and Hes-5 were determined by Western blotting analysis. The antibodies used here were listed as follows: primary antibody against Notch 1 (mN1A) was a mouse monoclonal IgG1 provided at 200 μg/ml (Cat. No. sc-32745) and purchased from Santa Cruz corporation. Anti-Notch 2 antibody (ab137665) was a rabbit polyclonal to Notch 2 and obtained from Abcam. Notch 3 (D11B8) was a rabbit monoclonal antibody and purchased from Abcam. Anti-Hes1 antibody (EPR4226) (ab108937) was a rabbit monoclonal (EPR4226) to Hes-1 and obtained from Abcam corporation. Anti-Hes-5 antibody (EPR15578) (ab194111) was a rabbit monoclonal (EPR15578) to Hes-5 and obtained from Abcam. The β-actin antibody was purchased from TransGenic Biotechnology (Beijing, China). The blots were developed using ECL Western blotting reagent (SuperSignal West Pico, Pierce Biotechnology, Rockford, IL, USA) and quantified using optical densitometry (Image J software, NIH).

Statistical analysis

The data were analyzed by SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). All results were expressed as mean ± standard deviation. $P < 0.05$ was considered statistically significant.

RESULTS

Baicalein inhibits the proliferation of cervical cancer cells

To identify the antitumor effects of baicalein in cervical cancer cells, baicalein was diluted at the final concentration of 0.1, 1.0, 10, 50, and 100 μM to treat human cervical cancer cells. Specifically, HeLa cells were treated with increasing concentrations of baicalein for 24 h. MTT assay was used to determine cell viability in each group. The inhibitory rate was calculated, and the results demonstrated that baicalein could inhibit the proliferation of HeLa cells at the concentration of 10, 50, and 100 μM for 24 h [Figure 1a].

Next, HeLa cells were treated with baicalein for different period of time, including 24, 48, and 72 h. As shown in Figure 1b, baicalein treatment inhibited cell viability in a dose- and time-dependent manner. All the data obviously demonstrated that baicalein had the antitumor effects in human cervical cancer cells.

SiHa cell line was a kind of human cervical squamous cell carcinoma, and we used SiHa as the other human cervical

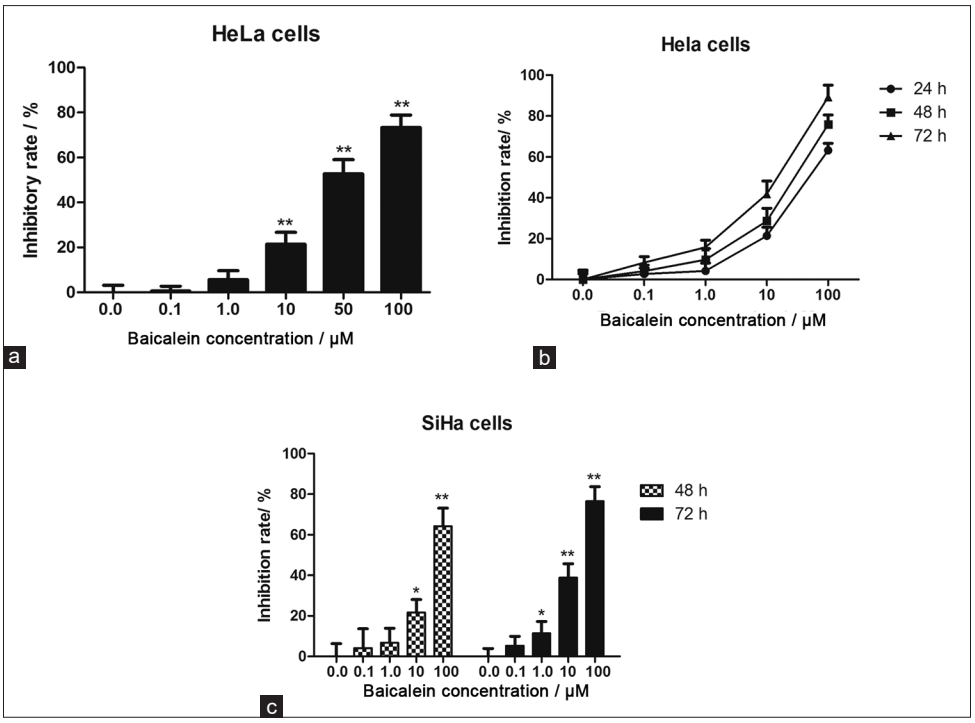

Figure 1: Baicalein inhibits the proliferation of cervical cancer cells. (a) HeLa cells were treated with different dose of baicalein for 24 h. Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. $^{**}P < 0.01$, compared with that untreated cells. (b) HeLa cells were treated with baicalein in a time- and dose-dependent manner. Baicalein was diluted as described in Materials and Methods. (c) SiHa cells were treated with increasing concentrations of baicalein for 48 and 72 h. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with untreated cells

cancer cell model. Then, SiHa cells were treated with baicalein at the final concentration of 0.1, 1.0, 10, and 100 μM for 48 and 72 h, respectively. As shown in Figure 1c, the results demonstrated that baicalein inhibited cell viability of SiHa cells in a time- and dose-dependent manner. All the data obviously suggested that baicalein had the antitumor effects in human cervical cancer cells and it suppressed the cell proliferation of cervical cancer cells in a time- and dose-dependent manner.

Baicalein treatment promotes cell apoptosis of human cervical cancer cells

We have identified that baicalein could inhibit cell proliferation of human cervical cancer cells. It has been reported that programmed cell death was mainly including cell apoptosis, cell autophagy, and necroptosis. In the present study, FACS assay was used to determine the cell apoptosis rate in baicalein-treated cervical cancer cells. As shown in Figure 2, HeLa cells and SiHa cells were treated with 10 and 100 μM of baicalein for 24 h. The results demonstrated that human cervical cancer cells were treated with 100 μM of baicalein for 24 h and cell apoptosis rate was significantly increased ($^{**}P < 0.01$, compared with untreated cells).

Baicalein treatment decreases the levels of Notch 1 in HeLa cells

Aberrant notch signaling has been observed in human cervical cancers. We wanted to know whether baicalein treatment affects the expression of notch signaling pathway

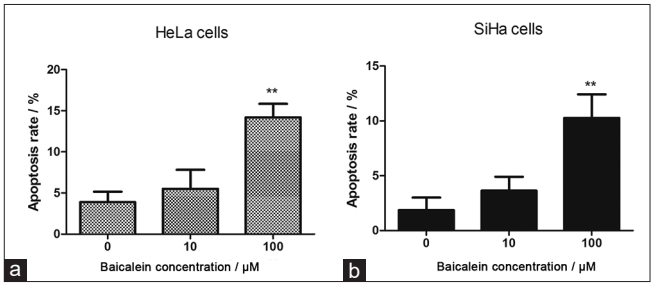

Figure 2: Baicalein treatment promotes cell apoptosis of human cervical cancer cells. (a) HeLa cells were treated with baicalein at the concentration of 10 and 100 μM for 24 h. Cell apoptosis rate of HeLa cells was shown in the histogram. $^{**}P < 0.01$, compared with untreated group. (b) SiHa cells were treated with baicalein for 24 h (10 and 100 μM). The cell apoptosis rate was shown in the histogram. $^{**}P < 0.01$, compared with untreated group

in human cervical cancers. In the experiment, HeLa cells were treated with 10 and 100 μM of baicalein for 24 h and the notch receptors were detected by Western blotting analysis. As shown in Figure 3, the expression level of Notch 1 was significantly decreased being treated with 100 μM of baicalein, suggesting Notch 1-related signaling pathway may involve in the progression of human cervical cancers.

Baicalein treatment significantly decreases the levels of Hes-1 and Hes-5 in cervical cancer cells

It is supported that notch target genes include the Hes-1 and Hes-5. Next, we detected the levels of Hes-1 and Hes-5 in

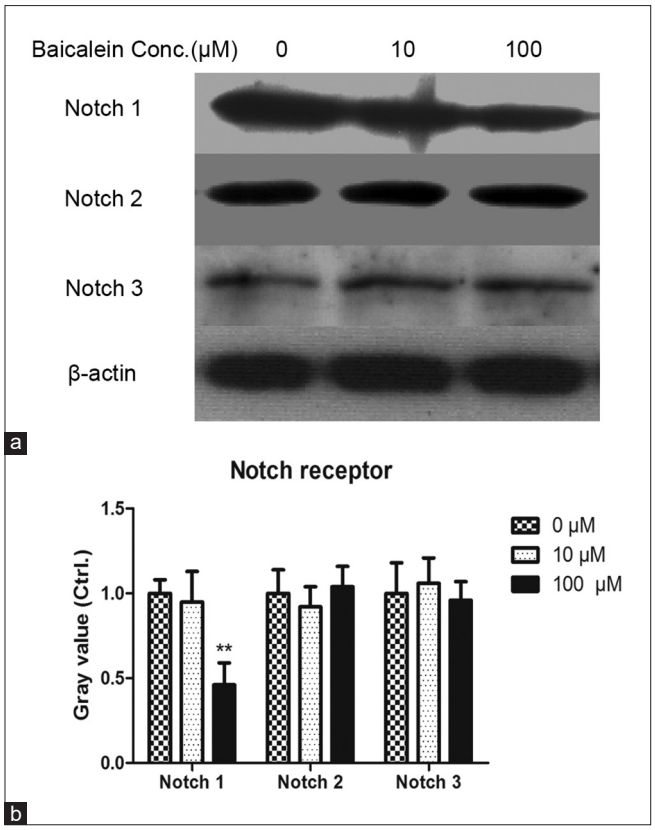

Figure 3: Baicalein treatment decreases the levels of Notch 1 in cervical cancer cells. (a) HeLa cells were treated with 10 and 100 μM of baicalein for 24 h. Notch 1, Notch 2, and Notch 3 levels were detected by Western blotting analysis. (b) The levels of Notch receptors were shown in the histogram. $^{**}P < 0.01$, compared with untreated group

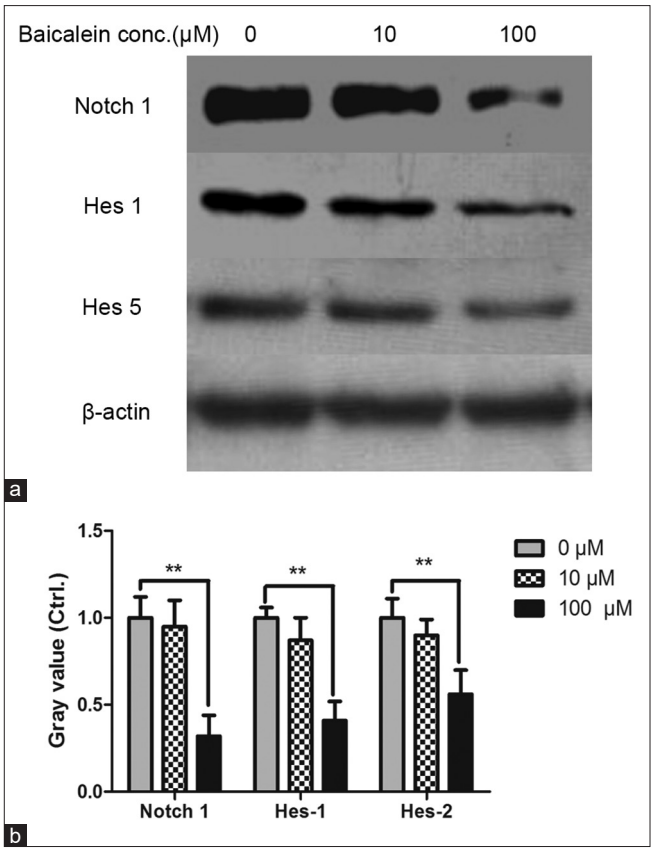

Figure 4: Baicalein treatment significantly decreases the levels of Hes-1 and Hes-5 in cervical cancer cells. (a) HeLa cells were treated with baicalein at the final concentration of 10 and 100 μM for 24 h. The levels of Notch 1, Hes-1, and Hes-5 were detected by Western blotting analysis. (b) The expression level of Notch 1, Hes-1, and Hes-5 was shown in the histogram $^{**}P < 0.01$

baicalein-treated HeLa cells. As shown in Figure 4, HeLa cells were treated with 100 μM of baicalein for 24 h, the levels of Hes-1 and Hes-5 were significantly decreased in HeLa cells. All the data obviously demonstrated that baicalein inhibited cell proliferation of human cervical cancer cells by regulating Notch 1/Hes-1 (Hes-5) signaling pathway.

DISCUSSION

Baicalein is a member of the flavone subclass of flavonoids, which is extracted from the root of the TCM plant, *S. baicalensis*.[24] In the present study, we tested the antitumor effects of baicalein in human cervical cancer cells, HeLa cells and SiHa cells. We found that baicalein significantly suppressed the proliferation of human cervical cancer cells in a time- and dose-dependent manner, suggesting baicalein had potent antitumor effects in the therapy of human cervical cancers. This was confirmed by several papers, in which the researchers found baicalein inhibited tumor cell proliferation and migration, such as gallbladder cancer cells,[25] colorectal cancer cells,[24,26] gastric cancer cells,[27] pancreatic cancer cells,[28] and liver cancer cells.[29]

Next, HeLa cells and SiHa cells were treated with baicalein for 24 h, and we found that baicalein induced cell apoptosis of human cervical cancer cells. The effective concentration of baicalein was 100 μM in HeLa and SiHa cells for 24 h. The effective concentration might be lower as the longer treatment time. This was consistent with the results from Peng *et al*.[30] They found that that baicalein induced apoptosis in a caspase-3-dependent pathway. Specifically, B-cell lymphoma 2 (Bcl-2) protein was downregulated, and the Bcl-2-associated X protein, Fas, Fas ligand, and caspase-8 were upregulated.

Furthermore, the molecular mechanism of baicalein on notch signaling pathways in human cervical cancer cells was investigated. The levels of notch receptors were detected by Western blotting analysis and the results demonstrated that the level of Notch 1 was significantly decreased as the increasing concentration of baicalein in HeLa cells; however, the expression levels of Notch 2 and Notch 3 were not obviously changed in baicalein-treated cells at the final concentration of 10 μM and 100 μM. Then, the target genes for Notch 1, such as Hes-1 and Hes-5 were also detected by Western blotting analysis. The data revealed that baicalein treatment significantly downregulated the expression levels of Notch 1, Hes-1, and Hes-5, suggesting that baicalein inhibited cell

viability of cervical cancer cells partly by regulating the Notch 1/Hes-1 or Hes-5 signaling pathway. Thus, in the present study, we found that baicalein treatment inhibited the proliferation of human cervical cancer cells via Notch 1/Hes signaling pathway, which could provide some new clues in the clinical therapy of human cervical cancers.

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Conflicts of interest

There are no conflicts of interest.

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