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The effect of baicalein on the expression of SATB1 in MDA-MB-231 cells*

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Abstract ***Objective:*** Baicalein had been proved to have anti-cancer activity *in vitro* and *in vivo*, including the inhibition of malignant proliferation, migration, adhesion and invasion of many kinds of cancer cells. The special AT-rich sequence binding protein 1 (SATB1) is a tissue-specific expression of nuclear matrix-binding protein and is reported to be a breast cancer "gene group organizer". Previous studies have shown that SATB1 is involved in the growth, metastasis and prognosis of breast cancer. The present study was aimed to investigate whether baicalein inhibits the proliferation and migration of MDA-MB-231 human breast cancer cells through down-regulation of the SATB1 expression. ***Methods:*** MDA-MB-231 cells were treated for 24 h, 48 h and 72 h with various concentrations of baicalein (0, 5, 10, 20, 40 and 80 μM) respectively. Then, the proliferation and migration of MDA-MB-231 cells following treatment with baicalein were determined using colorimetric 3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide (MTT) and wound healing assays. Thereafter, western blot analysis was performed to detect the changes of SATB1 protein expression in MDA-MB-231 cells. ***Results:*** Along with the prolongation of time and increase of drug concentration, inhibitory effect of baicalein on proliferation and migration of MDA-MB-231 cells gradually increased, in a time- and dose- dependent manner ($P < 0.05$). Meanwhile, after treated with baicalein in different concentrations for 48 h, the level of SATB1 protein expression of MDA-MB-231 cells decreased obviously, in a dose-dependent manner ($P < 0.05$). ***Conclusion:*** Baicalein inhibits breast cancer cell proliferation and suppresses its invasion and metastasis by reducing cell migration possibly by down-regulation of the SATB1 protein expression, indicating that baicalein is a potential therapeutic agent for human breast cancer.

Key words baicalein; special AT-rich sequence binding protein 1 (SATB1) ; breast cancer; proliferation; migration

Breast cancer is a common malignant tumor in women, which ranks the second in female cancer mortality in the world [1], and its morbidity increases year by year, producing a serious threat to women health. It was dissemination and proliferation of tumor cells at the secondary sites that results in patient death in most cases and brings about the major difficulties in clinical breast cancer treatment. There are nearly 50% of the breast cancer patients already have had distant metastasis [2–3] when they were first diagnosed. Therefore, it is especially important to prevent and control tumor metastasis of breast cancer.

Special AT-rich sequence binding protein 1 (SATB1) is a kind of tissue specific nuclear matrix binding protein, which is found predominantly in thymocytes as well as progenitor cells and epithelial basal layer, and almost displays no expression in other normal cells and tissues. As a global chromatin organizer and transcription factor, SATB1 has emerged as a key factor integrating higher-order chromatin architecture with gene regulation [4–6]. It plays an important role in T cell development, early erythroid differentiation, cell homeostasis and responses to all kinds of stimulation [4, 7–10].

Recent studies reported that SATB1 had abnormal high expression in a variety of tumor cells, which could control more than 1000 genes and promote tumor growth and metastasis, suggesting that SATB1 is a potentially important target for anti-tumor therapy.

Baicalein (5, 6, 7-trihydroxy-2-phenyl-4H-1-benzopyran-4-one, molecular weight, 270.24) is the mainly effective component extracted from the roots of Scutellaria baicalensis Georgi or Scutellaria Radix, widely used in Chinese herbal medicine (Fig. 1) [11]. It has been reported that baicalein could inhibit tumor cell proliferation, invasiveness and metastasis in human breast cancer, liver cancer and pancreatic cancer cell lines [12–14]. However, the specific molecular mechanism of its inhibition of tumor

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growth and metastasis is not clear. Our study hypothesizes a novel anti-tumor mechanism of baicalein, that baicalein may exert its anti-invasive and antimetastatic function by inhibiting the expression of SATB1 protein in human breast cancer MDA-MB-231 cells.

Materials and methods

Cell culture

The human breast cancer MDA-MB-231 cell line was purchased from Shanghai Institute of Biology (China). The cells were cultured in RPMI-1640 medium (USA) supplemented with 10% fetal bovine serum (FBS, USA), 100 U/mL penicillin and 100 μg/mL streptomycin. All cells were incubated in a humidified atmosphere of 5% CO_2 at 37 ℃. The medium was changed every other day. Cells were passaged when covering 80% of the bottle wall.

MTT assay

Cell viability and proliferation were determined by a colorimetric 3 (4, 5-dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide (MTT) assay according to the conventional protocols. MDA-MB-231 cells were seeded in 96-well culture plates at a density of 1×10^4 cells per well and treated with 200 μL serially diluted concentrations (0, 5, 10, 20, 40 and 80 μM) baicalein (purity of 99%; USA) at 37 ℃ for 24 h, 48 h and 72 h respectively. The blank control group was added in 200 μL RPMI-1640 medium, each concentration being set 6 parallel holes. Thereafter, The cells were then washed twice with PBS and cells were incubated with 20 μL MTT (Sigma, USA; 5 mg/mL) for 4 h. The optical density (OD) value of each hole was measured spectrophotometrically at 490 nm. The inhibitory rate of cell proliferation (inhibition ratio calculation, IR) was calculated. IR = (1 – experimental group mean OD value / control group mean OD value) × 100%. According to cell proliferation inhibition rate at different concentration of baicalein in 48 h, the 50% inhibition concentration (IC_{50}) of baicalein was calculated.

Wound healing assay

MDA-MB-231 cells were planted into a 6-well plate at a density of 2×10^5 cells per well and incubated with RPMI-1640 medium containing 10% FBS overnight to obtain a 80% confluent monolayer. The cell monolayer was wounded vertically after 24 h by a plastic tip (1 mm). The width of the wound area was recorded under the inverted microscope (40 ×). Each well was washed three times with PBS to remove cell debris, and then further incubated for 48 h in RPMI-1640 medium (2% FBS) with different concentrations of baicalein (0, 10 and 20 μM). Each group included 2 holes and the experiment was repeated for 3 times. The average distance of cell migration into the injured area was pictured and measured under inverted microscope (40 ×) at 0 h, 24 h and 48 h after baicalein was added.

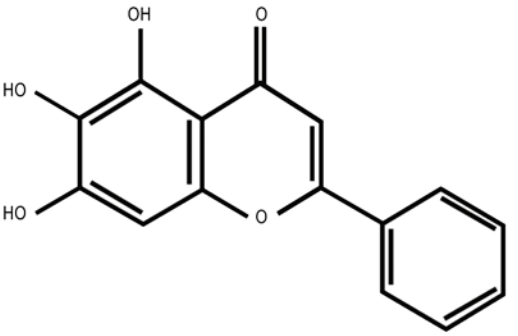

Fig. 1 Chemical structure of baicalein

Western blot analysis

After treatment with different concentrations of baicalein (0, 10, 20 and 40 μM), cells were collected. MDA-MB-231 cells were lysed with radio-immunoprecipitation assay lysis buffer, then the total cellular protein was extracted. The protein concentration in the supernatants was determined using BCA (Beijing Biosynthesis Biotechnology Co., Ltd, China) assay with a Varioskan multimode microplate spectrophotometer (USA). The equal amount of proteins was separated by 10% sodium dodecyl sulface-polyacrylamide gel electrophoresis and transferred onto the polyvinylidence fluoride (PVDF) membranes (0.45 μm PVDF; Millipore, USA) according to semi-dry transferred method. Subsequently, the membrane was blocked with skimmed milk powder (5% in Tris-buffered saline with TWEEN-20 buffer) at 37 ℃ for 90 min to block non-specific binding and were then incubated with primary antibodies against SATB1 (rabbit polyclonal antibody, 1.25:1000 dilution; Abcam, USA) and β-actin (rabbit polyclonal antibody, 1:1000 dilution; Santa Cruz, USA) for 4 ℃ overnight，followed by the secondary antibody (1:1000, horseradish peroxidase/hypothalamic regulatory peptides labeled, purchased from Beijing Biosynthesis Biotechnology Co., China) at 37 ℃ for 1 h, then the PVDF membranes was washed by PBS and colored by enhanced chemiluminnescence kit (ECL, Thermo Fisher Scientific Inc.). Detection was performed by the gel imaging system (Syngene, Frederick, USA). The experiment was repeated 3 times.

Statistical analysis

The values shown were mean ± standard deviations. The data was analyzed by SPSS 18.0 software and transferred into diagrams using GraphPad Prism. One-way ANOVA test was used to compare the difference among groups. The R × C chi-square test was used to compare the quantitative differences among groups. In all tests, *P*

Table 1 Inhibitory effects of baicalein on MDA-MB-231 cell migration (mean ± SD, n = 6)

Concentration (μM)	24 h		48 h		72 h	
	OD	IR (%)	OD	IR (%)	OD	IR (%)
0	0.406 ± 0.031	0 ± 3.31	0.603 ± 0.036	0 ± 2.11	0.981 ± 0.027	0 ± 1.19
5	0.482 ± 0.013	–22.8 ± 4.14	0.533 ± 0.051	13.2 ± 3.04	0.841 ± 0.063	14.3 ± 5.88
10	0.408 ± 0.052	–6.53 ± 1.55	0.507 ± 0.033	18.2 ± 3.91	0.824 ± 0.025	20.1 ± 1.94
20	0.338 ± 0.029	20.6 ± 8.87	0.356 ± 0.025	46.9 ± 2.99	0.465 ± 0.011	52.6 ± 6.93
40	0.316 ± 0.016	27.3 ± 4.82	0.283 ± 0.022	60.5 ± 2.56	0.212 ± 0.030	78.4 ± 2.55
80	0.264 ± 0.014	42.7 ± 4.17	0.207 ± 0.023	74.8 ± 1.56	0.193 ± 0.014	80.3 ± 1.20

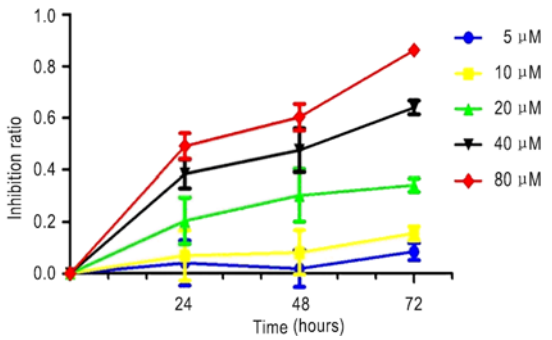

Fig. 2 Inhibition of MDA-MB-231 cells proliferation by baicalein in a time- and dose-dependent manner (mean ± SD, n = 6)

< 0.05 was considered statistically significant.

Results

Baicalein suppressed the proliferation of MDA-MB-231 cells

Compared with the control group, various concentrations (5, 10, 20, 40 and 80 μM) of baicalein inhibited MDA-MB-231 cell proliferation significantly at 24 h, 48 h, 72 h (P < 0.05). Along with the prolongation of time and increase of drug concentration, inhibitory effect of baicalein on proliferation of MDA-MB-231 cells gradually increased, in a time- and dose- dependent manner (P < 0.05; Table 1 and Fig. 2). But there were no obvious inhibiting effects of baicalein on proliferation of MDA-MB-231 cells in the concentration of 5 and 10 μM at 24 h. IC_{50} was 27.92 μM when treated with baicalein for 48 h.

Baicalein influenced migration of MDA-MB-231 cells *in vitro*

Detection of cell migration capacity was also a method to judge the metastatic potential of tumor cells. Under the inverted microscope, the cell morphology of MDA-MB-231 cells had no obvious change in the control group and 10 μM group, but the cells changed from the original long spindle shape to round ones in 20 μM group after treated with baicalein for 48 h. As shown in Fig. 3a, the number of cells which migrated into wound area of baicalein intervention group were significantly less than that of the control group (P < 0.05). As the drug concentration increased, the migration distance decreased significantly when compared with control group at 24 h and 48 h, which was also in a time- and dose-dependent fashion (P < 0.05). The inhibition rate was approximately 10.8%, and 88.7% at 48 h with 10 and 20 μM baicalein, respectively (Fig. 3b).

Baicalein inhibited the SATB1 protein expression of MDA-MB-231 cells

We used western blot analysis to investigate the inhibitory effect on SATB1 of baicalein in MDA-MB-231 cells. Compared with the control group, SATB1 protein expression decreased significantly in MDA-MB-231 cells after the cells were treated with relatively small concentrations of baicalein (10 and 20 μM) for 48 h (P < 0.01). The results demonstrated that baicalein could reduce the expression of SATB1 protein in MDA-MB-231 cells in a dose-dependent manner (10 and 20 μM groups, P < 0.01; 40 μM group, P < 0.05; Fig. 4).

Discussion

Abnormal growth and metastasis of cancer cells are important biological properties of malignant tumors. Invasion and metastasis is the main reason of cancer morbidity and mortality in millions of patients [15]. Screening effective anti-invasive and anti-metastatic drugs with little side effects bears the important theory significance and the value of clinical application to improve the curative effect, the prognosis of breast cancer and finally, the quality of life. Baicalein is baicalin aglycon compound extracted from traditional Chinese medicine scutellaria baicalensis georgi. More and more studies have shown that baicalein has potent anti-tumor properties [12, 15–16]. Its anti-tumor mechanism is mainly manifested in the inhibition of tumor cell proliferation, tumor invasion and metastasis, multidrug resistance of tumor cells and synergistic effect to chemotherapy drugs. Baicalein can inhibit the proliferation, invasion and migration ability and distant metastasis of many breast cancer cell lines, including MCF-7, MDA-MB-231, BT549, and 4T-1 cells. Various studies suggested that baicalein may achieve its anti-tumor function through down-regulating the pro-

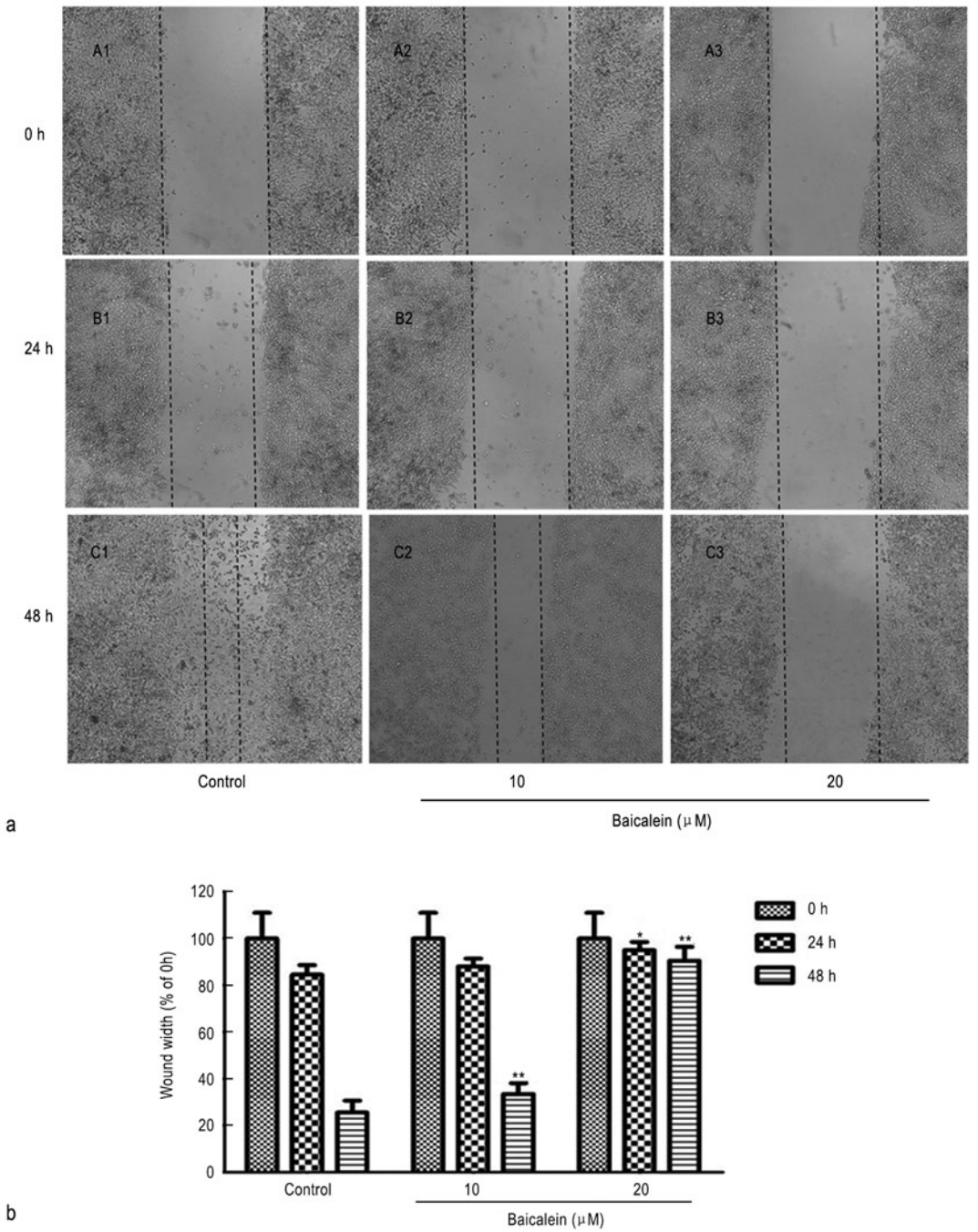

Fig. 3 Effect of baicalein on MDA-MB-231 cell migration *in vitro*, 40 × (current width / original width, mean ± SD, $n = 6$). (a) Photographs of wound cells treated with baicalein in different concentration at 0, 24 and 48 h. (b) Quantification of the wound healing assay. * $P < 0.05$ compared with control; ** $P < 0.01$ compared with control

tein expression of MMP-2, MMP-9 and uPA [12, 17–19], but the specific mechanism is unclear so far.

SATB1 was screened from human cDNA library by Dickinson *et al*, using matrix attachment regions sequences of immunoglobulin heavy chain L gene enhancer 3' as a probe. And it was found to be anchored on chromatin with its unique "cage-like" structure and could provide binding sites for many transcription factors. Therefore, SATB1 played an important role in gene transcription [7]. Han *et al* reported that SATB1 had abnormal high expression in breast cancer for the first time [19], and almost no expression in normal control tissues, which was

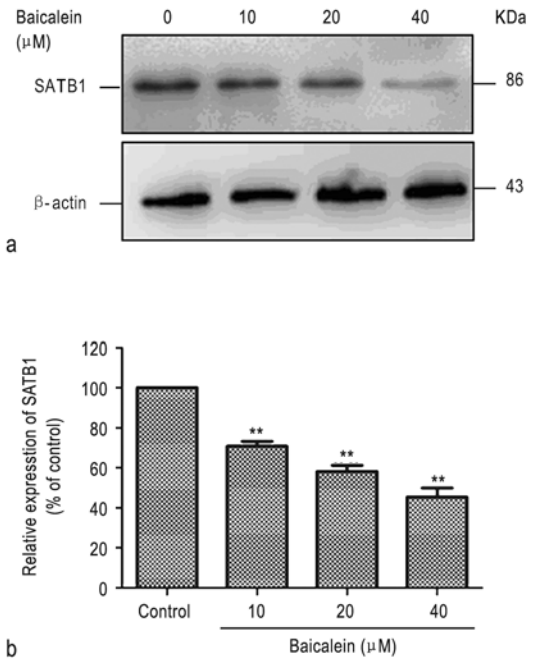

Fig. 4 Effect of baicalein on expression of SATB1 in MDA-MB-231 cells. (a) Western blot analysis of baicalein in the down-regulation of SATB1 expression in MDA-MB-231 cells. It showed that baicalein could inhibit the SATB1 expression in a dose-dependent manner; (b) Quantification of the western blot assay. * $P < 0.05$, ** $P < 0.01$, one-way ANOVA test

regarded as an independent adverse prognostic factor of breast cancer. SATB1 can promote growth and metastasis of breast cancer cell, and silencing the SATB1 expression in breast cancer MDA-MB-231 would change the invasive phenotype and inhibit tumor growth, indicating that SATB1 played a key role in tumor progression. Genomics research found that SATB1 can regulate the expression of more than 1000 genes which influenced the occurrence and development of tumor (including the ERBB2, MMP2, 3, 9, ABL1 and E-cadherin) and involved in 61 kinds of biological activities, such as cell proliferation, apoptosis, DNA, electron transport, protein expression, receptor activities, etc. SATB1 was positively correlated with the expression of a variety of biological and genetic markers, including cyclin D1, MMP-2, NF-kappaB, PCNA, and negatively correlated with the expression of APC and BRAF (V600E) [20–21]. Detection of SATB1 expression could be used as a new means to evaluate the gradation of breast cancer and is also expected to become a new therapeutic target in the future.

The MDA-MB-231 cells belong to the human breast cancer cell line, which are of high invasiveness. Recent study [20] reported that it also expressed SATB1 at high level. In our study, we found that baicalein had strong inhibitory effect on tumor cell malignant proliferation. Along with the prolongation of time and increase of drug concentration, inhibitory effect of baicalein on proliferation of MDA-MB-231 cells gradually increased, except the concentrations of 5 and 10 μM at 24 h. This result was consistent with Xu *et al* [22] speculated that low-dose baicalein may had no inhibitory effect on the proliferation of MDA-MB-231 cells, on the contrary there may be some growth-promoting effect. Wound healing assay confirmed that baicalein could inhibit cell migration ability of MDA-MB-231 cells at concentrations lower than 48 h IC_{50}. Small doses of baicalein intervention down-regulated the expression of SATB1, and the inhibitory effect was significantly enhanced as the drug concentration increased ($P < 0.01$), suggesting that down-regulation of SATB1 protein expression may be one of the mechanism of baicalein in inhibition of invasion and metastasis of breast cancer cells. However, this is only *in vitro* experiment, neither animal experiment nor positive control group was set in this study, so further research is needed.

Conclusion

Baicalein inhibits the MAD-MB-231 human breast cancer cells proliferation and suppresses its invasion and metastasis possibly by reducing cell migration through down-regulation of the SATB1 protein expression, indicating that baicalein is a potential therapeutic agent for human breast. The anti-tumor mechanisms of baicalein in breast cancer needed be investigated in further studies.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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