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Baicalin Regulates Proliferation, Apoptosis, Migration, and Invasion in Mesothelioma

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Background: Baicalin, one of the main bioactive components extracted from the traditional Chinese medicine baical Skullcap root, has an anti-tumor activity which had been studied in several cancers. However, its role in human mesothelioma remains unknown. In this study, we investigated the anti-tumor mechanisms of baicalin in the mesothelioma cell line MESO924.

Material/Methods: Effects of baicalin on mesothelioma were assessed by measuring cell viability, apoptosis, migration, invasion, inactivation of signaling intermediates, and cell-cycle alterations.

Results: Baicalin inhibited the proliferation, migration, and invasion of human mesothelioma cells and increased their apoptosis, all in a dose-dependent manner. Specifically, baicalin decreased the expression of p-EGFR, p-AKT, p-MAPK, p-S6, *Bcl-2*, and *VEGF* and increased the expression of *Bax* in mesothelioma cells. The suppressed mesothelioma cellular proliferation is due to the arrest of the S cell cycle by baicalin. Inhibition of the PI3K/AKT/mTOR signaling pathway by a PI3K/AKT/mTOR inhibitor augmented the anti-proliferation effects induced by baicalin. In addition, baicalin increased the sensitivity of MESO924 to the chemotherapeutic drugs doxorubicin, cisplatin, and pemetrexed.

Conclusions: These results highlight the roles of baicalin in inhibiting cell growth, migration, and invasion of mesothelioma cells while increasing apoptosis and sensitizing cells to chemotherapeutic agents through the PI3K/AKT/mTOR signaling pathway, which indicates that baicalin could be a useful drug for mesothelioma therapy.

MeSH Keywords: **Baicalin • Chinese Traditional Medicine • Mesothelioma • PI3K/AKT/mTOR Signaling Pathway**

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Background

Malignant mesothelioma (MESO) is a cancerous tumor with local invasion and high mortality, and it primarily occurs in people who are over-exposed to asbestos [1] and SV40 infections [2]. MESO is classified by histopathology into 3 categories – epithelioid, sarcomatoid, and mixed type – and the spindle type (sarcomatoid variant) is considered to have the worst prognosis [3]. As the traditional methods for MESO treatment are relatively limited and mainly include chemotherapy and radiotherapy, development of new anti-neoplastic drugs with low toxic adverse effects and high selectivity is required to improve the overall survival rates of patients.

Traditional Chinese medicine (TCM) has been accepted as a novel type of anti-neoplastic drug considering its profound impact on cancer treatment in recent decades [4–6]. Baicalin ($C_{21}H_{18}O_{11}$) is the major active component of Skullcap (Huang-Qin, a medicinal plant), an important traditional Chinese medicinal herb, which can be distilled from the root. As a natural metabolite product, it has been used as a therapeutic drug for various diseases for centuries because of its high medicinal value [7]. Preliminary results of ongoing clinical trials indicate that baicalin can be used as an anti-thrombosis and anti-hyperlipidemia drug due to its effects on reducing inflammation [8]. Previous studies showed that baicalin decreased cell viability and induced cell apoptosis by downregulation of *Bcl-2 and Bcl-6* and upregulation of *p53* and *Bax* in prostate cancer cells [9], and it inhibited HIF-1α expression in lung carcinoma [10]. In addition, Baicalin inhibited apoptosis of brain cells by downregulating the expression of *HIF-1α* in a dose-dependent manner [11]. Furthermore, as an inhibitor of the aryl hydrocarbon receptor (AhR), baicalin is considered an effective drug for the treatment of cancers induced by aberrant activation of AhR. For example, as the primary ingredient of Skullcap extracts, baicalin can inhibit the activation of AhR induced by cigarette smoking [12].

Although baicalin has been suggested to have tumor-inhibitory functions in several studies, the effects of baicalin on mesothelioma remain unknown. Hence, the present study investigated the effects of baicalin on mesothelioma cell proliferation, apoptosis, migration, and invasion and the impacts of sensitizing mesothelioma cells to chemotherapeutic agents. We also explored the potential molecular mechanisms of baicalin in treatment of mesothelioma. Our results indicate that baicalin could be a useful drug for mesothelioma therapy.

Material and Methods

Antibodies and reagents

Monoclonal antibodies to EGFR, AKT (AKT1/2/3), MAPK (MAPK1/2), S6, and GAPDH were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Monoclonal antibodies to p-EGFR (Tyr1068), p-AKT (AKT1/2/3) (S473), p-MAPK (MAPK1/2) (T202/Y204), and p-S6 (S235/236) were purchased from Cell Signaling Technology (Beverly, MA, USA). Baicalin was obtained from Sigma-Aldrich. Cisplatin, doxorubicin, and pemetrexed were obtained from Selleck Chemicals (TX, USA). LY294002 was obtained from Cell Signaling Technology (Beverly, MA, USA). Pemetrexed was reconstituted in distilled water, while baicalin, cisplatin, doxorubicin, and LY294002 were reconstituted in DMSO. The qRT-PCR primers of *Bcl-2*, *Bax*, vascular endothelial growth factor (*VEGF*), and *18S* were synthesized by Shanghai GenePharma Co. (Shanghai, China).

Cancer cell lines

MESO924 cells were established from surgical materials from previously untreated patients, as reported previously [13]. The MESO924 cells were established from epithelial-type mesotheliomas. MESO924 cells were kind gifts from Dr. Wen-Bin Ou (College of Life Sciences, Zhejiang Sci-Tech University), and were cultured in 1640 basic medium supplement with 10% fetal bovine serum. All cultures were supplemented with penicillin/streptomycin.

Protein lysate preparations and immunoblotting

The cell lysates were split in lysate agent for 30 min at 4°C and spun down by centrifuge at 13 500 rpm for 25 min at 4°C. Cell lysates were standardized for the protein content with a Bio-Rad protein assay and resolved by SDS-PAGE. Electrophoresis and immunoblotting were performed as previously described [13].

Cell proliferation and apoptosis assays

MESO924 cells were inoculated in 96-well plates and were cultured in 1640 basic medium containing 10% FBS. The cell proliferation tests were performed using the Cell Proliferation Assay Kit (Promega) in accordance with the manufacturer's instructions. Apoptosis assay was performed after incubation with baicalin for 48 h. The activity of caspase 3/7 was obtained with a Caspase-Glo luminescence assay in accordance with the manufacturer's instructions.

Apoptosis was assessed by the Alexa Fluor 488 annexin V/Dead Cell Apoptosis Kit (Thermo Fisher Scientific). Briefly, MESO924 cells were trypsinized and subjected to cold PBS washing after incubation with baicalin for 48 h and then treated with PI and

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488 Annexin V in 1×annexin-binding buffer for 15 min. Flow cytometry was used to analyze the stained cells. Then, the collected datas were analyzed with FlowJo software.

Cell-cycle analysis

MESO924 cells were trypsinized and subjected to cold PBS washing after incubation with baicalin for 48 h. Nuclear staining was conducted with PI reagent and the stained MESO924 cells were tested by flow cytometer. Data processing was carried out using FlowJo VX10.

In vitro wound-healing assay

Wound-healing assays were conducted as previously described with some modifications [14]. Briefly, MESO924 cells were trypsinized, seeded in 6-well plates, and cultured with 1640 basic medium supplemented with 10% FBS. Using the tip of a 200 µL pipette, a scratch was produced in the cultures that were close to fusion, then cultured for 48 h after baicalin treatment with different concentrations (50 and 100 µM). Cells were imaged at 48 h with a Leica DMI 3000B microscope (Leica Microsystems, Germany).

Cell migration and invasion assays

Cell migration and invasion were assessed through Transwell assays using a chamber system as previously described with some modifications [15]. Briefly, for migration assays, 45 000 cells were suspended in 250 µL of 1640 medium supplemented with 3% FBS and were seeded into the top chamber with 650 µL of 1640 medium supplement, with 15% FBS added to the bottom chamber, for 24 h. For invasion assays, briefly, Transwell inserts were coated with Matrigel, and 45 000 cells were plated into the upper chamber. After incubating for 48 h, the cancer cells in the top chamber were detached with cotton swabs. Then, 75% methanol was inserted into the membrane for 15 min and staining of cultured cells were conducted with 0.5% crystal violet in methanol for 20 min.

RNA isolation and qRT-PCR

RNA extraction from cells was conducted with a TIANGEN RNA extraction kit (DP430). cDNAs synthesis were performed with a M-MLV (Moloney murine leukemia virus) system (Promega, M1705), using 1 µg portion of the total RNA, and the reverse-transcribed DNA was subjected to PCR. The primers were as follows:
Bcl-2 forward, 5'- GCTACCTAAGAAAAACCTGG-3', and
Bcl-2 reverse, 5'-CAAGAAACAAGGTCAAAGGG-3';
Bax forward, 5'-GAGAGGTCTTTTTCCGAGTG-3', and
Bax reverse, 5'-GGTGAGGAGGCTTGAGGAGT-3';
VEGF forward, 5'-ATCTTCAAGCCGTCCTGTGT-3', and
VEGF reverse, 5'-GCATTCACATCTGCTGTGCT -3';
18S forward, 5'-TCGAGGCCCTGTAATTGGAA-3', and
18S reverse, 5'-CTTTAATATACGCTATTGGAGCTG-3'.

Statistical analysis

Data from the 2 groups were analyzed and compared using independent unpaired *t* tests, and data among multiple groups were compared by one-way analysis of variance (ANOVA), followed by a Tukey's multiple comparisons post hoc test. Significant differences were defined as * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

Results

Effects of baicalin on mesothelioma proliferation and cell cycle

Baicalin is a key flavonoid found in the roots of the plant *Scutellaria baicalensis*. The molecular weight of baicalin is 446.36 kDa. It is known to have different biological activities, such as anti-oxidative, anti-inflammatory, anti-tumor, and pro-apoptotic activities [16,17]. Therefore, in the present study, baicalin was used to assess its effects on human mesothelioma cells (MESO924).

Based on the anti-tumor roles of baicalin in different human cancers other than mesothelioma, we examined the role of baicalin on MESO924 *in vitro*. We first assessed the effects of baicalin on cell proliferation by cell viability assay in MESO924 cells after treatment with baicalin for 48 h (Figure 1A). The results showed baicalin decreased the viability of MESO924 in a dose-dependent manner when compared to the DMSO treatment as the control (Figure 1A). Then, we examined the cell cycle through flow cytometric analysis to determine whether baicalin could induce cell cycle arrest to exert its cytostatic effects on MESO924 cells. The results showed that treatment of MESO924 with baicalin was associated with a reduction in the G1 peak (80.63% for DMSO versus 64.94% for 50 µM baicalin and 57.01% for 100 µM baicalin) and an increase in the S peak (10.81% for DMSO, 24.89% for 50 µM baicalin, and 32.85% for 100 µM baicalin) when compared to the control group (Figure 1B). Together, these results suggested that the decreased MESO924 cells proliferation induced by baicalin might be caused by the cell cycle arrest.

The role of baicalin in the apoptosis of mesothelioma

To investigate whether baicalin could regulate the apoptosis of MESO924 cells, caspase 3/7 activity and flow cytometry assay were conducted. Results from flow cytometry showed that treatment of MESO924 with baicalin for 48 h showed a greater

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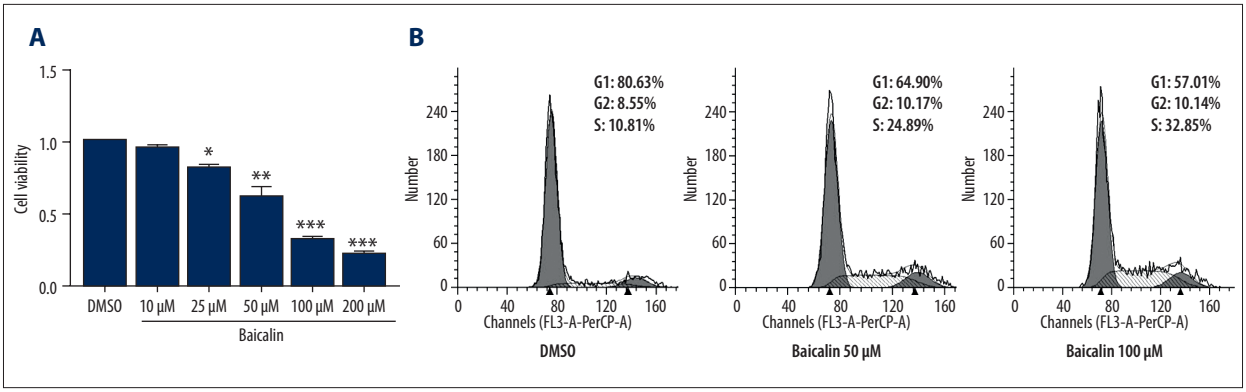

Figure 1. Cell viability and cell cycle assessment for MESO924 at 48 h after treatment with baicalin. (**A**) Cell viability assay was performed in MESO924 cells after incubation with baicalin for 48 h. The data were normalized to the DMSO treatment, and significant differences were defined as * $P<0.05$, ** $P<0.01$, *** $P<0.001$. (**B**) Cell-cycle analysis was performed after baicalin treatment for 48 h in MESO924 cells. MESO924 cells show substantial S-block after baicalin treatment when compared to DMSO.

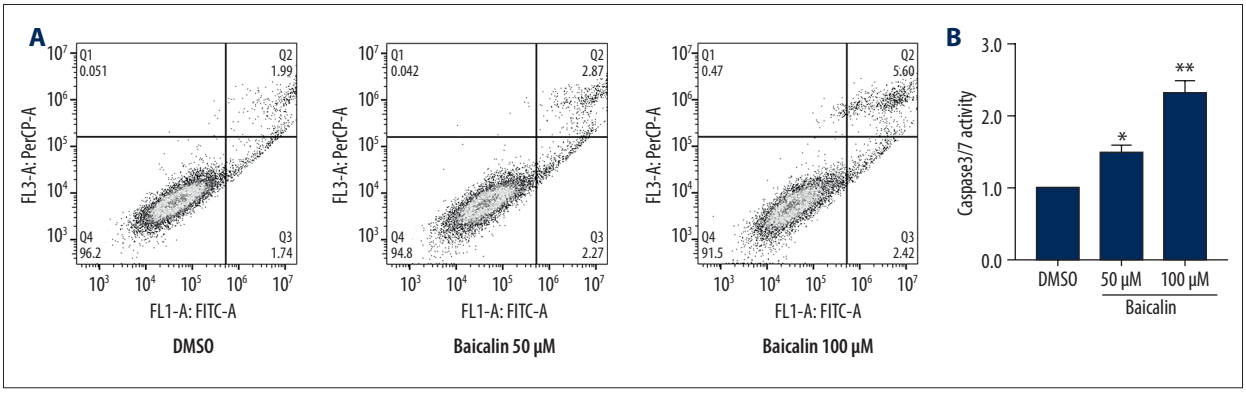

Figure 2. Baicalin induced apoptosis of MESO924 cells. (**A**) Apoptosis analyses following baicalin incubation for 2 days with PE Annexin V Apoptosis Detection Kit I. (**B**) Apoptosis was assessed in MESO924 cells at day 2 after incubation with baicalin. The activity of Caspase 3/7 was assessed using Caspase-Glo® luminescence assay.

increase in apoptotic cells (5.2% for 50 μM baicalin and 8.5% for 100 μM baicalin) compared to DMSO (3.8%, Figure 2A). Furthermore, we examined the effects of baicalin on apoptosis by measuring functional caspase 3/7 activation in MESO924 after the treatment with baicalin for 48 h. Treatment with baicalin (50 and 100 μM) resulted in a significant increase of caspase 3/7 activity in MESO924 (Figure 2B), indicating an increase of apoptosis. Our results suggested that baicalin administration is closely associated with MESO924 apoptosis.

Baicalin regulation of mesothelioma migration and invasiveness

Mesothelioma clinical spread is characterized by extension and invasive growth along the pleura, pericardium, or peritoneum. To determine the effects of baicalin on migration and invasion, we conducted wound-healing assay, Transwell migration, and invasion assays. Wound-healing assays demonstrated that baicalin led to an obvious suppression of wound healing at 48 h, whereas intact wound healing was observed in the control cells treatment with DMSO (Figure 3A). Similarly, Boyden chamber assays demonstrated that baicalin treatment resulted in a greater inhibition of migration than in the control group (Figure 3B). Furthermore, Boyden chamber assays demonstrated that incubation of MESO924 cells with baicalin resulted in a greater inhibition of invasion when compared to the control group (Figure 3C), indicating that baicalin might have potential effects to treat the metastatic mesothelioma.

Effects of baicalin on the PI3K/AKT/mTOR signaling pathway

The PI3K/AKT/mTOR pathway plays important roles in the proliferation, apoptosis, migration, and invasion of cancer cells [18]. Based on the observations that cell viability, migration, invasion, and anti-apoptosis inhibition occurred with baicalin treatment, we hypothesized that baicalin could regulate the MESO924 phenotype through the PI3K/AKT/mTOR signaling pathway.

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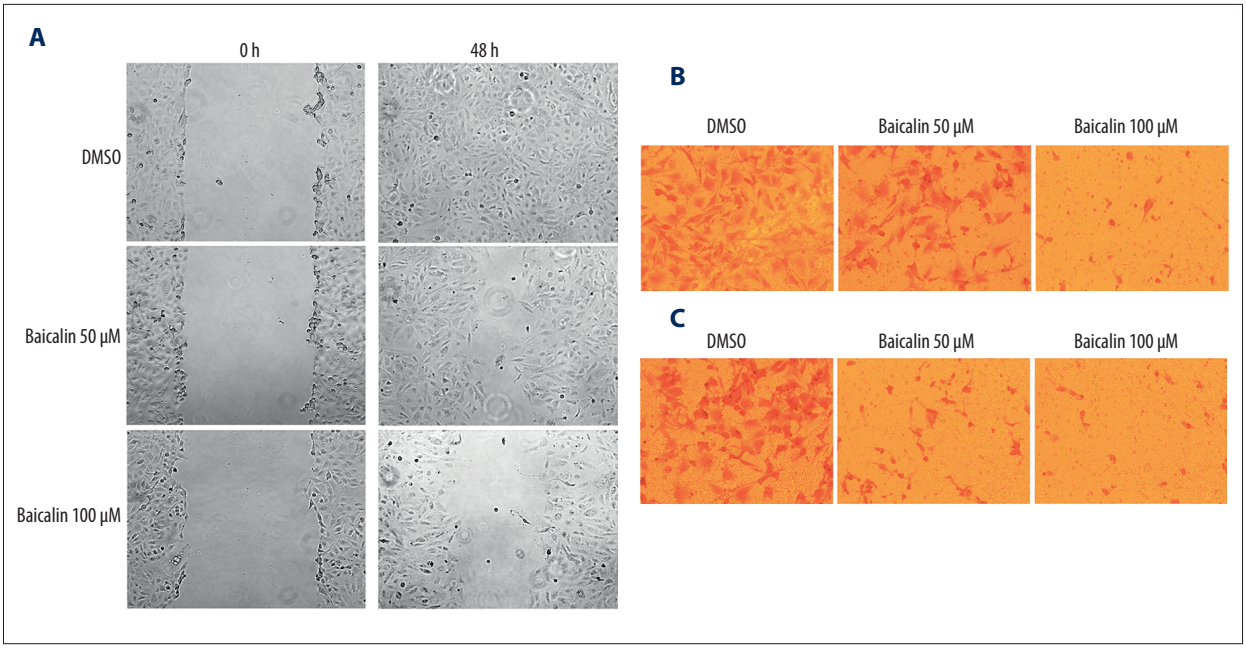

Figure 3. The regulatory effect of baicalin on the migration and invasion of MESO924 cells. (**A**) Baicalin inhibited migration of MESO924 after treatment with baicalin for 48 h, as demonstrated by *in vitro* wound healing assays. (**B**) Baicalin inhibited migration of MESO924 as assessed by Transwell migration assays after treatment with baicalin for 24 h. (**C**) Baicalin inhibited invasion of MESO924 cells as assessed by Matrigel invasion assays after treatment with baicalin for 48 h.

To investigate the function of baicalin in the PI3K/AKT/mTOR pathway, we conducted Western blotting assays to detect the expression of PI3K/AKT/mTOR signal-related proteins after treatment of MESO924 cells with baicalin (50 and 100 μM) for 6 h in serum-free medium. Western blotting results showed that baicalin had little effect on the expression of EGFR, AKT, MAPK, and S6. In contrast, baicalin induced the downregulation of p-EGFR, p-AKT, p-MAPK, and p-S6 in MESO924 cells in a dose-dependent manner when compared to the control group (Figure 4). These results suggest that the effect of baicalin in MESO924 phenotype cells could occur via the PI3K/AKT/mTOR signaling pathway.

PI3K/AKT/mTOR antagonist augments the anti-proliferation effects of baicalin on MESO924

Based on the observed anti-proliferation effect and downregulation of PI3K/AKT/mTOR signaling pathway activity induced by baicalin (Figures 1A, 4), we further conducted the cell viability assay to investigate whether PI3K/AKT/mTOR pathway inhibitor could regulate the sensitivity of MESO924 cells to baicalin. The PI3K/AKT/mTOR pathway was inactivated by a small-molecule inhibitor, LY294002. The results showed that baicalin obviously inhibited proliferation of MESO924 cells in a dose-dependent manner. Treatment of MESO924 cells with 10 μM LY294002 resulted in a ~25% decrease of cell viability at day 2. Interestingly, combined treatment with baicalin and LY294002 achieved a greater inhibition of the cell proliferation in a dose-dependent manner than that with either intervention alone (Figure 5). These results suggested that, at least partially, the reduction of PI3K/AKT/mTOR pathway activity might explain the effect of baicalin on mesothelioma cell proliferation and the synergetic effects when baicalin was used in combination with LY294002.

Synergistic effects of combined treatment of baicalin and chemotherapeutic drugs (doxorubicin, cisplatin, and pemetrexed) in mesothelioma

Blocking the PI3K/AKT/mTOR pathway can increase the sensitivity of tumor cells to chemotherapeutic drugs [18]. Due to the observation that downregulation of the PI3K/AKT/mTOR signaling pathway by baicalin could inhibit MESO924 proliferation (Figures 1A, 4), we hypothesized that synergistic inhibitory effects of proliferation on MESO924 might be observed after combined treatments with baicalin and chemotherapeutic drugs (Figure 6). To explore the synergetic effects of combined baicalin and chemotherapeutic drugs treatment, we conducted cell viability assays. Additional inhibitory effects on viability of MESO924 cells were observed through combined treatment of baicalin and chemotherapeutic drugs. Incubation of MESO924 with doxorubicin (2.5 μM), cisplatin (5 μM), and pemetrexed (0.1 μM) for 48 h resulted in ~40%, ~30%, and ~25% decrease of proliferation in MESO924 cells, respectively. Intriguingly, combined treatment with baicalin and chemotherapeutic drugs resulted in a greater reduction

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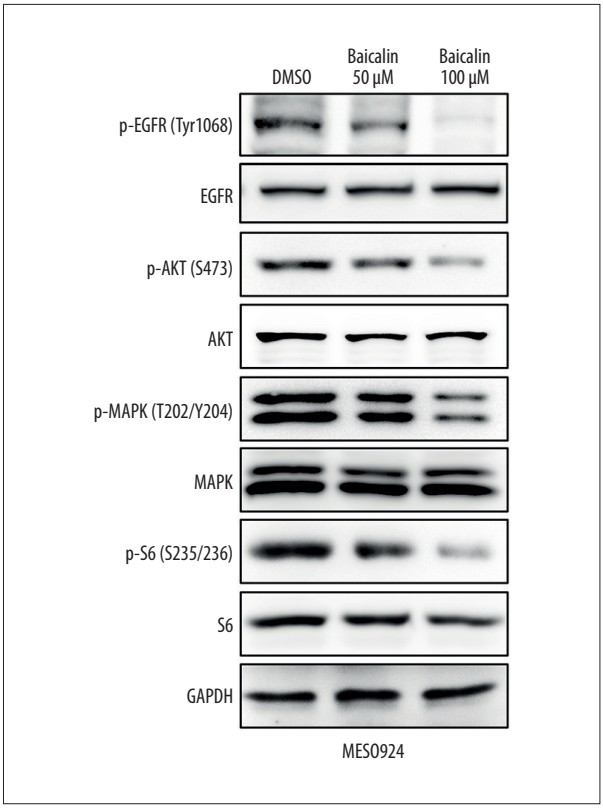

Figure 4. Effects of baicalin on the PI3K/AKT/mTOR signaling pathway. Expression of p-EGFR, p-AKT, p-MAPK, and p-S6 in MESO924 cells were assessed by immunoblotting after incubation with baicalin for 6 h in serum-free medium. GAPDH staining was used as a loading control.

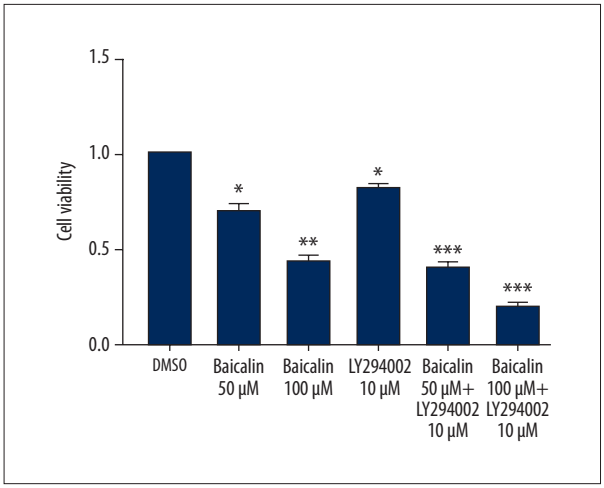

Figure 5. PI3K/AKT/mTOR antagonist augments proliferation inhibition of MESO924 induced by baicalin. Cell proliferation was assessed in MESO924 after incubation with LY294002 and baicalin for 48 h. The data were normalized to the DMSO treatment, and significant differences were defined as * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

in viability (~60–80% for doxorubicin, ~50–75% for cisplatin, and ~53–82% for pemetrexed) (Figure 6), indicating that the combination of doxorubicin/cisplatin and baicalin might have synergetic effects in treating mesothelioma.

Baicalin regulates the expression of *Bcl-2*, *Bax*, and *VEGF* in mesothelioma cells

The Bcl-2 family exerts an important role in apoptosis of cancer cells, and the 2 well-known genes *Bcl-2* and *Bax* that show anti-apoptotic and pro-apoptotic ability, respectively, belong to this family. Furthermore, various studies showed that *VEGF* increased the proliferation, migration, and invasion abilities and decreased the apoptosis of cancer cells [19,20]. To investigate the underlying mechanism by which baicalin treats mesothelioma, we performed qRT-PCR assays to assess the expression of *Bcl-2, Bax,* and *VEGF* after the treatment MESO924 cells with baicalin (Figure 7). Our results showed that the treatment of MESO924 cells with baicalin for 48 h induced down-regulation of *Bcl-2* and *VEGF* and upregulation of *Bax,* all in a dose-dependent manner (Figure 7). These results suggest that

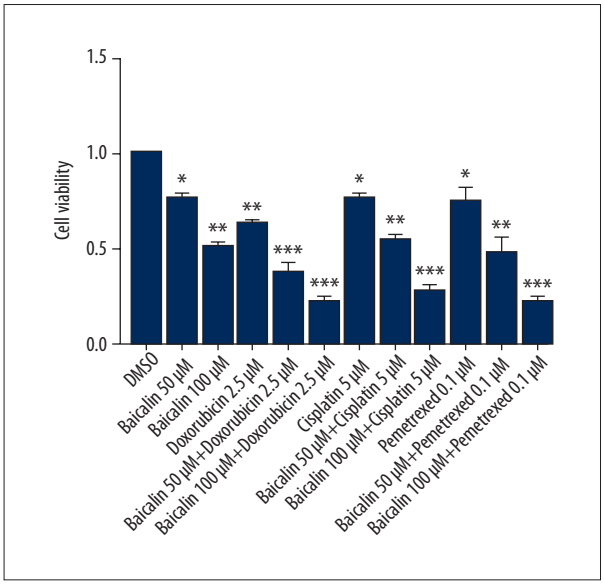

Figure 6. Synergistic effects were observed by the combined treatment with chemotherapeutic drugs (doxorubicin, cisplatin, and pemetrexed) and baicalin as indicated by cell viability in MESO924. Cell viability was assessed by a cell-titer assay in these cell lines, after incubation with chemotherapeutic drugs (doxorubicin, cisplatin, and pemetrexed) and baicalin for 48 h. The data were normalized to the DMSO treatment and significant differences were defined as * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

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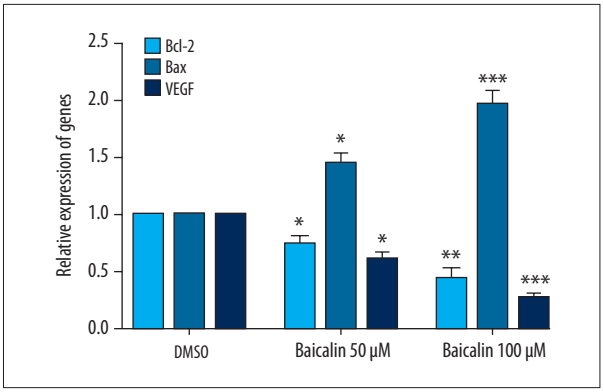

Figure 7. Baicalin treatment regulates expression of *Bcl-2*, *Bax*, and *VEGF* in human mesothelioma cells. The *Bcl-2*, *Bax*, and *VEGF* transcript levels in MESO924 were determined by qRT-PCR after incubation with baicalin for 48 h. The data were normalized to the DMSO treatment and significant differences were defined as * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

baicalin partially exert its functions of inhibiting the MESO924 phenotype cells through regulating the expression of Bcl-2, Bax, and VEGF.

Discussion

Although a variety of treatments have been developed, mesothelioma patients still have a high mortality rate. At present, standard therapies for mesothelioma are accompanied by many serious adverse effects. Therefore, new therapeutic agents for mesothelioma with lower adverse effects are needed. Due to its low adverse effects, TCM has been widely accepted as a major complementary and alternative therapy for cancer patients in China, and it has been used to treat cancer in China for several thousand years [21]. Interestingly, as an important Chinese herbal medicine, baicalin is used widely in the treatment of colon, cervical, lung, breast, liver, osteosarcoma, glioblastoma, skin, and colon cancer [22–30] because of its comprehensive anti-tumor effects. However, it was not clear whether baicalin could play an anti-tumor role in mesothelioma. In the present study, for the first time, we demonstrated that baicalin inhibited proliferation, cell cycle, migration, and invasion of human mesothelioma cells, and induced their apoptosis. Furthermore, our results suggested that these effects involved the PI3K/AKT/mTOR signaling pathway and its downstream effectors including Bcl-2, Bax, and VEGF.

Previous studies showed that continuous activation of AKT (the central mediator of the PI3K/AKT/mTOR pathway) was detected in MESO [31–33]. In the present study, our results indicated that baicalin induced inhibition of the PI3K/AKT/mTOR signaling pathway, decreased cell viability and cell migration, and increased cell apoptosis in MESO924 cells. Interestingly, the treatment of MESO924 cells with the PI3K/AKT/mTOR inhibitor LY294002 augmented the anti-proliferation effects induced by baicalin, consistent with the observation that the PI3K/AKT/mTOR signaling pathway exerts essential roles in the growth of MESO [34]. The results suggest that baicalin increases the anti-proliferation effects through blockage of the PI3K/AKT/mTOR signaling pathway. Although other inhibitors of the PI3K/AKT/mTOR signaling pathway were not assessed in the present study, our results suggest that treatment of MESO patients with baicalin could decrease the chemo-resistance phenotype of some therapeutic agents [35].

Bcl-2 family members, such as Bcl-2 and Bax, are the central regulators involved in apoptosis of cells. Interestingly, several studies showed that the levels of Bcl-2 and Bax play an important role in regulation of apoptotic signaling. Furthermore, some studies have demonstrated that both Bcl-2 and Bax exert important functions in the progression, development, and chemo-resistance of MESO [36,37]. Therefore, drugs that target Bcl-2/Bax might be effective in MESO treatment and alleviate chemotherapy resistance caused by over-expression of Bcl-2 [38]. In the present work, treatment of MESO924 cells with baicalin decreased the expression of *Bcl-2* (~25–60% reduction of mRNA) and increased the expression of *Bax* (~1.4–2-fold increase of mRNA) in a dose-dependent manner, suggesting that baicalin-induced apoptosis of MESO cells might be caused by the regulation of Bcl-2 and Bax. Additionally, the combined treatment of MESO924 cells with baicalin and chemotherapeutic drugs (doxorubicin and cisplatin) enhanced inhibition of the proliferation of MESO924 cells. Our results indicate that baicalin decreased the resistance of MESO to chemotherapeutic drugs (doxorubicin and cisplatin) and could provide a novel strategy for the treatment of drug-resistant mesothelioma.

As an effective angiogenic factor, VEGF is related to the occurrence and development of cancers. VEGF exerts a key role in the proliferation, apoptosis, migration and invasion of multiple myeloma by increasing the expression of MCL-1 and interleukin-6 (IL-6) and activating the PI3K/AKT/mTOR pathway [38,39]. Thus, agents targeting VEGF could improve the efficacy of anti-tumor drugs [19]. Previous studies showed that the anti-myeloma effects of thalidomide and lenalidomide were partly attributed to the downregulation of VEGF [40,41]. In this work, the treatment of MESO924 with baicalin decreased the expression of VEGF (~34–70% reduction of mRNA) in a dose-dependent manner, suggesting that baicalin-induced inhibition of proliferation, migration and invasion while increases of apoptosis on MESO924 might be caused by the attenuation of VEGF. Taken together, our results indicated that baicalin could be a potential anti-tumor drug for the treatment of metastatic mesothelioma.

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The present investigation achieved many important results; however, many limitations need to be considered. Firstly, our results showed that some classical functional genes related to tumorigenesis and disease progression were regulated by baicalin. Actually, baicalin might function via complicated intracellular effects to regulate the phenotype of mesothelioma, but in this study, we just focused on Bcl-2, Bax, and VEGF. Therefore, more studies are needed to investigate the other effectors of the action of baicalin against mesothelioma. Secondly, the results of our current work showed that the decreased PI3K/AKT/mTOR activity might be one of the mechanisms that regulated the phenotype of mesothelioma cells induced by baicalin. However, it is unclear whether other signaling pathways were involved in the change of mesothelioma phenotype induced by baicalin, and this needs to be investigated in further research. Thirdly, different types of MESO cell lines might respond to baicalin differently; however, we are unable to obtain additional cell lines besides MESO924, so additional research focused on other mesothelioma cell lines is needed.

Conclusions

Baicalin inhibited the proliferation, cell cycle, migration, and invasion of human mesothelioma MESO924 cells, and promoted apoptosis by downregulating the expression of *Bcl-2* and *VEGF* and upregulating the expression of *Bax*. In addition, Baicalin increased the sensitivity of MESO924 cells to chemotherapeutic drugs. Baicalin decreased the expression of p-EGFR, p-AKT, p-MAPK, and p-S6 in the PI3K/AKT signaling pathway in MESO924 cells. Blockage of the PI3K/AKT signaling pathway by a small molecule, LY294002, augmented the inhibition of proliferation induced by baicalin. Together, these findings show that baicalin might be a useful drug for the treatment of mesothelioma.

Conflict of interest

None.

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