Shikonin inhibits proliferation and induces apoptosis in glioma cells via downregulation of CD147

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Abstract. Shikonin, a traditional Chinese medicine, has been identified as being capable of inducing apoptosis in various tumors, including glioma, and is thus considered to be a promising therapeutic agent for tumor therapy. However, little is known about the molecular mechanism of shikonin in glioma. The present study investigated the influence of shikonin on the proliferation and apoptosis of glioma cells U251 and U87MG and explored the potential molecular mechanisms. It was identified that shikonin was able to induce apoptosis in human glioma cells in a time- and dose-dependent manner, and a decreased expression level of cluster of differentiation (CD)147 was observed in shikonin-treated U251 and U87MG cells. Knockdown of CD147 inhibited U251 and U87MG cell growth, whereas CD147 overexpression enhanced cell growth and decreased shikonin-induced apoptosis. Additionally, an increased expression level of CD147 suppressed the elevated production of reactive oxygen species and mitochondrial membrane potential levels induced by shikonin. The data indicated that shikonin was able to induce apoptosis in human glioma cells in a time- and dose-dependent manner, and a decreased expression level of cluster of differentiation (CD)147 was observed in shikonin-treated U251 and U87MG cells. Knockdown of CD147 inhibited U251 and U87MG cell growth, whereas CD147 overexpression enhanced cell growth and decreased shikonin-induced apoptosis. Additionally, an increased expression level of CD147 suppressed the elevated production of reactive oxygen species and mitochondrial membrane potential levels induced by shikonin. The data indicated that shikonin-induced apoptosis in glioma cells was associated with the downregulation of CD147 and the upregulation of oxidative stress. CD147 may be an optional target of shikonin-induced cell apoptosis in glioma cells.

Introduction

Glioma is one of the most common primary central nervous system tumors (1). Characterized by strong proliferation and intensive invasion, glioma cells always infiltrate into the surrounding normal brain tissues, which makes glioma difficult to eradicate completely by traditional surgical resection (2). Thus, comprehensive therapy, including radiotherapy and chemotherapy, is used in order to delay the recurrence and prolong survival rates (3,4). However, the prognosis of glioma remains poor, mainly due to its complex pathogenesis (5). To date, no effective treatment for glioma has been identified, and the search for new treatment options is thus necessary. Illuminating the possible molecular mechanism is also an important aspect for the treatment of glioma.

Shikonin, an active naphthoquinone extracted from the traditional Chinese herb Zicao, was originally used in traditional medicine as an ointment to treat wound healing, due to its anti-inflammatory properties (6,7). Recently, an increasing number of studies have indicated that shikonin may exert anti-tumor functions in cancer cells, including glioma (8-10). Marked inhibition of proliferation and the induction of apoptosis have been identified in shikonin-treated glioma cells, suggesting that shikonin may serve as a potential therapeutic agent for glioma (11,12). However, little is known about the molecular mechanism of shikonin in glioma.

Cluster of differentiation (CD), a transmembrane glycoprotein of the immunoglobulin superfamily, is upregulated in diverse human tumor cells and has been validated as a therapeutic target (13). Previous studies have demonstrated that CD147 is rarely expressed on normal brain cells but highly expressed on glioma cells, and that the expression of CD147 is significantly associated with World Health Organization tumor grade, indicating a potential role of CD147 in the occurrence and progress of glioma (14). An in vitro study further demonstrated that silencing of CD147 inhibits proliferation and induces apoptosis in glioma cells (15). However, whether CD147 is involved in shikonin-induced glioma cell apoptosis remains to be elucidated.

The present study hypothesized that CD147 may be an optional target of shikonin-induced cell apoptosis in glioma cells. It investigated the influence of shikonin on the
proliferation and apoptosis of glioma cells and examined the potential molecular mechanisms. The results may be of benefit in developing improved therapies for glioma.

Materials and methods

Cell culture. Human U251 and U87MG (ATCC® HTB14™, glioblastoma of unknown origin) cell lines were purchased from the American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle's medium (DMEM; high glucose) medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 1% penicillin/streptomycin, 2% L-glutamine and 10% fetal calf serum (FCS; HyClone; GE Healthcare Life Sciences, Logan, UT, USA) at 37°C in an atmosphere humidified with 5% CO₂. 

Cells in the logarithmic growth phase were collected for experimentation.

Monitoring cell proliferation using the xCELLigence system. U251 and U87MG cells were harvested, washed and resuspended in the DMEM with 10% FCS (HyClone; GE Healthcare Life Sciences, Logan, UT, USA) at 37°C in an atmosphere humidified with 5% CO₂. The cells were counted to 3x10⁵ cells/ml and cultured with different concentrations of shikonin (0.1, 0.5, 1, 2, 3 and 4 µM) for 24 h at 37°C. Subsequently, CCK-8 solution (10 µl/well; Beyotime Institute of Biotechnology, Haimen, China) was added and the plate placed into the xCELLigence system. Scans were run with sweeps every min for the first 6 h. Subsequent sweeps were taken every 30 min for 72 h.

Cell Counting Kit-8 (CCK-8) assay. U251 cells were plated on a 96-well plate at a concentration of 1x10⁵ cells/ml and cultured with different concentrations of shikonin (0.1, 0.5, 1, 2, 3 and 4 µM) for 24 h at 37°C. Subsequently, CCK-8 solution (10 µl/well; Beyotime Institute of Biotechnology, Haimen, China) was added and the plate was incubated at 37°C for 1 h. The cells were counted by absorbance measurements at a wavelength of 450 nm.

Cell apoptosis assay. U251 cells were plated at a seeding density of 1x10⁵ cells/ml in a 24-well plate and treated with different concentrations of shikonin (0.1, 0.5, 1, 2, 3 and 4 µM) for 24 h at 37°C. The cells were collected and washed twice in cold PBS. The cells were mixed in 100 µl 1X binding buffer and incubated with 5 µl Annexin V (BD Pharmingen; BD Biosciences, San Jose, CA, USA) at room temperature for 15 min in the dark. Subsequently, 5 µl propidium iodide (PI; BD Pharmingen; BD Biosciences) was added prior to detection by flow cytometry. The percentages of apoptotic cells were calculated using FlowJo 7.6.1 (FlowJo LLC, Ashland, OR, USA).

Knockdown and overexpression of CD147 by RNA interference. To knock down the expression of CD147 in U251 and U87MG cells, the trans-lentiviral pLKO System (Shanghai GeneChem Co., Ltd. Shanghai, China) was used to package the lentivirus and acquire the CD147 knockdown cells (U251-KD and U87MG-KD). The negative control cells were designated U251-NC and U87MG-NC. Polybrene (1 µg/ml; Shanghai GeneChem Co., Ltd.) was used to increase the transfection efficiency. The experiments were performed as previously described (16), with a multiplicity of infection (MOI) for U251 and U87MG cells of 10 and 20, respectively. To upregulate the expression of CD147 in cells, the overexpression lentivirus was produced by Shanghai GeneChem Co., Ltd. and transected into the U251 cells (MOI =5) and U87MG cells (MOI =10) to acquire the CD147 overexpression cells (U251-OE and U87MG-OE). According to the manufacturer’s protocols, the cells infected with the lentivirus were selected for puromycin resistance in DMEM containing 2 µg/ml puromycin (Sigma-Aldrich; Merck KGaA) for 7-10 days. Following selection, the cells were maintained in medium containing 1 µg/ml puromycin for 2 weeks until the final stable cell clones were harvested and verified by determining the expression of CD147 via flow cytometry.

Flow cytometric analysis. The expression of CD147 in U251 and U87MG cells was assessed by flow cytometry. Following transfection for 3 weeks, cells were fixed with 4% paraformaldehyde for 20 min at room temperature at a concentration of 1x10⁶ cells/ml. The cells were blocked with 1% bovine serum albumin (BSA; Beyotime Institute of Biotechnology) for 1 h at room temperature and incubated with CD147-FITC (BD Pharmingen; BD Biosciences) for 30 min at room temperature. Following washing with PBS, stained cells were analyzed on a Fluorescence Activated Cell Sorter flow cytometer (BD Accuri C6 with CFlow Version 1.0.264.15 software, BD Biosciences).

Western blot analysis. Cells (1x10⁶) were collected, washed twice with ice-cold PBS and lysed in 100 µl RIPA buffer (Beyotime Institute of Biotechnology) supplemented with protease inhibitor (Sigma-Aldrich; Merck KGaA) for 30 min. A Bicinchoninic Acid Protein Assay kit (Pierce; Thermo Fisher Scientific, Inc.) was used to determine the total protein concentration. Protein samples (30 µg/lane) were separated by 10% SDS-PAGE, and then transferred onto polyvinylidene fluoride membranes (Sigma-Aldrich; Merck KGaA). Following blocking with 5% milk for 1 h at room temperature, the membranes were incubated overnight at 4°C with mouse anti-human CD147 primary antibody (1:1,000; ab666, Abcam, Cambridge, MA, USA). β-tubulin was used as the loading control (1:2,000; ab44928, Abcam). The blots were extensively washed with PBS with 5% Tween-20 and incubated with goat anti-mouse horseradish peroxidase-conjugated secondary antibody (1:3,000; ab205719, Abcam) for 1 h at room temperature. Following washing, the blots were visualized with BeyoECL Plus reagent (Beyotime Institute of Biotechnology) using a ChemiDoc™ detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Protein expression was quantified using ImageJ v1.50i (National Institutes of Health, Bethesda, MD, USA).

Measuring intracellular reactive oxygen species (ROS). The redox-sensitive dye dichloro-dihydro-fluorescein diacetate
(DCFH-DA: Beyotime Institute of Biotechnology) was used to evaluate the levels of intracellular ROS. Briefly, 1x10⁶ cells were incubated with 1% Triton X-100 following staining with 2 μmol DCFH-DA in the dark at room temperature for 20 min. Subsequently, the cells were washed with PBS and resuspended in RPMI-1640 culture medium (Gibco; Thermo Fisher Scientific, Inc.) containing different concentrations of shikonin (0.5, 1 and 2 μM) at 37°C for 2 h. The fluorescence was measured at an excitation wavelength of 488 nm and an emission wavelength of 530 nm using a fluorescence spectrometer.

**Measuring mitochondrial membrane potential (MMP).** The fluorochrome dye Rh123 (Beyotime Institute of Biotechnology) was used to evaluate the alterations in MMP. Briefly, U251 cells were incubated with different concentrations of shikonin (0.5, 1 and 2 μM) at 37°C for 24 h. Following incubation, cells were collected and stained with 5 μg/ml Rh123 at 37°C for 10 min. Cells were harvested and washed twice, and resuspended in 500 μl RPMI-1640 culture medium. The fluorescence intensity of cells was quantitatively analyzed by flow cytometry (BD Accuri C6 with CFlow Version 1.0.264.15 software).

**Statistical analysis.** The results are presented as the mean ± standard deviation from ≥3 independent experiments. Comparisons between two groups with respect to ROS generation and MMP (U251NC vs. U251NC+S, U251KD vs. U251KD+S and U251OE vs. U251OE+S) was performed using a Student's t-test, and comparisons between multiple groups were performed using one-way analysis of variance followed by a Student-Newman-Keuls test. Data analyses were performed using Graph Pad Prism version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). P<0.05 was considered to indicate a statistically significant difference.

**Results**

**Shikonin inhibits U251 cell proliferation in a dose- and time-dependent manner.** An RTCA was used to assess the inhibitory effect of shikonin on U251 cell proliferation. The dynamic pattern of cell index (CI) curves for different shikonin concentrations on U251 cells are presented in Fig. 1A. The CI was further compared between the different concentration groups and the DMSO control group at the time point of 24 h, and it was identified that CI was decreased significantly in a dose-dependent manner (Fig. 1B). It was also investigated whether the effect of shikonin on cell viability was time-dependent. At a concentration of 1 μM, shikonin exhibited a significant inhibitory effect from 6 h, and the inhibitory rate gradually increased with time, indicating a time-dependent effect of shikonin in U251 cells (Fig. 1C). The half-maximal inhibitory concentration (IC₅₀) value of shikonin for U251 cells following 24 h of treatment was 2.39x10⁻⁶ M (Fig. 1D).

**Shikonin induces apoptosis in human glioma U251 cells.** To further investigate whether shikonin inhibited U251 cell growth through the induction of apoptosis, the percentage of apoptotic cells was calculated by flow cytometry using the Annexin V/PI double staining assay following treatment of the cells with various doses of shikonin. The representative flow cytometry data are presented in Fig. 2A. It was demonstrated that treatment with 0.5-4 μM shikonin for 24 h significantly increased the numbers of apoptotic cells compared with the control group (Fig. 2B), and the numbers of apoptotic cells increased in a dose-dependent manner. Shikonin-induced cell death was also tested by CCK-8. As presented in Fig. 2C, shikonin exhibited significant cell growth inhibition at each concentration, 0.5-4 μM, similar to the apoptosis data. These results indicated that the apoptotic pathway serves a pivotal role in the anti-proliferative effect of shikonin on U251 cells.

**CD147 expression is downregulated in glioma cells under shikonin treatment.** The expression of CD147 identified to be involved in tumor cell proliferation was examined to study the molecular mechanism underlying the inhibitory effects of shikonin on U251 and U87MG cell viability. As presented in Fig. 3A-D, fluorescence-activated cell sorting analysis for CD147 expression demonstrated a gradual decrease in U251 and U87MG cells treated with shikonin compared with the untreated cells. Western blot analysis confirmed that with the increase in shikonin concentration, the expression of CD147 was decreased (Fig. 3E-H).

**Knocking-down of CD147 inhibits glioma cell growth, whereas CD147 overexpression enhances cell growth in glioma cells.** To examine whether altered CD147 expression affects glioma growth, CD147 was knocked down and overexpressed in U251 and U87MG cells, respectively, using a lentivirus system. As indicated in Fig. 4A and B, following transfection with CD147 KD and CD147 OE viruses, CD147 was identified to be significantly knocked down in U251-KD cells and overexpressed in U251-OE cells. The cell viability of each cell line was evaluated by means of RTCA for 72 h. U251-KD cells had lower CI values when compared with the CI index values of U251-NC cells, which indicated a reduced cell viability (Fig. 4C and D). However, increased cell viability was observed in U251-OE cells, which exhibited relatively high CI values compared with those of U251-NC cells (Fig. 4C and D). Similar results were obtained in U87MG-KD and U87MG-OE cells (Fig. 4E-H). All the data suggested a positive role for CD147 in glioma cell proliferation.

**Overexpression of CD147 resists shikonin-induced U251 cell death.** It was further investigated whether CD147 was involved in shikonin-induced cell death in glioma cells. As presented in Fig. 5A and B, downregulation of CD147 exerted a significant inhibitory effect on U251 cell proliferation, similar to the cells treated with shikonin. Comparatively strong cell growth inhibition was observed in shikonin-induced U251 cells with the passage of time. When treated with shikonin, U251-NC cells demonstrated decreased CI values compared with those of CD147-overexpressing U251-OE cells (Fig. 5C and D).

**Shikonin induces apoptosis in U251 glioma cells through ROS generation and mitochondrial dysfunction via CD147 expression.** Shikonin has been reported to dysregulate cellular ROS levels and MMP, which ultimately induces cell apoptosis (17). Therefore, the intracellular ROS generation and...
Figure 1. Shikonin inhibits U251 cell proliferation in a time- and dose-dependent manner. Cells were plated at a seeding density of 2x10^4 cells in an E-Plate in Dulbecco's modified Eagle's medium containing a range of shikonin concentrations (0-4 µmol/l). Proliferation was monitored in real time using the xCELLigence system. Readings were taken every min for 6 h and then every 30 min up to 72 h, with readings expressed as cell index values. (A) The xCELLigence graph is representative of duplicate wells comparing the effect of different shikonin concentrations on U251 cell proliferation for 72 h. (B) Shikonin inhibited U251 cell proliferation in a dose-dependent manner. (C) Shikonin (1 µM) inhibited U251 cell proliferation in a time-dependent manner. (D) The IC_{50} values of shikonin for U251 cells were calculated. The data are presented as the mean ± standard deviation of three tests. **P<0.01 and ***P<0.001 vs. DMSO control group. DMSO, dimethyl sulfoxide.

Figure 2. Shikonin induces U251 cell death, partly by apoptosis. Cells were plated at a seeding density of 1x10^5 cells in 24-well plate and treated with different concentrations of shikonin for 24 h; cell death was detected using an Annexin V/PI staining assay. (A) Representative image of flow cytometry of U251 glioma cells. (B) Shikonin induced U251 cell apoptosis in a dose-dependent manner. (C) U251 cell proliferation was detected by Cell Counting Kit-8 assay. The data are presented as the mean ± standard deviation of three tests. **P<0.01 and ***P<0.001 vs. DMSO control group. DMSO, dimethyl sulfoxide; FITC, fluorescein isothiocyanate; PI, propidium iodide; S, shikonin.
Figure 3. Effects of shikonin on CD147 expression. Cells were plated at a seeding density of 1x10^5 cells in 24-well plate and treated with 0.5, 2 and 4 µM shikonin for 24 h, respectively. The expression of CD147 was detected using flow cytometry and western blotting. (A) Representative image of flow cytometry of U251 glioma cells. (B) Quantitative analysis of the FACS data demonstrated a significantly decrease in CD147 in shikonin-treated cells compared with untreated U251 cells. (C) Representative image of flow cytometry of U87MG glioma cells. (D) Quantitative analysis of the FACS data demonstrated a significantly decrease of CD147 in shikonin treated cells compared with untreated U87MG cells. (E) Representative image of western blotting of U251 glioma cells. (F) Western blot analysis revealed reduced CD147 expression in shikonin-treated U251 cells. (G) Representative image of western blotting of U87MG glioma cells. (H) Western blot analysis revealed reduced CD147 expression in shikonin-treated U87MG cells. The data are presented as the mean ± standard deviation of three tests. *P<0.05, **P<0.01 and ***P<0.001 vs. respective control group. CD, cluster of differentiation; NC, negative control; S, shikonin; FITC, fluorescein isothiocyanate; FACS, fluorescence-activated cell sorting.
MMP levels were detected in U251 glioma cells. Treatment with shikonin markedly increased intracellular ROS generation in a dose-dependent manner (Fig. 6A and B), as measured by fluorescence staining. Furthermore, it was identified that downregulation of CD147 in U251 glioma cells exhibited increased intracellular ROS and MMP levels, which further increased following the treatment with shikonin (Fig. 6C and D). However overexpression of CD147 was indicated to defend against the effect of shikonin on the intracellular ROS and MMP levels in U251 glioma cells (Fig. 6C and D). The statistical data are presented in Fig. 6E and F. All the data suggested that shikonin caused mitochondrial dysfunction, partly via regulation of CD147 expression.
Discussion

The present study identified the reduced cell proliferation and decreased expression level of CD147 caused by shikonin in U251 cells. Shikonin was able to induce apoptosis in human glioma U251 cells in a dose-dependent manner, and overexpression of CD147 resisted shikonin-induced U251 apoptosis. Additionally, an increased expression level of CD147 suppressed the elevated production of ROS and MMP levels induced by shikonin. The results of the present study indicated that shikonin-induced apoptosis in glioma cells was associated with the downregulation of CD147 and upregulation of oxidative stress.

As an anthraquinone compound derived from the roots of *Lithospermum erythrorhizon*, shikonin exhibits a broad spectrum of anti-tumor effects by inducing apoptosis in cancer of the gallbladder (18), pancreas (19), colon (20) and breast (21). The present study also demonstrated that shikonin inhibited glioma U251 cell proliferation in a dose- and time-dependent manner. At a concentration of 1 µM, shikonin exhibited a significant inhibitory effect from 6 h, and the IC$_{50}$ value of shikonin for U251 cells following 24 h treatment was 2.39x10$^{-6}$ M. Furthermore, treatment with 0.5-4 µM shikonin for 24 h significantly increased the number of apoptotic cells, and this result was similar to that for cell growth inhibition. These findings indicated that the apoptotic pathway serves a pivotal role in the anti-proliferative effect of shikonin on glioma cells. Lu *et al* (22) reported that shikonin induced glioma cell necroptosis in vitro, as PI positive cells following shikonin (20 µM) treatment did not exhibit apoptotic features, such as nuclear condensation or fragmentation. This may be due to the different concentrations of shikonin used in the studies. The present study identified that a high concentration of shikonin demonstrated characteristics of necrosis, whereas a low concentration demonstrated characteristics of apoptosis (data not shown). In spite of the large number of studies indicating that shikonin induces apoptosis in various human tumor cell lines (8,9,11), the molecular mechanism of the potential anti-glioma effect of shikonin is not completely understood.

Apoptosis, a type of programmed cell death, is regulated by multiple genes and protein factors (23); ROS generation has been demonstrated to serve a critical role in this process (24). As dysfunctional mitochondria are thought to be one of the principal sources of intracellular ROS (25), the present study detected the cellular ROS levels and MMP when treated with shikonin, and identified the disrupted MMP and increased intracellular ROS generation in glioma U251 cells. As in hepatoma cells, shikonin generates large amounts of intracellular ROS during the early stage of the apoptotic process (26). These findings demonstrated that shikonin may target mitochondria and cause mitochondrial dysfunction, further increasing ROS generation and ultimately inducing apoptosis.

A number of studies have demonstrated that CD147 serves an important role in glioma (15,27). The results of the present study also proved that CD147 was able to induce apoptosis in glioma cells. Yin *et al* (14) demonstrated that down-regulation of CD147 induces apoptosis in glioma. The present study hypothesized that CD147 may be an optional target of shikonin-induced cell apoptosis on glioma cells. CD147 expression was first detected when glioma cells were treated with shikonin, and it was identified that CD147 expression was downregulated in glioma cells under shikonin treatment.
Furthermore, overexpression of CD147 in glioma cells resisted shikonin-induced cell death and reduced the effect of shikonin on the intracellular ROS and MMP levels. In summary, the present study suggested that shikonin induced apoptosis in glioma via the regulation of CD147 expression.

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Availability of data and materials

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

Authors' contributions

NG and RM were responsible for the analysis and interpretation of data of the manuscript; NG, XCGa, DH and ZFH performed the majority of the experiments; XCGo and FLJ were responsible for design of the study and drafting of the manuscript. YN and NCJ contributed to the acquisition, analysis, and interpretation of data for the study and revised the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P and Ellison DW: The 2016 World Health Organization classification of tumors of the central nervous system. A summary. Acta Neuropathol 131: 803-820, 2016.

2. Aldape K, Zadeh G, Mansouri S, Reifenberger G and von Deimling A: Glioblastoma: Pathology, molecular mechanisms and markers. Acta Neuropathol 129: 829-848, 2015.

3. Hottinger AF, Aissa AB, Espeli V, Squiban D, Dunkel N, Vargaset MI, Hundsfelder T, Mach N, Schaller K, Weber DC, et al: Phase I study of sorafenib combined with radiation therapy and temozolomide as first-line treatment of high-grade glioma. Br J Cancer 110: 2655-2661, 2014.

4. Bush NA, Chang SM and Berger MS: Current and future strategies for treatment of glioma. Neurosurg Rev 40: 1-14, 2017.

5. Reifenberger G, Wirsching HG, Knobbe-Thomsen CB and Weller M: Advances in the molecular genetics of gliomas - implications for treatment of glioma. Neurosurg Rev 40: 1-14, 2017.

6. Andujar I, Rios JL, Giner RM and Recio MC: Pharmacological properties of shikonin - a review of literature since 2002. Planta Med 79: 1685-1697, 2013.

7. Zhang X, Cui JH, Meng QQ, Li SS, Zhou W and Xiao S: Advance in anti-tumor mechanisms of shikonin, alkalamin and their derivatives. Mini Rev Med Chem 18: 164-172, 2018.

8. Guo ZL, Li J, Ma YY, Qian D, Zhong JY, Jin MM, Huang P, Che LY, Pan B, Wang Y, et al: Shikonin sensitizes A549 cells to TRAIL-induced apoptosis through the JNK, STAT3 and AKT pathways. BMC Cell Biol 19: 29, 2018.

9. Durchschein C, Hufner A, Rinner B, Stallinger A, Deutsch A, Lohberger B, Bauer R and Kretschmer N: Synthesis of novel shikonin derivatives and pharmacological effects of cyclopropylacetetyl shikonin on melanoma cells. Molecules 23: E2820, 2018.

10. Zhang FY, Hu Y, Que ZY, Wang P, Liu YH, Wang ZH and Xue YX: Shikonin induces the migration and invasion of human glioblastoma cells by targeting phosphorylated β-Catenin and phosphorylated PI3K/Akt: A potential mechanism for the anti-glioma efficacy of a traditional Chinese herbal medicine. Int J Mol Sci 16: 23823-23848, 2015.

11. Zhang FL, Wang P, Liu YH, Liu LB, Liu XB, Li Z and Xue YX: Topoisomerase I inhibitors, shikonin and topotecan, inhibit growth and induce apoptosis of glioma cells and glioma stem cells. PLoS One 8: e68185, 2013.

12. Zhou Z, Lu B, Wang C, Wang Z, Piao T, Luo M, Meng F, Chi G, Luo Y and Ge P: RIP1 and RIP3 contribute to shikonin-induced DNA double-strand breaks in glioma cells via increase of intracellular reactive oxygen species. Cancer Lett 390: 77-90, 2017.

13. Xiong L, Edwards CR III and Zhou L: The biological function and clinical utilization of CD147 in human diseases: A review of the current scientific literature. Int J Mol Sci 15: 17411-17441, 2014.

14. Yin H, Shao Y and Chen X: The effects of CD147 on the cell proliferation, apoptosis, invasion, and angiogenesis in glioma. Neurol Sci 38: 129-136, 2017.

15. Li H, Xi Z, Dai X, Wu W, Li Y, Liu Y and Zhang H: CD147 and glioma: A meta-analysis. J Neurooncol 134: 145-156, 2017.

16. Guo N, Zhang K, Lv MH, Miao JL, Chen ZN and Zhu P: CD147 and CD98 complex-mediated homotypic aggregation attenuates the CyPA-induced chemotactic effect on Jurkat T cells. Mol Immunol 63: 253-263, 2015.

17. Wiencz B, Eichhorn T, Paulsen M and Effertth T: Shikonin directly targets mitochondria and causes mitochondrial dysfunction in cancer cells. Evid Based Complement Alternat Med 2012: 726025, 2012.

18. Zhai T, Hei Z, Ma Q, Liang H, Xu Y, Zhang Y, Jin L, Han C and Wang J: Shikonin induces apoptosis and G0/G1 phase arrest of gallbladder cancer cells via the JNK signaling pathway. Oncol Rep 38: 3473-3480, 2017.

19. Chen C, Xiao W, Huang L, Yu G, Ni J, Yang L, Wan R and Hu G: Shikonin induces apoptosis and necroptosis in pancreatic cancer via regulating the expression of RIP1/RIP3 and synergizes the activity of gemcitabine. Am J Transl Res 9: 5507-5517, 2017.

20. Liang W, Cui J, Zhang K, Xi H, Cai A, Li J, Gao Y, Hu C, Liu C, Li Y, Lu Y, et al: Shikonin induces ROS-based mitochondria-mediated apoptosis in colon cancer. Oncotarget 8: 109094-109106, 2017.

21. Shahsavari Z, Karami-Tehrani F and Salami S: Targeting cell necroptosis and apoptosis induced by Shikonin via receptor interacting protein kinases in estrogen receptor positive breast cancer cell line, MCF-7. Anticancer Agents Med Chem 18: 245-254, 2018.

22. Lu B, Gong X, Wang QZ, Ding Y, Wang C, Luo TF, Piao MH, Meng FK, Chi GF, Luo YN and Ge PF: Shikonin induces glioma cell necroptosis in vitro by ROS overproduction and promoting RIP1/RIP3 necroptosis formation. Acta Pharmacol Sin 38: 1543-1553, 2017.

23. Ondroušková E and Vojtěšek B: Programmed cell death in cancer cells. Klin Onkol 27 (Suppl 1): S7-S7, 2014.

24. Redža-Dutordoir M and Averill-Bates DA: Activation of apoptosis signalling pathways by reactive oxygen species. Biochim Biophys Acta 1863; 2977-2992, 2016.

25. Balaban RS, Nemoto S and Finkel T: Mitochondria, oxidants, and aging. Cell 120: 483-495, 2005.

26. Balaban SS, Nemoto S and Finkel T: Mitochondria, oxidants, and aging. Cell 120: 483-495, 2005.

27. Fei L, Li S, Fei Z and Chen ZN: The roles of CD147 in the progression of gliomas. Expert Rev Anticancer Ther 15: 1351-1359, 2015.

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