

Umbelliferone inhibits proliferation and metastasis via modulating cadherin/ β -catenin complex-aided cell-cell adhesion in glioblastoma cells

Wei Ma^{1,A–C}, Hangyu Shi^{2,C,D}, Xinya Dong^{2,B,C,E}, Yongqiang Shi^{2,B–D}, Luyi Zhang^{2,A,B,D}, Bin Jiang^{2,A,F}

¹ Department of Neurosurgery, The Second Affiliated Hospital of Air Force Medical University, Xi'an, China

² Department of Neurosurgery, The Affiliated Children's Hospital of Xi'an Jiaotong University, Xi'an Children's Hospital, China

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;

D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online)

Adv Clin Exp Med. 2025;34(8):1383–1392

Address for correspondence

Bin Jiang

E-mail: jiangbin1984@outlook.com

Funding sources

Natural Science Basic Research Program of Shaanxi Province (grant No. 2022JM-595).

Conflict of interest

None declared

Received on January 29, 2024

Reviewed on April 2, 2024

Accepted on August 22, 2024

Published online on January 10, 2025

Abstract

Background. Glioblastoma multiforme (GBM) is the most aggressive brain tumor malignancy in adults, accounting for nearly 50% of all gliomas. Current medications for GBM frequently lead to drug resistance.

Objectives. Umbelliferone (UMB) is found extensively in many plants and shows numerous pharmacological actions against inflammation, degenerative diseases and cancers. However, its anticancer effects on GBM cells have not yet been explored.

Materials and methods. This research intended to assess the antitumor efficacy of UMB and the molecular mechanism of cell–cell adhesion proteins in human U-87 GBM cells. The cytotoxicity assay, intracellular reactive oxygen species (ROS), cell adhesion proteins, and cell apoptosis actions of UMB were assessed using 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT), dichlorodihydrofluorescein diacetate (DCFH-DA), 4',6-diamidino-2-phenylindole (DAPI), acridine orange/ethidium bromide (AO/EB), and western blot.

Results. The findings revealed that UMB reduced the proliferation of GBM cells and cell adhesion proteins, while augmenting apoptosis through the elevation of cellular ROS. Bcl-2 family protein levels of Bcl-2 and Bcl-XL were mitigated; conversely, the pro-apoptotic proteins Bad and Bim were elevated upon treatment with UMB in a quantity-dependent way. Furthermore, UMB-treated GBM cells suppressed N-cadherin, β -catenin, Slug, and matrix metalloproteinase 2 (MMP-2) expression, whereas they showed enhanced TIMP protein and E-cadherin levels.

Conclusions. Our findings suggest that UMB can prevent proliferation and metastasis and stimulate apoptosis in GBM cells.

Key words: glioblastoma, proliferation, apoptosis, umbelliferone, cell adhesion proteins

Cite as

Ma W, Shi H, Dong X, Shi Y, Zhang L, Jiang B. Umbelliferone inhibits proliferation and metastasis via modulating cadherin/ β -catenin complex-aided cell-cell adhesion in glioblastoma cells. *Adv Clin Exp Med.* 2025;34(8):1383–1392. doi:10.17219/acem/192547

DOI

10.17219/acem/192547

Copyright

Copyright by Author(s)

This is an article distributed under the terms of the Creative Commons Attribution 3.0 Unported (CC BY 3.0) (<https://creativecommons.org/licenses/by/3.0/>)

Background

Gliomas are the predominant kind of malignancy in the central nervous system and are accountable for a significant number of human deaths.¹ Gliomas comprise 24.7% of all primary brain tumors and 74.6% of all malignancies, according to the American Brain Tumor Association (ABTA).² Glioma patients have an extremely low survival rate; with current treatments, the average survival duration for low-grade gliomas is less than 60 months, and for advanced phases, it is fewer than 15 months.³ As a recurrent brain tumor in adults, glioblastoma multiforme (GBM) is an extremely aggressive malignancy owing to its highly invasive and vascularizing nature.⁴ Currently, available standard therapy for GBM is surgical resection with subsequent radiotherapy and temozolomide chemotherapy.⁵ Because of the level of distortion and chemoresistance, GBM certainly relapses despite recent advancements in these treatments.⁶ Thus, it is urgently required to advance innovative therapeutic approaches for the management and improvement of GBM survival rates.

Reactive metabolic byproducts, such as reactive oxygen species (ROS), greatly influence both harmful and positive actions. Reactive oxygen species in cells act as secondary messengers in signaling cascades that are a threat to usual physiological actions, including development and differentiation.^{7,8} Conversely, hypergeneration of ROS can impair biomolecules,⁹ which leads to cell integrity harm followed by cell pathology. Recently, ROS have been shown to promote tumorigenesis, angiogenesis and metastasis.¹⁰ However, extreme accumulation of ROS has been recognized to bring about apoptotic cell death.¹¹ Metastasis is the primary cause of cancer-associated mortality,¹² along with catastrophic organ dysfunction following the establishment and uncontrolled progression of exogenous cancer cells surrounded by normal tissue. Adhesion and cell–cell interaction are crucial tools for effective tissue function and homeostasis by describing compartmentalization and polarity in cells.¹³ Among the numerous adhesion molecules, the key group is the cadherins superfamily, which are transmembrane proteins that are essential in the development of adherens junctions.¹⁴ Matrix metalloproteinases (MMPs) are endopeptidases in a zinc-dependent family that are responsible for destroying constituents of the extracellular matrix (ECM),¹⁵ tumor cell incursion, angiogenesis, and the subduing of antitumor immune surveillance.¹⁶ There are natural endogenous secreted proteins comprising tissue inhibitors of metalloproteinases (TIMPs) that constrain the actions of MMPs.¹⁷ Hence, TIMPs control cellular functions, including glioma proliferation, movement and survival, by regulating ECM deprivation through communication with MMPs.¹⁸

Natural herbal constituents have been shown to be vital sources of medicines and models for drug design. The well-known 7-hydroxycoumarin umbelliferone (UMB) is extensively found in numerous popular plants from

the Umbelliferae family comprising garden angelica, coriander, carrot, etc. It displays numerous pharmacological actions against microbial infections, inflammation, degenerative diseases, and cancers.¹⁹ It employs anticancer activities against colon cancer,²⁰ laryngeal cancer²¹ and liver cancer.²²

Objectives

This report was intended to explore the anticancer efficacy of UMB in terms of cytotoxicity, apoptosis and metastasis in the most commonly studied GBM cells.

Materials and methods

Chemicals

Umbelliferone, Dulbecco's modified Eagle's medium (DMEM), foetal bovine serum (FBS), antibiotics, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2'-7'-dichlorodihydrofluorescein diacetate (DCFH-DA), 6-diamidino-2-phenylindole (DAPI), sodium dodecyl sulphate (SDS), dimethyl sulphoxide (DMSO), and phosphate-buffered saline (PBS) were acquired from Gibco (Waltham, USA). The primary and secondary antibodies for Western blot analysis were purchased from Beyotime Biotechnology (Beijing, China). Analytical-grade biochemicals and solvents were used.

Cell culture

Human U-87 GBM cells were acquired from Shanghai Aiyuan Biotechnology Co., Ltd (Shanghai, China) and cultured in DMEM medium, which contained 10% FBS, streptomycin (100 µg/mL) and penicillin (100 U/mL) in a 5% CO₂ atmosphere with below 95% humidity at 37°C.

Cell proliferation assay

Human glioma cell viability was evaluated using the MTT test.²³ Briefly, U-87 GBM cells were sowed into 96-well plates (1×10⁵ cells/well) and cultured at 37°C in a 5% CO₂ wet incubator. Once they were incubated overnight, the medium was separated, washed with PBS in the cells and incubated through different concentrations of UMB (5–60 µM/mL) for 1 day. Then, a 10 µL solution of MTT was supplemented to the treated cells and sustained for another 4 h. Subsequently, crystals of formazan were dissolved by treating with DMSO (150 µL). The optical density (OD) was determined at 490 nm using a multifunctional plate reader (BD Biosciences, Franklin Lakes, USA). Cell proliferation was measured as a percentage of viability against control GBM cells (100%). The IC₅₀ value was determined using the method shown below:

cell viability inhibition (%) = (control OD – test OD) × 100.

Determination of intracellular ROS

Human glioma cells were sowed in 6-well plates each and kept for 1 day; then, different concentrations of UMB (30 or 40 $\mu\text{M}/\text{mL}$) were added. Next, the control and treated cells were stained with 10 μM of DCFH-DA and preserved for 30 min at 37°C. The stained cells were washed with ice-cold PBS twice to remove any excess dye. The excitation and emission fluorescence were measured using a PR 4100 microplate reader, which is a next-generation 8-channel absorbance microplate reader (Bio-Rad Laboratories India Pvt. Ltd., Gurgaon, India), as described previously.²⁴

Evaluation of apoptosis using dual staining with AO/EB

The apoptotic morphology of the human U87 cells after exposure to UMB was evaluated using acridine orange/ethidium bromide (AO/EB) staining.²⁵ Glioblastoma multiforme cells were supplemented with different concentrations of UMB (30 or 40 $\mu\text{M}/\text{mL}$) and kept for 1 day. The UMB-supplemented and control cells were treated with an AO/EB (100 $\mu\text{g}/\text{mL}$ each dye) mixture. All groups were kept in the dark at room temperature for 20 min, ensuring unattached dye was separated using PBS and detected using a fluorescence microscope (model BX51; Olympus Corp., Tokyo, Japan).

Apoptosis assessed using DAPI staining

Human GBM cells were sowed at 1×10^5 cells per plate and added to UMB (30 or 40 $\mu\text{M}/\text{mL}$) and then fixed with 4% paraformaldehyde for 10 min at 37°C. These preserved GBM cells underwent DAPI staining to evaluate the nucleus changes related to apoptotic cell death using a method described previously.²⁶ Then, all samples were fixed on a glass slip and examined through a BX51 fluorescence microscope (Olympus Corp.).

Western blotting analysis

Human GBM cells were supplemented with 30 or 40 $\mu\text{M}/\text{mL}$ of UMB and cultivated for 1 day. The cell lysates were set with lysis buffer in ice-cold conditions, ensuring protease inhibitors, and western blot analysis was performed. Concisely, protein measurement was achieved using a Protein BCA Assay Kit (Pierce Chemical Co., Rockford, USA). After these were quantified, they were electrophoretically dispersed and moved into a polyvinylidene fluoride (PVDF) film. At that point, the film was blocked with a probe preserved at room temperature for 1 h, treated with primary antibodies in a 1:1,000 dilution (Bcl-XL, Bcl-2, Bim, Bad, N-cadherin, E-cadherin, β -catenin, MMP-2, Slug, TIMP-1, and TIMP-2), and set aside overnight at 4°C. Then, horseradish peroxidase (HRP)-conjugated secondary antibodies were added. The protein bands were successively stained

and then visualized. The protein band quantification was determined using densitometry with ImageJ software (National Institutes of Health (NIH), Bethesda, USA) and normalized to GAPDH expression.

Statistical analyses

The statistical analysis of the data from each group was conducted using GraphPad Prism v. 8.0.2 (GraphPad Software, San Diego, USA) and IBM SPSS v. 25.0 (IBM Corp., Armonk, USA). Measurement data were presented as medians (min–max). As the sample size was too small to verify normal data distribution, the differences between the groups were analyzed using the nonparametric Kruskal–Wallis test with Dunn's post hoc test. Subsequently, significant differences among multiple groups were examined using the Kruskal–Wallis test, and Dunn's post hoc test was employed for multiple comparisons. A statistically significant data divergence was considered when $p < 0.05$. All tests in this study were bilateral.

Results

Tables 1–3 show the results of comparing variables among groups.

Antiproliferative and cytotoxic effects of UMB on glioma cells

Human U-87 GBM cell viability was assessed using the MTT test with different concentrations (5–60 $\mu\text{M}/\text{mL}$) of UMB. The data revealed that UMB reduced viability through its cytotoxic and antiproliferative actions on GBM cells in a dose-dependent way. Umbelliferone treatment at a concentration of less than 10 μM did not expressly alter the antiproliferation and cytotoxicity results for GBM cells. Conversely, higher concentrations of UMB (30 and 40 $\mu\text{M}/\text{mL}$) substantially reduced ($p < 0.05$) the viability of both U-87 cells compared to an untreated control. Using MTT assay, we detected the IC₅₀ values of the GBM cells. Recognized based on the figure of the inhibitory concentration data of 30 and 40 $\mu\text{M}/\text{mL}$, UMB was taken for further trials (Table 1, Fig. 1).

Impact of UMB on intracellular ROS accumulation in human GBM cells

The formation of intracellular ROS is related to numerous stimuli, and it can trigger cell death and cell cycle arrest. Intracellular ROS levels were elevated in U-87 cells after being supplemented with 30 or 40 $\mu\text{M}/\text{mL}$ UMB in an amount-dependent way, in contrast to the control. To clearly distinguish the accumulation of ROS, the DCFH-DA-labeled cells were examined under a BX51 fluorescence microscope (Olympus Corp.). The intensity

Table 1. Groups compared with each other regarding MTT results

Variables	Control	5 μ M	10 μ M	20 μ M	30 μ M	40 μ M	50 μ M	60 μ M	Test value (H)**	p-value*
MTT	99.96 (90.95–108.95)	89.82 (81.73–97.89)	78.61 (71.53–85.67)	70.55 (64.17–76.87)	61.04 (55.54–66.52)	52.17 (47.46–56.84)	37.72 (34.33–41.11)	28.21 (25.66–30.74)	45.08	0.005

Data were presented as median (min–max); *p-value was generated from Kruskal–Wallis test with Dunn's post hoc test; **degrees of freedom (df) = 7. MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; H – total test values.

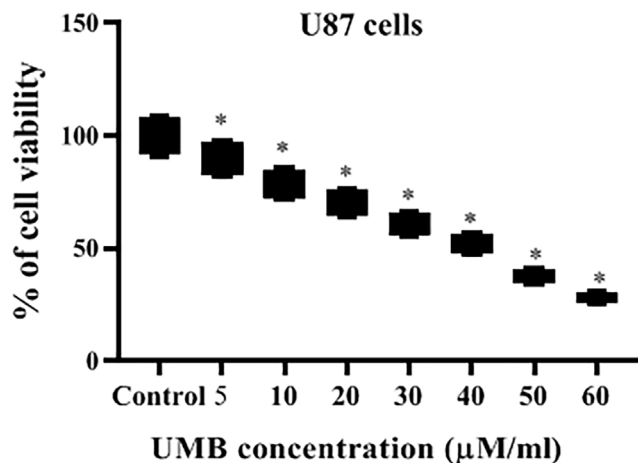


Fig. 1. Umbelliferone inhibits human GBM cell proliferation. Human U-87 GBM cells were supplemented with various concentrations (5–60 μ M/mL) of UMB for 1 day. Cell viability was determined using an MTT assay. Results are presented as medians (min–max) for triplicate trials. Significant differences compared to the untreated control are denoted by * ($p < 0.05$). The p-value was generated from the Kruskal–Wallis test with Dunn's post hoc test

GBM – glioblastoma multiforme; UMB – umbelliferone; MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

of ROS was greatly augmented with 40 μ M/mL of UMB (Table 2,3, Fig. 2).

Umbelliferone-induced apoptosis of human GBM cells revealed using dual staining with AO/EB

Apoptotic alterations in U-87 cells were visualized using dual staining with AO/EB. The GBM control cells exhibited evenly stained viable green cells (Table 2,3, Fig. 3A). The UMB-treated GBM cells presented higher apoptosis variations compared to the controls in a dose-dependent mode. Umbelliferone-treated (30 or 40 μ M/mL) U-87 cells revealed apoptotic cells, chromatin condensation and membrane blebbing in what seemed like light greenish-yellow dots. Supplementation with UMB at a dose of 40 μ M/mL exposed late apoptotic alterations in GBM cells, which presented as an orange-red color.

Table 2. Groups compared with each other regarding other parameters

Variables	Control (n = 6)	30 μ M (n = 6)	40 μ M (n = 6)	Test value (H)**	p-value*
ROS	7.03 (6.40–7.66)	23.40 (21.29–25.51)	34.90 (31.76–38.04)	15.15	<0.001
AO/EB staining	1.75 (1.59–1.91)	34.19 (31.11–37.27)	50.49 (45.95–55.03)	15.17	<0.001
DAPI staining	3.90 (3.55–4.25)	35.02 (31.87–38.17)	51.07 (46.47–55.67)	15.17	<0.001
Bcl-2	1.00 (0.91–1.09)	0.62 (0.56–0.68)	0.47 (0.43–0.51)	15.23	<0.001
Bcl-XL	1.00 (0.91–1.09)	0.68 (0.62–0.74)	0.50 (0.46–0.55)	15.22	<0.001
Bad	1.00 (0.91–1.09)	1.36 (1.24–1.48)	2.25 (2.05–2.45)	15.20	<0.001
Bim	1.00 (0.91–1.09)	1.40 (1.27–1.53)	1.96 (1.78–2.14)	15.20	<0.001
E-cadherin	1.00 (0.91–1.09)	1.37 (1.25–1.49)	2.15 (1.96–2.34)	15.20	<0.001
N-cadherin	1.00 (0.91–1.09)	0.80 (0.73–0.87)	0.65 (0.59–0.71)	15.20	<0.001
β -catenin	1.00 (0.91–1.09)	0.62 (0.56–0.68)	0.45 (0.41–0.49)	15.23	<0.001
MMP-2	1.00 (0.91–1.09)	0.80 (0.73–0.87)	0.53 (0.48–0.58)	15.20	<0.001
Slug	1.00 (0.91–1.09)	0.65 (0.59–0.71)	0.37 (0.34–0.40)	15.23	<0.001
TIMP-1	1.00 (0.91–1.09)	1.58 (1.44–1.72)	2.30 (2.09–2.51)	15.20	<0.001
TIMP-2	1.00 (0.91–1.09)	1.20 (1.09–1.31)	2.01 (1.83–2.19)	15.01	<0.001

Data were presented as median (min–max); *p-value was calculated using Kruskal–Wallis test with Dunn's post hoc test; **degrees of freedom (df) = 2; ROS – reactive oxygen species; AO/EB – acridine orange/ethidium bromide; DAPI – 4',6-diamidino-2-phenylindole; Bcl-2 – B-cell leukemia/lymphoma 2 proteins; Bcl-XL – B-cell lymphoma-extra-large; Bad – BCL2-associated death promoter; Bim – BCL-2-interacting mediator of cell death; MMP-2 – matrix metalloproteinases 2; Slug – SNAIL family of transcriptional repressors; TIMP-1 – tissue inhibitor of metalloproteinase-1; TIMP-2 – tissue inhibitor of metalloproteinase-2.

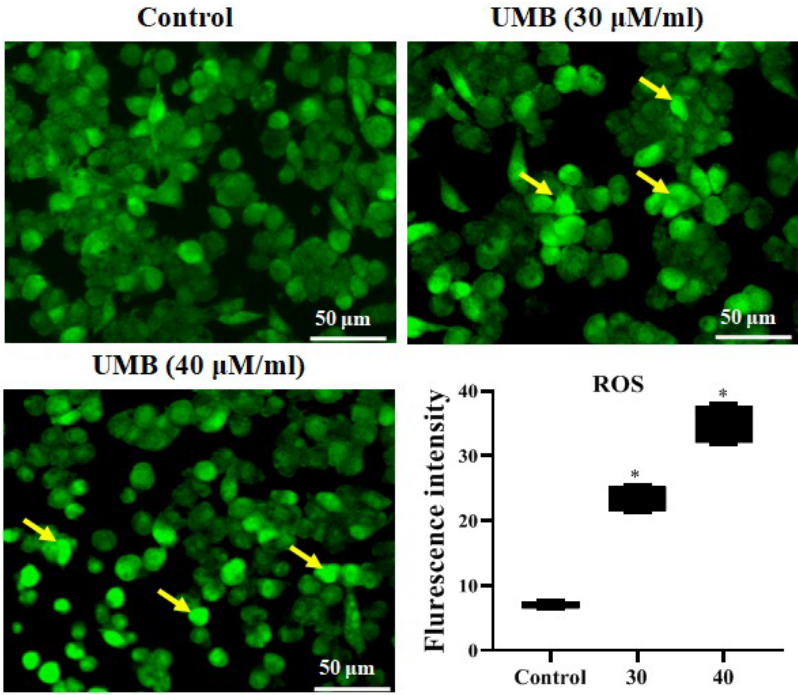


Fig. 2. Umbelliferone enhances the accumulation of ROS in glioma cells. Scale bar = 50 µm. U-87 cells with untreated control (green florescence) showing weak background florescence; arrow mark represents clearly visible DCF florescence. Cells treated with UMB (30 or 40 µM/mL) for 1 day showing bright DCF florescence. Results are presented as medians (min–max) for triplicate trials. Significant differences compared to the untreated control are denoted by * ($p < 0.05$). The p-value was calculated using the Kruskal–Wallis test with Dunn’s post hoc test

ROS – reactive oxygen species; DCFH – DA – 2’–7’-dichloro-dihydrofluorescein diacetate; UMB – umbelliferone.

Table 3. The results of the Dunn’s post hoc test

Explained variable	C vs 30 µM	C vs 40 µM	30 µM vs 40 µM
ROS	$p < 0.155$	$p < 0.001$	$p < 0.155$
AO/EB staining	$p < 0.154$	$p < 0.001$	$p < 0.154$
DAPI staining	$p < 0.154$	$p < 0.001$	$p < 0.154$
Bcl-2	$p < 0.154$	$p < 0.001$	$p < 0.154$
Bcl-XL	$p < 0.153$	$p < 0.001$	$p < 0.153$
Bad	$p < 0.154$	$p < 0.001$	$p < 0.154$
Bim	$p < 0.154$	$p < 0.001$	$p < 0.154$
E-cadherin	$p < 0.154$	$p < 0.001$	$p < 0.154$
N-cadherin	$p < 0.154$	$p < 0.001$	$p < 0.154$
β-catenin	$p < 0.153$	$p < 0.001$	$p < 0.153$
MMP-2	$p < 0.154$	$p < 0.001$	$p < 0.154$
Slug	$p < 0.153$	$p < 0.001$	$p < 0.153$
TIMP-1	$p < 0.154$	$p < 0.001$	$p < 0.154$
TIMP-2	$p < 0.174$	$p < 0.001$	$p < 0.174$

ROS – reactive oxygen species; AO/EB – acridine orange/ethidium bromide; DAPI – 4’,6-diamidino-2-phenylindole; Bcl-2 – B-cell leukemia/lymphoma 2 proteins; Bcl-XL – B-cell lymphoma-extra-large; Bad – BCL2-associated death promoter; Bim – BCL2-interacting mediator of cell death; MMP-2 – matrix metalloproteinases 2; Slug – SNAIL family of transcriptional repressors; TIMP-1 – tissue inhibitor of metalloproteinase-1, TIMP-2 – tissue inhibitor of metalloproteinase-2.

Umbelliferone-triggered apoptosis of human GBM cells displayed using DAPI staining

Human U-87 GBM cells stained with DAPI revealed typical viable cells with normal nuclei (Table 2,3, Fig. 3B). Treatment of the glioma cells with UMB stimulated

apoptosis that intensified the nuclear morphology and fragmentation of the nuclear bodies compared to the control cells. When UMB (30 or 40 µM/mL) was added to the glioma cells, they exhibited chromatin reduction, membrane blebbing, destruction of the nuclear envelope, and cellular collapse. These effects underlined that UMB-triggered apoptosis occurs in a quantity-dependent way.

Studies on the effects of UMB on Bcl-2 family protein expression

In human GBM cells supplemented with UMB (30 or 40 µM/mL), pro-apoptotic Bad and Bim levels were elevated, whereas anti-apoptotic Bcl-2 and Bcl-XL mitigated their protein levels. These results established the apoptotic action of UMB in a quantity-dependent way (Table 2,3, Fig. 4).

Influence of UMB on metastatic protein expression

For U-87 human GBM cells supplemented with UMB (30 or 40 µM/mL), E-cadherin protein expression was elevated, whereas N-cadherin, β-catenin, MMP-2, and Slug showed attenuated protein expression. These results confirmed the apoptotic action of UMB in a concentration-dependent way (Table 2,3, Fig. 5).

Protein expression analysis of TIMP-1 and TIMP-2

TIMP-1 and TIMP-2 expressions were downregulated in untreated glioma cells. The GBM cells supplemented

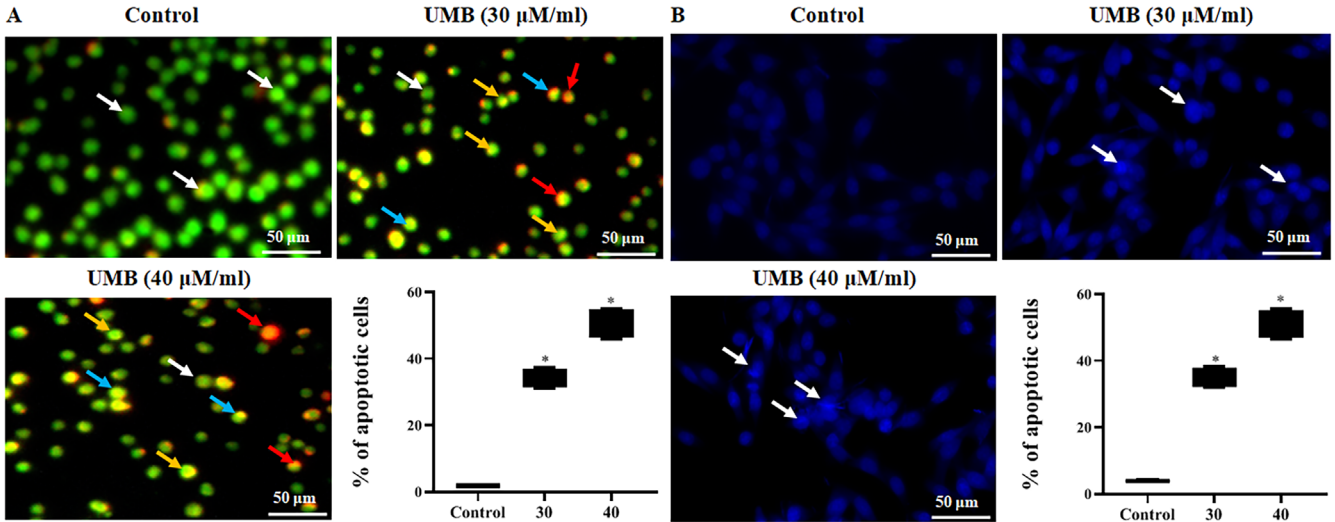


Fig. 3. Umbelliferone triggered apoptosis in glioma cells. U-87 cells were either untreated controls or U-87 cells treated with 30 or 40 μM/mL UMB for 24 h. Scale bar = 50 μm. The evaluation of apoptosis was performed using the dual staining of: (A) AO/EB staining; white arrow indicates green fluorescence, the orange arrow indicates apoptotic bodies, the blue arrow indicates apoptotic cells, and the yellow arrow indicates necrotic cells; (B) DAPI staining examined under a fluorescence microscope. The control cells showed a dark background as normal cells, but for the UMB-treated cells, the white arrow indicates bright fluorescence. Results are presented as medians (min–max) for triplicate trials. Significant differences compared to the untreated control are denoted by * (p < 0.05). The p-value was calculated using the Kruskal–Wallis test with Dunn’s post hoc test

ROS – reactive oxygen species; DCFH DA – 2’–7’- dichlorodihydrofluorescein diacetate; UMB – umbelliferone.

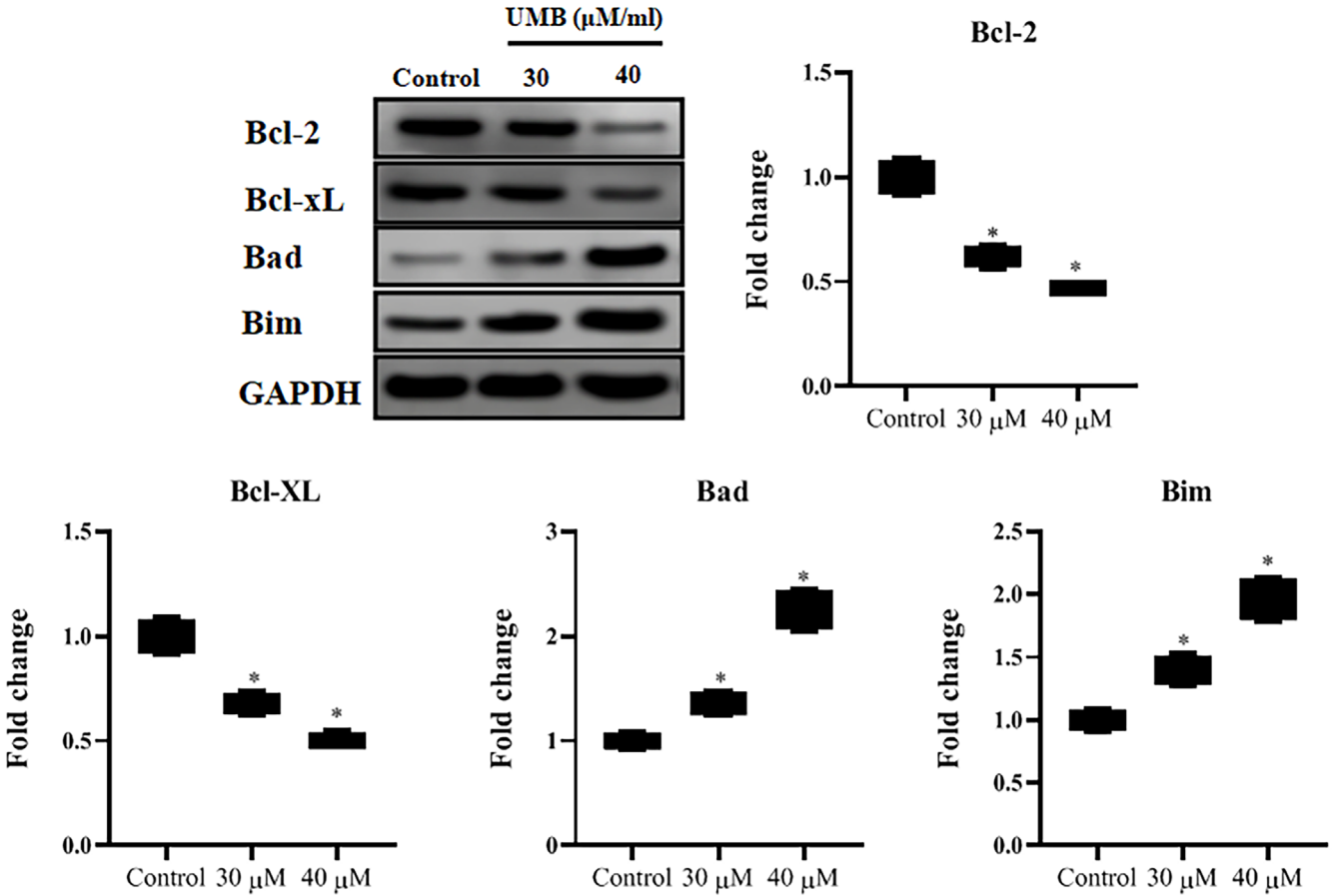


Fig. 4. Influence of UMB on the Bcl-2 family protein expression in human GBM cells. Umbelliferone (30 or 40 μM/mL) was added to the U-87 cells for 24 h, and protein levels were studied using western blot. Results are presented as medians (min–max) for triplicate trials. Significant differences compared to the untreated control are denoted by * (p < 0.05). The p-value was calculated using the Kruskal–Wallis test with Dunn’s post hoc test

GBM – glioblastoma multiforme; UMB – umbelliferone.

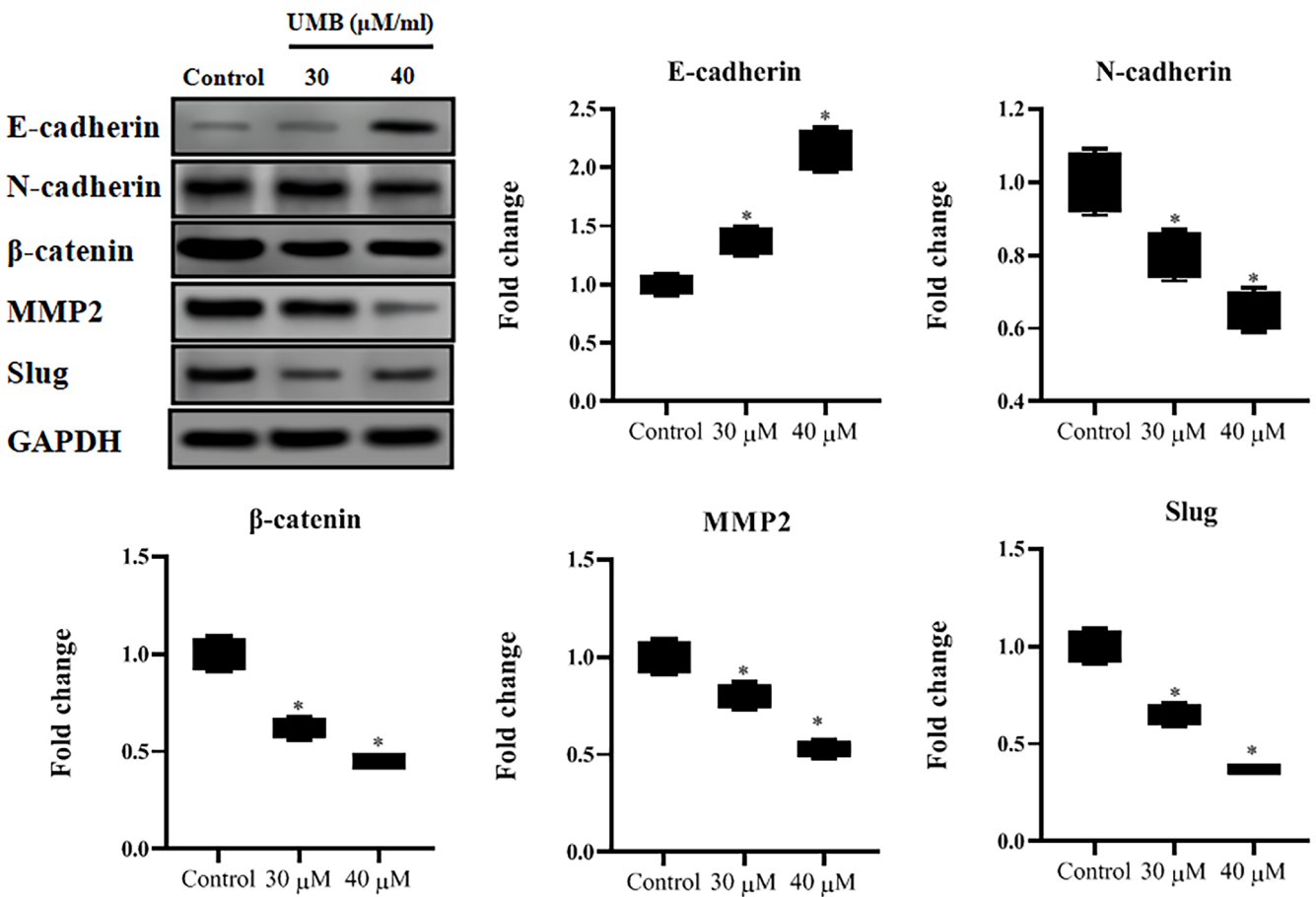


Fig. 5. Influence of UMB on metastatic protein expression in human GBM cells. Umbelliferone (30 or 40 μ M/mL) was added to glioma cells for 1 day. Results are presented as medians (min–max) for triplicate trials. Significant differences compared to the untreated control are denoted by * ($p < 0.05$). The p-value was calculated using the Kruskal–Wallis test with Dunn’s post hoc test

GBM – glioblastoma multiforme; UMB – umbelliferone.

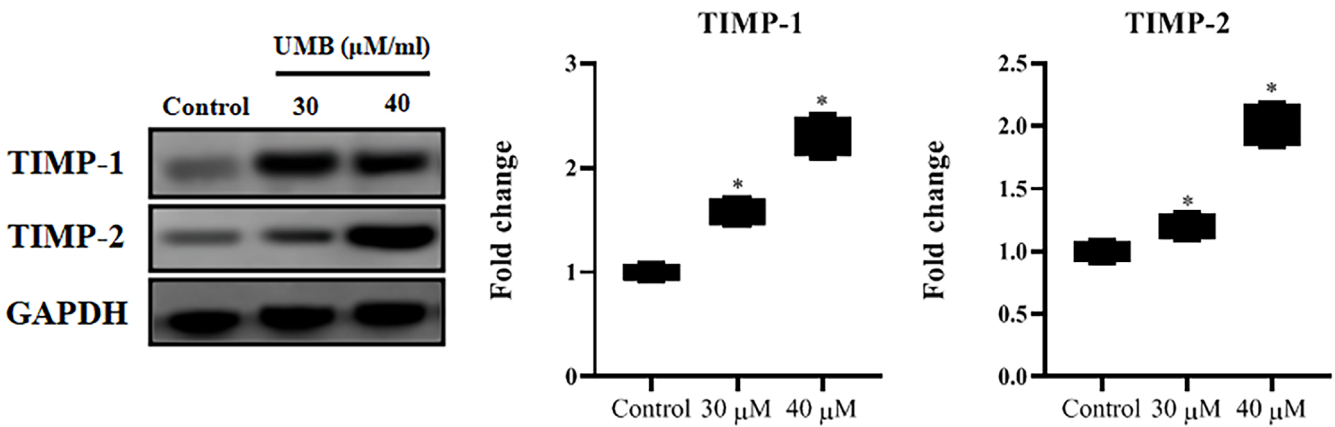


Fig. 6. Influence of UMB on TIMP-1 and TIMP-2 protein levels in human GBM cells. Umbelliferone (30 or 40 μ M/mL) was added to U-87 cells for 24 h. Results are presented as medians (min–max) for triplicate trials. Significant differences compared to the untreated control are denoted by * ($p < 0.05$). The p-value was calculated using the Kruskal–Wallis test with Dunn’s post hoc test

GBM – glioblastoma multiforme; UMB – umbelliferone; TIMP-1 – tissue inhibitor of metalloproteinase-1; TIMP-2 – tissue inhibitor of metalloproteinase-2.

with 30 or 40 μ M/mL UMB upregulated TIMP-1 and TIMP-2 protein expression in a quantity-dependent way (Table 2,3, Fig. 6).

Discussion

Malignant carcinomas are a growing problem in the medical community due to delayed diagnosis, elevated metastasis, augmented resistance, and the massive

complexity of currently existing medications. The GBM is the most common glioma, with the highest grade of malignancy among brain tumors, the least-favorable prognosis and the greatest likelihood of metastasis and recurrence.^{1,3} Temozolomide is the most beneficial medication accepted for GBM treatment, but its drawback is drug resistance, which frequently happens in medical use, eventually causing treatment failure.⁵ At present, several treatments are available for GBM; however, their effectiveness is partly owing to their broad ramifications. As such, researchers are mainly focused on the development of innovative medications with reduced complications. Over 50% of cancer medicines are produced from natural ingredients.

Umbelliferone, also known as 7-hydroxycoumarin, is an extensive natural component of the coumarin family. It has been documented to have analgesic, anti-inflammatory, antinociceptive, and bronchodilatory actions.^{27,28} Umbelliferone has been demonstrated to display anticancer and immunomodulatory actions by constraining tumor progression of sarcoma 180 in mice models.²⁹ Kielbus et al.²¹ stated that UMB repressed laryngeal cancer cell proliferation and migration. They reported that UMB mitigated the proliferation and migration of laryngeal RK33 malignant cells in a quantity-dependent way.²¹ Umbelliferone has also been tested for anticancer activities against 7,12-dimethylbenz(a)anthracene-prompted mammary carcinomas in rats.³⁰ To the best of our knowledge, the current study is the first report to exhibit the cytotoxic, apoptotic and antimetastatic prompting actions of UMB in human U-87 GBM cells in vitro. The MTT assay showed that treatment with UMB mitigated cell proliferation in a dose-dependent way in glioma cells, which indicates that UMB might be an active antitumor candidate, as shown in this research.

It has been stated that stable levels of ROS are vital for preserving typical physiological roles.^{7,8} The generation of intracellular ROS accompanies oxidative stress, which ultimately leads to the process of apoptosis. Additional ROS production in a biological system is harmful to proteins and DNA, which may eventually cause cell death. When there are upsurges in ROS generation, morphological variations occur and cause late apoptotic modifications, as evidenced by the dual staining of AO/EB in the current research. Next, to confirm that UMB triggered apoptosis, DAPI staining was performed. The fluorescent dye DAPI attaches to the A-T-rich DNA section. U-87-stained cells were examined under a fluorescent microscope, and the images presented fragmented DNA after treatment with UMB. Our findings revealed that elevated ROS generation occurred following UMB administration at different concentrations (30 or 40 $\mu\text{M/mL}$), in contrast to the control GBM cells, in a quantity-dependent manner. This finding also confirmed the ROS-stimulating action of UMB on U-87 cells.

To clarify the outcomes, we assessed the level of apoptotic protein expression following supplementation of glioma cells with UMB. The proportion of pro-apoptotic and

anti-apoptotic proteins is vital during cell destiny determination. Our findings revealed that UMB attenuated the level of anti-apoptotic (Bcl-XL and Bcl-2) proteins and elevated the level of pro-apoptotic (Bad and Bim) proteins in a dose-dependent way. Anti-apoptotic and pro-apoptotic proteins also offer the creation of discrete channels for the permeabilization of the mitochondrial membrane.³¹ Finally, we showed that the mediator molecules for cell death by UMB are Bim and Bad, which are involved in the crucial mechanisms of cell apoptosis in the mitochondrial pathway.

The current study revealed that UMB-treated glioma cells enhanced E-cadherin and TIMPs while mitigating the protein levels of N-cadherin, β -catenin, MMP-2, and Slug, compared to untreated GBM control cells. Numerous researchers have assessed the arrays of classical cadherin expression in GBM. The expression of N-cadherin in GBM has been examined in different patient cohorts, with nearly 60–80% of cases.³² E-cadherin levels have been further showed as rare or lacking in gliomas.³³ Slug protein is a well-considered transcriptional repressor, which can directly attach to and curb the E-cadherin promoter and other cell–cell adhesion within epithelial cells.³⁴ The cytoplasmic domain of cadherin also contributes to cell–cell adhesion by alleviating the cadherin/catenin complex and binding this complex to the actin cytoskeleton at the membrane. It controls an extensive variety of typical physiological developments, comprising embryo enlargement, cell propagation, differentiation, gene expression, and apoptosis.³⁵ Preceding reports have established that the TIMP gene family members are differentially expressed in numerous tumors.^{36,37} As MMPs are greatly associated with the prognosis of multiple cancers, TIMPs, at least theoretically, may affect diagnosis in a few malignancy cases as precise inhibitors. In brain tumors, including GBM, several reports have explored the disparity in the expression of TIMPs.³⁸ In the current study, UMB enhanced the protein expression levels of TIMP-1 and TIMP-2 in GBM cells in a dosage-dependent way.

Limitations

As a limitation, microRNA interference level molecular studies were not performed.

Conclusions

This report showed that UMB subdues the proliferation of human U-87 GBM cells through the stimulation of ROS-mediated apoptosis and its cytotoxic effects. Additionally, it could also suppress metastasis of the GBM cells by modulating the cadherin/ β -catenin complex-mediated cell–cell adhesion in human glioblastoma cells. These outcomes suggest that UMB may prove to be a key component of antitumor agents or antitumor lead molecules. However, further research endeavors involving the precise molecular mechanisms of its antimetastatic effects are urgently required.

Supplementary data

The supplementary materials are available at <https://doi.org/10.5281/zenodo.12804976>. The package includes the following files:

Supplementary Fig. 1. Results of Kruskal–Wallis test as presented in Fig. 1.

Supplementary Fig. 2. Results of Kruskal–Wallis test as presented in Fig. 2.

Supplementary Fig. 3. Results of Kruskal–Wallis test as presented in Fig. 3.

Supplementary Fig. 4. Results of Kruskal–Wallis test as presented in Fig. 4.

Supplementary Fig. 5. Results of Kruskal–Wallis test as presented in Fig. 5.

Supplementary Fig. 6. Results of Kruskal–Wallis test as presented in Fig. 6.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Wei Ma  <https://orcid.org/0009-0000-2793-1065>

Hangyu Shi  <https://orcid.org/0009-0005-8832-1646>

Xinya Dong  <https://orcid.org/0009-0008-9413-8663>

Yongqiang Shi  <https://orcid.org/0009-0009-7240-0488>

Luyi Zhang  <https://orcid.org/0009-0008-8173-1468>

Bin Jiang  <https://orcid.org/0000-0001-8438-0001>

References

- Molinaro AM, Taylor JW, Wiencke JK, Wrensch MR. Genetic and molecular epidemiology of adult diffuse glioma. *Nat Rev Neurol*. 2019;15(7):405–417. doi:10.1038/s41582-019-0220-2
- Souza TKF, Nucci MP, Mamani JB, et al. Image and motor behavior for monitoring tumor growth in C6 glioma model. *PLoS One*. 2018;13(7):e0201453. doi:10.1371/journal.pone.0201453
- Delgado-Martín B, Medina MÁ. Advances in the knowledge of the molecular biology of glioblastoma and its impact in patient diagnosis, stratification, and treatment. *Adv Sci (Weinh)*. 2020;7(9):1902971. doi:10.1002/advs.201902971
- Bleeker FE, Molenaar RJ, Leenstra S. Recent advances in the molecular understanding of glioblastoma. *J Neurooncol*. 2012;108(1):11–27. doi:10.1007/s11060-011-0793-0
- Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol*. 2009;10(5):459–466. doi:10.1016/S1470-2045(09)70025-7
- Furnari FB, Fenton T, Bachoo RM, et al. Malignant astrocytic glioma: Genetics, biology, and paths to treatment. *Genes Dev*. 2007;21(21):2683–2710. doi:10.1101/gad.1596707
- Sena LA, Chandel NS. Physiological roles of mitochondrial reactive oxygen species. *Mol Cell*. 2012;48(2):158–167. doi:10.1016/j.molcel.2012.09.025
- Covarrubias L, Hernández-García D, Schnabel D, Salas-Vidal E, Castro-Obregón S. Function of reactive oxygen species during animal development: Passive or active? *Dev Biol*. 2008;320(1):1–11. doi:10.1016/j.ydbio.2008.04.041
- Bellance N, Lestienne P, Rossignol R. Mitochondria: From bioenergetics to the metabolic regulation of carcinogenesis. *Front Biosci (Landmark Ed)*. 2009;14(11):4015–4034. doi:10.2741/3509
- Sullivan LB, Chandel NS. Mitochondrial reactive oxygen species and cancer. *Cancer Metab*. 2014;2(1):17. doi:10.1186/2049-3002-2-17
- Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. *Biochim Biophys Acta Mol Cell Res*. 2016;1863(12):2977–2992. doi:10.1016/j.bbamcr.2016.09.012
- Bacac M, Stamenkovic I. Metastatic cancer cell. *Annu Rev Pathol Mech Dis*. 2008;3(1):221–247. doi:10.1146/annurev.pathmechdis.3.121806.151523
- Coopman P, Djiane A. Adherens junction and E-cadherin complex regulation by epithelial polarity. *Cell Mol Life Sci*. 2016;73(18):3535–3553. doi:10.1007/s00018-016-2260-8
- Hulpiau P, Van Roy F. Molecular evolution of the cadherin superfamily. *Int J Biochem Cell Biol*. 2009;41(2):349–369. doi:10.1016/j.biocel.2008.09.027
- Lu P, Takai K, Weaver VM, Werb Z. Extracellular matrix degradation and remodeling in development and disease. *Cold Spring Harb Perspect Biol*. 2011;3(12):a005058. doi:10.1101/cshperspect.a005058
- Ahir BK, Engelhard HH, Lakka SS. Tumor development and angiogenesis in adult brain tumor: Glioblastoma. *Mol Neurobiol*. 2020;57(5):2461–2478. doi:10.1007/s12035-020-01892-8
- Arpino V, Brock M, Gill SE. The role of TIMPs in regulation of extracellular matrix proteolysis. *Matrix Biol*. 2015;44–46:247–254. doi:10.1016/j.matbio.2015.03.005
- Ries C. Cytokine functions of TIMP-1. *Cell Mol Life Sci*. 2014;71(4):659–672. doi:10.1007/s00018-013-1457-3
- Mazimba O. Umbelliferone: Sources, chemistry and bioactivities review. *Bull Fac Pharm Cairo Univ*. 2017;55(2):223–232. doi:10.1016/j.bfopcu.2017.05.001
- Muthu R, Thangavel P, Selvaraj N, Ramalingam R, Vaiyapuri M. Synergistic and individual effects of umbelliferone with 5-fluorouracil on the status of lipid peroxidation and antioxidant defense against 1,2-dimethylhydrazine induced rat colon carcinogenesis. *Biomed Prevent Nutr*. 2013;3(1):74–82. doi:10.1016/j.bionut.2012.10.011
- Kielbus M, Skalik-Kozłowska K, Grabarska A, et al. 7-substituted coumarins inhibit proliferation and migration of laryngeal cancer cells in vitro. *Anticancer Res*. 2013;33(10):4347–4356. PMID:24123002.
- Yu SM, Hu DH, Zhang JJ. Umbelliferone exhibits anticancer activity via the induction of apoptosis and cell cycle arrest in HepG2 hepatocellular carcinoma cells. *Mol Med Rep*. 2015;12(3):3869–3873. doi:10.3892/mmr.2015.3797
- Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods*. 1983;65(1–2):55–63. doi:10.1016/0022-1759(83)90303-4
- Gao H, Liu Z, Xu W, et al. Pterostilbene promotes mitochondrial apoptosis and inhibits proliferation in glioma cells. *Sci Rep*. 2021;11(1):6381. doi:10.1038/s41598-021-85908-w
- Kasibhatla S, Amarante-Mendes GP, Finucane D, Brunner T, Bossy-Wetzel E, Green DR. Acridine orange/ethidium bromide (AO/EB) staining to detect apoptosis. *Cold Spring Harb Protoc*. 2006;2006(3):pdb.prot4493. doi:10.1101/pdb.prot4493
- Zheng D, Zhu G, Liao S, et al. Dysregulation of the PI3K/Akt signaling pathway affects cell cycle and apoptosis of side population cells in nasopharyngeal carcinoma. *Oncol Lett*. 2015;10(1):182–188. doi:10.3892/ol.2015.3218
- Leal LKAM, Ferreira AAG, Bezerra GA, Matos FJA, Viana GSB. Antinociceptive, anti-inflammatory and bronchodilator activities of Brazilian medicinal plants containing coumarin: A comparative study. *J Ethnopharmacol*. 2000;70(2):151–159. doi:10.1016/S0378-8741(99)00165-8
- Lino CS, Taveira ML, Viana GSB, Matos FJA. Analgesic and antiinflammatory activities of *Justicia pectoralis* Jacq and its main constituents: Coumarin and umbelliferone. *Phytother Res*. 1997;11(3):211–215. doi:10.1002/(SICI)1099-1573(199705)11:3<211::AID-PTR72>3.0.CO;2-W
- Stefanova TH, Nikolova NJ, Toshkova RA, Neychev HO. Antitumor and immunomodulatory effect of coumarin and 7-hydroxycoumarin against sarcoma 180 in mice. *J Exp Ther Oncol*. 2007;6(2):107–115. PMID:17407969.

30. Maucher A, Von Angerer E. Antitumour activity of coumarin and 7-hydroxycoumarin against 7, 12-dimethylbenz[a]anthracene-induced rat mammary carcinomas. *J Cancer Res Clin Oncol*. 1994;120(8):502–504. doi:10.1007/BF01191806
31. Youle RJ, Strasser A. The BCL-2 protein family: Opposing activities that mediate cell death. *Nat Rev Mol Cell Biol*. 2008;9(1):47–59. doi:10.1038/nrm2308
32. Noh MG, Oh SJ, Ahn EJ, et al. Prognostic significance of E-cadherin and N-cadherin expression in gliomas. *BMC Cancer*. 2017;17(1):583. doi:10.1186/s12885-017-3591-z
33. Howng SL, Wu CH, Cheng TS, et al. Differential expression of Wnt genes, β -catenin and E-cadherin in human brain tumors. *Cancer Lett*. 2002;183(1):95–101. doi:10.1016/S0304-3835(02)00085-X
34. Eger A, Aigner K, Sonderegger S, et al. DeltaEF1 is a transcriptional repressor of E-cadherin and regulates epithelial plasticity in breast cancer cells. *Oncogene*. 2005;24(14):2375–2385. doi:10.1038/sj.onc.1208429
35. Ratheesh A, Yap AS. A bigger picture: Classical cadherins and the dynamic actin cytoskeleton. *Nat Rev Mol Cell Biol*. 2012;13(10):673–679. doi:10.1038/nrm3431
36. Böckelman C, Beilmann-Lehtonen I, Kaprio T, et al. Serum MMP-8 and TIMP-1 predict prognosis in colorectal cancer. *BMC Cancer*. 2018;18(1):679. doi:10.1186/s12885-018-4589-x
37. Azevedo Martins JM, Rabelo-Santos SH, Do Amaral Westin MC, Zeferino LC. Tumoral and stromal expression of MMP-2, MMP-9, MMP-14, TIMP-1, TIMP-2, and VEGF-A in cervical cancer patient survival: A competing risk analysis. *BMC Cancer*. 2020;20(1):660. doi:10.1186/s12885-020-07150-3
38. Guo H, Sun Z, Wei J, et al. Expressions of matrix metalloproteinases-9 and tissue inhibitor of metalloproteinase-1 in pituitary adenomas and their relationships with prognosis. *Cancer Biother Radiopharm*. 2019;34(1):1–6. doi:10.1089/cbr.2018.2589