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Research article

Ursolic acid inhibits glioblastoma through suppressing TGFβ-mediated epithelial-mesenchymal transition (EMT) and angiogenesis

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ABSTRACT

Found in many fruits and plants, Ursolic acid (UA), a pentacyclic triterpene that occurs naturally, is recognized for its anti-cancer effects, especially in combating glioblastoma. However, the intricate molecular mechanisms underpinning its anti-tumor actions are still not fully understood, despite the recognition of these effects. By examining the functions of epithelial-mesenchymal transition (EMT) and angiogenesis, crucial for glioblastoma progression, and their regulation through Transforming Growth Factor Beta (TGFβ) – a key marker for glioblastoma, our research aims to fill this knowledge gap. This study explores how ursolic acid can block the progression of glioblastoma by precisely targeting TGFβ-triggered EMT and angiogenesis. The findings show that UA successfully blocks the spread, movement, and invasion of glioblastoma cells. Accompanying this, there is a significant reduction in the expression of TGFβ and crucial EMT indicators like snail and vimentin. Furthermore, UA shows a reduction in angiogenesis that depends on the dosage, highlighted by decreased vascular endothelial growth factor (VEGF) in human umbilical vein endothelial cells (HUVECs). Interestingly, increased TGFβ expression in U87 and U251 glioblastoma cell lines was found to weaken UA's anti-tumor properties, shedding more light on TGFβ's critical function in glioblastoma's pathology. Supporting these laboratory results, UA also showed considerable inhibition of tumor growth in a glioblastoma xenograft mouse model. Overall, our research emphasizes Ursolic acid's promise as a new treatment for glioblastoma and clarifies its action mechanism, mainly by inhibiting TGFβ signaling and thereby EMT and angiogenesis.

1. Introduction

Malignant gliomas rank among the most common and deadly brain tumors. Glioblastoma, distinguished by its rapid proliferation and aggressive invasion, stands as the deadliest variant, often resulting in a grim outlook for affected individuals. Despite extensive research into treatments like surgical intervention, chemotherapy, radiation, and combined approaches over recent decades, the median survival duration for glioblastoma patients rarely surpasses 18 months [1,2]. This underscores the critical need for novel

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therapeutic discoveries in the battle against glioblastoma.

Emerging clinical and laboratory studies have highlighted the role of epithelial-mesenchymal transition (EMT) in the development and spread of various cancers. EMT in cancer cells is characterized by the transition from epithelial to mesenchymal traits, which enhances their capacity for proliferation, migration, and invasion. This transformation enables the cancer cells to spread into surrounding tissues and vasculature or to separate from their original location. Key indicators of EMT include the upregulation of mesenchymal markers such as vimentin and snail, leading to the breakdown of cellular connections, thereby facilitating increased mobility and invasiveness [3,4]. Although the role of EMT in glioblastoma remains a subject of debate, the neuro-epithelial perspective has gained support from studies demonstrating EMT-like behaviors in glioblastoma, which are linked to its aggressive features, including enhanced migration and invasion both in laboratory settings and animal models [5,6]. Targeting EMT pathways has shown promise as an effective strategy for glioblastoma treatment [7,8]. Various factors, including cytokines and growth factor signals, can influence EMT, with transforming growth factor-beta (TGFβ) identified as a key player in EMT induction [9–11]. The relationship between elevated TGFβ levels and reduced survival rates in cancer patients suggests that blocking TGFβ to deactivate EMT could offer a novel avenue for glioblastoma therapy [12–15].

Angiogenesis, crucial for the growth and spread of solid tumors, involves the creation of new blood vessels through a complex series of steps including the breakdown of the extracellular matrix, proliferation of endothelial cells, and the organization of these cells into tubes. The role of angiogenesis in both the development and metastasis of glioblastoma is increasingly recognized, with the degree of angiogenesis in tumors being linked to patient outcomes. The concept of "anti-angiogenic therapy" was introduced by Dr. Folkman and colleagues in 1971 and has since become a cornerstone in cancer treatment strategies [16–18]. Recent research confirms that targeting angiogenesis is an effective approach to combating tumors. Angiogenesis is governed by various molecular mechanisms and signaling pathways, with substantial evidence pointing to TGFβ as a significant promoter of cancer progression due to its angiogenic properties [12,19]. Furthermore, TGFβ is instrumental in angiogenesis through its impact on vascular endothelial growth factor (VEGF) [20,21], marking it as a key target for glioblastoma treatment.

Plants have been recognized for their anti-cancer capabilities across a range of human malignancies, a fact that has been exploited in traditional medicine. Ursolic acid, a triterpenoid compound found in numerous plants, is known for its wide array of biological and pharmacological effects, including its role as an antioxidant, anti-inflammatory agent, and neuroprotector. Specifically, its anti-cancer activities against various cancer cell types, such as osteosarcoma, colorectal, esophageal, hepatocellular carcinoma, and breast cancer, have been documented [22–26]. Despite known effects on cell proliferation and apoptosis in multiple cancer types, the precise impact and mechanisms of ursolic acid in glioblastoma remain to be fully understood.

In our investigation, the U87 and U251 human glioblastoma cell lines served as models to assess the impact of ursolic acid on cell proliferation, migration, and invasion. We delved into whether these effects were attributable to ursolic acid's modulation of EMT and angiogenesis through the alteration of TGFβ expression in glioblastoma. It was observed that TGFβ overexpression in glioblastoma cells reduced the efficacy of ursolic acid on EMT and angiogenesis. Ultimately, we found that ursolic acid curtailed tumor growth in a glioblastoma xenograft mouse model, supporting its potential as an anti-glioblastoma agent and elucidating its action mechanisms.

2. Method and materials

2.1. Chemicals, reagents and antibodies

Ursolic acid (UA) was sourced from Shilan Science and Technology Ltd., Tianjin, China, solubilized in DMSO, and kept at 4 °C for storage. The procurement of antibodies was as follows: TGFβ (#3709), vimentin (#5741), and GAPDH (#3700) from Cell Signaling Technology, Beverly, MA; VEGF (ab69479), CD31 (ab9498), and snail (ab69479) were acquired from Abcam, Cambridge, MA.

2.2. Cell culture

The cell lines U87 (glioblastoma, verified via STR profiling, ATCC version, catalog #1101HUM-PUMC000208, ATCC), U251, and HA1800 (normal human astrocytes) were sourced from the Chinese Academy of Medical Sciences, Beijing, China. These cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM) enriched with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin-streptomycin (HyClone, USA). Human Umbilical Vein Endothelial Cells (HUVECs, ATCC catalog No. PCS-100-013) were grown in Endothelial Cell Medium (ECM, ScienCell, USA) supplemented with 1% endothelial cell growth supplement (ECGS, ScienCell, USA) and 10% FBS, in a controlled environment at 37 °C and 5% CO_2.

2.3. Cell viability assay

Cells were plated in 96-well plates (5 × 10^3 cells/well) and allowed to attach for 24 h at 37 °C. Post-attachment, cells underwent various treatments as specified. Cell viability was then assessed by adding CCK-8 solution (10 μl; Dojindo, Japan) to each well, followed by measuring absorbance at 450 nm with a microplate reader.

2.4. Cell migration and invasion assay

For these assays, 2 × 10^4 cells were suspended in serum-free medium and placed into the upper chamber, with or without a 100 μl Matrigel (BD Biosciences, CA, USA) coating. Following a 24-h incubation, cells that did not migrate or invade were cleared from the

upper chamber using a cotton swab. The cells that reached the lower chamber were fixed with methanol, stained with 0.1% crystal violet, and images were captured in three randomly selected 100 × magnification fields per well. The cells were then counted using Image Pro Plus software.

2.5. Capillary tube formation assay

To assess angiogenic potential, HUVECs at 1 × 10ˆ5 cells/well were placed on 24-well plates pre-coated with 200 μl Matrigel (BD Biosciences, USA). Capillary-like structures were examined under an inverted microscope (Olympus BX-UCB; Olympus, Melville, NY), and the number of tube-like structures was quantified using Image-Pro Plus software.

2.6. Transfection

To induce TGFβ overexpression, cells were transfected with the TGFβ-flag or flag-only plasmid (pcDNA3.1-3 × Flag, GeneCopoeia, Rockville, Maryland, USA) using Lipofectamine 3000, following the supplier's guidelines.

2.7. Western blotting

Protein samples were prepared using Pro-prepTM protein Extraction Solution (iNtRON Biotechnology, Korea), as per the provided protocol. Samples were then heated for 5 min and loaded onto 10% SDS-polyacrylamide gels (30 μg protein per sample). After electrophoresis, proteins were transferred to a PVDF membrane, which was incubated with primary antibodies overnight at 4 °C, followed by a 1-h incubation with horseradish peroxidase-conjugated secondary antibodies (1:1000 dilution). Detection was performed using the Amersham ECL plus kit (Pierce, Rockford, IL, USA), with GAPDH as the normalization control.

3. ELISA

VEGF levels in the culture supernatants of HUVECs were determined using ELISA kits (R&D Systems) as per the manufacturer's instructions. Duplicate samples were added to wells of specially prepared plates. Following a wash step, a horseradish peroxidase-conjugated detection antibody was applied. Tetramethylbenzidine served as the substrate for color development. Absorbance was read at 450 nm using a microplate reader, and VEGF concentrations were calculated with SoftMax Pro software, setting the assay's detection limit at 5 pg/ml.

3.1. Immunohistochemistry

Tissue sections were first heated at 37 °C for an hour and then deparaffinized. Antigen retrieval was conducted in citrate buffer under microwave heating for 10 min. Sections were treated with 3% H2O2 in methanol to quench peroxidase activity, followed by a 10-min block with normal goat serum to prevent non-specific binding. Primary antibodies were applied at room temperature for an hour. This was followed by incubation with biotinylated anti-rabbit/mouse IgG and peroxidase-conjugated streptavidin, each for 10 min. Images were acquired using microscopy and analyzed with Image Pro-Plus software.

3.2. U87 xenograft mouse model ursolic acid treatment

U87 cells (5 × 10ˆ6) were subcutaneously injected into the dorsum of 6–8 week old female nude mice. After 7 days, mice were assigned into two groups (5 mice each) and received either oral doses of UA (50 mg/kg/day) or vehicle (normal saline). Tumor dimensions and mouse weight were recorded every five days. Tumor volume was calculated as $1/2 \times \text{length} \times \text{width}^2$. The animals were procured from Beijing Huafukang Biotechnology Co., Ltd. (Beijing, China). All procedures adhered to ethical guidelines comparable to the U.K.'s Animals (Scientific Procedures) Act, 1986, the European Communities Council Directive 2010/63/EU, or the NIH-OLAW, including ARRIVE guidelines adherence. Euthanasia aligned with AVMA Guidelines, using Lethabarb Euthanasia Injection (200 mg/kg body weight) for humane endpoint.

3.3. Statistical analysis

Data are presented as mean ± standard deviation from a minimum of three separate experiments. Independent samples Student's t-test was utilized to compare mean values between two groups, while one-way ANOVA followed by Tukey's post-hoc test was employed for multiple comparisons, utilizing GraphPad Prism software for analysis. A p-value <0.05 was deemed to indicate statistical significance.

4. Results

4.1. UA's effects on glioblastoma proliferation, migration, and invasion

Initially, we assessed the effects of various UA concentrations on U87, U251, and HA1800 cell viability using the CCK-8 assay after

24 h. Glioblastoma cells showed a dose-responsive decline in viability (Fig. 1A–B), while HA1800, the normal human astrocyte cell line, was unaffected (Fig. 1C). Further, to investigate UA's influence on cell movement, migration, and invasion assays were conducted at lower UA concentrations (5 μM and 10 μM) to avoid cell mortality. Compared to controls, UA treatment led to fewer glioma cells penetrating the transwell membrane's bottom, indicating reduced migratory and invasive capabilities. Notably, UA had no impact on the migration of HA1800 cells in similar tests (Fig. 1D–G). These outcomes highlight UA's potent inhibitory action on glioblastoma cell proliferation and invasion.

4.2. Impact of UA on TGFβ and EMT marker expression in glioblastoma cells

Given TGFβ′s role as a cancer-promoting protein in various cancers, including glioblastoma, inhibiting its expression presents a promising treatment strategy. Similarly, curbing the EMT process is considered a viable therapeutic option for glioblastoma. Our study delved into UA's ability to modulate TGFβ and EMT markers in U87, U251, and HA1800 cells. A dose-dependent decrease in TGFβ, vimentin, and snail levels was observed in U87 and U251 cells treated with UA, as shown in Fig. 1H. However, these changes were not detected in HA1800 cells. These findings underscore UA's critical role in regulating TGFβ and EMT processes in glioblastoma cells, contrasting with its non-effectiveness in normal astrocyte cells.

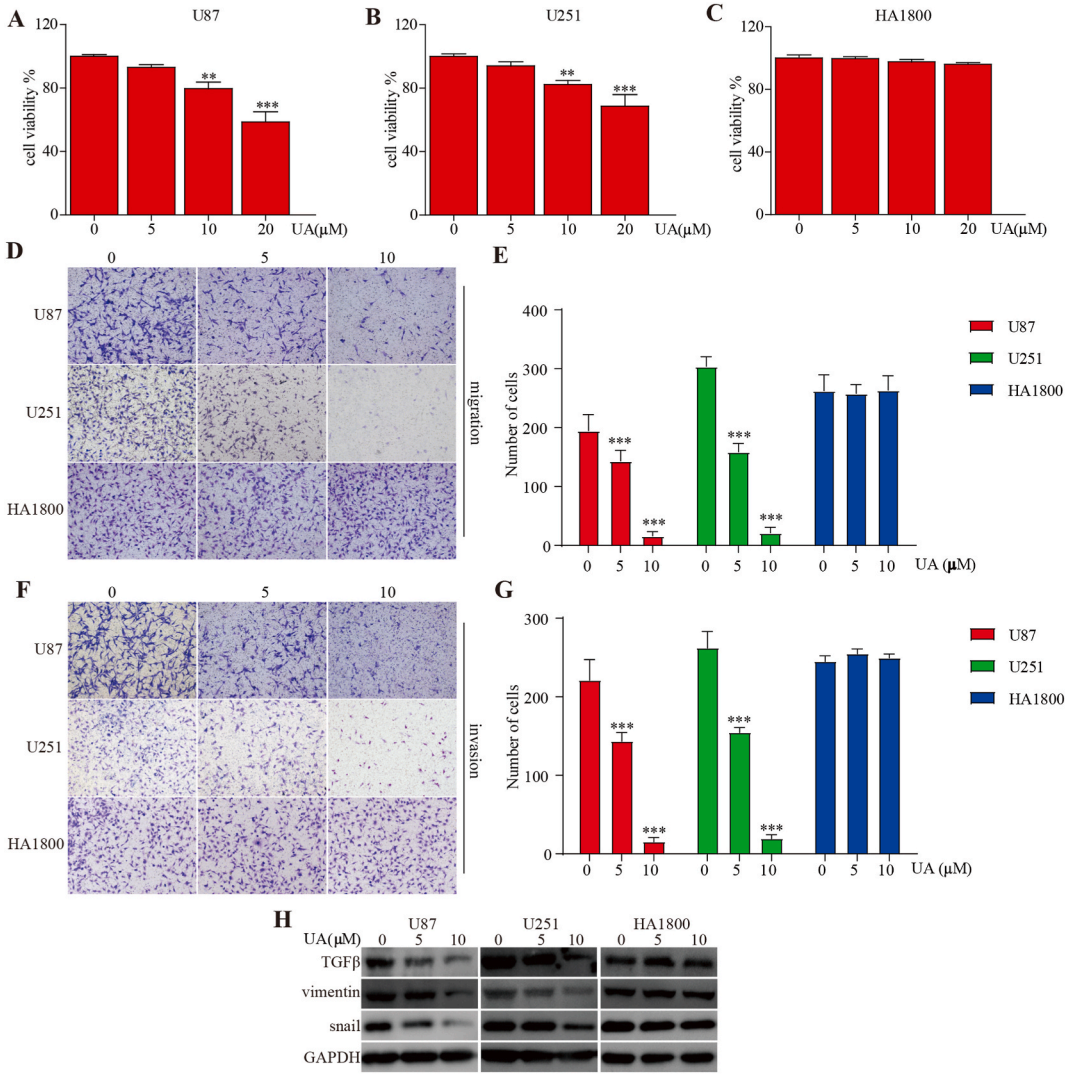

Fig. 1. UA inhibited cell proliferation, invasion and downregulated TGFβ and EMT markers in the glioblastoma cells. Glioblastoma cells U87, U251, and HA1800 were exposed to varying UA concentrations for 24 h. **(A–C)** The CCK8 assay assessed cell viability. **(D–G)** Transwell assays measured the migration and invasion of U87, U251, and HA1800 cells post 24-h UA treatment (scale bar = 100 μm). **(H)** Western blot analysis investigated the protein quantities of TGFβ and EMT markers. *P < 0.05, compared with control (0 μM).

4.3. TGFβ overexpression and its interaction with UA in glioblastoma cells

Investigating the role of TGFβ in UA's anti-cancer activity, we engineered U87 and U251 cells to overexpress TGFβ via transfection with a TGFβ cDNA plasmid (Fig. S1), using vector-only cells as controls. The results, depicted in Fig. 2A–B, demonstrated that elevated TGFβ levels increased glioblastoma cell proliferation and reduced UA's suppressive impact on this proliferation. Moreover, TGFβ overexpression enhanced cell migration and invasion, effects that were lessened with UA application (Fig. 2C–F). The study also indicated that TGFβ's increase bolstered EMT marker levels, such as vimentin and snail, while UA's presence counteracted the upsurge of these markers (Fig. 2G). These outcomes imply that UA's anti-cancer capabilities partially stem from its ability to counteract TGFβ-induced EMT processes in glioblastoma cells.

5. UA's effect on angiogenesis and VEGF regulation in HUVECs

Exploring UA's effect on endothelial cell proliferation, CCK-8 assays were conducted on HUVECs treated with 5 μM and 10 μM UA for 24 h. Fig. 3A illustrates UA's dose-dependent inhibition of HUVEC viability. In addition, UA's impact on angiogenesis was assessed through tube formation assays, revealing a significant suppression of vascular structure formation at increasing UA doses (Fig. 3B–C). The investigation extended to measuring VEGF protein levels in UA-treated HUVECs, noting a dose-responsive decrease in VEGF

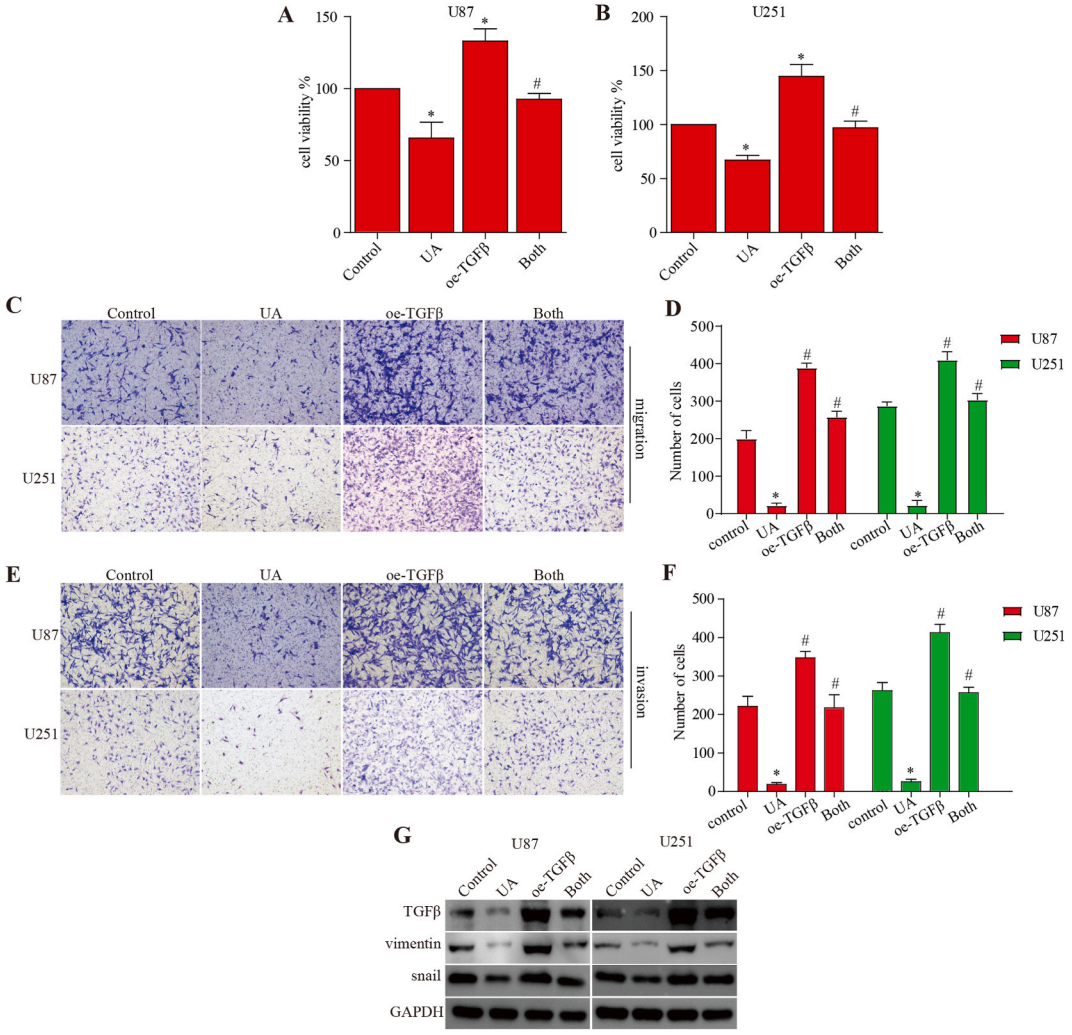

Fig. 2. Over-expression of TGFβ abolished UA-mediated cell proliferation, invasion and EMT. U87 and U251 cells, transfected with either TGFβ or a control vector for 18 h, were collected. **(A–B)** 5000 cells were placed in a 96-well plate and then treated with either 10 μM UA or a control vehicle for 24 h. The CCK-8 assay determined cell growth rates. **(C–F)** 10,000 cells were introduced into a transwell chamber, and their ability to invade was assessed using transwell migration and invasion assays (scale bar = 100 μm). **(G)** Protein levels were analyzed through Western blotting. Control: transfection vector; ursolic acid: transfection vector + ursolic acid; oe-TGFβ: transfection TGFβ; Both: transfection TGFβ + ursolic acid. *P < 0.05 vs control. $^{\#}$P < 0.05, compared with either ursolic acid treatment or TGFβ transfection alone.

expression (Fig. 3D).

5.1. Influence of TGFβ overexpression on UA's anti-angiogenic activity

In an attempt to understand the specific role of TGFβ in the process of angiogenesis influenced by ursolic acid (UA), human umbilical vein endothelial cells (HUVECs) were genetically modified to overexpress TGFβ using corresponding plasmids. Vector-transfected cells served as the control group for these experiments (Fig. 3E). The results indicated that heightened TGFβ levels not only encouraged angiogenesis and the production of vascular endothelial growth factor (VEGF) but also negated the anti-angiogenic effects of UA, including its suppression of VEGF expression in HUVECs (Fig. 3F–H).

5.2. UA reduces glioblastoma growth in U87 xenograft mice

To explore UA's potential against glioblastoma in a living organism, a U87 xenograft mouse model was utilized. Post 25 days of UA treatment, a significant reduction in tumor growth was recorded (Fig. 4A–B), with no detrimental effects on the overall body weight of the mice (Fig. 4C). Analysis of tumor tissues revealed that UA treatment led to decreased levels of TGFβ, vimentin, snail, and VEGF, along with reduced vascular density (Fig. 4D–E). These observations confirm UA's capacity to inhibit both epithelial-mesenchymal transition (EMT) and angiogenesis in vivo, thereby impeding glioblastoma progression.

6. Discussion

This research demonstrates that ursolic acid (UA) curbs the growth, movement, and penetration of glioblastoma cells by weakening the epithelial-mesenchymal transition (EMT) mechanism. Furthermore, UA impedes the formation of new blood vessels in tumors and reduces the levels of vascular endothelial growth factor (VEGF). We found that UA's suppression of EMT and angiogenesis can be attributed to the decreased expression of transforming growth factor-beta (TGFβ). In addition, the same mechanisms that UA employs to inhibit glioma were consistent in an intracranial glioblastoma mouse model, mirroring in-vitro findings.

The exploration of natural compounds offers a promising path for creating novel anticancer agents, with growing interest in identifying effective natural treatments for gliomas. Ursolic acid (UA), identified in a wide range of traditional medicinal herbs as a pentacyclic triterpenic acid, has diverse biological activities such as anti-inflammatory, antioxidant, and anticancer properties through several pathways [27–29]. For example, UA has been shown to reduce inflammation in fat tissue by triggering the Akt-GLUT4 signaling pathway in elderly rats [30]. Moreover, combining UA with empagliflozin has proven effective in mitigating diabetic nephropathy through the reduction of oxidative stress [31]. Regarding glioma therapy, UA derivatives are known to provoke cell death in glioblastoma cells by suppressing cAMP and reducing temozolomide (TMZ) resistance through the lowering of O(6)-methylguanine-DNA methyltransferase (MGMT) levels [32,33]. Nevertheless, given the complexity of its anticancer actions, more research is needed to thoroughly grasp UA's impact on glioblastoma. Our research has shown that UA blocks the EMT process and the development of new blood vessels in U87 and U251 cells by reducing TGFβ levels. While UA presents significant potential as a treatment option, extensive studies are crucial to confirm its safety and efficacy for human use.

TGFβ serves critical functions in development, maintaining tissue balance, healing wounds, and modulating immune activities as a

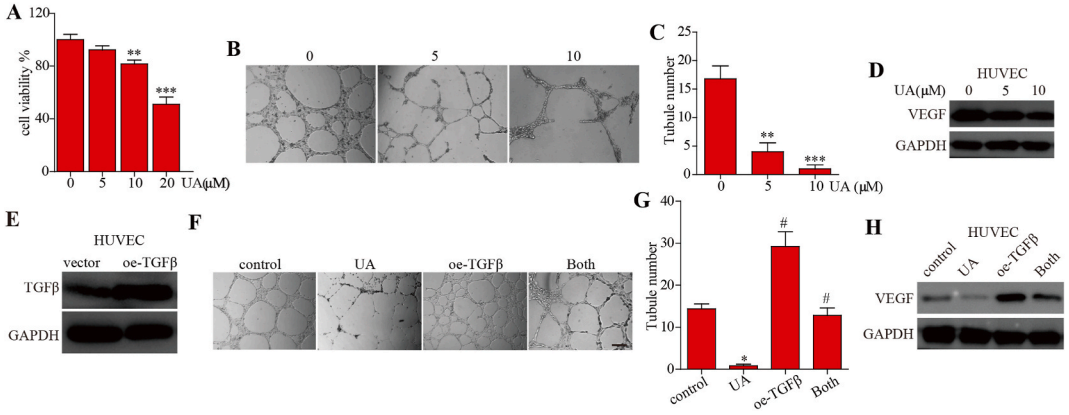

Fig. 3. The effect of UA on angiogenesis in HUVECs. HUVEC were treated with the indicated concentration of UA **(A)** Cell proliferation under various UA treatments was evaluated using the CCK8 assay. **(B, C)** UA's influence on HUVEC angiogenesis was quantified through tube formation assays. **(D)** VEGF protein levels were identified via Western blot analysis. **(E)** Post-TGFβ overexpression, TGFβ protein levels in HUVECs were measured. **(F–I)** TGFβ overexpression counteracted the anti-angiogenic effects of UA in HUVECs. HUVECs, after 18 h of TGFβ or control plasmid transfection, were treated with 10 μM UA or left untreated for 24 h. Tube counts (F, G) and VEGF levels (H, I) were determined through Western blot and ELISA. Control: transfection vector; ursolic acid: transfection vector + ursolic acid; oe-TGFβ: transfection TGFβ; Both: transfection TGFβ + ursolic acid. *P < 0.05 vs control. #P < 0.05, compared with either ursolic acid treatment or TGFβ transfection alone.

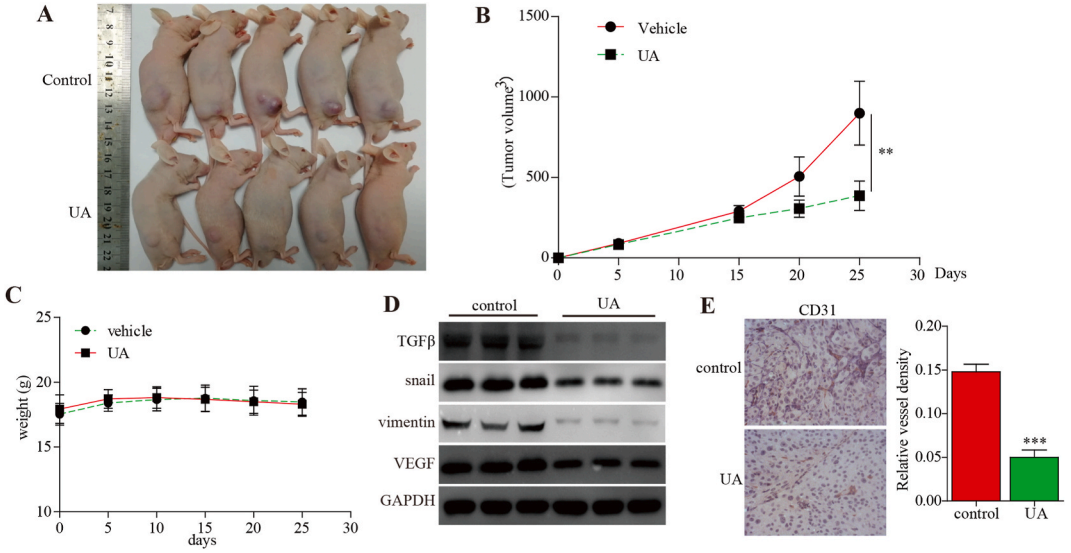

Fig. 4. UA decreased glioblastoma growth in xenograft mouse model. 7 days post-inoculation, mice were randomly assigned to two groups, each comprising five mice. Mice received oral administration of either a vehicle (normal saline) or UA (50 mg/kg/day). **(A)** The reduction in xenografted U87 tumor sizes was documented. Tumor volumes **(B)** and body weights **(C)** were recorded every five days. At the end of the experiments, tumor tissues were harvested for analysis, with protein lysates examined via Western blot **(D)** and CD31 levels assessed through immunohistochemistry **(E)** (scale bar = 50 μm). Each western blotting image represented at least three independent results*$P < 0.05$, compared with control.

versatile cytokine. Dysregulated TGFβ signaling is linked to the emergence and advancement of various cancers, particularly glioblastoma, positioning it as a viable candidate for therapy. Current molecular studies have revealed a connection between elevated TGFβ levels and the EMT process [9], with the regulation of tumor angiogenesis by TGFβ expression also documented [19]. Our research shows that UA suppresses EMT and tumor angiogenesis in glioblastoma by reducing TGFβ levels.

The significance of epithelial-mesenchymal transition (EMT) in malignant glioma, often dubbed an "EMT-like process" due to glioma's distinct traits, continues to be contested. Many reports have highlighted its strong link to the aggressiveness of these tumors. This link indicates that regardless of the unconventional expression of epithelial markers in gliomas, the EMT process, or its variant specific to glioma, is key to tumor growth and malignancy [8,34,35]. In particular, activators, mainly from the Snail family, have been recognized and confirmed to initiate and alter the EMT process, notably increasing the migration and invasion potential of glioma cells, as shown in both laboratory and animal studies [36,37]. Furthermore, growing evidence suggests that blocking the EMT process could be a strategic approach to halt glioblastoma's progression and dissemination. For instance, substances like curcumin, resveratrol, berberine, and sulforaphane have been shown to curtail glioblastoma cell migration and invasion by obstructing the EMT process [38–40]. Additionally, UA's inhibition of EMT in gastric cancer cells has been documented [41]. In our latest research, we found that UA produces anti-EMT effects by lowering TGFβ levels.

Angiogenesis is fundamental for the development and survival of malignant tumors. Bevacizumab, targeting VEGF-A, has been effective against several cancers clinically, yet its use in glioma therapy is still experimental, hindered by significant side effects in patients with glioma [42]. Consequently, the search for angiogenesis-targeting agents persists. Earlier research indicates that UA hinders angiogenesis, thereby suppressing non-small cell lung and melanoma cancer cells [43]. Additionally, studies have linked TGF-β activity to the stimulation of angiogenesis in tumors, facilitated by elevated VEGF levels [21]. Our current study corroborates these findings, demonstrating UA's dual anti-EMT and anti-angiogenic actions through TGFβ downregulation in both laboratory and animal experiments. Furthermore, endothelial cells, especially HUVECs, are critical in tumor angiogenesis and are commonly used as models to explore this phenomenon. While anti-angiogenic tumor treatments slightly impact normal angiogenesis, the level of angiogenesis in tumors significantly exceeds physiological norms. Our experiments revealed that UA curbs endothelial cell growth and tumor angiogenesis without notable adverse effects in mice, shown by the minimal weight differences between control and treated groups.

Our research utilized U87 and U251 cells, drawing on prior studies for selection. Notably, U87 is wild-type for the P53 gene, whereas U251 exhibits a P53 mutation. The role of P53 as a crucial tumor suppressor gene in glioma makes it pertinent to assess UA's impact on both cell lines.

Although our study sheds light on ursolic acid's inhibition of glioblastoma, especially via TGFβ-mediated suppression of EMT and angiogenesis, it's important to recognize its limitations. A major limitation is the use of a subcutaneous tumor model. While useful for examining tumor growth and drug response, this model doesn't fully mimic the intricacies and microenvironment of glioblastoma in situ. Glioblastoma models in situ, featuring tumor development within the brain, offer a more precise depiction of the disease and its interactions with brain tissue and the blood-brain barrier. Thus, although our results are encouraging, they warrant cautious interpretation since observations in a subcutaneous model may not directly apply to an in situ context. Future research employing in situ

glioblastoma models is essential to validate our findings further and comprehend the clinical implications of ursolic acid for glioblastoma treatment.

This research employed transient transfection to achieve TGFβ overexpression. Although transient transfection is less efficient than stable methods such as lentiviral transfection, it suited our experimental goals of studying short-term effects. We recognize that stable transfection could yield more reliable and lasting gene expression for in-depth studies on TGFβ′s prolonged role in glioblastoma. Hence, while transient transfection met this study's requirements, its limitations for long-term gene expression underscore a potential avenue for future investigations.

In summary, our findings reveal that UA inhibits EMT and angiogenesis in glioblastoma, evidenced in both laboratory and animal models. Moreover, we pinpoint the TGFβ-driven EMT and angiogenesis as prospective therapeutic targets for UA in glioblastoma treatment. Thus, UA presents itself as a promising natural agent in glioblastoma therapy, underscoring its potential as a target for future research and therapeutic development.

Ethics declarations

The experimental protocol was established, according to the ethical guidelines of the Declaration of Helsinki and mouse experiments were approved by the Committee on the Ethics of Animal Experiments of Army General Hospital (Approval Number: No.2021-12) in accordance with the Guide for the Care and Use of Laboratory Animals.

Data availability statement

Data included in article/supp. Material/referenced in article.

CRediT authorship contribution statement

Bo Hei: Writing – original draft, Software. **Ru-en Liu:** Writing – review & editing, Supervision, Funding acquisition, Data curation. **Meihua Li:** Writing – review & editing, Supervision, Investigation, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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