Culture supernatant of *Toxoplasma gondii* tachyzoites inhibits the proliferation and metastasis of bladder cancer cells

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Abstract: *Toxoplasma gondii*, an opportunistic protozoan, can be cultivated from numerous human cell lines. Bladder cancer is the most common type of tumor in the urinary system and has a high incidence and mortality rate. Protozoan parasites have recently gained popularity as a research topic for cancer therapy. The present study aimed to evaluate the effect of *T. gondii* tachyzoite culture supernatants on bladder cancer cells. Bladder cancer 5637 cells were infected with two different genotypes of *T. gondii* tachyzoite culture supernatants, RH and ToxoDB#9. The effects of *T. gondii* tachyzoite culture supernatant on cell proliferation were detected using a Cell Counting Kit-8 assay. The effects on cell migration and invasion were evaluated using wound healing and Transwell assays. The effect on the apoptosis rate of the cells was assessed using a TUNEL assay, while the protein levels of Bax and Bcl-2 were analyzed by western blotting. The results indicated that the culture supernatant of *T. gondii* tachyzoites altered the morphology of bladder cancer 5637 cells, inhibited cell proliferation, reduced cell migration and invasion and promoted apoptosis. The ToxoDB#9-type experimental group showed higher efficacy than the RH-type experimental group in inhibiting bladder cancer cell proliferation, reducing cell migration and invasion and promoting apoptosis. These results indicated that the culture supernatant of *T. gondii* tachyzoites could be a promising therapeutic agent for the treatment of bladder cancer.

Introduction

According to the most recent predictions from the International Organization for Research on Cancer, there will be >20 million new cases of cancer (which includes nonmelanoma skin cancers) and 9.7 million cancer-associated deaths (including nonmelanoma skin cancers) worldwide in 2022 (1). According to demographic forecasts, the annual incidence of new cancer cases globally will exceed 35 million by 2050 (1). Bladder cancer is the tenth most frequent malignancy worldwide and is ~4 times more likely to occur in men than in women (2). In China, lung cancer has the highest incidence and mortality (3). Bladder cancer is one of the main cancer types in China (4). Bladder cancer is a prevalent urological malignancy with a poor prognosis in the advanced/metastatic stage. Bladder cancer patients are typically treated with a variety of severe treatments, involving radical cystectomy, chemotherapy, immunological therapy and radiotherapy. However, these treatments frequently result in problems and undesirable effects, such as postoperative infection, impaired urination and weakened immune system (5).

Toxoplasma gondii is an opportunistic protozoan parasite that can infect warm-blooded animals, birds and humans (6). When infected, oocysts break in the gut, releasing sporozoites into the lumen. Sporozoites infect enterocytes and differentiate into tachyzoites, which are highly proliferative, mobile and spread quickly throughout the body, eliciting an immediate immunological response (7). *T. gondii* primarily infects humans through the following ways: eating food contaminated with the parasite's eggs or oocysts; breaking through skin mucous membranes and coming into contact with soil contaminated with the parasite's eggs or oocysts; coming into contact with animal excrement contaminated with the parasite eggs or oocysts; and vertical transmission from the mother to the fetus via the placenta (8). Depending on the person, *T. gondii* infection might have different health implications (9). When healthy individuals get *T. gondii*, the majority of them have concealed infections, which frequently resolve on their own and lack clear clinical signs and symptoms. Some infected people may have mild symptoms such as low-grade fever, headache, swelling of superficial lymph nodes, and occasionally pneumonia, pleurisy and myocarditis. However, those who have hypoplasia or immunodeficiency may experience more severe infection symptoms, including the possibility of systemic toxoplasmosis (10). In extreme situations, toxoplasmic encephalitis may potentially result in death (11). Toxoplasma infections in pregnant women can have particularly severe consequences

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for the fetus. Infections occurring during the early and middle stages of pregnancy may result in miscarriage, stillbirths, or congenital malformations. Conversely, infections that arise during the later stages of pregnancy can lead to preterm delivery and may be associated with hydrocephalus, meningoencephalitis, visual disturbances, or epilepsy in newborns within a few months to several years post-delivery (12). African countries have the greatest average incidence rate of infection of *T. gondii* (61.4%), followed by Oceania (38.5%), South America (31.2%), Europe (29.6%), the United States and Canada (17.5%) and Asia (16.4%) (13); thus, *T. gondii* has a high prevalence globally. *T. gondii* infection is more prevalent among immunocompromised patients, pregnant women, blood donors, women of reproductive age and newborns, compared with the general population (14). Therefore, special attention needs to be paid to these groups. Some studies have shown that patients with cancer are more frequently positive for *T. gondii* than healthy individuals; however, whether *T. gondii* causes cancer is unclear (15-20).

Recent studies have explored the potential anticancer properties of *T. gondii*, demonstrating its ability to invade and destroy various human cancer cell lines, such as breast cancer cells (21), colon cancer cells (22) and lung cancer cells (23). *T. gondii* has been formerly categorized into three primary lineages based on its virulence: Types I, II and III (24). ToxoDB #59 and #2 are the most prevalent in America, #1 is the most common in Africa, #9 is the most prevalent genotype in China (17,25,26). However, the effect of *T. gondii* on bladder cancer cells is uncertain. Therefore, the aim of the present study was to examine the influence of *T. gondii* on bladder cancer cells and the outcomes will fill a gap in the investigation of *T. gondii* in bladder cancer and provide novel ideas for medication development in bladder cancer.

Materials and methods

Cell lines and cell culture. Bladder cancer 5637 cells were purchased from Wuhan Punosai Life Technology Co. Ltd. The cell lines were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin solution (Beijing Solarbio Science & Technology Co., Ltd.). Cells were cultured in a humidified incubator at 37˚C with 5% CO_2.

Preparation of culture supernatant of T. gondii tachyzoites. A total of three six-week-old male BALB/c mice (weight, 20-22 g (Beijing Viton Lihua Lab Animal Technology Co. Ltd.) were used. These mice were accommodated in a hygienic, temperature-regulated cage (Temperature: 18~29˚C; Humidity: 40~70%) setting with a 12-h light-dark cycle. They were given unrestricted access to a laboratory-standard diet and water. Daily monitoring of animal health and behavior was undertaken. The exclusion criteria encompassed mortality during the research duration; however, this study did not witness any such. *T. gondii* tachyzoites (Laboratory of Dali University, China) was administered intraperitoneally to three randomly selected BALB/c mice ($4x10^6$ per mouse) and peritoneal fluid was collected after 72 h. The peritoneal fluid was centrifuged at 1,400 x g for 8 min at 37˚C, the supernatant was discarded and the sample was washed twice with PBS. The supernatant was filtered through CF-11 cellulose column, the column was loaded into a sterile syringe with PBS and the hole was sealed with filter paper at the bottom. The height of the column was 3 cm and the liquid was kept on the surface of the column. The suspension of *T. gondii* was quickly passed through the column and the filtrate was collected and centrifuged at 3,000 x g for 8 min on 37˚C to remove the supernatant and the precipitate was the *T. gondii*. These were collected and counted microscopically before being resuspended in RPMI-1640 (including 10 ml/l fetal bovine serum, Thermo Fisher Scientific, Inc.). To determine the optimal concentration of tachyzoite culture supernatant infecting bladder cancer cells, the quantity of tachyzoites was adjusted to 2, 4 and $8x10^7$/ml and then tachyzoites were inoculated into 6-well plates for 24 h. The supernatant was collected and filtered through 0.22-μm pore size filter membranes. Then, two sets of flasks (each containing three groups) containing bladder cancer 5637 cells were inoculated with *T. gondii* tachyzoite culture supernatants at 10:1, 100:1, 1,000:1 and 2,000:1. Thereafter, culture supernatants were harvested at various days until bladder cancer 5637 cells were destroyed and tachyzoites were counted. Finally, the appropriate ratio was bladder cancer: *T. gondii*=10:1. At the culmination of the experiment, all mice were sacrificed via CO_2 inhalation (flow rate was regulated at 30% of the cage volume per min) and mortality was confirmed by the absence of respiration or cardiac activity. All animal experiments received approval from the Institutional Animal Care and Use Committee of the University of Dali, Yunnan, China (approval no. 2024-0833).

Cell morphology. The changes of cell size, shape and nuclear morphology of bladder cancer cells before and after infection with the supernatant of tachyzoite culture of *T. gondii* were observed by contrast microscope.

Cell proliferation assay. A Cell Counting Kit-8 (CCK-8; Beijing Solarbio Science & Technology Co., Ltd.) was used to evaluate the proliferation of bladder cancer cells. Bladder cancer 5637 cells were seeded in 96-well plates at a density of $1x10^6$ cells/well and treated with the culture supernatant of *T. gondii* tachyzoites for 24, 48 and 72 h at 37˚C. Following the treatment, 10 μl CCK-8 solution was added to each well and cells were incubated for 1 h, according to the manufacturer's protocol. The optical density (OD) was measured at 450 nm using a microplate reader (Multiskan GO; Thermo Fisher Scientific, Inc.). Proliferation inhibition rate (%)=(average OD value of control group-average OD value of experimental group)/average OD value of control group x100%.

Wound healing assay. A marker was used to draw lines on the backs of the 6-well plates. Bladder cancer cells ($1x10^6$, no FBS) were inoculated into a 6-well plate at 37˚C in a 5% CO_2 atmosphere. A 10-μl micropipette tip was used to draw lines on the 6-well plates until the cells reached 80-90% confluence. After washing twice with PBS, the 6-well plates were observed under a fluorescence microscope and images were captured to record the scratch width at 0 h. The control group cells were added to 2 ml serum-free culture medium, while the experimental group cells were added to 2 ml *T. gondii* tachyzoite

Table I. Cell inhibition rate assessed using a Cell Counting Kit-8 after treatment of bladder cancer cells with *T. gondii* culture supernatant at 24, 48 and 72 h (n=6).

	24 h		48 h		72 h	
Group	OD450	Inhibition rate (%)	OD450value	Inhibition rate (%)	OD450value	Inhibition rate (%)
Control	0.76±0.04	-	1.12±0.10	-	2.11±0.12	-
RH	0.69±0.03[a]	9.68	1.06±0.08	5.59	1.72±0.07[a]	18.57
ToxoDB#9	0.62±0.03[a]	18.88	0.87±0.03[a]	22.70	1.27±0.10[a]	35.24

[a]P<0.01 vs. control. OD, optical density.

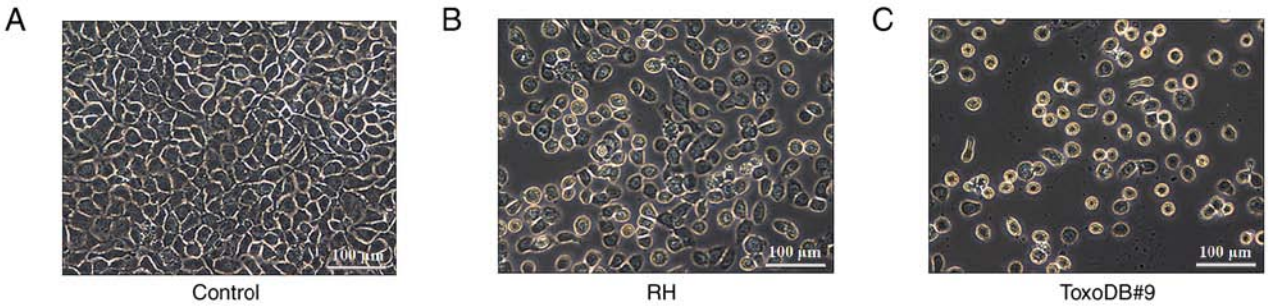

Figure 1. Cell morphology. (A) Morphology of bladder cancer 5637 cells. (B) Co-culture of *T. gondii* tachyzoite culture supernatant of the RH strain with bladder cancer 5637 cells. (C) Co-culture of *T. gondii* tachyzoite culture supernatant of ToxoDB#9 strain with bladder cancer 5637 cells.

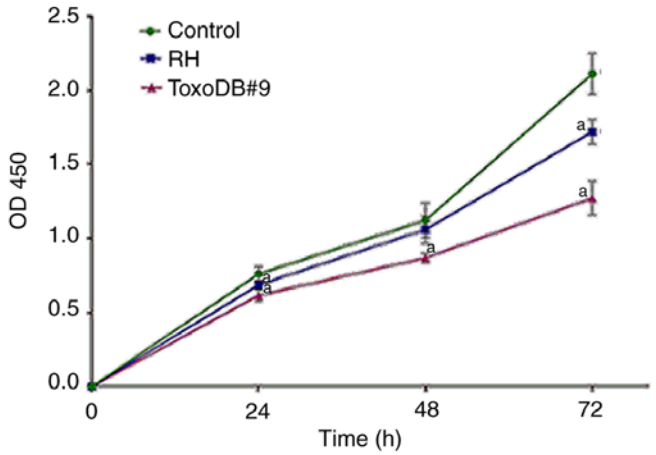

Figure 2. *T. gondii* tachyzoite culture supernatant inhibits the proliferation of bladder cancer 5637 cells. OD450 values of *T. gondii* tachyzoite culture supernatant at 24, 48 and 72 h in bladder cancer 5637 cells. [a]P<0.01 vs. control group. OD, optical density.

culture supernatant at 37˚C in a 5% CO_2 atmosphere. After 24 and 36 h, all plates were removed for washing with PBS and then images were captured. ImageJ 1.46 (National Institutes of Health) was used to determine migration rate.

Transwell migration and invasion assays. Typically, 1x10^6 bladder cancer 5637 cells and *T. gondii* tachyzoite culture supernatants + bladder cancer 5637 cells (10:1) in serum-free media were separately seeded into the upper chambers with (invasion) or without (migration) Matrigel coating (diluted in RPMI-1640; Corning, Inc.; 4˚C for 3 h) and the bottom chambers were filled with 600 μl supplemented RPMI-1640 medium. After 36 h, the cells that had migrated and invaded through to the bottom of the inserts were fixed with methanol and stained with 0.1% crystal violet for 30 min at room temperature. In five random fields of view, the numbers of cells that had migrated/invaded were observed under an inverted light microscope (magnification, x200) and images captured and quantified.

Cell apoptosis assay. A TUNEL Apoptosis Detection Kit was obtained from Shanghai Biyuntian Biotechnology Co., Ltd. *T. gondii* tachyzoite culture supernatants were added when bladder cancer cells reached 60-70% confluency. After 48 h, the cells were fixed with 500 μl 4% paraformaldehyde (Beijing Solarbio Science & Technology Co., Ltd.) and incubated with a highly permeable immunostaining solution for 5 min at room temperature. The TUNEL detection solution (50 μl) was added to each well, followed by incubation for 5 min. The apoptotic rate was determined using fluorescence microscopy.

Western blotting. The primary antibodies used for western blotting were as follows: Rabbit anti-Bax (cat. no. ab32503; 1:1,000; Abcam), rabbit anti-Bcl-2 (cat. no. ab112; 1:1,000; Beyotime Institute of Biotechnology) and rabbit anti-β-actin (cat. no. ab209857; 1:1,000; Abcam). The protein concentration was determined using the BCA method. Equal amounts of protein (40 μg/lane) were applied to a 10% SDS-PAGE for electrophoresis and transferred to a PVDF membrane (Beijing Solarbio Science & Technology Co., Ltd.). The membrane was

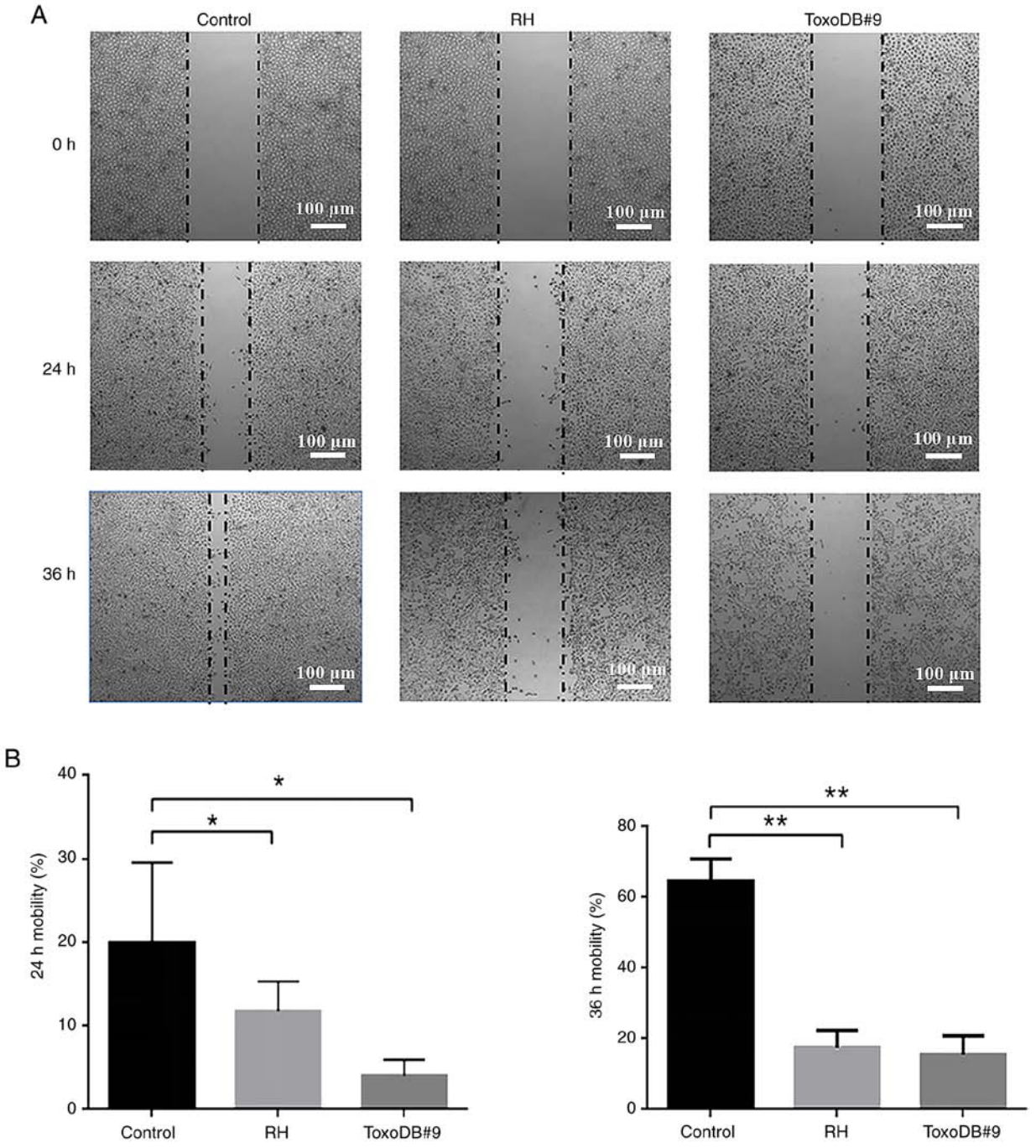

Figure 3. *T. gondii* tachyzoite culture supernatant inhibits the migration of bladder cancer 5637 cells. (A) Migration was assessed using a scratch assay after treatment of bladder cancer 5637 cells with *T. gondii* tachyzoite culture supernatant at 0, 24 and 36 h. (B) Quantitative analysis of the number of migrating cells in (A). *P<0.05, **P<0.01 vs. control.

transferred to a blocking solution (5% BSA; Beijing Solarbio Science & Technology Co., Ltd.) for 1 h on a shaking bed at room temperature and incubated with the primary antibody at 4˚C overnight and secondary antibodies (cat. no. ab2307391; 1:10,000; Jackson; Horseradish Peroxidase) at 37˚C for 1 h. Finally, the membrane was visualized using an ECL Luminescence Assay Kit (Shanghai Biyuntian Biotechnology Co., Ltd.). Western blot density was assessed using ImageJ 1.46 (National Institutes of Health).

Statistical analysis. SPSS Statistics 26.0 (IBM Corp.) was used for statistical analysis. ImageJ 1.46 (National Institutes of Health) was used for densitometry analysis. Comparisons between groups were assessed using one-way ANOVA and LSD as a post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Morphology of bladder cancer 5637 cells altered by culture supernatant of T. gondii tachyzoites. After 24 h, the morphology of bladder cancer 5637 cells was observed (Fig. 1A). The cells in the control groups were tightly arranged and evenly sized and exhibited consistent morphology, with

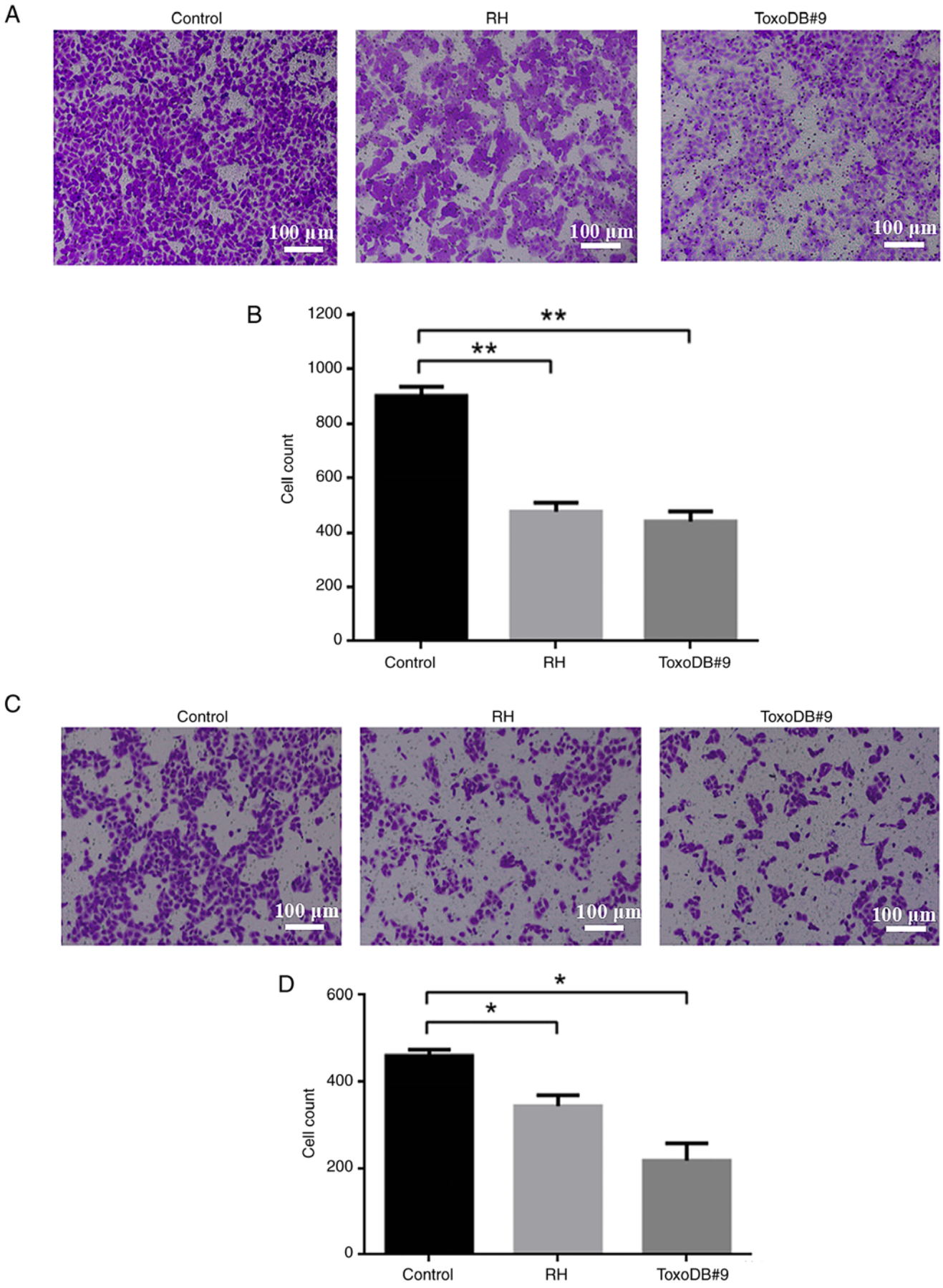

Figure 4. *T. gondii* tachyzoite culture supernatant inhibits the migration and invasion of bladder cancer 5637 cells *in vitro*. (A) Migration was assessed using a Transwell assay following treatment of bladder cancer 5637 cells with *T. gondii* tachyzoite culture supernatant. (B) Quantitative analysis of the number of migrating cells in (A). (C) Cell invasion was assessed using a Transwell assay following treatment of bladder cancer 5637 cells with *T. gondii* tachyzoite culture supernatant. (D) Quantitative analysis of the number of invading cells in (C). *P<0.05, **P<0.01 vs. control group.

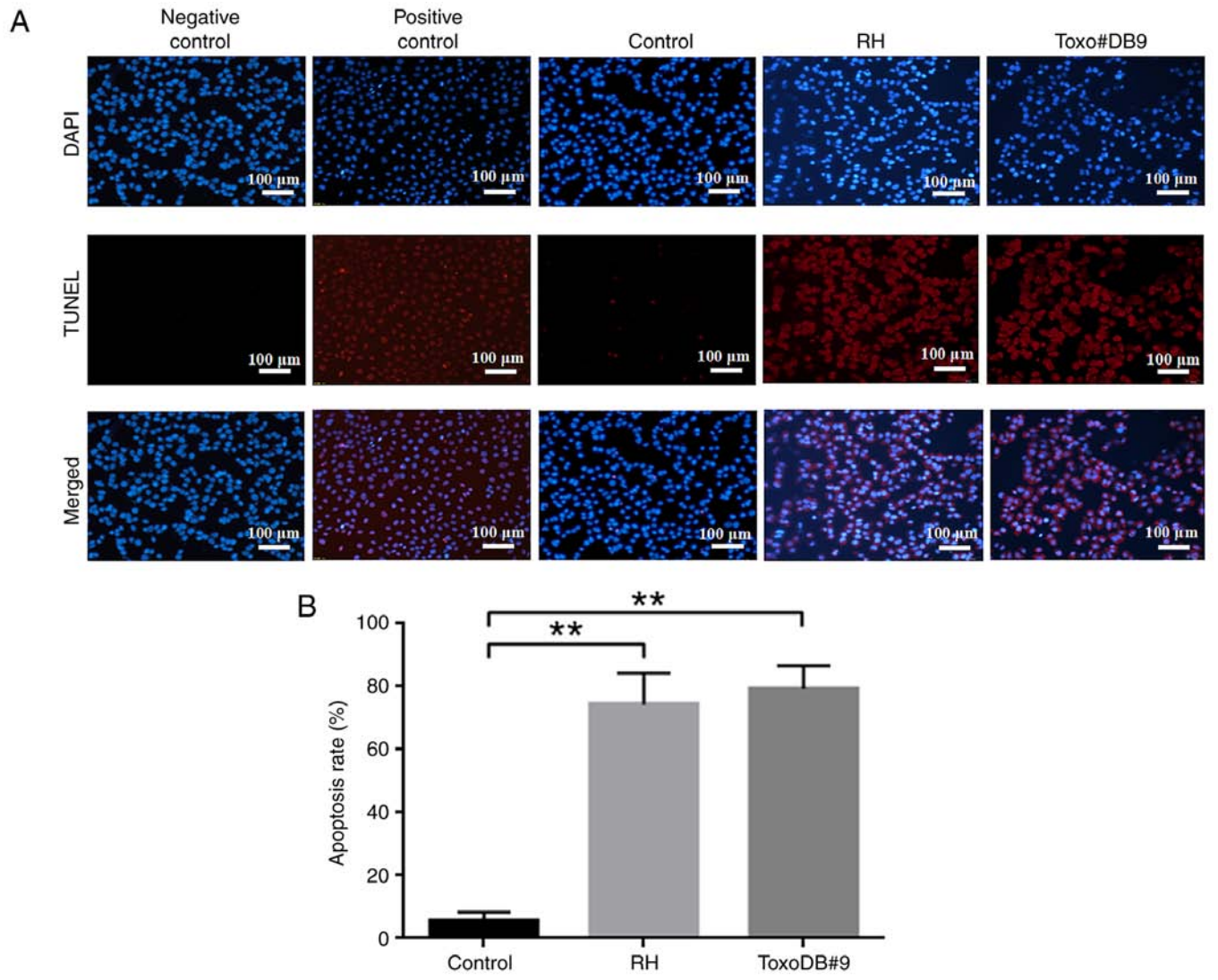

Figure 5. *T. gondii* tachyzoite culture supernatant promotes apoptosis in bladder cancer 5637 cells. (A) Cells were treated with *T. gondii* tachyzoite culture supernatant for 48 h, followed by Apoptosis Detection kit staining and analysis under a fluorescence microscope. (B) Quantification of the number of apoptotic cells in (A). **P<0.01 vs. control group.

a large proportion of cell nuclei and cytoplasm. The experimental groups exhibited a decrease in cell quantity, reduced cell volume, wrinkled cells, decreased nuclear-cytoplasmic ratios and apoptosis, including nuclear condensation and rupture (Fig. 1B and C). The results indicated that *T. gondii* tachyzoite culture supernatant was able to alter the morphology of bladder cancer 5637 cells.

T. gondii tachyzoite culture supernatant inhibits proliferation of bladder cancer 5637 cells in vitro. To assess whether *T. gondii* tachyzoite culture supernatant can inhibit proliferation of bladder cancer 5637 cells *in vitro*, CCK-8 assay was used to detect the effects of *T. gondii* tachyzoite culture supernatant on the proliferation of bladder cancer 5637 cells. The OD values were measured at 24, 48 and 72 h and growth curves were plotted based on the results (Fig. 2). After *T. gondii* culture supernatant acted on bladder cancer 5637 cells, proliferation was inhibited to different degrees. At 72 h, the inhibition rates of the RH experimental group and ToxoDB#9 experimental group reached 18.57 and 35.24%, respectively and the inhibition rate of *T. gondii* culture supernatant stimulated at the same time in the ToxoDB#9 experimental group was higher than that of the RH experimental group (P<0.01; Table I). For the selection of *T. gondii* culture supernatant doses in subsequent experiments, the concentrations were based on the results of the pre-experiment and the MOI of *T. gondii* tachyzoite culture supernatant with bladder cancer 5637 cells was 10:1. These experiments helped to determine the appropriate range of *T. gondii* tachyzoite culture supernatant concentrations that would not cause excessive cell death, while still eliciting a biological response.

T. gondii tachyzoite culture supernatant inhibits the migration of bladder cancer 5637 cells in vitro. After *T. gondii* tachyzoites culture supernatant was applied to bladder cancer 5637 cells, the cell migration rates of the control, RH experimental and ToxoDB#9 experimental groups were 19.92±8.62, 11.80±3.18 and 4.02±1.73% at 24 h and 64.60±5.60, 17.57±4.24 and 15.47±4.82% at 36 h, respectively (Fig. 3A). At the same time, the cell migration rates of the RH and ToxoDB#9

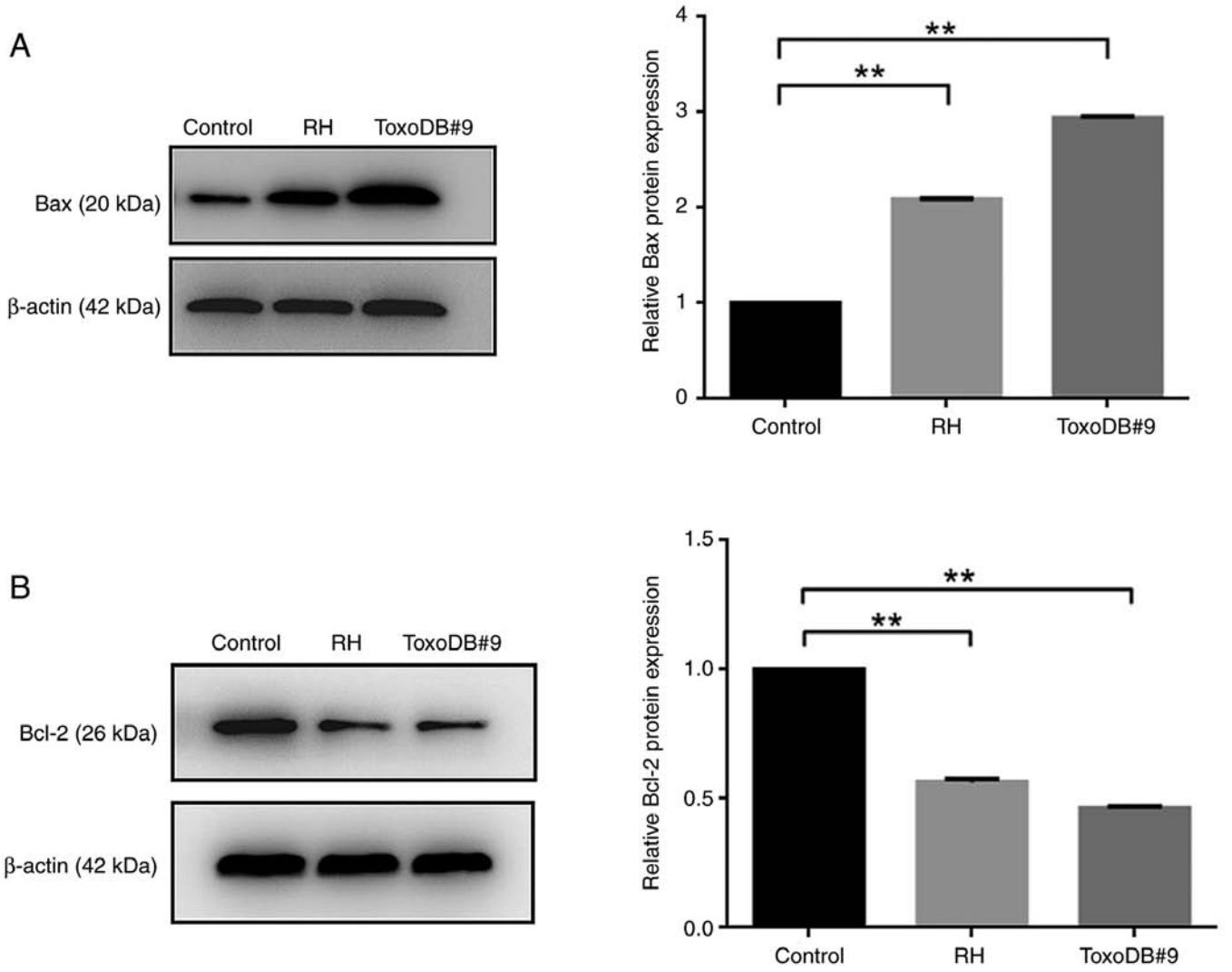

Figure 6. *T. gondii* tachyzoite culture supernatants affect apoptosis in bladder cancer 5637 cells. (A) Western blotting was used to detect Bax protein expression in the control and experimental groups. (B) Western blotting was used to detect Bcl-2 protein expression in the control and experimental groups. **P<0.01 vs. control group.

experimental groups were significantly lower than those of the control group (Fig. 3B). The results indicated that *T. gondii* tachyzoite culture supernatant inhibited the migration of bladder cancer 5637 cells and the inhibition rate of *T. gondii* tachyzoite ToxoDB#9-type strain culture supernatant was higher than that of the RH-type strain.

T. gondii tachyzoite culture supernatant inhibits the migration and invasion of bladder cancer 5637 cells in vitro. Following the action of *T. gondii* culture supernatant on bladder cancer 5637 cells, the number of migrating cells in the control, RH experimental and ToxoDB#9 experimental groups were 899.33±28.19, 476.00±25.96 and 439.00±31.38 (Fig. 4A and B) and the number of invading cells were 459.67±11.61, 343.33±20.74, 217.33±32.42 (Fig. 4C and D). The number of migrating and invading cells was reduced in both experimental groups compared with that in the control group. The results indicated that the culture supernatant of *T. gondii* tachyzoite inhibited the migration and invasion of bladder cancer 5637 cells. On the other hand, ToxoDB#9 experimental group has fewer migrating and invading cells compared with RH experimental group, which means that the culture supernatant of *T. gondii* ToxoDB#9 genotype was more inhibitory than RH genotype to the migration and invasion of bladder cancer cells.

T. gondii tachyzoite culture supernatant promotes apoptosis in bladder cancer 5637 cells. The present study evaluated the effect of *T. gondii* tachyzoite culture supernatant on the induction of apoptosis in bladder cancer 5637 cells. The apoptosis rates of the control, RH experimental and ToxoDB#9 experimental groups were 5.71±2.37, 74.35±5.88 and 79.29±6.54%, respectively, after the action of *T. gondii* tachyzoite culture supernatant on 5637 bladder cancer cells (Fig. 5A and B). Compared with that in the control group, the apoptosis rate was higher in the RH and ToxoDB#9 experimental groups, which indicated that the culture supernatant of *T. gondii* tachyzoite promoted the apoptosis of bladder cancer 5637 cells.

T. gondii tachyzoites culture supernatants promotes apoptosis in bladder cancer 5637 cells by modulating apoptotic proteins. Upon the action of *T. gondii* tachyzoite culture supernatant on bladder cancer 5637 cells, the expression levels of Bax in the

RH and ToxoDB#9 experimental groups were significantly increased, the expression levels of Bcl-2 were significantly decreased and the Bcl-2/Bax ratio were decreased compared with those in the control group (Fig 6A and B), suggesting *T. gondii* tachyzoite culture supernatant may promote the apoptosis of bladder cancer by regulating apoptotic genes.

Discussion

Bladder cancer is the most prevalent malignant neoplasm affecting the urinary system and is characterized by its propensity for frequent recurrence and metastasis, often resulting in a poor prognosis (27). The primary treatment modalities for this condition typically involve surgical intervention in conjunction with radiotherapy or chemotherapy; however, the efficacy of these approaches remains limited. Consequently, there is a pressing demand for the development of novel therapeutic strategies that are both safe and efficacious for the management of bladder cancer. The landscape of cancer treatment is continuously evolving, with a growing emphasis on biological therapy due to its reduced toxicity profile and minimal adverse effects. Notably, the existing literature from both domestic and international sources suggests that *T. gondii* or its secreted metabolites may exert a dual influence on the initiation and progression of malignant tumors (28).

Xu *et al* (29) used CRISPR/CRISPR associated protein 9 technology to create an uracil auxotroph *T. gondii* RH strain with orotidine 5'-monophosphate decarboxylase gene deletion (RH-Δompdc). The authors revealed that inserting RH-Δompdc immediately into the 4T1 tumor prompts anti-infection and antitumor immunity in mice, inhibiting tumor growth and metastasis, promoting tumor-bearing survival and increasing IL-12 and IFN-γ secretion in serum and the tumor microenvironment. Ye *et al* (21) reported that *T. gondii* transcriptionally regulates various signaling pathways by modifying hub genes such as BRCA1, MYC and IL-6, which can suppress breast tumor development and migration (21). Zhu *et al* (30) used ultracentrifugation to isolate exosomes that from uninfected dendritic cells (DCs) and *T. gondii* Me49-infected DCs and found that the exosomes inhibit polarization of macrophages to M2 phenotype and regulate suppressor of cytokine signaling 1 expression by functional microRNA-155-5p to inhibit colorectal cancer. Ismail *et al* (31) reported that *T. gondii*-derived antigen changes the tumor microenvironment of the Ehrlich solid carcinoma mouse model and increases the immunotherapeutic efficacy of cyclophosphamide. Eissa *et al* (32) demonstrate a prophylactic antineoplastic activity of autoclaved Toxoplasma vaccine against Ehrlich solid carcinoma. Zhou *et al* (33) report that *T. gondii* rhoptry protein 18 reduces human glioma cell apoptosis via a mitochondrial route, targeting host cell P2X1. Mani *et al* (34) report that *T. gondii* rGRA6Nt is a unique and successful protein enhancer when used in vaccinations with non-replicable cancer cells to effectively activate immune defenses, particularly against the cancer cells used in the vaccine. All these studies have shown that *T. gondii* and its secretions have antitumor effects. However, it is not known whether *T. gondii* and its secretions can inhibit the growth of bladder cancer. Therefore, the present experiment was designed to explore the role of *T. gondii* tachyzoite culture supernatant in bladder cancer 5637 cells.

In the present study, an *in vitro* study was conducted using the human bladder cancer 5637 cell line. The cells were cultured in the presence of varying concentrations of *T. gondii* tachyzoite culture supernatant. The initial observations revealed that the *T. gondii* tachyzoite culture supernatant had an effect on the proliferation, migration, invasion and apoptosis of 5637 cells. To elucidate the underlying mechanisms, a series of molecular analyses were performed. Western blotting and quantitative PCR assays demonstrated that the supernatant induced apoptosis in the bladder cancer cells, as evidenced by the increased expression of pro-apoptotic proteins such as Bax and cleaved caspase-3 and a corresponding decrease in the levels of anti-apoptotic proteins such as Bcl-2. Furthermore, it was observed that the *T. gondii* tachyzoite culture supernatant upregulated the expression of tumor suppressor genes and downregulated oncogene expression, indicating a potential tumor-suppressive role of *T. gondii* tachyzoite culture supernatant. Notably, supernatant appeared to impede the migration and invasion of 5637 cells, as measured by wound healing and Transwell assays. The present study suggested that *T. gondii* tachyzoite culture supernatant may be able to limit the metastatic spread of bladder cancer, which is an important part of cancer progression.

In conclusion, the results of the present study demonstrated that *T. gondii* tachyzoite culture supernatant had the ability to inhibit the proliferation, migration and invasion and promote the apoptosis of bladder cancer 5637 cells. These findings suggested that *T. gondii* or its secretions could be a novel source of therapeutic agents for the treatment of bladder cancer. However, there are still some limitations to the present study. Although the culture supernatant of *T. gondii* tachyzoites was found to inhibit the proliferation, migration and invasion and promote the apoptosis of bladder cancer 5637 cells, the exact mechanism is not known and the present study lacked *in vivo* experiments. Therefore, future studies should focus on identifying the specific components of the supernatant responsible for these effects and evaluating their efficacy *in vivo*, as well as assessing any potential adverse effects that may arise from the use of *T. gondii*-derived treatments.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

SD designed and performed the experiments, analyzed the data and wrote the manuscript. YY, TW, LZ and HL performed experiments and data analyses. SD and YL designed and supervised the project, acquired the funding and revised the

manuscript. LZ and HL confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Medical Ethics Committee of Dali University (approval number: 2024-0833).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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