

Tryptophan 2, 3-dioxygenase promotes proliferation, migration and invasion of ovarian cancer cells

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Abstract. Tryptophan 2,3-dioxygenase (TDO2) is a key rate-limiting enzyme in the kynurenine pathway and promotes tumor growth and escape from immune surveillance in different types of cancer. The present study aimed to investigate whether TDO2 serves a role in the development of ovarian cancer. Reverse transcription-quantitative PCR and western blotting were used to detect the expression of TDO2 in different cell lines. The effects of TDO2 overexpression, TDO2 knockdown and TDO2 inhibitor on ovarian cancer cell proliferation, migration and invasion were determined by MTS, colony formation and Transwell assays. The expression of TDO2 in ovarian cancer tissues, normal ovarian tissues and fallopian tube tissues were analyzed using the gene expression

data from The Cancer Genome Atlas and Genotype-Tissue Expression project. Immune cell infiltration in cancer tissues was evaluated using the single sample gene set enrichment analysis algorithm. The present study found that Ras^{V12}-mediated oncogenic transformation was accompanied by the upregulation of TDO2. In addition, it was demonstrated that TDO2 was upregulated in ovarian cancer tissues compared with normal ovarian tissues. TDO2 overexpression promoted proliferation, migration and invasion of ovarian cancer cells, whereas TDO2 knockdown repressed these phenotypes. Treatment with LM10, a TDO2 inhibitor, also repressed the proliferation, migration and invasion of ovarian cancer cells. The present study indicated that TDO2 can be used as a new target for the treatment of ovarian cancer.

Introduction

Ovarian cancer is the seventh commonest type of cancer in women and the eighth most common cause of cancer death worldwide, with a 5-year survival rate <45%. The incidence has increased with increased life expectancy, especially in developing countries. As the symptoms of early-stage ovarian cancer are mild and non-specific, most cases are diagnosed at an advanced stage and require aggressive cytoreductive surgery, followed by platinum and taxane-based chemotherapy (1). At present, ~85% of patients with advanced ovarian cancer who have achieved full remission following surgery and chemotherapy develop recurrent disease. The median survival time of patients with advanced ovarian cancer ranges only 12–24 months (2,3).

The kynurenine pathway is an important pathway for tryptophan metabolism; >95% of the tryptophan in the body is metabolized through the kynurenine pathway (4,5). In this pathway, tryptophan is first metabolized into the intermediate N-formyl kynurenine by one of three first-step enzymes, namely, indoleamine 2,3-dioxygenase 1 (IDO1), indoleamine 2,3-dioxygenase 2 (IDO2) and tryptophan 2,3-dioxygenase (TDO2) and then converted into kynurenine

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Abbreviations: TDO2, tryptophan 2,3-dioxygenase; IDO1, indoleamine 2,3-dioxygenase 1; IDO2, indoleamine 2,3-dioxygenase 2; RT-qPCR, reverse transcription-quantitative PCR; NK, natural killer cells; Th1, T-helper type 1 cells; iDCs, immature dendritic cells; Tregs, regulatory T cells; Th2, T-helper type 2 cells; γδT, gamma delta T cells; aDCs, activated dendritic cells; TFH, follicular helper T cells; TCM, central memory T cell

Key words: tryptophan 2,3-dioxygenase, ovarian cancer, proliferation, migration, invasion

by arylformamidase (6). Kynurenine subsequently undergoes a series of enzymatic reactions and is converted into 3-hydroxykynurenine, 3-hydroxyanthranilic acid, 2-amino-3-carboxymuconatesemialdehyde and quinolinic acid, after which it is finally converted to nicotinamide adenine dinucleotide for metabolic use. The metabolites of the kynurenine pathway are involved in multiple physiological activities, mainly in the nervous and the immune systems (4,5). The first-step enzymes, namely IDO1, IDO2 and TDO2, are the most important rate-limiting enzymes in the kynurenine pathway (7). At the amino acid level, IDO1 and IDO2 have ~43% sequence similarity, whereas TDO2 has little similarity with IDO1 and IDO2 (8,9). Studies have shown that one or more of these enzymes are upregulated to different degrees in tumor tissues (10-16). Upregulation of these enzymes leads to tryptophan depletion and kynurenine accumulation, thereby impairing the anticancer immune response (17-23). The expression and roles of IDO1 in ovarian cancer have been extensively investigated (24-29). Inaba *et al* (30) reported that higher expression of IDO1 in ovarian cancer tissues is associated with shorter survival time. Furthermore, overexpression of IDO1 in the human ovarian carcinoma cell line SKOV3 promotes xenograft tumor growth (30), whereas knockdown of IDO1 in SKOV3 cells suppresses xenograft tumor growth (31). The tumor-promoting effect of IDO2 in SK-IDO-xenografted mice is blocked by oral administration of the IDO inhibitor 1-methyl-tryptophan (30). The present study aimed to investigate the expression and roles of TDO2 in ovarian cancer.

Materials and methods

Plasmid and reagents. The TDO2 overexpression plasmid was designed by Sino Biological Inc. Briefly, the coding sequence of pCMV3-TDO2 was amplified using 5'-TAATAC GACTCACTATAGGG-3' and 5'-TAGAAGGCACAGTCG AGG-3' as primers and inserted into the *Kpn*I/*Xba*I site of pCMV3-untagged vector (Sino Biological Inc.). LM10, which is an effective TDO inhibitor, was purchased from Selleck Chemicals. The cells were treated with LM10 at a final concentration of 500 µM (32).

Cell culture. The human ovarian epithelial cell lines T29 and T29H were provided by Dr Jinsong Liu (School & Hospital of Stomatology, Wenzhou Medical University, China) (33,34). Briefly, isolated human surface ovarian epithelium cells were infected sequentially by retroviruses containing SV40 T/t antigens and hTERT genes to generate T29 cells. The immortalized, but non-oncogenic T29 cells were further transformed by introducing an oncogenic H-RAS^{V12} in a pLNCX retroviral vector to form the T29H cell. The SKOV3 cell line was provided by Dr Xueqiong Zhu (The Second Affiliated Hospital of Wenzhou Medical University, China). The OVSAHO (JCRB1046) cells were from JCRB Cell Bank. T29, T29H and OVSAHO cells were maintained in high-glucose Dulbecco's modified Eagle's medium (DMEM; HyClone; Cytiva) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin (Gibco; Thermo Fisher Scientific, Inc.). Human ovarian cell line SKOV3 cells were maintained in

Roswell Park Memorial Institute-1640 medium (RPMI-1640; HyClone; Cytiva) supplemented with 10% FBS and 1% penicillin-streptomycin. Cells were cultured in an incubator in a 5% CO₂ humidified atmosphere at 37°C. All cell lines were mycoplasma free and cells passaged at the Digestive Cancer Center, The First Affiliated Hospital of Wenzhou Medical University for >6 months following receipt were authenticated by genetic profiling using polymorphic short-tandem repeat loci (35).

Small interfering (si)RNA and transfection. siRNAs were purchased from Gema Gene. The siRNA sequences were: siTDO2, 5'-CGUUUAUCGCGUUAUAUACCGGUATT-3' (sense), 5'-UACGCGUAUUAUACCGCGAUUAACGTT-3' (anti-sense); siNC (negative control siRNA), 5'-AAUUCUCCG AACGUGUCACGUTT-3' (sense) and 5'-ACGUGACACGUU CGGAGAAUUTT-3' (anti-sense).

Cells were seeded into 6-well plates at 3x10⁵/well. The plasmids were transfected 2.5 µg/well and the siRNAs were transfected at a final concentration of 50 nM using Lipofectamine® 2000 Reagent (Invitrogen; Thermo Fisher Scientific, Inc.), following the manufacturer's instructions. The cells were then collected 48 h after transfection for subsequent analysis, including western blotting and reverse transcription-quantitative (RT-q) PCR.

Western blotting. Total protein was extracted from T29, T29H, OVSAHO and SKOV3 cells using RIPA lysis buffer (Beyotime Institute of Biotechnology). After mixing with SDS lysis buffer and boiling, the protein content in cell lysates were measured using BCA Protein Assay kit (Beyotime Institute of Biotechnology). Equal amounts of protein (30 µg) were loaded onto 10% SDS-PAGE and separated via electrophoresis, then separated proteins were transferred to PVDF membranes. After blocking in 5% skimmed milk at room temperature for 1 h, membranes were incubated overnight at 4°C with primary antibodies against TDO2 (cat. no. H00006999-A01; Abnova; 1:1,000) and anti-GAPDH (cat. no. 2118S; Cell Signaling Technology, Inc; 1:1,000). The membranes were washed with TBST, followed by incubation with goat anti-mouse IgG (cat. no. ab6789; Abcam; 1:10,000) or goat anti-rabbit IgG (cat. no. ab6721; Abcam; 1:10,000) antibodies at room temperature for 1 h. Subsequently, the bands were detected with ECL plus reagents (GE Healthcare). Densitometric analysis was performed using ImageJ software (version 1.8.0; National Institutes of Health). All experiments were repeated at least three times.

RT-qPCR. Total RNA was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.), incubated with RNase-free DNase I (Promega Corporation) for 30 min and reverse transcribed using the M-MLV reverse transcription kit (Promega Corporation) according to the manufacturer's protocol. qPCR was subsequently performed using a SYBR Green PCR Master mix (Vazyme Biotech Co., Ltd.) on an ABI PRISM 7300 Sequence Detection system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The following thermocycling conditions were used for qPCR: 95°C for 30 sec followed by 40 cycles of 95°C for 10 sec and 60°C for 30 sec. The following primer sequences were used:

TDO2: 5'-TCCTCAGGCTATCACTACCTGC (forward) and 5'-ATCTTCGGTATCCAGTGTGG-3' (reverse) (36); GAPDH: 5'-GCAAATTCCATGGCACCGTC-3' (forward) and 5'-CCTGGAAGATGGTGATGGGA-3' (reverse). The $\Delta\Delta Ct$ method was used to measure the relative expression levels of the subject genes. ΔCq was obtained by subtracting the Cq (threshold cycle) value of GAPDH from that of the subject gene and $\Delta\Delta Cq$ was calculated by subtracting the ΔCq of the control sample from that of the subject sample. The fold change was calculated as $2^{-\Delta\Delta Cq}$ and the relative expression level of the control sample was defined as 1 (37). All experiments were performed independently and repeated three times.

Cell proliferation assay. Following cell transfection for 24 h, the T29H, OVSAHO or SKOV3 cells were seeded in 96-well plates with 2×10^3 cells at each time point, CellTiter 96 Aqueous One Solution Cell Proliferation Assay (MTS) kit (Promega Corporation) was added to the corresponding plates and incubated for 3 h at 37°C according to the manufacturer's instructions. MTS is bioreduced by cells into a formazan product that is soluble in tissue culture medium. The absorbance of the formazan at 490 nm can be measured directly from the 96-well assay plates using a Multiskan Spectrum microplate reader (Thermo Fisher Scientific, Inc.) (38).

For cell number counting assays, cells were seeded into 96-well plates with 1×10^4 /well. Cells were counted at 12, 24, 48 and 72 h after transfection or drug treatment using a Countess 3 Automated Cell Counter (Thermo Fisher Scientific, Inc.).

Colony formation assay. Following cell transfection for 24 h, the cells were digested with trypsin and resuspended with the cell medium and inoculated into a six-well plate with 200 cells/well and 2 ml of the complete medium was added. The six-well plate was placed in the incubator for further cultivation. After 18 days, the cells were fixed with absolute methanol for 20 min, stained with 0.5% crystal violet at room temperature for 20 min and the sample was rinsed and images captured for counting.

Cell migration and invasion assay. Transwell inserts of 8 μ m-pore plain (to assess migration) or Matrigel-coated (to assess invasion; Costar; Corning, Inc.) were placed in the wells of 24-well culture plates and 500 μ l of DMEM or RPMI-1640 containing 10% FBS was added to the lower chamber. The T29H, OVSAHO, or SKOV3 cells were washed once with Hanks' Balanced Salt Solution (Invitrogen; Thermo Fisher Scientific, Inc.) 12 h after transfection, resuspended in 100 μ l serum-free medium (8×10^4 cells) and added to the upper chamber. After 12 h of incubation at 37°C with 5% CO₂, the cells on the top side of the filter were manually removed with a cotton swab. The cells adherent to the bottom surface of the insert were fixed in cold absolute methanol for 10 min and then stained with 0.01% crystal violet in 20% ethanol at room temperature. After 10 min, the filters were washed thoroughly in water and images were captured under a DMI3000 M inverted manual microscope (Leica Microsystems GmbH). The number of migratory cells was recorded using an optical

microscope at x100 magnification. The average number of migrated cells was assessed by counting five randomly selected microscopic fields. The experiment was performed in triplicate.

Measurement of kynurenone. Supernatants from the control group and knockdown TDO2 group cells were collected at 500 x g for 15 min at 4°C. Levels of kynurenone in the supernatant were determined using the human kynurenone ELISA kit (CUSABIO Biotech Co., Ltd.; cat. no. CSB-E13659h).

Activities of caspase-3/7. The activities of caspase-3/7 were measured using a caspase-3/7 activity apoptosis assay kit (Sangon Biotech Co., Ltd.), according to the manufacturer's instructions.

The Cancer Genome Atlas (TCGA) and The Genotype-Tissue Expression (GTEx) dataset analysis. RNA sequencing data of ovarian serous cystadenocarcinoma were obtained from TCGA database (<https://tcga-data.nci.nih.gov/tcga/>). RNA sequencing data of normal ovarian tissues and fallopian tube tissues were obtained from GTEx project (<http://gtexportal.org>). The expression data were log₂ (TPM+1) transformed. Bindea *et al* (39) examined the spatio-temporal dynamics of 24 different tumor-infiltrating immune cells. The relative quantities of these 24 immune cell types in ovarian cancer were evaluated by using the R software (www.R-project.org; version 3.6.2) GSVA (version 1.34.0) package (40). Correlation between the expression of TDO2 and the relative quantity of immune cells was calculated by the signature gene sets of the immune cells.

Statistical analysis. Data from three independent experiments are presented as the mean \pm standard error of the mean. Differences were analyzed using a two-tailed unpaired Student's t-test. Univariate hazard ratios with 95% confidence intervals were calculated using the Cox proportional hazards regression and significance was calculated using Wald's test. Statistical analysis was performed using SPSS software (version 20.0; IBM Corp.). P<0.05 was considered to indicate a statistically significant difference.

Results

Upregulation of tryptophan 2,3-dioxygenase in ovarian cancer. To investigate whether TDO2 serves a role in ovarian cancer development, the expression of TDO2 in ovarian cancer tissues, normal ovarian tissues and normal fallopian tube tissues was compared. As shown in Fig. 1A, the *TDO2* mRNA level was significantly higher in ovarian cancer tissues compared with normal ovarian tissues and fallopian tube tissues. It was then evaluated whether TDO2 was upregulated in a genetically defined model of human ovarian cancer. The T29 cells were derived from primary human ovarian surface epithelial cells by stable transfection with the SV40 T/t antigens and hTERT. The immortalized but non-oncogenic T29 cells were further transformed by introducing oncogenic HRasV12 to generate the T29H cell line, which resembles natural ovarian cancer in several aspects. It was found that the mRNA level of *TDO2* was

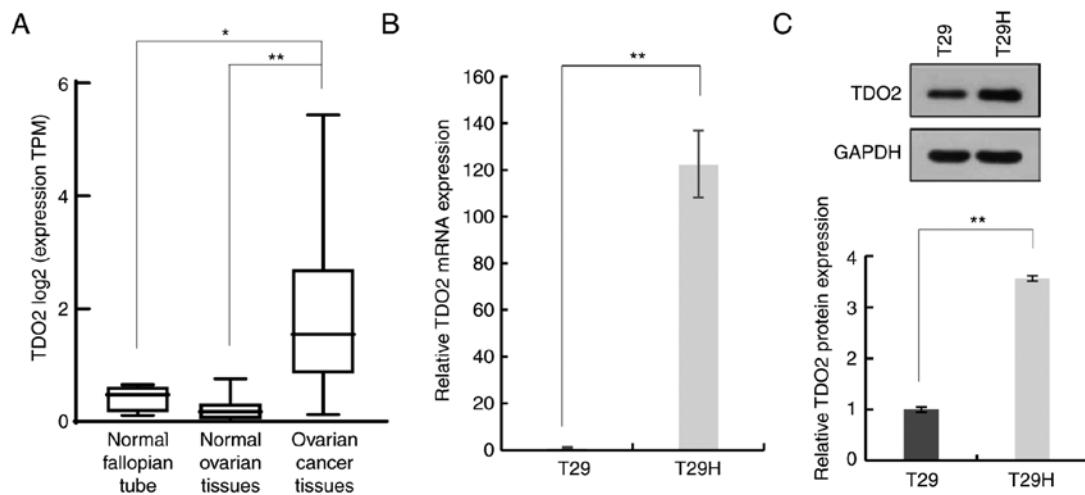


Figure 1. Upregulation of *TDO2* in ovarian cancer cells; comparison of *TDO2* mRNA expression. (A) Comparison of *TDO2* mRNA expression in 374 cases of ovarian cancer, 88 normal ovarian tissues and 5 normal fallopian tube tissues. (B) Quantification of *TDO2* levels in T29 and T29H cells using real-time polymerase chain reaction. (C) Representative western blotting of the *TDO2* protein in T29 and T29H cells. *P<0.05; **P<0.01 by Student's t-test. *TDO2*, tryptophan 2,3-dioxygenase.

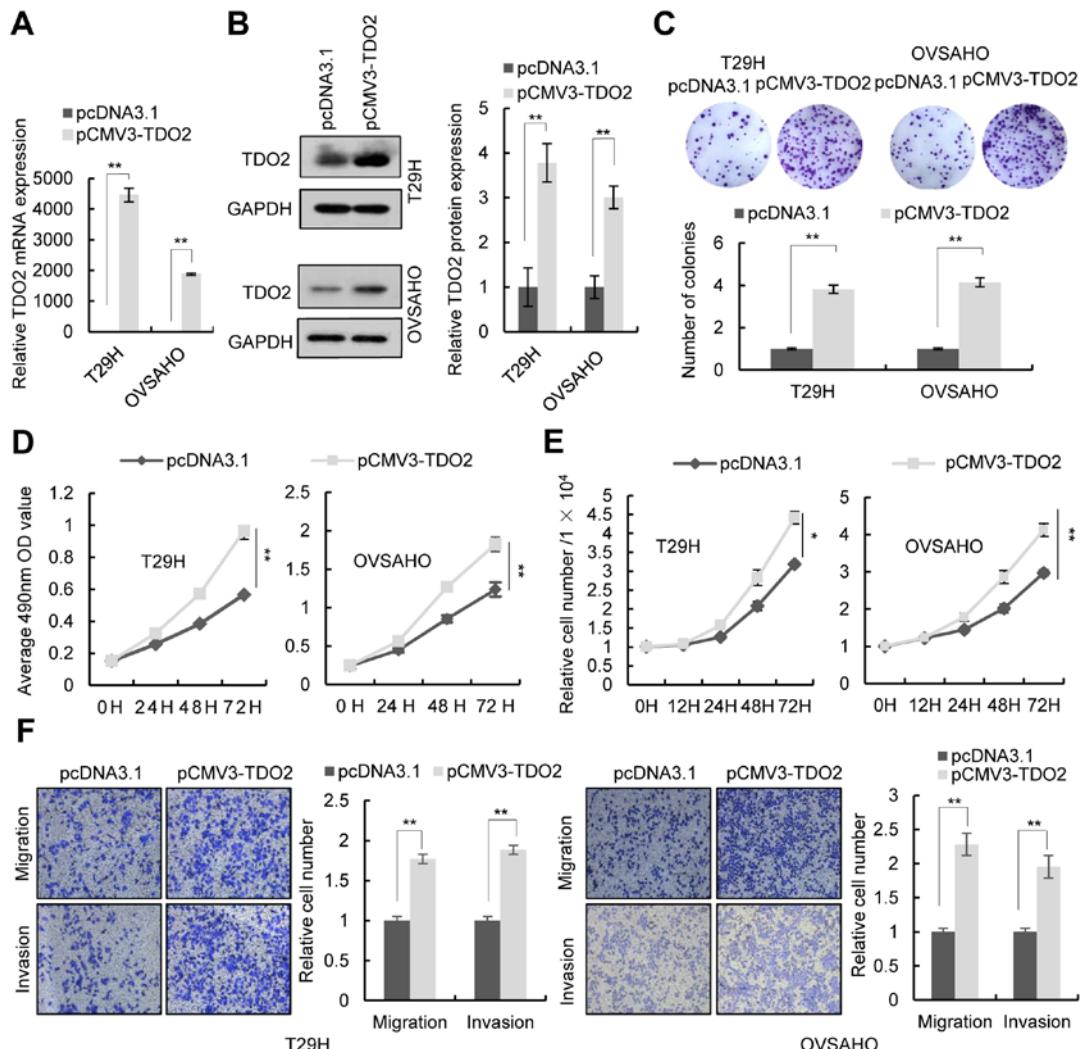


Figure 2. Effect of TDO2 overexpression on proliferation, migration and invasion of ovarian cancer cells. T29H and OVSAHO cells transfected with pCMV3-TDO2-untagged (pCMV3-TDO2) or pcDNA3.1. (A) *TDO2* mRNA levels quantified 48 h post-transfection using real-time polymerase chain reaction. (B) Representative western blotting of *TDO2* protein levels 48 h post-transfection. (C) Representative images of colony formation assay (top) and the number of colonies (bottom). (D) Cell proliferation assays were performed at different time points following transfection; (E) Cell counts 12, 24, 48 and 72 h after transfection; (F) Representative images of cell migration and invasion assays (left) and quantification (right) from three independent experiments. Magnification, x100. Data are shown as mean ± standard deviation from three independent experiments. *P<0.05; **P<0.01 by Student's t-test. *TDO2*, tryptophan 2,3-dioxygenase.

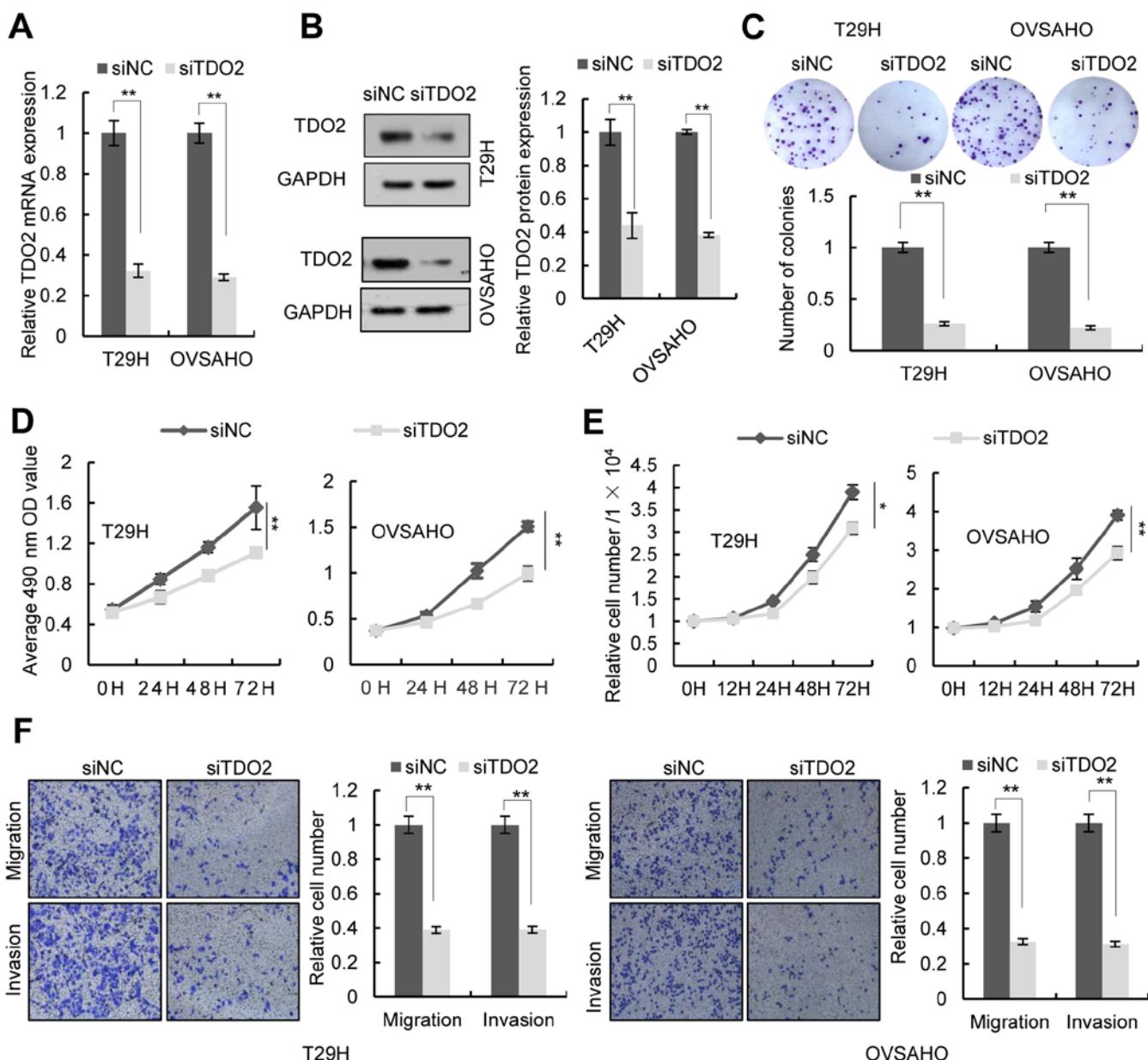


Figure 3. Effect of TDO2 knockdown on proliferation, migration and invasion of ovarian cancer cells. T29H and OVSAHO cells transfected with siTDO2 or siNC. (A) The expression of *TDO2* determined using reverse transcription-quantitative PCR and (B) western blotting 48 h after transfection. (C) Representative images of colony formation assay (top) and the number of colonies (bottom). (D) Cell proliferation assays were performed at different time points after transfection. (E) Cell counts 12, 24, 48 and 72 h after transfection. (F) Representative images of cell migration and invasion assays (left) and the quantification (right) from three independent experiments. Magnification, $\times 100$. Data are shown as mean \pm standard deviation from three independent experiments. * $P<0.05$; ** $P<0.01$ by Student's t-test. TDO2, tryptophan 2,3-dioxygenase; si, short interfering; NC, negative control.

>100-fold higher in T29H cells compared with T29 cells (Fig. 1B). Western blotting revealed that the TDO2 protein was ~3-fold higher in T29H cells compared with T29 cells (Fig. 1C). Taken together, these data indicated that TDO2 is upregulated in ovarian cancer cells.

Regulation of proliferation, migration and invasion in ovarian cancer cells by tryptophan 2,3-dioxygenase. To investigate the function of TDO2 in ovarian cancer cells, the *TDO2* overexpression plasmid was transfected into the human ovarian cancer cell lines T29H, OVSAHO and SKOV3. RT-qPCR and western blotting confirmed the overexpression of TDO2 in these cell lines

(Figs. 2A and B and S1A and B). Colony formation assays, MTS assays and cell number counts revealed that TDO2 overexpression in T29H, OVSAHO and SKOV3 promoted cell proliferation (Figs. 2C-E and S1C-E). Transwell assays indicated that TDO2 overexpression promoted the migration and invasion of ovarian cells (Figs. 2F and S1F). It was then investigated whether TDO2 knockdown affected the proliferation, migration and invasion of ovarian cancer cells. Transfection of a *TDO2*-specific siRNA led to decreased expression of TDO2 in T29H, OVSAHO and SKOV3 cells (Figs. 3A and B and S1A and B). Colony formation assays, MTS assays, and cell number counts showed that knockdown of TDO2 decreased cell proliferation (Figs. 3C-E and S1C-E).

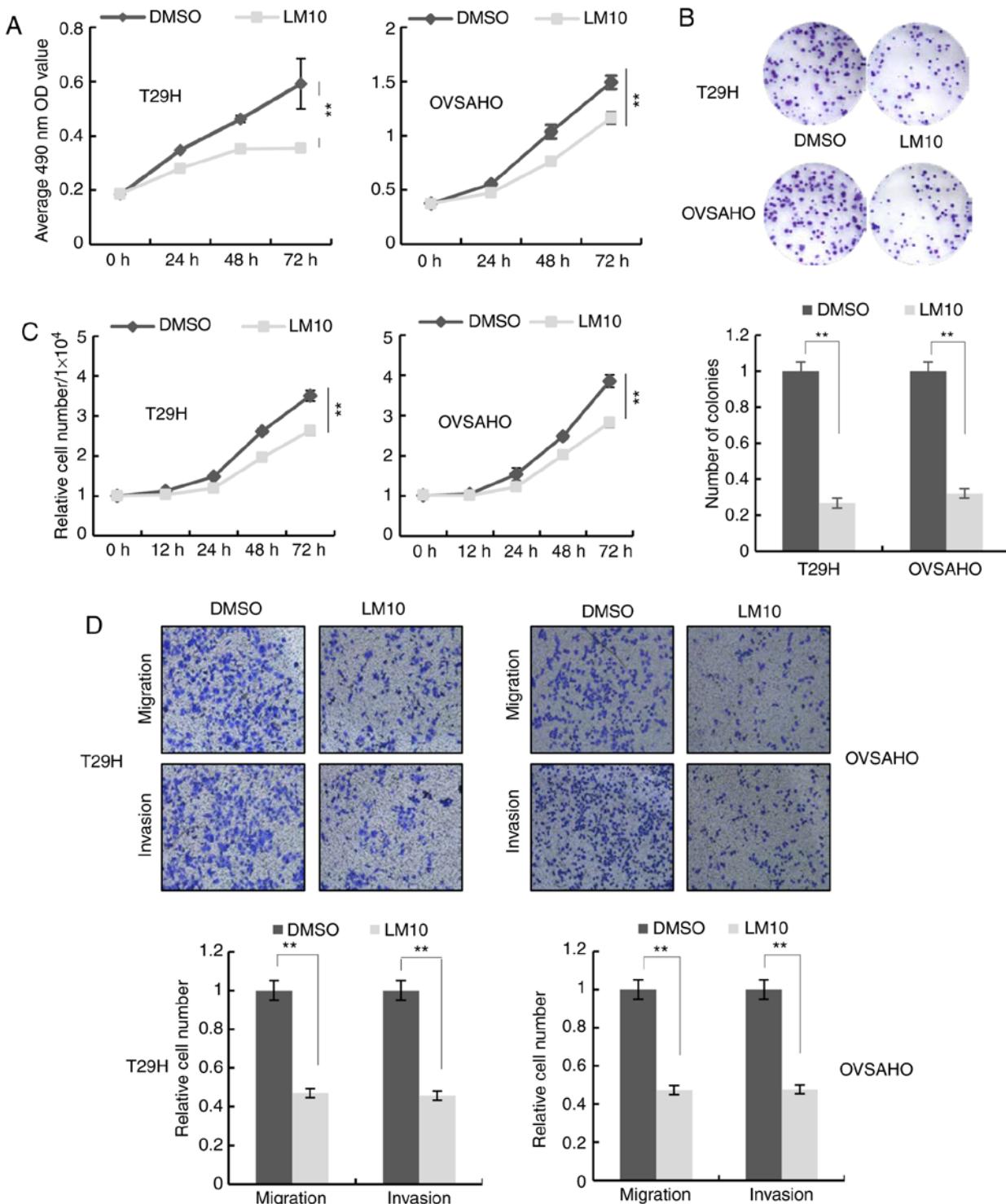


Figure 4. Effect of LM10 on proliferation, migration and invasion of ovarian cancer cells. T29H and OVSAHO cells were treated with 500 μ M LM10 or an equivalent volume of Dulbecco's modified Eagle's medium. (A) Cell proliferation assays performed at different time points. (B) Representative images of colony formation assay (top) and the number of colonies (bottom). (C) Cell counts 12, 24, 48 and 72 h after treatment. (D) Representative images of cell migration and invasion assays (top) and quantification (bottom) from three independent experiments. Magnification, $\times 100$. Data are shown as mean \pm standard deviation from three independent experiments. ** $P<0.01$ by Student's t-test. TDO2, tryptophan 2,3-dioxygenase.

Transwell assays indicated that TDO2 knockdown reduced cell migration and invasion (Figs. 3F and S1F). TDO2 knockdown did not increase the activity of caspase-3/7, indicating that the decrease of MTS signal and cell numbers in TDO2 knockdown cells was not a result of cell apoptosis (Fig. S2).

Inhibition of proliferation, migration and invasion of ovarian cancer cells by the tryptophan 2,3-dioxygenase inhibitor, LM10. LM10, a TDO2 inhibitor, reportedly exhibits anti-cancer activity by reversing the tumoral immune resistance caused by TDO2 overexpression (15). It was therefore examined whether LM10 could inhibit the proliferation, migration and invasion of

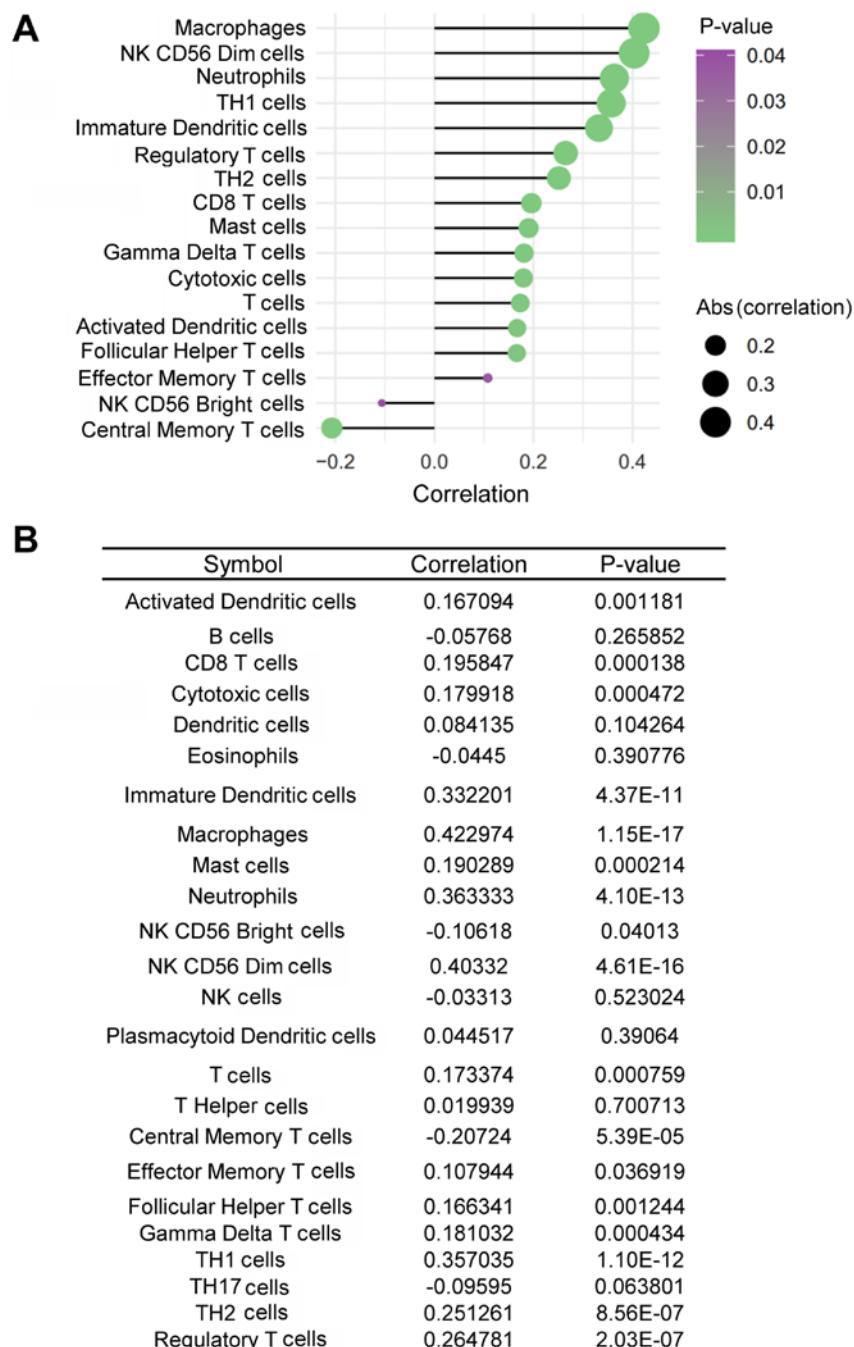


Figure 5. Association of TDO2 expression with the infiltration of different immune cells of patients with ovarian cancer. (A and B) Association of TDO2 expression with the infiltration of different immune cells in ovarian cancer tissues. TDO2, tryptophan 2,3-dioxygenase.

ovarian cancer cells. MTS assays, colony formation assays and cell number counting revealed that treatment with LM10 inhibited cell proliferation (Figs. 4A-C and S1C-E). Transwell assays indicated that LM10 significantly repressed cell migration and invasion (Figs. 4D and S1F).

Association of tryptophan 2,3-dioxygenase expression with pathological characteristics in patients with ovarian cancer. TDO2 is capable of inhibiting anti-CD3-driven T-cell proliferation and inducing CD8⁺ T-cell death (41,42). It was therefore assessed whether the expression of TDO2 was inversely associated with immune cell infiltration in ovarian cancer tissues. TDO2 expression was positively,

rather than negatively, associated with the infiltration of CD8⁺ T cells in ovarian cancer tissues (Fig. 5A and B). In addition, TDO2 expression was also positively associated with the infiltration of other immune cells, including macrophages, CD56dim natural killer (NK) cells, neutrophils, T-helper type 1 (Th1) cells, immature dendritic cells (iDCs), regulatory T cells (Tregs), T-helper type 2 (Th2) cells, mast cells, gamma delta T ($\gamma\delta$ T) cells, cytotoxic T cells, activated dendritic cells (aDCs), follicular helper T (TFH) cells and effector helper T cells in ovarian cancer tissues, whereas it was negatively associated with the infiltration of CD56bright NK cells and central memory T (TCM) cells (Fig. 5A and B).

Discussion

TDO2 has been found to be upregulated in different types of cancers including Merkel cell carcinoma, breast carcinoma, bladder carcinoma, colorectal carcinoma, hepatocarcinoma and melanoma (15,43,44). However, its expression and role in ovarian cancer remain to be elucidated. The present study found that TDO2 was upregulated in ovarian cancer tissues compared with that in the normal ovarian tissues. Overexpression of TDO2 promoted the proliferation, migration and invasion of ovarian cancer cells, whereas knockdown of TDO2 suppressed these phenotypes. In addition, inhibiting the activity of TDO2 with LM10 suppressed the proliferation of ovarian cancer cells. Kynurenine is reported to promote proliferation, migration and invasion of cancer cells (6,13). The results of the present study showed that knockdown of *TDO2* by ~70% did not significantly reduce the levels of kynurenine (Fig. S3), suggesting that TDO2 may promote cancer cell proliferation, migration and invasion in a kynurenine-independent mechanism. Taken together, these data suggested that *TDO2* functions as an oncogene in ovarian cancer and that LM10 may be a candidate drug for ovarian cancer treatment.

TDO2 has been shown to inhibit the proliferation and activation of T cells in the tumor microenvironment, thereby allowing tumors to escape immune surveillance (9,45). Pilote *et al* (15) reported that cancer cells with increased expression of TDO2 exhibited increased immune tolerance in mice and grew rapidly and the oral TDO2 inhibitor LM10 reversed this immune tolerance and inhibited tumor growth by activating anti-tumoral immunity. Consistent with these studies, the present study found that TDO2 expression was positively associated with Treg infiltration in ovarian cancer tissues. By contrast, the expression levels of TDO2 were also positively associated with CD8⁺ T-cell infiltration. These conflicting observations suggested that further studies are needed to determine whether TDO2 can negatively regulate the functions of CD8⁺ T cells. In addition, TDO2 expression was also positively associated with the infiltration of other immune cells, including macrophages, CD56dim NK cells, neutrophils, Th1 cells, iDCs, Th2 cells, mast cells, γδT cells, aDCs and TFH cells but negatively associated with the infiltration of CD56bright NK and TCM cells in ovarian cancer tissues. Future efforts should be directed to address the effects of TDO2 on these immune cells.

In summary, the findings of the present study indicated that TDO2 can promote proliferation, migration and invasion of ovarian cancer cells and that TDO2 expression is associated with immune cell infiltration in ovarian cancer tissues. Future studies should construct different animal models to investigate the *in vivo* role of TDO2 in ovarian cancer and to elucidate the underlying molecular mechanisms.

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Availability of data and materials

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

Authors' contributions

KFT conceived and devised the study. KFT, YX, RO, WC, XW and FT designed the experiments and performed the analysis. YZ drafted the manuscript and revised the manuscript critically for important content. FT drafted the original manuscript. YZ, FT, JJ, LC, JD, XC, QH and SZ performed the experiments and analyzed the data. KFT, YX, RO, WC and XW contributed reagents and materials. KFT, YX and YZ confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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