lncRNA ZNF667-AS1 inhibits ovarian cancer progression by interacting with the TNF signaling pathway

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Abstract. Ovarian cancer (OC) is the most lethal gynecological malignancy and poses a significant public health burden. The present study explored the function and mechanism of long noncoding RNA (lncRNA) ZNF667-AS1 in OC progression. The present study conducted a multifaceted evaluation, including transcriptomic analyses, to examine the expression and prognostic value of lncRNA ZNF667-AS1 in OC via the Cancer Genome Atlas and Genotype-Tissue Expression data. *In vitro* experiments on OC cell lines were used to investigate the functional effect of ZNF667-AS1 via cell proliferation, migration and invasion assays and RNA sequencing and western blotting were used to explore the implicated molecular pathways. ZNF667-AS1 was significantly underexpressed in OC tissues and cell lines. Its expression levels were positively associated with improved patient prognosis and affected both tumor behavior and tumor microenvironment interactions. Functional analysis confirmed the tumor-suppressive role of ZNF667-AS1 and revealed a marked decrease in proliferation, migration and invasion in ZNF667-AS1-overexpressing cells. Additionally, ZNF667-AS1 was identified as a key regulator in the tumor necrosis factor signaling pathway, which suggests a strong link between ZNF667-AS1 expression and OC progression. The present study identified ZNF667-AS1 as a potential biomarker for OC prognosis and treatment and illustrated its significant regulatory effects on the TNF pathway and its broader implications in cancer pathobiology.

Introduction

Gynecological cancers accounted for ~94,000 new cases annually in the United States between 2012 and 2016 (1,2). Globally, gynecological cancers account for >3.6 million new cases and 1.3 million mortalities per year, which represents >40% of female cancer incidence and 30% of female cancer mortality worldwide (3,4). Ovarian cancer (OC) is the most lethal gynecological malignancy, with the highest mortality rate among these types of cancer (3). Despite advances in diagnostics and therapies, OC prognosis remains poor because of the lack of sensitive and specific biomarkers for early detection and prognosis. Novel OC biomarkers are urgently needed to improve clinical outcomes.

Long noncoding RNAs (lncRNAs), RNA transcripts >200 nucleotides long with limited protein-coding potential, are critical regulators of tumor pathobiology (5). By modulating gene expression and key oncogenic pathways, lncRNAs can function as tumor suppressors or oncogenes and influence cancer cell chemoresistance, the epithelial-mesenchymal transition, proliferation and other malignant traits (6-8). Studies have linked dysregulated lncRNA expression to the pathogenesis and progression of various types of cancer, lncRNA CASC15 drives the OC epithelial-mesenchymal transition by regulating microRNA (miR)-23b and SMAD3 (9), similarly, lncRNA-CDC6 and RMRP are key regulators in breast and bladder cancer, respectively (10,11). Additionally, lncRNA 01123 is an oncogenic lncRNA that promotes hepatocellular carcinoma metastasis and proliferation through the miR-34a-5p/TUFT1 pathway (12).

ZNF667-AS1 is a cancer-associated lncRNA located at chromosomal region 19q13.43 in humans and it is silenced early in malignant transformation due to promoter DNA hypermethylation and loss of expression during cellular immortalization (13). ZNF667-AS1 is normally expressed in healthy cells, but its expression is lost upon cellular immortalization. This silencing is due to promoter DNA hypermethylation following transformation. Studies have shown that

downregulation of ZNF667-AS1 occurs in different types of cancer, which demonstrates a potential tumor-suppressive role (14,15).

Analysis of The Cancer Genome Atlas (TCGA) revealed significant downregulation of ZNF667-AS1 in OC and its expression was significantly associated with ovarian cancer prognosis. However, the functional significance and mechanistic roles of ZNF667-AS1 in OC pathogenesis are not fully defined. The present study employed a multifaceted experimental approach, including reverse transcription-quantitative (RT-q) PCR, RNA sequencing, fluorescence *in situ* hybridization (FISH), cell function assays and western blotting, to elucidate the tumor-suppressive functions and molecular mechanisms of lncRNA ZNF667-AS1 in OC, which pave the way for its development as a novel therapeutic target.

Materials and methods

Pan-cancer analysis. Expression data for ZNF667-AS1 across 33 types of cancer and corresponding normal tissues were extracted from TCGA (http://sangerbox.com) and genotype-tissue expression databases (http://sangerbox.com) and subsequent pan-cancer analyses of ZNF667-AS1 concerning overall survival (OS), disease-specific survival (DSS), immune infiltration (TIMER, EPIC, IPS, MCPcounter, xCELL, QUANTISEQ and CIBERSORT), genomic heterogeneity and stemness (DNA, EREG-METH, DMPs, ENHs, RNAss and EREG-EXPs) were conducted via the SangerBox web tool (http://sangerbox.com) (16).

Cell cultures. The OC cell line SKOV3 used in the present study was purchased from Procell Life Science & Technology Co., Ltd. The IOSE80 and OVCA433 cells were a gift from the First Affiliated Hospital of the Anhui Medical University (Anhui, China). SKOV3, IOSE80 and OVCA433 cells were cultured in McCoy's 5A medium (Procell Life Science & Technology Co., Ltd), RPMI-1640 medium, or RPMI-1640 medium (Procell Life Science & Technology Co., Ltd), respectively, supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.). The cells were maintained at 37˚C in a humidified 5% CO_2 incubator and the medium was renewed every 2 days.

Cell transfection. *In vitro* transfection of OC cells was performed using Lipofectamine® 2000 reagent (Thermo Fisher Scientific, Inc.) with a PCDH vector expressing lncRNA ZNF667-AS1 (2.5 µg of lncRNA ZNF667-AS1 and 5 µl of Lipofectamine® 2000 reagent), ZNF667-AS1 small interfering (si)RNA (10 µl of siRNA and 5 µl of Lipofectamine® 2000 reagent), or corresponding negative controls (Wuhan Miaoling Biotech Science Co., Ltd.) and the transfection mass of the control group was the same as that of the experimental group. The sequences of plasmid were shown in Table SI. Transfections were conducted at 60% confluence in 6-well plates following the manufacturer's protocol. Following transfection, the experiment was continued for 24-48 h in a 37˚C cell incubator.

RT-qPCR. Total RNA was extracted from $2x10^6$ cells using TRIzol® reagent (Thermo Fisher Scientific, Inc.) and detection of RNA concentration and purity (260/280) was performed using nucleic acid protein analyzer. RNA was reverse transcribed into cDNA with a Promega reverse transcription system (Promega Corporation). RT-qPCR was performed with SYBR Green Master Mix (Vazyme Biotech Co., Ltd.) on a real-time PCR detection system: 95˚C predenaturation 30 sec, 40 cycles of 95˚C 10 sec and 60˚C 30 sec. Relative lncRNA ZNF667-AS1 levels were quantified by the $2^{-\Delta\Delta Cq}$ method and normalized to the level of GAPDH (17). The RT-qPCR primers used are listed in Table SI. In studies that do not involve metabolic regulation, GAPDH, as a widely expressed steward gene, is an internal reference gene with stable expression and is often used in lncRNA studies (18). RNA extraction, cDNA synthesis, and qPCR performed according to the manufacturer's protocols and these experiments were replicated three times.

5-Ethynyl-2'-deoxyuridine (EdU) assay. Cell proliferation was assessed using the EdU incorporation assay. Briefly, cells grown on coverslips in 24-well plates were incubated with EdU medium for 2 h at 37˚C. Following fixation in 4% paraformaldehyde at room temperature for 15 min and permeabilization in 0.3% Triton X-100 (Biosharp Life Sciences), EdU labelling was performed via a click reaction cocktail for 30 min, following the manufacturer's instructions (Beyotime Institute of Biotechnology). The cells were counterstained with Hoechst 33342 (Beyotime Institute of Biotechnology) at room temperature for 10 min and visualized under a fluorescence microscope (magnification, x100).

Colony formation assay. The cells were seeded in 6-well plates and cultured for 10-14 days at 37˚C with 5% CO_2. Colonies were fixed in 4% paraformaldehyde at room temperature for 15 min, stained with 0.1% crystal violet solution (Beyotime Institute of Biotechnology) at room temperature for 15 min and counted manually. A total of three wells in each group were counted and used as separate experiments.

Scratch wound healing assay. Confluent cell monolayers in 6-well plates were scratched with a 200 µl pipette tip to create wound gaps, washed three times with PBS and incubated in serum-free medium. Wound closure was monitored by taking phase contrast images at 0 and 24 h post wounding using an inverted microscope (magnification, x100).

Transwell assay. Cell migratory and invasive capacities were evaluated via Transwell assays. For invasion, the upper chamber was precoated with Matrigel matrix at 37˚C for 2 h (BD Biosciences). Cells in serum-free medium were added to the upper chamber, while complete medium supplemented with 10% FBS as a chemoattractant was added to the lower well. Following 24-48 h of incubation, the cells on the lower surface were fixed in 4% paraformaldehyde at room temperature for 15 min and stained with crystal violet solution (Beyotime Institute of Biotechnology). The cells were imaged and quantified under a light microscope (magnification, x100). For the migration assays, the upper chamber was without the Matrigel matrix.

RNA sequencing (RNA-seq). RNA extracted from three pairs of stably transfected ZNF667-AS1 overexpressing and control OC cells was subjected to RNA-seq analysis by Genergy

A — Overall survival

CancerCode	P-value	Hazard Ratio (95%CI)
TCGA-GBMLGG (N=619)	1.4e-6	1.73 (1.38,2.15)
TCGA-SKCM (N=444)	7.1e-4	1.11 (1.05,1.18)
TCGA-SKCM-M (N=347)	7.5e-4	1.12 (1.05,1.20)
TCGA-STAD (N=372)	7.7e-3	1.16 (1.04,1.29)
TCGA-LGG (N=474)	0.01	1.51 (1.08,2.10)
TCGA-STES (N=547)	0.02	1.11 (1.02,1.22)
TCGA-THCA (N=501)	0.09	2.19 (0.90,5.32)
TCGA-COADREAD (N=368)	0.13	1.14 (0.96,1.36)
TCGA-COAD (N=278)	0.14	1.15 (0.96,1.38)
TCGA-LIHC (N=341)	0.14	1.07 (0.98,1.17)
TCGA-UCEC (N=166)	0.33	1.09 (0.92,1.29)
TCGA-SKCM-P (N=97)	0.34	1.09 (0.92,1.29)
TCGA-ESCA (N=175)	0.35	1.08 (0.92,1.27)
TCGA-CHOL (N=33)	0.42	1.15 (0.82,1.61)
TCGA-PRAD (N=492)	0.45	1.73 (0.41,7.28)
TCGA-LAML (N=144)	0.46	1.04 (0.94,1.15)
TCGA-READ (N=90)	0.50	1.19 (0.71,2.01)
TCGA-LUSC (N=468)	0.58	1.02 (0.95,1.09)
TCGA-SARC (N=254)	0.82	1.01 (0.91,1.13)
TCGA-ACC (N=77)	0.82	1.04 (0.73,1.49)
TCGA-UVM (N=74)	7.3e-7	0.69 (0.58,0.81)
TCGA-KICH (N=64)	1.7e-3	0.58 (0.40,0.84)
TCGA-KIPAN (N=855)	2.5e-3	0.89 (0.83,0.96)
TCGA-PAAD (N=172)	0.01	0.83 (0.72,0.96)
TCGA-CESC (N=273)	0.02	0.87 (0.77,0.98)
TCGA-KIRC (N=515)	0.02	0.86 (0.76,0.98)
TCGA-THYM (N=117)	0.02	0.56 (0.34,0.94)
TCGA-PCPG (N=170)	0.03	0.27 (0.08,0.90)
TCGA-OV (N=407)	0.04	0.90 (0.81,0.99)
TCGA-HNSC (N=509)	0.06	0.92 (0.84,1.00)
TCGA-KIRP (N=276)	0.20	0.92 (0.81,1.04)
TCGA-DLBC (N=44)	0.26	0.76 (0.47,1.24)
TCGA-LUAD (N=490)	0.31	0.95 (0.87,1.05)
TCGA-UCS (N=55)	0.42	0.93 (0.79,1.11)
TCGA-BLCA (N=398)	0.42	0.97 (0.90,1.04)
TCGA-MESO (N=84)	0.67	0.97 (0.86,1.10)
TCGA-BRCA (N=1044)	0.69	0.98 (0.88,1.09)
TCGA-GBM (N=144)	0.74	0.95 (0.72,1.27)
TCGA-TGCT (N=128)	0.75	0.80 (0.21,3.13)

log2 (Hazard Ratio (95%CI))

B — Disease-specific survival

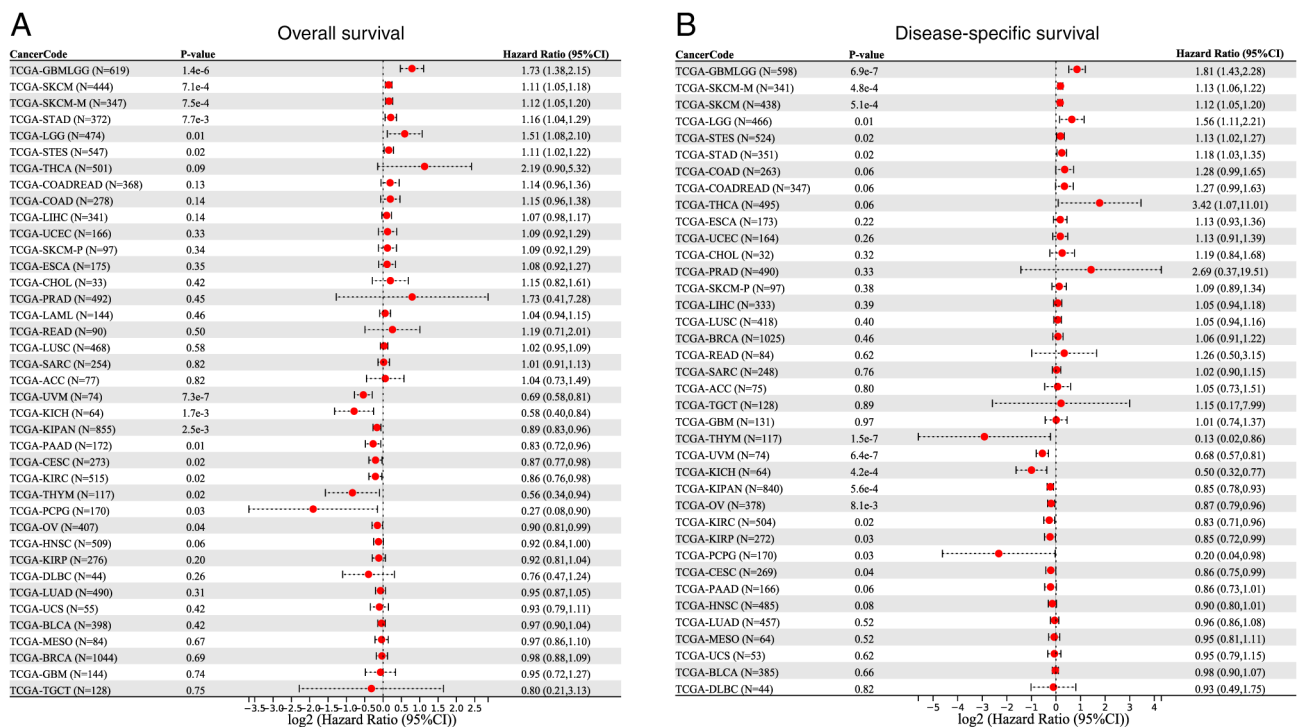| CancerCode	P-value	Hazard Ratio (95%CI)
TCGA-GBMLGG (N=598)	6.9e-7	1.81 (1.43,2.28)
TCGA-SKCM-M (N=341)	4.8e-4	1.13 (1.06,1.22)
TCGA-SKCM (N=438)	5.1e-4	1.12 (1.05,1.20)
TCGA-LGG (N=466)	0.01	1.56 (1.11,2.21)
TCGA-STES (N=524)	0.02	1.13 (1.02,1.27)
TCGA-STAD (N=351)	0.02	1.18 (1.03,1.35)
TCGA-COAD (N=263)	0.06	1.28 (0.99,1.65)
TCGA-COADREAD (N=347)	0.06	1.27 (0.99,1.63)
TCGA-THCA (N=495)	0.06	3.42 (1.07,11.01)
TCGA-ESCA (N=173)	0.22	1.13 (0.93,1.36)
TCGA-UCEC (N=164)	0.26	1.13 (0.91,1.39)
TCGA-CHOL (N=32)	0.32	1.19 (0.84,1.68)
TCGA-PRAD (N=490)	0.33	2.69 (0.37,19.51)
TCGA-SKCM-P (N=97)	0.38	1.09 (0.89,1.34)
TCGA-LIHC (N=333)	0.39	1.05 (0.94,1.18)
TCGA-LUSC (N=418)	0.40	1.05 (0.94,1.16)
TCGA-BRCA (N=1025)	0.46	1.06 (0.91,1.22)
TCGA-READ (N=84)	0.62	1.26 (0.50,3.15)
TCGA-SARC (N=248)	0.76	1.02 (0.90,1.15)
TCGA-ACC (N=75)	0.80	1.05 (0.73,1.51)
TCGA-TGCT (N=128)	0.89	1.15 (0.17,7.99)
TCGA-GBM (N=131)	0.97	1.01 (0.74,1.37)
TCGA-THYM (N=117)	1.5e-7	0.13 (0.02,0.86)
TCGA-UVM (N=74)	6.4e-7	0.68 (0.57,0.81)
TCGA-KICH (N=64)	4.2e-4	0.50 (0.32,0.77)
TCGA-KIPAN (N=840)	5.6e-4	0.85 (0.78,0.93)
TCGA-OV (N=378)	8.1e-3	0.87 (0.79,0.96)
TCGA-KIRC (N=504)	0.02	0.83 (0.71,0.96)
TCGA-KIRP (N=272)	0.03	0.85 (0.72,0.99)
TCGA-PCPG (N=170)	0.03	0.20 (0.04,0.98)
TCGA-CESC (N=269)	0.04	0.86 (0.75,0.99)
TCGA-PAAD (N=166)	0.06	0.86 (0.73,1.01)
TCGA-HNSC (N=485)	0.08	0.90 (0.80,1.01)
TCGA-LUAD (N=457)	0.52	0.96 (0.86,1.08)
TCGA-MESO (N=64)	0.52	0.95 (0.81,1.11)
TCGA-UCS (N=53)	0.62	0.95 (0.79,1.15)
TCGA-BLCA (N=385)	0.66	0.98 (0.90,1.07)
TCGA-DLBC (N=44)	0.82	0.93 (0.49,1.75)

log2 (Hazard Ratio (95%CI))

Figure 1. Relationship between lncRNA ZNF667-AS1 expression and OS and DSS of pan-cancer. (A) lncRNA ZNF667-AS1 and OS of pan-cancer. (B) lncRNA ZNF667-AS1 and DSS of pan-cancer. lncRNA, long noncoding RNA; OS, overall survival; DSS, disease-specific survival; CI, confidence interval.

Bio-Technology (Shanghai) Co. Differentially expressed genes were identified and analyzed for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment. The gene set for the KEGG pathway analysis was obtained from the Molecular Signatures Database (curated gene sets, canonical pathways, KEGG analysis for human gene symbols; v2022.1; https://www.gsea-msigdb.org/gsea/index.jsp).

Fluorescence in situ hybridization (FISH). FISH was performed using a Shanghai GenePharma Co., Ltd. kit, according to the manufacturer's protocol. OC cells grown on coverslips were hybridized with a Cy3-labelled ZNF667-AS1 probe, counterstained with DAPI and visualized via confocal microscopy (magnification, x400). The ZNF667-AS1 probe was designed and synthesized by Shanghai GenePharma Co., Ltd.

Western blotting. Total protein was extracted from the cells with RIPA lysate (Beyotime Institute of Biotechnology) and determined by BCA. The proteins (20-100 μg) were subjected to SDS-PAGE (10%) and then transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked with 5% skimmed milk for 1 h at room temperature and then incubated with primary and secondary antibodies. Bound antibodies were detected via enhanced chemiluminescence (ECL). The specific antibodies employed in this experiment were GAPDH (cat. no. 200306-7E4; OriGene Technologies, Inc.), Tubulin (cat. no. 250009; OriGene Technologies, Inc.), PCNA (cat. no. 10205-2-AP; Proteintech Group, Inc.), MMP2 (cat. no. 10373-2-AP; Proteintech Group, Inc.), MMP9 (cat. no. 10375-2-AP; Proteintech Group, Inc.), rabbit second antibody (cat. no. SA00001-2; Proteintech Group, Inc.) and mouse second antibody (cat. no. SA00001-1; Proteintech Group, Inc.). All primary antibodies were diluted 1:1,000 and incubated at 4˚C for 12 h, while goat anti-rabbit and anti-mouse IgG H&L (HRP) second antibodies were diluted 1:5,000 and incubated at room temperature for 1 h. Finally, ImageJ (National Institutes of Health) was used to calculate the gray value of the protein strip.

Statistical analysis. The data are presented as the means ± standard deviation. Statistical comparisons between groups were conducted via unpaired Student's t-test in GraphPad Prism 8.0 (Dotmatics) and R version 4.1.2 (http://www.R-project.org/). $P<0.05$ was considered to indicate a statistically significant difference.

Results

lncRNA ZNF667-AS1 in pan-cancer analysis. The present study explored the prognostic significance of lncRNA ZNF667-AS1 in different types of cancer by using data from the SangerBox website. The analysis revealed that high expression of lncRNA ZNF667-AS1 was associated with poor prognosis in several types of cancer, particularly glioblastoma (GBM) and skin cutaneous melanoma. For example, in GBM patients, elevated lncRNA ZNF667-AS1 is associated with reduced OS, as shown in Fig. 1A, where a forest plot demonstrates a significant difference ($P<0.05$) between the high- and low-expression groups. By contrast, in uveal melanoma and OC, high expression of lncRNA ZNF667-AS1 is linked to an improved prognosis and the forest plot in Fig. 1B demonstrated improved outcomes in patients with elevated expression.
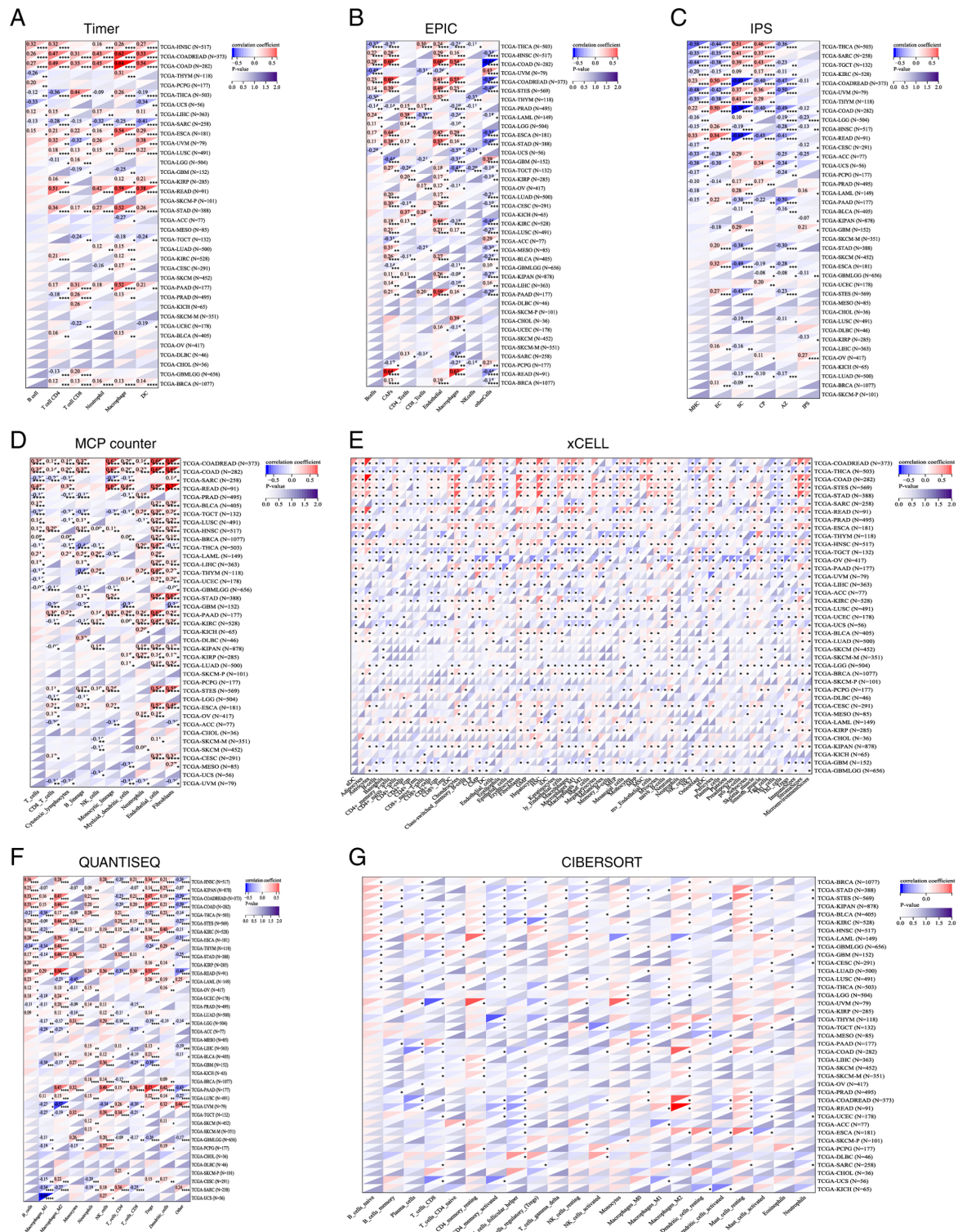
Figure 2. Relationship between lncRNA ZNF667-AS1 expression and pan-cancer immune cells: (A) Timer, (B) EPIC, (C) IPS, (D) MCPcounter, (E) xCELL, (F) QUANTISEQ, (G) CIBERSORT. *P<0.05, **P<0.01, ***P<0.001. lncRNA, long noncoding RNA; IPS, immune score.

The present study subsequently analyzed the correlation between lncRNA ZNF667-AS1 and various immune cell types by using the SangerBox website. To investigate the infiltration levels of immune cells, the present study employed several algorithms, including TIMER, EPIC, IPS, MCPcounter, xCELL, QUANTISEQ and CIBERSORT. These algorithms assess the composition and activity of immune cells in the tumor microenvironment from different perspectives. For example, TIMER focuses on estimating tumor-infiltrating lymphocytes, whereas MCPcounter distinguishes the abundance of different immune cell subpopulations. As shown in Fig. 2, multiple algorithms indicated that higher expression of lncRNA ZNF667-AS1 was associated with increased immune cell infiltration in tumors such as colon adenocarcinoma and
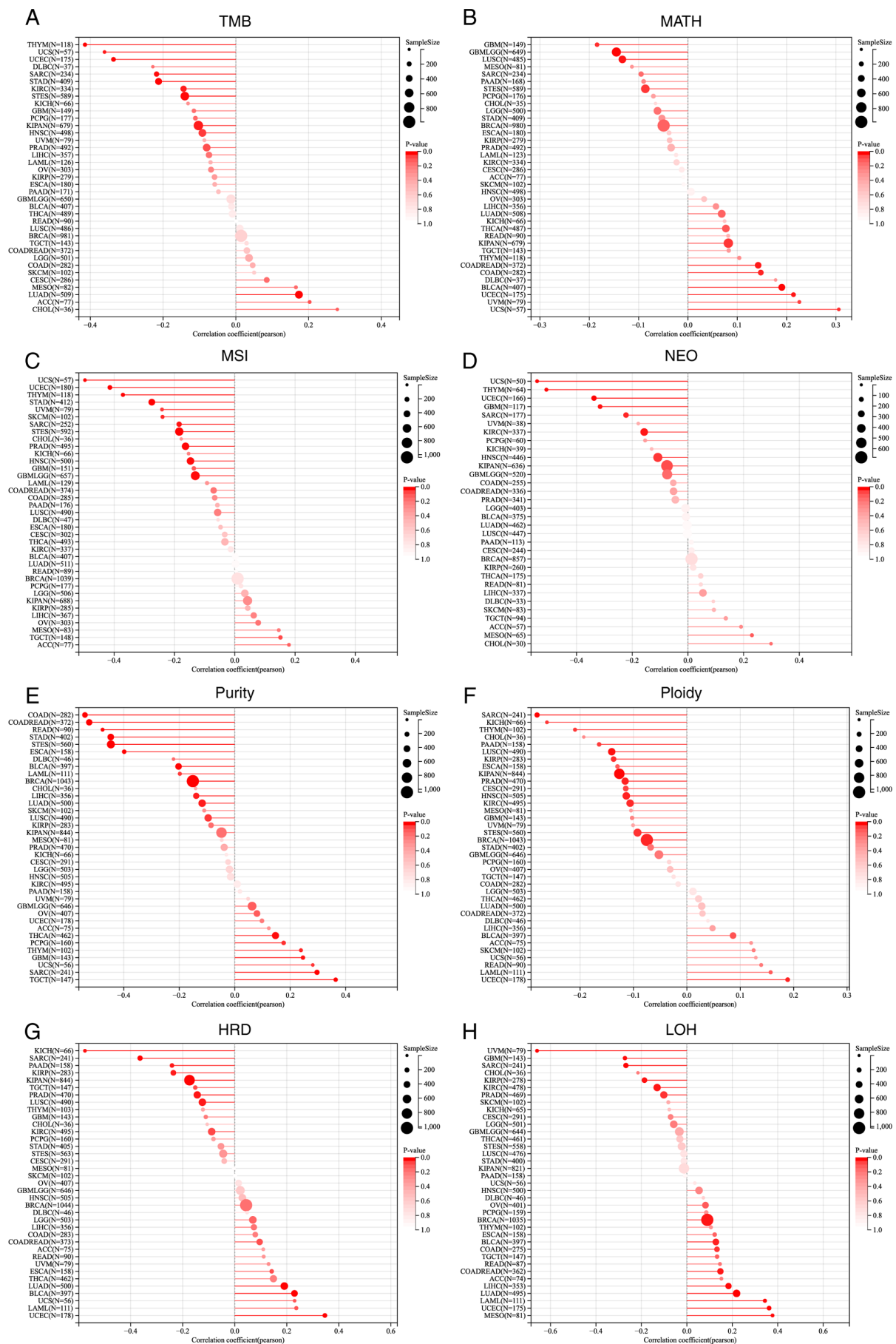
Figure 3. Relationship between lncRNA ZNF667-AS1 expression and pan-cancer various heterogeneity metrics: (A) TMB, (B) MATH, (C) MSI, (D) NEO, (E) PURITY, (F) PLOIDY, (G) HRD, (H) LOH. lncRNA, long noncoding RNA; TMB, tumor mutation burden; MATH, variant-allele tumor heterogeneity; MSI,microsatellite instability; NEO, neopeptide; HRD, homologous recombination deficiency; LOH, loss of heterozygosity.
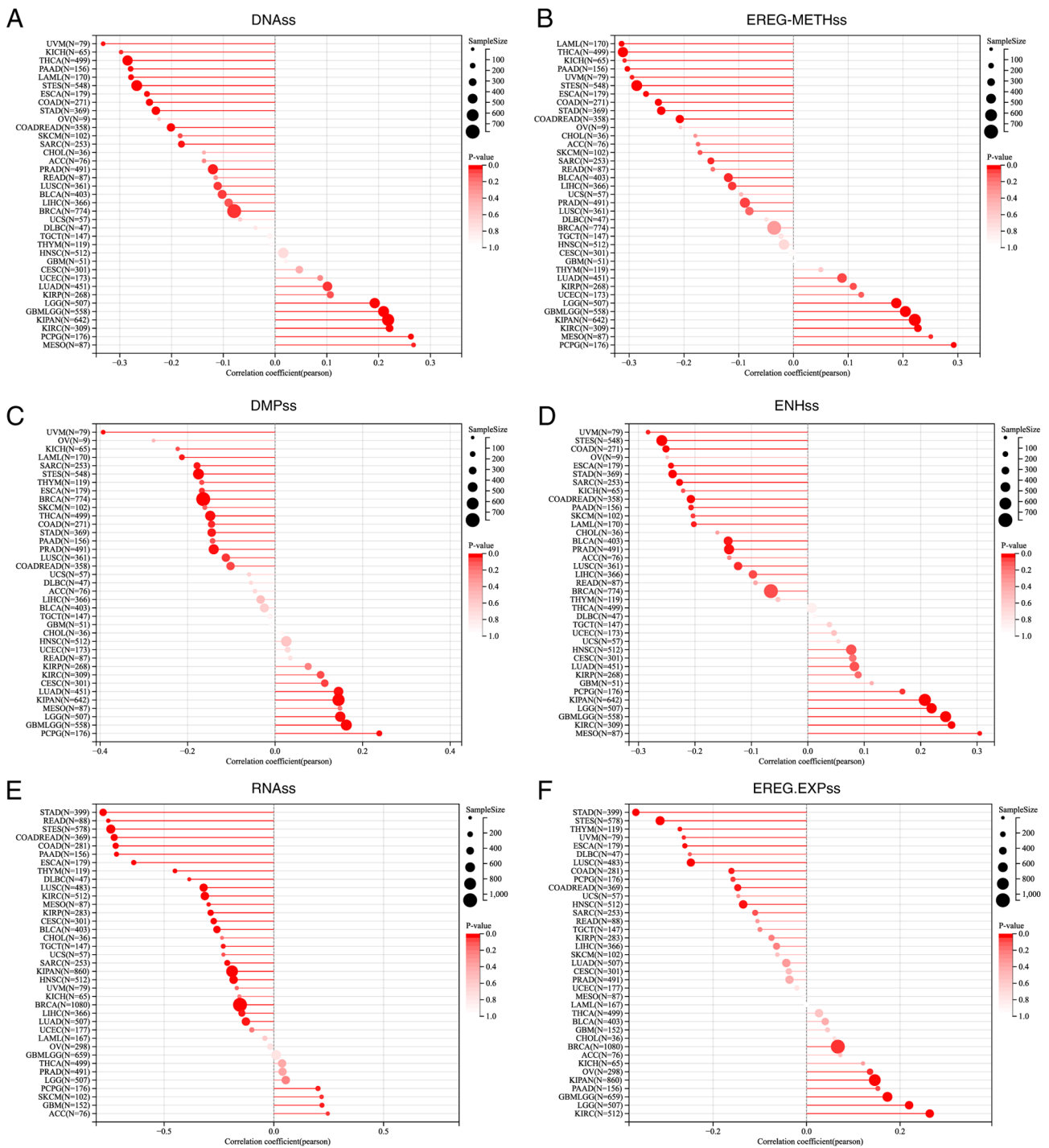
Figure 4. Relationship between lncRNA ZNF667-AS1 expression and pan-cancer tumor stemness: (A) DNAss, (B) EREG-METHss, (C) DMPss, (D) ENHss, (E) RNAss, (F) EREG-EXPss. lncRNA, long noncoding RNA.

thyroid carcinoma, whereas lower expression was associated with decreased immune cell infiltration in tumors such as brain lower grade glioma. These findings indicated a potential role for this lncRNA in regulating the tumor immune microenvironment. The present study further investigated the effect of genomic heterogeneity on the expression of lncRNA ZNF667-AS1. The findings indicated that lncRNA ZNF667-AS1 had a complex relationship with various metrics of genomic heterogeneity, including mutations, copy number variations and chromosomal structural variations in different types of cancer. Fig. 3 illustrated the intricate correlations

between lncRNA ZNF667-AS1 expression and these heterogeneous parameters and highlights its potential role as a marker of tumor evolution and treatment response.

Tumor stemness, a key factor in cancer biology, refers to the ability of tumor cells or tissues to maintain or acquire stem cell characteristics. This information is crucial for understanding tumor biology, predicting disease prognosis and developing new anticancer treatments. As shown in Fig. 4, in most types of cancer, lncRNA ZNF667-AS1 expression was negatively associated with tumor stemness and this relationship was confirmed by several methods, including DNA,
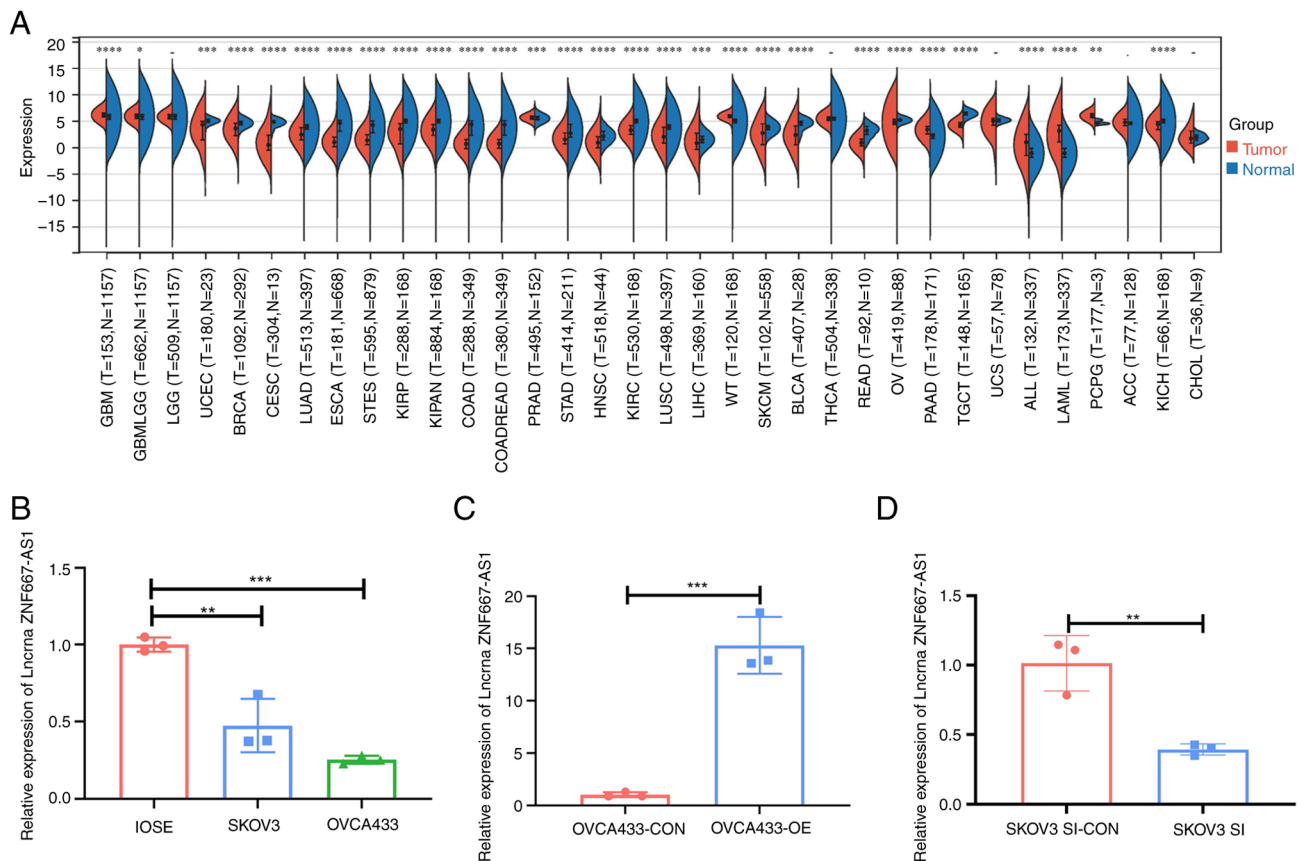
Figure 5. Expression of the lncRNA ZNF667-AS1 in pan-cancer and OC cell lines. (A) Comparison of the expression of the lncRNA ZNF667-AS1 between different types of cancer. (B) The expression level of lncRNA ZNF667-AS1 between IOSE80 normal ovarian cell and SKOV3, OVCA433 OC cell lines. (C and D) The efficiency of ZNF667-AS1 overexpression (P<0.001) and knockdown (P<0.05) was detected by reverse transcription-quantitative PCR in the indicated cells transfected with plasmids and siRNAs. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. lncRNA, long noncoding RNA; OC, ovarian cancer; OE, overexpression; si/SI, small interfering.

EREG-METH, DMPs, ENHs, RNAss and EREG-EXPs. These results showed that lncRNA ZNF667-AS1 may prevent tumor cells from acquiring stem-like properties.

Expression of the lncRNA ZNF667-AS1 is downregulated in human OC tissues. Analysis of TCGA data using the SangerBox platform revealed downregulation of lncRNA ZNF667-AS1 across multiple types of cancer, including OC (Fig. 5A). The present study validated this finding in ovarian cell lines; lower ZNF667-AS1 levels occurred in the SKOV3 and OVCA433 cancer lines than in normal IOSE80 cells. ZNF667-AS1 expression was particularly diminished in OVCA433 cells (Fig. 5B).

lncRNA ZNF667-AS1 mediates cell proliferation in vitro. To elucidate the functional role of ZNF667-AS1 in OC, the present study generated ZNF667-AS1-overexpressing OVCA433 cells and ZNF667-AS1-knockdown SKOV3 cells via plasmid transfection and siRNA strategies, respectively. Successful overexpression and knockdown were confirmed by RT-qPCR (Fig. 5C and D). A colony formation assay revealed significantly reduced proliferation in ZNF667-AS1-overexpressing OVCA433 cells compared with controls. Conversely, ZNF667-AS1 knockdown enhanced SKOV3 cell proliferation (Fig. 6A-D). The EdU experiment showed consistent results (Fig. 6E-H). In addition, western blotting revealed that

overexpression of ZNF667-AS inhibited the expression of the proliferation marker PCNA (Fig. 7E).

Alterations in the expression of lncRNA ZNF667-AS1 affect OC cell migration and invasion in vitro. The present study further examined the effects of ZNF667-AS1 on OC cell motility via Transwell and wound healing assays. Wounds were introduced into layers of SKOV3-SI, OVCA433-OE and corresponding control cells by scratching and the cells were subsequently cultured for 24 h. Overexpression of the lncRNA ZNF667-AS1 significantly decreased the spreading potential of OVCA433 cells, whereas downregulation of the lncRNA ZNF667-AS1 significantly increased the spreading potential of SKOV3 cells (Fig. 7A and B). The Transwell assay revealed a similar trend with respect to the invasion and migration abilities of SKOV3 and OVCA433 cells (Fig. 7C and D). In addition, overexpression of ZNF667-AS inhibited the expression of the migration and invasion markers MMP2 and MMP9 (Fig. 7E).

lncRNA ZNF667-AS1 mediates the TNF signaling pathway to affect ovarian cancer functions. The FISH results (Fig. 8A and B) revealed that lncRNA ZNF667-AS1 was mainly expressed in the nuclei of SKOV3 and OVCA433 cells. To elucidate the molecular mechanisms by which ZNF667-AS1 functions in OC, RNA-seq was performed on
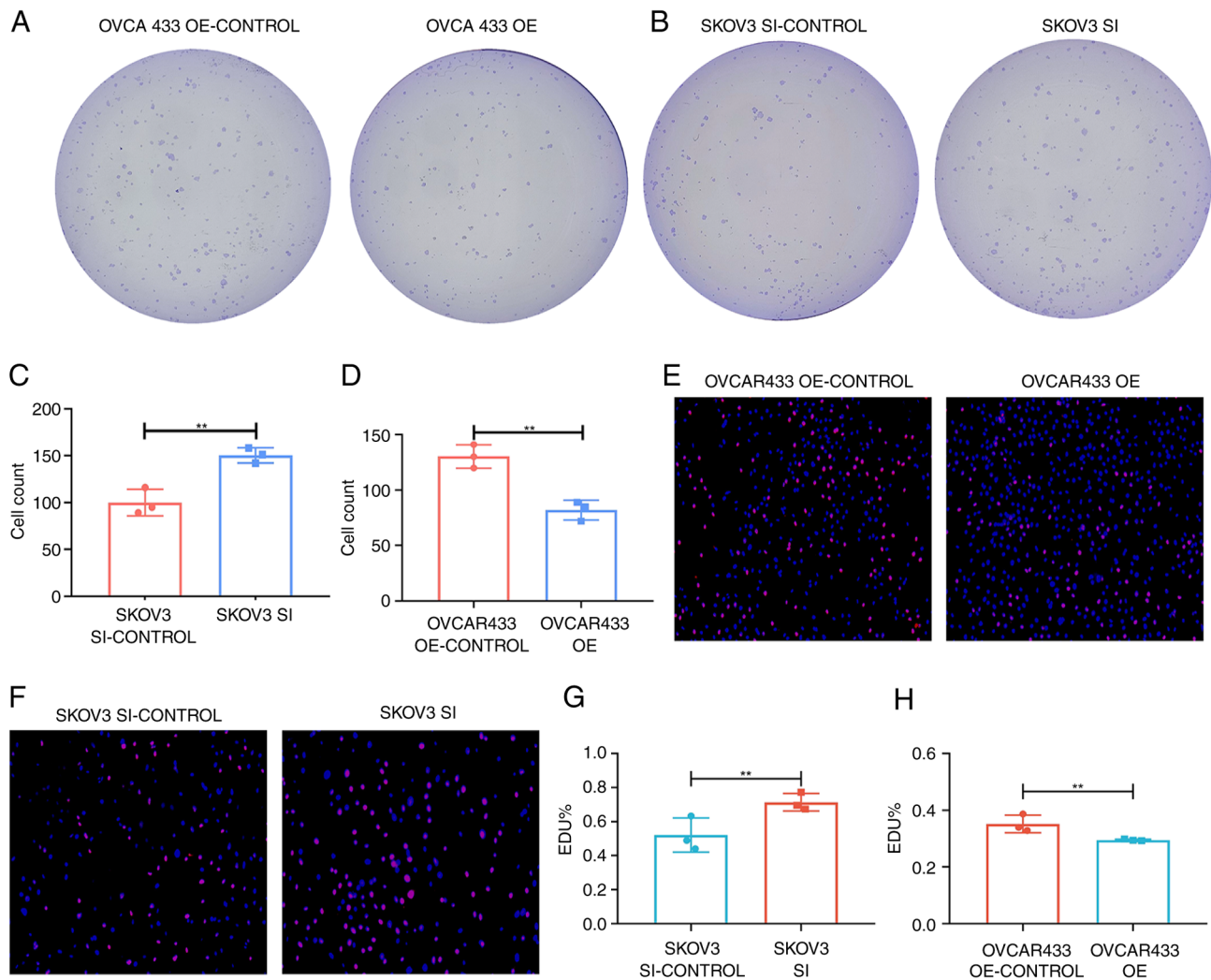
Figure 6. lncRNA ZNF667-AS1 suppressed OC cell proliferation *in vitro*. (A-D) Colony formation assays showing that upregulation of ZNF667-AS1 suppressed cell proliferation in the OVCA433 cell line (P<0.01) and that knockdown of ZNF667-AS1 promoted cell proliferation in the SKOV3 cells (P<0.01). (E-H) EdU assays showing that upregulation of ZNF667-AS1 suppressed cell proliferation in the OVCA433 cell line (P<0.01) and that knockdown of ZNF667-AS1 promoted cell proliferation in the SKOV3 cells (P<0.01; magnification, x100). **P<0.01. lncRNA, long noncoding RNA; OC, ovarian cancer; EdU, 5-Ethynyl-2'-Deoxyuridine.

ZNF667-AS1-overexpressing and control OVCA433 cells. Differentially expressed genes were identified using volcano plot and heatmap analyses (Fig. 8C and D). KEGG pathway enrichment analysis revealed significant enrichment of differentially expressed genes in the TNF pathway (Fig. 8E and F). TNF-α is expressed mainly on the cell membrane, but its signaling process involves gene expression regulation in the nucleus (19) and ZNF667-AS1 is expressed mainly in the nucleus. Western blotting confirmed that overexpression of ZNF667-AS1 decreased the expression of TNF (Fig. 7E).

Missense mutations were the predominant type among the differentially expressed genes, with NOTCH4 being the most frequently mutated gene in OC (Fig. 8G). These results suggested that ZNF667-AS1 was a key regulator of the TNF signaling cascade in OC.

Discussion

The advent of targeted cancer therapies has revolutionized cancer treatment by selectively modulating deregulated oncogenic signaling pathways to improve patient survival. While progress has been made in identifying OC biomarkers and in targeted therapeutics, further research is necessary to develop more effective diagnostic, prognostic and treatment strategies. Identifying key OC biomarkers is a critical step towards improving clinical outcomes.

A growing body of evidence shows that lncRNA expression is closely associated with cancer pathogenesis, metastasis and clinical outcomes (20-22). By modulating gene expression programs and key oncogenic signaling cascades, such as the PI3K/AKT pathway, lncRNAs can act as tumor suppressors or oncogenes (23,24). Studies have reported frequent downregulation of the lncRNA ZNF667-AS1 in various types of malignancy, which suggests a potential tumor-suppressive role (13,25). Silencing of ZNF667-AS1 occurs early in carcinogenesis and is sustained throughout cancer progression to invasive and metastatic disease (26). Furthermore, low ZNF667-AS1 expression is significantly associated with an advanced tumor stage and poor prognosis in several types of cancer (27).
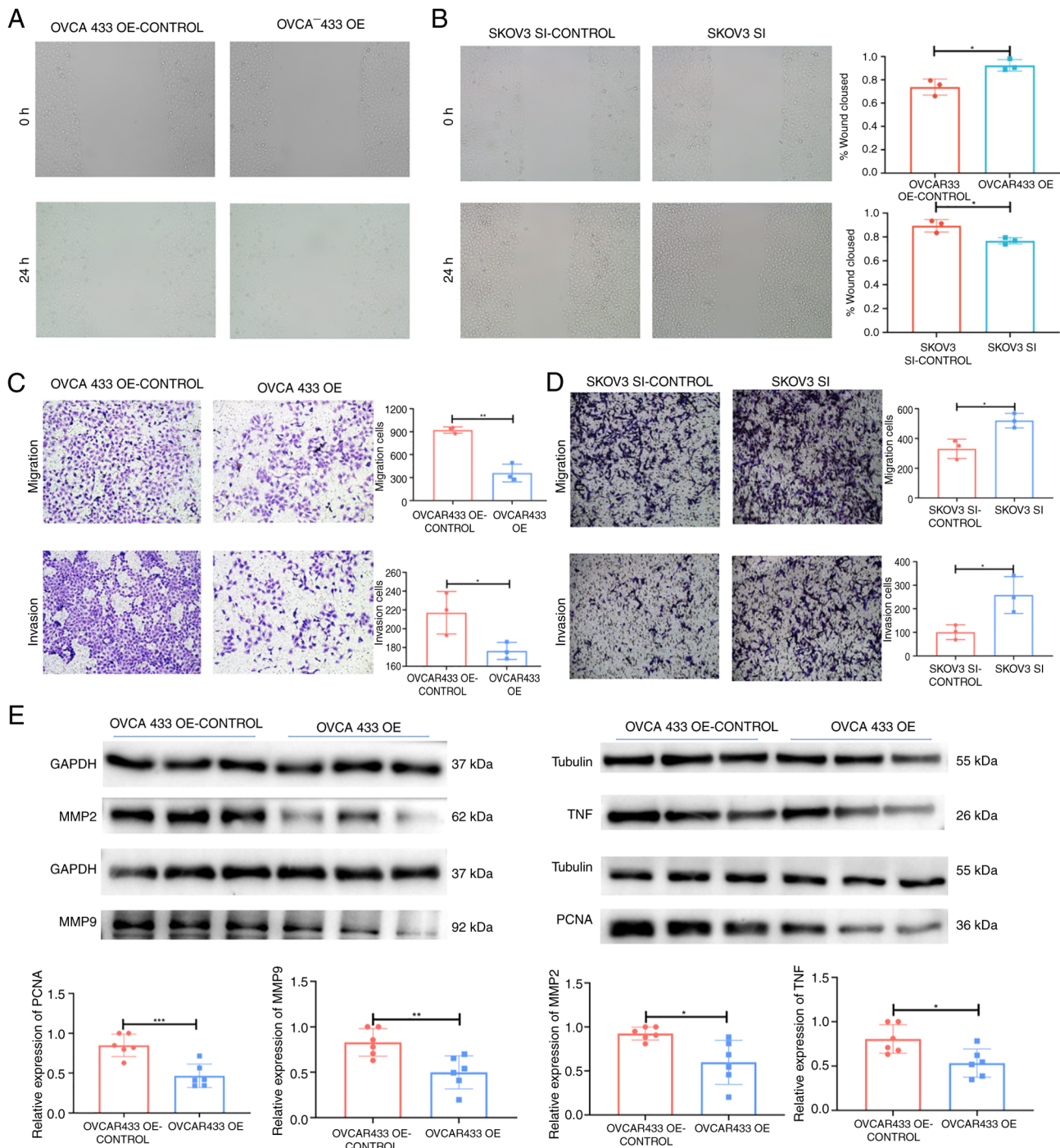
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Figure 7. lncRNA ZNF667-AS1 inhibited OC cell migration, invasion *in vitro* and western blotting validation. (A and B) Transwell assay showing that upregulation of ZNF667-AS1 suppressed cell migration (P<0.01) and invasion (P<0.05) in the OVCA433 cell line and that knockdown of TPTEP1 promoted cell migration (P<0.05) and invasion (P<0.05) in the SKOV3 cell line (magnification, x100). (C and D) Wound-healing assay showing that upregulation of ZNF667-AS1 suppressed cell migration in the OVCA433 cell line (P<0.05) and that knockdown of ZNF667-AS1 promoted cell migration in the SKOV3 cell line (P<0.05) (magnification, x100). (E) western blotting showed the association between ZNF667-AS1 expression level and tumor indexes of MMP2, MMP9, PCNA and TNF. *P<0.05, **P<0.01, ***P<0.001. lncRNA, long noncoding RNA; OC, ovarian cancer; PCNA, proliferating cell nuclear antigen.

The pan-cancer analysis of the present study revealed widespread downregulation of ZNF667-AS1 in nearly half of the types of cancer examined. Notably, the prognosis for certain solid tumors, such as OC, showed improved prognosis when ZNF667-AS1 was expressed. Along with the associations between ZNF667-AS1 and genomic heterogeneity and stemness, these data highlighted its potential as a pan-cancer prognostic marker.

Functionally, The present study found that overexpression of ZNF667-AS1 significantly inhibited OC cell proliferation, migration and invasion *in vitro*, whereas knockdown of ZNF667-AS1 promoted these oncogenic properties. These complementary results demonstrated that ZNF667-AS1 is a potential therapeutic target in OC. Additionally, protein markers for proliferation, invasion and migration, including PCNA, MMP2 and MMP9, tended to decrease following ZNF667-AS1 overexpression.
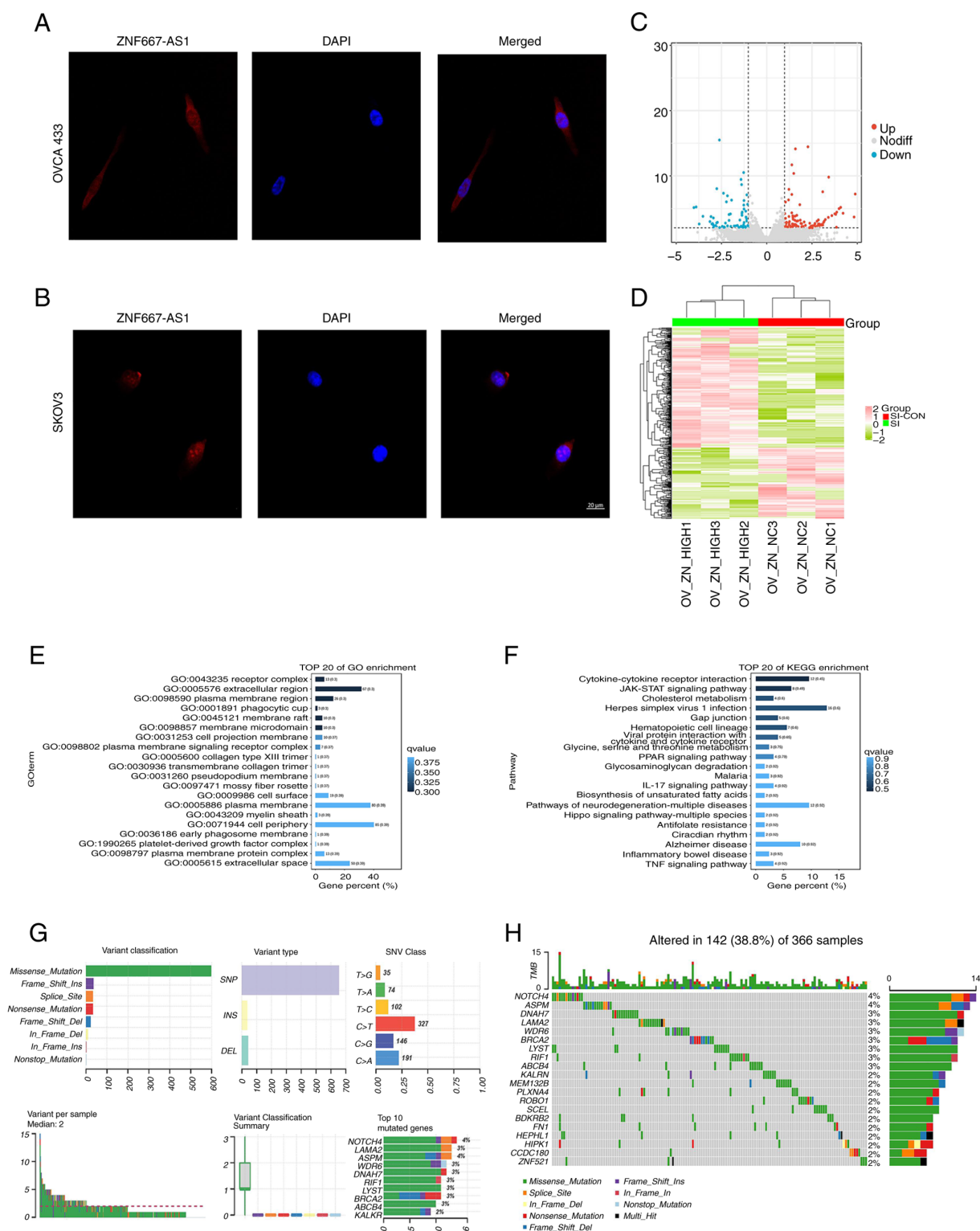
Figure 8. Distribution of the lncRNA ZNF667-AS1 in OC cell lines and RNA-seq analysis. (A and B) The expression level of the lncRNA ZNF667-AS1 was assessed in SKOV3 and OVCA433 cells by FISH (magnification, x400). (C and D) Differential gene volcano and heatmap of lncRNA ZNF667-AS1 overexpression and control in OVCA433 cell line. (E and F) The result of GO and KEGG analysis of differentially expressed genes. (G and H) Overview of the mutation profiles of differential gene in OC samples. lncRNA, long noncoding RNA; OC, ovarian cancer; FISH, fluorescence *in situ* hybridization; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

TNF-α is a crucial cytokine and TNF-α can kill target cells and regulate adaptive immunity to protect the body (28). The upregulation of TNF-α, a proinflammatory cytokine frequently detected in various malignancies, is associated with an increased incidence of OC (29). Additionally, IL-15

agonists (such as ALT-803) can upregulate TNF-α expression and restore natural killer cell function in the ascites of OC patients (30). In the present study, pathway enrichment analysis revealed that TNF signaling pathway was one of the top pathways modulated by ZNF667-AS1 in OC, as validated

by western blotting. These results suggested that ZNF667-AS1 may regulate ovarian cancer progression by interacting with the TNF signaling pathway.

The present study had certain limitations, including a reliance on *in vitro* experiments alone. Future *in vivo* studies are warranted to validate the tumor-suppressive functions of ZNF667-AS1 in OC models. Additionally, further research is needed to delineate the specific mechanisms by which ZNF667-AS1 interacts with and regulates TNF signaling in OC.

The present study revealed that ZNF667-AS1 was an important regulator of OC progression, potentially by modulating TNF signaling. These findings shed light on the functional and mechanistic roles of lncRNA ZNF667-AS1 in OC pathogenesis and may facilitate the development of novel diagnostic, prognostic, or therapeutic approaches that leverage ZNF667-AS1 in the clinical management of ovarian cancer.

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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

FFW and TWG performed the study and drafted the article. FFW, TCZ and XYM conducted cell culture, data analysis and interpretation. FFW and TWG contributed to the study design. All authors discussed the results and agreed to be accountable for all aspects of the work. All authors read and approved the final version of the manuscript. TG and FW confirm the authenticity of all the raw data.

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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