Original Article

Contribution of sphingomyelin phosphodiesterase acid-like 3B to the proliferation, migration, and invasion of ovarian cancer cells

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Background: Cancer has the highest mortality rate among gynecological cancers and poses a serious threat to women's lives. However, the treatment options for ovarian cancer are still limited, and exploring effective targeted biomarkers is particularly important for predicting and treating ovarian cancer. Therefore, it is necessary to explore the molecular mechanisms of the occurrence and development of ovarian cancer.

Methods: This investigation encompassed the analysis of gene expression profiles, measurement of transcription levels of potential target genes in peripheral blood samples from ovarian cancer patients and characterization of the ovarian cancer-related secretory protein sphingomyelin phosphodiesterase acid-like 3B (SMPDL3B). Through bioinformatics analysis, potential target genes were identified, and their association with overall survival (OS) and progression-free survival (PFS) in ovarian cancer patients was assessed utilizing relevant databases. Subsequently, differences in target gene expression in ovarian cancer tissue samples were validated through protein blotting and quantitative real-time PCR (qRT-qPCR). Cell proliferation assays using the cell count kit-8 (CCK-8) method, as well as transwell chamber assay and pre coated matrix gel chamber assay were employed to elucidate the role of SMPDL3B in ovarian cancer cell migration and invasion.

Results: This study revealed a substantial upregulation of *SMPDL3B* in the serum of ovarian cancer patients, correlating with an unfavorable prognosis. High *SMPDL3B* expression was linked not only to increased proliferation of ovarian cancer cells, but also enhanced migration and invasion. Remarkably, the knockdown the human alkaline ceramidase 2 (*ACER2*) gene in cancer cells with heightened *SMPDL3B* expression significantly inhibited cell proliferation, migration, and invasion induced by *SMPDL3B* activation ($P<0.05$), highlighting the functional interplay between *ACER2* and *SMPDL3B* in ovarian cancer.

Conclusions: In summary, this study proposes *SMPDL3B* as a prognostic marker for ovarian cancer, with implications for potential therapeutic intervention targeting the ACER2-SMPDL3B axis.

Keywords: Ovarian cancer; sphingomyelin phosphodiesterase acid-like 3B (*SMPDL3B*); ACER2-SMPDL3B axis

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Introduction

Ovarian cancer is the third most common malignant cancer affecting the female reproductive system, following cervical and uterine cancers. It is associated with highest mortality rate among all gynecological cancers, posing a serious threat to women's lives (1,2). Notably, family history and genetic syndromes are key risk factors for ovarian cancer (3,4). At present, transvaginal ultrasound and blood sampling for cancer antigen 125 (CA-125) tumor marker assessment are the primary diagnostic methods for ovarian cancer. However, the aggressive metastasis and challenges in achieving radical cure make ovarian cancer particularly formidable. Moreover, the asymptomatic nature of ovarian cancer in its early stages results in over three-quarter of patients being diagnosed at an advanced stage, contributing to poor prognoses (5). Effective identification of prognostic biomarkers holds the potential to enhance ovarian cancer diagnosis and treatment, and thus there this an urgent need to identify and develop these biomarkers.

Eukaryotic cell membranes mainly comprise sphingolipids and cholesterol, which play essential roles in regulating cellular signaling pathways (6). Sphingomyelin phosphodiesterase acid-like 3B (*SMPDL3B*) is involved in Toll-like receptor signaling and adenosine triphosphate (ATP) polypeptide regulation (7). Additionally, *SMPDL3B* is responsible for the terminal differentiation of glomerular cells, activation of integrin, and the migration and survival of specialized podocytes (8,9). Studies have indicated that *SMPDL3B* levels regulate cellular lipid content and macrophage flexibility and are implicated in radiation-induced renal podocyte injury through the induction of sphingolipid ceramide (9-11). Serving both as a hydrolase and a sphingomyelin phosphodiesterase, *SMPDL3B* regulates sphingolipid differentiation (12). However, its impact on ovarian cancer remains unclear. Previous data support the regulatory role of human alkaline ceramidase 2 (*ACER2*) in cellular ceramide hydrolysis and cancer cell proliferation. *ACER2* upregulation is associated with tumor growth, while its knockdown suppresses cell growth and migration, increasing *SMPDL3B* expression levels. Notably, *SMPDL3B* has been shown to drive the proliferation, migration, and invasion of hepatocellular carcinoma through the *ACER2* pathway (7) and is a potential marker for prostate cancer screening and prediction (13).

In this study, we aimed to identify target genes that are markedly upregulated in ovarian cancer and associated with poor prognosis through bioinformatics analysis. Furthermore, we sought to characterize the biological functions of ovarian cancer cells and the potential mechanisms underlying ovarian cancer pathogenesis in order to offer new insights for the clinical diagnosis and treatment of this challenging disease. We present this article in accordance with the TRIPOD and MDAR reporting checklists (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-309/rc).

Highlight box

Key findings

- High *SMPDL3B* expression was linked not only to increased proliferation of ovarian cancer cells, but also enhanced migration and invasion.
- *SMPDL3B* functioned as a downstream target of *ACER2* in ovarian cancer.

What is known and what is new?

- Effective identification of prognostic biomarkers holds the potential to enhance ovarian cancer diagnosis and treatment.
- SMPDL3B as a novel diagnostic marker for ovarian cancer. Our new finding is that SMPDL3B promoted ovarian cancer cell migration and invasion.

What is the implication, and what should change now?

- *SMPDL3B* gene expression was negatively associated with the overall survival and progression-free survival of patients with ovarian carcinoma.
- Elevated *SMPDL3B* contributes to the proliferation, migration, and invasion of ovarian carcinoma cells.
- *SMPDL3B* may function downstream of *ACER2*.

Methods

Materials and instruments

The materials and instruments used in our study were the following: high-glucose Dulbecco's Modified Eagle Medium (DMEM) (cat. no. CM1001; Zhongkemaichen Beijing Science and Technology, Beijing, China), fetal bovine serum (FBS) (cat. no. CS002; Zhongkemaichen Beijing Science and Technology, Beijing, China), Lipofectamine 3000 (cat. no. L3000075; Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), TRIzol (cat. no. 15596026; Invitrogen), penicillin and streptomycin (cat. no. 132-98-9; Sigma-Aldrich, St. Louis, MI, USA), cell count kit-8 (CCK-8) (cat. no. CA1210; Soleibo Technology, Beijing, China), HiScript II Q RT SuperMix for qPCR (+g DNA wiper; cat. no. R233-01; Nanjing Vazyme Biotech Co., Ltd., Nanjing, China), ChamQ Universal SYBR qPCR Master Mix Kit

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(cat. no. Q312-02/03; Vazyme Biotech), a Type-371 CO_2 Incubator (cat. no. V143463; Thermo Fisher Scientific), and a LightCycler 480 real-time polymerase chain reaction (RT-PCR) machine (Roche Diagnostics, Basel, Switzerland).

Bioinformatics analysis

The genome-wide expression and survival data for ovarian carcinoma cases were obtained from The Cancer Genome Atlas (TCGA) database, and the ovarian carcinoma genome expression was obtained from the Genotype-Tissue Expression (GTEx) database. Additionally, we extracted information on 2,059 secreted proteins detected in peripheral blood from the MetaSecKB database. Employing the "limma" package of R software (The R Foundation for Statistical Computing), we determined the differential expression of the 2,059 secreted proteins, which culminated in the selection of target genes of interest. Subsequently, we used data from 426 cancer samples and 88 control samples to draw box plots of the identified genes via R package "ggplot2". Finally, we determined the correlation of overall survival (OS) and progression-free survival (PFS) with the identified genes using clinical data from TCGA.

Cell lines and cell culture

The human ovarian carcinoma cell lines used in our study included the following: Caov-3 cells [cat. no. CTCC-400-0069; China Center for Type Culture Collection (CCTCC), Wuhan, China], characterized as human poorly differentiated ovarian clear cell carcinoma cells; SK-OV-3 cells (cat. no. CTCC-001-0011; CCTCC); and HEY cell lines (cat. no. CTCC-400-0122; CCTCC). The three cell types tested negative for *Mycoplasma*, bacteria, yeast, and fungi. The cells were cultured in DMEM supplemented with 10% (v/v) FBS and 100 U/mL of penicillin/streptomycin and were maintained in a 5% CO_2 incubator at 37 ℃. The short tandem repeat (STR) validation and *Mycoplasma* detection results for all cell lines were confirmed.

Primary cell culture and extracellular vesicle extraction

Fresh tumor tissue was dissected from surrounding adipose and connective tissues and subjected to two rounds of washing with a designated solution. Subsequently, the tissue was sliced into thin sections with a blade to precipitate the shedding of numerous epithelial cells. After the shed cells were washed, they were introduced into a complete culture medium to procure a refined upper layer of cells. These cells are then cultured in a suitable vessel at 37 ℃ under 5% CO_2, with regular medium renewal occurring every 2–3 days. After 7–10 days, the epithelial cells had consistently formed into a monolayer.

Upon achieving a tumor cell growth convergence rate of 85–95%, the culture medium was harvested to isolate the cell culture supernatant. This supernatant underwent a sequential process involving membrane filtration to remove larger particles, followed by ultrafiltration and centrifugation to concentrate the extracellular vesicles. The resulting centrifuged ultrafiltration solution was combined with a 10–12 w/v% polyethylene glycol solution at a 1:1 ratio based on volume, thoroughly mixed, and subsequently subjected to centrifugation at 4 ℃. The resultant precipitate constituted the isolated extracellular vesicle.

Construction of stable knockdown ovarian cancer cell lines

Within 24 hours after cell passage, the complete medium was replaced with serum/antibiotics-free medium in a six-well plate with about an 80% cell density. Transfection reagent Lipofectamine 3000 was mixed with both empty plasmids [short hairpin RNA (shRNA), Thermo Fisher Scientific] and the knockdown plasmids (sh-SMPDL3B#1, sh-SMPDL3B#2, sh-ACER2 + SMPDL3B; Thermo Fisher Scientific) to form cation complexes. The transfection medium was replaced with fresh complete medium after 6–8 hours of incubation with the cation complexes in the six-well plate. The cells were then screened in DMEM medium supplemented with G418 (cat. no. GL0001; Baiao Leibo Technology, Beijing, China) for 3 weeks after 48 hours of transfection. Following this period, selected clones were imaged under a fluorescence microscope, and surviving cell clusters were isolated to establish stable knockdown cell lines. The knockdown efficiency was determined via western blot analysis.

CCK-8 cell proliferation assay

Subsequent to trypsinization, 1,000 cells were seeded into 96-well plates and then subjected to the aforementioned transfection process. On the first day posttransfection, a 10-µL aliquot of medium and 10 µL of CCK-8 mixture were added to the transfected cells, which were then incubated for 1 hour at 37 ℃. The proliferation kinetics of the cells were measured and graphed through the measurement of absorbance via optical density (OD) at 450 nm. The

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experiment was repeated three times independently.

Cell migration and invasion assays

Prior to the cell invasion experiment, Matrigel was embedded in the upper chamber of the transwell. Approximately 2×10^4 cells suspended in 100 µL of serum-free medium were added to the upper chamber, while the lower chamber was supplemented with 600 µL of DMEM containing 20% FBS to induce chemotaxis, prompting tumor cells to migrate toward the nutrient-rich lower chamber. The quantification of cells entering the lower chamber could provide an assessment of the migration ability of the tumor cells.

Following a 24-hour incubation, cells on the surface of the upper chamber were removed. Subsequently, the remaining cells were fixed with 4% paraformaldehyde for 15 minutes at room temperature, stained with 0.1% crystal violet overnight, and washed with water. The stained cells were then visualized and quantified using a microscope. This experiment was repeated three times.

Cell scratch assay

Upon reaching confluency in a six-well plate, the cells were trypsinized, and a 200-µL pipette tip was used to create a uniform break in the monolayer after the cells were completely confluent. Subsequently, the disrupted cells were then cultured in medium devoid of FBS. To monitor cell migration, the cells were visually assessed and imaged under a microscope at 0, 24, and 48 hours after scratch formation. The extent of migration was quantified by measuring and documenting the distance covered across the blank area. The experiment was repeated three times.

RNA extraction and quantitative RT-PCR assay

The extraction of total RNA from cells was carried out using TRIzol reagent. Reverse transcription was performed as per the manufacturer's instructions using the HiScript II Q RT SuperMix for qPCR (+g DNA wiper) kit (Vazyme Biotech). Following reverse transcription, quantitative RT-PCR experiments were performed with the ChamQ Universal SYBR qPCR Master Mix kit (Vazyme Biotech). The obtained values were normalized to the internal reference gene *GAPDH* for each parallel experiment. The quantitative RT-PCR primers for *SMPDL3* in this study were as follows: forward—5'-TTTTCCTGGCTAACTGGGGAG-3'; reverse—5'-GGATACCTTGTAGTCAGGGTCAA-3'. Meanwhile, those for *GAPDH* were as follows: forward—5'-GGAGCGAGATCCCTCCAAAAT-3'; reverse—5'-GGCTGTTGTCATA CTTCTCATGG-3'.

Western blotting

The cells were washed with ice-cold phosphate-buffered saline (PBS) thrice before being collected into a 1.5-mL Eppendorf tube. Subsequently, the cells were lysed in radioimmunoprecipitation assay (RIPA) buffer for 10 minutes on ice. Following this, the lysed cells were centrifugated at 14,000 rpm for 10 minutes at 4 ℃ to remove the cell debris, and the supernatants were transferred to a new Eppendorf tube. The protein concentration was detected with bicinchoninic acid (BCA) kit, after which the protein samples were uniformly diluted. The diluted cell lysates were mixed with 2× Laemmli sample buffer, boiled for 10 min at 95 ℃, and subsequently loaded into the wells of a sodium dodecyl sulfate-polyacrylamide (SDS) gel for electrophoresis before being transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were incubated with 5% dry milk diluted in PBS for 1 hour at room temperature and later subjected to successive incubations with specific primary and corresponding secondary antibodies for 1 hour each at room temperature. Finally, they were imaged with a chemiluminescence imaging system.

Anti-SMPDL3B antibody (1:1,000 dilution; cat. no. ab30912) and anti-ACER2 antibody (1:1,000 dilution; cat. no. ab282276) were purchased from Abcam (Cambridge, UK), anti-GAPDH antibody (1:2,500 dilution; cat. no. ab9485) was purchased from Cell Signaling Technology (CST; Danvers, MA, USA), goat anti-rabbit/mouse immunoglobin G (IgG) horseradish peroxidase–conjugated antibody was purchased from Zhongshan Jinqiao Biotechnology (Beijing, China), and SMPDL3B shRNA plasmid (m) was purchased from Santa Cruz Biotechnology (cat. no. sc-76526-SH; Santa Cruz, CA, USA).

Clinical samples of patients with ovarian cancer

Twenty samples of ovarian tissue and adjacent normal tissue were selected from gynecological surgeries conducted at Fujian Provincial Hospital between February 2020 and October 2020. All patients with ovarian cancer had primary cancer and were aged between 51 and 69 years, with an average age of 58.95±6.83 years. The inclusion criteria

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for patients were as follows: (I) confirmation of ovarian cancer tissue diagnosis through postoperative pathological examination; (II) absence of any prior adjuvant therapies such as radiotherapy, chemotherapy, and immunotherapy before surgery; and (III) availability of comprehensive clinical data. Meanwhile, the exclusion criteria were the following: (I) concurrent malignant cancers, (II) severe liver and renal dysfunction, (III) severe immunodeficiency, and (IV) severe infectious diseases. All patients received surgical treatment, and during the operation, cancer tissue and corresponding paracancerous tissue (more than 2.5 cm distant from the cancer tissue) were collected. All samples were snap-frozen in liquid nitrogen and stored within a −80 ℃ refrigerator. This study was reviewed and approved by the Ethics Review Committee of Fujian Provincial Hospital (approval No. K2020-01-031) and was conducted according to the declaration of Helsinki (as revised in 2013). All enrolled patients signed informed consent form.

Statistical analysis

The statistical analysis and visualizations were performed using R v. 3.6.1 and GraphPad Prism 8.0 software (GraphPad Software, Inc., La Jolla, CA, USA). The obtained data are presented as the mean ± standard deviation (SD). Analysis of variance (ANOVA) was employed for comparisons among multiple groups, while measurement data were analyzed with the *t*-test and were compared with ANOVA and the Bonferroni multiple comparison test. Correlations between variables were determined via Pearson correlation coefficients. To assess the differences in the OS between the two groups, Kaplan-Meier analysis was conducted. All P values were calculated as two-sided, and those less than 0.05 were defined as statistically significant. All experiments were conducted as three independent parallel experiments.

Results

SMPDL3B as a novel diagnostic marker for ovarian cancer

We downloaded genome-wide expression and survival data of ovarian cancer cases, normal ovarian tissue expression data from the GTEx database, and 2,059 secreted proteins in tissue samples from the MetaSecKB database. Using the "limma" package in R software, we extracted the differential expression results for the 2,059 secreted proteins and depicted the results in a volcano plot. The selected cutoff value was [|log2 fold change (FC)| >1 and P<0.05], with red denoting a significantly high expression, green denoting significantly low expression, and gray denoting nonsignificant genes (*Figure 1A*). We selected target genes meeting the expected requirements [$\log_2$FC >4.2 and −$\log_{10}$(P value) >47], including *SMPDL3B*, *PTGS1*, and *IL4I1* (*Figure 1A*).

Using the "ggplot2" package in R, we generated a box plot depicting the expression of the *SMPDL3B* gene in 426 cancer samples and 88 control samples, revealing a significant increase in *SMPDL3B* (*Figure 1B*). According to the clinical data from TCGA, we constructed forest maps illustrating the OS and PFS of *SMPDL3B*, *PTGS1*, and *IL4I1*. Our analysis revealed that *SMPDL3B* exhibited the most positive correlation with the OS and PFS of ovarian cancer among the three genes, leading to the selection of *SMPDL3B* as the final target gene (*Figure 1C,1D*). To determine the functional significance of SMPDL3B in ovarian cancer, Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed on the co-expressed genes of *SMPDL3B*, as shown in Figure S1.

SMPDL3B gene expression was negatively associated with the OS and PFS of patients with ovarian carcinoma

To investigate the connection between the expression level of *SMPDL3B* and the clinical prognosis of ovarian carcinoma, we first drew a line graph using the clinical data of patients with ovarian cancer and the expression of *SMPDL3B* from TCGA database. The data revealed a negative correlation between the expression of the *SMPDL3B* and the survival rate of patients with ovarian cancer (*Figure 2A*). Subsequently, statistical analysis was conducted to investigate the association between *SMPDL3B* gene, OS, and PFS in ovarian carcinoma. As shown in *Figure 2B,2C* the OS and PFS of ovarian cancer patients in the *SMPDL3B* high-expression group were significantly worse than those in the low-expression group, indicating that the prognosis of ovarian cancer was significantly negatively associated with *SMPDL3B* (P<0.05).

SMPDL3B was upregulated in ovarian cancer

To explore the expression levels of the *SMPDL3B* gene in patients with ovarian cancer, we conducted Western blot analysis of *SMPDL3B* protein levels in ovarian cancer

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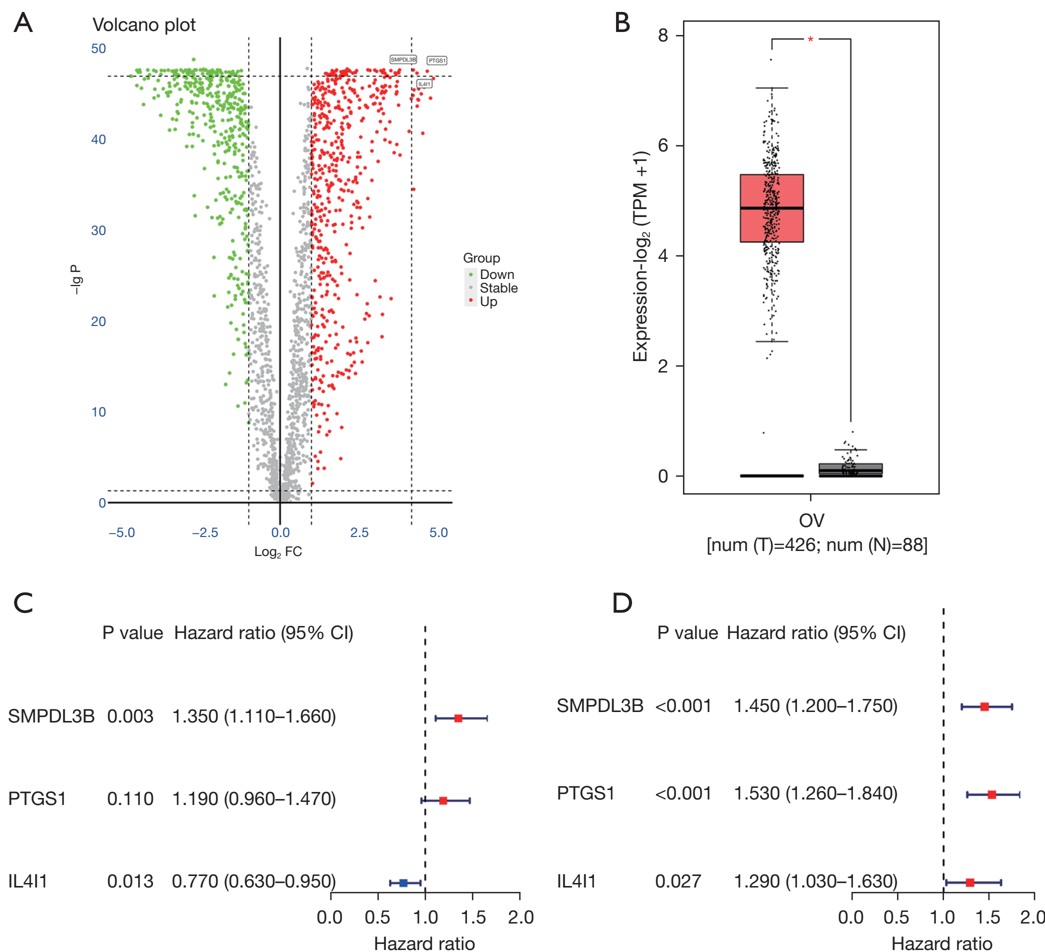

Figure 1 SMPDL3B as a potential prognostic marker for ovarian cancer. (A) Analysis of secreted proteins in TCGA-OV via a volcano plot revealed genes with a significantly high expression gene (in red) and low expression (in green) based on the selected cutoff values ($|\log_2 FC|$ >1 and P<0.05), with *SMPDL3B*, *PTGS1*, and *IL4I1* identified as target genes meeting specific criteria ($\log_2$FC >4.2 and $-\log_{10}$(P value) >47). (B) The box plot of *SMPDL3B* gene expression in 426 cancer samples and 88 control samples from TCGA-OV highlights the significant upregulation of *SMPDL3B* in cancer samples, *, P<0.05. (C) Forest plot depicting the OS analysis of *SMPDL3B*, *PTGS1*, and *IL4I1*. (D) Forest plot depicting the PFS survival analysis of *SMPDL3B*, *PTGS1*, and *IL4I1*. *SMPDL3B*, sphingomyelin phosphodiesterase acid-like 3B; TCGA-OV, The Cancer Genome Atlas-Ovarian Cancer; PTGS1, prostaglandin-endoperoxide synthase 1; *IL4I1*, interleukin-4-induced-1; OS, overall survival; PFS, progression-free survival; OV, ovarian cancer; TPM, transcripts per million; CI, confidence interval.

tissues and adjacent normal tissues obtained from 20 patients with ovarian cancer. Intriguingly, our data showed significantly higher translation levels of *SMPDL3B* in the ovarian cancer tissues compared to the normal tissues (*Figure 3A,3B*; P<0.0001). Furthermore, we performed quantitative RT-PCR to evaluate the expression levels of *SMPDL3B* in the ovarian cancer lesions, with the data demonstrating an increase in *SMPDL3B* gene expression in ovarian cancer tissues, consistent with the results of the Western blot experiments (*Figure 3C*; P<0.01). Subsequently, primary cells were isolated from the ovarian cancer tissues and cultured for 0.5, 1, 2, 4, and 8 hours, after which the supernatant was collected to extract exosomes for the assessment of *SMPDL3B* protein levels via Western blotting. Additionally, as the culture time of the primary cells obtained from ovarian cancer tissues elapsed, the *SMPDL3B* protein levels increased (*Figure 3D,3E*; P<0.05). Taken together, these results suggest that the transcription and translation levels of *SMPDL3B* are increased in ovarian carcinoma and are positively correlated with the culture time of primary cells

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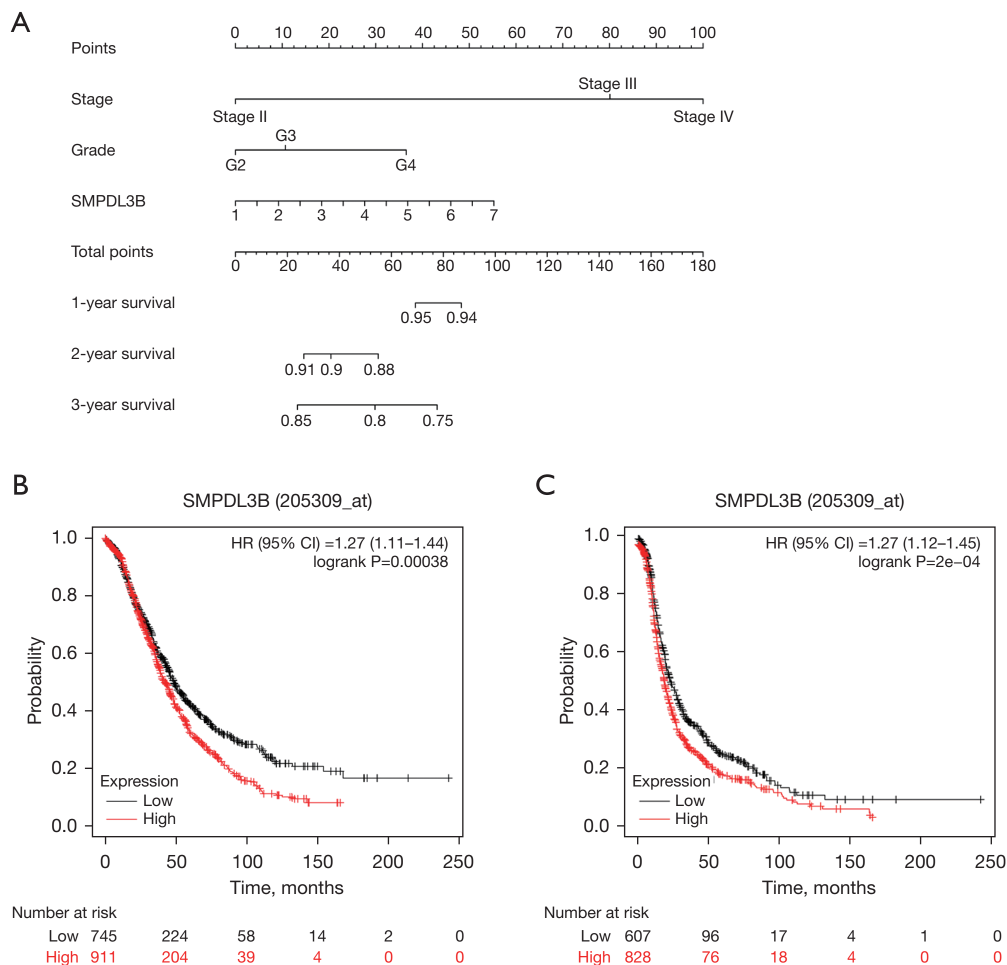

Figure 2 Correlation of SMPDL3B expression with survival in patients with ovarian cancer. (A) Nomogram analysis regarding the relationship between clinical grade and stage in relation to *SMPDL3B* expression level and patient survival; (B) analysis TCGA-OV OS according to *SMPDL3B* expression levels; (C) analysis of TCGA-OV PFS according to *SMPDL3B* expression levels. *SMPDL3B*, sphingomyelin phosphodiesterase acid-like 3B; TCGA-OV, The Cancer Genome Atlas-Ovarian Cancer; OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval.

derived from ovarian cancer.

SMPDL3B promoted ovarian cancer cell proliferation

We conducted quantitative RT-PCR experiments to measure the messenger RNA (mRNA) levels of *SMPDL3B* in Caov-3, SK-OV-3, and HEY cell lines (*Figure 4A*; P<0.0001). Due to the high expression level of *SMPDL3B*, we selected the SK-OV-3 and HEY cell lines for subsequent functional experiments. Furthermore, we constructed *SMPDL3B* low-expression cell lines using knockout assays based on these two cell lines (*Figure 4B,4C*; P<0.0001). In addition, CCK-8 cell proliferation assays showed that specific *SMPDL3B* shRNA transfection significantly reduced the proliferation of SK-OV-3 and HEY cells (*Figure 4D,4E*; P<0.05). Based on these compelling results, we could conclude that the *SMPDL3B* gene promotes the proliferation of ovarian cancer cells within ovarian cancer cell lines.

SMPDL3B promoted ovarian cancer cell migration and invasion

Based on the previous data demonstrating heightened SMPDL3B levels in ovarian carcinoma patients and correlations with poor OS and PFS time, we hypothesized

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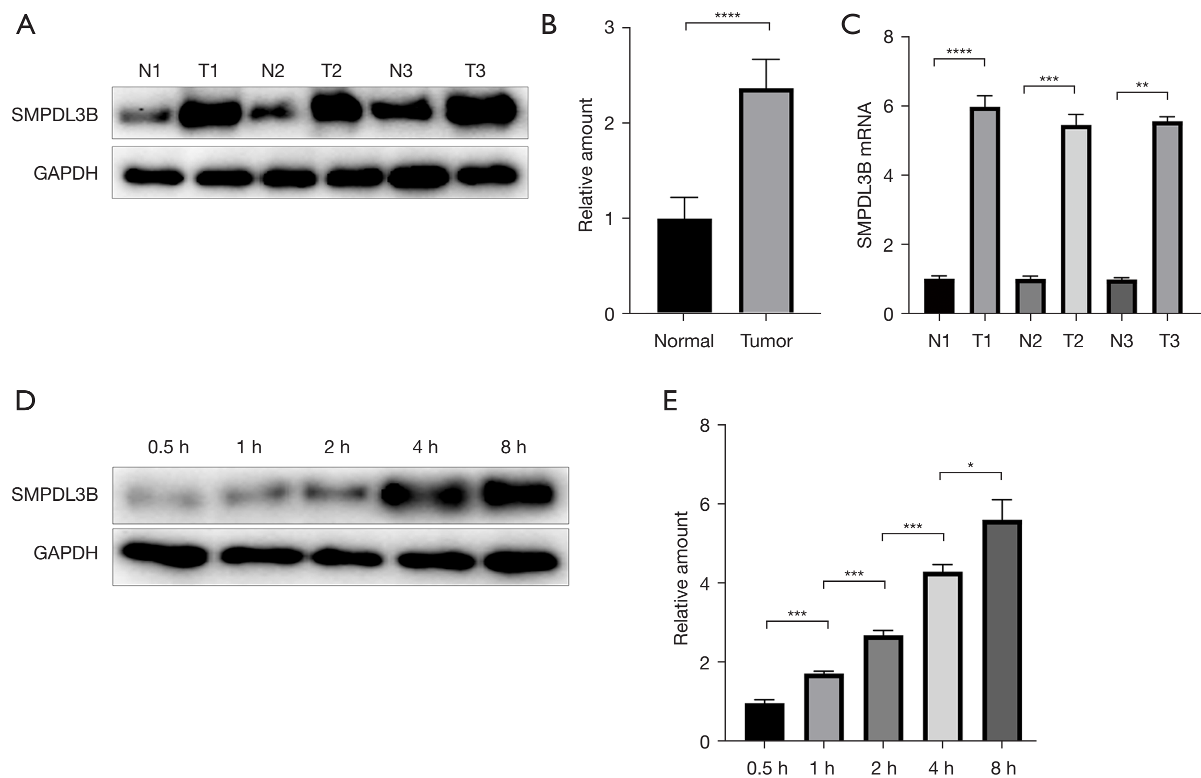

Figure 3 The expression level of SMPDL3B was upregulated in ovarian cancer. (A,B) Western blot analysis revealed significantly higher protein levels of SMPDL3B in ovarian carcinoma lesions compared to adjacent nontumor tissues; n=20. (C) qRT-PCR verified elevated transcription levels of SMPDL3B in tumor tissues from three groups of patients with ovarian cancer in comparison to adjacent nontumor tissues; n=20. (D,E) Western blot assay was used to measure the secretion of SMPDL3B protein over the indicated cell culture time; n=3. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001. SMPDL3B, sphingomyelin phosphodiesterase acid-like 3B; qRT-PCR, quantitative real-time polymerase chain reaction.

that *SMPDL3B* may promotes the migration and invasion of carcinoma cells. To investigate the functional role of *SMPDL3B* in the migration and invasion of ovarian cancer cells, we constructed *SMPDL3B* gene stable knockdown cells derived from SK-OV-3 and HEY cell lines. The results from scratch experiments showed that decreased *SMPDL3B* expression significantly inhibited the migration of ovarian cancer cells, with SMPDL3B#2 demonstrating a notable inhibitory effect on the proliferation of SK-OV-3 and HEY (*Figure 5A,5B*; P<0.01). Furthermore, transwell assay was used to assess the function of the *SMPDL3B* gene in cell migration, with the data indicating that decreased *SMPDL3B* expression reduced ovarian cancer cell migration (*Figure 5C,5D*; P<0.01). Additionally, the Transwell Matrigel experiment confirmed that reduced *SMPDL3B* expression inhibited the invasion of ovarian cancer cells, with SMPDL3B#2 demonstrating a substantial inhibitory effect on the invasion of SK-OV-3 and SMPDL3B#1 exerting a significant inhibitory effect on the invasion of HEY (*Figure 5E,5F*; P<0.01). Collectively, our results point to the role of *SMPDL3B* in promoting the migration and invasion of ovarian cancer cells.

SMPDL3B functioned as a downstream target of ACER2 in ovarian cancer

Recent a study has clarified the role of *ACER2* in the regulation of cellular ceramide hydrolysis. It has been observed that *ACER2* positively regulates the expression of *SMPDL3B*, a known carcinogenic gene, at the mRNA level, and that *SMPDL3B* acts downstream of *ACER2* (11). This suggests that *SMPDL3B* may serve as a downstream target of *ACER2*, holding importance in the progression of ovarian cancer. In our study, *ACER2* knockdown led to

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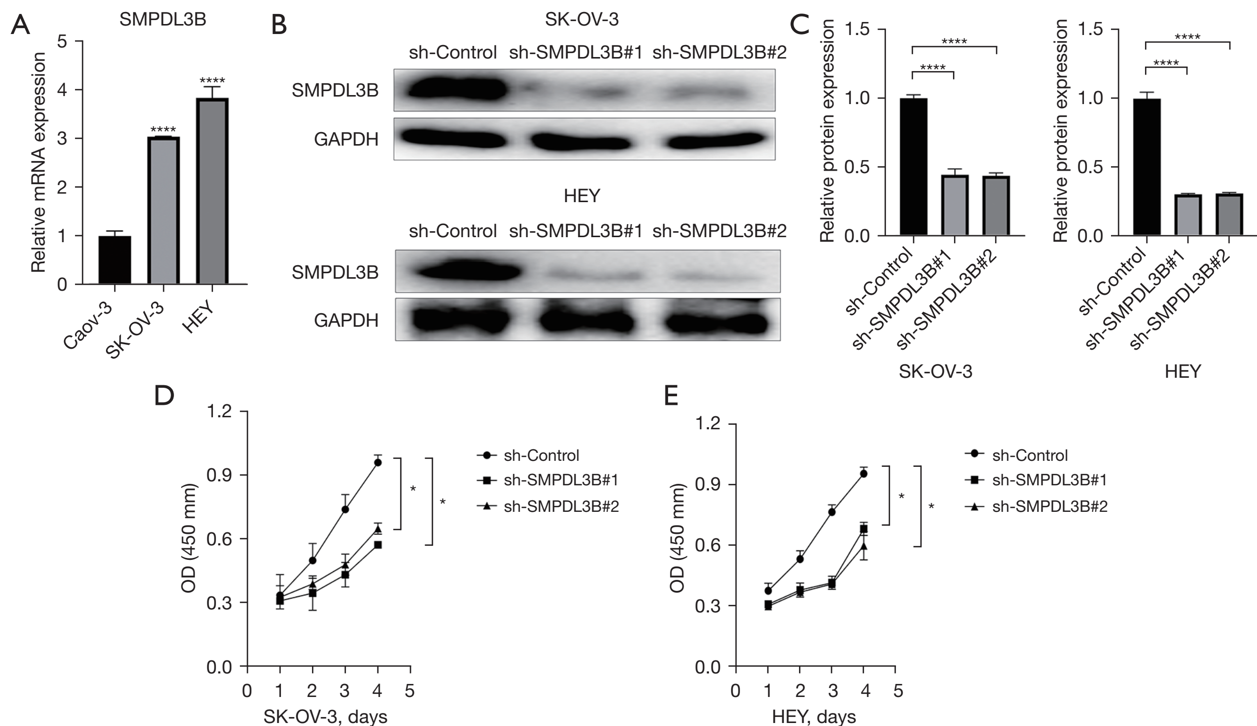

Figure 4 *SMPDL3B* promoted the proliferation of ovarian cancer cells. (A) qRT-PCR assay for measuring the mRNA production of *SMPDL3B* gene in the Caov-3, SK-OV-3, and HEY cell lines; n=3. (B,C) Western blot analysis of *SMPDL3B* protein levels following shRNA knockdown in the SK-OV-3 and HEY cell lines; n=3. (D,E) CCK-8 cell proliferation assay assessing the impact of *SMPDL3B* knockdown on the proliferation of the SK-OV-3 and HEY cell lines; n=3. *, P<0.05; ****, P<0.0001. *SMPDL3B*, sphingomyelin phosphodiesterase acid-like 3B; qRT-PCR, quantitative real-time polymerase chain reaction; CCK-8, cell count kit-8; OD, optical density.

a downregulation of SMPDL3B protein, while conversely, overexpression of *SMPDL3B* alleviated the inhibitory effects of *ACER2* (*Figure 6A*; P<0.05). Furthermore, overexpression of *ACER2* induced an upregulation of *SMPDL3B* protein expression, and interference with *SMPDL3B* resulted in a reduction of this upregulation (*Figure 6B*; P<0.01). The results of CCK-8, transwell and cell scratch assays showed that downregulation of *ACER2* significantly inhibited cell proliferation, migration and invasion. And overexpression of *SMPDL3B* could counteract the inhibitory effect of *ACER2* downregulation on these cellular processes (*Figure 6C-6F*; P<0.05). Overall, these findings suggest that ACER2 may have therapeutic potential as a target in ovarian cancer cases characterized by high SMPDL3B expression.

Discussion

The findings of this study strongly suggest that *SMPDL3B* is highly expressed in the tissue of patients with ovarian cancer and is associated with poor prognosis, pointing to *SMPDL3B* as a potential prognostic marker for ovarian cancer. Furthermore, the data demonstrated that reducing *SMPDL3B* levels considerably inhibited the proliferation, migration, and invasion of ovarian cancer cells, supporting its potential as a therapeutic target. Our findings further show that *SMPDL3B* may be involved in the signaling pathway upstream of *ACER2*. Our experiments also revealed that *ACER2* knockdown inhibited ovarian cancer, potentially due to the high expression level of *SMPDL3B* induced by increasing cancer cell proliferation, migration, and invasion. In addition, the function of *SMPDL3B* is not limited to this. GO and KEGG pathway analysis shows that *SMPDL3B* is associated with various cell structures, participating in intracellular body activity and ubiquitin mediated protein hydrolysis processes. In addition, *SMPDL3B* also participates in autophagy in the cell cycle, suggesting that high expression of *SMPDL3B* in ovarian cancer may help cancer cells escape protein hydrolysis and autophagy pathways, thereby causing ovarian cancer cell proliferation. Therefore, our next step will focus on the

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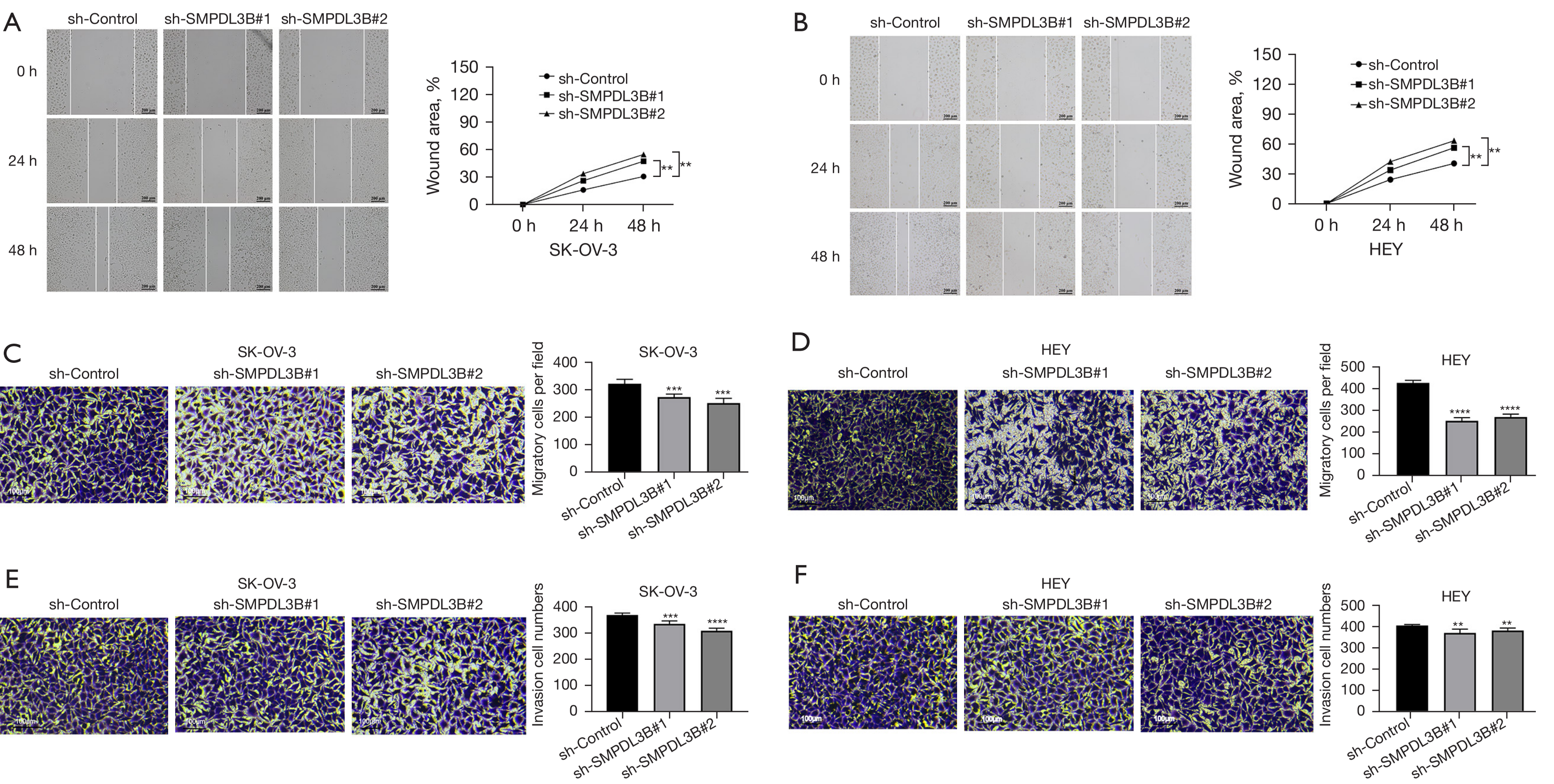

Figure 5 SMPDL3B promoted ovarian cancer cell migration and invasion. (A,B) Cell scratch assay assessing the migration of SK-OV-3 and HEY cells following SMPDL3B knockdown (200 μm). (C,D) Migration assay further confirming the reduced migration of SK-OV-3 and HEY cells after *SMPDL3B* knockdown (100 μm). (E,F) Invasion assay evaluating the decreased invasion of SK-OV-3 and HEY cells following *SMPDL3B* knockdown (100 μm). Staining method: crystal violet stained. **, P<0.01; ***, P<0.001; ****, P<0.0001. SMPDL3B, sphingomyelin phosphodiesterase acid-like 3B.

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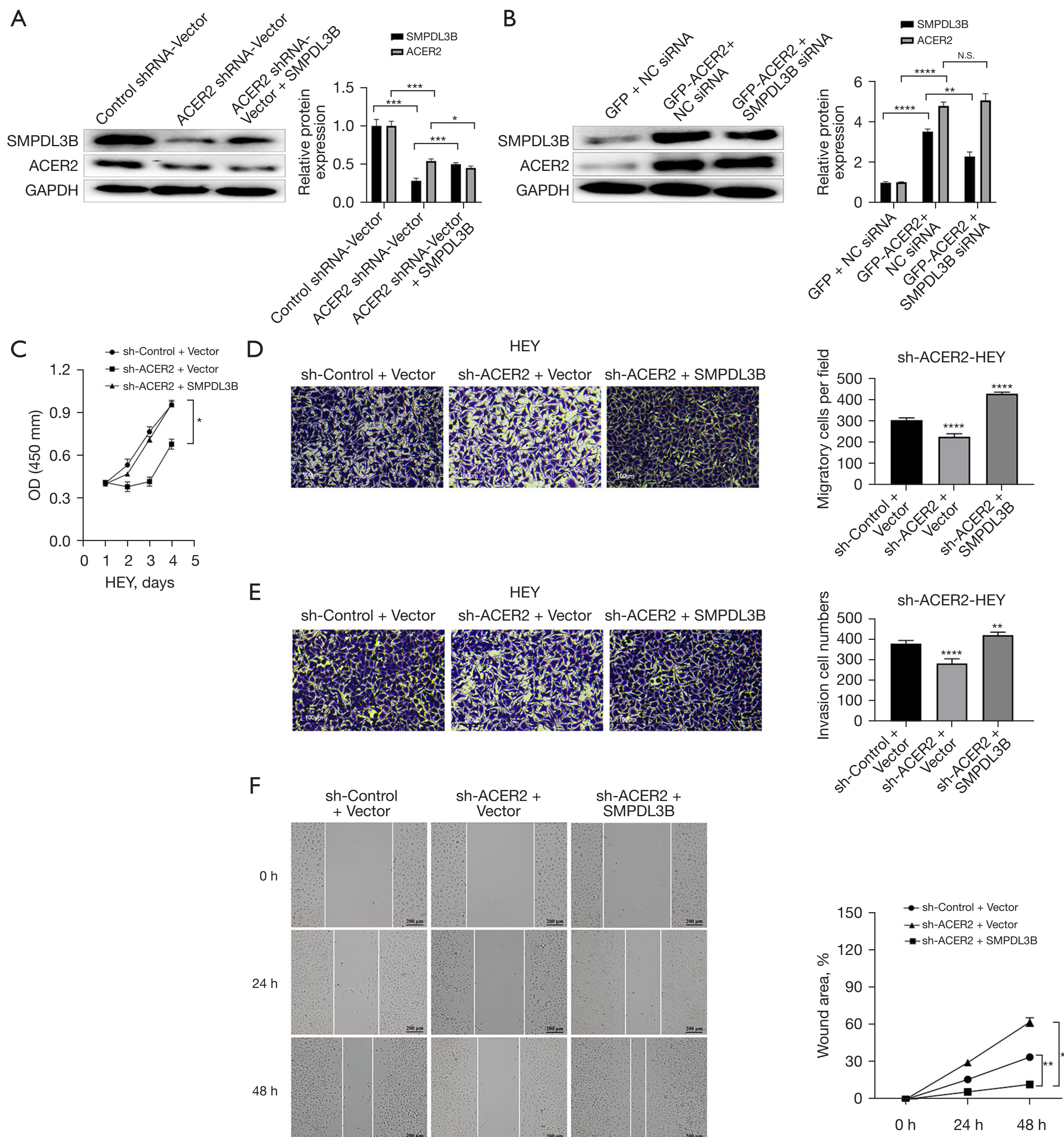

Figure 6 Role of SMPDL3B downstream of ACER2 in ovarian cancer cells. (A) Western blotting showing the protein level of *SMPDL3B* after ACER2 knockdown; n=3. (B) Western blot analysis of *SMPDL3B* protein levels following the overexpression of *ACER2*; n=3. N.S indicates no statistical significance. (C) CCK-8 cell proliferation assay assessing the effect of *ACER2* knockdown on the proliferation of HEY cells; n=3. (D) Migration assay assessing the migration of HEY ovarian cancer cells after *ACER2* knockdown (100 μm); n=3. (E) Invasion experiment evaluating the invasion of HEY ovarian cancer cells after *ACER2* knockdown (100 μm); n=3. (F) Cell scratch assay measuring the migration of HEY ovarian cancer cells after *ACER2* knockdown (200 μm); n=3. Staining method: crystal violet stained. *, P<0.5; **, P<0.01 ***, P<0.001; ****, P<0.0001; N.S., not significant. *ACER2*, alkaline ceramidase 2; *SMPDL3B*, sphingomyelin phosphodiesterase acid-like 3B; CCK-8, cell count kit-8; OD, optical density; GFP, green fluorescent protein.

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key role of *SMPDL3B* in ovarian cancer cell proliferation, and verify its specific mechanisms involved in cell cycle autophagy and protein hydrolysis through experiments. This can be achieved by gene knockout, overexpression, or inhibition of *SMPDL3B* expression to observe changes in ovarian cancer cell proliferation and validate its effectiveness as a drug target. Thus, *ACER2* may be a potential therapeutic target in patients with ovarian cancer who have high levels of the *SMPDL3B* protein. Given the high mortality rates associated with ovarian cancer, there is an urgent need to identify effective prognostic markers and thereby improve diagnosis and treatment (12,13). Our study highlights the limitations of current detection methods, such as imaging examination, endocrine examination, and serum tumor markers detection, particularly their poor specificity. In contrast, the proposed *SMPDL3B* gene has the potential to serve as a new prognostic marker for diagnosing ovarian cancer, offering promise for enhancing the efficacy of diagnosis and treatment in women afflicted by this challenging disease.

Our finding of the substantial elevation of *SMPDL3B* levels in ovarian cancer tumor tissues also points to the important function of *SMPDL3B* in ovarian cancer. Importantly, this observation aligns with similar results detected in other solid and nonsolid tumors, such as prostate cancer, hepatocellular carcinoma, and acute myeloid leukemia (7,14,15). Notably, there is evidence associating high *SMPDL3B* expression *in vivo* with poor prognosis in prostate cancer, while inhibition of *SMPDL3B* has been demonstrated to have a detrimental effect on the growth of acute myeloid leukemia cells. Our data thus indicate that *SMPDL3B* may serve as a robust prognostic indicator and potential therapeutic target for human cancers. However, comprehensive investigations are required to fully elucidate its importance in other type of carcinomas.

The *SMPDL3B* was initially identified as a glycosylphosphatidylinositol (GPI)-anchored factor (16,17), and subsequent discoveries have shed light on its crucial biological functions. Notably, as a phosphodiesterase, *SMPDL3B* plays a vital role in lipid regulation and fluidity (9,18). Moreover, it has been implicated in cellular activities including cellular immunity and cell injury (10,11,19). Recent findings have indicated that increased *SMPDL3B* levels are associated with poor survival in patients with prostate carcinoma and that knockdown of *SMPDL3B* also reduces the migration of PC317 cells, a human prostate cancer cell line (15). These findings provide a new perspective regarding the role of *SMPDL3B*, particularly in malignancies. Despite these discoveries, the specific role of *SMPDL3B* in malignancies, particularly in the development of ovarian carcinoma, has remained largely unknown. We hypothesized that the *SMPDL3B* gene may serve as a new diagnostic marker for the detection of ovarian cancer. Subsequently, through bioinformatics methods, we found there to be an increased expression of *SMPDL3B* in ovarian cancer lesions compared to adjacent nontumor tissues. Additionally, through statistical analysis of databases, we found that increased expression of *SMPDL3B* was associated with the prognosis of patients with cancer, suggesting its potential use as a prognostic indicator for cancer determination. Importantly, we also proved that downregulation of *SMPDL3B* inhibited the proliferation, migration, and invasion of ovarian carcinoma cells, suggesting that *SMPDL3B* may be a novel target for ovarian carcinoma treatment.

In addition to its role in hydrolyzing sphingomyelin, *SMPDL3B* is known to recognize ATP as a potential substrate (20). Considering the stimulation of carcinoma cell metabolism by ATP consumption (21) and the ability of *SMPDL3B* to hydrolyze ATP in promoting the proliferation of carcinoma cells, we believe this may constitute another crucial mechanism through which *SMPDL3B* promotes the emergence and progression of ovarian carcinoma. Additionally, *SMPDL3B* has been shown to block Toll-like receptor-regulated responses, thus inhibiting innate immune responses (10). We hypothesize that *ACER2* induces tumorigenesis through *SMPDL3B*, as there is substantial evidence supporting the contribution of innate cells in the host defense against cancer cells. Taken together, these data strongly support the notion that *SMPDL3B* is upregulated in ovarian cancer.

Sphingolipids are essential components of cell membranes and are critically involved in the pathogenesis of carcinoma. Endogenous and biologically active sphingolipid ceramides have been shown to inhibit the proliferation of numerus tumors. Sphingosine is produced from ceramide hydrolyzation by *ACER2*. Additionally, *ACER2* also regulates cellular activities, such as autophagy, DNA damage, and apoptosis (22,23), and there is pertinent evidence indicating that *ACER2* can promote tumor cell growth (24). Although *ACER2* is recognized as an important component in inducing cellular ceramide hydrolysis to influence carcinoma cell replication, the specific mechanisms related to ovarian carcinoma are not well established. Notably, high levels of *ACER2* have been reported in hepatocellular carcinoma, and it has been

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found that the ACER2-SMPDL3B complex promotes the development of hepatocellular carcinoma by promoting ceramide hydrolysis and the production of the blood-derived chemical signal sphingosine-1-phosphate (S1P) (9). Additionally, several studies have demonstrated that *ACER2* is associated with the proliferation, apoptosis, DNA damage, and autophagy in various cancer cells (25-28).

Despite the insights garnered from this investigation, there were some limitations to our study. First, some issues in examining the function and mechanism of *SMPDL3B* in ovarian cancer pathogenesis should be noted. The relatively small number of tissue samples used in the study and the reliance on TCGA as the only resource for confirming SMPDL3B's role as a prognostic indicator in ovarian carcinoma are both valid concerns. Recruiting a larger cohort of patients with ovarian cancer in future studies will be essential to conducting a more comprehensive analysis of the relationship between *SMPDL3B* levels and patient outcomes, including OS and disease-free survival. Additionally, further investigation into the expression level changes of the *SMPDL3B* gene in ovarian carcinoma tissues would be beneficial. Furthermore, while our study provides evidence supporting the role of *SMPDL3B* as a prognostic marker and a potential therapeutic target in ovarian carcinoma, we recognize the need for a deeper exploration into the underlying mechanisms. It is indeed crucial to conduct more sophisticated experiments such as protein–protein interaction studies, RNA sequencing, and mass spectrometry to better understand how *SMPDL3B* promotes the progression of ovarian carcinoma. By acknowledging these limitations and the need for further research, our study sets the stage for important future investigations that will contribute to a more thorough understanding of *SMPDL3B* and its significance in ovarian carcinoma.

In the field of oncology, research on ovarian cancer has always been a focus of attention for researchers. Our study found that *SMPDL3B* affects the proliferation, migration, and invasion of ovarian cancer. This study not only reveals the important role of *SMPDL3B* in the development of ovarian cancer, but also delves into its correlation with the tumor microenvironment, providing new ideas for future drug development. Firstly, *SMPDL3B* is a protein closely related to cellular signaling and gene expression regulation. In ovarian cancer, the expression level of *SMPDL3B* is often abnormally elevated, which is closely related to its key role in cancer cell proliferation, migration, and invasion. By regulating relevant signaling pathways, *SMPDL3B* can significantly promote the malignant transformation of ovarian cancer cells, thereby accelerating tumor development. The tumor microenvironment is a complex ecosystem that includes tumor cells, immune cells, stromal cells, and various bioactive molecules. These components interact with each other and together affect the occurrence, development, and metastasis of tumors. *SMPDL3B* indirectly alters the homeostasis of the tumor microenvironment by influencing the biological behavior of tumor cells. Meanwhile, some factors in the tumor microenvironment can also regulate the expression of *SMPDL3B*, forming an interactive cycle. This interaction is of great significance for the progression of ovarian cancer. On the one hand, overexpression of *SMPDL3B* may lead to tumor cell resistance to treatment, reducing treatment efficacy; On the other hand, changes in the tumor microenvironment may also promote the spread and metastasis of tumor cells, further exacerbating the condition. In response to this issue, future drug development goals should focus on the following aspects: firstly, developing drugs that can specifically inhibit the activity of *SMPDL3B* to block its role in the development of ovarian cancer; The second is to study how to inhibit the progression of ovarian cancer by regulating the tumor microenvironment, such as by enhancing the activity of immune cells or altering the secretion function of stromal cells; The third is to explore a combined treatment plan that combines the above two strategies to improve treatment effectiveness and reduce side effects.

Of course, achieving these goals requires in-depth research and extensive experimental verification by researchers. However, I believe that with the continuous progress of science and technology and the accumulation of clinical data, we will definitely be able to find more effective treatment strategies, bringing better hope for the survival of ovarian cancer patients.

Conclusions

The results from this study are significant, as they demonstrated increased *SMPDL3B* levels in primary ovarian carcinoma lesion compared to nontumor tissues. Furthermore, the evidence suggests that elevated *SMPDL3B* contributes to the proliferation, migration, and invasion of ovarian carcinoma cells. Notably, the identification of *ACER2* as an upregulator of *SMPDL3B* in ovarian carcinoma cells implies that *SMPDL3B* may function downstream of *ACER2*. This highlights the potential of

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the ACER2/SMPDL3B signaling pathway in regulating the emergence and progression of ovarian carcinoma. The implications of these results are substantial and justify further research into the ACER2/SMPDL3B pathway and its specific role in the pathogenesis of ovarian carcinoma. These investigations have the potential to identify novel prognostic markers and therapeutic targets for ovarian cancer, offering new avenues for improving patient outcomes.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was reviewed and approved by the Ethics Review Committee of Fujian Provincial Hospital (approval No. K2020-01-031) and was conducted according to the declaration of Helsinki (as revised in 2013). All enrolled patients signed informed consent form.

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