# Actin-related protein 2/3 complex subunit 1B promotes ovarian cancer progression by regulating the AKT/PI3K/mTOR signaling pathway

**Miao Ke[1#], Huimin Zhu[2#], Yu Lin[1#], Ying Zhang[1], Tao Tang[3], Yuhao Xie[4], Zhe-Sheng Chen[4], Xiaoyu Wang[1], Yuan Shen[1]**

[1]Department of Obstetrics and Gynecology, The First Affiliated Hospital of Jinan University, Guangzhou 510630, Guangdong Province, China;
[2]Department of Cardiology, The First Affiliated Hospital of Jinan University, Guangzho 510630, Guangdong Province, China;
[3]Department of Obstetrics and Gynecology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong 518172, China;
[4]College of Pharmacy and Health Sciences, St. John's University, New York 11439, New York, USA

## ABSTRACT

**Background and Objectives:** Actin-related protein 2/3 complex subunit 1B (ARPC1B) is an essential subunit of the actin-related protein 2/3 (Arp2/3) complex. While there have been numerous research reports on Arp2/3 in relation to tumors, there needs to be more research on ARPC1B and its role in tumors, particularly at the pan-cancer level. **Methods:** Utilizing data from the cancer genome atlas (TCGA) and genotype-tissue expression (GTEx) databases, we analyzed ARPC1B expression differences in normal, tumor, and adjacent tissues, investigating its correlation with prognosis and clinical stages in various cancers. We conducted gene enrichment analysis and explored ARPC1B's connection to the tumor immune microenvironment and its impact on anti-tumor drug resistance. In addition, *in vivo* and *in vitro* experiments have also been carried out to find the mechanism of ARPC1B on ovarian cancer (OV) proliferation and invasion. **Results:** ARPC1B was highly expressed in 33 tumor types, suggesting its role as a tumor-promoting factor. Its expression correlated with poor prognosis and served as a clinical staging marker in over 10 tumor types. ARPC1B is implicated in various biological processes and signaling pathways, uniquely associated with tumor immunity, indicating immunosuppressive conditions in high-expression cases. High ARPC1B expression was linked to resistance to six anti-tumor drugs. Further experiments showed that ARPC1B can affect the proliferation, apoptosis, migration, and invasion of OV cells through the AKT/PI3K/mTOR pathway. **Conclusion:** ARPC1B is a biomarker for immune suppression, prognosis, clinical staging, and drug resistance, providing new insights for cancer therapeutics.

**Key words**: actin-related protein 2/3 complex subunit 1B, The Cancer Genome Atlas, Genotype-Tissue Expression, tumor, resistance

#These authors contributed equally to this work.

**Address for Correspondence:**
Yuan Shen, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Jinan University, No. 613 West Huangpu Ave, Tianhe District, Guangzhou 510630, Guangdong Province, China.
Email: missyy@126.com.
Xiaoyu Wang, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Jinan University, No. 613 West Huangpu Ave, Tianhe District, Guangzhou 510630, Guangdong Province, China.
Email: twxy163@163.com

**Access this article online**

**Website:** www.intern-med.com

**DOI:** 10.1515/jtim-2024-0025



## INTRODUCTION

In various physiological and pathological states of the human body, the remodeling of the actin skeleton plays a crucial role. This process involves the participation of various actin-related proteins through complex interactions.[1] A series of previous studies have found that actin cytoskeleton remodeling plays a crucial role in immune cell function, and its changes can lead to autoimmunity or primary immunodeficiency (PID).[2,3] Among the various actin-related proteins, the actin-related protein 2/3 (Arp2/3) complex has garnered widespread attention.[4] The absence of the Arp2/3 complex results in reduced lamellipodia formation, chemotaxis defects, and diminished cell migration capability. This, in turn, gives rise to innate and adaptive immune abnormalities, ultimately leading to immune disorders.[5] Actin-related protein

2/3 complex subunit 1B (ARPC1B) is a protein encoded by the ARPC1B gene in humans.[6] This gene encodes one of the seven subunits of the human Arp2/3 complex.[7] This subunit can enhance the branching of actin filaments and influence the Arp2/3 complex's capacity to assemble actin,[8] which plays a pivotal role in the alterations of actin filament dynamics mediated by the Arp2/3 complex.

The Arp2/3 complex is a critical regulator of various biological activities. Previous studies have shown that Arp2/3 subunits are highly expressed in various types of tumors, including lung cancer,[9] breast cancer,[10] glioma,[11] gastric cancer[12] and colorectal cancer.[13] Interestingly, recent studies have shown that the Arp2/3 complex is overexpressed not only in tumor cells, but also in cancer-associated fibroblasts, a component of the tumor microenvironment (TME).[14,15] This overexpression is associated with the transition to invasive stages of cancer progression, suggesting complex interactions between tumor cells and the surrounding microenvironment. This interaction may have important implications for cancer development and treatment strategies. In addition, recent studies have found that this complex can affect mitochondrial dynamics and thereby change the metabolic state of tumor cells, leading to the occurrence of tumor drug resistance.[16] However, the role of ARPC1B, a key component of the Arp2/3 complex, has not been extensively studied in depth. Current research tends to suggest that ARPC1B is associated with platelet function and immune system defects,[17,18] but its role in tumors is not clear.

As research in the field of oncology advances, our understanding of cancer, a complex disease, is continually evolving. Notably, the exploration of the (TME has opened new avenues for cancer research.[19,20] The TME comprises a variety of cell types, with tumor-infiltrating immune cells constituting a significant portion.[21] Despite the widespread investigation of the TME in recent years, there is still a need for further research into the dynamic adjustment and interaction of its components. Concurrently, the critical role of immune cells in tumor initiation and progression has given rise to innovative treatment methods like immune checkpoint blockade.[22,23] However, the issue of drug resistance in tumor cells remains a pervasive challenge in clinical cancer treatment, significantly limiting the effectiveness of various therapeutic approaches.[24] Interestingly, previous studies have shown that the AKT/PI3K/mTOR pathway plays a key role in a variety of processes including cell growth, proliferation and survival.[25] In cancer, this pathway is frequently disrupted, which promotes tumor development and progression. In the TME, the AKT/PI3K/mTOR pathway interacts with factors such as immune cells, stromal cells, and extracellular matrix, which can affect or be affected by the pathway.[26,27] Dysregulation of this pathway in the TME can promote tumor growth, angiogenesis, and resistance to therapy, making it an important target in cancer treatment strategies.[28,29] It's worth noting that while there has been significant progress in studying the treatment and drug resistance of individual tumor types, there is a dearth of research exploring commonalities across different cancers at a broader, pan-cancer level.

In this study, we conducted an analysis of ARPC1B gene expression differences in 33 different cancer types using data from multiple databases. We examined how ARPC1B expression varies across each cancer and its impact on patient prognosis in a pan-cancer context. Additionally, we performed gene enrichment analysis and TME correlation analysis. We also established a TME signature score system to investigate the role of the ARPC1B gene in a variety of cancers. Furthermore, we assessed the connection between ARPC1B and immune infiltrating cells, immune-related genes, major histocompatibility complex (MHC) family genes, chemokines, and chemokine receptors using data from multiple databases. Our aim was to elucidate the relationship between the ARPC1B gene and tumor immunity. Finally, we analyzed the relationship between this gene and tumor drug sensitivity. Further experiments showed that ARPC1B can affect the proliferation, apoptosis, migration and invasion of ovarian cancer (OV) cells through the AKT/PI3K/mTOR pathway. It is important to note that, among the 33 cancer types, we placed special emphasis on ovarian serous cystadenocarcinoma (OV) and conducted a detailed analysis of the unique role of the ARPC1B gene in this specific cancer.

## MATERIALS AND METHODS

### *Gene expression analysis in pan-cancer*

Through the UCSC XENA website (https://xenabrowser.net/datapages/),[30] 33 types of tumor samples, normal tissue RNA sequencing (RNA-seq), and related clinical data were downloaded from The Cancer Genome Atlas (TCGA) database[31] and Genotype-Tissue Expression (GTEx) database[32] to analyze the expression of ARPC1B in a variety of cancer samples, normal tissues, and adjacent tissues, and to reveal the relationship between the gene and the clinical stages of various tumors. Statistical analysis was conducted by R software v4.1.1, "ggplot2", "ggpubr" and "ggradar" packages were used for visualization. The two sets of data were detected by *t*-test, and $P < 0.05$ was considered to have statistically significant genomic changes.

The changes in the ARPC1B status of cancer patients are obtained from the online cBioPortal database of cancer genomics (http://www.cbioportal.org/).[33] The

genome changes of ARPC1B include "amplification", "deep deletion", "missense mutation" and "structural abnormality". In addition, we also analyzed the correlation between the copy number of ARPC1B and promoter methylation in each tumor, using the "ggplot2" package for plotting.

### *Survival and prognostic analysis of ARPC1B*

To investigate the relationship between ARPC1B and the prognosis of various cancers, we performed a univariate Cox regression analysis, considering overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI) indicators. For Kaplan-Meier survival analysis, patients were stratified into high and low expression groups based on the ARPC1B expression cutoff value in various tumors using the "survminer" and "survival" packages. A *P*-value less than 0.05 was considered statistically significant.

### *Gene enrichment analysis*

To explore the gene function of ARPC1B and its biological effects in various cancers, gene set variation analysis (GSVA) enrichment analysis was performed to evaluate the correlation between this ARPC1B and 50 hallmark pathways based on the MsigDB database (http://software.broadinstitute.org/gsea/msigdb/index.jsp) HALLMARK pathway dataset.[34] Among all cancers, we paid special attention to OV, so we conducted the correlation analysis between ARPC1B and key pathways in OV. In this study, we believe that *P*-value < 0.05 is statistically significant. GSVA analysis uses R software v4.1.1, "GSVA" package for scoring, "ggplot2" and "ggpubr" for plotting.

In addition, to further explore the role of this gene in OV, we analyzed the correlation between ARPC1B and all mRNAs, and performed gene set enrichment analysis (GSEA)-GO, GSEA-KEGG GSEA-Reactome analysis based on the results of correlation analysis, P-value < 0.05 was considered statistically significant. Gene correlation analysis results are presented in the form of heat maps using the "pheatmap" package, and GSEA analysis is performed using the "clusterpofiler" package.

### *TME analysis*

Estimate algorithm is an algorithm to estimate the matrix and immune cells in malignant tumor tissue based on expression data.[35] It can calculate matrix scores and immunity based on indicators such as the abundance of matrix and tumor infiltrating immune cells in tumor samples score. To explore the correlation between the ARPC1B gene and the TME, we used the R language "ESTIMATE" package to calculate the correlation between the gene and tumor tissue's Stromalcore, ImmuneScore, ESTIMATEScore (the sum of the first two), and TumorPurity. In addition, we also focused on the analysis of the relationship between ARPC1B in OV and the TME.

The TME gene sets were downloaded and the scores of each gene set in TCGA pan-cancer were calculated using the method from the published paper.[36]

### *Correlation analysis between ARPC1B and tumor immune infiltrating cells*

Based on the previous enrichment analysis and TME analysis results, we found that the ARPC1B gene is closely related to the immune response in the occurrence and development of a variety of cancers. To further explore the role of this gene in tumor immunity, we conducted a relationship analysis between a variety of cancers and immune cell infiltration based on this gene. CIBERSORT was originally mainly used for the analysis of TME, but currently, it is used more and more for the characteristics of immune infiltration in non-tumor tissue analysis. Immune cell infiltration scores are downloaded from the ImmuCellAI database (http://bioinfo.life.hust.edu.cn/ImmuCellAI#!/),[37] which contains 32 types of tumors (without acute myeloid leukemia [LAML]) and 24 types of immune cell infiltration status. Use CIBERSORT to calculate the correlation, and the results are displayed in the form of a heat map.

The Ttumor immune estimation resource (TIMER) database (https://cistrome.shinyapps.io/timer/)[35] is an integrated web server used to evaluate the abundance of tumor infiltration immune cells (TIIC) in different cancer types. We also downloaded the pan-cancer immune infiltration data from the TIMER2 database to calculate the correlation between gene expression and each immune cell gene, and the results were displayed in heat maps.

Previous studies believe that tumor cells can stimulate immune checkpoint targets,[38] lose MHC/human leukocyte antigen (HLA) class I molecules,[39] and affect immune cell recruitment to escape from being cleared by the immune system,[40,41] To further study the specific role of ARPC1B in tumor immunity, we further analyzed the correlation between this gene and immunosuppressive genes, MHC genes, chemokine/chemokine receptors at the pan-cancer level, and OV was toked as an example for analyzing the relationship between ARPC1B and 5 immune checkpoints. The visualization of all heat maps in this step was conducted by the "ggplot2" package. The correlation between ARPC1B and immune checkpoints in OV is presented as a circle diagram, visualized by the "circlize" package.

### *Cell culture and gene transfection*

The SKOV3 and A2780 cell lines were procured from

Procell Life Science& Technology Co., Ltd. situated in Wuhan, China. The cells were cultured in DMEM (KeyGen, China) supplemented with 10% FBS (Thermo fisher, USA) and 1% penicillin/streptomycin (100 U/mL) for SKOV3 cells and A2780 cells. The cells were maintained in a standard culture environment with 5% $CO_2$ at 37°C. In the lentiviral transduction experiment, we utilized lentiviral constructs obtained from OBiO in Shanghai, China, to create knockdown (shARPC1B) and control (shNC) vectors for the ARPC1B gene. Subsequently, we used 5 μg/mL puromycin to select for stable expression of these two constructs in OV cells.

### *Cell counting kit-8 (CCK-8)*

ES-2 and OVCAR-3 cells were seeded into 96-well plates at a density of $5 \times 10^4$ cells/well and subjected to treatment with shNC and shARPC1B for 0 h, 24 h, 48 h, and 72 h. Following treatment, the cells were exposed to 10 μL of CCK-8 (Beyotime, China) at 37°C for 2 h, and the absorbance values were measured at 450 nm using a microplate reader (Bio-Rad, USA).

### *Ethynyldeoxyuridine (EdU) assay*

The current investigation evaluated the cell proliferation status, which indicates the percentage of cells undergoing DNA replication, by employing an EdU detection kit (RiboBio, China). The incorporation rate of EdU was determined by calculating the proportion of cells incorporating EdU to those stained with Hoechst 33,342. Each experimental group was evaluated by enumerating a minimum of 500 cells.

### *Transwell assay*

For the invasion assay, the upper chamber was pre-coated with Matrigel (BD Bioscience, China), and cells were seeded in the serum-free medium. The complete medium was subsequently added to the lower chamber. After 24 h of culture, non-migrating or non-invading cells were removed from the upper chamber using cotton swabs. The cells in the lower chamber were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. The migrated or invaded cells were then counted under a microscope.

### *Western blot*

Proteins were extracted from cells using RIPA lysis buffer (Beyotime, China). Subsequently, the proteins were separated on a 10% SDS-PAGE gel and transferred onto a 0.45 μm PVDF membrane. The membrane was blocked with a 5% skim milk solution in TBST at room temperature for 1 h. Following blocking, the membrane was incubated overnight at 4°C with the primary antibody, and subsequently with an HRP-conjugated secondary antibody for 2 h at room temperature. The protein bands were visualized and quantified using the ECL kit (Beyotime, China) and Image J software, respectively. Each experiment was performed thrice. The following antibodies (Sigma) were used for the Western blot analysis: anti-ARPC1B (Beyotime, China), anti-GAPDH (Beyotime, China), and anti-rabbit IgG (Beyotime, China).

### *Wound healing*

ES-2 and OVCAR3 cells were seeded in a 6-well culture plate. Transfection was performed using ARPC1B shRNA. The cells were allowed to grow until fully confluent. A scratch was made on the culture plate using a P200 pipette tip. Images were captured at 0 h and 24 h using a Japanese Olympus inverted microscope. Cell migration was analyzed using the Image J software, and each experiment was repeated three times.

### *Apoptosis assay*

The cell culture and cell transfection were similar to the previous experiments. After transfection for 72 h, the control and transfection group cells were digested with EDTA-free trypsin and washed twice with PBS solution. According to the manufacturer's guidelines, we used the Annexin V-APC/PI apoptosis detection kit (Beyotime, China) to conduct the apoptosis assay. Finally, a flow cytometer, and FlowJo software, were used to detect the cell and analyze the results.

### *Statistical analysis*

In this article, a Student's *t*-test was performed for the gene expression data of tumor samples downloaded from TCGA and GTEx databases. Except for the correlation between ARPC1B and tumor drug resistance using Spearman correlation analysis, other correlation tests were all expressed by Pearson coefficient. All analyses were performed using R software (version 4.1.1, www.r-project.org) loaded with R packages (ggplot2, ggradar, ggpubr, pheatmap, clusterpofiler, GSVA, survmine, suivival, ESTIMATE, and circlize packages). The results of $P < 0.05$ were considered statistically significant and provided credibility for data analysis.

## RESULT

### *The expression of ARPC1B in pan-cancer*

In this study, we analyzed 33 tumor specimens to delineate the expression profile of the ARPC1B gene across diverse cancers, drawing upon data from the TCGA and GTEx databases (Figure 1A). Our results demonstrate significant upregulation of ARPC1B in various tumor types, particularly in (OV, while its expression is comparatively lower in kidney chromophobe (KICH), LAML, lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), and thyroid carcinoma (THCA) when set against normal tissue benchmarks. Furthermore, a radar chart was

employed to concisely illustrate the mean expression levels of ARPC1B in the 33 tumor categories included in the TCGA dataset (Figure 1B). The notable overexpression of ARPC1B in OV and other malignancies highlights its potential oncogenic role. In contrast, increased expression of ARPC1B was observed in normal spleen, bone marrow, and blood samples (Figure 1C). Additionally, ARPC1B expression across various cell lines was investigated (Figure 1D). The subcellular localization of ARPC1B, analyzed using the Human Protein Atlas (HPA) database, was assessed in cell lines such as A-431, U-251MG, and U-2OS (Figure 1E). Subsequent analysis of ARPC1B protein levels, specifically in (OV tissues using the HPA database, revealed a significant elevation compared to normal tissues (Figure 1F).

### *Correlation between ARPC1B and pan-cancer characteristics*

In this comprehensive study, we elucidate the relationship between ARPC1B gene expression and the clinical stage across a spectrum of tumor types. Our rigorous analysis covered ten distinct tumor types, namely adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA), (KICH, kidney renal clear cell carcinoma (KIRC), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), (OV, pancreatic adenocarcinoma (PAAD), stomach adenocarcinoma (STAD), and uveal melanoma (UVM). Our findings demonstrate a notable positive correlation between ARPC1B expression and advanced clinical stages (Supplementary Figure 1). The research further identifies a significant linkage of gene mutations, amplifications, mRNA methylation modifications, and copy number alterations with the onset and progression of these tumors.

Expanding our research scope, we investigated the presence of similar ARPC1B alterations across a broader array of 33 cancer types. The data revealed that ARPC1B amplification plays a major role in tumor progression in esophageal carcinoma (ESCA), diffuse large b-cell lymphoma (DLBC), (PAAD, cholangiocarcinoma (CHOL), uterine carcinosarcoma (UCS), (ACC, (KIRC, and (KIR.C Conversely, genomic mutations were found to be a primary contributing factor to alterations in (UVM, with significant implications in colon adenocarcinoma (COAD) and uterine corpus endometrial carcinoma (UCEC). A critical finding was the identification of deep deletion of ARPC1B as a key factor in (LAML and (THCA (Figure 2A). We also investigated the mutation rate of ARPC1B in (OV tumors, uncovering a mutation rate of 0.37%, which is visually represented in a lollipop plot (Figure 2B). The somatic mutation landscape of the OV cohort was further illustrated using an Oncoplot, underscoring the high frequency of mutations in TP53, TTN, and CSMD3, with a more prevalent occurrence of ARPC1B mutations in the low-expression group (Figure 2C).

A significant positive correlation was established between ARPC1B expression and copy number variations in 27 tumor types, juxtaposed with a noteworthy negative correlation observed in thymoma (THYM, Supplementary Figure 2A). An inverse relationship was also noted between the level of gene expression and mRNA methylation modification across various tumor types (Supplementary Figure 2B).

Delving into the prognostic implications, our study reveals that high promoter methylation of ARPC1B is associated with diminished OS in KIRC (Supplementary Figure 2C), lower grade glioma (LGG, Supplementary Figure 2D), and UVM (Supplementary Figure 2E), whereas it correlates with improved survival outcomes in pheochromocytoma and paraganglioma (PCPG, Supplementary Figure 2F). Prognostic factor index (PFI) analysis further delineates the role of ARPC1B methylation as a protective factor in breast invasive carcinoma (BRCA), LGG, PCPG, THYM, and UVM, in contrast to its risk factor status in testicular germ cell tumors (TGCT, Supplementary Figure 3A). Additionally, heightened ARPC1B methylation was identified as a protective factor in DSS analysis (Supplementary Figure 3B).

### *Correlation between ARPC1B and pan-cancer prognosis and survival*

In an effort to elucidate the prognostic significance of ARPC1B expression in cancer, we conducted Kaplan-Meier survival analyses across nine distinct tumor types. Our study revealed a statistically significant correlation between elevated ARPC1B expression and reduced OS probabilities in ACC (Figure 3A), glioblastoma (GBM, Figure 3B), KICH (Figure 3C), KIRC (Figure 3D), LGG, Figure 3E), LIHC (Figure 3F), LUAD (Figure 3G), OV (Figure 3H), and UVM (Figure 3I). This trend was further substantiated by the analysis of DSS, where increased ARPC1B expression emerged as a significant risk factor in ACC, COAD, DLBC, GBM, KIRC, LGG, OV, rectum adenocarcinoma (READ), and UVM (Supplementary Figure 4A).

Furthermore, we examined the impact of ARPC1B expression on PFI across different cancer types. Kaplan-Meier curves indicated a notable association between higher ARPC1B levels and poorer PFI in ACC, cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), GBM, KIRC, LGG, PRAD, and UVM (Supplementary Figure 4B).

### *ARPC1B and immune infiltrating cells*

The aforementioned analysis results demonstrated a strong correlation between ARPC1B and immune responses in

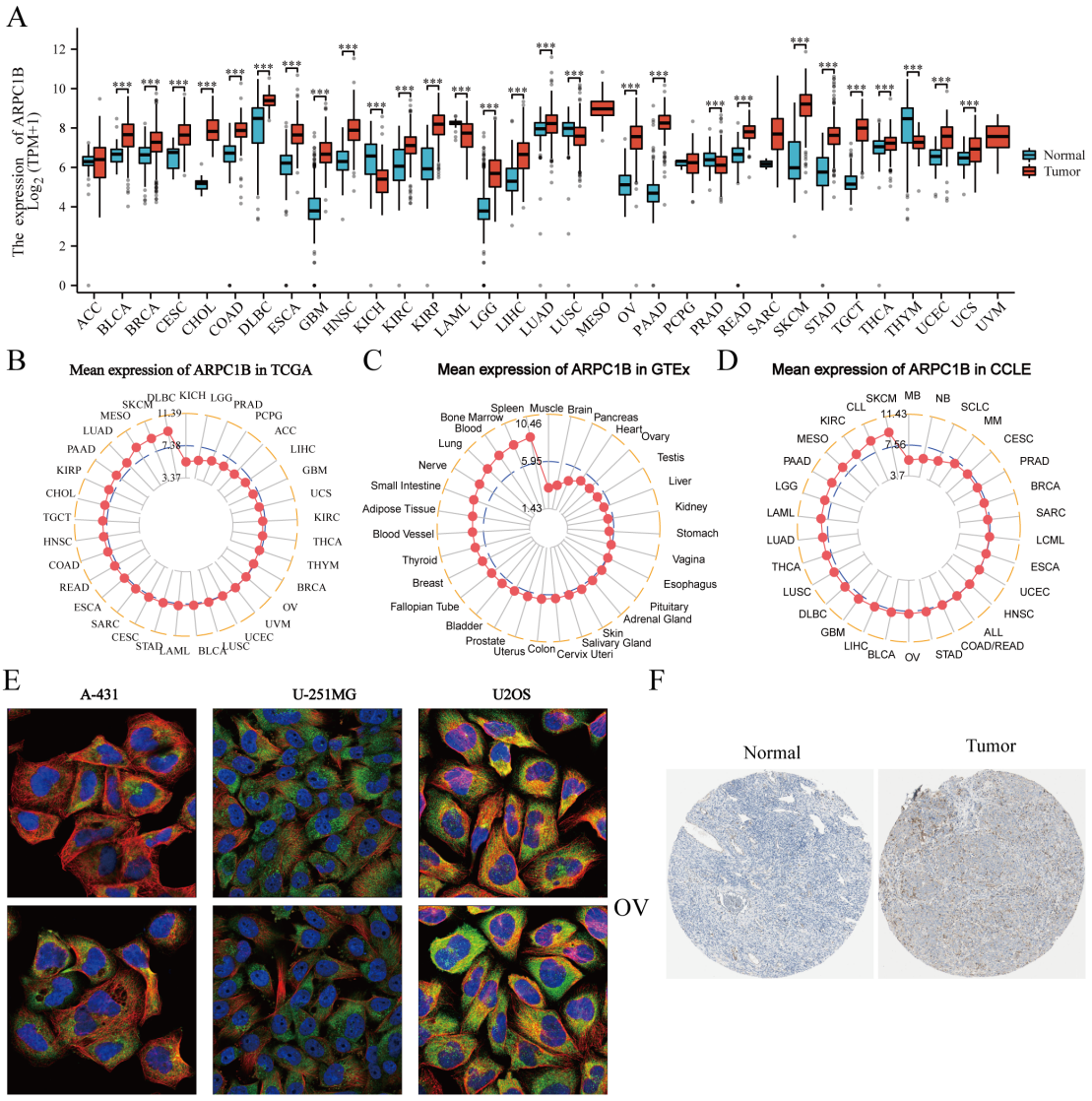


**Figure 1: ARPC1B expression levels and localization. (A) Analysis of the TCGA and GTEx databases indicated higher expression of ARPC1B in several tumors, including OV, and lower expression in KICH, LAML, LUSC, PRAD, and THCA compared to normal tissues. See Supplementary Table 1 for full TCGA disease abbreviations. (B) A radar chart illustrates the varying average expression levels of the ARPC1B gene in 33 TCGA tumors. (C) Demonstrates the expression levels of the ARPC1B gene in normal tissues, especially in the spleen, bone marrow, and blood, reflecting the gene's importance in normal physiological conditions. (D) Examines the expression of the ARPC1B gene in different cell lines. (E) Detailed analysis of the subcellular localization of ARPC1B in various cell lines, including A-431, U-251MG, and U-2OS, using the HPA database, revealing its specific intracellular localization. Green: ARPC1B, Blue: nucleus, Red: microtubules. (F) Analysis using the HPA database reveals higher ARPC1B protein expression in OV compared to normal tissue, suggesting a potential role in its development. Wilcoxon rank-sum test was used for analyzing two groups, and the Kruskal-Wallis test for multiple groups. Data are expressed as mean ± standard deviation. Significance markers: ns, $P \geq 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. ARPC1B: Actin-related protein 2/3 complex subunit 1B; TCGA: the cancer genome atlas; GTEx: genotype-tissue expression; ACC: adrenocortical carcinoma; BLCA: bladder urothelial carcinoma; BRCA: breast invasive carcinoma; CESC: cervical squamous cell carcinoma; CHOL: cholangiocarcinoma; COAD: colon adenocarcinoma; DLBC: lymphoid neoplasm diffuses large B cell lymphoma; ESCA: esophageal carcinoma; GBM: glioblastoma multiforme; HNSC: head and neck squamous cell carcinoma; KICH: kidney chromophobe; KIRC: kidney renal clear cell carcinoma; KIRP: kidney renal papillary cell carcinoma; LAML: acute myeloid leukemia; LGG: brain lower grade glioma; LIHC: liver hepatocellular carcinoma; LUAD: lung adenocarcinoma; LUSC: lung squamous cell carcinoma; MESO: mesothelioma; OV: ovarian serous cystadenocarcinoma; PAAD: pancreatic adenocarcinoma; PCPG: pheochromocytoma and paraganglioma; PRAD: prostate adenocarcinoma; READ: rectum adenocarcinoma; SARC: sarcoma; SKCM: skin cutaneous melanoma; STAD: stomach adenocarcinoma; TGCT: testicular germ cell tumors; THCA: thyroid carcinoma; THYM: thymoma; UCEC: uterine corpus endometrial carcinoma; UCS: uterine carcinosarcoma; UVM: uveal melanoma; TCGA: the cancer genome atlas; GTEx: genotype-tissue expression; CCLE: cancer cell line encyclopedia; CLL: chronic lymphoblastic leukemia; ALL: acute lymphoblastic leukemia; LCML: chronic myeloid leukemia; MM: multiple myeloma; SCLC: small cell lung cancer; NB: neuroblastoma; MB: medulloblastoma; HPA: human protein atlas.**

various types of tumors. Consequently, we delved deeper into how ARPC1B interacts with immune cells within different tumor environments. Utilizing the ImmuCellAI database, we discovered that at the pan-cancer level, the expression of ARPC1B exhibits a notable correlation with various immune cell infiltrations, particularly in

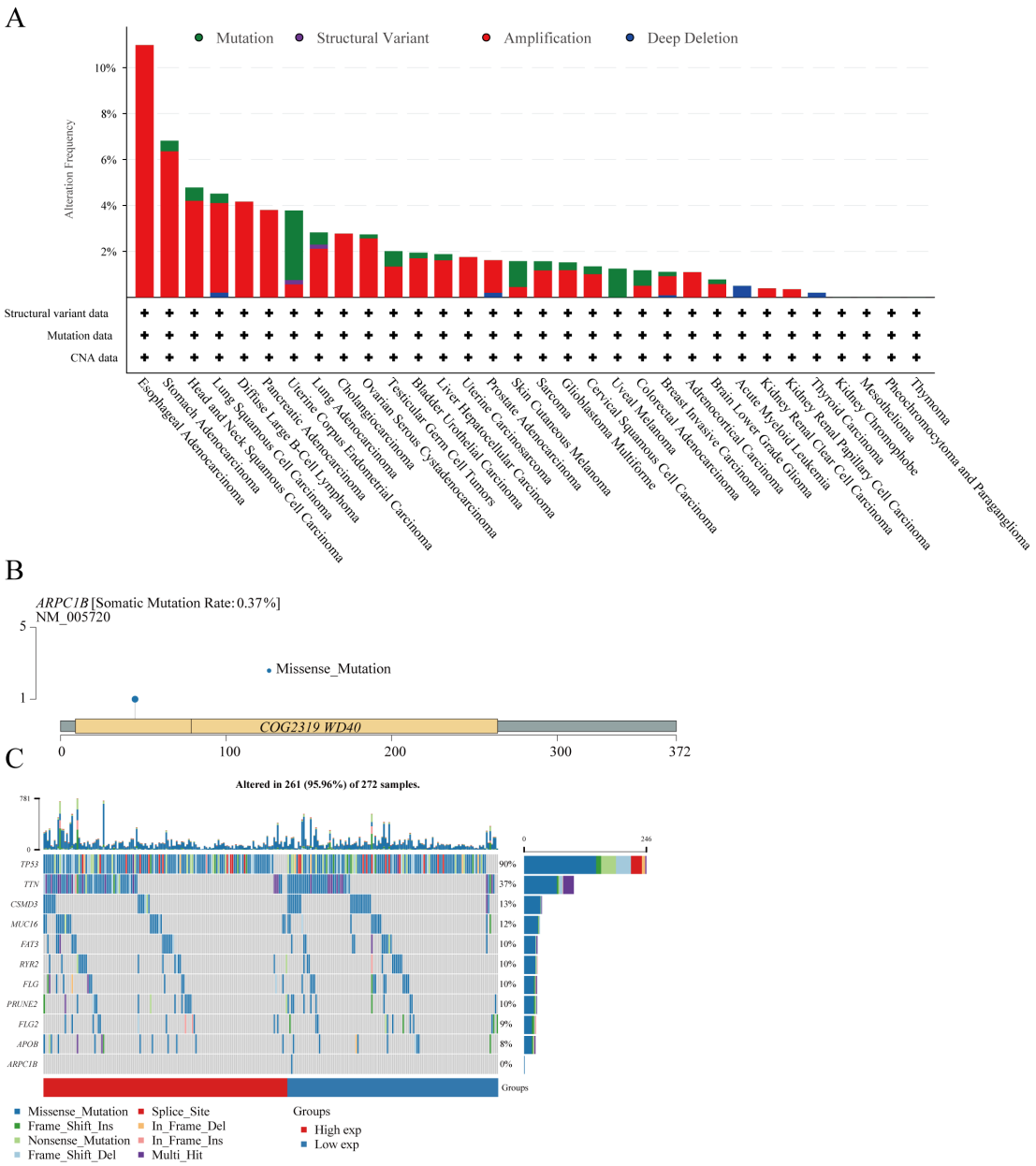


**Figure 2: Genomic Alterations and Mutation Profile in Tumors. (A) ARPC1B amplification and its influence on various cancers, highlighting key differences in genomic alterations. (B) Mutation rate of ARPC1B in OV tumors, represented in a lollipop plot. (C) Somatic mutation landscape in OV, with a focus on ARPC1B and other significant gene mutations. ARPC1B: Actin-related protein 2/3 complex subunit 1B; OV: ovarian serous cystadenocarcinoma.**

LGG and sarcoma (SARC), where it shows a high degree of positive correlation with infiltration scores, macrophages, and regulatory T (Treg) cell infiltrations. In most tumors, ARPC1B exhibits a negative correlation with neutrophil infiltration (Figure 4A). Moreover, examining the correlation of immune cell infiltration based on the TIMER2 database revealed that the expression level of ARPC1B exhibits a significant positive correlation with tumor-associated fibroblasts and macrophages in various tumors (Figure 4B).

Furthermore, our observations reveal a robust correlation between ARPC1B and immune suppressor genes, including *LGALS9*, *TGFB1*, and *CSF1R*, across various tumor types (Supplementary Figure 5A). Moreover, from a pan-cancer perspective, ARPC1B expression consistently displays a positive correlation with the expression of *HLA-E*, as well as the expression levels of chemokines such as *CXCL16*, *CCL5*, *CCL3*, and chemokine receptors like *CCR1*, *CCR5*, and *CXCR3* (Supplementary Figure 5B-D). These collective findings suggest that individuals with elevated ARPC1B gene expression may exhibit an immune-suppressed state.

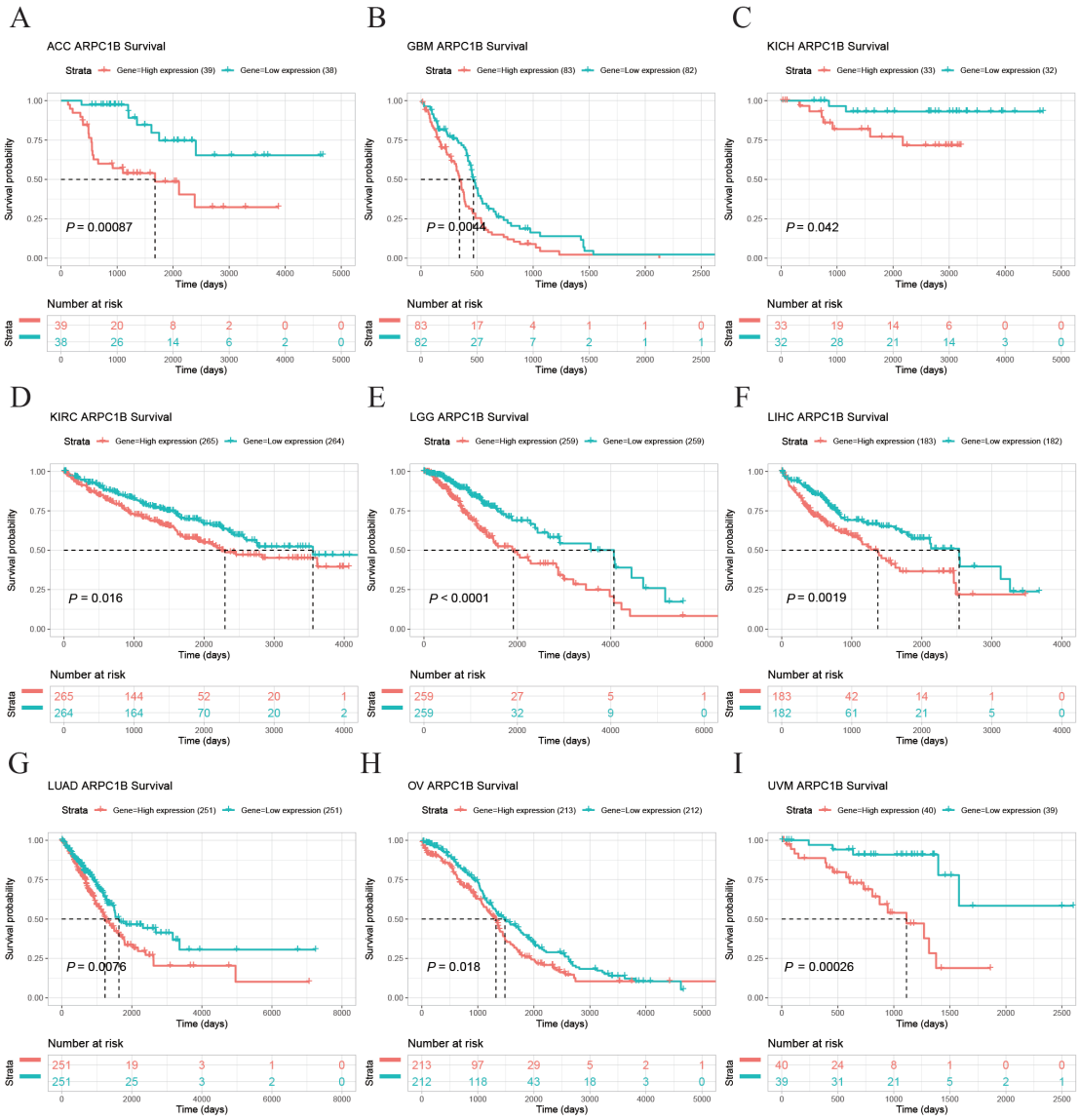


**Figure 3: Analysis of the correlation between ARPC1B expression and OS rates in patients with nine different types of tumors. (A) ACC. (B) GBM. (C) KICH. (D) KIRC. (E) LGG. (F) LIHC. (G) LUAD. (H) OV. (I) UVM. ARPC1B: Actin-related protein 2/3 complex subunit 1B; ACC: adrenocortical carcinoma; GBM: glioblastoma multiforme; KICH: kidney chromophobe; KIRC: kidney renal clear cell carcinoma; LGG: brain lower grade glioma; LIHC: liver hepatocellular carcinoma; LUAD: lung adenocarcinoma; OV: ovarian serous cystadenocarcinoma; UVM: uveal melanoma; OS: overall survival.**

In our study, we conducted an analysis of the association between ARPC1B and immune checkpoint genes in OV. Our findings indicate a positive correlation between ARPC1B and multiple immune checkpoint genes (Supplementary Figure 5E). This observation suggests that ARPC1B may have a modulatory effect on the immune response, potentially contributing to the promotion of cancer. These discoveries underscore the potential significance of ARPC1B in the regulation of immune responses, which could have implications for tumor development and the immune status of patients. As a result, these findings offer valuable insights for future investigations into the role of ARPC1B in tumor immunology.

## *ARPC1B and TME analysis*

The TME, comprising stromal cells, tumor cells, and immune cells, has been widely recognized in the academic community.[36] Its significance lies in its role in the advancement and control of cancer, exerting substantial influence on tumor proliferation, metastasis, resistance to therapeutic drugs, and treatment effectiveness. We investigated the relationship between ARPC1B and Stromalcore, ImmuneScore, ESTIMATEScore, and tumor purity in various tumors (Figure 5A). Notably, the results indicate that in various tumors, the expression of ARPC1B exhibits a significant positive correlation with Stromalcore, ImmuneScore, and ESTIMATEScore, while showing a

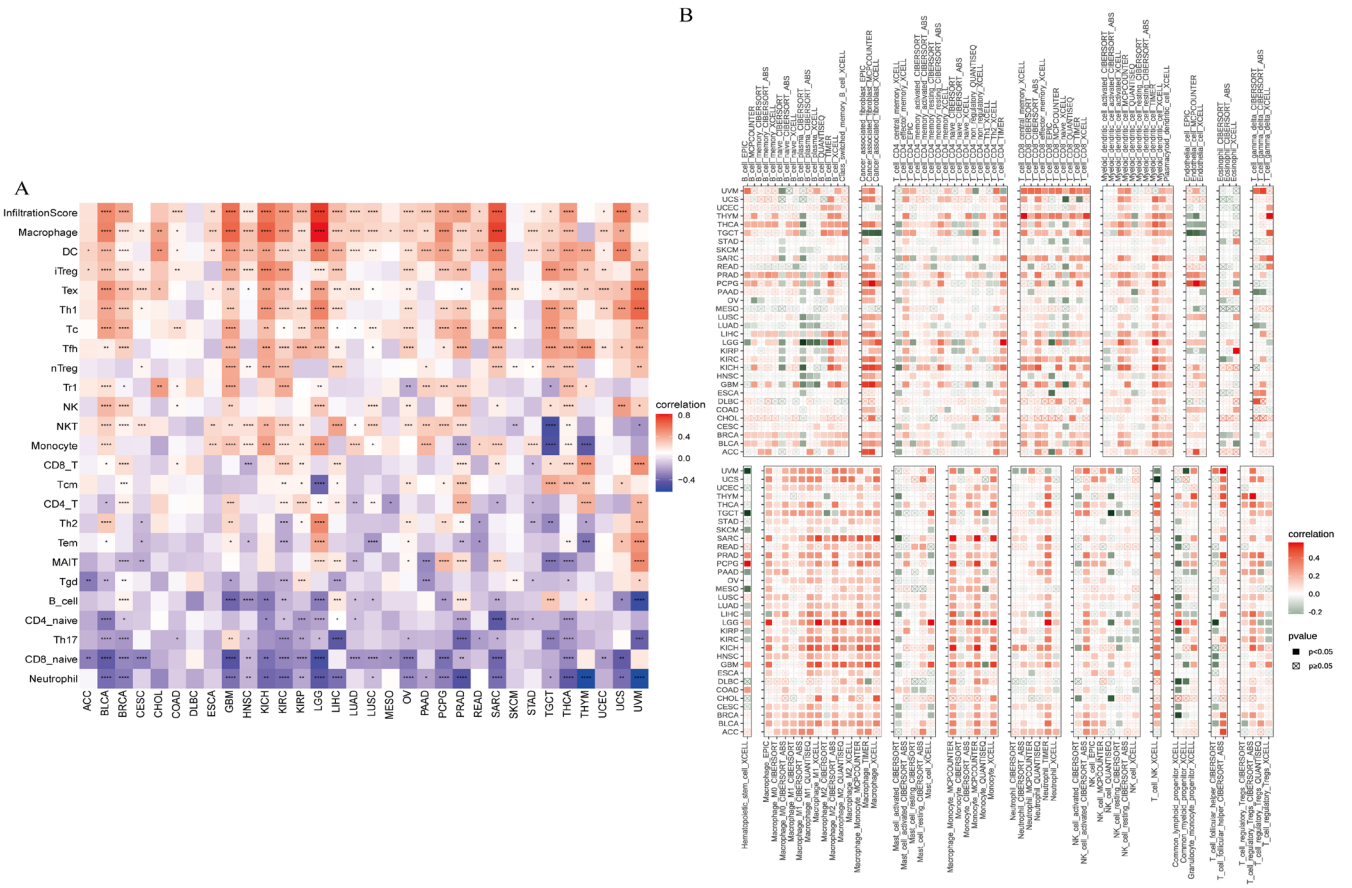


**Figure 4: Correlation between ARPC1B and immune cell infiltration. (A) Results of immune infiltration analysis based on ImmuCellAI database. (B) Results of immune infiltration analysis based on TIMER2 database. Significance markers: ns, $P \geq 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. ARPC1B: Actin-related protein 2/3 complex subunit 1B; ACC: adrenocortical carcinoma; BLCA: bladder urothelial carcinoma; BRCA: breast invasive carcinoma; CESC: cervical squamous cell carcinoma; CHOL: cholangiocarcinoma; COAD: colon adenocarcinoma; DLBC: lymphoid neoplasm diffuses large B cell lymphoma; ESCA: esophageal carcinoma; GBM: glioblastoma multiforme; HNSC: head and neck squamous cell carcinoma; KICH: kidney chromophobe; KIRC: kidney renal clear cell carcinoma; KIRP: kidney renal papillary cell carcinoma; LAML: acute myeloid leukemia; LGG: brain lower grade glioma; LIHC: liver hepatocellular carcinoma; LUAD: lung adenocarcinoma; LUSC: lung squamous cell carcinoma; MESO: mesothelioma; OV: ovarian serous cystadenocarcinoma; PAAD: pancreatic adenocarcinoma; PCPG: pheochromocytoma and paraganglioma; PRAD: prostate adenocarcinoma; READ: rectum adenocarcinoma; SARC: sarcoma; SKCM: skin cutaneous melanoma; STAD: stomach adenocarcinoma; TGCT: testicular germ cell tumors; THCA: thyroid carcinoma; THYM: thymoma; UCEC: uterine corpus endometrial carcinoma; UCS: uterine carcinosarcoma; UVM: uveal melanoma; TIMER: immune estimation resource.**

negative correlation with tumor purity.

The heatmap illustrates the association between the expression of ARPC1B and various factors related to the TME. In most cancer cases, a strong positive correlation is observed between ARPC1B expression levels and terms related to antigen processing machinery, DNA replication, base excision repair, nucleotide excision repair, mismatch repair, and antigen processing machinery (Figure 5B). Furthermore, through additional analysis, we identified significant differences in processes such as $CD8^{+}$ T effector, immune checkpoint, antigen presentation process, pan-fibroblast TGFb reaction signature (Pan-F-TBRS), and epithelial-interstitial transformation between the ARPC1B high expression group and low expression group in OV (Figure 5C).

## *Functional enrichment analysis of ARPC1B*

Additionally, we conducted a correlation analysis of the ARPC1B gene in OV. We used heatmaps to visually represent the top 50 genes exhibiting the most significant positive and negative correlations individually (Supplementary Figure 6A-B). Furthermore, a GSEA was performed on all the genes found to be correlated. The results of GSEA-GO, GSEA-KEGG, and GSEA-Reactome analyses unveiled a strong association between the expression of ARPC1B and various pathways and biological processes in OV, including innate immune response, oxidative phosphorylation, and centrifugal degranulation (Figure 6A-C).

The enrichment scores of various hallmark pathways were analyzed using GSVA, and the corresponding ARPC1B expression levels were visualized as a heatmap sourced

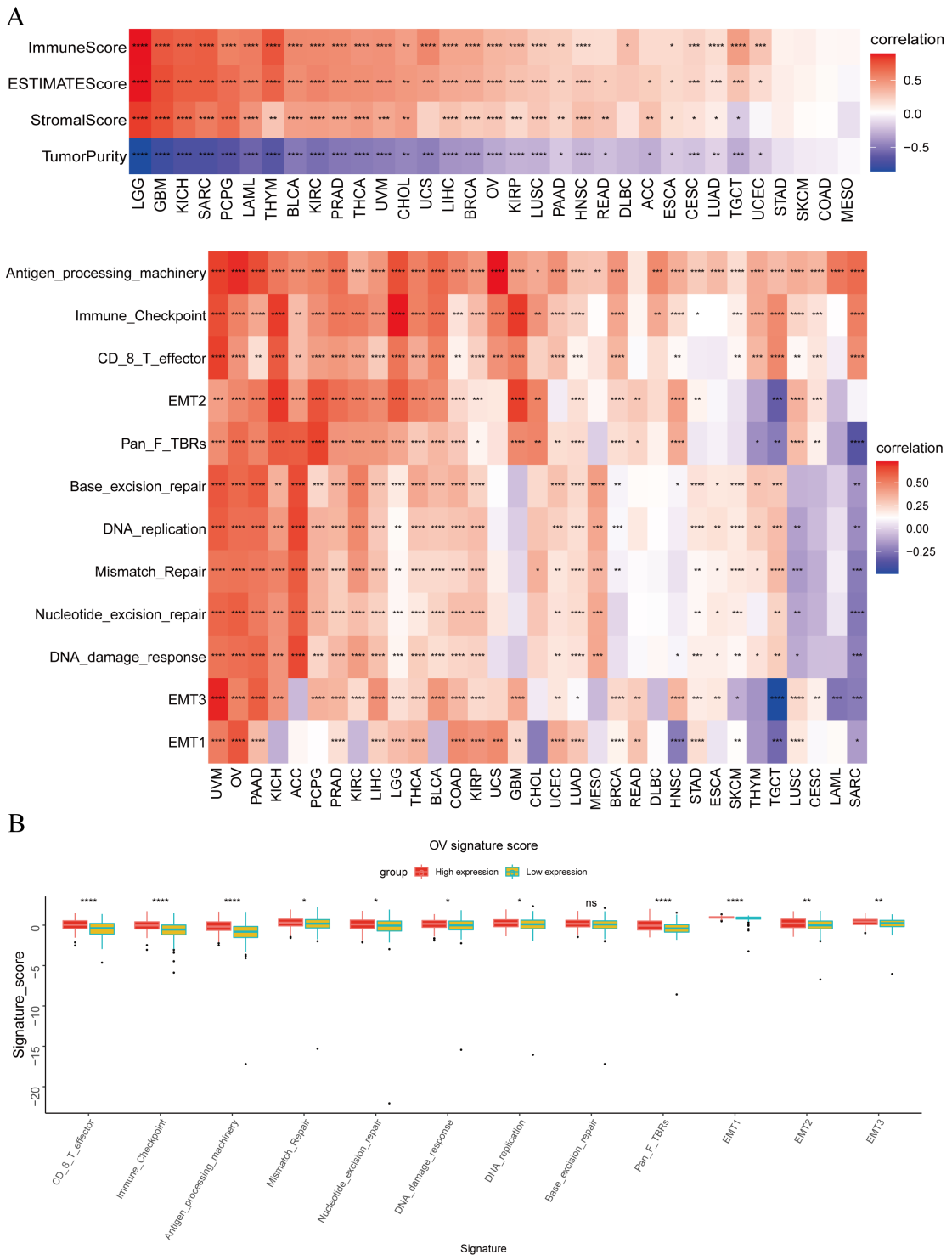


**Figure 5. The correlation between ARPC1B and tumor microenvironment. (A) The relationship between ARPC1B and Stromalcore, ImmuneScore, ESTIMATEScore, tumor purity, and TME-relatedin pan-cancer. (B) The difference between the ARPC1B high and low expression group and each signature score in OV. Significance markers: ns, $P \geq 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. ARPC1B: Actin-related protein 2/3 complex subunit 1B; ACC: adrenocortical carcinoma; BLCA: bladder urothelial carcinoma; BRCA: breast invasive carcinoma; CESC: cervical squamous cell carcinoma; CHOL: cholangiocarcinoma; COAD: colon adenocarcinoma; DLBC: lymphoid neoplasm diffuses large B cell lymphoma; ESCA: esophageal carcinoma; GBM: glioblastoma multiforme; HNSC: head and neck squamous cell carcinoma; KICH: kidney chromophobe; KIRC: kidney renal clear cell carcinoma; KIRP: kidney renal papillary cell carcinoma; LAML: acute myeloid leukemia; LGG: brain lower grade glioma; LIHC: liver hepatocellular carcinoma; LUAD: lung adenocarcinoma; LUSC: lung squamous cell carcinoma; MESO: mesothelioma; OV: ovarian serous cystadenocarcinoma; PAAD: pancreatic adenocarcinoma; PCPG: pheochromocytoma and paraganglioma; PRAD: prostate adenocarcinoma; READ: rectum adenocarcinoma; SARC: sarcoma; SKCM: skin cutaneous melanoma; STAD: stomach adenocarcinoma; TGCT: testicular germ cell tumors; THCA: thyroid carcinoma; THYM: thymoma; UCEC: uterine corpus endometrial carcinoma; UCS: uterine carcinosarcoma; UVM: uveal melanoma**

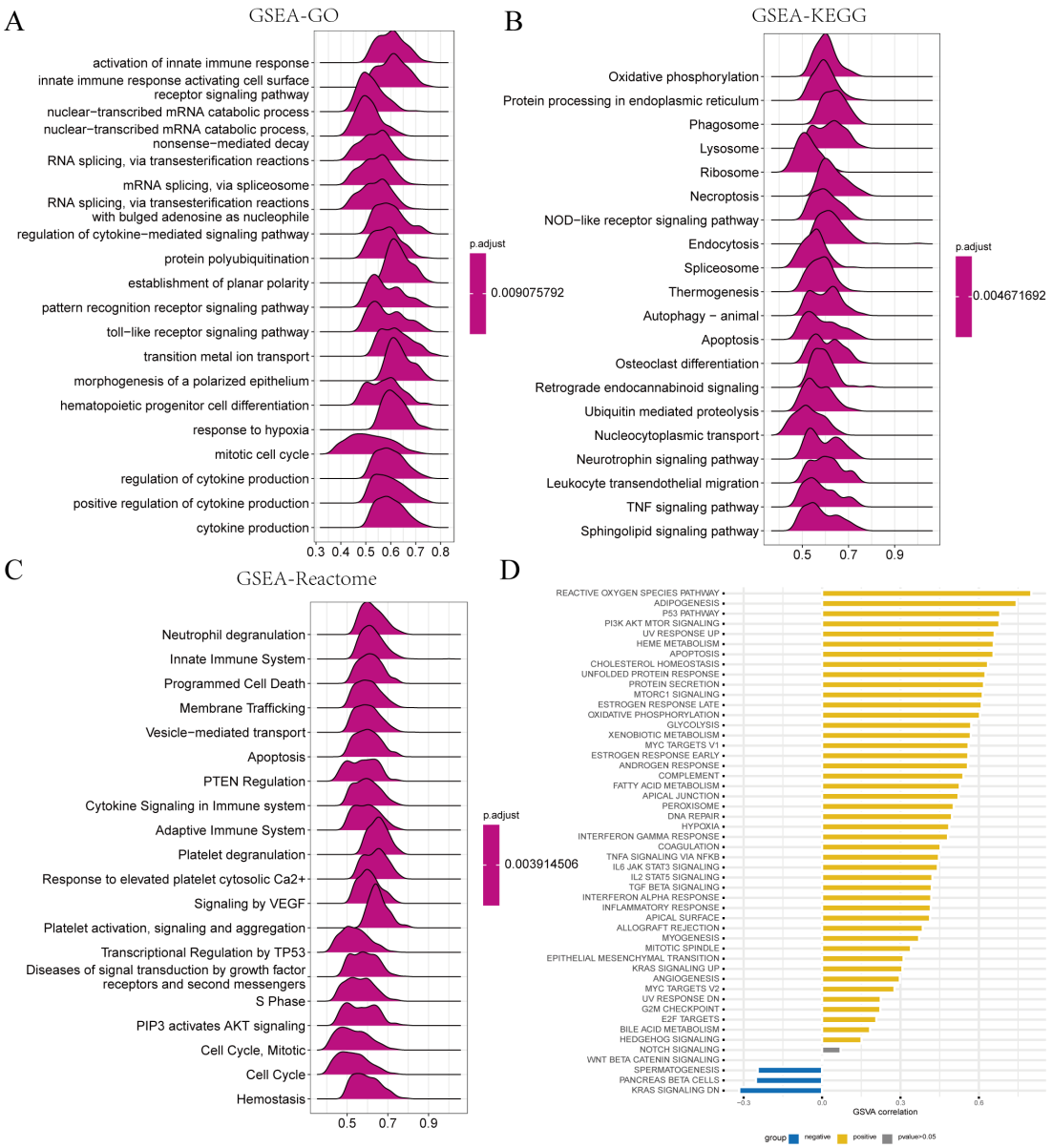


**Figure 6. Functional enrichment analysis of ARPC1B. (A) The result of functional enrichment of GSEA-GO. (B) The result of functional enrichment of GSEA-KEGG. (C) The result of functional enrichment of GSEA-Reactome. (D) Correlation between ARPC1B in OV and 50 hallmark star pathways. ARPC1B: Actin-related protein 2/3 complex subunit 1B; OV: ovarian serous cystadenocarcinoma; GSEA: gene set enrichment analysis.**

from the MsigDB database. In OV, we observed that the expression of ARPC1B was significantly positively correlated with 45 pathways, including the reactive oxygen species pathway (ROS pathway), adipogenesis, P53 pathway, and PI3K AKT MTOR signaling, while it was significantly negatively correlated with pathways related to spermatogenesis, pancreas beta cells, and KRAS DN signaling (Figure 6D).

## *The suppression of cell proliferation, migration, and enhancement of apoptosis in OV is achieved through the inhibition of ARPC1B*

From the above results, we found that high expression of ARPC1B is associated with a poor prognosis. This suggests that ARPC1B may be a potential target for the treatment of OV. our research has confirmed the potential involvement of ARPC1B in the progression of OV. To further investigate this issue, we first analyzed the expression of ARPC1B in different cell lines using the Cancer Cell Line Encyclopedia (CCLE) database (Figure 7A). Additionally, we validated the results in various tumor cell lines through Western blot. The findings revealed that, compared to normal ovarian cells, A2780 and SKOV3 exhibited the highest expression of ARPC1B (Figure 7B). Subsequently, we conducted shRNA-mediated silencing experiments on A2780 and SKOV3 cells. The effectiveness

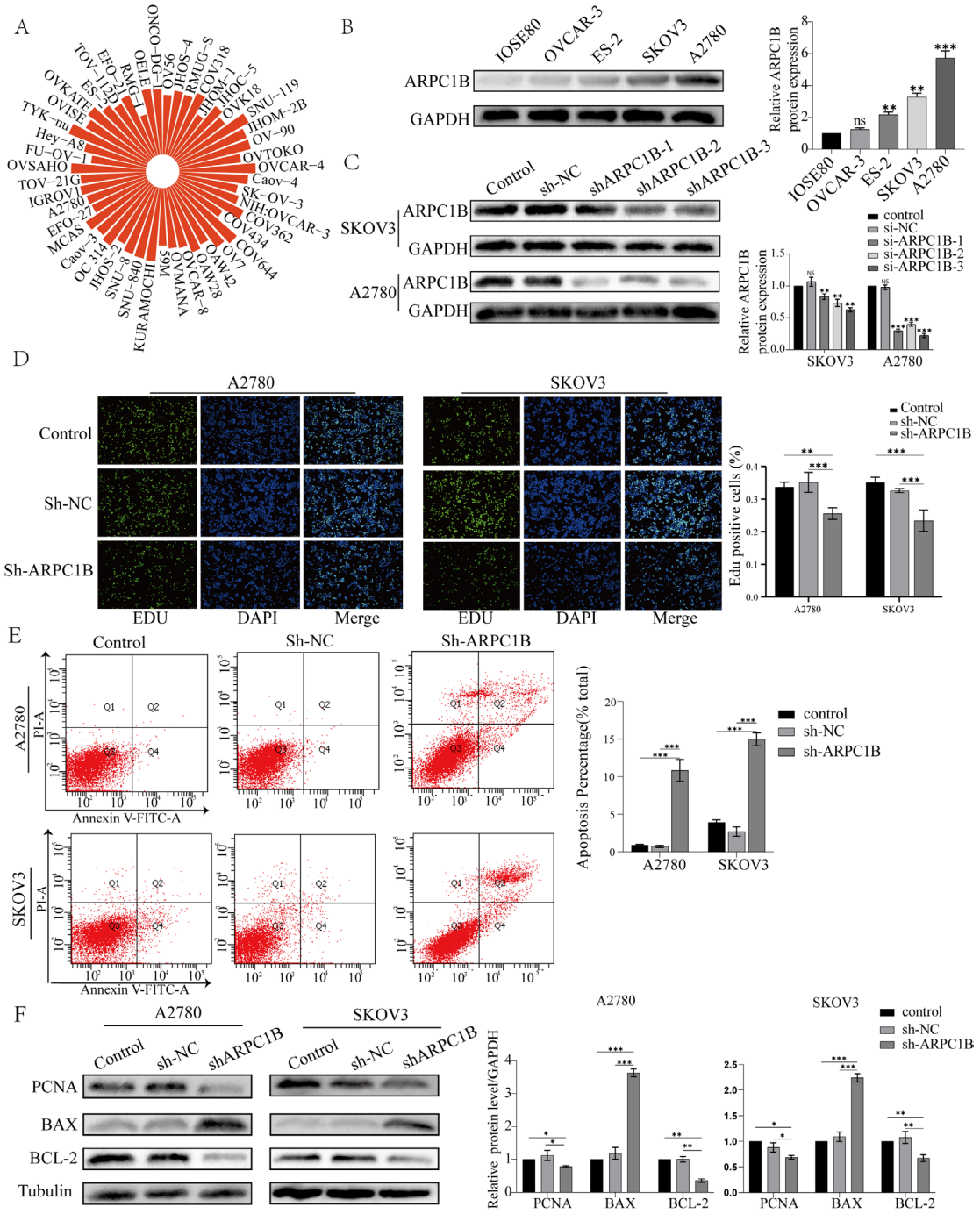


**Figure 7: ARPC1B promotes OV cell proliferation and migration and inhibits apoptosis. (A) The expression distribution of mRNA in different cell lines. (B) Expression of ARPC1B in different cell lines was analyzed through WB. (C) The transfection efficiency of sh-ARPC1B in A2780 and SKOV3 cells was evaluated. (D) The effect of c on cancer cell proliferation was assessed using the EdU assay. (E) The apoptosis assay showed that apoptosis of OV cells increased significantly after ARPC1B knockdown. (F) The impact of ARPC1B on cell proliferation and apoptosis biomarkers. scale bar: 200 $\mu$m. Significance markers: ns, $P \geq 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. ARPC1B: Actin-related protein 2/3 complex subunit 1B; OV: ovarian cancer; WB: Western blot.**

of ARPC1B-shRNA was confirmed through Western blot analysis (Figure 7C).

Following this, we measured cell viability using EdU experiments. Our research results indicated that inhibiting ARPC1B significantly reduced the proliferation of OV cells (Figure 7D). To further study the regulatory role of the ARPC1B gene in OV cells, we analyzed apoptotic cells using flow cytometry. Our apoptosis experiment results showed that knocking down ARPC1B expression significantly increased the apoptosis rate of A2780 and SKOV3 cells (Figure 7E). Furthermore, through the

examination of BCL2, BAX, and PCNA, it was observed that sh-ARPC1B significantly increased cell apoptosis and inhibited cell proliferation (Figure 7F).

Moreover, wound healing experiments provided further evidence, demonstrating that the reduction of ARPC1B decreased the migration ability of A2780 and SKOV3 cells (Figure 8A). Additionally, transwell experiments demonstrated that after ARPC1B knockdown, the migration and invasion abilities of A2780 and SKOV3 cells significantly decreased (Figure 8B). Furthermore, through the aforementioned GSEA analysis, we observed the enrichment of ARPC1B in the PI3K/AKT pathway. We found that silencing ARPC1B significantly reduced the protein levels of p-AKT, p-PI3K, and p-mTOR (Figure 8C). These results indicate that ARPC1B positively regulates the PI3K/AKT/mTOR pathway in OV cells.

## DISCUSSION

The Arp2/3 complex, as a key player in actin nucleation, has been extensively studied in the context of cancer.[43,44] However, the role of ARPC1B, one of its crucial constituent subunits, in tumorigenesis remains poorly understood. Currently, most research on ARPC1B has primarily focused on blood-related and immune-related disorders.[45,46] Nevertheless, recent studies have begun to shed light on its potential involvement in cancer. For instance, research has indicated that estrogen receptor Beta can inhibit pseudopodia formation through the mTOR-Arpc1b/EVL signaling pathway, thereby preventing the progression of signet ring cell gastric cancer in young patients.[47] Furthermore, Liu *et al.* found that ARPC1B might play a role in UVM's glucose metabolism and immune response, making it a potential clinical prognostic marker.[48] In a randomized controlled trial conducted by Zhou *et al.* in 2017, low-frequency non-synonymous variants of the ARPC1B gene were shown to potentially increase the risk of breast cancer in Chinese women.[49] Notably, a study by Nicolas Molinie *et al.* suggested that the branched actin structures generated by the Arp2/3 complex are necessary for the G1-S transition in the cell cycle. When an adequate number of ARPC1B-containing branches form in the cell cortex, it triggers the cell to enter the S phase. Additionally, in breast cancer tissues, the Arp2/3 complex containing ARPC1B exhibits higher expression levels compared to normal tissues, suggesting its pivotal role in breast cancer progression.[50] Building on this, previous studies have also established that ARPC1B interacts with PAK1, a crucial player in tumor-related signal transduction pathways.[51,52] Collectively, these research findings underscore the significant, yet incompletely elucidated, role of ARPC1B in cancer.

In this study, based on the analysis of data from 33 different tumor and normal tissue samples obtained from the TCGA and GTEx databases, we observed that ARPC1B is highly expressed across various cancers, indicating its potential as a tumor-promoting gene. Further investigation into the characteristics of these tumors revealed that ARPC1B can serve as a marker for clinical staging and prognosis in a wide range of cancer types. It represents a risk factor in 19 different tumors, including OV, and may emerge as a common target for clinical treatment across multiple cancer types in the future. Additionally, we noted that the alterations in the ARPC1B gene vary among different tumors. For instance, in UVM, ARPC1B primarily undergoes genomic mutations, while in ESCA, DLBC, and other cancers, changes in gene copy number are more prevalent. Furthermore, we observed a clear positive correlation between methylation modifications of ARPC1B and various tumors, suggesting that the epigenetics of this gene may also hold promise as a potential target for future cancer treatments.

The results of the gene function enrichment analysis further underscore the role of ARPC1B in tumorigenesis and cancer development. We observed a significant association between ARPC1B and various signaling pathways, with a particular emphasis on immune responses. Immune responses play a pivotal role in numerous types of cancer. In a healthy context, the immune system is tasked with recognizing and eliminating tumor cells within the TME. However, tumor cells often employ mechanisms to evade immune surveillance and evade removal. Our research revealed that ARPC1B gene expression has the highest correlation with ImmuneScore within the TME, highlighting its strong association with immune cell infiltration in TME. Subsequent analysis of tumor immune infiltration disclosed a noteworthy relationship between ARPC1B expression and various immune cell types across different cancers, particularly in LGG and SARC. ARPC1B expression exhibited significant correlations with Treg cells, macrophages, neutrophils, and tumor-associated fibroblasts in these TMEs.

Both Treg cells and T helper 17 cells (Th17 cells) originate from the differentiation of $CD4^+$ T cells. Past research has indicated that the TME influences the balance of differentiation between these two cell types.[53] Among these, Treg cells are a type of classic immune cell that can be involved in the initiation and progression of tumors by influencing tumor immunity and facilitating immune evasion.[54] In addition, we also observed that the enrichment analysis results indicate a strong correlation between the expression of ARPC1B in OV and the activation of the PI3K-AKT-mTOR signaling pathway. This finding aligns with the results reported by Salmond *et al.*,

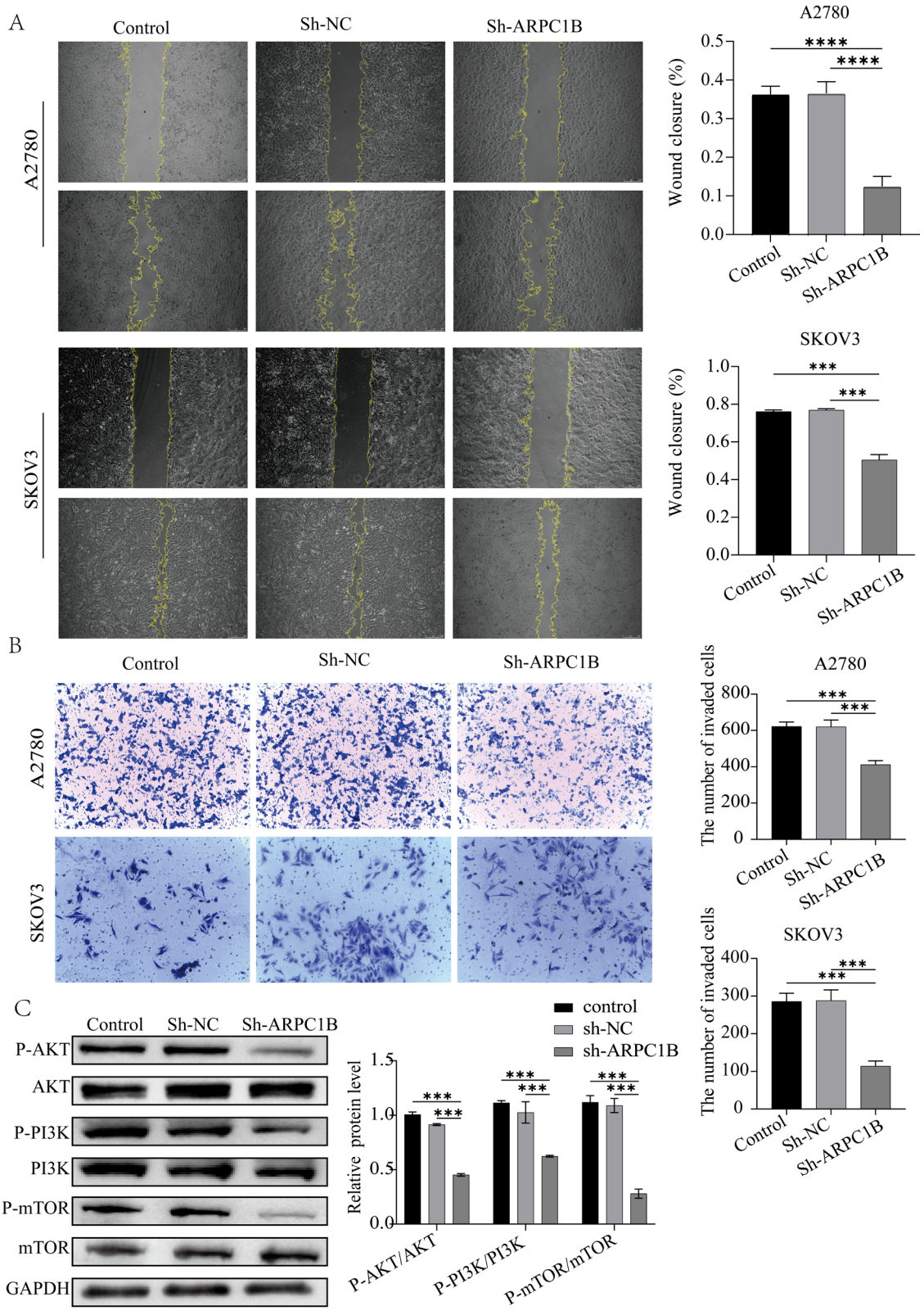


**Figure 8: ARPC1B promotes OV cell migration and invasion. (A) The wound healing assay revealed that ARPC1B enhanced the migratory activity of OV cells. (B) The Transwell assay demonstrated that ARPC1B enhanced the migration and invasion capabilities of OV cells. (C) Western blot analysis was performed to assess the protein expression levels of PI3K, p-PI3K, AKT, p-AKT, mTOR, and p-mTOR in stable ARPC1B knockdown OV cells. GAPDH was used as a loading control. scale bar: 200 μm. Significance markers: ns, $P \geq 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. ARPC1B: Actin-related protein 2/3 complex subunit 1B; OV: ovarian cancer.**

suggesting that ARPC1B might influence $CD4^+$ T cells by modulating energy metabolism, thereby reshaping the TME and contributing to tumorigenesis.[55] On the other hand, macrophages, another type of infiltrating cells, play a pivotal role in orchestrating chronic inflammation and associated pathologies.[56] Previous studies have suggested that they have contradictory two-way effects on the occurrence and development of tumors.[57,58] Weiss *et al.* pointed out that tumor cells induce macrophages to produce itaconic acid, which changes the PH environment of TME and promotes tumor growth.[59] In addition, macrophages can secrete an immunosuppressive medium that blocks immune checkpoints of T cells and NK cells (including PD-L1 and VISTA), which helps tumor cells escape the immune system's surveillance.[60,61] This conclusion is consistent with the conclusion that we observed in OV that ARPC1B is highly positively correlated with the expression of multiple immune checkpoint genes. Cancer-associated fibroblasts (CAF) are currently considered to be the main source of immunosuppressive activity in TME.[62] Our ARPC1B gene enrichment study at the pan-cancer level found that a variety of tumors were highly positively correlated with the IL6-JAK-STAT3 signaling pathway. The pathway has been shown to play a key role in the activation of tumor-associated macrophage and Treg cell immunosuppressive genes driven by CAF.[63,64]

Next, we found in the gene correlation analysis at the pan-cancer level that the expression of ARPC1B had positively correlated with the expression of immunosuppressive genes such as *LGALS9*, *TGFB1*, and *CSF1R* in a variety of tumors. In the analysis of the correlation with the expression of MHC family genes, we found that ARPC1B is positively correlated with the expression of *HLA-E* genes in almost all tumors (32 types), and the latest research points out that *HLA-E* is a highly conservative MHC-I molecule which played an important role in suppressing immune clearance mediated by NK and $CD8^+$ T cells, which may become a new target for immune checkpoint blocking therapy.[65,66] Similarly, we also found that the expression level of ARPC1B is generally positively correlated with the expression levels of chemokines *CXCL16*, *CCL5*, *CCL3*, and chemokine receptors *CCR1*, and *CCR5*. *CCL5* and *CCL3* can act on *CCR5* receptors in common, to play a role in promoting blood vessels and lymphangiogenesis in tumors,[67] and they can both play a role in recruiting Tregs and macrophages in tumors,[68,69] and by affecting CAF or triggering cell signaling pathways such as PI3K /AKT eventually lead to changes in TME and promote tumor progression.[70,71] In addition, studies have pointed out that CXCL16 and CCR1 can also bind to their response ligands/receptors to exert similar tumor-promoting effects.[72,73] However, it is worth noting that some studies have pointed out the anti-cancer effects of chemokines,[74,75] how to control the chemokine ligand receptors, and the mode of action of immune infiltrating cells in tumors, is a problem worth thinking about.

In addition, our research explored the relationship between the ARPC1B gene and tumor drug resistance. It is worth mentioning that in existing studies, some chemokines were thought to cause tumor resistance, such as CCL3-mediated multiple myeloma and acute lymphoblastic leukemia.[76,77] Similarly, CCL5 activates Akt/PKB→NF-κB or STAT3 pathways to increase apoptosis resistance and drug resistance, *etc.*[78-80] This evidence may provide new ideas for solving tumor drug resistance, which is worthy of further exploration.

Notably, in these bioinformatics-based analyses above we have focused on exploring alterations in TME in OV. Because of the nature of Bulk mRNA-Seq, it is possible that this sequencing information also contains transcriptomic information of TME cells, and therefore we have also included in the discussion the exploration of the impact of the AKT/PI3K/mTOR pathway on TME. In further experimental validation, we found that ARPC1B could promote the proliferation and invasion of OV cells and inhibit their apoptosis through the AKT/PI3K/mTOR pathway. AKT/PI3K/mTOR has been involved as a therapeutic target in more and more studies nowadays in the development of clinical treatments for tumors due to its important role in cellular activity, and many drugs can affect the course of OV by influencing the activation of this pathway, such as glucocorticoids,[81] endosomal nano curcumin and oxaliplatin,[82] but our study seems to be the first to elucidate this endogenous mechanism of action of ARPC1B-AKT/PI3K/mTOR. In addition, combined with what has been explored in the bioinformatics section, it is reasonable to believe that ARPC1B's ability to extensively regulate the AKT/PI3K/mTOR pathway in tumor cells, as well as in the TME, is an important rationale for it to become a potent tumor therapeutic target in the future.

However, this study also has these limitations and deficiencies. First, although bioinformatics analysis provided us with information about the role of ARPC1B in different tumors, *in vivo*, and *in vitro* experiments are still needed to prove our findings. Similarly, our research pointed out the relevance of this gene to some biological processes, but the specific rules of action between them need to be further explored. Secondly, the number of samples contained in the databases used in this article was limited, which may cause bias in the analysis results. Finally, although our research pointed out that ARPC1B was associated with the prognosis and clinical stages of patients with pan-cancer, the existing analysis results are not sufficient to prove whether the high correlation between

ARPC1B, TME, and tumor immunity is the cause of these clinical features.

## CONCLUSIONS

Overall, our research revealed the mechanism of the ARPC1B gene at the pan-cancer level and deeply analyzed the cross-linking effect of this gene with TME and tumor immune system in a variety of tumors including OV. At the same time, it also can predict the prognosis, clinical staging, and tumor drug resistance which indicates its potential clinical significance. Here, we are hoping to broaden the understanding of the role of the Arp2/3 complex in tumors, which can provide new ideas for the treatment of various tumors.

## Acknowledgments

The authors of the TCGA and GTEx public datasets are highly appreciated.

## Author Contributions

Resources, Yu Lin; Data curation, Ying Zhang; Cell experiments, Miao Ke and Huimin Zhu; Writing and original draft preparation, Miao Ke; Writing, review and editing, Huimin Zhu, Yu Lin, Tao Tang, Yuhao Xia and Zhe-Sheng Chen; Visualization, Yuan Shen; Project administration, Xiaoyu Wang, Yuan Shen and Zhe-Sheng Chen. All authors have read and agreed to the published version of the manuscript.

## Informed Consent

Not applicable.

## Ethical Approval

Not applicable.

## Source of Funding

This research was supported by Science and Technology Projects in Guangzhou (202201020044) and the Medical Joint Fund of Jinan University (NO. 34422052).

## Conflicts of Interest

The authors declare no conflict of interest.

## Data Availability Statement

The original tumor data were downloaded from the TCGA and GTEx databases.

## REFERENCES

1. Fletcher DA, Mullins RD. Cell mechanics and the cytoskeleton. Nature 2010;463:485-492.
2. Wagh K, Wheatley BA, Traver MK, Hussain I, Schaefer BC, Upadhyaya A. Bcl10 is associated with actin dynamics at the T cell immune synapse. Cell Immunol 2020;356:104161.
3. Papa R, Penco F, Volpi S, Gattorno M. Actin remodeling defects leading to autoinflammation and immune dysregulation. Front Immunol 2020;11:604206.
4. Chakrabarti R, Lee M, Higgs HN. Multiple roles for actin in secretory and endocytic pathways. Curr Biol 2021;31:R603-618.
5. Schaks M, Giannone G, Rottner K. Actin dynamics in cell migration. Essays Biochem 2019;63:483-495.
6. Welch MD, DePace AH, Verma S, Iwamatsu A, Mitchison TJ. The human Arp2/3 complex is composed of evolutionarily conserved subunits and is localized to cellular regions of dynamic actin filament assembly. J Cell Biol 1997;138:375-384.
7. Tyler JJ, Allwood EG, Ayscough KR. WASP family proteins, more than Arp2/3 activators. Biochem Soc Trans 2016;44:1339-1345.
8. Abella JV, Galloni C, Pernier J, Barry DJ, Kjær S, Carlier MF, *et al*. Isoform diversity in the Arp2/3 complex determines actin filament dynamics. Nat Cell Biol 2016;18:76-86.
9. Zhang L, Zhang Y, Lei Y, Wei Z, Li Y, Wang Y, *et al*. Proline-rich 11 (PRR11) drives F-actin assembly by recruiting the actin-related protein 2/3 complex in human non-small cell lung carcinoma. J Biol Chem 2020;295:5335-5349.
10. Li Y, Qiu J, Pang T, Guo Z, Su Y, Zeng Q, *et al*. Restoration of Arpin suppresses aggressive phenotype of breast cancer cells. Biomed Pharmacother 2017;92:116-121.
11. Zhou T, Wang CH, Yan H, Zhang R, Zhao JB, Qian CF, *et al*. Inhibition of the Rac1-WAVE2-Arp2/3 signaling pathway promotes radiosensitivity via downregulation of cofilin-1 in U251 human glioma cells. Mol Med Rep 2016;13:4414-4420.
12. Kaneda A, Kaminishi M, Sugimura T, Ushijima T. Decreased expression of the seven ARP2/3 complex genes in human gastric cancers. Cancer Lett 2004;212:203-210.
13. Yan P, Liu J, Zhou R, Lin C, Wu K, Yang S, *et al*. LASP1 interacts with N-WASP to activate the Arp2/3 complex and facilitate colorectal cancer metastasis by increasing tumour budding and worsening the pattern of invasion. Oncogene 2020;39:5743-5755.
14. Wesolowska N, Avilov I, Machado P, Geiss C, Kondo H, Mori M, *et al*. Actin assembly ruptures the nuclear envelope by prying the lamina away from nuclear pores and nuclear membranes in starfish oocytes. Elife 2020;9:e49774.
15. Molinie N, Gautreau A. The Arp2/3 regulatory system and its deregulation in cancer. Physiol Rev 2018;98:215-238.
16. Romani P, Nirchio N, Arboit M, Barbieri V, Tosi A, Michielin F, *et al*. Mitochondrial fission links ECM mechanotransduction to metabolic redox homeostasis and metastatic chemotherapy resistance. Nat Cell Biol 2022;24:168-180.
17. Volpi S, Cicalese MP, Tuijnenburg P, Tool ATJ, Cuadrado E, Abu-Halaweh M, *et al*. A combined immunodeficiency with severe infections, inflammation, and allergy caused by ARPC1B deficiency. J Allergy Clin Immunol 2019;143:2296-2299.
18. Kahr WHA, Pluthero FG, Elkadri A, Warner N, Drobac M, Chen CH, *et al*. Loss of the Arp2/3 complex component ARPC1B causes platelet abnormalities and predisposes to inflammatory disease. Nat Commun 2017;8:14816.
19. Kang J, La Manna F, Bonollo F, Sampson N, Alberts IL, Mingels C, *et al*. Tumor microenvironment mechanisms and bone metastatic disease progression of prostate cancer. Cancer Lett 2022;530:156-169.
20. Hinshaw DC, Shevde LA. The tumor microenvironment innately modulates cancer progression. Cancer Res 2019;79:4557-4566.

21. Hui L, Chen Y. Tumor microenvironment: sanctuary of the devil. Cancer Lett 2015;368:7-13.
22. Li B, Chan HL, Chen P. Immune checkpoint inhibitors: basics and challenges. Curr Med Chem 2019;26:3009-3025.
23. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science 2015;348:69-74.
24. Vasan N, Baselga J, Hyman DM. A view on drug resistance in cancer. Nature 2019;575:299-309.
25. Barra F, Evangelisti G, Ferro Desideri L, di Domenico S, Ferraioli D, Vellone VG, *et al*. Investigational PI3K/AKT/mTOR inhibitors in development for endometrial cancer. Expert Opin Investig Drugs 2019;28:131-142.
26. Thomas S, Thurn KT, Raha P, Chen S, Munster PN. Efficacy of histone deacetylase and estrogen receptor inhibition in breast cancer cells due to concerted down regulation of Akt. PLoS One 2013;8:e68973.
27. Meeks JJ, Lerner SP. Molecular landscape of non-muscle invasive bladder cancer. Cancer Cell 2017;32:550-551.
28. Amer J, Salhab A, Jaradat N, Abdallah S, Aburas H, Hattab S, *et al*. Gundelia tournefortii inhibits hepatocellular carcinoma progression by lowering gene expression of the cell cycle and hepatocyte proliferation in immunodeficient mice. Biomed Pharmacother 2022;156:113885.
29. Chen Y, Chen C, Fang J, Su K, Yuan Q, Hou H, *et al*. Targeting the Akt/PI3K/mTOR signaling pathway for complete eradication of keloid disease by sunitinib. Apoptosis 2022;27:812-824.
30. Zhu Q, Yang H, Cheng P, Han Q. Bioinformatic analysis of the prognostic value of the lncRNAs encoding snoRNAs in hepatocellular carcinoma. Biofactors 2019;45:244-252.
31. Colaprico A, Silva TC, Olsen C, Garofano L, Cava C, Garolini D, *et al*. TCGAbiolinks: an R/Bioconductor package for integrative analysis of TCGA data. Nucleic Acids Res 2016;44:e71.
32. Consortium G. The Genotype-Tissue Expression (GTEx) project. Nat Genet 2013;45:580-585.
33. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, *et al*. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013;6:pl1.
34. Ferreira MR, Santos GA, Biagi CA, Silva Junior WA, Zambuzzi WF. GSVA score reveals molecular signatures from transcriptomes for biomaterials comparison. J Biomed Mater Res A 2021;109:1004-1014.
35. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, *et al*. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res 2017;77:e108-e110.
36. Zeng D, Li M, Zhou R, Zhang J, Sun H, Shi M, *et al*. Tumor microenvironment characterization in gastric cancer identifies prognostic and immunotherapeutically relevant gene signatures. Cancer Immunol Res 2019;7:737-750.
37. Miao YR, Zhang Q, Lei Q, Luo M, Xie GY, Wang H, *et al*. ImmuCellAI: a unique method for comprehensive T-cell subsets abundance prediction and its application in cancer immunotherapy. Adv Sci (Weinh) 2020;7:1902880.
38. Antonangeli F, Natalini A, Garassino MC, Sica A, Santoni A, di Rosa F. Regulation of PD-L1 expression by NF-κB in cancer. Front Immunol 2020;11:584626.
39. Burr ML, Sparbier CE, Chan KL, Chan YC, Kersbergen A, Lam EYN, *et al*. An evolutionarily conserved function of polycomb silences the MHC class I antigen presentation pathway and enables immune evasion in cancer. Cancer Cell 2019;36:385-401.
40. DeNardo DG, Ruffell B. Macrophages as regulators of tumour immunity and immunotherapy. Nat Rev Immunol 2019;19:369-382.
41. Sarvaria A, Madrigal JA, Saudemont A. B cell regulation in cancer and anti-tumor immunity. Cell Mol Immunol 2017;14:662-674.
42. Yang W, Soares J, Greninger P, Edelman EJ, Lightfoot H, Forbes S, *et al*. Genomics of Drug Sensitivity in Cancer (GDSC):a resource for therapeutic biomarker discovery in cancer cells. Nucleic Acids Res 2013;41:D955-961.
43. von Loeffelholz O, Purkiss A, Cao L, Kjaer S, Kogata N, Romet-Lemonne G, *et al*. Cryo-EM of human Arp2/3 complexes provides structural insights into actin nucleation modulation by ARPC5 isoforms. Biol Open 2020;9:bio054304.
44. Latario CJ, Schoenfeld LW, Howarth CL, Pickrell LE, Begum F, Fischer DA, *et al*. Tumor microtubes connect pancreatic cancer cells in an Arp2/3 complex-dependent manner. Mol Biol Cell 2020;31:1259-1272.
45. Turro E, Astle WJ, Megy K, Gräf S, Greene D, Shamardina O, *et al*. Whole-genome sequencing of patients with rare diseases in a national health system. Nature 2020;583:96-102.
46. Kuijpers TW, Tool ATJ, van der Bijl I, de Boer M, van Houdt M, de Cuyper IM, *et al*. Combined immunodeficiency with severe inflammation and allergy caused by ARPC1B deficiency. J Allergy Clin Immunol 2017;140:273-277.
47. Wang X, Xia X, Xu E, Yang Z, Shen X, Du S, *et al*. Estrogen receptor beta prevents signet ring cell gastric carcinoma progression in young patients by inhibiting pseudopodia formation via the mTOR-Arpc1b/EVL signaling pathway. Front Cell Dev Biol 2020;8:592919.
48. Liu J, Lu J, Li W. A comprehensive prognostic and immunological analysis of a six-gene signature associated with glycolysis and immune response in uveal melanoma. Front Immunol 2021;12:738068.
49. Zhou W, Jiang Y, Zhu M, Hang D, Chen J, Zhou J, *et al*. Low-frequency nonsynonymous variants in FKBPL and ARPC1B genes are associated with breast cancer risk in Chinese women. Mol Carcinog 2017;56:774-780.
50. Molinie N, Rubtsova SN, Fokin A, Visweshwaran SP, Rocques N, Polesskaya A, *et al*. Cortical branched actin determines cell cycle progression. Cell Res 2019;29:432-445.
51. Vadlamudi RK, Li F, Barnes CJ, Bagheri-Yarmand R, Kumar R. p41-Arc subunit of human Arp2/3 complex is a p21-activated kinase-1-interacting substrate. EMBO Rep 2004;5:154-160.
52. Rane CK, Minden A. P21 activated kinase signaling in cancer. Semin Cancer Biol 2019;54:40-49.
53. Corral-Jara KF, Rosas da Silva G, Fierro NA, Soumelis V. Modeling the Th17 and tregs paradigm: implications for cancer immunotherapy. Front Cell Dev Biol 2021;9:675099.
54. Tanaka A, Sakaguchi S. Regulatory T cells in cancer immunotherapy. Cell Res 2017;27:109-118.
55. Salmond RJ. mTOR regulation of glycolytic metabolism in T cells. Front Cell Dev Biol 2018;6:122.
56. Netea MG, Balkwill F, Chonchol M, Cominelli F, Donath MY, Giamarellos-Bourboulis EJ, *et al*. A guiding map for inflammation. Nat Immunol 2017;18:826-831.
57. Zhao S, Mi Y, Guan B, Zheng B, Wei P, Gu Y, *et al*. Tumor-derived exosomal miR-934 induces macrophage M2 polarization to promote liver metastasis of colorectal cancer. J Hematol Oncol 2020;13:156.
58. Tarique AA, Logan J, Thomas E, Holt PG, Sly PD, Fantino E. Phenotypic, functional, and plasticity features of classical and alternatively activated human macrophages. Am J Respir Cell Mol Biol 2015;53:676-688.
59. Weiss JM, Davies LC, Karwan M, Ileva L, Ozaki MK, Cheng RY, *et al*. Itaconic acid mediates crosstalk between macrophage metabolism and peritoneal tumors. J Clin Invest 2018;128:3794-3805.
60. Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour-associated macrophages as treatment targets in oncology. Nat Rev Clin Oncol 2017;14:399-416.
61. Pérez-Ruiz E, Melero I, Kopecka J, Sarmento-Ribeiro AB, García-Aranda M, De Las Rivas J. Cancer immunotherapy resistance based on immune checkpoints inhibitors: targets, biomarkers, and remedies. Drug Resist Updat 2020;53:100718.
62. Barrett RL, Puré E. Cancer-associated fibroblasts and their influence on tumor immunity and immunotherapy. Elife 2020;9:e57243.
63. Kato T, Noma K, Ohara T, Kashima H, Katsura Y, Sato H, *et al*. Cancer-associated fibroblasts affect intratumoral CD8+ and FoxP3+ T cells via IL6 in the tumor microenvironment. Clin Cancer Res 2018;24:4820-4833.

64. Zhou J, Wang XH, Zhao YX, Chen C, Xu XY, Sun Q, *et al*. Cancer-associated fibroblasts correlate with tumor-associated macrophages infiltration and lymphatic metastasis in triple negative breast cancer patients. J Cancer 2018;9:4635-4641.
65. Gornalusse GG, Hirata RK, Funk SE, Riolobos L, Lopes VS, Manske G, *et al*. HLA-E-expressing pluripotent stem cells escape allogeneic responses and lysis by NK cells. Nat Biotechnol 2017;35:765-772.
66. Pereira BI, Devine OP, Vukmanovic-Stejic M, Chambers ES, Subramanian P, Patel N, *et al*. Senescent cells evade immune clearance via HLA-E-mediated NK and CD8+ T cell inhibition. Nat Commun 2019;10:2387.
67. Korbecki J, Grochans S, Gutowska I, Barczak K, Baranowska-Bosiacka I. CC chemokines in a tumor: a review of pro-cancer and anti-cancer properties of receptors CCR5, CCR6, CCR7, CCR8, CCR9, and CCR10 ligands. Int J Mol Sci 2020;21:7619.
68. Jiao X, Nawab O, Patel T, Kossenkov AV, Halama N, Jaeger D, *et al*. Recent advances targeting CCR5 for cancer and its role in immuno-oncology. Cancer Res 2019;79:4801-4807.
69. Walens A, DiMarco AV, Lupo R, Kroger BR, Damrauer JS, Alvarez JV. CCL5 promotes breast cancer recurrence through macrophage recruitment in residual tumors. Elife 2019;8:e43653.
70. Rubinstein-Achiasaf L, Morein D, Ben-Yaakov H, Liubomirski Y, Meshel T, Elbaz E, *et al*. Persistent inflammatory stimulation drives the conversion of MSCs to inflammatory CAFs that promote pro-metastatic characteristics in breast cancer cells. Cancers (Basel) 2021;13:1472.
71. Nie Y, Huang H, Guo M, Chen J, Wu W, Li W, *et al*. Breast Phyllodes tumors recruit and repolarize tumor-associated macrophages via secreting CCL5 to promote malignant progression, which can be inhibited by CCR5 inhibition therapy. Clin Cancer Res 2019;25:3873-3886.
72. Adekoya TO, Richardson RM. Cytokines and chemokines as mediators of prostate cancer metastasis. Int J Mol Sci 2020;21:4449.
73. Karash A, Mazzoni MR, Gilchrist A. Pharmacological intervention at CCR1 and CCR5 as an approach for cancer: help or hindrance. Curr Top Med Chem 2014;14:1553-1573.
74. Yang X, Lu Y, Hang J, Zhang J, Zhang T, Huo Y, *et al*. Lactate-modulated immunosuppression of myeloid-derived suppressor cells contributes to the radioresistance of pancreatic cancer. Cancer Immunol Res 2020;8:1440-1451.
75. Schaller TH, Batich KA, Suryadevara CM, Desai R, Sampson JH. Chemokines as adjuvants for immunotherapy: implications for immune activation with CCL3. Expert Rev Clin Immunol 2017;13:1049-1060.
76. He ZC, Li XY, Guo YL, Ma D, Fang Q, Ren L, *et al*. Heme oxygenase-1 attenuates the inhibitory effect of bortezomib against the APRIL-NF-κB-CCL3 signaling pathways in multiple myeloma cells: Corelated with bortezomib tolerance in multiple myeloma. J Cell Biochem 2019;120:6972-6987.
77. Chen YL, Tang C, Zhang MY, Huang WL, Xu Y, Sun HY, *et al*. Blocking ATM-dependent NF-κB pathway overcomes niche protection and improves chemotherapy response in acute lymphoblastic leukemia. Leukemia 2019;33:2365-2378.
78. Xiang P, Jin S, Yang Y, Sheng J, He Q, Song Y, *et al*. Infiltrating CD4+ T cells attenuate chemotherapy sensitivity in prostate cancer via CCL5 signaling. Prostate 2019;79:1018-1031.
79. Cui ZY, Park SJ, Jo E, Hwang IH, Lee KB, Kim SW, *et al*. Cordycepin induces apoptosis of human ovarian cancer cells by inhibiting CCL5-mediated Akt/NF-κB signaling pathway. Cell Death Discov 2018;4:62.
80. Chen K, Wang Y, Hou Y, Wang Q, Long D, Liu X, *et al*. Single cell RNA-seq reveals the CCL5/SDC1 receptor-ligand interaction between T cells and tumor cells in pancreatic cancer. Cancer Lett 2022;545:215834.
81. Karvonen H, Arjama M, Kaleva L, Niininen W, Barker H, Koivisto-Korander R, *et al*. Glucocorticoids induce differentiation and chemoresistance in ovarian cancer by promoting ROR1-mediated stemness. Cell Death Dis 2020;11:790.
82. Seyed Hosseini E, Alizadeh Zarei M, Tarrahimofrad H, Zamani J, Haddad Kashani H, Ahmad E, *et al*. Synergistic effects of dendrosomal nanocurcumin and oxaliplatin on oncogenic properties of ovarian cancer cell lines by down-expression of MMPs. Biol Res 2023;56:3.


**How to cite this article:** Ke M, Zhu H, Lin Y, Zhang Y, Tang T, Xie Y, *et al*. Actin-related protein 2/3 complex subunit 1B promotes ovarian cancer progression by regulating the AKT/PI3K/mTOR signaling pathway. J Transl Intern Med 2024; 12: 406-423.