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ORIGINAL RESEARCH

ARRDC3 as a Diagnostic and Prognostic Biomarker for Epithelial Ovarian Cancer Based on Data Mining

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Purpose: The dysregulation of arrestin domain containing 3 (ARRDC3) has an important effect on oncogenesis and tumor progression in many cancers, including renal cell carcinoma and breast cancer. However, the role of ARRDC3 in ovarian cancer (OC) has not been reported.

Methods: The present study explored the diagnostic and prognostic roles of ARRDC3 in ovarian cancer using GEPIA, ONCOMINE, GEO, and Kaplan–Meier Plotter databases for training and validation. Then, we conducted a stratified analysis for clinicopathological factors using Kaplan–Meier Plotter and GEPIA databases. To further explore the mechanisms, we also used the MIST database to visualize the protein–protein interaction network of ARRDC3 associated with OC. The gene–gene interaction network was visualized by GeneMANIA plugin in Cytoscape 3.8.0 software, and the associated co-expression genes of ARRDC3 were analyzed by the cBioPortal database. The 100 top co-expression genes chosen for gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were used by the DAVID website.

Results: A significant difference in ARRDC3 mRNA expression was found between OC and normal ovary tissues. ARRDC3 could potentially be implicated in the diagnosis of OC. A high ARRDC3 mRNA expression level was associated with poor overall survival and progression-free survival. However, no significance was reported in respect to post progression survival. Except for histology, which had no prognostic value for PPS in stratified analysis, stratified analysis of other factors had prognostic value for OS, PFS, and PPS. Interestingly, we found a positive correlation between ARRDC3 expression and CD8+ T cells, macrophages, neutrophils, and dendritic cells, indicating that ARRDC3 might be associated with immune infiltration of these immune cells. Co-expression genes enrichment analysis found that they were involved in the Renin-angiotensin system pathway.

Conclusion: Differentially expressed ARRDC3 might be a potential prognostic and diagnostic marker in Ovarian Cancer.

Keywords: biomarker, diagnosis, prognosis, ovarian neoplasms

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Introduction

Ovarian cancer (OC) is one of the most common malignancies worldwide, but it is the most lethal gynecological malignancies.[1,2] Globally, the age-standardized incidence of OC has been increasing, with the largest increase observed in Brazil. In particular, the incidence of OC has risen significantly in most countries in recent birth cohorts.[1] In the absence of effective early screening measures, epithelial Ovarian Cancer are often found to have advanced cases.[3] The five-year relative survival for OC reaches 46–47% according to SEER database and World Ovarian Cancer Coalition. Targeted drugs and immunosuppressive agents have made a breakthrough in the treatment of OC, and can prolong the survival time in some patients; however, the overall effect is not satisfactory. Even

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with PARP inhibitors therapy, tumor-free survival is only extended in some patients.[4–6] The pathogenesis and metastasis of OC are very complex, involving processes such as repetitive wounding of the ovarian surface epithelium, cross-talk signaling events and interactions between ovarian cancer cells and various stromal cells, in which many genes are involved and are altered.[7] New drug targets for OC may be identified by screening gene networks for changes related to tumor pathogenesis and metastasis.

Arrestin Domain Containing 3 (ARRDC3) is a protein-coding gene, also known as thioredoxin-binding protein-2-like inducible membrane protein, which includes beta-3 adrenergic receptor binding, which acts as an adaptor protein.[8] It regulates cell proliferation and PPAR gamma activation.[8] It also regulates cell-surface expression of adrenergic receptors and probably also other G protein-coupled receptors.[8–10] ARRDC3 plays a role in NEDD4-mediated ubiquitination and endocytosis of activated ADRB2 and subsequent ADRB2 degradation.[11] ARRDC3 has been studied for disease occurrence and progression. In this respect, ARRDC3 was found to be overexpressed in placental tissues from patients with preeclampsia.[12] It also plays an important function in the pathogenesis of preeclampsia.[12] ARRDC3 regulates the apoptosis of hepatic stellate cells in non-alcoholic fatty liver disease and non-alcoholic steatohepatitis, which shows that it plays a role in the development of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis.[13]

ARRDC3 also plays an important function in tumorigenesis and progression. Down-expression of ARRDC3 was first observed in breast cancer, and aberrant expression of ARRDC3 was correspondingly observed in multiple malignant tumors, including renal cell carcinoma, prostate cancer, cervical cancer, and colorectal cancer.[14–16] Given previous studies on ARRDC3 in tumors, therefore, we launched a study aiming to detect a correlation between ARRDC3 and OC.

Materials and Methods

Expression and Transcription Analysis

First, transcription and gene expression levels were collected using Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/index.html, accessed May 6, 2020),[17] which were validated using the datasets from GEO and ONCOMINE.[18,19] Then, transcription was stratified analyzed based on clinical stage. Other settings were as following: $|\log2FC|\geq2$, $P\leq0.05$.

Diagnostic and Survival Analysis

First, diagnostic receiver operating characteristic curves were drawn using the ONCOMINE (https://www.oncomine.org/resource/login.html, accessed May 6, 2020) website and GSE14407 dataset.[18,19] The Profiling dataset was obtained from the Gene Expression Omnibus website (GEO, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?a cc=gse14407, accessed May 5, 2020).[19] Then, overall survival (OS), progression-free survival (PFS), and post-progression-free survival (PPS) were calculated using the Kaplan–Meier Plotter website (http://kmplot.com/analysis/, accessed May 12, 2020).[20] In addition, low and high expression groups were set at a cut-off of the median expression level. After that, we performed a prognostic analysis in subgroups of patients with OC, stratified by pathological type, tumor stage, pathological grade, TP53 gene mutation, and degree of debulking with settings using database default

Genomic Alterations Analysis

Genes alterations were analyzed by the cBio Cancer Genomics Portal (cBioPortal https://www.cbioportal.org/), which is an open resource. The integrated data sets can be downloaded from the website and directly used for literature publication.[21]

The relationships between immune cells

The relationships between immune cells and ARRDC3 in OC were explored using the Tumor Immune Estimation Resource (TIMER https://cistrome.shinyapps.io/timer/), which precomputed the levels of immune subsets of six tumour infiltrates in 10,897 of the 32 cancers.[22]

Gene–Gene Interaction (GGI), Co-Expression Gene, and Protein–Protein Interaction (PPI) Analysis

First, the gene–gene interaction network was generated by the GeneMANIA plugin cytoscape 3.8.0.[23] The co-expression genes of ARRDC3 in ovarian cancer were analyzed using cBioPortal with settings using database default. Then, ARRDC3 and the top 100 co-expression genes were selected for Gene Ontology Consortium (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment using the Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david-d.ncifcrf.gov/) website, and the results were visualized using R3.5.1.[24] After that, PPI network was analyzed using the Molecular Interaction Search Tool (MIST, https://fgrtools.hms.harvard.edu/MIST/) with settings using default.[25]

Statistical Analysis

Data were analyzed by using GraphPad Prism8.2 software and expressed as mean standard deviation (SD). Use *t*-test to analyze the data difference between the two groups. Statistical significance was considered as $P<0.05$.

Results

mRNA Expression of ARRDC3 in Patients with OC

As shown in Figure 1A, the mRNA expression levels of ARRDC3 in ovarian cancer tissues were much lower than those in the normal tissues. A total of 426 epithelial ovarian cancer patients and 88 normal ovary tissue samples were included in the GEPIA database ($p\leq0.05$, $|\log_2 FC|\geq2$). After a significant difference in ARRDC3 mRNA expression was found between ovarian cancer and normal ovary tissues, we analyzed the associations between ARRDC3 mRNA expression and cancer clinical stages in the ovarian cancer patients with GEPIA. As shown in Figure 1B, the ARRDC3 mRNA expression level was strongly associated with cancer clinical stages, and patients who were in late stages trended to have a lower ARRDC3 mRNA expression. A total of 12 ovarian normal surface epithelial cells and 12 ovarian cancer epithelial cells were included in the GEO database, and the mRNA expression of ARRDC3 in ovarian cancer epithelial cells was lower than in ovarian normal surface epithelial cells (P=0.0023, 95% CI: 0.6345–2.556, Figure 1C). There were 29 epithelial ovarian cancer patients and 5 normal tissues included in the ONCOMINE database. This result was consistent with the results in the GEO and GEPIA databases (P=0.0268, 95% CI: −1.459–0.09508, Figure 1D).

Diagnostic Value of mRNA Expression of ARRDC3 in Ovarian Cancer Patients

A total of 12 Ovarian normal surface epithelial cells and 12 ovarian cancer epithelial cells were included in the GEO database, and there were 29 epithelial ovarian cancer patients and 5 normal tissue samples included in the ONCOMINE database. The diagnostic analysis of ARRDC3 for ovarian cancer in the GEO and ONCOMIE database showed significance for ovarian cancer diagnosis (all $P\leq0.05$, Figure 1E and F). A greater area under the curve was observed for the GEO database (0.8819, Figure 1E).

Prognostic Value of ARRDC3 mRNA Expression in Ovarian Cancer Patients

Furthermore, the prognostic values of ARRDC3 mRNA expression in ovarian cancer patients were analyzed using the Kaplan-Meier plotter. As shown in Figure 2, mRNA expressions of ARRDC3 were significantly associated with the prognosis of ovarian cancer patients. The relationship between ARRDC3 mRNA expressions and OS, PFS, and PPS in ovarian cancer patients was analyzed. The results showed that higher mRNA expressions of ARRDC3 were associated with poorer OS and PFS in ovarian cancer patients (Figure 2A HR=1.48, 95% CI: 1.2–1.82, and P=2.2e-04; Figure 2D HR=1.26, 95% CI: 1.04–1.53, and P=1.7e-02). However, there was no correlation between the expression level of ARRDC3 and PPS (Figure 2G HR=1.17, 95% CI: 0.9–1.52, and P=2.3e-01). In addition, we also conducted stratification of ARRDC3 for OS, PFS and PPS analysis, respectively. The stratification was based on pathological type, clinical stage, pathological grade, TP53 gene mutation, and degree of debulking. All the exhaustive results of the stratified analysis are shown in Figures 2–6.

The results by stratified survival analysis of ARRDC3 in ovarian cancer by pathological type of OS, PFS, PPS are shown in Figure 2. The OS of the high expression group of serous carcinoma was poorer than that of the low expression group (Figure 2B HR=1.4 (1.11–1.77) P=0.0047). But there was no difference between the two groups in endometrial carcinoma (Figure 2C HR=2.45 (0.25–23.6), P=0.42). The PFS of the high expression group of serous carcinoma was poorer than that of the low expression group (Figure 2E HR=1.39 (1.13–1.7) P=0.0019). But there was no difference between the two groups in endometrial carcinoma (Figure 2F HR=0.34 (0.11–1.09), P=0.057). The PPS of the high-expression group of serous carcinoma was not significant compared with the low-expression group (Figure 2H HR=1.12 (0.85–1.48), P=0.41).

The results by stratified survival analysis of ARRDC3 in ovarian cancer by clinical stage of OS, PFS, PPS are shown in Figure 3. The OS of the high expression group of late-stage patients was poorer than that of the low expression group (Figure 3B HR=1.47 (1.17–1.86), P=0.00091). But there was no difference between the low expression group and high expression group of early-stage patients (Figure 3A HR=0.27 (0.06–1.21), P=0.066). The PFS of the high expression

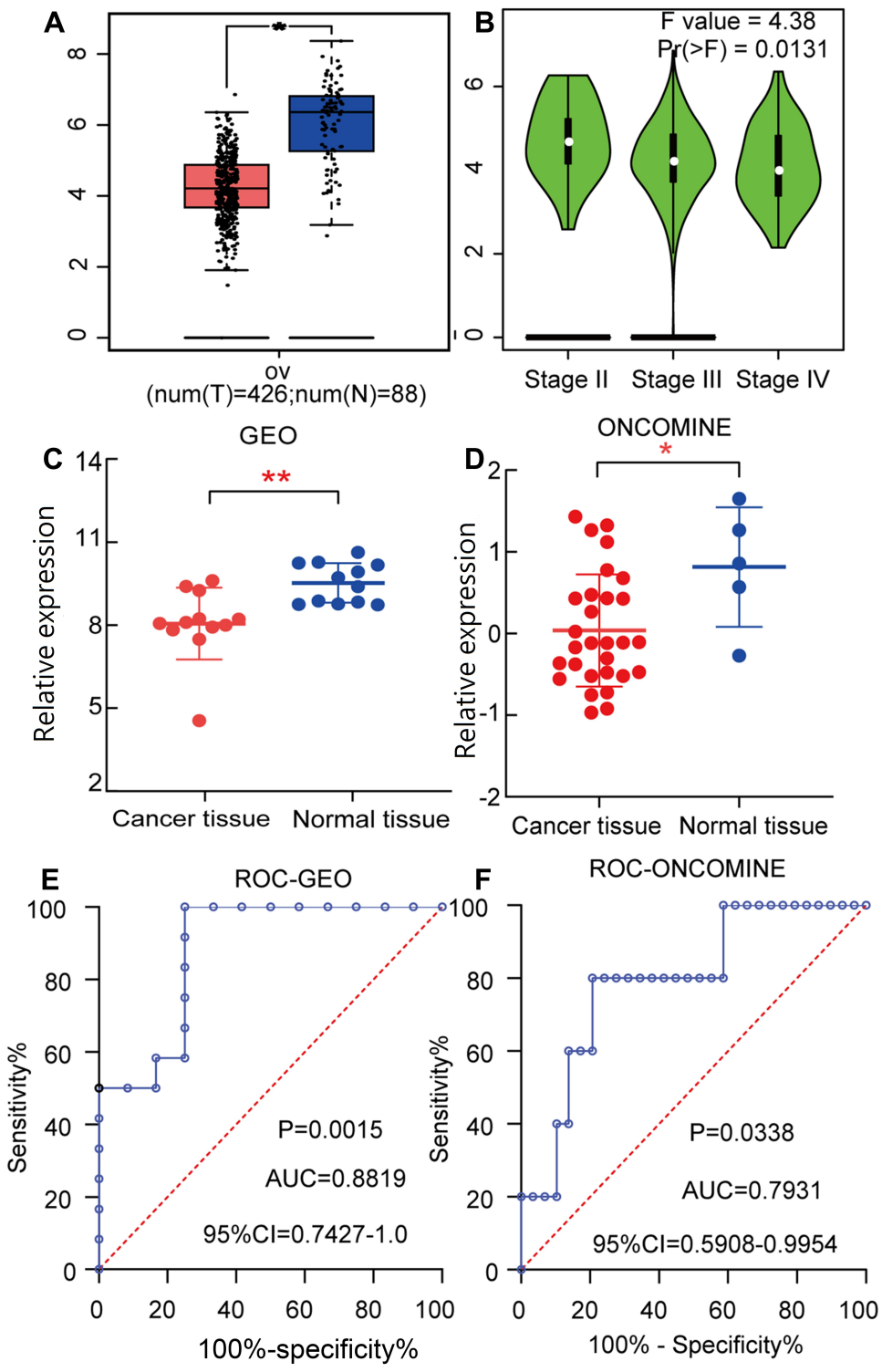

Figure 1 Analysis of differential expression, disease progression and diagnostic implications of *ARRDC3* in ovarian cancer. (**A** and **B**) Differential expression and disease progression of *ARRDC3* in ovarian cancer, respectively; (**C** and **D**) validation of differential expression of *ARRDC3* in GEO and ONCOMINE databases, respectively; (**E** and **F**) diagnostic implications of *ARRDC3* in GEO and ONCOMINE databases, respectively.

Notes: *P <0.05; **P<0.01.

group of late-stage patients was poorer than that of the low expression group (Figure 3D HR=1.39 (1.14–1.69), P=0.0011). But there was no difference between the low expression group and high expression group of early-stage patients (Figure 3C HR=0.55 (0.25–1.21), P=0.13). The PPS of the high expression group of early-

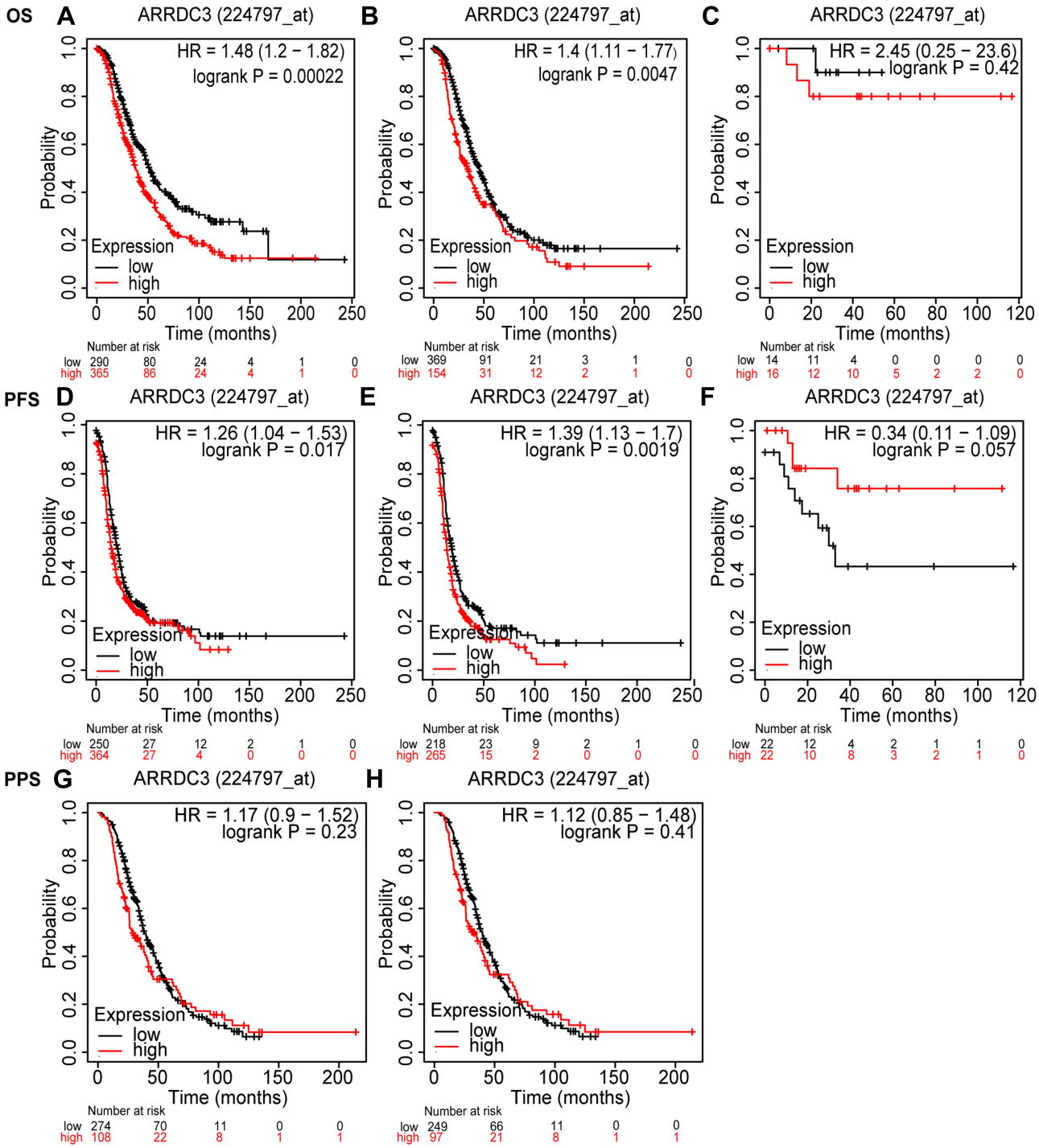

Figure 2 Survival analysis of *ARRDC3* in ovarian cancer by OS, PFS, PPS as well as stratified analysis by serous carcinoma and endometrioid carcinoma types. (**A**–**C**) Survival analysis of *ARRDC3* in ovarian cancer by as well as stratified analysis by serous carcinoma and endometrioid carcinoma types, respectively; (**D**–**F**) survival analysis of *ARRDC3* in ovarian cancer by PFS well as stratified analysis by serous carcinoma and endometrioid carcinoma types, respectively; (**G** and **H**) survival analysis of *ARRDC3* in ovarian cancer by PPS well as stratified analysis by serous carcinoma type, respectively.

stage patients was better than that of the low expression group, but there was no difference between the low expression group and high expression group of late-stage patients ((Figure 3E HR=0.12 (0.01–0.94) P=0.016; Figure 3F HR=1.24 (0.97–1.58), P=0.091))

The results by stratified survival analysis of ARRDC3 in ovarian cancer by pathological grade of OS, PFS, PPS are shown in Figure 4. The OS of the high expression group of low- and high-grade patients was poorer than that of the low expression group ((Figure 4A HR=1.94

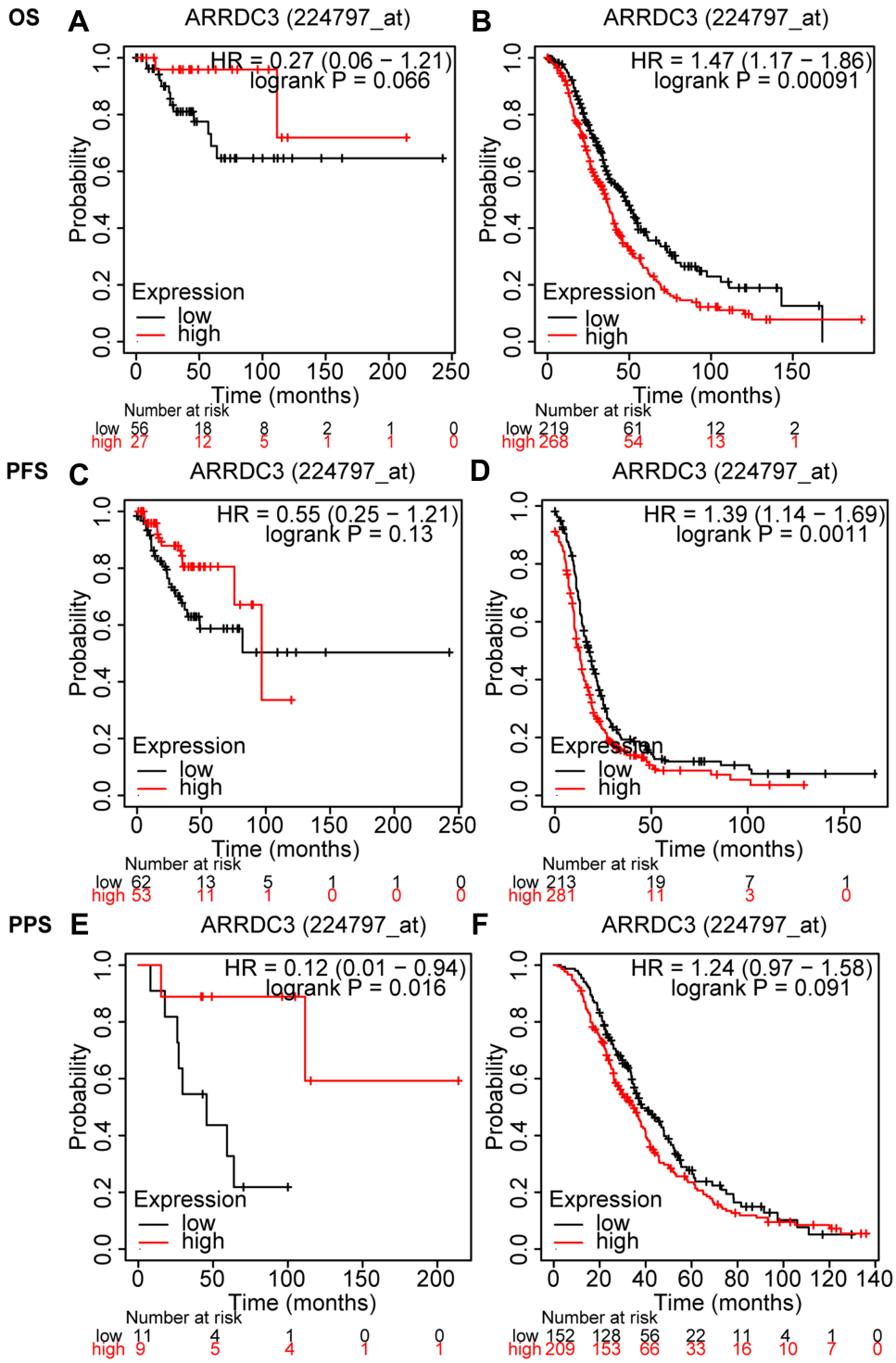

Figure 3 Stratified survival analysis of *ARRDC3* in ovarian cancer by clinical stage of OS, PFS, PPS. (**A** and **B**) Stratified survival analysis of *ARRDC3* in ovarian cancer of early stage and late stage of OS, respectively; (**C** and **D**) stratified survival analysis of *ARRDC3* in ovarian cancer of early stage and late stage of PFS, respectively; (**E** and **F**) stratified survival analysis of *ARRDC3* in ovarian cancer of early stage and late stage of PPS, respectively.
Notes: Early stage=1+2 stage; late stage=3+4 stage.

(1.14–3.28), P=0.012; Figure 4B HR=1.63 (1.26–2.1), P=0.00015)). The PFS of the high expression group of low- and high-grade patients was poorer than that of the low expression group ((Figure 4C HR=1.7 (1.1–2.64), P=0.016; Figure 4D HR=1.55 (1.21–1.99), P=0.00055)). The PPS of the high expression group of low-grade patients was better than that of the low expression group (Figure 4E HR=0.48 (0.29–0.77), P=0.002). However, the

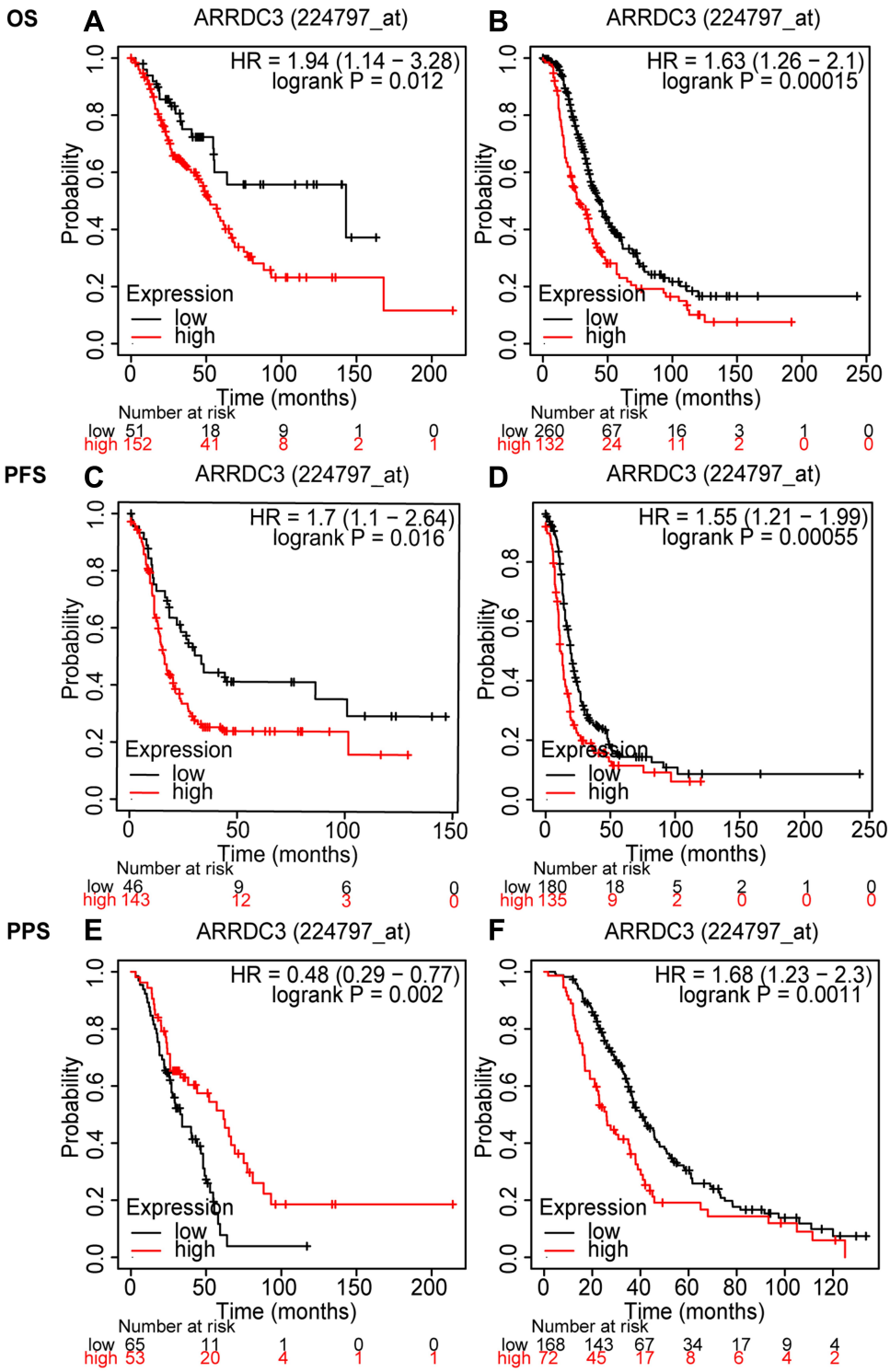

Figure 4 Stratified survival analysis of *ARRDC3* in ovarian cancer by pathological grade of OS, PFS, PPS. (**A** and **B**) Stratified survival analysis of *ARRDC3* in ovarian cancer of low grade and high grade of OS, respectively; (**C** and **D**) stratified survival analysis of *ARRDC3* in ovarian cancer of low grade and high grade of PFS, respectively; (**E** and **F**) stratified survival analysis of *ARRDC3* in ovarian cancer of low grade and high grade of PPS, respectively.
Notes: Low grade =1+2 grade; high grade =3 grade.

PPS of the high expression group of high-grade patients was poorer than that of the low expression group (Figure 4F HR=1.68 (1.23–2.3), P=0.0011).

The results by stratified survival analysis of ARRDC3 in ovarian cancer by TP53 mutation of OS, PFS, PPS are shown in Figure 5. The OS, PFS, PPS of the high

expression group of mutation type patients were poorer than that of the low expression group ((Figure 5A HR=1.83 (1.2–2.78), P=0.0044; Figure 5C HR=1.78 (1.17–2.7) P=0.0063; Figure 5E HR=1.66 (1.08–2.53), P=0.018)). The OS, PPS of the high expression group of wild-type patients were not significant with that of the low expression group ((Figure 5B HR=1.8 (0.61–5.36), P=0.28; Figure 5F HR=0.44 (0.12–1.66) P=0.21)). The

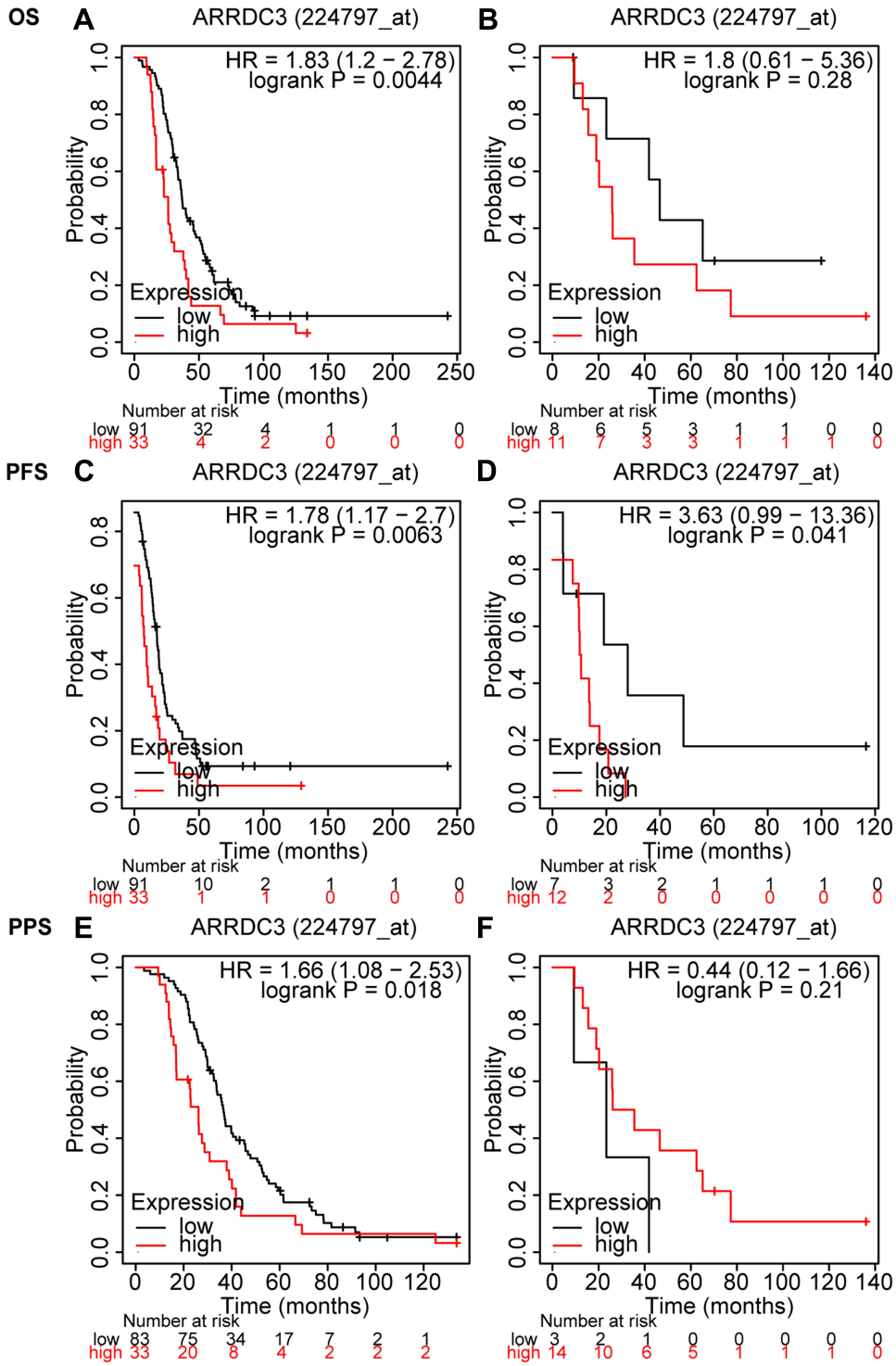

Figure 5 Stratified survival analysis of *ARRDC3* in ovarian cancer by TP53 mutation of OS, PFS, PPS. (**A** and **B**) Stratified survival analysis of *ARRDC3* in ovarian cancer of mutation type and wild type of OS, respectively; (**C** and **D**) stratified survival analysis of *ARRDC3* in ovarian cancer of mutation type and wild type of PFS, respectively; (**E** and **F**) stratified survival analysis of *ARRDC3* in ovarian cancer of mutation type and wild type of PPS, respectively.

PFS of the high expression group of wild-type patients was poorer than that of the low expression group ((Figure 5D HR=3.63 (0.99–13.36), P=0.041)).

The results by stratified survival analysis of ARRDC3 in ovarian cancer by debulking degree of OS, PFS, PPS are shown in Figure 6. The OS, PFS of the high expression group of optimal and suboptimal patients were poorer than that of the low expression group ((Figure 6A HR=1.78 (1.04–3.05), P=0.034; Figure 6B HR=1.63 (1.17–2.26) P=0.0032; Figure 6C HR=1.43 (1.04–1.99), P=0.029; Figure 6D HR=1.84 (1.35–2.52) P=9.2e-05)). The PPS of the high expression group of suboptimal patients was poorer than that of the low expression group (Figure 6F HR=1.43 (1.02–1.99), P=0.035). However, the PPS of the high expression group of optimal patients was not significant with the low expression group (Figure 6E HR=0.84 (0.54–1.29), P=0.42).

ARRDC3 Alterations in OC

The types and frequency of ARRDC3 alterations were analyzed in ovarian cancer using the cBioPortal based on sequencing data from ovarian cancer patients in the TCGA database. The result showed that ARRDC3 was altered in 11 of 311 (4%) ovarian patients (Figure 7A). Deep deletion was the most common type of ARRDC3 alteration observed in ovarian cancer.

The Relationship Between ARRDC3 and Immune Cell Subtypes in OC

To further understand the correlation between ARRDC3 and diverse immune cell subsets, we used the Timer database to analyze the relationship between ARRDC3 and a variety of immune cell subsets in ovarian cancer. The results are shown in Figure 7B. ARRDC3 expression showed a weakly positive correlation with CD8+ T cells, macrophages, neutrophils, and dendritic cells in ovarian cancer. Other immune cell subsets showed no significant relationship with ARRDC3.

Biological Interaction Network of ARRDC3 in OC

After determining the potential prognostic value of ARRDC3 in ovarian cancer, we wanted to further explore its possible mechanism. To explore the internal mechanism of ARRDC3 involved in ovarian cancer, firstly, we investigated gene–gene regulation. The network of gene–gene interactions for ARRDC3 in ovarian cancer was drawn using the GeneMANIA plugin in cytoscape 3.8.0. The result is shown in Figure 7C; ARRDC3 is the seed gene (indicated with black), and all other genes are automatically identified as altered in ovarian cancer. Different colors in the network edge indicate the bioinformatics methods.

Secondly, we used MIST to analyze the PPI of ARRDC3 in OC. The result is shown in Figure 7D; ARRDC3 is located at the center and each ellipse is a protein that interacts with ARRDC3. Different interacting proteins or molecules were given a score and rated "High" because the interaction was supported by multiple experimental methods and/or demonstrated in multiple publications. The node color represents the Genotype-Tissue Expression (GTEx) Median TPMs in the ovary.

Lastly, we used cBioPortal to determine the ARRDC3 co-expression genes in OC. GO and KEGG enrichment analyses were performed on the top 100 genes and ARRDC3 using DAVID, and the results were visualized by R3.5.1. In the GO enrichment analysis (Figure 8A), biological processes such as GO:0033554 (cellular response to stress), GO:0008104 (protein localization), GO:0046907 (intracellular transport), GO:0015031 (protein transport), GO:0045184 (establishment of protein localization), GO:0006974 (response to DNA damage stimulus), GO:0006259 (DNA metabolic process), GO:0006281 (DNA repair), GO:0006605 (protein targeting), GO:0006886 (intracellular protein transport), GO:0034613 (cellular protein localization) and GO:0070727 (cellular macromolecule localization) were regulated by the co-expression genes in OC. Cellular components, including GO:0005643 (nuclear pore) and GO:0046930 (pore complex), were associated with the co-expression genes of ARRDC3 in OC. In addition, co-expression genes also affected the molecular functions, such as GO:0008565 (protein transporter activity), GO:0008094 (DNA-dependent ATPase activity), and GO:0005385 (zinc ion transmembrane). In KEGG analysis, only one pathway (Has 04614: Renin-angiotensin system) was associated with the functions of co-expression genes in OC (Figure 8B).

Discussion

This study showed that GEPIA, GEO ONCOMINE databases indicate mRNA expression levels of ARRDC3 in ovarian cancer tissues were much lower than those in the normal tissues. In addition, GEPIA database indicated that ARRDC3 mRNA expression level was negatively associated with cancer clinical stages, which demonstrating patients who were in late stages trended to have a lower

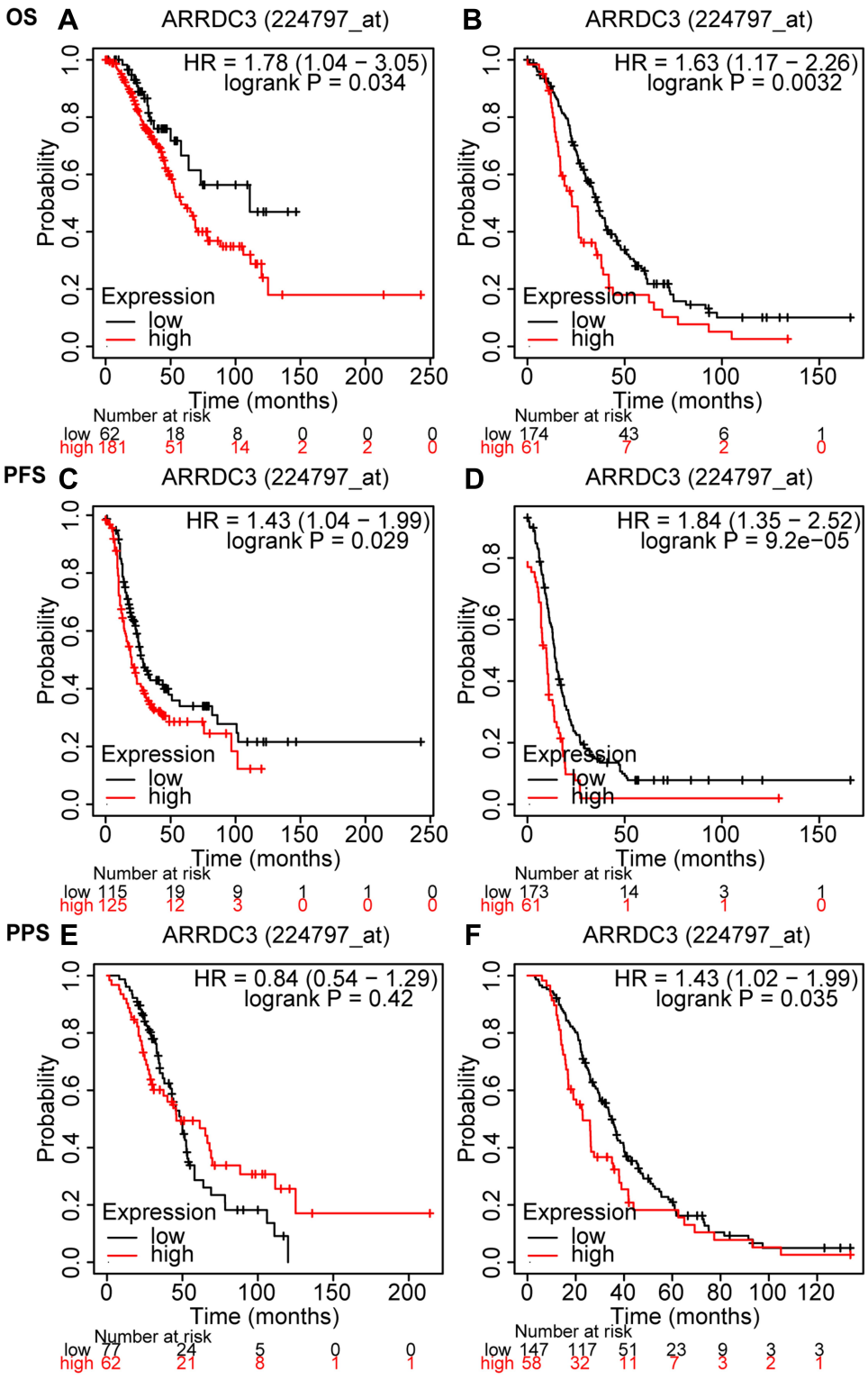

Figure 6 Stratified survival analysis of *ARRDC3* in ovarian cancer by debulking degree of OS, PFS, PPS. (**A** and **B**) Stratified survival analysis of *ARRDC3* in ovarian cancer of optimal and suboptimal of OS, respectively; (**C** and **D**) stratified survival analysis of *ARRDC3* in ovarian cancer of optimal and suboptimal of PFS, respectively; (**E** and **F**) stratified survival analysis of *ARRDC3* in ovarian cancer of optimal and suboptimal of PPS, respectively.

ARRDC3 mRNA expression. Additionally, Kaplan-Meier plotter results suggested higher mRNA expressions of ARRDC3 were associated with poorer OS and PFS in ovarian cancer patients. This result indicated that ARRDC3 may play an oncogene role in OC prognosis. However, this oncogene role is consistent with its

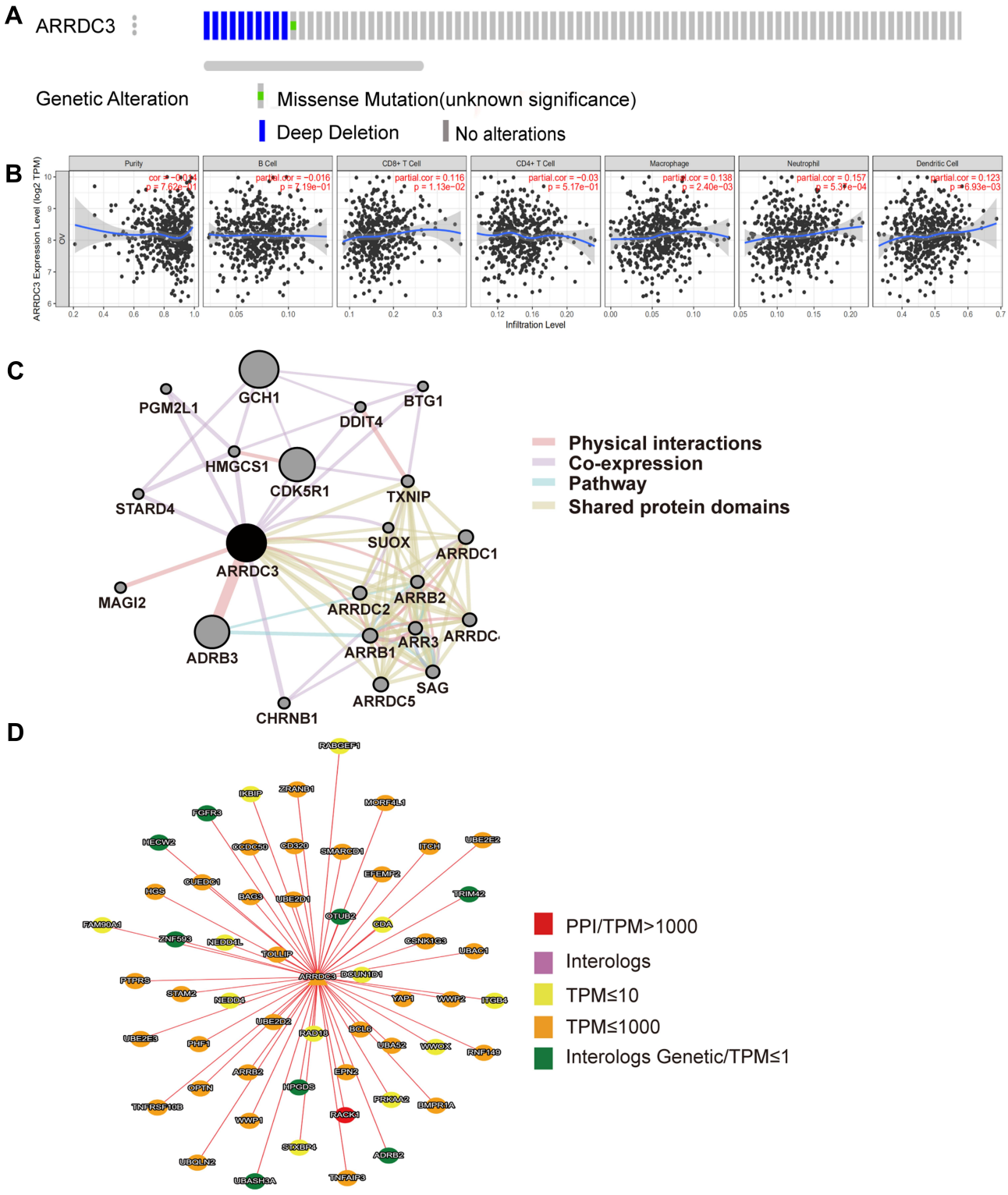

Figure 7 The mutation, immune and interaction network of *ARRDC3* in ovarian cancer. (**A**) Genetic alterations, including missense mutation and deep deletion of *ARRDC3* in ovarian cancer; (**B**) The correlation analysis between *ARRDC3* expression and infiltrate level of diverse immune cell types in ovarian cancer; (**C**) The gene–gene interaction network of *ARRDC3* with other related genes in physical interaction, co-expression, pathway and shared protein domains aspects; (**D**) The protein–protein interaction network of *ARRDC3* with other proteins with PPI/TPM>1000, interologs, TPM≤10, TPM≤1000, and interologs genetic/TPM≤1.

expressions in tumor tissues but controversial with its clinical stage. Therefore, we postulate that tumor stage alone may not be play a predominant role in patient survival but other factors may influence the prognosis. However, these potentially factors were not revealed by the present study and therefore, need to be clarified in the future.

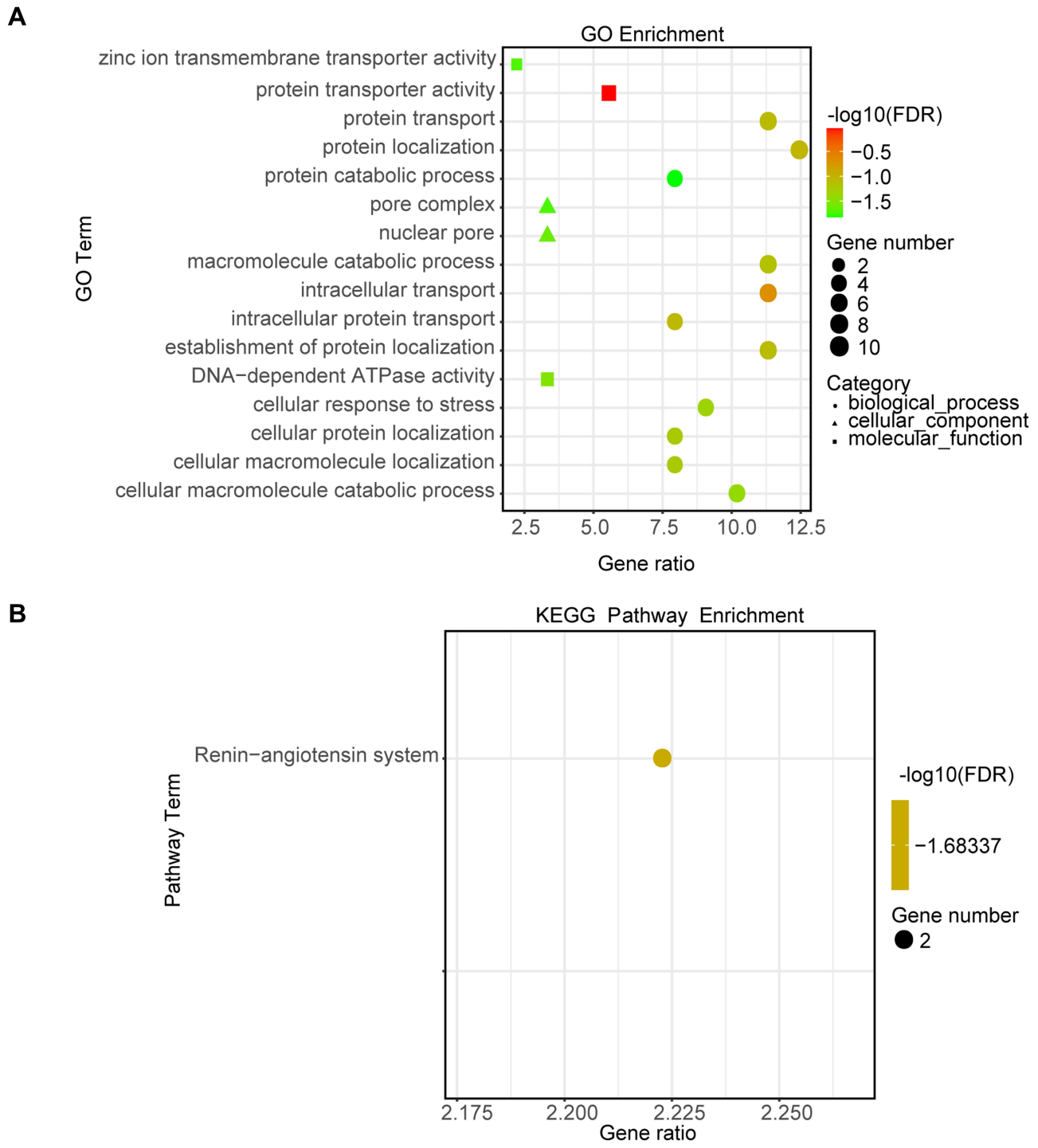

Figure 8 Enrichment analysis of the *ARRDC3* and its co-expression related genes in cBioPortal database. (**A**) Enrichment analysis of gene ontology terms predicted by *ARRDC3* and its co-expression related genes, including biological processes, cellular components and molecular functions; (**B**) Enrichment analysis of KEGG pathway predicted by *ARRDC3* and its co-expression related genes.

ARRDC3, a member of the arrestin family of proteins, regulates G protein-mediated signalling. It is reported that ARRDC3 interacts with neural precursor development downregulated protein 4 (NEDD4), recruits NEDD4 to the activated beta2-adrenergic receptor (beta2AR), and then promotes its ubiquitination.[11] Previous reports have reported that the expression of ARRDC3 is associated with various diseases, including inflammatory disease and malignant tumors.[12–16,26] However, information relating to the association between ARRDC3 and OC remained unknown.

In our study, we performed an analysis on the correlations between ARRDC3 and OC patients. The mRNA expression levels of ARRDC3 in OC tissues were much lower than those in normal tissue in our data, which showed the potential diagnostic value of ARRDC3 in OC. Our results also showed that higher mRNA expressions of ARRDC3 were associated with poorer OS and poorer PFS in ovarian cancer patients. Our results were similar to those found in previous reports. In these reports, down-expression of ARRDC3 was also found in other cancers, such as prostate cancer and breast cancer, consistent with the results from our data. Moreover, down-expression of ARRDC3 has been found to be associated with the grade, metastasis, and invasion of these cancers by negatively regulating β-4 integrin (ITGβ4).[27,28] Additionally, abnormal ARRDC3 methylation has been observed in invasive ductal carcinomas (IDCs) and is strongly related to lymph node status and the grade of IDCs.[29] ARRDC3 also regulates the typical JNK signaling pathway underlying breast cancer invasion.[30] Subsequent studies have found that ARRDC3 is negatively regulated by miR-182-5p,[31] which promotes the degradation of ARRDC3 mRNA in prostate cancer. ARRDC3 acts as a tumor suppressor gene in colorectal cancer and bonds and degrades the oncogene YAP, which plays a vital role in the development of cancer through the Hippo pathway.[14] Similar functions and mechanisms have been reported in renal clear cell carcinoma.[15] Moreover, in our data, the PPI network of ARRDC3 in OC also showed that ITGβ4 and YAP1 were the interaction proteins. Therefore, we speculate that ARRDC3 may also play a tumor suppressor role in OC through a similar mechanism.

Our stratified analysis results showed that some clinicopathological factors were related to patient prognosis, including pathological type, tumor stage, pathological grade, TP53 gene mutation, and degree of debulking. A large number of studies have already confirmed that these factors were prognostic factors for patients with OC. The TP53 gene mutation has been broadly recognized as a risk factor for the prognosis of epithelial OC.[32,33]

Interestingly, in our study, we used the Timer database to analyze the relationship and found a positive correlation between ARRDC3 expression and CD8+ T cells, macrophages, neutrophils, and dendritic cells in OC, which indicates that ARRDC3 might be associated with infiltration of these immune cells. Similarly, in pyloric screw gastritis, it has been reported that ARRDC3 might be associated with immune infiltration of neutrophils and the severity of gastritis. Here, ARRDC3 promoted gastric inflammation, characterized by a CXCR2-dependent influx of neutrophils (CD45+, CD11b+, Ly6C-, Ly6G+), whose migration was induced by ARRDC3-dependent production of CXCL2.[26]

There were some limitations of our study that are worthy of noting. Although our study is the first to present evidence for the importance and potential functions of ARRDC3 in ovarian cancer, the results were based on online public databases and functional experiments and mechanistic exploration was not carried out. Secondly, sample sizes for the corresponding studies in the GEO and ONCOMINE databases were relatively small, and prognostic studies have only single-factor findings. Therefore, large-scale clinical samples, including various ethnic groups and a multi-factor analysis, are required. Thirdly, the results from our study were only at the mRNA level, and the protein level will need to be verified in subsequent functional and mechanistic experiments.

Conclusion

Taken together, our study is the first to present evidence for the importance and potential functions of ARRDC3 in ovarian cancer. The results primarily indicate that ARRDC3 might have a potential association with OC risk, to some extent, and may affect OC through the renin-angiotensin system signaling pathway. Further studies with a larger sample size, functional experiments and mechanism exploration are necessary to confirm the association.

Abbreviations

ARRDC3, dysregulation of arrestin domain containing 3; OC, ovarian cancer; OS, overall survival; PFS, progression-free survival; PPS, post-progression survival; GO, gene ontology; KEGG, Kyoto encyclopedia of genes and genomes; GEPIA, gene expression profiling interactive analysis; DAVID, database for annotation, visualization and integrated discovery; GEO, gene expression omnibus; MIST, molecular interaction search tool; TIMER, tumor immune estimation resource; GTEx, genotype-tissue expression; TCGA, the cancer genome atlas; PPAR, peroxisome proliferator Activated Receptor; NEDD4, neural precursor development downregulated protein 4; ADRB2, beta2-adrenergic receptor; PPI, protein–protein interaction; ITGβ4, β-4 integrin; IDCs, invasive ductal carcinomas; YAP, yes associated protein; CXCR2, C-X-C motif

chemokine receptor 2; CXCL2, C-X-C motif chemokine ligand 2.

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Disclosure

The authors report no conflicts of interest in this work.

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