Overexpression of CAPG Is Associated with Poor Prognosis and Immunosuppressive Cell Infiltration in Ovarian Cancer

Senwei Jiang, Yuebo Yang, Yu Zhang, Qingjian Ye, Jiao Song, Min Zheng, and Xiaomao Li

1Department of Gynecology, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou 510000, China
2Department of Gynecology, Sun Yat-sen University Cancer Center, Guangzhou 510000, China

Correspondence should be addressed to Xiaomao Li; lixmao@mail.sysu.edu.cn

Received 22 December 2021; Revised 9 January 2022; Accepted 18 January 2022; Published 9 February 2022

1. Introduction

Ovarian cancer (OC) is the most lethal gynecologic malignancy worldwide [1]. Most patients are not diagnosed until they reach advanced stages, contributing to a 5-year overall survival (OS) rate of less than 30% [2]. A combination of surgery and chemotherapy is the classic treatment for ovarian cancer [3]. However, most of stage III–IV patients that have an initial complete response to surgery and chemotherapy will ultimately experience disease progression and resistance to the first-line treatment regimen [4]. Therefore, we urgently need to find novel, more efficient therapies for ovarian cancer.

The primary adjuvant treatment for ovarian cancer has advanced from chemotherapy to targeted molecular therapy [5]. Recently, immunotherapies, such as vaccination, adoptive cellular therapy, and checkpoint inhibitors, have become an attractive therapeutic strategy [6]. Although immune checkpoint blockade (ICB) therapies such as cytotoxic T lymphocyte-associated antigen 4 (CTLA4) and programmed death-1/ligand1 (PD-1/PD-L1) inhibitors showed promising antitumor effects in many cancers, they only exhibited a
partial response and poor clinical efficacy in advanced OC [7, 8]. An increasing number of studies have found that the weakness of immunotherapy is dominantly due to the immunosuppressive tumor microenvironment (TME) [9]. Therefore, in order to improve the efficacy of immunotherapies for OC, there is an urgent need for a more comprehensive understanding of immune-related gene expression and immunocyte infiltration in respect to the disease.

The gelsolin protein superfamily is a conserved family of proteins. These proteins have specific roles in actin filament remodeling, cell motility, apoptosis control, phagocytosis regulation, and gene expression regulation [10]. The CAPG protein, Gelsolin-Like (CAPG) gene encodes a member of the gelsolin/villin protein superfamily. CAPG, also known as Macrophage Capping Protein, reversibly blocks the barbed ends of F-actin filaments in a Ca2+ in a phosphoinositide-regulated manner without severing pre-formed actin filaments [11]. CAPG is hypothesized to play a role in regulating cytoplasmic or nuclear structures through interactions with actin, such as cell differentiation, membrane ruffling, and cell motility [12]. Although the function of CAPG has not been extensively studied, it has been reported that CAPG is upregulated in various malignancies, suggesting its potential role as a tumor driver, particularly contributing to cancer cell invasion and metastasis [13–16]. A previous study shows that bone marrow-derived macrophages of CAPG knockout (KO) mice exhibited distinct motility defects [17]. In addition, CAPG plays a unique role in receptor-mediated ruffling, phagocytosis, and vesicle rocketing of macrophages [18]. Moreover, a previous study exhibits that CAPG has a potential regulating effect in the polarization of tumor-associated macrophages (TAMs) in glioma [19]. CAPG has also been reported to be specifically upregulated in Tregs during chronic helminth infection [20]. These findings indicate that CAPG may not only function as an oncogene but can prospectively be used as a predictive biomarker for cancer patient prognosis and the immunotherapy efficacy.

Here, we investigated CAPG expression in ovarian cancer by utilizing patient data from various public databases. Our results showed that patients with high tumoral CAPG expression had significantly shorter 5-year OS. By performing multidimensional database analysis, we evaluated the coexpression and functional networks related to CAPG in ovarian cancer. Inflammatory response, chemokine and cytokine signaling, cell adhesion, and Toll-like receptor signaling pathways were enriched in the CAPG-related phenotype. Moreover, we investigated the correlation of CAPG with tumor-infiltrating immune cells in ovarian cancer microenvironments. We found that CAPG expression was positively correlated with infiltrating levels of type 1 Tregs, natural Tregs, induced Tregs, TAMs, and TExs while being negatively correlated with infiltrating levels of NKTs and neutrophils. Furthermore, CAPG expression had strong correlations with markers of Tregs, TAMs, MDCs, and TExs. Our results not only shine a light on the important role of CAPG in ovarian cancer but also provide insight into the underlying mechanisms behind CAPG and tumor-immune interactions.

2. Materials and Methods

2.1. TCGA Dataset. The mRNA expression data (379 samples) and clinical information were downloaded from the Cancer Genome Atlas (TCGA) database (https://cancergenome.nih.gov). The following samples were excluded: (1) repeated sequencing results and (2) insufficient survival information. A total of 376 ovarian cancer patients with complete clinical information (i.e., age, sex, primary tumor site, metastatic state at diagnosis, survival time, and survival time) were included in our analysis. Follow-up was started at the time of diagnosis, and OS time was censored at the last date the patient was known to be alive. The expression profiles were extracted from transcriptome RNA High-throughput sequence (HTSeq) data of the ovarian cancer samples. Raw data were processed into Fragments Per Kilobase of transcript per Million mapped reads (FPKM) for further analyses.

2.2. Oncomine Database. Oncomine (https://www.oncomine.org) is a cancer microarray database and web-based data-mining platform [21]. Oncomine is often used for validation the expression level of genes in cancers and paired normal tissues in bioinformatic research [22]. Cancer vs. normal analysis was determined according to the following threshold: p value of 1E-4, fold change of 2.0, and gene rank of top 10%. CAPG expression in OC was based on Bonome Ovarian, Yoshihara Ovarian, TCGA Ovarian, and Lu Ovarian datasets.

2.3. GEPIA Database. The Gene Expression Profiling Interactive Analysis (GEPIA) database (http://gepia2021.cancer-pku.cn/) is an interactive web that includes tumor and normal samples from TCGA and the GTEx projects [23]. GEPIA was used to generate cancer vs. normal dot plot based on CAPG expression in 33 different types of cancer. In addition, the Pearson and Spearman methods were used to determine the gene expression correlation coefficient based on TCGA-OV data. CAPG was used for the y-axis, and other genes of interest are represented on the x-axis.

2.4. UALCAN Database. UALCAN (http://ualcan.path.uab.edu) uses TCGA RNA-seq data and Clinical Proteomic Tumor Analysis Consortium (CPTAC) proteomic expression data from different cancer types [24]. UALCAN was used to analyze how the clinical characteristics of ovarian cancer patients were related to CAPG gene expression, total protein expression, and phosphoprotein expression. The t-test was used to estimate the significance of difference in gene expression levels between groups.

2.5. Kaplan-Meier Plotter Database. The Kaplan-Meier plotter (http://kmplot.com/analysis/) is capable to assess the effect of genes (mRNA, miRNA, and protein) on survival in cancers [25]. Sources for the databases include GEO and TCGA. Sources for the analysis include TCGA, GSE9891, GSE65986, GSE63885, GSE26712, and other GEO datasets. The relationship between CAPG expression levels and prognosis of ovarian cancer patients was analyzed using the Kaplan-Meier plotter. Patients were split by autoselect best
cutoff; the follow-up threshold was 60 months. Excluding biased arrays, 1656 patients were enrolled in overall survival (OS) analysis, and 1435 patients were enrolled in progression-free survival (PFS) analysis.

2.6. LinkedOmics Database. LinkedOmics (http://www.linkedomics.org/login.php) is a publicly available portal that includes multimetrics data from all 32 TCGA cancer types and 10 CPTAC cancer cohorts [26]. CAPG coexpression was analyzed statistically using Pearson’s correlation in 303 patients with HiSeq RNA sequencing. Function module of LinkedOmics performs analysis of Gene Ontology, KEGG/ Panther pathways, miRNA-target enrichment, and transcription factor-target enrichment by the gene set enrichment analysis (GSEA). The genes were also classified using Gene Ontology (GO) according to biological processes, cellular components, and molecular functions. The rank criterion was false discovery rate (FDR) < 0.05, and 500 simulations were performed; enriched gene sets are postprocessed by affinity propagation methods to reduce redundancy.

2.7. GeneMANIA Database. GeneMANIA (http://genemania.org/) finds other genes that are related to a set of input genes [27]. A Gene-Gene Interaction (GGI) network composed of 50 CAPG coexpression genes was constructed. These nodes represent genes closely related to CAPG in terms of physical interactions, shared protein domains, prediction, colocalization, pathway, coexpression, and genetic interactions.

2.8. NetworkAnalyst Database. NetworkAnalyst 3.0 (https://www.networkanalyst.ca/) can create cell type- or tissue-specific protein-protein interaction (PPI) networks, gene regulatory networks, and gene coexpression networks as well as networks for disease, drug, and chemical studies [28]. We use NetworkAnalyst to carry out ovary-specific PPI, TF-gene interactions, and protein–chemical interaction analysis on the CAPG coexpression module. CAPG coexpression module including the top 100 significant genes was positively and negatively correlated with CAPG in TCGA-OV. In these networks, nodes represent individual genes/proteins/chemicals, while the edges which connect nodes correspond to a known, curated interaction between a given pair of nodes.

2.9. TIMER Database. TIMER (Tumor IMMune Estimation Resource, http://timer.cistrome.org/) is a comprehensive resource for systematical analysis of immune infiltrates across diverse cancer types [29]. In the outcome module, Cox proportional hazard model’s covariates were age, stage, purity, and CAPG expression, then present the normalized coefficient of the infiltrate for each model across multiple cancer types in a heat map. Correlations between CAPG expression and gene markers of tumor-infiltrating immune cells were explored via correlation modules by Spearman’s method; correlations were adjusted by tumor purity and age. CAPG was used for the y-axis with gene symbols, and related marker genes are represented on the x-axis as gene symbols. The gene expression level was displayed with Log2 RSEM.

2.10. ImmuCellAI Database. ImmuCellAI (Immune Cell Abundance Identifier, http://bioinfo.life.hust.edu.cn/ImmuCellAI#!/) is a tool to estimate the abundance of 24 immune cells from gene expression dataset including RNA-Seq and microarray data [30]. We use ImmuCellAI to analyze the correlations of CAPG expression with immune cell abundance of TCGA-OV datasets. Moreover, immune infiltration score and immune checkpoint blockade (ICB) response prediction of OC patients were assessed by ImmuCellAI.

2.11. Statistical Analysis Database. The expression level of the CAPG gene in patients with OC was evaluated by using box plots. The cutoff value of CAPG expression was selected by ROC curve and Youden’s index. The Wilcoxon signed-rank test and logistic regression were performed to analyze the association between clinical features and CAPG expression in OC. The Kaplan-Meier analysis was performed to draw survival curves. The univariate Cox analysis is used to screen potential prognostic factors, and multivariate Cox analysis is used to verify the prognostic factors. All statistical analyses were performed using SPSS software (version 19.0) or GraphPad Prism 6; a p value less than 0.05 is considered statistically significant.

3. Results

3.1. The CAPG Expression Levels in Different Types of Human Cancers. CAPG expressions in tumor and normal tissues of patients across multiple cancer types were analyzed via Oncomine. The results showed that CAPG expression was higher in numerous solid tumors when compared to the normal tissues most notably in brain cancer, breast cancer, ovarian cancer, and pancreatic cancer (Figure 1(a)). To confirm these results, we examined CAPG expression across multiple malignancies in the GEPIA database and found that CAPG levels were also significantly higher in BRCA (breast invasive carcinoma), GBM (glioblastoma multiforme), OV (ovarian serous cystadenocarcinoma), and PAAD (pancreatic adenocarcinoma) when compared to adjacent normal tissues (Figure 1(b)).

3.2. Elevated Expression of CAPG in Ovarian Cancer. Although OC is the most lethal gynecologic malignancy worldwide, CAPG expression and its potential prognostic impact on OC have not been thoroughly evaluated. To evaluate CAPG expression in ovarian cancer, we retrieved data from Oncomine containing multiple ovarian cancer cohorts generated by independent studies. We observed that in all the cohorts analyzed, CAPG mRNA level was significantly higher in tumor tissues than in normal tissues (Figure 2(a)). Further subgroup analysis of CPTAC samples in the UALCAN database showed that CAPG protein expression was significantly changed in OC subgroup analysis in respect to disease stage, tumor grade, and age (Figure 2(b)).

3.3. Baseline Characteristics of Patients. The RNA-seq data for a total of 376 ovarian cancer patients was acquired from TCGA-OV database. The detailed clinical features are listed
**Figure 1:** CAPG expression level in cancers. (a) Increased or decreased CAPG in datasets of different cancers compared with normal tissues in the Oncomine database. Cell color is determined by the best gene rank percentile for the analyses within the cell. (b) Human CAPG expression levels in different tumor types and paired normal tissues from the GEPIA database. Log2 (TPM + 1) scale.
Figure 2: Continued.
in Table S1. Among the 376 participants, 90 were ≤50 years old (24.2%) and 286 were >50 years old (53.7%). All the patients’ cancer histological types were serous cystadenocarcinoma (100%). In terms of FIGO stage, 1 patient was stage I (0.3%), 22 were stage II (5.9%), 293 were stage III (77.9%), 57 were stage IV (15.1%), and 3 were not available (0.8%). The 5-year vital status included 146 alive (38.8%) and 230 dead (61.2%).

3.4. Correlation between CAPG Expression and Clinical Features. The association identified between CAPG expression and clinical features in TCGA-OV is summarized in Table 1. ICB response prediction and immune infiltration score were analyzed by ImmuCellAI. CAPG expression levels were significantly correlated with 5-year vital status (\( p = 0.028 \)), ICB response prediction (\( p = 0.003 \)), and immune infiltration score (\( p = 6.33e - 05 \)). However, CAPG expression was not significantly correlated with other clinical features such as FIGO stage, lymphatic invasion, and residual tumor size.

Univariate Cox analysis showed that high CAPG expression (HR = 1.359, 95% confidence interval \( [1.025 - 1.801, \ p = 0.033] \), residual tumor size (HR = 1.294, 95% CI = 1.123 - 1.490, \( p = 3.56e - 04 \)), and platinum-free interval (HR = 2.233, 95% CI = 1.907 - 2.615, \( p = 2.07e - 23 \)) were unfavorable predictors; however, chemotherapy (HR = 0.276, 95% CI = 0.178 - 0.427, \( p = 8.11e - 09 \)) and primary therapy outcomes (HR = 0.321, 95% CI = 0.215 - 0.479, \( p = 2.57e - 08 \)) were favorable predictors (Table 2). Further multivariate Cox analysis demonstrated that CAPG expression (HR = 1.713, 95% CI = 1.196 - 2.454, \( p = 0.003 \)), residual tumor size (HR = 1.393, 95% CI = 1.141 - 1.700, \( p = 1.11e - 03 \)), primary therapy outcomes (HR = 0.430, 95% CI = 0.268 - 0.689, \( p = 4.59e - 04 \)), and platinum-free interval (HR = 2.110, 95% CI = 1.741 - 2.558, \( p = 2.85e - 14 \)) were independent prognostic factors for OC.

3.5. CAPG Expression Is Survival-Associated. Kaplan-Meier survival analysis was used to assess the association between CAPG expression and the survival outcomes of TCGA-OV cohorts. Although the two groups’ disease-free survival (DFS) showed no significant difference \( (n = 376, \ p = 0.668) \), the high CAPG expression group had significantly shorter overall survival (OS) \( (n = 376, \ p = 0.032) \) compared to the low expression group (Figure 2(c)). Moreover, we used the Kaplan-Meier plotter database to verify our results. Consistently, in these databases, the high-expression group had significantly shorter OS \( (n = 1657, \ p = 0.0048) \) and DFS \( (n = 1436, \ p = 0.027) \) than the low-risk group (Figure 2(d)).
transcription regulator activity, zinc ion binding, RNA binding, and protein domain-specific binding.

3.7. GSEA Identifies CAPG-Related Signaling Pathways. We performed gene set enrichment analysis (GSEA) on the CAPG coexpression datasets to identify Gene Ontology and signaling pathways that were differentially activated in TCGA-OV cohort. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis showed the top 5 significant enrichment pathways which are antigen processing and presentation, oxidative phosphorylation, lysosome, Th1 and Th2 cell differentiation, and chemokine signaling. Panther pathway analysis showed significantly enriched pathways which are B cell activation, toll receptor signaling, and DNA replication (Figure 4(a)). The gene sets related to cytokine-cytokine receptor interaction, cell adhesion molecules, Th1 and Th2 cell differentiation, oxidative phosphorylation, amino sugar and nucleotide sugar metabolism, and the toll-like receptor signaling pathway showed differential enrichment in the CAPG expression phenotype (Figure 4(b)). These results suggest that there is a widespread impact of CAPG on the immune regulation, cell mobility, and metabolism.

### Table 1: Correlations between CAPG expression and clinicopathological features of patients with ovarian cancer.

| Clinical characteristics            | CAPG low (n = 206) | CAPG high (n = 170) | p value |
|-----------------------------------|-------------------|---------------------|---------|
| Race                              |                   |                     |         |
| Asian                             | 6                 | 5                   | 0.972   |
| Non-Asian                         | 195               | 159                 |         |
| Subdivision                       |                   |                     |         |
| Left/right                        | 49                | 52                  | 0.172   |
| Bilateral                         | 143               | 110                 |         |
| Stage                             |                   |                     |         |
| I + II                            | 13                | 10                  | 0.76    |
| III + IV                          | 181               | 159                 |         |
| Grade                             |                   |                     |         |
| G1 + G2                           | 26                | 17                  | 0.384   |
| G3                                | 172               | 150                 |         |
| Lymphatic invasion                |                   |                     |         |
| Yes                               | 53                | 47                  | 0.292   |
| No                                | 21                | 27                  |         |
| Venous invasion                   |                   |                     |         |
| Yes                               | 36                | 27                  | 0.147   |
| No                                | 17                | 23                  |         |
| Residual tumor size               |                   |                     |         |
| >10 mm                            | 56                | 40                  | 0.562   |
| ≤10 mm                            | 130               | 107                 |         |
| Chemotherapy                      |                   |                     |         |
| Yes                               | 188               | 158                 | 0.55    |
| No                                | 18                | 12                  |         |
| Targeted molecular therapy        |                   |                     |         |
| Yes                               | 22                | 10                  | 0.097   |
| No                                | 184               | 160                 |         |
| Hormone therapy                   |                   |                     |         |
| Yes                               | 20                | 11                  | 0.256   |
| No                                | 186               | 159                 |         |
| Immunotherapy                     |                   |                     |         |
| Yes                               | 4                 | 6                   | 0.341   |
| No                                | 202               | 164                 |         |
| Radiation therapy                 |                   |                     |         |
| Yes                               | 16                | 9                   | 0.338   |
| No                                | 190               | 161                 |         |
| Platinum-free interval            |                   |                     |         |
| ≥6 months                         | 111               | 86                  | 0.533   |
| <6 months                         | 52                | 47                  |         |
| Primary therapy outcomes          |                   |                     |         |
| SD + PD                           | 142               | 112                 | 0.372   |
| CR + PR                           | 24                | 25                  |         |
| New tumor event after initial treatment |   |                     |         |
| None/locoregional                 | 82                | 75                  | 0.399   |
| Progression/recurrence            | 124               | 95                  |         |
| Vital status (5 years)            |                   |                     |         |
| Dead                              | 96                | 98                  | 0.028   |
| Alive                             | 110               | 71                  |         |
| ICB response prediction           |                   |                     |         |
| Response                          | 139               | 89                  | 0.003   |
| Not response                      | 67                | 81                  |         |
| Immune infiltration score         |                   |                     |         |
| Low                               | 149               | 89                  | 6.33 E−05|
| High                              | 57                | 81                  |         |

Italic values indicate p < 0.05.
8 Disease Markers

Table 2: Univariate and multivariate Cox regression analyses of clinical characteristics associated with 5-years OS.

| Clinical characteristics                                      | Univariate analysis | Multivariate analysis |
|---------------------------------------------------------------|---------------------|-----------------------|
| Age (≤50 vs. >50)                                             | 1.390 0.971–1.988   | 1.391 0.898–2.157     |
| Race (Asian vs. non-Asian)                                    | 1.304 0.482–3.525   | 0.601                 |
| Subdivision (left/right vs. bilateral)                        | 0.949 0.685–1.314   | 0.751                 |
| Stage (I + II vs. III + IV)                                   | 1.459 0.918–2.321   | 0.11                  |
| Grade (G1 + G2 vs. G3)                                        | 1.263 0.930–1.715   | 0.135                 |
| Lymphatic invasion (Y vs. N)                                  | 1.463 0.845–2.533   | 0.174                 |
| Venous invasion (Y vs. N)                                     | 0.978 0.510–1.878   | 0.948                 |
| Residual tumor size (>10 mm vs. ≤10 mm)                       | 1.294 1.123–1.490   | 3.56 E–04             |
| Chemotherapy (Y vs. N)                                       | 0.276 0.178–0.427   | 8.11 E–09             |
| Targeted molecular therapy (Y vs. N)                          | 0.573 0.320–1.029   | 0.062                 |
| Hormone therapy (Y vs. N)                                     | 0.821 0.511–1.319   | 0.416                 |
| Immunotherapy (Y vs. N)                                      | 0.473 0.175–1.273   | 0.138                 |
| Radiation therapy (Y vs. N)                                   | 0.721 0.418–1.242   | 0.238                 |
| Primary therapy outcomes (SD + PD vs. CR + PR)                | 0.321 0.215–0.479   | 2.57 E–08             |
| New tumor event after initial treatment (Y vs. N)             | 1.150 0.843–1.568   | 0.377                 |
| Platinum-free interval (≥6 months vs. <6 months)              | 2.233 1.907–2.615   | 2.07 E–23             |
| ICB response prediction (R vs. NR)                            | 0.793 0.589–1.068   | 0.127                 |
| Infiltration score (low vs. high)                             | 0.820 0.604–1.111   | 0.201                 |
| CAPG expression (high vs. low)                                | 1.359 1.025–1.801   | 0.033                 |

Abbreviations: HR: hazard ratio; CI: confidence interval; Y: yes; N: no; SD: stable disease; PD: progressive disease; CR: complete response; PR: partial response; R: response; NR: not response. Italic values indicate \( p < 0.05 \).

3.8. Regulators of CAPG Networks in Ovarian Cancer. To further explore the CAPG regulators in respect to OC, we analyzed microRNAs (miRNAs) and transcription factor (TF) enrichment of CAPG coexpressed genes by the Linkedomics database (Table S3). The top 5 most significant miRNAs were MIR-519, MIR-181, MIR-93, MIR-302, and MIR-372. The most enriched TFs were ETS2, PU1, E2F, IRF, NFIB, and YY1.

The protein-protein interaction (PPI) network was assembled based on CAPG coexpressed genes in the TCGA-OV cohort by GeneMANIA. Analysis showed that the 50 most significant coexpressed genes plays roles in phagocytosis, leukocyte migration, and the negative regulation of immune system processes (Figure 5(a)). Next, the CAPG coexpression network was assembled based on ovary-specific data collected from the DifferentialNet database [31] by NetworkAnalyst (Figure 5(b)). The top 5 hub proteins were DEXH-Box Helicase 9 (DHX9), Homeodomain Interacting Protein Kinase 2 (HIPK2), Spi-1 Proto-Oncogene (SP11), Fibronectin 1 (FN1), and Rac Family Small GTPase 2 (RAC2). Further, a graph of TF-miRNA coregulatory interactions of the CAPG coexpressed genes was constructed based on the RegNetwork database [32] (Figure 5(c)). From this, the top five TFs identified were Spi-1 Proto-Oncogene (SP11), Myelocytomatosis Oncogene (MYC), MYC-Associated Factor X (MAX), YIN-YANG1 (YY1), and BCL6 Corepressor (BCOR).

3.9. Identifies Potential Target Drugs of CAPG Networks. To gain insight into potential target drugs based on our established CAPG coexpressed gene network, we examined protein-chemical interactions from the Comparative Toxigenomics Database (CTD) [33]. Excluding hazardous chemicals, the top 5 drugs were valproic acid, JQ1, tretinoin, vorinostat, and vitamin E (Figure 5(d)). Valproic acid (valproate, VPA) has been tested in the treatment of AIDS and numerous cancers due to its histone-deacetylase-inhibiting effects [34]. It can also resensitize cisplatin-resistant ovarian cancer cells [35]. JQ1 inhibits tumor growth when used in combination with cisplatin, and it suppresses the JAK/STAT signaling pathway in OC [36]. Tretinoin (ATRA), an annexin A2 signaling pathway inhibitor, can inhibit OC proliferation and invasion [37]. Vorinostat (suberoylanilide hydroxamic acid, SAHA) was the first histone deacetylase inhibitor approved by the FDA. It can enhance the activity of the chemotherapy drug, olaparib, by targeting homologous recombination DNA repair in OC [38]. Van Impe et al. reported that a CapG single-domain antibody or nanobody could strongly reduce breast cancer metastasis [39]. Based on these things, these drugs show promising potential as novel therapies against OC via CAPG networks that warrants further evaluation.

3.10. CAPG Expression Is Correlated with Immune Infiltration Level in Ovarian Cancer. Tumor immune phenotypes are independent predictors of the outcome of immunotherapy treatment and OS in ovarian cancer patients [40]. In the ImmuCellAI database, CAPG expression also had a significant positive correlation with an abundance of type 1 regulatory T cell (Tr1, \( p = 1.8e−04 \), natural
Figure 3: Continued.
regulatory T cell (nTreg, \( p = 7e - 11 \)), induced regulatory T cell (iTreg, \( p = 4e - 05 \)), tumor-associated macrophage (TAM, \( p = 0.0024 \)), and exhausted T (Tex, \( p = 4.8e - 06 \)). Meanwhile, CAPG expression had a negative correlation with an abundance of natural killer T cell (NKT, \( p = 0.0041 \)) and neutrophil (\( p = 6.6e - 04 \)) (Figure 6(a)).

Cox analysis with multiple algorithms (TIMER, CIBERSORT, quanTIseq, XCell, MCP-counter, and EPIC) was used to examine immune infiltration levels across TCGA-OV data, with the covariate being CAPG expression. This analysis showed that infiltration levels of naïve B cell, M2 macrophage, EC (endothelial cell), and CAF (cancer-associated fibroblast) were unfavorable predictors, while infiltration levels of CD8+ T cell central memory, CD4+ T memory cell, T follicular helper cell (Tfh), B cell, plasma B cell, M1 macrophage, and plasmacytoid dendritic cell were favorable predictors (Figure 6(b)). Further Kaplan-Meier plots showed that the differences in OS were stratified by both the estimated level of immune cell infiltration and CAPG expression level in respect to TCGA-OV cohort. Survival analysis showed that CD8+ T, CD4+ T memory, Tfh, plasma B cell, M1 macrophage, and CAF had a statistically significant positive association with OS while naïve B cell and M2 macrophage had a negative association (Figure 6(c)). These findings strongly suggest that CAPG plays a specific role in promoting infiltration of immunosuppressive cells in ovarian cancer.

### 3.11. Correlation between CAPG Expression and Immune Marker Genes

Moreover, we focused on the correlation between CAPG and immune markers in various immunosuppressive cells present in OC. These immune marker genes included those for T helper 2 cell (Th2), Treg, TAM, M2 macrophage, MDSC, EC, CAE, and Tex. The correlation adjustment in respect to purity or age was done in TIMER (Table 3).

Interestingly, we found that expression of FOXP3, CD25, CCR8, and TGFβ in respect to Treg; CCL2 and CD68 in respect to TAM; CD163, VSIG4, and MS4A4A in respect to M2 macrophage; CD33 and CD11b in respect to MDSC; and PDL1, CTLA4, LGALS3, TIM3, GZMB, 2B4, and TIGIT in respect to Tex was significantly correlated with CAPG expression in OC (Figures 7(a)–7(e)). Further GEPIA database analysis showed a correlation between CAPG and markers of Treg, TAM, M2 macrophage, MDSC, and Tex which are similar to those in TIMER (Table 4).

Therefore, these results further confirmed that CAPG may participate in the recruitment of immunosuppressive cells to ovarian cancer, leading to an exhausted T cell phenotype and eventually tumor progression.

### 4. Discussion

The overexpression of CAPG is associated with poorer prognosis in multiple cancers. For instance, CAPG was found to be upregulated in bladder cancer and associated with clinical aggressiveness and worse prognosis [16]. Additionally, high CAPG levels significantly correlated with shorter relapse-free survival as well as enhanced paclitaxel resistance in breast cancer patients [12]. Although CAPG overexpression
has been reported in 18/47 (38%) of OC patients [41]. CAPG expression and its potential prognostic impact on OC have not been thoroughly evaluated. Our study found that CAPG gene expression was significantly higher in OC than in normal ovarian tissues and that patients with high CAPG expression had significantly shorter OS in TGGA-OV cohort (n = 1376, p = 0.032) and Kaplan-Meier plotter database (n = 1657, p = 0.0048). Cox analysis confirmed that CAPG expression (HR = 1.713, 95% CI = 1.196–2.454, p = 0.003) was an independent risk factor for OS in OC.

Several studies found that CAPG participates in a variety of cell functions and pathways. Gau et al. reported that CAPG participates in a variety of cell functions and pathways. Gau et al. reported that CAPG participates in a variety of cell functions and pathways.
CAPG was one of the key regulators of actin cytoskeleton/cell adhesion and cell migration associated with the loss of BRCA1 function in OC via quantitative proteomics study [42]. Bahassi et al. investigated CAPG involvement in tumor cell motility and cytoskeletal dynamics in a clinically derived human fibrosarcoma cell line [43]. Parikh et al.'s findings suggest that specific motility deficits in macrophages, dendritic cells, and neutrophils render CAPG(-/-) mice more susceptible to Listeria infection [17]. Witke et al. reported that the loss of CAPG in bone marrow macrophages profoundly inhibits macrophage colony-stimulating factor (CSF-) stimulated ruffling [18]. Renz et al. noted that increased expression of the CAPG protein triggers an increase in cell motility in invasive breast cancer [44]. To probe the signaling events in controlling abnormal CAPG expression, we tested the CAPG coexpression network. Consistent with the above studies, we found that CAPG's function enriched in respect to cell-cell adhesion, immune system process, cytokine production, immune response, and cell activation. High CAPG expression was associated with cell adhesion, inflammatory response, and chemokine and cytokine signaling pathways.

For mining regulators potentially responsible for CAPG dysregulation, we found that CAPG in OC is associated with
Figure 6: Correlations of CAPG expression with immune infiltration level in OC. (a) Low/high CAPG expression with immune cell abundance in TCGA-OV by ImmuCellAI. (b) The multivariable Cox proportional hazard model of CAPG expression and immune infiltration level of multiple immune. Red indicates significant positive association, blue indicates significant negative association, and gray denotes a nonsignificant result. (c) Kaplan-Meier plots show the difference of OS stratified by both the immune infiltration level and CAPG expression level.
familial network can be viewed as a functional module which associates with tumorigenesis [46]. Various studies indicate that the Myc c-gene regulatory programs [47]. Yang et al. reported that N-Myc and STAT Interactor (NMI) and CAPG were upregulated in glioblastoma, functioning as an in-flammatory programs [47]. Yang et al. reported that N-Myc and STAT Interactor (NMI) and CAPG were upregulated in glioblastoma, functioning as an inflammatory regulator of tumor progression. Some studies have shown that overexpression of DHX9 is associated with aggressive tumor behavior and decreased survival in patients with glioblastoma [46]. The Myc family network can be viewed as a functional module which acts to convert environmental signals into specific gene regululatory programs [47]. Yang et al. reported that N-Myc and STAT Interactor (NMI) and CAPG were upregulated in glioblastoma, functioning as an inflammatory response. The Myc family network can be viewed as a functional module which acts to convert environmental signals into specific gene reguulatory programs [47]. Yang et al. reported that N-Myc and STAT Interactor (NMI) and CAPG were upregulated in glioblastoma, functioning as an inflammatory response [48]. Sheng et al. showed that cisplatin-mediated miR-145 downregulation increased PD-L1 expression via targeting the c-Myc transcription factor, thereby inducing T cell response. The Myc family network can be viewed as a functional module which acts to convert environmental signals into specific gene regulatory programs [47]. Yang et al. reported that N-Myc and STAT Interactor (NMI) and CAPG were upregulated in glioblastoma, functioning as an inflammatory response. The Myc family network can be viewed as a functional module which acts to convert environmental signals into specific gene regulatory programs [47]. Yang et al. reported that N-Myc and STAT Interactor (NMI) and CAPG were upregulated in glioblastoma, functioning as an inflammatory response [48]. Sheng et al. showed that cisplatin-mediated miR-145 downregulation increased PD-L1 expression via targeting the c-Myc transcription factor, thereby inducing T cell response.
immune responses more di
ture DCs, MDSCs, TAMs, and CAFs, which make antitumor
cytokines that recruit suppressive cells such as Tregs, imma-
major obstacle for cancer immunity. Tumor cells can secrete
ing pathways in tumor cells help produce a suppressive tumor
the genomic analysis of tumor samples. These altered signal-
apoptosis in OC [49]. Jimenez-Sanchez et al. investigated
associated immune cell exclusion with the amplification of
Myc target genes in treatment-naive OC [50]. Our results,
partly in line with the findings in the above studies, showed
that CAPG participated in cancer progression and immune
regulation with genes like Myc. However, the mechanisms
behind these interactions require further investigation.
The tumor microenvironment is the noncancerous cells
present in and around a tumor having a strong influence on
the genomic analysis of tumor samples. These altered signal-
ing pathways in tumor cells help produce a suppressive tumor
microenvironment enriched for inhibitory cells, posing a
major obstacle for cancer immunity. Tumor cells can secrete
cytokines that recruit suppressive cells such as Tregs, imma-
ture DCs, MDSCs, TAMs, and CAFs, which make antitumor
immune responses more difficult to instigate and sustain [51, 
52]. Nelson reported that ovarian tumors are often infiltrated
by CD4+ CD25+ FoxP3+ regulatory T cells, which leads to
the suppression of antitumor immunity [53]. Cui et al. dem-
strated that MDSCs inhibited T cell activation and
enhanced ovarian cancer stem cell gene expression, sphere
formation, and metastasis [54]. Zhou et al. reported that eox-
somes released from TAMs transfer miRNAs that induce a
Treg/Th17 imbalance and generate an immune-suppressive
microenvironment that facilitates OC progression and metas-
tasis [55]. Ji et al. showed that IL-8 secreted from CAFs could
stimulate malignant growth and increased OC cisplatin resis-
tance [56]. The above suppressive cells can cause dysfunction
in effector T cells, causing a state called “T cell exhaustion.” It
is characterized by progressive loss of function, changes in
transcriptional profiles, and sustained expression of inhibitory
receptors [57]. PD1, CTLA4, LAG3, TIM3, and GZMB are cru-
ial genes that regulate T cell exhaustion and are associated
with inefficient control of tumors [58]. PD1+ TIM3+ CD8+
T cells present all features of functional exhaustion and corre-
late with poor disease outcome [59]. Our results demonstrate
that CAPG expression was significantly positively correlated
with immunosuppressive cell (Tregs, TAMs, MDSCs, and
CAFs) infiltration and T cell exhaustion markers (PD-1,
CTLA4, TIM3, GZMB, 2B4, and TIGIT). Together, these find-
ings suggest that the CAPG plays a crucial role in immune
response regulation and T cell exhaustion in OC.
In summary, we showed that high CAPG expression is
 correlated with clinical progression and can be considered an
independent risk factor for OS in patients with OC. CAPG
can regulate a variety of immune-related signaling pathways
in OC, which may recruit immunosuppressive cells to create
immunosuppressive microenvironment, leading to an
exhausted T cell phenotype. The importance of this study is
that we discovered that CAPG may serve as an important
Data Availability

The authors certify that all the original data in this research could be obtained from a public database. All data generated or analyzed during this study are included in this article.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors’ Contributions

Senwei Jiang and Yuebo Yang contributed to the conceptualization; Xiaomao Li contributed to the supervision; Senwei Jiang and Qingqian Ye contributed to the data curation; Senwei Jiang and Yu Zhang contributed to the methodology; Yuebo Yang and Min Zheng contributed to the resources; Senwei Jiang contributed to the software; Senwei Jiang contributed to the visualization; Senwei Jiang contributed to the original draft; Yuebo Yang contributed to the review and editing; Qingqian Ye, Jiao Song, Min Zheng, and Xiaomao Li contributed to the funding acquisition. Senwei Jiang and Yuebo Yang contributed equally to this work.

Acknowledgments

This work was supported by the Nature Science Foundation of Guangdong Province (grant number 2021A1515011542), Chinese Society of Clinical Oncology Foundation (grant number Y-2019AZQN-1049), Guangdong Basic and Applied Basic Research Foundation (grant number 2020A1515111091), and National Natural Science Foundation of China (grant number 81872434).

Supplementary Materials

TABLE S1: clinical characteristics of TCGA-OV patients. TABLE S2: CAPG positively/negatively correlated significant genes. TABLE S3: the miRNA target and transcription factor networks of CAPG in OC. Abbreviations: ES: enrichment score; NES: normalized enrichment score; FDR: false discovery rate. (Supplementary Materials)

References

[1] R. L. Siegel, K. D. Miller, and A. Jemal, “Cancer statistics, 2020,” CA: A Cancer Journal for Clinicians, vol. 70, pp. 7–30, 2020.
[2] S. Lheureux, C. Gourley, I. Vergote, and A. M. Oza, “Epithelial ovarian cancer,” Lancet, vol. 393, no. 10177, pp. 1240–1253, 2019.
[3] C. Yang, B. R. Xia, Z. C. Zhang, Y. J. Zhang, G. Lou, and W. L. Jin, "Immunotherapy for ovarian cancer: adjuvant, combination, and neoadjuvant," Frontiers in Immunology, vol. 11, article 577869, p. 2595, 2020.
[4] F. De Felice, L. Vertechy, E. Giudice et al., “Evolution of Clinical Trials in Ovarian Cancer Management over the Past 20 Years: Never Settle Down, Always Go Beyond," Journal of Oncology, vol. 2021, Article ID 1682532, 2021.
[5] J. A. Beaver, R. L. Coleman, R. C. Arend et al., “Advancing drug development in gynecologic malignancies,” Clinical Cancer Research, vol. 25, no. 16, pp. 4874–4880, 2019.
[6] Y. Zhou, C. L. Chen, S. W. Jiang et al., “Retrospective analysis of the efficacy of adjuvant CIK cell therapy in epithelial ovarian cancer patients who received postoperative chemotherapy,” Oncoimmunology, vol. 8, no. 2, article e1528411, 2019.
[7] C. Valero, M. Lee, D. Hoey et al., "Response rates to anti-PD-1 immunotherapy in microsatellite-stable solid tumors with 10 or more mutations per megabase," *JAMA Oncology*, vol. 7, no. 5, pp. 739–743, 2021.

[8] H. Ji, M. Ren, T. Liu, and Y. Sun, "Prognostic and immunological significance of CXCR2 in ovarian cancer: a promising target for survival outcome and immunotherapeutic response assessment," *Disease Markers*, vol. 2021, Article ID 5350232, 2021.

[9] F. De Felice, C. Marchetti, I. Palaia et al., "Immunotherapy of ovarian cancer: the role of checkpoint inhibitors," *Journal of Immunology Research*, vol. 2015, Article ID 191832, 2015.

[10] P. Silacci, L. Mazzolai, C. Gaucci, N. Stergiopulos, H. L. Yin, and H. Ji, M. Ren, T. Liu, and Y. Sun, "Disease Markers*, vol. 724, 2010.

[11] J. Wei, L. Feng, and L. Wu, "Integrated analysis identified CAPG as a prognostic factor correlated with immune infiltrates in lower-grade glioma," *Clinical and Translational Medicine*, vol. 10, no. 2, p. e51, 2020.

[12] L. E. Layland, J. Mages, C. Loddenkemper et al., "Pronounced phenotype in activated regulatory T cells during a chronic helminth infection," *Journal of Immunology*, vol. 184, pp. 713–724, 2010.
[38] P. A. Konstantinopoulos, A. J. Wilson, J. Saskowski, E. Wass, and D. Khabele, "Suberoylanilide hydroxamic acid (SAHA) enhances olaparib activity by targeting homologous recombination DNA repair in ovarian cancer," Gynecologic Oncology, vol. 133, pp. 599–606, 2014.

[39] K. Van Impe, J. Bethynse, S. Cool et al., "A nanobody targeting the F-actin capping protein CapG restrains breast cancer metastasis," Breast Cancer Research, vol. 15, no. 6, pp. 1–15, 2013.

[40] M. Hornburg, M. Desbois, S. Lu et al., "Single-cell dissection of cellular components and interactions shaping the tumor immune phenotypes in ovarian cancer," Cancer Cell, vol. 39, no. 7, pp. 928–944, 2021.

[41] J. Glaser, M. H. Neumann, Q. Mei et al., "Macrophage capping protein CapG is a putative oncogene involved in migration and invasiveness in ovarian carcinoma," BioMed Research International, vol. 2014, Article ID 379847, 2014.

[42] D. M. Gau, J. L. Lesnock, B. L. Hood et al., "BRCA1 deficiency in ovarian cancer is associated with alteration in expression of several key regulators of cell motility - a proteomics study," Cell Cycle, vol. 14, pp. 1884–1892, 2015.

[43] E. M. Bahassi, S. Karyala, C. R. Tomlinson, M. A. Sartor, M. Medvedovic, and R. F. Hennigan, "Critical regulation of genes for tumor cell migration by AP-1," Clinical & Experimental Metastasis, vol. 21, no. 4, pp. 293–304, 2004.

[44] M. Renz, B. Betz, D. Niederaecher, H. G. Bender, and J. Langowski, "Invasive breast cancer cells exhibit increased mobility of the actin-binding protein CapG," International Journal of Cancer, vol. 122, pp. 1476–1482, 2008.

[45] R. Puca, L. Nardinocchi, G. Pistritito, and G. D’Orazi, "Overexpression of HIPK2 circumvents the blockade of apoptosis in chemo-resistant ovarian cancer cells," Gynecologic Oncology, vol. 109, pp. 403–410, 2008.

[46] P. S. Patel, K. J. Abraham, K. K. N. Guturi et al., "RNF168 regulates R-loop resolution and genomic stability in BRCA1/2-deficient tumors," Journal of Clinical Investigation, vol. 131, no. 3, article e140105, 2021.

[47] C. Grandori, S. M. Cowley, L. P. James, and R. N. Eisenman, "The Myc/Max/Mad network and the transcriptional control of cell behavior," Annual Review of Cell and Developmental Biology, vol. 16, pp. 653–699, 2000.

[48] Q. Yang, R. Wang, B. Wei et al., "Candidate biomarkers and molecular mechanism investigation for glioblastoma multi-forme utilizing WGCNA," BioMed Research International, vol. 2018, Article ID 4246703, 2018.

[49] Q. Sheng, Y. Zhang, Z. Wang, J. Ding, Y. Song, and W. Zhao, "Cisplatin-mediated down-regulation of miR-145 contributes to up-regulation of PD-L1 via the c-Myc transcription factor in cisplatin-resistant ovarian carcinoma cells," Clinical and Experimental Immunology, vol. 200, pp. 45–52, 2020.

[50] A. Jimenez-Sanchez, P. Cybulska, K. L. Mager et al., "Unraveling tumor-immune heterogeneity in advanced ovarian cancer uncovers immunogenic effect of chemotherapy," Nature Genetics, vol. 52, pp. 582–593, 2020.

[51] M. R. Junttila and F. J. de Sauvage, "Influence of tumour micro-environment heterogeneity on therapeutic response," Nature, vol. 501, pp. 346–354, 2013.

[52] S. A. Richard, "Explicating the pivotal pathogenic, diagnostic, and therapeutic biomarker potentials of myeloid-derived suppressor cells in glioblastoma," Disease Markers, vol. 2020, Article ID 8844313, 2020.

[53] B. H. Nelson, "The impact of T-cell immunity on ovarian cancer outcomes," Immunological Reviews, vol. 222, pp. 101–116, 2008.

[54] T. X. Cui, I. Kryczek, L. Zhao et al., "Myeloid-derived suppressor cells enhance stemness of cancer cells by inducing microRNA101 and suppressing the corepressor CtBP2," Immunity, vol. 39, pp. 611–621, 2013.

[55] J. Zhou, X. Li, X. Wu et al., "Exosomes released from tumor-associated macrophages transfer miRNAs that induce a Treg/Th17 cell imbalance in epithelial ovarian cancer," Cancer Immunology Research, vol. 6, pp. 1578–1592, 2018.

[56] Z. Ji, W. Tian, W. Gao, R. Zang, H. Wang, and G. Yang, "Cancer-associated fibroblast-derived interleukin-8 promotes ovarian cancer cell stemness and malignancy through the Notch3-mediated signaling," Frontiers in Cell and Developmental Biology, vol. 9, article 684505, 2021.

[57] C. U. Blank, W. N. Haining, W. Held et al., "Defining T cell exhaustion," Nature Reviews Immunology, vol. 19, no. 11, pp. 665–674, 2019.

[58] E. J. Wherry and M. Kurachi, "Molecular and cellular insights into T cell exhaustion," Nature Reviews Immunology, vol. 15, no. 8, pp. 486–499, 2015.

[59] J. Fucikova, J. Rakova, M. Hensler et al., "TIM-3 dictates functional orientation of the immune infiltrate in ovarian cancer," Clinical Cancer Research, vol. 25, pp. 4820–4831, 2019.