Comprehensive Analysis of Prognostic Value of MEX3A and Its Relationship with Immune Infiltrates in Ovarian Cancer

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MEX3A is a critical RNA-binding ubiquitin ligase that is upregulated in various types of cancer. However, the correlations of MEX3A with prognosis and its molecular mechanism in ovarian cancer (OC) remain unclear. The expression level, prognostic values, and the genetic variations of MEX3A were analyzed via Gene Expression Profiling Interactive Analysis (GEPIA) Oncomine, Kaplan–Meier plotter, and cBioPortal. We used the LinkedOmics database to investigate the functions of MEX3A coexpressed genes and performed visualizing gene interaction network analysis on the GeneMANIA website. The correlations between MEX3A and cancer immune infiltration were analyzed by the Tumor Immune Estimation Resource (TIMER) site and the TISIDB database. Furthermore, in vitro analysis was performed to evaluate the biological functions of MEX3A in OC cells. Our study showed that the expression of the MEX3A in OC was higher than in normal tissues; it had the greatest prognostic value in OC, and strong physical interaction with PABPC1, LAMTOR2, KHDRBS2, and IGF2BP2, which indicated the association between MEX3A and immune infiltration. We also found that MEX3A was negatively related to infiltrating levels of several types of immune cells, including macrophages, neutrophils, dendritic cells (DCs), B cells, and CD8+ T cells. Additionally, in vitro experiments demonstrated that MEX3A promotes proliferation and migration in OC cells. Taken together, MEX3A might influence the biological functions of OC cells by regulating the immune infiltration in the microenvironment as a prognostic biomarker and a potential therapeutic target.

1. Introduction

Ovarian cancer (OC) is a common gynecological malignancy with high mortality. More than 70% of patients with OC are diagnosed with advanced-stage cancer (III and IV) [1]. Although the development of surgery and chemotherapy in ovarian cancer has been advanced in recent decades, the benefits of traditional treatment are limited [2]. Recently, immunotherapy has offered a novel and promising therapeutic strategy. Still, immunotherapy, which has been developing rapidly resulting in major breakthroughs in many areas, cannot achieve a good treatment effect because of a special tumor immune microenvironment [3]. Like many other solid tumors, OC is immunogenic, and the imbalance between immune activation and immune suppression can lead to tumorigenesis and cancer progression. Thus, it is necessary to select and identify reliable immune-related biomarkers and novel targets for immunotherapy strategies necessary to diagnose OC early.

MEX3A is an important component of the Mex3 family, which has a conserved region of about 70 amino acids, including MEX3A, MEX3B, MEX3C, and MEX3D [4]. MEX3A is a kind of RNA-binding protein (RBPs), which has the highly conserved RNA-binding domain and a C-terminal RING finger domain that are involved in posttranscriptional regulatory mechanisms [5]. Recently, MEX3A has been reported as a novel biomarker promoting proliferation and migration in various cancers such as pancreatic ductal adenocarcinoma (PDA), liver cancer, and colorectal cancer [6–8]; yet, its role in OC is still unclear.

In this study, we investigated the mRNA expression, mutation patterns, and prognosis value of MEX3A in OC for the first time based on large database analyses including Oncomine, GEPIA, cBioPortal, PrognoScan, and the
Kaplan–Meier plotter. We also explored the function of the coexpression genes with MEX3A to clarify the potential mechanism in OC by GO and KEGG. In addition, we revealed the potential relationship between the expression of MEX3A and immune infiltration in the OC microenvironment via TIMER and TISIDB. We have further demonstrated that MEX3A enhanced tumor proliferation and migration in vitro. Collectively, our findings revealed the important role of MEX3A and provided a novel target and a valuable insight into the underlying mechanism between MEX3A and tumor-immune interactions in OC.

2. Materials and Methods

2.1. Oncomine Analysis. The MEX3A mRNA expression level was analyzed in OC by the Oncomine platform (http://www
oncine.org/), a publicly accessible, online cancer microarray database with 715 data sets and 86,733 samples that allow for a powerful genome-wide expression analysis [9]. We selected a P value of 0.01 and a fold change of 2 as the threshold, and ranked genes in the top 10% as significant.

2.2. GEPIA Analysis. Gene Expression Profiling Interactive Analysis (GEPIA) is an interactive web used to analyze the RNA sequencing expression, including The Cancer Genome Atlas (TCGA) tumor sample information and Genotype-Tissue Expression (GTEx) normal sample information. GEPIA provides a series of key interactive and customizable functions by using a standard processing pipeline (http://gepia.cancer-pku.cn) [10].

2.3. cBioPortal Analysis. The cBioPortal for Cancer Genomics (http://cbioportal.org) provides an online resource to explore, visualize, and analyze complex cancer genomics and clinical profile data from TCGA [11]. In this study, the cBioPortal was used to access genetic variations in MEX3A (amplifications, deep deletions, and missense mutations), DNA copy number alterations, and mRNA expression z-scores (RNA Seq V2 RSEM). The tab OncoPrint shows an overview of genetic alterations for each sample in MEX3A. Besides, coexpression datasets were analyzed according to the online instructions of cBioPortal, and the R package was used for further enrichment analysis.

2.4. LinkedOmics. LinkedOmics (http://www.linkedomics.org/login.php) is a publicly available web tool used to provide multiomics data of 32 TCGA cancer types [12]. We used the linkInterpreter module to derive biological insights into coexpressed gene enrichment by using Pearson's correlation coefficient. These genes were presented in volcano plots and heat maps.

2.5. Functional Enrichment Analysis. To further explore the functions of MEX3A, Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were performed in R statistical computing environment.

2.6. GeneMANIA. GeneMANIA (http://www.genemania.org) is a flexible, friendly web interface that is used for visualizing gene interaction networks and evaluating gene function [13]. It enables analysis of gene lists and prioritizes the marked genes for functional assays associated with MEX3A. The sources of the edge of the network, which represent the following bioinformatics methods, namely, physical interaction, coexpression, colocation, genetic interaction, and website prediction, were set.

2.7. TIMER Database Analysis. To obtain the MEX3A expression and correlation between MEX3A and immunity cells in TCGA datasets, an online analytical tool called “Tumor Immune Estimation Resource (TIMER)” was used. TIMER is an online database used for evaluating the relationship between clinical associations, mutation, SCNA, and infiltration of different immune cells (B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells) in diverse cancer types [14]. The survival module also showed the Kaplan–Meier plotter and provided the multivariable Cox regression analysis of clinical factors (age, stage, and tumor purity). Once all conditions were defined, TIMER outputs revealed the Cox regression results, including hazard ratios (HR), 95% confidence intervals (CI), and statistical significance (P < 0.05) automatically.

2.8. TISIDB Analysis. TISIDB (http://cis.hku.hk/TISIDB) is a user-friendly web portal, which contains a summary of 988 immune-related antitumor genes for 30 TCGA cancer types [15]. The associations between gene expression and immune features, including lymphocytes, immunomodulators, subtypes, and chemokines, were calculated by high-throughput data analysis. In this research, we used the TISIDB web to analyze the correlations between MEX3A expression and clinical stages, lymphocytes, and subtype immunomodulators in OC.

2.9. Kaplan–Meier Plotter Analysis. The Kaplan–Meier plotter (http://www.kmplot.com) is a common tool for biomarkers used to assess survival and prognosis, which includes gene expression data and survival information of 1,816 clinical tissue samples from OC patients [16]. The overall survival (OS) and progression-free survival (PFS) of OC patients were determined by dividing two groups (high vs. low expression) of patients by median. In addition, we further investigated OS and PFS of different histological subtypes (endometrioid and serous) in MEX3A by using the Kaplan–Meier method. These data were evaluated with a hazard ratio (HR), 95% confidence intervals (CI), and log-rank P value.

2.10. PrognoScan Database Analysis. The relationship between MEX3A expression and prognosis in OC was analyzed by the PrognoScan database (http://www.abren.net/PrognoScan/), such as OS and PFS [17]. The threshold was adjusted to a Cox P value < 0.05 or corrected P value < 0.5.

### Table 1: Survival analysis of MEX3A mRNA in multiple cancers.

| Dataset     | Endpoint             | Probe ID       | Number | Corrected P value | COX P value | In (HR) HR (95% CI-low CI-up) |
|-------------|---------------------|----------------|--------|-------------------|-------------|------------------------------|
| GSE9891     | Overall survival    | 226346_at      | 278    | 0.013633          | 0.007141    | 0.27                         | 1.31 (1.08-1.60)   |
| GSE9891     | Overall survival    | 227512_at      | 278    | 0.114667          | 0.022613    | 0.25                         | 1.28 (1.04-1.59)   |
| GSE17260    | Overall survival    | A_24_P857404   | 110    | 0.004934          | 0.031824    | -0.136                       | 0.72 (0.53-0.97)   |
| GSE17260    | Overall survival    | A_32_P96036    | 110    | 0.001922          | 0.097382    | -1.23                        | 0.78 (0.58-1.05)   |
| GSE17260    | Progress-free survival | A_32_P96036  | 110    | 0.044430          | 0.338936    | -0.11                        | 0.90 (0.72-1.12)   |

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Figure 2: Correlation of MEX3A expression with immune infiltration level in OC. (a) Correlation of MEX3A expression with immune infiltration level in OC (TIMER). Spearman’s correlation of MEX3A with lymphocytes and immunomodulators (TISIDB). (b) Heat maps of correlations between MEX3A expression and TILs by TISIDB. (c) Scatterplots of correlations between MEX3A expression and top 4 TILs. (d) Heat maps of correlations between MEX3A expression and immunostimulators. (e) Scatterplots of correlations between MEX3A expression and top 4 immunostimulators. (f) Heat maps of correlations between MEX3A expression and immunoinhibitors. (g) Scatterplots of correlations between MEX3A expression and top 4 immunoinhibitors. (h) Heat maps of correlations between MEX3A expression and MHC molecules. (i) Scatterplots of correlations between MEX3A expression and top 4 MHC molecules. Red and blue represent positive and negative correlations. The color intensity is directly proportional to the strength of the correlations. *P < 0.05, **P < 0.01, and ***P < 0.001.
2.11. Cell Culture and Transfection. The ES2 cells were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco’s Modified Eagle’s Medium (DMEM; HyClone; GE Healthcare Life Sciences) with 10% FBS at 37°C and 5% CO₂. ES2 cells (to 70% confluency) were seeded on 6-well plates transfected with MEX3A siRNAs designed and synthesized by GenePharma (Shanghai, China), which were transfected into the cells using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific) according to the protocols for the interference expression of MEX3A; the cells were cultured for 48 h or 72 h for further assays.

2.12. Cell Counting Kit-8 (CCK-8) Assay and Colony Formation Assays. Transfected cells were seeded on a 96-well plate at a density of 2 × 10³ cells/well. The CCK-8 solution (10 μl; Dojindo Laboratories, Kumamoto, Japan) was then added to each well of the plate. The plate was incubated for 2 h in the incubator, and the absorbance at each wavelength of 450 nm was measured using an automatic enzyme-linked immune detector.

For the colony formation assay, transfected cells were seeded into 6-well plates. One week later, the cells were fixed with 4% paraformaldehyde and stained with 0.5% (w/v) crystal violet. Then, cell clones were photographed and counted. These experiments were performed in triplicate.

2.13. 5-Ethynyl-2-Deoxyuridine (EdU) Staining Assay. Collected cells were seeded on 24-well plates at a density of 1 × 10⁴ cells/well and incubated for 24 h. According to the protocol of the EdU Kit (BeyoClick™ EDU Cell Proliferation Kit with Alexa Fluor 488; Beyotime, Shanghai, China), after transfection, EdU was added 1:1,000 in the cell medium for 2 h at 37°C. Cells were fixed with 4% paraformaldehyde.

**Figure 3:** Gene-gene interaction network between MEX3A and correlated genes (GeneMANIA). Each node represents a gene. The node size indicates the strength of the interaction. The connecting lines between nodes represent the type of gene-gene interaction, and the line color represents the type of interaction. Node colors represent the possible biological functions of each gene.
Figure 4: Genes differentially expressed in correlation with MEX3A (LinkedOmics). (a) Correlations between MEX3A and genes differentially expressed in OC. (b) Heat maps showing genes positively and negatively correlated with MEX3A in OC (Top 50). (c) Heat maps showing genes negatively correlated with MEX3A in OC. Red indicates positively correlated genes, and blue indicates negatively correlated genes. (d) Barplot representing enriched functions of the upregulated genes coexpressed with MEX3A. (e) Barplot representing enriched pathways of the upregulated genes coexpressed with MEX3A. (f) Barplot representing enriched functions of the downregulated genes coexpressed with MEX3A. (g) Barplot representing enriched pathways of the downregulated genes coexpressed with MEX3A.

Figure 4: Genes differentially expressed in correlation with MEX3A (LinkedOmics). (a) Correlations between MEX3A and genes differentially expressed in OC. (b) Heat maps showing genes positively and negatively correlated with MEX3A in OC (Top 50). (c) Heat maps showing genes negatively correlated with MEX3A in OC. Red indicates positively correlated genes, and blue indicates negatively correlated genes. (d) Barplot representing enriched functions of the upregulated genes coexpressed with MEX3A. (e) Barplot representing enriched pathways of the upregulated genes coexpressed with MEX3A. (f) Barplot representing enriched functions of the downregulated genes coexpressed with MEX3A. (g) Barplot representing enriched pathways of the downregulated genes coexpressed with MEX3A.
for 15 min and treated with 0.3% Triton-X for 10 min at room temperature. Then, the cells were incubated for 30 min with a Click reaction cocktail in the dark. Nuclei were stained with Hoechst 33342 for 10 min. Photographs were taken in three randomly selected fields with an Olympus (Tokyo, Japan) microscope to analyze proliferation rates. Each experiment was performed at least three times.

2.14. Transwell Assay and Wound Healing Assay. Cells (4 × 10^4 cells/well) were incubated in 100 μl culture medium and seeded on the Transwell inserts (Corning Glass Works; Corning, NY, USA) with 8 μm pores to determine the migration ability of the cells. A 600 μl culture medium was added to the lower chamber. After 48 h, the inserts were fixed with 95% ethanol, and 0.5% (w/v) crystal violet was used for staining. Migrated cells were counted in five nonoverlapping locations.

To analyze wound healing, we seeded transfected cells on 6-well plates. When the cell density reached 80-100%, we scraped cells at the bottom of the wells using a sterile 200 μl pipette tip to form a linear gap and culture treated cells with FBS-free DMEM. After 24 h, images of the wells were taken with an inverted fluorescence microscope. All assays were repeated at least three times.

2.15. Quantitative Real-Time PCR. Total RNA was extracted using the TRIzol Reagent (Invitrogen; Thermo Fisher Scientific). According to the manufacturer’s instructions, the concentration of total RNA was measured using Thermo Fisher Scientific NanoDrop ND-100. CDNA was synthesized using the SYBR PrimeScript RT-PCR Kit (Takara Bio, Inc., Japan). Real-time PCR was carried out using a Thermal Cycler Dice™ Real-Time system Tp800 (Takara Bio, Inc.). The primer sequences designed for MEX3A and β-actin are as follows (5′-3′): MEX3A, forward, TGGAGAACTAGGATGTTTCGGG, and reverse, GAGGCAGAGTTGATCGAGACTC; and β-actin, forward, CATGTACGTTGCTATCCAGGC, and reverse, CTCCTTAATGTCACGCACGAT. The mRNA expression of the target gene was analyzed using the 2^-ΔΔCt method.

2.16. Western Blotting. Total protein was obtained from cells using ice-cold RIPA buffer mixed with protease inhibitor cocktails (Roche), and concentration was assayed by a BCA assay. Fifty micrograms of denatured protein was separated by 10% SDS-PAGE and transferred onto PVDF membranes. After blocking with 5% skimmed milk for 1 h at room temperature, the membranes were incubated with antibodies against MEX3A (1:1000; ab79046; Abcam) overnight at 4°C, followed by incubation with a secondary antibody (1:3,000; #A0208; Beyotime, Beijing, China) at room temperature for 1 h. The ECL detection kit was used to detect protein signals.

2.17. Immunohistochemical (IHC) Staining. MEX3A expression was assessed by IHC assay, using previously described protocol [18]. Anti-MEX3A antibody (ab79046; Abcam) was used at a 1:50 dilution at 4°C overnight. Rabbit immunoglobulin G (1:1000; ab6721; Abcam) was used as a negative control. Aperio Scanning System (Aperio Group, LLC) was employed to scan the slides, and Aperio Image Scope.
Table 2: The expression of 771 related genes with MEX3A in cBioPortal.

| Gene       | LogFC | entrezID |
|------------|-------|----------|
| LAMTOR2    | >10   | 28956    |
| RAB25      | >10   | 57111    |
| UBQLN4     | 7.9   | 56893    |
| LMNA       | 7.86  | 4000     |
| SSR2       | 6.9   | 6746     |
| ARHGEF2    | 6.31  | 9181     |
| RFXP4      | 6.27  | 339403   |
| KHDC4      | 5.86  | 22889    |
| SEMA4A     | 7.73  | 64218    |
| SL22A44     | 7.68  | 9673     |
| SCARN4A    | 6.19  | 677771   |
| SNORA80E   | 6.19  | 677823   |
| SYT1       | 6.14  | 23208    |
| RIT1       | 5.73  | 6016     |
| PMF1       | 6.64  | 11243    |
| PMF1-BGLAP | 6.64  | 100527963 |
| GON4L      | 5.68  | 54856    |
| BGLAP      | 5.68  | 54856    |
| PAQR6      | 5.9   | 79957    |
| SMG5       | 5.9   | 23381    |
| CCT3       | 5.84  | 7203     |
| GLMP       | 5.84  | 112770   |
| TMEM79     | 5.84  | 84283    |
| VHLL       | 5.84  | 391104   |
| ASH1L-AS1  | 4.95  | 645676   |
| DAP3       | 4.95  | 7818     |
| MSTD1      | 4.95  | 55154    |
| YY1AP1     | 4.95  | 55249    |
| MSTD2P8    | 4.73  | 100129405 |
| TSACC      | 5.73  | 128229   |
| ASH1L      | 4.13  | 55870    |
| C1ORF61    | 5.67  | NA       |
| RHBG       | 5.67  | 57127    |
| POU5F1P4   | 4.16  | 645682   |
| LRRC71     | 6.98  | 149499   |
| TRIM46     | 4.2   | 80128    |
| DPM3       | 4.14  | 54344    |
| EFNA1      | 4.14  | 1942     |
| GBA        | 4.14  | 2629     |
| GBP1P1     | 4.14  | 2630     |
| KRTCAP2    | 4.14  | 200185   |
| MXI1       | 4.14  | 4580     |
| MUC1       | 4.14  | 4582     |
| SLC59A1     | 4.14  | 55974    |
| THBS3      | 4.14  | 7059     |
| ARHGEF11   | 6.9   | 9826     |
| HDGF       | 5.98  | 3068     |
| INSRR      | 5.98  | 3645     |

Table 2: Continued.

| Gene       | LogFC | entrezID |
|------------|-------|----------|
| ISG20L2    | 5.98  | 81875    |
| MRPL24     | 5.98  | 79590    |
| NTRK1      | 5.98  | 4914     |
| PEAR1      | 5.98  | 375033   |
| PRCC        | 5.98  | 5546     |
| RNNAD1      | 5.98  | 51093    |
| SH2D2A      | 5.98  | 9047     |
| FAM189B     | 3.99  | 10712    |
| SCAMP3      | 3.99  | 10067    |
| CLK2        | 3.79  | 1196     |
| FDPS        | 3.79  | 2224     |
| HCN3        | 3.79  | 57657    |
| PKLR        | 3.79  | 5313     |
| RUSC1       | 3.79  | 23623    |
| RUSC1-AS1   | 3.79  | 284618   |
| IQGAP3      | 5.39  | 128239   |
| MEF2ID      | 5.39  | 4209     |
| CRABP2      | 5.9   | 1382     |
| CYCSP52     | 6.82  | 360155   |
| ET3V        | 6.82  | 2117     |
| ET3V3       | 6.82  | 440695   |
| EFNA4       | 4.19  | 1945     |
| ZBTB7B      | 4.19  | 51043    |
| ADAM15      | 4.02  | 8751     |
| DCST1       | 4.02  | 149095   |
| DCST1-AS1   | 4.02  | 100505666 |
| DCST2       | 4.02  | 127579   |
| EFNA3       | 4.02  | 1944     |
| BCAN        | 5.31  | 63827    |
| GPATCH4     | 5.31  | 54865    |
| HAPLN2      | 5.31  | 60484    |
| NAXE        | 5.31  | 128240   |
| NES         | 5.31  | 10763    |
| TTC24       | 5.31  | 164118   |
| FCRL4       | 6.64  | 83417    |
| FCRL5       | 6.64  | 83416    |
| CKS1B       | 4.05  | 1163     |
| FLAD1       | 4.05  | 80308    |
| LENS        | 4.05  | 55891    |
| ADAR        | 4.58  | 103      |
| KCNN3       | 3.88  | 3782     |
| PRXIP1      | 3.88  | 57326    |
| PMV1        | 3.88  | 10654    |
| PYGO2       | 3.88  | 90780    |
| SHC1        | 3.88  | 6464     |
| CD5L        | 6.54  | 922      |
| FCR1        | 6.54  | 115350   |
| FCR2        | 6.54  | 79368    |
| FCR3        | 6.54  | 115352   |
| Gene       | LogFC | entrezID |
|------------|-------|----------|
| KIRREL1    | 6.54  | 55243    |
| CHRN2      | 4.49  | 1141     |
| SHE        | 4.23  | 126669   |
| TDRD10     | 4.23  | 126668   |
| UBE2Q1     | 4.23  | 55585    |
| CREB3L4    | 3.9   | 148327   |
| CRT2       | 3.9   | 200186   |
| DENND4B    | 3.9   | 9909     |
| JTB        | 3.9   | 10899    |
| RAB13      | 3.9   | 5872     |
| RPS27      | 3.9   | 6232     |
| SLC39A1    | 3.9   | 27173    |
| NUP210L    | 3.65  | 91181    |
| CD1A       | 6.43  | 909      |
| CD1B       | 6.43  | 910      |
| CD1C       | 6.43  | 911      |
| CD1D       | 6.43  | 912      |
| CD1E       | 6.43  | 913      |
| LIN10704   | 6.43  | 646268   |
| OR10K2     | 6.43  | 391107   |
| OR10T2     | 6.43  | 128360   |
| IL6R       | 4.01  | 3570     |
| C1ORF189   | 3.73  | NA       |
| C1ORF43    | 3.73  | NA       |
| HAX1       | 3.73  | 10456    |
| TPM3       | 3.73  | 7170     |
| UBAp31     | 3.73  | 9898     |
| OR10K1     | 5.43  | 391109   |
| OR10R2     | 5.43  | 343406   |
| GATA2D2B   | 3.28  | 57459    |
| SLC27A3    | 3.28  | 11000    |
| PSMD4      | 2.95  | 5710     |
| AQP10      | 3.65  | 89872    |
| ATP8R2     | 3.65  | 57198    |
| C2CD4D     | 3.05  | 100191040|
| C2CD4D-AS1 | 3.05  | 100132111|
| LING04     | 3.05  | 339398   |
| MRPL9      | 3.05  | 65005    |
| OA2Z       | 3.05  | 51686    |
| ROC        | 3.05  | 6097     |
| TDRK1H     | 3.05  | 11022    |
| THEM5      | 3.05  | 284486   |
| INTS3      | 3.17  | 65123    |
| SNAPIN     | 3.17  | 23557    |
| LYSMD1     | 2.87  | 388695   |
| PIP5K1A    | 2.87  | 8394     |
| SCN1M       | 2.87  | 79005    |
| TMOD4      | 2.87  | 29765    |
| TNFAIP8L2  | 2.87  | 79626    |

| Gene                  | LogFC | entrezID   |
|-----------------------|-------|------------|
| TNFAIP8L2-SCNM1       | 2.87  | 100534012  |
| VPS72                 | 2.87  | 6944       |
| MND2A                 | 4.84  | 4332       |
| LCE2B                 | 3.49  | 26239      |
| LCE2C                 | 3.49  | 353140     |
| LCE2D                 | 3.49  | 353141     |
| THEM4                 | 2.96  | 117145     |
| SEMAS6C               | 2.8   | 10500      |
| ILF2                  | 3.07  | 3608       |
| NPR1                  | 3.07  | 4881       |
| OR10X1                | 5.31  | 128367     |
| OR10Z1                | 5.31  | 128368     |
| OR6K6                 | 5.31  | 128371     |
| OR6N1                 | 5.31  | 128372     |
| OR6N2                 | 5.31  | 81442      |
| OR6P1                 | 5.31  | 128366     |
| OR6Y1                 | 5.31  | 391112     |
| TCHH                  | 3.2   | 7062       |
| TCHHL1                | 3.2   | 126637     |
| CGN                   | 2.88  | 57530      |
| PLEKHO1               | 2.88  | 51177      |
| VPS45                 | 2.88  | 11311      |
| ZNF687                | 2.88  | 57592      |
| LCE3A                 | 3.36  | 353142     |
| LCE3B                 | 3.36  | 353143     |
| LCE3C                 | 3.36  | 353144     |
| LCE3D                 | 3.36  | 84648      |
| LCE3E                 | 3.36  | 353145     |
| HORMAD1               | 2.6   | 84072      |
| CHTOP                 | 2.98  | 26097      |
| ANP32E                | 2.8   | 81611      |
| APH1A                 | 2.8   | 51107      |
| C1ORF54               | 2.8   | NA         |
| CA14                  | 2.8   | 23632      |
| CIART                 | 2.8   | 148523     |
| MRPS21                | 2.8   | 54460      |
| POGZ                  | 2.8   | 23126      |
| NBPF18P               | 3.09  | 441908     |
| RPTN                  | 3.09  | 126638     |
| SI00A10               | 3.09  | 6281       |
| SI00A11               | 3.09  | 6282       |
| ANXa9                 | 2.66  | 8416       |
| MINDY1                | 2.66  | 55793      |
| PRUNE1                | 2.66  | 58497      |
| GOLPH3L               | 2.54  | 55204      |
| CRCIT1                | 3.23  | 54544      |
| LCE5A                 | 3.23  | 254910     |
| CELF3                 | 2.89  | 11189      |
| RIIAD1                | 2.89  | 284485     |
| Gene          | LogFC | entrezID |
|--------------|-------|----------|
| S100A1       | 2.89  | 6271     |
| S100A13      | 2.89  | 6284     |
| S100A14      | 2.89  | 57402    |
| TUF1         | 2.89  | 7286     |
| C1ORF68      | 3.41  | NA       |
| LCE2A        | 3.41  | 353139   |
| LCE4A        | 3.41  | 199834   |
| ACKR1        | 4.73  | 2532     |
| AIM2         | 4.73  | 9447     |
| CADM3        | 4.73  | 57863    |
| CADM3-AS1    | 4.73  | 100131825|
| FCR1A        | 4.73  | 2205     |
| IFI16        | 4.73  | 3428     |
| OR10J3       | 4.73  | 441911   |
| OR6K2        | 4.73  | 81448    |
| OR6K3        | 4.73  | 391114   |
| PYHIN1       | 4.73  | 149628   |
| SPTA1        | 4.73  | 6708     |
| GABPB2       | 2.6   | 126626   |
| OTUD7B       | 2.81  | 56957    |
| PI4KB        | 2.81  | 5298     |
| PSMB4        | 2.81  | 5692     |
| RFX5         | 2.81  | 5993     |
| S100A2       | 2.81  | 6273     |
| SELENBP1     | 2.81  | 8991     |
| SNX27        | 2.81  | 81609    |
| CRNN         | 3.12  | 49860    |
| PRPF3        | 2.66  | 9129     |
| ADAMTSL4     | 2.54  | 54507    |
| ADAMTSL4-AS1 | 2.54  | 574406   |
| MCL1         | 2.54  | 4170     |
| KPRP         | 2.37  | 448834   |
| LCE1F        | 2.37  | 353137   |
| S100A16      | 2.73  | 140576   |
| BNIPL        | 2.59  | 149428   |
| C1ORF56      | 2.59  | NA       |
| CDC42SE1     | 2.59  | 56882    |
| CERS2        | 2.59  | 29956    |
| MLLT11       | 2.59  | 10962    |
| FLG          | 3.01  | 2312     |
| FLG-AS1      | 3.01  | 339400   |
| FLG2         | 3.01  | 388698   |
| CTSS         | 2.48  | 1520     |
| ENSA         | 2.48  | 2029     |
| LCE1A        | 3.14  | 353131   |
| LCE1B        | 3.14  | 353132   |
| LCE1C        | 3.14  | 353133   |
| LCE1D        | 3.14  | 353134   |
| LCE1E        | 3.14  | 353135   |
| Gene               | LogFC | entrezID  |
|-------------------|-------|-----------|
| LY9               | 4.05  | 4063      |
| ACP6              | 2.42  | 51205     |
| ANKRD20A12P       | 2.42  | 100874392 |
| ANKRD34A          | 2.42  | 284615    |
| ANKRD35           | 2.42  | 148741    |
| BCL9              | 2.42  | 607       |
| BOLA1             | 2.42  | 51027     |
| CD160             | 2.42  | 11126     |
| CHD1L             | 2.42  | 9557      |
| EMBP1             | 2.42  | 647121    |
| FAM72B            | 2.42  | 653820    |
| FAM72D            | 2.42  | 728833    |
| FCGR1A            | 2.42  | 2209      |
| FCGR1B            | 2.42  | 2210      |
| FCGR1CP           | 2.42  | 100132417 |
| FMO5              | 2.42  | 2330      |
| GJA5              | 2.42  | 2702      |
| GJA8              | 2.42  | 2703      |
| GNHR2             | 2.42  | 114814    |
| GPR89A            | 2.42  | 653519    |
| GPR89B            | 2.42  | 51463     |
| HIST2H2AA3        | 2.42  | 8337      |
| HIST2H2AB         | 2.42  | 317772    |
| HIST2H2AC         | 2.42  | 8338      |
| HIST2H2BA         | 2.42  | 337875    |
| HIST2H2BC         | 2.42  | 337873    |
| HIST2H2BE         | 2.42  | 8349      |
| HIST2H2BF         | 2.42  | 440689    |
| HIST2H3A          | 2.42  | 333932    |
| HIST2H3D          | 2.42  | 653604    |
| HIST2H4A          | 2.42  | 8370      |
| HJV               | 2.42  | 148738    |
| ITGA10            | 2.42  | 8515      |
| LINCO0623         | 2.42  | 728855    |
| LINCO0624         | 2.42  | 100289211 |
| LINCO0869         | 2.42  | 57234     |
| LINCO1138         | 2.42  | 388685    |
| LINCO2591         | 2.42  | 388692    |
| LIXIL             | 2.42  | 128077    |
| LOC102723769      | 2.42  | 102723769 |
| LOC653513         | 2.42  | 653513    |
| LOC728989         | 2.42  | 728989    |
| LSP1P5            | 2.42  | 645166    |
| NBPF10            | 2.42  | 100132406 |
| NBPF11            | 2.42  | 200030    |
| NBPF12            | 2.42  | 149013    |
| NBPF13P           | 2.42  | 644861    |
| NBPF14            | 2.42  | 25832     |
| NBPF15            | 2.42  | 284565    |

| Gene               | LogFC | entrezID  |
|-------------------|-------|-----------|
| NBPFF17P          | 2.42  | 401967    |
| NBPFF20           | 2.42  | 100288142 |
| NBPFF25P          | 2.42  | 101929780 |
| NBPFF8            | 2.42  | 728841    |
| NBPFP9            | 2.42  | 400818    |
| NUDT17            | 2.42  | 200035    |
| PDE4DIP           | 2.42  | 767846    |
| PI3A1P1           | 2.42  | 171423    |
| PDZK1             | 2.42  | 5174      |
| PDZK1P1           | 2.42  | 100034743 |
| PEX11B            | 2.42  | 8799      |
| PFN1P2            | 2.42  | 767846    |
| PIAS3             | 2.42  | 10401     |
| POLR3C            | 2.42  | 10623     |
| POLR3GL           | 2.42  | 84265     |
| PPIAL4A           | 2.42  | 653505    |
| PPIAL4D           | 2.42  | 645142    |
| PPIAL4E           | 2.42  | 730262    |
| PPIAL4G           | 2.42  | 644591    |
| PRKAB2            | 2.42  | 5565      |
| RBM8A             | 2.42  | 9939      |
| RNF115            | 2.42  | 27246     |
| SEC22B            | 2.42  | 9554      |
| SRGAP2-AS1        | 2.42  | 100873165 |
| TXNIP             | 2.42  | 10628     |
| ELF3              | 3.31  | 1999      |
| PIK3C2B           | 3.31  | 5287      |
| CFAAP45           | 3.6   | 25790     |
| CRP               | 3.6   | 1401      |
| FCRL6             | 3.6   | 343413    |
| IGSF9             | 3.6   | 57549     |
| SLAMF8            | 3.6   | 56833     |
| SLAMF9            | 3.6   | 89886     |
| SNHG28            | 3.6   | 284677    |
| TAGLN2            | 3.6   | 8407      |
| VSIG8             | 3.6   | 391123    |
| FAM72C            | 2.12  | 554282    |
| ADAMTS4           | 4.31  | 9507      |
| APOA2             | 4.31  | 336       |
| ARHGAP30          | 4.31  | 257106    |
| B4GALT3           | 4.31  | 8703      |
| CFAP126           | 4.31  | 257177    |
| DEDD              | 4.31  | 9191      |
| DUSP12            | 4.31  | 11266     |
| F11R              | 4.31  | 50848     |
| FCER1G            | 4.31  | 2207      |
| FCGR2A            | 4.31  | 2212      |
| FCGR2B            | 4.31  | 2213      |
| Gene      | LogFC | entrezID |
|-----------|-------|----------|
| CENPL     | 3.24  | 91687    |
| DARS2     | 3.24  | 55157    |
| DCAF8     | 3.24  | 50717    |
| GAS5      | 3.24  | 60674    |
| GAS5-AS1  | 3.24  | 100506046|
| IPO9      | 3.24  | 55705    |
| LINCO0628 | 3.24  | 127841   |
| PEA15     | 3.24  | 8682     |
| RC3H1     | 3.24  | 149041   |

Table 2: Continued.

| Gene      | LogFC | entrezID |
|-----------|-------|----------|
| ITLN1     | 3.9   | 55606    |
| KIFAP3    | 3.73  | 321523   |
| LSEC4     | 3.73  | 268687   |
| MRQH9     | 3.73  | 80133    |
| NOS1AP    | 3.31  | 9722     |
| PRX1      | 3.73  | 5396     |
| SLAMF1    | 3.73  | 51744    |
| SLAMF6    | 3.31  | 114836   |
| SLAMF8    | 3.31  | 5396     |
| SLAMF9    | 3.73  | 2327     |
| SOD2      | 3.31  | 321523   |
| SOD2      | 3.31  | 9722     |
| USP21     | 3.73  | 114836   |
| USP21     | 3.31  | 9722     |

Table 2: Continued.

| Gene      | LogFC | entrezID |
|-----------|-------|----------|
| ATP1A2    | 3.58  | 83540    |
| ATP1A4    | 3.31  | 9722     |
| BLACAT1   | 3.05  | 101669762|
| CCDC190   | 3.58  | 339512   |
| LOC100422212 | 3.58 | 100422212|
| NUF2      | 3.58  | 83540    |
| SH2D1B    | 3.58  | 117157   |
| SPATA46   | 3.58  | 284680   |
| UAP1      | 3.58  | 6675     |
| UHMK1     | 3.58  | 127933   |
| LAMC2     | 2.52  | 3918     |
| NMNAT2    | 2.52  | 23057    |
| ASTN1     | 3.05  | 460      |
| BLACAT1   | 3.05  | 101669762|
| FLJ13156  | 3.05  | 403150   |
| CD84      | 3.31  | 8832     |
| DDR2      | 3.31  | 4921     |
| HSD17B7   | 3.31  | 51478    |
| LRRN2     | 3.31  | 10446    |
| NOS1AP    | 3.31  | 9722     |
| SLAMF6    | 3.31  | 114836   |
| FMO2      | 3.73  | 2327     |
| FMO3      | 3.73  | 2328     |
| FMO6P     | 3.73  | 388714   |
| GORAB     | 3.73  | 92344    |
| KIFAP3    | 3.73  | 22920    |
| MRQH9     | 3.73  | 80133    |
| PRX1      | 3.73  | 5396     |
| ANGPTL1   | 2.73  | 9068     |
| FAM20B    | 2.73  | 9917     |
| Gene     | LogFC | entrezID |
|----------|-------|----------|
| LAMC1    | 2.73  | 3915     |
| RALGPS2  | 2.73  | 55103    |
| TOR3A    | 2.73  | 64222    |
| ETNK2    | 2.88  | 55224    |
| FOSL2    | 2.88  | 2355     |
| GOLT1A   | 2.88  | 127845   |
| IPO9-AS1 | 2.88  | 100873949|
| KISS1    | 2.88  | 3814     |
| LGR6     | 2.88  | 59352    |
| NFKB1    | 2.88  | 9887     |
| PLEKHA6  | 2.88  | 22874    |
| PTPRVP   | 2.88  | 148713   |
| REN      | 2.88  | 5972     |
| SNRPE    | 2.88  | 6635     |
| SOX13    | 2.88  | 9580     |
| ZC3H11A  | 2.88  | 9877     |
| ABL2     | 2.6   | 27       |
| PLB1     | 2.6   | 151056   |
| CNTN2    | 3.09  | 6900     |
| DSYT     | 3.09  | 25778    |
| KLHDC8A  | 3.09  | 55220    |
| KLHL20   | 3.09  | 27252    |
| NUAK2    | 3.09  | 81788    |
| RBBP5    | 3.09  | 5929     |
| TMEM81   | 3.09  | 388730   |
| ATP1B1   | 3.95  | 481      |
| BLZF1    | 3.95  | 8548     |
| C10ORF112| 3.95  | NA       |
| C10ORF21 | 3.95  | 57821    |
| LINC00626| 3.95  | 79100    |
| LINC00970| 3.95  | 101978719|
| METTL18  | 3.95  | 92342    |
| NME7     | 3.95  | 29922    |
| SCYL3    | 3.95  | 57147    |
| SELD     | 3.95  | 6402     |
| SELF     | 3.95  | 6402     |
| SELP     | 3.95  | 6402     |
| SLC19A2  | 3.95  | 10560    |
| ARL8A    | 2.73  | 127829   |
| C10ORF21 | 2.73  | NA       |
| C10ORF220| 2.73  | NA       |
| CSR1P1   | 2.73  | 1465     |
| GLUL     | 2.73  | 2752     |
| GPR37L1  | 2.73  | 9283     |
| LINC00272| 2.73  | 388719   |
| LINC00303| 2.73  | 284573   |
| LINC01344| 2.73  | 400799   |
| NAV1     | 2.73  | 89796    |
| PTPN7    | 2.73  | 5778     |

| Gene     | LogFC | entrezID |
|----------|-------|----------|
| RP510P7  | 2.73  | 376693   |
| SOAT1    | 2.73  | 6646     |
| TEDDM1   | 2.73  | 127670   |
| TEX35    | 2.73  | 84066    |
| ZNF648   | 2.73  | 127665   |
| NUCKS1   | 2.9   | 64710    |
| OCLM     | 2.9   | 10896    |
| ODR4     | 2.9   | 54953    |
| PBX1     | 2.9   | 5087     |
| PDC      | 2.9   | 5132     |
| PRG4     | 2.9   | 10216    |
| RAB29    | 2.9   | 8934     |
| SLCO4A1  | 2.9   | 254428   |
| TPR      | 2.9   | 7175     |
| ARPC5    | 2.59  | 10092    |
| LINCO1686| 2.59  | 284648   |
| NPL      | 2.59  | 80896    |
| RG51     | 2.59  | 85397    |
| RG5L1    | 2.59  | 353299   |
| RNASL    | 2.59  | 6041     |
| TSEN15   | 2.59  | 116461   |
| FMO1     | 3.14  | 2326     |
| FMO4     | 3.14  | 2329     |
| LMX1A    | 3.14  | 4009     |
| TOP1P1   | 3.14  | 7151     |
| ANKR36BP1| 3.54  | 84832    |
| CD247    | 3.54  | 919      |
| CREG1    | 3.54  | 8804     |
| DPT      | 3.54  | 1805     |
| DUSP27   | 3.54  | 92235    |
| F5       | 3.54  | 2153     |
| FAM7B    | 3.54  | 149297   |
| FMO9P    | 3.54  | 116123   |
| GPA33    | 3.54  | 10223    |
| ILD2     | 3.54  | 387597   |
| LINCO1363| 3.54  | 101928484|
| LOC100505918| 3.54| 100505918|
| MAEL     | 3.54  | 84944    |
| PO6K     | 3.54  | 57645    |
| PO2F1    | 3.54  | 5451     |
| SFT2D2   | 3.54  | 375035   |
| TADA1    | 3.54  | 117143   |
| TXB19    | 3.54  | 9095     |
| TIPRL    | 3.54  | 261726   |
| Gene       | LogFC | entrezID |
|------------|-------|----------|
| XCL1       | 3.54  | 6375     |
| XCL2       | 3.54  | 6846     |
| BRINP2     | 2.73  | 57795    |
| CRYZL2P    | 2.73  | 730102   |
| GS1-279B7.1| 2.73  | 100288079|
| IVNS1ABP   | 2.73  | 10625    |
| LINCO1741  | 2.73  | 101928778|
| RASAL2-AS1 | 2.73  | 100302401|
| RN6-79P    | 2.73  | 100873779|
| SWT1       | 2.73  | 54823    |
| TRMT1L     | 2.73  | 81627    |
| ZBED6      | 2.73  | 100381270|
| APOBEC4    | 2.47  | 403314   |
| DHX9       | 2.47  | 1660     |
| LHX4       | 2.47  | 89884    |
| LHX4-AS1   | 2.47  | 100527964|
| SHCBP1L    | 2.47  | 81626    |
| ERO1B      | 1.97  | 56605    |
| ANKRD45    | 2.92  | 339416   |
| BRINP3     | 2.92  | 339479   |
| COPA       | 2.92  | 1314     |
| EEF1AKNMT  | 2.92  | 51603    |
| GPR52      | 2.92  | 9293     |
| LEMD1      | 2.92  | 93273    |
| LEMD1-AS1  | 2.92  | 284576   |
| LOC100505716| 2.92| 100505716|
| MYOC       | 2.92  | 4653     |
| NCSRN      | 2.92  | 23385    |
| NHLH1      | 2.92  | 4807     |
| PEX19      | 2.92  | 5824     |
| PLA2G4A    | 2.92  | 5321     |
| PRRC2C     | 2.92  | 23215    |
| RXRG       | 2.92  | 6258     |
| SCARN3A    | 2.92  | 677679   |
| SUMO1P3    | 2.92  | 474338   |
| TEX50      | 2.92  | 730159   |
| TMCC2      | 2.92  | 9911     |
| VAMP4      | 2.92  | 8674     |
| VANGL2     | 2.92  | 57216    |
| ADORA1     | 2.58  | 134      |
| AXDND1     | 2.58  | 126859   |
| CHI3L1     | 2.58  | 1116     |
| CHIT1      | 2.58  | 1118     |
| FAM163A    | 2.58  | 148753   |
| LAX1       | 2.58  | 54900    |
| LINCO1350  | 2.58  | 101929093|
| MYBPH      | 2.58  | 4608     |
| MYOG       | 2.58  | 4656     |
| NIBAN1     | 2.58  | 116496   |
| PM20D1     | 2.58  | 1488111  |
| PPP1CB     | 2.58  | 5500     |
| RNF2       | 2.58  | 6045     |
| SEC16B     | 2.58  | 898666   |
| TDRD5      | 2.58  | 163589   |
| TOR1AIP1   | 2.58  | 26092    |
| TOR1AIP2   | 2.58  | 163590   |
| COLGALT2   | 2.35  | 23127    |
| RGL1       | 2.35  | 23179    |
| SMG7-AS1   | 2.35  | 284649   |
| ADCY10     | 3.21  | 55811    |
| GPR161     | 3.21  | 234332   |
| MPC2       | 3.21  | 25874    |
| MPZL1      | 3.21  | 9019     |
| RCSD1      | 2.31  | 92241    |
| SLCA41AP   | 3.21  | 22950    |
| SUPT7L     | 3.21  | 9913     |
| SUGCT      | >10   | 79783    |
| MTR        | 1.99  | 4548     |
| CIORF53    | 2.73  | 4235     |
| CDK18      | 2.73  | 5129     |
| COP1       | 2.73  | 64326    |
| LHX9       | 2.73  | 56956    |
| LOC100505795| 2.73| 100505795|
| NEK7       | 2.73  | 140609   |
| PPP1R12B   | 2.73  | 4660     |
| PTGS2      | 2.73  | 5743     |
| SLC9C2     | 2.73  | 284525   |
| NCF2       | 2.24  | 4688     |
| PLEKYG2    | 1.56  | 64857    |
| ZFP36      | 1.56  | 7538     |
| BTG2       | 2.44  | 7832     |
| CACNA1E    | 2.44  | 777      |
| CEP350     | 2.44  | 9857     |
| CTSE       | 2.44  | 1510     |
| EDEM3      | 2.44  | 80267    |
| FLJ23867   | 2.44  | NA       |
| FMO4       | 2.44  | 2331     |
| HMCN1      | 2.44  | 83872    |
| LINC01136  | 2.44  | 730227   |
| LINC01699  | 2.44  | 100287948|
| MAPP2A2    | 2.44  | 60677    |
| QSOX1      | 2.44  | 5768     |
| TNN1       | 2.44  | 7135     |
| MTH1H1     | 1.92  | 645745   |
| LRRC52     | 2.95  | 440699   |
software (version 10.2.2.2317, Aperio Technologies) was used for further quantitative analysis.

2.18. Statistical Analyses. Survival analysis was analyzed using the Kaplan–Meier method. GO enrichment analysis and KEGG enrichment analysis were performed under an R computing environment. Statistical analyses were performed using GraphPad Prism 7.0 (GraphPad Software, La Jolla, CA, USA). Comparisons were performed by a two-tailed Student’s t-test. P values < 0.05 were considered statistically significant. Data were expressed as mean ± standard deviation (SD).

| Gene     | LogFC | entrezID  |
|----------|-------|-----------|
| MRPL33   | 2.95  | 9553      |
| TNFSF18  | 2.95  | 8995      |
| GMFG     | 1.52  | 9535      |
| MED29    | 1.52  | 55588     |
| PAF1     | 1.52  | 54623     |
| SAMD4B   | 1.52  | 55095     |
| SMG7     | 2.14  | 9887      |
| ACTN2    | 1.85  | 88        |
| EDARADD  | 1.85  | 128178    |
| GPR137B  | 1.85  | 7107      |
| HEATR1   | 1.85  | 55127     |
| LGALS8   | 1.85  | 3964      |
| LGALS8-AS1 | 1.85 | 100287902 |
| NID1     | 1.85  | 4811      |
| RYR2     | 1.85  | 6262      |
| DENND1B  | 2.56  | 163486    |
| GLRX2    | 2.56  | 51022     |
| RO60     | 2.56  | 6738      |
| SLC45A3  | 2.56  | 85414     |
| SRGAP2   | 2.56  | 23380     |
| SRGAP2B  | 2.56  | 647135    |
| SRGAP2C  | 2.56  | 653464    |
| SUCO     | 2.56  | 51430     |
| UBE2T    | 2.56  | 29089     |
| DN3M     | 2.31  | 26052     |
| IER5     | 2.31  | 51278     |
| KIAA1614 | 2.31  | 57710     |
| LINCO0260| 2.31  | 84719     |
| LOC284581| 2.31  | 284581    |
| MR1      | 2.31  | 3140      |
| PAX8     | 2.31  | 7849      |
| PAX8-AS1 | 2.31  | 654433    |
| SNORA77  | 2.31  | 677843    |
| STX6     | 2.31  | 10228     |
| LRATD1   | 4.05  | 151354    |
| C19ORF47 | 1.94  | NA        |
| ARID4B   | 1.79  | 51742     |
| CHRM3    | 1.79  | 1131      |
| CHRM3-AS1| 1.79  | 100873984 |
| GGP51    | 1.79  | 9453      |
| TBCE     | 1.79  | 6905      |
| KLRF2    | 2.05  | 100431172 |
| LINCO1132| 1.67  | 100506810 |
| ALDH9A1  | 2.73  | 223       |
| DCAF6    | 2.73  | 55827     |
| KIAA0040 | 2.73  | 9674      |
| LINCO1351| 2.73  | 101929120 |
| LOC440700| 2.73  | 440700    |
| LRRCS2-AS1| 2.73| 400794    |
3. Result

3.1. High Expression Level and Prognostic Value of MEX3A in Ovarian Cancer by Bioinformatics Analyses. Firstly, to determine differences in MEX3A expression in tumor and normal tissues, the MEX3A mRNA levels in different tumors and normal tissues of various cancer types were analyzed using the Oncomine database. The database, which had a total of 241 unique samples for MEX3A, and a total of 22 cancers, including brain and CNS cancer, breast cancer, colorectal cancer, and ovarian cancer, showed that MEX3A mRNA levels were significantly upregulated in various cancers, and MEX3A expression in OC was high on top 5 (Figure S1a). GEPIA analysis also revealed similar results (Figure S1b).

Next, we found that MEX3A expression in OC significantly increased between 426 cases of OC and 88 cases of normal ovarian tissues via GEPIA (Figure 1(a)). In order to clarify the results, the expression differences in OC tissues (40 samples from Renji Hospital) and normal ovarian tissues (25 samples from Renji Hospital) were also validated by IHC staining (Figure 1(c)).

In addition, we investigated whether MEX3A was associated with prognosis in OC patients by using the Kaplan–Meier plotter and PrognoScan. The Kaplan–Meier plotter and PrognoScan databases showed that OC patients with high MEX3A expression experienced poor OS and PFS (Figures 1(b) and 1(d), Table 1). In order to explore the prognostic value of different histologies, the database revealed that higher MEX3A expression was correlated with shorter OS and PFS both in patients with endometrioid and serous cancers (Figures S1c–e). Collectively, MEX3A can be considered as an independent prognostic biomarker linked to a poor survival rate in OC.

3.2. MEX3A Expression Is Correlated with Immune Infiltration Level in OC. To better understand the underlying mechanism of MEX3A in OC, we further investigated the relationships between MEX3A and the immune system. Tumor-infiltrating immune cells (TIICs) are an important part of the tumor microenvironment and which are independent predictors of cancer survival. It is unclear whether targeting MEX3A could influence the recruitment numbers of TIICs to impact the prognosis of cancers. Through TIMER analysis, we found that most immune cells were negatively correlated with MEX3A expression (Figure 2(a)). MEX3A expression had a negative correlation with B cells, CD8+ T cells, neutrophils and dendritic cells (DCs), and macrophages. However, the expression of MEX3A had weak associations with CD4+ T cells in OC. Subsequently, we used the TISIDB database to further analyze the relationship between MEX3A expression and immune regulation. Figures 2(b) and 2(c) show the correlation between MEX3A expression and TILs, which corresponded to the results reported above. Immunomodulators can be further divided into immunoinhibitors, immunostimulators, and major histocompatibility complex (MHC) molecules. Furthermore, we assessed the correlation between MEX3A expression and diverse immunomodulators. Figures 2(d) and 2(e) indicate the correlations between MEX3A expression and immunostimulators, and the greatest correlations include C10orf54 (Spearman’s $\rho = -0.505$, $P < 2.2e-16$), TNFRSF18 (Spearman’s $\rho = -0.439$, $P < 2.2e-16$), TNFRSF14 (Spearman’s $\rho = -0.438$, $P < 2.2e-16$), and TNFRSF13C (Spearman’s $\rho = 0.384$, $P < 2.2e-16$). Figures 2(f) and 2(g) indicate correlations between MEX3A levels and immunoinhibitors, where the strongest include IL10RB (Spearman’s $\rho = -0.45$, $P < 2.2e-16$), IDO1 (Spearman’s $\rho = -0.444$, $P < 2.2e-16$), VTCN1 (Spearman’s $\rho = -0.435$, $P < 2.2e-16$), and HAVCR2 (Spearman’s $\rho = -0.425$, $P < 2.2e-16$). Correlations between MEX3A expression and MHC molecules were also explored, and the greatest correlations include B2M (Spearman’s $\rho = -0.49$, $P < 2.2e-16$), HLA-DMA (Spearman’s $\rho = -0.5$, $P < 2.2e-16$), HLA-DPA1 (Spearman’s $\rho = -0.451$, $P < 2.2e-16$), and HLA-DPB1 (Spearman’s $\rho = -0.452$, $P < 2.2e-16$) (Figures 3(h) and 3(i)). Therefore, MEX3A may be involved in negative immune regulation.

3.3. Enrichment Analysis of Coexpression Genes Correlated with MEX3A in OC. Next, we analyzed mRNA sequencing data from OC patients in TCGA by using the function module of LinkedOmics. As shown in the volcano plot (Figure 2(a)), 2596 genes (dark red dots) showed significant positive correlations with MEX3A, and 3050 genes (dark green dots) showed significant negative correlations (FDR < 0.01). The 50 significant gene sets (such as ACTBL2, C12orf43, CCDC56, CCL27, CPNE8, FAM78B, KCTD17, and LAMB4) positively and negatively correlated with MEX3A are shown in the heat map (Figures 2(b) and 2(c)). These results indicated an important influence of MEX3A on the transcriptome level. Besides, GO term analysis showed that high expressed genes in correlation with MEX3A were mainly located in the chromatin centrosome and nuclear chromosome part, where they mostly participated in mRNA processing, covalent chromatin modification, and histone modification. Poor expressed genes were mainly located in the endosome membrane, secretory granule membrane, and side of the membrane and were involved in immune-related processing, including neutrophil and T cell activation and regulation of lymphocyte activation (Figures 4(d) and 4(e)). KEGG pathway analysis showed the most important enrichment in the herpes simplex virus 1 infection of high expressed genes and cytokine-cytokine receptor interaction of poor expressed genes (Figures 4(f) and 4(g)). These data pointed out that MEX3A might promote tumor progression by regulating immune cell response in the tumor microenvironment.

3.4. Genomic Alterations of MEX3A in OC. Based on the above analysis, MEX3A is closely related to tumor immunology. In order to better understand the potential immune mechanism of MEX3A in cancer, genetic variations of MEX3A retrieved from the TCGA database (489 cases, Nature 2011) were analyzed by using the cBioPortal database. The results showed mRNA expression changes in 60 cases (16%), amplification in 38 cases (10%), a mutation in 1 case (0.3%), and multiple alterations in 19 cases (5%), in which amplification was the most common type (Figure 5(a)). Further, the expression of 771 genes was
Figure 6: Continued.
positively related to MEX3A and was increased with the amplification of MEX3A. Among these genes, LAMTOR2 had the most frequent alterations (Table 2). LAMTOR2 is essential for macrophage and dendritic cell (DC) homeostasis via mediating immune responses [19, 20]. Significantly enriched GO analysis showed that these genes encoded proteins that were mainly localized to the cornified envelope (Figures 5(b) and 5(c)). They were primarily involved in immunoglobulin binding, IgG binding, and RAGE reporter binding.

3.5. Construction of a Gene-Gene Interaction Network. To further explore the potential mechanism of MEX3A in promoting OC progression, we constructed a gene-gene interaction network by using the GeneMANIA database. Their functions were also analyzed. MEX3A were surrounded by 20 nodes representing genes that were greatly correlated with the family in terms of physical interactions, coexpression, prediction, colocalization, pathway, genetic interactions, and shared protein domains. From the results (Figure 3), we found that PABPC1, a kind of shuttling protein from the cytoplasm to nucleus in most eukaryotes, was correlated with MEX3A for physical interactions. PABPC1 is important for protein translation initiation and decay by binding to regulatory proteins [21]. In addition, KHDRBS2 was associated with IGF2BP2 and MEX3A in terms of shared protein domains. KHDRBS2 is also an RNA-binding protein that is tyrosine phosphorylated by Src during mitosis [22]. IGF2BP2 was colocalized with STRA6. Further functional analysis revealed that most proteins were greatly correlated with skeletal system development and genitalia development.

3.6. MEX3A Promoted Ovarian Cell Proliferation, Migration, and Invasion In Vitro. To further evaluate the biological functions of MEX3A on ovarian cancer, the expression of MEX3A in different cell lines was tested, and in vitro studies were performed (Figure 6(a)). The ES2 cell line was chosen for further study. We silenced MEX3A expression by siRNA, and a nontargeting siRNA was used as a control. The efficiency was evaluated by Western blotting and RT-PCR (Figures 6(b) and 6(c)). We first studied its influence on OC growth by using CCK8 assay, clone formation assay, and EdU assay. Compared with the normal control group, MEX3A knockdown partly suppressed the proliferation of OC cells (P < 0.05, Figure 6(d)). Similarly, the colony number was significantly smaller than that of the control group (P < 0.05, Figure 6(e)). EdU is a thymidine nucleoside analogue, which is involved in DNA replication when targeting proliferating cells. The proliferation activity of ES2 cells can be analyzed with the number of red/blue fluorescence spots. Figure 6(f) shows that compared with the control group, knockdown of MEX3A significantly inhibits the EdU uptake rate, which also indicates suppressed proliferation ability. Next, we assessed the role of MEX3A knockdown on the migration ability of OC. Transwell assay and wound healing assay were performed, and the results showed that MEX3A knockdown significantly inhibited cell migration in ES2 cells in comparison to the control group (Figures 6(g) and 6(h)). Collectively, these results indicated that MEX3A could promote the proliferation and migration in OC cells.

4. Discussion

OC is usually detected during the late stages; thus, few patients are eligible for timely treatment. Identifying sensitive and specific biomarkers for improving diagnosis and accurately evaluating prognosis continues to be an important research focus. In this study, we explored a novel gene—MEX3A—which is an RNA-binding protein or an E3 ubiquitin ligase acting posttranscriptional regulation, associated with the diagnosis and prognosis of OC. MEX3A has important roles in biological processes. Its expression is associated with intestinal homeostasis by regulating intestinal differentiation and promoting high expression of intestinal stem cell markers (LGR5, BMI1, and MSI1) [23, 24]. Moreover, a few studies have evaluated the effect of MEX3A on tumors. Abnormal activation of MEX3A can promote tumor cell proliferation, metastasis, and migration in gastric cancer and pancreatic ductal adenocarcinoma, breast cancer, and osteosarcoma [6, 25–27]. For example, MEX3A may act as a tumor promoter for breast cancer by regulating PIK3CA. Also, MEX3A could combine RIG-I to promote its ubiquitylation.
and proteasome-dependent degradation, which is beneficial for tumorigenesis [28].

However, the mechanisms of the MEX3A function have yet to be elucidated in OC. To the best of our knowledge, this is the first study that reported the role of MEX3A in OC through bioinformatics analysis of public sequencing data to guide future research in OC.

First, the results of the prognostic analysis showed that upregulation of MEX3A mRNA expression had the greatest correlation with poor OS and PFS in OC patients. In addition, we performed a series of in vitro experiments, which proved the inhibition of OC development by MEX3A knockdown. Hence, we speculate that MEX3A is extremely important as a prognostic indicator in OC patients and can be used as a predictor of tumor proliferation and metastasis. These results are consistent with bladder cancer, lung adenocarcinoma, and glioma [29–31]. Liang et al. found that MEX3A could enhance the instability of LAMA2 mRNA to promote lung adenocarcinoma metastasis by the PI3K/AKT pathway. In addition, they reported that MEX3A exerted its ubiquitination role to induce glioma tumorigenesis.

To explore the specific mechanisms of MEX3A in OC, a comprehensive bioinformatic analysis of MEX3A has been performed. Copy number variations (CNVs) have major genomic implications in human diseases, especially cancer, which can lead to phenotypic differences [30]. We found that the major CNV type of MEX3A was amplification, which was associated with shorter survival. Besides, neighboring gene networks close to MEX3A generally showed different degrees of amplification in OC. The genes coexpressed with MEX3A were subjected to functional and pathway enrichment analyses, and the results indicated that they were mainly involved in the immune response processes during tumorigenesis and progression of ovarian cancer.

We also constructed a gene-gene interaction network. The results suggested that MEX3A interacted intensively with other genes, such as PABPC1 and LAMTOR2. PABPC1 has been reported to bind the poly(A) tails of mRNAs, regulating the stability and biofunction of lncRNAs, which have critical roles in OC progression [31, 32]. PABPC1 could promote the binding of hnRNPLL (a plasma cell-specific RBP) to the immunoglobulin mRNA and regulate switching from mIgH to sIgH in plasma cells [33]. Yu et al. reported that PABPC1 could involve innate immune surveillance by regulating the activity of NK cells [34]. LAMTOR2, a regulator/LAMTOR complex member, activates AKT/mTOR to regulate dendritic cell homeostasis [20]. They implied that MEX3A might have an essential role in immunity by combining with PABPC1. Therefore, these results suggested that MEX3A and its related genes together regulate OC progression by a complex regulatory network.

Another important aspect of this study was that MEX3A expression was related to immune infiltration in OC. Our results demonstrated a moderate to a strong relationship between MEX3A expression level and infiltration level of macrophages, neutrophils, dendritic cells (DCs), B cells, and CD8+ T cells. Furthermore, immune cell activation and immunomodulators have been known for reducing mortality rates in patients with OC. In our study, we assessed the correlation between MEX3A and the immune system via the TIMER and TISIDB database, finding that MEX3A had the greatest correlation with lymphocytes (such as B cells, CD8+ T cells, neutrophils, and dendritic cells (DCs) and macrophages), immune inhibitors (such as IL10RB, IDO1, VTCN1, and HAVCR2), immunostimulators (such as C10orf54, TNFRSF18, TNFRSF14, and TNFRSF13C), and MHC molecules (such as B2M HLA-DMA HLA-DPA1, and HLA-DPB1). Therefore, MEX3A, which is associated with these immune-related genes, may provide a new target in immune therapy for OC.

The present study has several limitations. First, most results on the transcriptional level may reflect some aspects of immune infiltration. Also, reported findings need to be confirmed with larger clinical samples and experimental data using molecular biology techniques. Finally, we plan to further deepen our understanding of the underlying mechanism of immunomodulators related to MEX3A in our future work.

In conclusion, this study demonstrated that high MEX3A expression was correlated with poor prognosis and increased immune infiltration levels in macrophages, neutrophils, dendritic cells (DCs), B cells, and CD8+ T cells in OC. Our study provides a novel insight into the potential role of MEX3A as a cancer biomarker from the perspective of tumor immunology.

Data Availability

Previously reported [RNA-Seq] TCGA data were used to support this study and are available at GEPIA (doi: 10.1093/nar/gkx247), cBioPortal (doi: 10.1126/sci-signal.2004088), LinkedOmics (doi: 10.1093/nar/gkx1090), and the Kaplan–Meier plotter (doi: 10.1530/ERC-11-0329). These prior studies (and datasets) are cited at relevant places within the text as references [9–11, 15]. There is no research data used to support this study.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors’ Contributions

Panpan Zhang and Tong Su contributed equally to this work.

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Supplementary Materials

Figure S1: the mRNA expression levels of MEX3A in various cancers and prognostic values of MEX3A in ovarian cancer in the Kaplan–Meier plotter. (a) The expression of MEX3A in various cancers from Oncomine. The threshold was designed with the following parameters: fold change = 2 and P value = 0.01. The color intensity (red or blue) is directly proportional to the significance level of upregulation or downregulation. (b) The MEX3A expression levels in different tumor types from the TCGA database were determined...
by GEPIA. (c, d) Prognostic significance of MEX3A in serous ovarian carcinoma. (e, f) Prognostic significance of MEX3A in endometrioid carcinoma. *P < 0.05, **P < 0.01, and ***P < 0.001. (Supplementary Materials)

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