Low Expression of RILPL2 Predicts Poor Prognosis and Correlates with Immune Infiltration in Endometrial Carcinoma

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Abstract

Increasing numbers of biomarkers have been identified for various cancers. However, biomarkers associated with endometrial carcinoma (EC) remain largely to be explored. In the current research, we downloaded the RNA-seq data and corresponding clinicopathological features from the Cancer Genome Atlas (TCGA) database. We conducted expression analysis, which resulted in identification of RILPL2 as a novel diagnostic biomarker in EC. The dysregulation of RILPL2 in EC was also validated in multiple datasets. The correlations between clinical features and RILPL2 expression were assessed by logistic regression analysis. Then, Kaplan-Meier analysis, univariate, and multivariate Cox regression analysis were performed to estimate prognostic values of RILPL2 in the TCGA cohort, which unveiled that increased level of RILPL2 was remarkably associated with better prognosis and could be severed as an independent prognostic biomarker in patients with EC. Moreover, correlation analysis of RILPL2 and tumor-infiltrating immune cells (TIICs) indicated that RILPL2 might play a critical role in regulating immune cell infiltration in EC and is related to immune response. Besides, high methylation level was a significant cause for RILPL2 low expression in EC. Subsequently, weighted gene co-expression network analysis (WGCNA) and enrichment analysis were conducted to explore the RILPL2-involved underlying oncogenic mechanisms, and the results indicated that RILPL2 mainly regulated cell cycle. In conclusion, our findings provided evidence that downregulation of RILPL2 in EC is an indicator of adverse prognosis and RILPL2 may act as a promising target for the therapeutics of EC.

1. Background

Endometrial carcinoma (EC) is the fourth most widespread malignant tumors among females in the world behind breast cancer, lung cancer, and colorectal cancer [1], with increasing incidence and morbidity over the past years [2]. To differentiate the prognosis and its clinical course, Bokhman et al. divided EC into type I and type II. Type I EC is summarized with estrogen-dependent pathogenesis, low-grade lesions and better clinical outcome, while type II EC is regarded as non-estrogen-dependent pathogenesis, poorly differentiated grade and poor prognosis [3]. Although most ECs are generally low stage and low grade, with favourable prognosis, the high-grade group accounts for a disproportionate number of EC deaths [4]. Despite EC still occurs more commonly in older women, it also is being diagnosed in younger and younger women [5]. Therefore, it is urgent to exploring potential indicators that are directly associated with diagnosis in the early stage, therapy, prognosis after treatment and detection of asymptomatic recurrence in endometrial carcinoma.

Rab interacting lysosomal protein like 2 (RILPL2) is a protein-coding gene, encoding a protein that contains a Rab-interacting lysosomal protein-like domain similar to that of Rab-interacting lysosomal protein (RILP). RILP is a Rab7/Rab34 effector, which interacts with dynein-dynactin to directly regulate the movement of late endosomes and lysosomes along microtubules [6]. Unlike RILP, RILPL2 does not consist of a Rab7 junction region, which prevents its ectopic expression from the lysosomal compartment [7]. Although RILPL2 does not affect lysosomal trafficking, it may still act as a functional Rab effector due to its interaction with activated Rab34 and Rab36 [8, 9]. The analysis of data obtained from online
databases suggested that RILPL2 expressed lower level in breast cancer tissues than in adjacent tissues, and that RILPL2 low expression was prominently positively associated with TNM stage and differentiated grade [10]. Furthermore, previous studies indicated that RILPL2 functioned in viral replication and could be employed as a potential target for the therapy of HCV [11]. However, the correlation between RILPL2 and the clinical features of EC has not been studied.

In this research, we firstly analyze the expression level of RILPL2 and summarize its prognostic roles, biological features, and its correlation with the tumor immune infiltration in EC patients mainly according to the public data obtained from the Cancer Genome Atlas (TCGA) database. Summarily, our study suggests that RILPL2 may be a novel predictive indicator for diagnosis and prognosis and promising therapeutic target for EC.

2. Methods

2.1. Collection of the TCGA and the GEO data

Transcriptome RNA-seq data of 575 samples (containing 23 normal samples and 552 tumor samples) and the corresponding clinical characteristics were downloaded from the TCGA database (https://TCGAData.nci.nih.gov/TCGA/), which acted as a public repository that includes high-throughput microarray experimental data [12]. EC sequencing data were generated by the Illumina HiSeq_RNA-Seq platform. Then, we conducting pre-processing of clinicopathologic data to eliminate cases with missing or defective information. As a result, the clinical information of 524 patients were reserved for the following analysis.

The GSE17025 dataset was obtained from the Gene Expression Omnibus (GEO) database (www.ncbi.nlm.nih.gov/geo) [13], which was used to validate the expression of RILPL2. The GSE17025 dataset contained high-through data of 91 EC samples, and 12 were atrophic endometrium samples from postmenopausal women.

2.2. Collection of EC specimens

To validate the aberration of RILPL2 in EC compared to normal tissues, we further collected 15 EC tissues and paired adjacent tissues in the Third People's Hospital of Nantong in 2020. Ethical approval for the study was granted by the Clinical Research Ethics Committee, the Third People's Hospital of Nantong, and the current study was performed in conform to the Declaration of Helsinki.

2.3. Total RNA extraction and quantitative real-time PCR analysis

To extract total RNA from EC tissues, TRizol reagent (Thermo Fisher Scientific) was applied. Next, Agilent Bioanalyzer 2100 (Agilent Technologies) with RNA 6000 Nano kit was used to assess the integrity of extracted total RNA. All procedures of real-time quantitative RT-PCR (qRT-PCR) are described as previously [14]. Primer sequences for qRT-PCR were presented as follows: RILPL2: ACGTGTATGACATCTCCTACCTG
ACCGGGACGACTTTGAAGT (reverse); GAPDH: CGCTCTCTGTCTCCTCCTGT (forward), CATGGTGGAATCATATTTG (reverse).

### 2.4. Validation of RILPL2 protein level

Human Protein Atlas (HPA), which was on the ground of integration of multi-omics, put insight into characterizing all the human proteins [15]. In this study, the level of RILPL2 protein in human endometrium and EC samples were compared after acquiring data from the HPA database. Besides, to further validate the protein level of RILPL2, the CPTAC analysis in UALCAN was applied [16].

### 2.5. Analysis of RILPL2 expression in EC samples with various clinicopathologic features

As for the data downloaded from the TCGA database, we utilized “ggplot” package in R language (v. 3.6.2) to analyze the RNA-seq data and corresponding clinical features. The relevance between the expression of RILPL2 and various clinicopathologic characteristics were evaluated and emerged by boxplots. The difference of variables was tested using the Wilcoxon signed-rank test or Kruskal-Wallis test. A logistic regression analysis of RILPL2 level and other clinical features was performed via R language. $P < 0.05$ was representative of statistically significant.

### 2.6. Survival analysis dependent on RILPL2 expression

Definitions and outcomes of overall survival (OS), disease-free survival (DFS), and disease-specific survival (DSS) of the TCGA cohort were obtained from the TCGA-Clinical Data Resource (CDR) Outcome. Kaplan-Meier curves were exhibited through the “survival” and “survminer” package in R, $P < 0.05$ was seemed to be statistically significant. Univariable and multivariable Cox regression analysis were performed orderly to screen the prognosis-related factors among the available clinical features, together with the RILPL2 expression profile via the “coxph function” in the “survival” package.

### 2.7. Correlation analysis of RILPL2 and tumor-infiltrating immune cells

ESTIMATE, a method which utilizes the gene expression profiles to speculate the infiltrations of stromal and immune cells in tumor tissues [17], was applied to assess the level of immune cell content (immune score), the stromal cell infiltration (stromal score), the stromal-immune comprehensive score (ESTIMATE score) and the tumor purity in each EC sample.

The TIMER tool, an online platform (https://cistrome.shinyapps.io/timer/) for comprehensively analyzing the molecular features of tumor-infiltrating immune cells [18], was applied to study and visualize the correlations between the expression of RILPL2 and the proportion of 6 tumor-infiltrating immune cells (TIICs), namely B cells, CD8 $+$ T cells, CD4 $+$ Tcells, macrophages, dendritic cells (DCs) and neutrophils.

CIBERSORT (http://cibersort.stanford.edu/), a analytical tool, which was applied to precisely assess the infiltrations of TIICs according to transcriptome data [19]. According to the infiltration level of 22 immune
cell subtypes reported by the CIBERSORT algorithm, the “vioplot” package was applied to visualize the difference of 22 TIICs in high and low RILPL2 expression EC samples.

2.8. Methylation and mutation analysis

On the basis of DNA methylation data downloaded from the TCGA database, we assessed the difference of methylation degree of RILPL2 by comparing EC with normal tissues. Furthermore, we analyzed the correlations between RILPL2 methylation and its expression level in EC. Besides, the genetic alterations of RILPL2 gene in the TCGA dataset were analyzed by cBioPortal tool [20].

2.9. WGCNA analysis

First, as previously described, differentially expressed genes (DEGs) between EC and normal specimens in the TCGA dataset were screened out using the “limma” package with threshold of adjusted P-value < 0.05 and |log2FC| >1 [21]. Next the expression profile of these DEGs was sent to establish a gene co-expression network by the weighted gene co-expression network analysis (WGCNA) package in R language as previously described [22]. To matching the correlations with the nature regulation relationship, we built a scale-free topology to emphasize the strong correlations and attenuate the weak correlations with the soft threshold power of $\beta = 3$ (scale free $R^2 = 0.85$). After that, the topological overlap matrix (TOM) was calculated on the basis of adjacency matrices. Finally, we applied the dynamic tree cut algorithm to classify genes according to their expression patterns and merge gene modules. Gene significance (GS) was deemed as the correlation coefficient between transcriptome expression and module traits. The module eigengene (ME) was calculated as a summary profile for all genes in a module. Module significance (MS) was defined as the correlation coefficient between a module and traits. Module membership (MM) was defined by the correlation coefficient of the module eigengene and transcriptome data. When gene with MM > 0.80, it was supposed to be the modules’ representative gene, which might possessed potential crucial functions.

2.10. Enrichment and PPI analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were applied to uncover the potential mechanisms of these genes in the turquoise module in DAVID tool (https://david-d.ncifcrf.gov/). Besides, the key genes were sent into the STRING database (https://string-db.org/) to construct the protein-protein interaction (PPI) network of gene encoding proteins. We set the minimum interaction score as 0.4 (medium confidence).

3. Results

3.1. Analysis RILPL2 expression in EC patients

By analyzing the data from TCGA, the expression of RILPL2 in the paracancerous samples was remarkably higher than that in the tumor specimens ($P < 0.001$, Fig. 1A). Based on GSE17025 dataset, we observed that RILPL2 was also decreased in EC samples compared with paracancerous samples ($P <$
0.001, Fig. 1B). In addition, we further validated RILPL2 expression using our recruited cohort, and the result showed that RILPL2 in tumor samples was obviously lower compared with normal control (P = 0.013, Fig. 1C). Moreover, the expression level of RILPL2 protein in human endometrium and EC specimens were compared calculating the data obtained from the HPA database, and the result showed that RILPL2 protein was loss in EC samples (Fig. 1D). To further validate the protein level of RILPL2, the CPTAC analysis in UALCAN was applied. Not surprisingly, the result validated the downregulated RILPL2 in EC samples again (P < 0.001, Fig. 1E).

3.2. Association between RILPL2 expression and clinicopathologic features

A correlation analysis was performed between RILPL2 expression and corresponding clinical characteristics. As presented in Fig. 2, decreased expression of RILPL2 remarkably related to the multiple factors, including age (P = 0.033, Fig. 2A), grade (P < 0.001, Fig. 2B), the tumor histological type (P < 0.001, Fig. 2C), and clinical stage (P < 0.001, Fig. 2D). Moreover, as exhibited in Table 1, logistic regression analysis utilizing the median of RILPL2 expression as a classification of the dependent variable indicated that reduced RILPL2 expression was significantly correlated with high grade (Grade 2 vs. Grade 1, P = 0.005; Grade 3 vs. Grade 1, P < 0.001; Grade 4 vs. Grade 1, P < 0.001), histological type (Mix vs. Endometrial, P = 0.042; Serous vs. Endometrial, P < 0.001) and pathological stage (Stage II vs. Stage I, P < 0.001; Stage III vs. Stage I, P < 0.001; Stage IV vs. Stage I, P < 0.001).

| Clinical characteristics | Odds ratio | P value |
|--------------------------|------------|---------|
| Age ≤ 60 vs. >60         | 0.99 (0.98–1.01) | 0.483   |
| Grade 2 vs. 1            | 0.42 (0.23–0.77)  | 0.005   |
| 3 vs. 1                  | 0.17 (0.10–0.28)  | < 0.001 |
| 4 vs. 1                  | 0.06 (0.01–0.26)  | < 0.001 |
| Histology endometrial vs. mix | 0.40 (0.16–0.95) | 0.042   |
| endometrial vs. serous   | 0.40 (0.10–0.28)  | < 0.001 |
| Stage II vs. I           | 0.57 (0.31–1.03)  | 0.064   |
| III vs. I                | 0.34 (0.22–0.53)  | < 0.001 |
| IV vs. I                 | 0.22 (0.09–0.51)  | < 0.001 |

3.3. Prognostic value of RILPL2 in EC patients and Cox regression analysis
Based on the fact of RILPL2 downregulation in EC, we further focused on its prognostic role in patients with EC. Kaplan-Meier analysis of the TCGA dataset demonstrated that the patients with high RILPL2 expression led to significantly better OS (P < 0.001) DFS (P < 0.001), and DSS (P < 0.001) in EC patients compared to the low RILPL2 expression group (Fig. 3A-C). Univariate Cox analysis revealed that RILPL2 (HR = 0.578, 95%CI: 0.463–0.721, P < 0.001) were low-risk factor, while age, stage, histological type, and grade were high-risk factors (Fig. 3D). Furthermore, multivariate Cox analysis showed that RILPL2 (HR = 0.747, 95%CI: 0.574–0.972, P = 0.030) was independently related to OS (Fig. 3E), which implied that RILPL2 could be an independent prognostic predictor for EC.

3.4. The relevance of RILPL2 expression with immune infiltration

Based on the median expression value of RILPL2, 552 EC specimens obtained from the TCGA database were classified into high and low expression cohorts (H-RILPL2 and L-RILPL2 groups). It indicated that the L-RILPL2 group had a higher immune score and stromal score than the H-RILPL2 group via ESTIMATE analysis, while the tumor purity score was inferior (Fig. 4A).

The TIMER analysis exhibited that RILPL2 expression had remarkably positive correlations with B cells, CD4+ T cells, macrophages, and DCs in EC (Fig. 4B). Moreover, we evaluated the correlations between RILPL2 expression and immune markers of different TIICs subtypes in EC tissues using TIMER database. The analysis exhibited that RILPL2 expression were correlated with the expression of marker genes of CD8+ T cells, T cells (general), B cells, monocyte, TAMs, M2 macrophage, neutrophils, NK cells, DCs, Th1 cells, Th2 cells, Th17 cells, Treg cells, and T cell exhaustion to varying degrees. Whereas, It revealed unrelated relationship between the expression of RILPL2 and the expression of gene markers for M1 macrophage and Tfh cell in EC samples (Table 2).
Table 2
Correlation analysis between RILPL2 and related gene markers of immune cells.

| Description       | Gene markers | R value | P value |
|-------------------|--------------|---------|---------|
| CD8 + T cell      | CD8A         | 0.141   | 0.001   |
|                   | CD8B         | 0.105   | 0.014   |
| T cell (general)  | CD3D         | 0.211   | < 0.001 |
|                   | CD3E         | 0.198   | < 0.001 |
|                   | CD2          | 0.193   | < 0.001 |
| B cell            | CD19         | 0.246   | < 0.001 |
|                   | CD79A        | 0.157   | < 0.001 |
| Monocyte          | CD86         | 0.247   | < 0.001 |
|                   | CSF1R        | 0.319   | < 0.001 |
| TAM               | CCL2         | 0.135   | 0.002   |
|                   | CD68         | 0.157   | < 0.001 |
|                   | IL10         | -0.111  | 0.010   |
| M1 macrophage     | NOS2         | -0.021  | 0.631   |
|                   | IRF5         | 0.025   | 0.562   |
|                   | PTGS2        | 0.072   | 0.092   |
| M2 macrophage     | CD163        | 0.128   | 0.003   |
|                   | VSIG4        | 0.213   | < 0.001 |
|                   | MS4A4A       | 0.215   | < 0.001 |
| Neutrophils       | CEACAM8      | 0.005   | 0.915   |
|                   | ITGAM        | 0.294   | < 0.001 |
|                   | CCR7         | 0.182   | < 0.001 |
| Natural killer cell| KIR2DL1     | 0.071   | 0.096   |
|                   | KIR2DL3      | 0.066   | 0.125   |
|                   | KIR2DL4      | 0.196   | < 0.001 |
|                   | KIR3DL1      | 0.022   | 0.615   |
|                   | KIR3DL2      | 0.035   | 0.419   |
| Description       | Gene markers | R value | P value |
|-------------------|--------------|---------|---------|
| KIR3DL3           | 0.079        | 0.066   |         |
| KIR2DS4           | 0.028        | 0.517   |         |
| Dendritic cell    | HLA-DPB1     | 0.325   | < 0.001 |
|                   | HLA-DQB1     | 0.218   | < 0.001 |
|                   | HLA-DRA      | 0.333   | < 0.001 |
|                   | HLA-DPA1     | 0.301   | < 0.001 |
|                   | CD1C         | 0.419   | < 0.001 |
|                   | NRP1         | 0.288   | < 0.001 |
|                   | ITGAX        | 0.262   | < 0.001 |
| Th1 cell          | TBX21        | 0.155   | < 0.001 |
|                   | STAT4        | 0.150   | < 0.001 |
|                   | STAT1        | -0.053  | 0.214   |
|                   | IFNG         | 0.026   | 0.540   |
|                   | TNF          | 0.028   | 0.516   |
| Th2 cell          | GATA3        | -0.032  | 0.452   |
|                   | STAT6        | 0.208   | < 0.001 |
|                   | STAT5A       | 0.271   | < 0.001 |
|                   | IL13         | -0.020  | 0.641   |
| Tfh cell          | BCL6         | 0.024   | 0.582   |
|                   | IL21         | 0.012   | 0.773   |
| Th17 cell         | STAT3        | 0.200   | < 0.001 |
|                   | IL17A        | 0.013   | 0.758   |
| Treg cell         | FOXP3        | 0.119   | 0.005   |
|                   | CCR8         | 0.086   | 0.046   |
|                   | STAT5B       | 0.141   | 0.001   |
|                   | TGFB1        | 0.032   | 0.453   |
| T cell exhaustion | PDCD1        | -0.010  | 0.808   |
|                   | CTLA4        | 0.169   | < 0.001 |
### Description

| Description | Gene markers | R value | P value |
|-------------|--------------|---------|---------|
| LAG3        | -0.041       | 0.334   |
| HAVCR2      | 0.244        | < 0.001 |
| GZMB        | -0.032       | 0.454   |

To distinguish the variance of the distribution of 22 TIICs between the two groups, CIBERSORT algorithm was applied to analyze EC cases from the TCGA database. The violin plot manifested the ratio differentiation of 22 TICs between EC tumor specimens with L- or H- RILPL2 expression. T cells CD4 memory activated, T cells follicular helper, T cells regulatory, Dentritic cells resting, and Dentritic cells activated were primary immune cells having a significant relationship with RILPL2 expression (Fig. 4C). The proportion of all these 5 immune cells was increased in H-RILPL2 group compared with L-RILPL2 group.

### 3.5. Methylation and genetic alterations of RILPL2 in EC

The DNA methylation level of RILPL2 was obtained from the TCGA database, and the differentially expressed methylation levels of RILPL2 between EC and paracancerous specimens were analyzed. The methylation level of RILPL2 in the normal samples was notably lower than that in the tumor samples (P < 0.001, Fig. 5A). Besides, the total methylation level of RILPL2 gene and methylation level of each site all showed significantly negative correlation with its expression (P < 0.001, Fig. 5B, Fig. S1), which indicated that high methylation level was a significant cause for RILPL2 low expression in EC. Besides, low amplification and mutation rates of RILPL2 were found in EC patients (Fig. 5C). Thus, genetic alterations might not be crucial for the dysregulation of RILPL2 in EC.

### 3.6. Analysis of the potential mechanisms of RILPL2

In order to build a weighted co-expression network and identify modules and genes related to RILPL2, 2,573 DEGs between EC and paracancerous tissues from the the TCGA database were submitted to WGCNA. After a series of adjustments for WGCNA parameters, the DEGs were clarified into 9 modules by average linkage hierarchical clustering (Fig. S2A-B, Fig. 6A-B). Among these modules, the turquoise module hinted the highest negative correlation with RILPL2 expression (Cor=-0.45, P < 0.001) (Fig. 6C), which might indicated the RILPL2-involved potential oncogenic mechanisms. Subsequently, forty-two genes in the turquoise module were reserved as key genes (GS > 0.2 and MM > 0.8) (Fig. 6D).

To further understand the potential oncogenic mechanisms associated to these hub genes, we next conduct GO and KEGG analysis to perform. “chromosome segregation”, “chromosome region”, and “DNA-dependent ATPase activity” were the significantly important GO terms for cellular components, biological processes and molecular functions, respectively (Fig. 7A). “cell cycle” was the most significant pathway in the KEGG pathway analysis (Fig. 7B). Besides, we established a PPI network with these hub genes and found that a large numbers of cell cycle related genes play key roles (Fig. 7C).
4. Discussion

EC is one of the most widespread malignant tumors in female's genital tract in the world and accounts for approximately 74,000 deaths per year worldwide [23, 24]. The incidence of EC is increasing in the range of 10 to 20 per 100,000 women annually, and the age of onset is younger than in prior years [5]. The combination of surgery, radiotherapy, and chemotherapy is the current gold standards for treatment of EC patients. However, the 5-year survival rate is approximately 45%-60% and 15%-25% for stage III and stage IV EC, respectively, which led to the majority of EC-related deaths [25]. Hence, it is of critical importance to identify credible biomarkers for early diagnosis, early initiation of treatments and improved prognosis of EC.

In this work, sequential data filtering was performed from the TCGA database, which determined the identification of the key gene RILPL2. The relationship between RILPL2 expression and clinical factors was analyzed by logistic regression analysis and clinical relevance filtering. Besides, we used Kaplan-Meier analysis, as well as univariate and multivariate Cox analysis to assess the associations between RILPL2 expression and survival outcomes. Not only that, we analyzed the relevance of RILPL2 expression with immune cells infiltration. Finally, the WGCNA analysis was conducted to perform the underlying mechanisms of RILPL2.

Previous research indicated that RILPL2 widely expressed in most tissues, including brain, heart, lung, liver, kidney, pancreas, and placenta [7]. To our best knowledge, no available studies have revealed the expression of RILPL2 and its underlying prognostic impact in EC. However, relative studies indicated that RILPL2 showed a significant correlation with breast cancer by conducting series of literatures of RILPL2. Chen et al. revealed that RILPL2 expression in breast cancer tissues is lower than that in paracancerous tissues, and that RILPL2 upregulation is associated with prolonged prognosis. Overexpression of RILPL2 inhibited breast cancer cell proliferation and metastasis in vitro and in vivo. Besides, the interaction of exogenous RILPL2 with TUBB3 resulted in the downregulation of breast cancer cell proliferation and migration and upregulation of PTEN expression by promoting destabilization of TUBB3 [10].

The present study focused on the potential role of RILPL2 in EC. We identified that RILPL2 was expressed differently in normal tissues and EC tissues. Kaplan-Meier analysis exhibited that high level of RILPL2 was notably related to better prognosis in patients with EC. From the result of univariate and multivariate Cox analysis, we found that RILPL2 was a high-risk factor and could be served as an independent indicator to forecast the clinical outcome of patients with EC. Besides, The ESTIMATE, TIMER, and CIBERSORT analysis both suggested RILPL2 might play a critical role in regulating immune cell infiltration in EC and is related to immune response. The fraction of immune cell infiltration plays crucial roles in tumor growth, metastasis, and therapeutic resistance [26]. For example, CD8 + cytotoxic T cell serves as crucial role to destroy tumor cells in many cancers [27]. Macrophages are classified into two classifications with various specific functions: M1, with a proinflammatory function, and M2, with an anti-inflammatory or wound healing effect [28, 29]. Recent years, bioinformatics-dependent identification of immune-related biomarkers has been emerging, and increasing numbers of genes are found to be
associated with tumor immunity [30, 31]. Our current research suggested that the expression of RILPL2 may regulate immune infiltration of multiple TIICs in the immune microenvironment of EC specimens, thus directly and/or indirectly regulating immune monitor and influencing progression of tumor.

5. Conclusion

In summary, accumulating evidence on the tumorigenesis and development of RILPL2 in EC were acknowledged and preliminarily presents its potential as a promising diagnostic and prognostic indicator. Our results suggest that RILPL2 is decreased in EC and low RILPL2 expression is related to grade of malignancy and poor prognosis. In addition, our research also indicates that RILPL2 is closely correlated with immune infiltration in EC. Overall, these findings provide a theoretical basis for next underlying studies to validate the function of RILPL2 in EC.

Abbreviations

EC: endometrial carcinoma; RILPL2: Rab interacting lysosomal protein like 2; RILP: Rab-interacting lysosomal protein; TCGA: the Cancer Genome Atlas; GEO: Gene Expression Omnibus; qRT-PCR: real-time quantitative RT-PCR; HPA: Human Protein Atlas; OS: overall survival; DFS: disease-free survival; DSS: disease-specific survival; CDR: Clinical Data Resource; TIICs: tumor-infiltrating immune cells; DCs: dendritic cells; DEGs: differentially expressed genes; WGCNA: weighted gene co-expression network analysis; TOM: topological overlap matrix; GS: gene significance; ME: module eigengene; MS: module significance; MM: module membership; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: protein-protein interaction.

Declarations

Ethics approval and consent to participate

Ethical approval for the study was granted by the Clinical Research Ethics Committee, the Third People's Hospital of Nantong, and the current study was performed in conform to the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and material

All data are included in the article.

Competing interests

The authors declare no conflict of interest.
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**Authors' contributions**

HZ conceived the study and participated in the study design, performance, and manuscript writing. JL, MX, ZW and JL conducted the bioinformatics analysis. HZ revised the manuscript. All authors read and approved the final manuscript.

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Figures

![Figure 1](image)

**Figure 1**

Expression analysis of RILPL2 in EC. (A-C) Differential expression of RILPL2 in tumor and adjacent samples from the TCGA dataset, the GSE17025 dataset, and our recruited cohort. (D) Representative images shows the samples stained with RILPL2 from the HPA database. (E) Differential expression of RILPL2 protein in tumor and adjacent samples from the CPTAC dataset.
Figure 2

Correlation of RILPL2 expression of and clinicopathologic variables. (A) age, (B) grade, (C) histological type, and (D) stage.
Figure 3

Prognostic value of RILPL2 in EC patients in the TCGA database. (A) OS, overall survival. (B) DFS, disease free survival. (C) DSS, disease specific survival. (D) univariate analysis of RILPL2. (E) multivariate analysis of RILPL2.
Figure 4

The correlation of TIICs infiltration with RILPL2 expression. (A) Comparison of stromal scores, immune scores, ESTIMATE scores, and tumor purity between the L-RILPL2 and H-RILPL2 groups according to the ESTIMATE tool. (B) TIMER analysis of the correlation between the expression of RILPL2 and 6 immune cells in EC. (C) The proportion of 22 immune cells in EC tissues in the L-RILPL2 and H-RILPL2 groups.
Figure 5

Methylation and mutation analysis of RILPL2 in EC. (A) Methylation level of RILPL2 in EC from the TCGA dataset. (B) Correlation between RILPL2 methylation level and its expression in EC. (C) The variety and proportion of samples with genetic alterations of RILPL2 in EC.

Figure 6
Exploration for genes correlated with RILPL2 in EC. (A) Clustering dendrogram of EC patients from the TCGA dataset. (B) A total of 2,573 DEGs were clustered according to the dissimilarity measure (1-TOM) and were classified into 9 modules. (C) A correlation heatmap between module eigengenes and clinical features of EC. (D) Scatter plot of turquoise module eigengenes.

Figure 7

Latent oncogenic mechanisms of RILPL2 in EC. (A) GO analysis of genes in turquoise module eigengenes, top 10 terms were exhibited. (B) KEGG analysis of gene in turquoise module eigengenes, top 10 terms were exhibited. (C) PPIs of hub genes constructed by STRING tool.

Supplementary Files

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