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Expression and bioinformatics analysis of RPL38 protein and mRNA in gastric cancer

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ABSTRACT

We aimed to clarify the expression of RPL38 in gastric cancer, explore the relationship between the expression level of RPL38 and the clinicopathological parameters and prognosis of gastric cancer patients, and explore whether RPL38 has the potential to be used as a therapeutic target for gastric cancer and a biomarker for assessing prognosis. The mRNA and protein expression of RPL38 in gastric cancer tissues and normal tissues were compared by TIMER, Kaplan-Meier plotter, CCLE and UALCAN databases, respectively. Next, the relationship between the expression level of RPL38 in gastric cancer tissues and clinicopathological features was analysed using the UALCAN database. The Kaplan-Meier plotter database was then used to predict the prognostic value of RPL38 in gastric cancer patients, and overall survival curves were plotted based on the follow-up information of clinical specimens. The relationship between RPL38 expression and the level of immune infiltration in gastric cancer was explored using the TIMER database. Finally, co-expression analysis as well as enrichment analysis of RPL38 was performed using LinkedOmics database and GSEA, respectively. Through comprehensive bioinformatics analysis and immunohistochemistry experiments, we comprehensively concluded that RPL38 was highly expressed in gastric cancer. Univariate analysis showed that TNM stage (P=0.008), radiotherapy (P=0.02), and RPL38 expression level (P=0.0006) were associated with prognosis. Multifactorial analysis showed that RPL38 expression level (P=0.019), TNM stage (P=0.015) and radiotherapy (P=0.039) were independent risk factors affecting the prognosis of gastric cancer. Gastric cancer patients with high expression of RPL38 had poorer OS. In addition, RPL38 was associated with immune infiltration in gastric cancer. RPL38 is highly expressed in gastric cancer tissues, and RPL38 protein plays an important role in the development of gastric cancer, which is one of the important factors in assessing the prognosis of gastric cancer patients.

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Introduction

Gastric cancer is one of the most common malignant tumours worldwide, ranking third in global cancer-related mortality (1). Of concern, nearly half of the new cases and deaths occur in China (2), and surgical resection remains the only potentially curative treatment for gastric cancer (3). The results of related clinical studies show that the recurrence rate of stage I gastric cancer is less than 10%, the recurrence rate of stage II gastric cancer is about 20%-30%, the recurrence rate of stage III gastric cancer is about 40%-50%, and the recurrence rate of stage IV gastric cancer is as high as about 90% (4). With the popularity of gastroscopy, the number of cases of early gastric cancer has gradually increased in China in recent years. Intramucosal and submucosal cancers with good biological behaviours perform minimally invasive endoscopic mucosal or submucosal dissection, but due to the insidious onset of most GCs, many patients are diagnosed in the middle or late stages, and metastasis, recurrence and drug resistance are the main causes of patient treatment failure and death (5). Environment, diet, obesity, heredity, genetic mutation and Helicobacter Pylori (HP) long-term infection (6), gastric mucosal intestinal epithelial hyperplasia (7), Epstein-Barr virus (EBV) infection (8) are risk factors for gastric carcinogenesis. Heterogeneous proliferation of epithelial and glandular epithelial cells in the gastric mucosa caused by various factors over a long period of time, i.e., low-grade intraepithelial neoplasia-high-grade intraepithelial neoplasia-invasive carcinoma, is the process of the disease spectrum of most gastric carcinomas (9), which is accompanied by the participation of multiple genes and manifested in a series of morphological and functional changes, with obvious heterogeneity in molecular phenotypes and biological behaviours, so the early detection of pre-cancerous lesions is an important means of preventing and controlling GC (10-12). Therefore, for the basic research of early diagnosis of gastric cancer, actively exploring the genes that affect the abnormal expression of GC in the process of GC occurrence, evolution, invasion, and metastasis, searching for potential new drug therapeutic targets and prognostic indicators, and exploring the interaction relationship between different genes are one of the important contents of the basic and applied research fields of gastric cancer at present.

RPL38 belongs to the L38E ribosomal protein family.

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It is located in the cytoplasm. Alternative splice variants have been identified and both encode the same protein. Like the genes encoding ribosomal proteins, this gene has multiple processed pseudogenes scattered throughout the genome, one of which is located in the promoter region of the type 1 angiotensin II receptor gene (13). Sahin et al. identified RPL38 (14), which is expressed predominantly in the pancreatic ductal epithelium. They also demonstrated that RPL38 is highly expressed in a panel of pancreatic cancer cell lines and may be available for tumour targeting or as a tumour marker. Zheng et al (15) identified RPL38 as a differentially expressed gene that may play a role in the prognosis of pancreatic ductal adenocarcinoma. In this study, we explored the role of RPL38 in GC development and progression.

In this paper, we performed bioinformatics and immunohistochemistry analysis of RPL38 expression in GC and its prognostic significance. Firstly we analysed the expression of RPL38 in GC by TIMER, Kaplan-Meier plotter, CCLE and UALCAN databases. Secondly, we analysed the relationship between the expression level of RPL38 and clinicopathological features using the UANCAL database, and then we predicted the prognostic value of RPL38 in gastric cancer patients using the Kaplan-Meier plotter database, which was validated and analysed on clinical specimens. In addition the relationship between RPL38 expression and the level of immune infiltration was explored using the TIMER database. Finally, co-expression analysis as well as enrichment analysis of RPL38 was performed using LinkedOmics database and GSEA, respectively.

Materials and Methods

Bioinformatics prediction methods

The differential expression of RPL38 in all TCGA tumours compared to normal tissues was investigated using the TIMER (https://cistrome.shinyapps.io/timer/) database. In addition, we analysed the relationship between RPL38 expression and the abundance of immune infiltration; protein expression of RPL38 in gastric cancer was obtained using the UALCAN (http://ualcan.path.uab.edu) database. We analysed the mRNA levels of RPL38 in several cancer cell lines by the CCLE (http://portals.broadinstitute.org/ccle) database; the use of the GEPIA database (http://gepia.cancer-pku.cn/) was a function of survival analysis. The relationship between RPL38 expression in gastric cancer and overall survival was obtained; differential gene expression analysis in tumour, normal and metastatic tissues and the relationship between RPL38 expression and survival in gastric cancer was investigated by the Kaplan-Meier plotter (http://kmplot.com/analysis/) database; In this paper, we obtained the co-expressed genes of RPL38 through LinkedOmics database and performed GO and KEGG analysis. Subsequently, we analysed the Gene Ontology biological process (GO_BP), Gene Ontology cellular component (GO_CC) and Gene Ontology molecular function (GO_MF), and KEGG pathway by "LGSEA". Where the grade criterion was FDR<0.05, a total of 500 simulations were performed.

Immunohistochemical (IHC) staining

We conducted immunohistochemical analysis of RPL38 protein expression in gastric cancer tissue and normal gastric tissue, using HPA052543 as the antibody.

Correlation between RPL38 and clinicopathological or survival rates of gastric cancer

We extracted survival information for each sample in TCGA. Then, we selected several indicators: OS, disease-specific survival (DSS), DFS and progression-free interval (PFS) to elucidate the association between RPL38 expression and prognosis of gastric cancer patients. Survival curves were performed using the R packages "survminer" and "survival". Then, Cox analysis was performed using R package "survival" to determine the correlation between RPL38 and survival. The R packages "ggpubr" and "limma" were used for clinicopathological correlation analysis.

Statistical analysis

Correlations between OS or PFS and gene mutations and clinical characteristics were analysed using Cox proportional risk models. All statistical tests were two-sided and differences of $P<0.05$ were considered statistically significant. All statistical analyses were performed using Statistic Package for Social Science (SPSS) version 23.0 (IBMCorporation, Armonk, NY, USA) or GraphPad Prism 5.0 (GraphPadSoftware, LaJolla, CA, USA).

Results

TIMER database analysis of RPL38 expression levels in pan-cancer

We used the TIMER database to analyse the expression differences of RPL38 in all TCGA-hosted tumours. The results showed that the expression of RPL38 in bladder uroepithelial carcinoma, breast invasive carcinoma, colon cancer, oesophageal cancer, hepatocellular carcinoma, gastric cancer, renal smectic cell carcinoma, and head and neck carcinoma was significantly higher than that in normal tissues (Figure 1).

CCLE database analysis of RPL38 expression levels in gastric cancer and systemic tumour cell lines

To further validate the expression of RPL38 in different human tumours, we used the CCLE database to analyse the mRNA sequence data at the cellular level. As shown, RPL38 was highly expressed in tumours such as myeloma, leukaemia, and colorectal cancer carcinoma, while it was lowly expressed in tumours such as brain cancer, prostate cancer, and adrenocortical cancer (Figure 2). These data indicate that RPL38 has different expression levels in different tumours, implying that RPL38 may play multiple functions in various tumours.

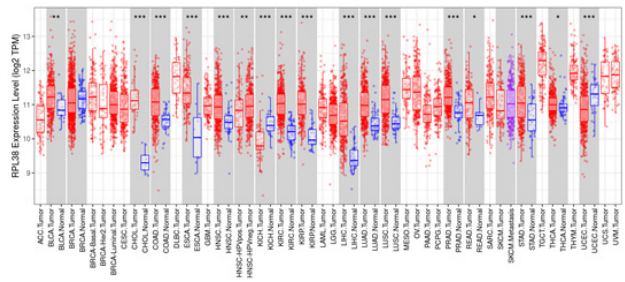

Figure 1. Expression levels of RPL38 in pan-cancer.

Kaplan-Meier plotter database to analyse the expression level of RPL38

We used Kaplan-Meier plotter database to analyse the mRNA expression of RPL38 in gastric cancer tissues, normal tissues. The results showed that the expression of RPL38 was significantly elevated in gastric cancer (P<0.001) (Figure 3).

UALCAN database to analyse the relationship between RPL38 expression and clinicopathological features

In order to investigate whether the expression level of RPL38 was related to the development of gastric cancer, we analysed the correlation between the expression of RPL38 and the clinicopathological features of gastric cancer using the UALCAN database. For TP53 mutation, the expression of RPL38 in the mutant type was not significantly different from that of the wild type (P>0.05) (Figure 4A). For gastric cancer tumour stage, the expression of RPL38 in stage4 presented a significant difference (P<0.05) from stage 1, stage 2 and stage 3, while there was no significant difference between stage 1, stage 2 and stage 3 (Figure 4C). As shown, for gender, the expression of RPL38 in male gastric cancer tissues was not significantly different from that of female gastric cancer tissues (P>0.05) (Figure 4D). For age, there was no significant difference in the expression of RPL38 in gastric cancer tissues between 21-40 and 41-60 years old, and there was no significant difference in the expression of RPL38 in gastric cancer tissues between 61-80 and 81-100 years old. There was a significant difference in the expression of RPL38 in the gastric cancer tissues between 21-40 years old, and 41-60 years old compared with 61-80 years old and 81-100 years old (P<0.05) (Figure 4E). According to the tumour grade and whether or not they were infected with H. pylori, there was no significant correlation between the expression of RPL38 in gastric cancer tissues and the tumour grade and whether or not they were infected with H. pylori (P>0.05) (Figure 4F, G). According to the status of lymph node metastasis, there was no significant difference in RPL38 expression in NO, N1, N2 and N3 gastric cancer tissues (P>0.05) (Figure 4H).

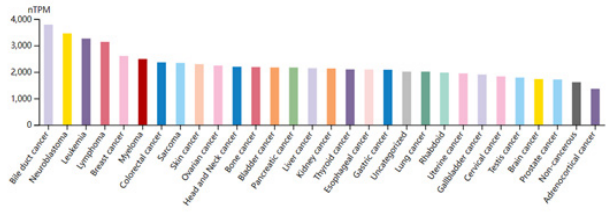

Figure 2. Expression levels of RPL38 in different tumour cell lines.

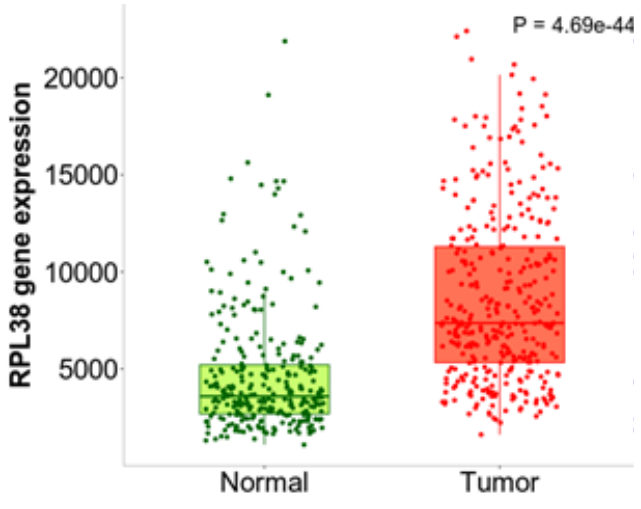

Figure 3. Kaplan-Meier plotter analysis of RPL38 expression levels in gastric cancer tissues, normal tissues.

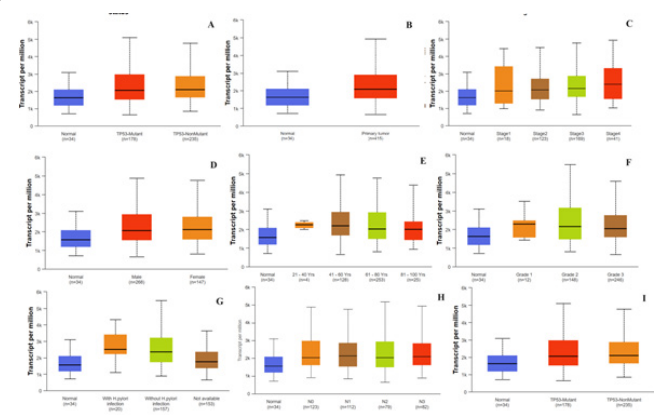

Figure 4. Relationship between RPL38 gene expression level and clinicopathological and molecular features of gastric cancer patients analysed by UALCAN database.

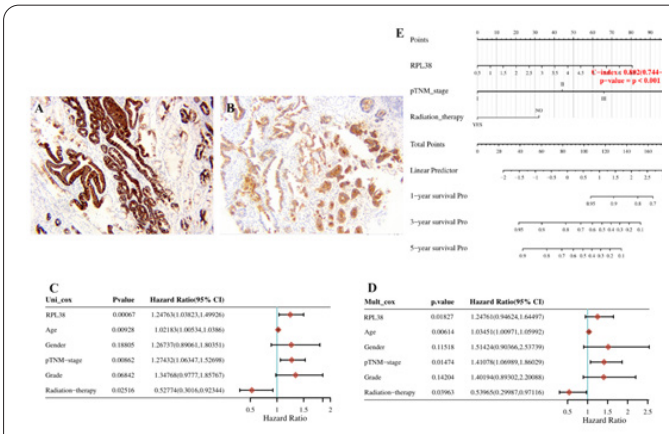

Figure 5. Construction of immunohistochemically detected RPL38 expression and prognostic characteristics of gastric cancer. (A) normal tissue (B) gastric cancer tissue (C) univariate analysis (D) multivariate Cox regression analysis (E) a column-line plot consisting of risk scores and other clinical indicators for predicting 1, 3, and 5-year OS in gastric cancer patients.

Prognostic diagnostic role of RPL38 in gastric cancer

To assess the expression of RPL38 at the protein level, IHC results were obtained and analysed from HPA. The results showed that RPL38 IHC staining was weak in normal gastric tissues (Figure 5A), while higher intensity RPL38 IHC staining was detected in gastric cancer tumour tissues (Figure 5B). We further applied univariate and multivariate Cox regression models to investigate the prognostic diagnostic role of RPL38 in gastric cancer. The results of univariate analysis showed that RPL38 (95% CI: 1.247 (1.038-1.499), P<0.0001) had prognostic value for OS in gastric cancer (Figure 5C). In multivariate stepwise Cox regression analysis, RPL38 (95% CI:1.248(0.946-1.649), P<0.05) still maintained its prognostic value (Figure 5D). The results indicated that RPL38 expression was an independent prognostic factor for gastric cancer. Thereafter, RPL38, STAGE and whether or not they received

radiotherapy were visualised in the column line graphs. The column line graphs of 1, 3 and 5-year OS in the cohort are shown in Figure 5E.

High RPL38 expression is associated with poor prognosis in gastric cancer patients

We first analysed the relationship between RPL38 expression and OS in gastric cancer patients using the Kaplan-Meier Plotter database, and the results showed that high RPL38 expression levels were associated with shorter OS in gastric cancer patients (P<0.001). Survival association analysis including OS, DSS, DFS and PFS was used to further validate the correlation between RPL38 expression and the prognosis of gastric cancer. The results of KM mapper showed that individuals with gastric cancer patients with a high level of RPL38 expression had a shorter survival time (P=0.002). Furthermore, DSS analysis showed a correlation between high RPL38 expression and poor outcomes in gastric cancer patients (P=0.003). Gastric cancer patients with high RPL38 expression had worse PFS (P<0.001) (Figure 6).

TIMER database analysis of the correlation between RPL38 expression and markers of different immune cell subpopulations

We explored the correlation between RPL38 expression and the level of immune infiltration in gastric cancer by TIMER database. The results showed that RPL38 expression was significantly negatively correlated with CD4$^+$T cells (P<0.01, r=-0.19), macrophages (P<0.001, r=-0.238), neutrophils (P<0.001, r=-0.197), and dendritic cells (P<0.001, r=-0.215). rPL38 expression was significantly correlated with B cells (P>0.05, r=0.057) and CD8$^+$T cells (P>0.05, r=-0.066) with no significant correlation (Figure 7).

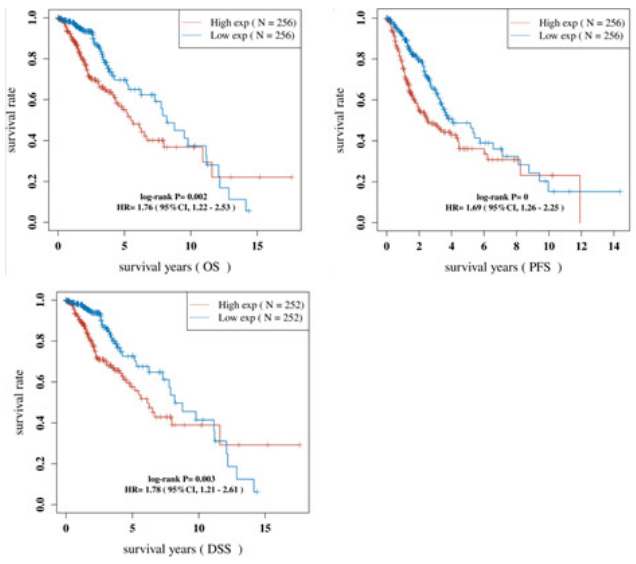

Figure 6. Survival profile of RPL38 gene.

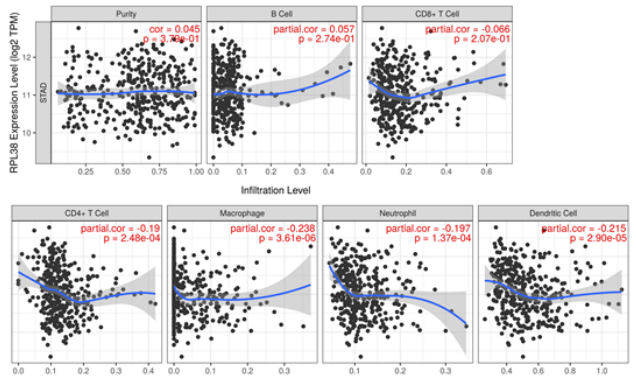

Figure 7. Relationship between RPL38 expression and the level of immune infiltration in gastric cancer.

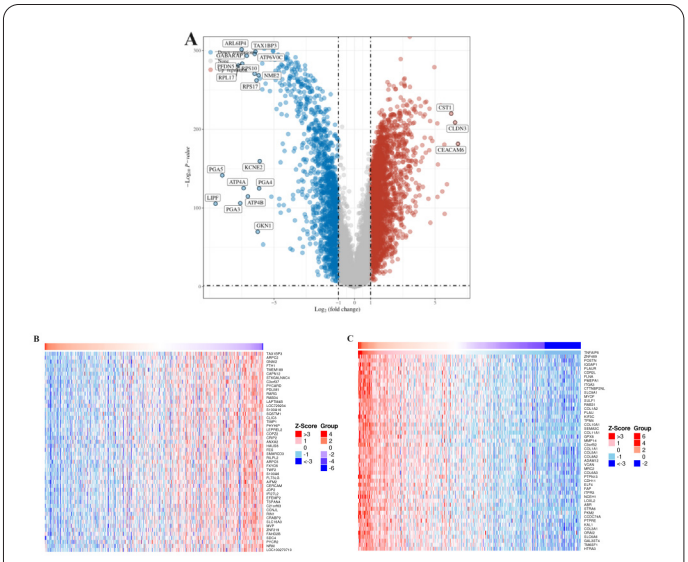

Figure 8. Co-expressed genes of RPL38 in gastric cancer.

LinkedOmics database analysis of RPL38 gene co-expression analysis

We assessed the co-expression pattern of RPL38 in a gastric cancer cohort using the LinkedOmics functional module. The results showed that 1569" of the genes (shown as red dots) were significantly positively correlated with RPL38 expression, and 4973 genes (shown as blue dots) were significantly negatively correlated with RPL38 expression (Figure 8A). And the 50 most correlated genes with RPL38 expression were shown in the heatmap, respectively, with Figure 8B as positively correlated genes and Figure 8C as negatively correlated genes. According to the LinkedOmics database analysis, TAX1BP3 was the most correlated gene with RPL38 in gastric cancer.

LinkedOmics database analysis of enrichment of RPL38-related co-expressed genes

Based on the results of the co-expressed gene analysis of RPL38, the GO term analysis of GSEA showed that the genes related to RPL38 were mainly located in the side of the cell membrane, mitochondria, replication forks, cytoplasmic parts and tertiary granules. By KEGG pathway analysis, its enrichment occurred in the P53 signalling pathway, cell differentiation, lipids and atherosclerosis (Figure 9).

Discussion

GC is one of the most heterogeneous malignant tumours of the gastrointestinal tract, which is difficult to diagnose at an early stage and is prone to metastasis, recurrence, and drug resistance at a later stage, resulting in a high number of gastric cancer diagnoses and deaths worldwide (16). In addition to the risk factors of Helicobacter pylori infection, genetic factors, and high levels of dietary nitro-

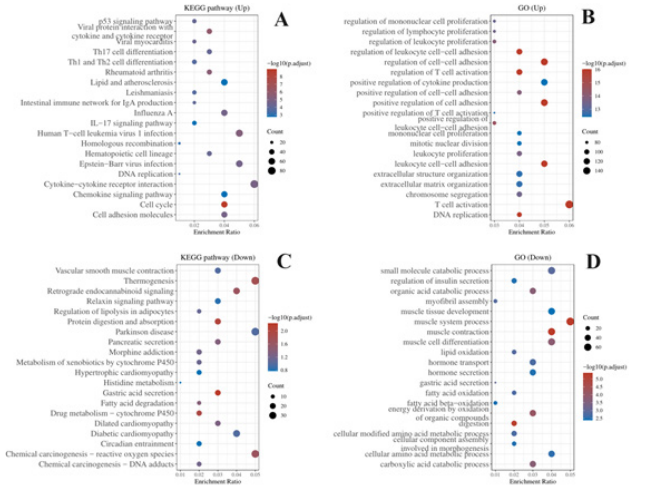

Figure 9. Enrichment analysis of RPL38 co-expressed genes using the LinkedOmics database.

samines (17), most GCs undergo an abnormal proliferation of intestinal epithelial chemotaxis and intraepithelial neoplasia, which is accompanied by the involvement of many genes at the molecular level and manifests itself in a series of morphological and functional changes. Different molecular genetic features and abnormal signalling pathway activation affect the biological behaviour and heterogeneous progression of gastric cancer, which is the biological basis of metastatic recurrence and drug resistance in gastric cancer patients. Exploring new tumourigenic markers and prognostic markers in the process of gastric cancer development and progression, as well as exploring the possible correlation between different proteins, are very important for early intervention in gastric pre-cancerous lesions, prevention and control of gastric cancer, and targeted drug therapy. Research on its mechanism and prognosis has become a hotspot of concern, and different scholars have different conclusions on the analysis of prognostic factors of gastric cancer.YANG et al. (18) reported that tissue typing and the absence of lymph node metastasis were independent prognostic factors. Kono et al. (19) reported that age and gender were independent prognostic factors. Tumour development, progression and metastasis is a complex process of multi-step, multi-factorial, multistage and multi-genetic alterations with multiple factors involved.RPL38 expression in gastric cancer tissues has been reported to be less common, which is mainly related to the relative rarity of gastric cancer.

In this study, we explored the role of RPL38 in gastric cancer for the first time and confirmed that RPL38 is highly expressed in gastric cancer, and predicted that the high expression of RPL38 may be closely related to the poor prognosis of gastric cancer patients. To obtain a reliable result, we explored the expression of RPL38 using several online databases. The results showed that both mRNA and protein RPL38 were highly expressed in gastric cancer.

We then further applied univariate and multivariate Cox regression models to investigate the independent influencing factors of gastric cancer, and the results showed that RPL38 expression, age, radiotherapy, and TNM grade were independent prognostic factors of gastric cancer. Subsequently, we explored the prognostic potential of RPL38 in gastric cancer using the Kaplan-Meier and GEPIA databases, both of which suggested that gastric cancer patients with high RPL38 expression had a shorter OS, and finally also confirmed these results on immunohistochemistry-based analysis. Together, these findings elucidate the expression of RPL38 as a potential biomarker for predicting the prognosis of gastric cancer.

In addition, the relationship between RPL38 expression and the level of immune infiltration in gastric cancer is an important aspect of this study. Here, we extensively evaluated the expression of RPL38 in gastric cancer and its relationship with the level of immune infiltration. The results showed that the expression of RPL38 was significantly negatively correlated with $CD4^+$T cells, macrophages, neutrophils and dendritic cells. In this study, through the analysis of GO and KEGG pathways enriched for RPL38 and its related genes, we hypothesised that RPL38 may affect gastric cancer development through these regulatory pathways.

Despite the above studies on the value of RPL38 in gastric cancer, we still have some limitations. First, most of the data we analysed were downloaded from online databases and lacked experimental validation in terms of experimental zoology and molecular biology. Second, the gastric cancer samples we studied were limited and not representative of all gastric cancer populations, and further validation using techniques such as real-time PCR or Western blot is needed. Finally, this study is a retrospective study and needs to be supported by further prospective findings.

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Conflict of Interests

The authors declared no conflict of interest.

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