Exploring the Interplay Between CCND1 Expression, Methylation and Clinicopathological Factors in Gastric Cancer: A Meta-Analysis

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Abstract: Aim: The role of CCND1 in gastric cancer (GC) was analyzed through bioinformatics to provide scientific basis for the early diagnosis and treatment of GC. Methods: The expression of CCND1 in (GC) was analyzed using R (3.6.3), Ualcan, and HPA, and to evaluate the relationship between CCND1 and clinicopathological features in patients of GC, so as to evaluate the value of CCND1 in the diagnosis of GC. At the same time, the correlation between CCND1 gene changes and its methylation with prognosis in patients of GC are explored by cBioPortal website, MethSurv and evaluate the role of CCND1 in immune cell infiltration. Results: The expression of CCND1 was abnormally high in GC, and was closely correlated with patient age (P=0.018), histological type (P=0.031), reflux history (P=0.036), and Barrett's esophagus (P=0.042). The sensitivity and specificity of CCND1 in the diagnosis of GC are 0.848 and 0.900, respectively, with positive predictive value of 0.944 and negative predictive value of 0.750. CCND1 gene mutation and amplification are the most common genetic changes in GC, and are not associated with patient prognosis. CCND1 methylation is associated with overall survival and disease-free survival of gastric cancer patients, while the prognosis of CCND1 hypermethylated gastric cancer patients are poor. CCND1 plays an important role in immune infiltration. Conclusions: In GC, the high expression of CCND1 is related to the clinicopathological features of patients and will become a biomarker for the diagnosis of gastric cancer. Moreover, hypermethylation of CCND1 has been shown to be associated with poor prognosis and plays a key role in immune infiltration.

Keywords: Gastric Cancer; Cyclin D1; Bioinformatics; Prognostic Value; Immune Infiltration; Clinicopathological Features

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1 Introduction

Gastric cancer (GC) is a major concern in the digestive system, known for its high mortality rates and poor prognosis [1]. Despite being a leading cause of cancer-related death in East Asia and Eastern Europe, early diagnosis remains a challenge due to the lack of noticeable symptoms in its early stages [2]. The 5-year survival rate for advanced GC is less than 20% [3]. The CCND1 gene, located on chromosome 11q13, has been the focus of much attention for its role in cell cycle regulation [4, 5]. CCND1 expression levels can be detected in precancerous gastric lesions and have been shown to increase as the severity of the lesions increases. As such, CCND1 may serve as an early warning signal for gastric cancer. This study aims to comprehensively evaluate the role and diagnostic value of CCND1 in GC using R (3.6.3) and bioinformatics analysis platforms. The results of this study will provide a better

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understanding of the importance of CCND1 in GC development and offer new avenues for early screening, leading to improved treatment options, clinical outcomes, and quality of life for GC patients.

2 Materials and Methods

2.1 CCND1 Gene Differential Expression Analysis

The mRNA expression levels of CCND1 in TCGA and GTEx datasets were analyzed through a comprehensive process. First, the RNAseq data in TPM format was downloaded from UCSC Xena (https://xenabrowser.net/datapages/), and then was uniformly processed by Toil [6]. Next, the data of gastric cancer and its corresponding normal tissues were extracted from both TCGA and GTEx datasets. In addition, the mRNA expression levels of CCND1 in the STAD project were analyzed using the Ualcan [7] online analysis tool. The RNAseq data in the level 3 HTSeq-FPKM format was obtained from TCGA (https://portal.gdc.cancer.gov/) and paired samples were kept for analysis. To understand the expression of CCND1 protein in normal tissue and GC tissue, the immunohistochemical results of glandular cells from the Human Protein Atlas (HPA, https://proteinatlas.org/) [8] were collected and analyzed. All the data in this study are obtained from the open database and did not require approval from an ethics committee.

2.2 Correlation Analysis of CCND1 and Clinicopathological Features

The RNAseq data of the STAD project in level 3 HTSeq-FPKM format are obtained from the TCGA database, then standardized to TPM format and converted to log2. A sample group of 375 GC patients, including relevant clinicopathological information, is carefully selected. Further analysis, including chi-square tests and Fisher's exact tests, is conducted to examine the relationship between CCND1 expression and various clinicopathological parameters such as age, sex, pathological stage, race, TNM stage, histological type, histological grade, reflux history, Barrett's esophagus, and H. pylori infection in GC patients. This is done to gain insights into the association between the expression level of CCND1 and different clinicopathological characteristics.

2.3 Potential Diagnostic Value of CCND1

The RNAseq data from TCGA and GTEx in TPM format are processed using the UCSC XENA platform (https://xenabrowser.net/datapages/) and Toil pipeline [6], and the GC and normal tissue samples from TCGA and GTEx are transformed into log2 format. Statistical analysis and visualization are performed using R (3.6.3), including the pROC (1.17.0.1) and ggplot2 (3.3.3) packages. The diagnostic performance of CCND1 in gastric cancer is evaluated using receiver operating characteristic (ROC) curves.

2.4 Genetic Changes and Prognostic Value Analysis of CCND1

The cBioPortal for Cancer Genomics [9, 10] is a comprehensive platform that provides easy access to cancer genomics data for researchers and clinicians. With over 5000 tumor samples from 20 different cancers, the platform simplifies complex procedures and enables visual analysis across various genomic data types, such as somatic mutations, gene copy number changes, DNA methylation, mRNA and miRNA expression, non-synonymous mutations, protein enrichment, and phosphorylated protein enrichment, without requiring bioinformatics expertise. In this study, the genomic data of GC patients was selected and analyzed using the "Mutations" module of cBioPortal to evaluate the CCND1 mutation in GC. The "Comparison/Survival" template was also utilized to assess the correlation between CCND1 genetic changes and the prognosis of GC patients, in order to evaluate the prognostic value of CCND1 genetic changes.

2.5 Relationship Between CCND1 Methylation and Prognosis

MethSurv [11], a cutting-edge platform for cancer research, provides access to over 7,358 methylation data profiles from 25 different types of cancer, available from both TCGA and GDAC Firehose data sets. With its user-friendly interface, MethSurv allows researchers to easily visualize DNA methylation data and its relationship with important clinical factors such as survival status, patient characteristics, and clinicopathological characteristics. In this study, we utilized MethSurv's "single CpG" analysis module to examine the correlation between the

methylation level of the CCND1 gene and the prognosis and survival outcomes of GC patients.

2.6 Relationship Between CCND1 Expression and Immune Cell Infiltration and Prognosis

To explore the connection between CCND1 and immune cell infiltration in GC, we employed the Single-Sample Gene Set Enrichment Analysis (ssGSEA) algorithm using the GSVA package in R (3.6.3) [12]. The markers of 24 types of immune cells were taken from a previous study in Immunity [13]. The Timer database [14, 15], which is based on the TCGA data analysis results, includes 10897 samples from 32 cancer types and has six main analysis modules for dynamic analysis and visualization of the relationship between immune infiltration and various factors. The "Survival" module was utilized to assess the prognostic value of immune cell infiltration in GC patients.

2.7 Other Statistical Analyses

In this study, we employed statistical methods using the R software version 3.6.3 to evaluate the expression of CCND1 in GC samples. Mann-Whitney U test was used to compare the expression of CCND1 in unpaired GC samples and to determine the correlation between CCND1 expression and 24 types of immune cells in GC by group. To assess CCND1 expression in paired GC samples, we performed paired sample t test. Furthermore, we used chi-square test and Fisher's exact test to analyze the relationship between CCND1 expression and clinicopathological characteristics of GC patients.

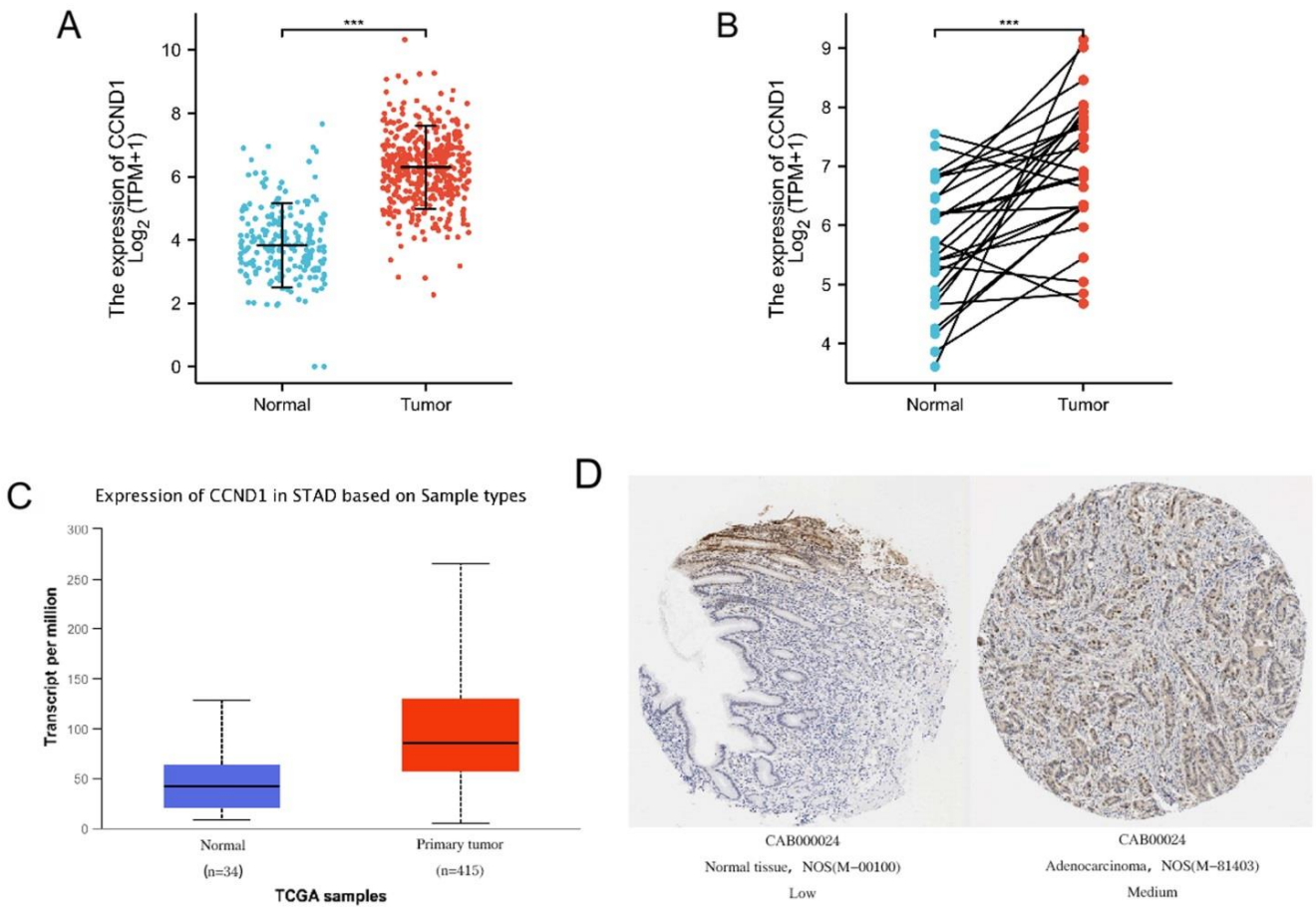

Figure 1 The expression of CCND1mRNA and protein in GC

(A) the expression of CCND1 in GC and normal tissues; (B) the expression of CCND1 in GC and its paired adjacent tissues; (C) Ualcan online analysis of the mRNA expression of CCND1 in TCGA database, (D) the protein expression of CCND1 in GC based on HPA

3 Results

3.1 Up-regulated Expression of CCND1 mRNA and Protein

The results of the unpaired sample analysis demonstrate that the expression level of CCND1 mRNA in gastric cancer tissue is significantly higher compared to the normal control group (Figure 1A). The comparison between the expression level of CCND1 in gastric cancer tissue and adjacent normal tissue reveals a noticeable upregulation of CCND1 mRNA in gastric cancer tissue (Figure 1B). Further analysis of the expression of CCND1 mRNA in gastric adenocarcinoma was performed using Ualcan, and the results of the immunohistochemical staining of glandular cells from the HPA database were collected. This analysis confirms that the expression of CCND1 protein is also upregulated in gastric cancer (Figure 1C, D). These results collectively suggest that the expression of both CCND1 mRNA and protein increases when gastric tissue cells transform into cancerous cells.

3.2 Relationship Between CCND1 and Clinicopathological Characteristics

Grouping of 187 GC patients based on the median expression level of CCND1 mRNA results in a low expression group and a high expression group. Our analysis shows that there is a correlation between the expression of CCND1 in GC patients and various factors such as age (P=0.018), histological type (P=0.031), reflux history (P=0.036), and Barrett's esophagus (P=0.042). However, no significant association was observed with other clinicopathological factors such as histological grade (P=1.000), pathological stage (P=0.791), gender (P=0.221), race (P=0.252), or H. pylori infection (P=1.000) (refer to Table 1).

Table 1 Relationship between CCND1 and clinicopathological variables in gastric cancer patients

Characteristic	**Low expression of CCND1**	**High expression of CCND1**	***P***
n	187	188	
T stage, n (%)			0.501
T1	12 (3.3%)	7 (1.9%)	
T2	39 (10.6%)	41 (11.2%)	
T3	88 (24%)	80 (21.8%)	
T4	46 (12.5%)	54 (14.7%)	
N stage, n (%)			0.856
N0	57 (16%)	54 (15.1%)	
N1	48 (13.4%)	49 (13.7%)	
N2	34 (9.5%)	41 (11.5%)	
N3	38 (10.6%)	36 (10.1%)	
M stage, n (%)			0.988
M0	164 (46.2%)	166 (46.8%)	
M1	13 (3.7%)	12 (3.4%)	
Pathologic stage, n (%)			0.791
Stage I	25 (7.1%)	28 (8%)	
Stage II	59 (16.8%)	52 (14.8%)	
Stage III	71 (20.2%)	79 (22.4%)	
Stage IV	18 (5.1%)	20 (5.7%)	
Gender, n (%)			0.221
Female	73 (19.5%)	61 (16.3%)	
Male	114 (30.4%)	127 (33.9%)	
Race, n (%)			0.252
Asian	43 (13.3%)	31 (9.6%)	
Black or African American	4 (1.2%)	7 (2.2%)	
White	117 (36.2%)	121 (37.5%)	
Age, n (%)			0.018
<=65	94 (25.3%)	70 (18.9%)	
>65	92 (24.8%)	115 (31%)	
Histological type, n (%)			0.031
Diffuse Type	43 (11.5%)	20 (5.3%)	

Characteristic	Low expression of CCND1	High expression of CCND1	*P*
Mucinous Type	11 (2.9%)	8 (2.1%)	
Not Otherwise Specified	91 (24.3%)	116 (31%)	
Papillary Type	2 (0.5%)	3 (0.8%)	
Signet Ring Type	6 (1.6%)	5 (1.3%)	
Tubular Type	33 (8.8%)	36 (9.6%)	
Histologic grade, n (%)			1.000
G1	5 (1.4%)	5 (1.4%)	
G2	69 (18.9%)	68 (18.6%)	
G3	110 (30.1%)	109 (29.8%)	
Reflux history, n (%)			0.036
No	77 (36%)	98 (45.8%)	
Yes	25 (11.7%)	14 (6.5%)	
Antireflux treatment, n (%)			0.06
No	65 (36.3%)	77 (43%)	
Yes	24 (13.4%)	13 (7.3%)	
H. pylori infection, n (%)			1.000
No	68 (41.7%)	77 (47.2%)	
Yes	8 (4.9%)	10 (6.1%)	
Barretts esophagus, n (%)			0.042
No	95 (45.7%)	98 (47.1%)	
Yes	12 (5.8%)	3 (1.4%)	

*Notice: There is statistically significant difference (P<0.05)

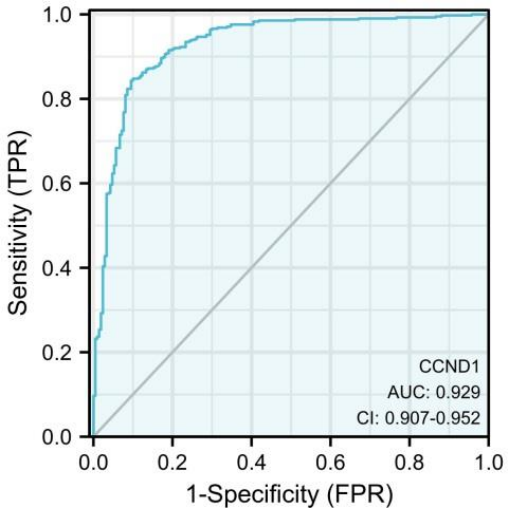

Figure 2 The Diagnostic value of ROC working Curve in evaluating CCND1 in GC

3.3 Diagnostic Value of CCND1 Differential Expression Level

The results of this study demonstrate the accuracy of CCND1 as a biomarker for gastric cancer, with an area under the curve (AUC) value of 0.929 (95%CI: 0.907-0.952). A critical value of 5.228 was chosen, resulting in true positive predictions for 351 patients and true negative predictions for 189 patients. The negative and positive predictive values, specificity, sensitivity, and Youden's index were found to be 0.750, 0.944, 0.900, 0.848, and 0.748 respectively (Figure 2). These results suggest that CCND1 may be a valuable tool for distinguishing gastric cancer from normal gastric tissue.

3.4 Relationship Between CCND1 Genetic Changes and Survivals

The results of the study reveal the presence of genetic alterations in different subtypes of gastric cancer, including mutations, amplifications, and deletions in esophagogastric adenocarcinoma and primarily mutations and amplifications in undifferentiated stomach adenocarcinoma (Figure 3A). A total of 35 cases (8%) out of 440 GC patients were found to have changes in CCND1. The majority of the mutations were amplifications, with three missense mutations (of unknown significance) and one deep deletion (Figure 3B). By mapping the CCND1 mutation sites in GC, three mutation sites were identified: A81T, D192Y, and A271T (Figure 3C, D). Further analysis was conducted to determine the impact of CCND1 genetic changes on the prognosis of GC patients, but no relationship was found between CCND1 genetic changes and the overall survival (OS) or disease-free survival (DFS) of GC patients (Figure 3E, F) (P=0.776 for OS and P=0.929 for DFS).

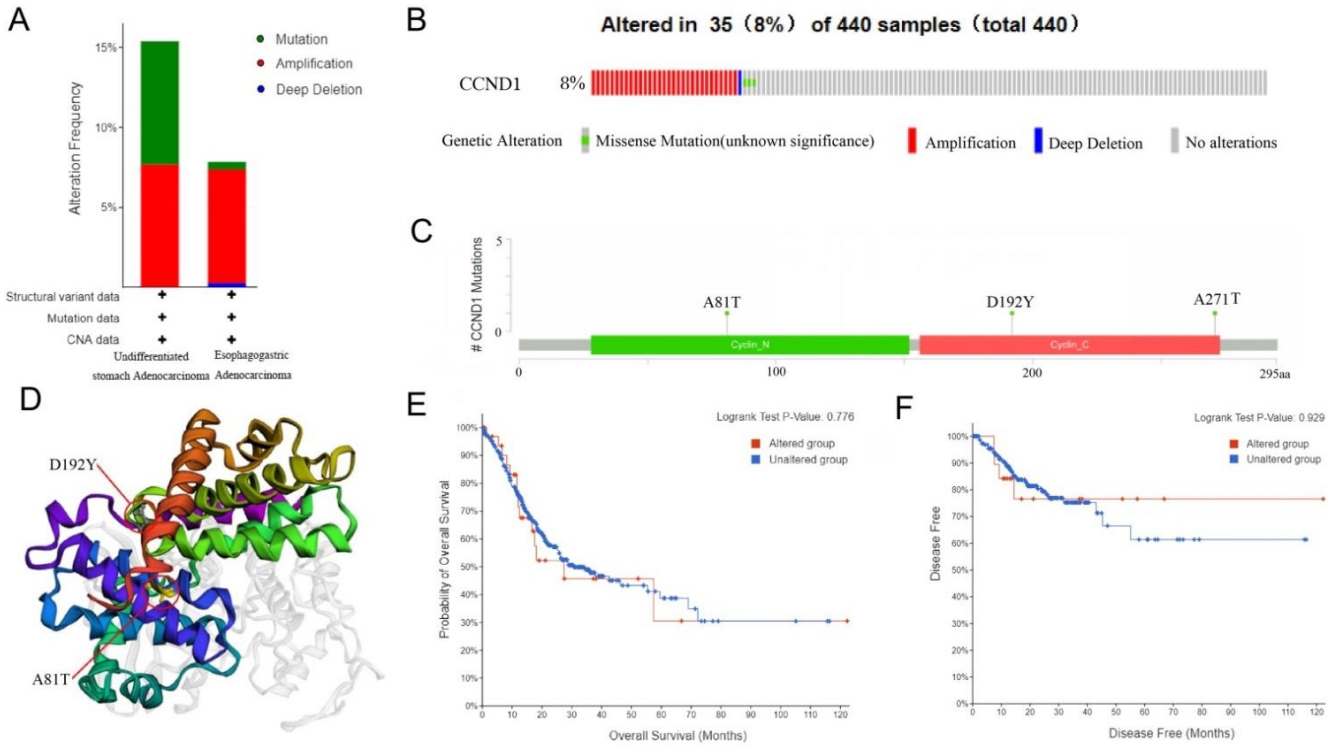

Figure 3 cBioPortal online analysis of the distribution of CCND1 genetic changes in GC patients and its effect on survivals.

CCND1 genetic changes in different subtypes of GC (B) Frequency and distribution of CCND1 genetic changes in 440 patients with GC (C, D) Plane map and 3D map distribution of common CCND1 mutations (E, F) Prognostic value of CCND1 genetic changes in GC patients (including overall survival and disease-free survival)

3.5 Relationship Between CCND1 Methylation Level and Prognosis

The analysis reveals that patients in the group with low CCND1 methylation have better overall survival compared to those with high CCND1 methylation. Nine CpG sites located within the CpG island have been identified to have a significant association with prognosis, including cg12266049, cg25060573, cg02694676, cg10539418, cg23753457, cg03735308, cg07149785, cg07781399, and cg09551996 (Table 2).

Table 2 Prognostic value of CCND1 methylation in GC patients

CPG island	Low expression of CCND1	High expression of CCND1	HR	Log rank P
cg12266049	295	100	1.47	0.033
cg25060573	215	180	1.535	0.009
cg02694676	283	112	1.482	0.027
cg10539418	296	99	1.524	0.022
cg23753457	220	175	1.598	0.0042
cg03735308	283	112	1.531	0.015
cg07149785	132	263	1.747	0.002
cg07781399	159	236	1.506	0.017
cg09551996	99	296	1.798	0.0067

*Notice: There is statistically significant difference (P<0.05)

3.6 Relationship Between CCND1 Expression and Tumor Immune Infiltrating Cells

According to the expression levels of CCND1 mRNA in 355 GC patients, 187 were classified as having low expression and the remaining 188 as having high expression. The data was analyzed and visualized using R (3.6.3) and the GSVA package (Figure 4A). The expression of CCND1 was found to be associated with B lymphocytes, Mast cells, pDC cells, Tgd cells, Tfh cells, and DC cells in GC. In addition, the low expression group showed a significant increase in the levels of B cells, Mast cells, pDC cells, Tfh cells, Tgd cells, and DC cells compared to the high expression group. To examine the correlation between CCND1 expression and immune infiltration and cumulative survival rate in GC patients, the TIMER database was used. The results showed that CCND1 expression was negatively correlated with the infiltration of CD4 T cells (r=-0.165, P=1.51e-03), macrophages (r=-0.255, P=6.95e-07), and DC cells (r=-0.112, P=3.09e-02). Additionally, macrophage infiltration was found to be associated with time-dependent changes in cumulative survival in GC patients (P=0.004) (Figure 4B). These findings suggest that CCND1 may play a role in regulating immune infiltration and survival in GC.

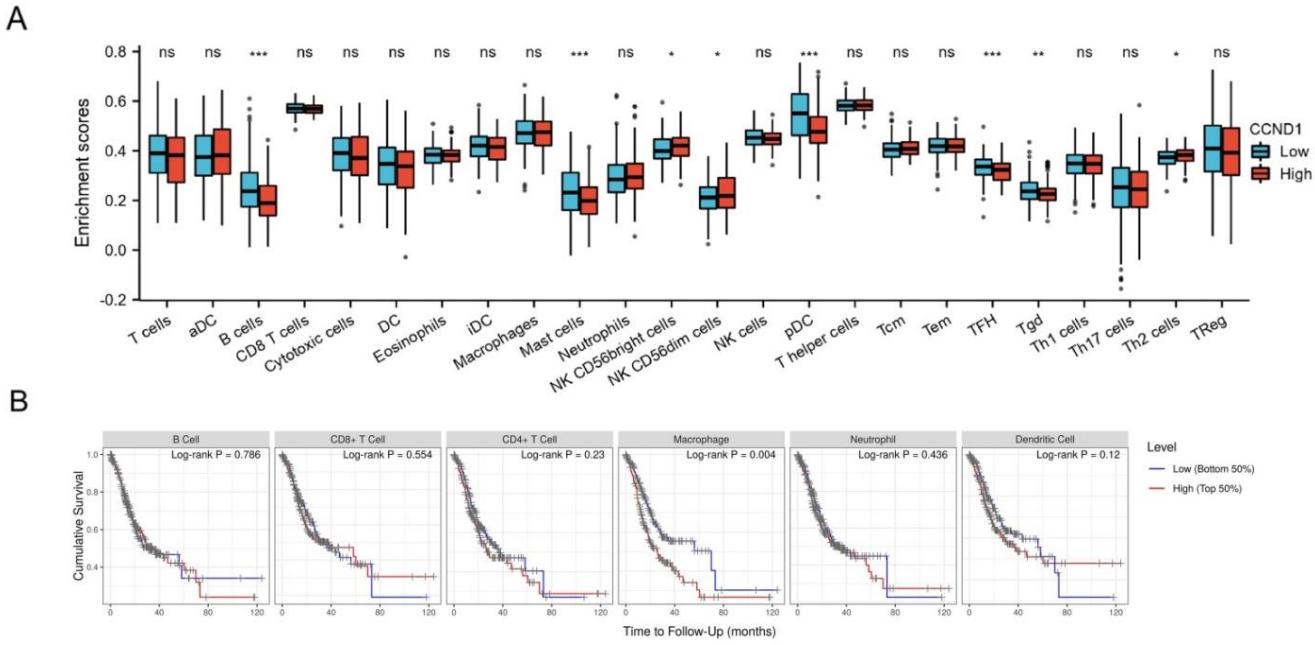

Figure 4 The relationship between the expression of CCND1 and the level of immune infiltration and prognosis in GC

(A) Analysis of the relationship between the expression of CCND1 and 24 kinds of immune cells infiltration in GC.
(B) Kaplan-Meier curve is drawn by Timer database to evaluate the effect of CCND1 expression and immune cell infiltration on the survival rate of GC patients

4 Discussion

The expression levels of CCND1 mRNA and protein in GC tissues were found to be higher than those in normal tissues, which may be attributed to the levels of miR-623 (1), miR-193a-3p [16], and AURKB activation [17]. Some studies have shown that the expression of CCND1 in normal human tissues is so low that it cannot be detected by immunohistochemical staining [18], and this finding is consistent with the results of this study. It was also discovered that the expression of CCND1 in GC patients was significantly correlated with age, histological classification, reflux history, and Barrett's esophagus. Some of these findings are in line with the results of Takano et al. [19].

These results suggest that CCND1 may have the potential to become a biomarker for distinguishing GC tissue from normal gastric tissue and for providing better diagnoses and treatments for clinical patients. Gene mutations are a common cause of carcinogenesis [20], and the most common genetic abnormality affecting CCND1 is DNA

amplification, which increases the transcription of the gene and the expression of its protein [21]. However, there is no obvious connection between genetic changes and the prognosis of GC. Changes in epigenetics also play a role in the occurrence and development of GC. DNA methylation, the earliest known and identified epigenetic change, is an important factor that changes gene expression and leads to uncontrolled cell proliferation [22].

This analysis found that patients with lower CCND1 methylation had better prognoses. As a result, the level of CCND1 methylation may serve as a new biomarker for prognosis and have valuable guidance in predicting the overall survival (OS) of GC patients. Tumor immune cell infiltration is considered to be "non-specific" and related to sentinel lymph node status and the survival rate of patients in various human cancers [23, 24]. Studies have proven a correlation between the level of immune cell infiltration and the occurrence, development, and prognosis of GC [25]. In this study, the R (3.6.3) GSVA package was used to explore the relationship between CCND1 expression and immune infiltration in GC. It was found that B lymphocytes, Mast cells, pDC cells, Tgd cells, TFH cells, NK CD 56 bright cells, NK CD 56 dim cells, Th2 cells, and DC cells were all associated with CCND1 expression. The Timer database also revealed that CCND1 expression was negatively correlated with immune infiltration of CD4 T cells, macrophages, and DC cells. There is a relationship between macrophages and the prognosis of GC patients, which is consistent with the results of Xiao et al. [26].

The correlation between CCND1 expression and certain clinical characteristics of gastric cancer (GC) patients requires further validation through the use of updated bioinformatics databases. Additionally, the findings in this paper have not yet been substantiated through experimental evidence.

5 Conclusions

In this study, abnormally high expression of CCND1 was observed in GC and it was found to have a close association with the patient's age, histological type, reflux history, and Barrett's esophagus. The hypermethylation of CCND1 was also linked to poor prognosis. Furthermore, the gene is believed to have the potential to serve as a biomarker for the diagnosis of GC and could play a role in regulating tumor immune infiltration.

Abbreviations

GC, gastric cancer

CCND1, the gene is closely related to cell cycle regulation and located on chromosome 11q13.

Authors' Contributions

Jianing Yang, Shuying Li, designed the study, wrote the manuscript, and contributed equally to this work; Ke Zhang, participated in the design of the study, and performed the statistical analysis. All authors read and approved the final manuscript.

Competing Interests

The authors report no conflicts of interest.

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