

# Clinical significance and biological roles of cyclins in gastric cancer

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**Background and aim:** Cyclins have been reported to be overexpressed with poor prognosis in several human cancers. However, limited numbers of studies evaluated the expressions and prognostic roles of cyclins in gastric cancer (GC). We aim to evaluate the expressions and prognostic roles of cyclins. Also, further efforts were made to explore biological function of the differentially expressed cyclins.

**Methods:** Cyclins expressions were analyzed by OncoPrint and The Cancer Genome Atlas datasets, and the prognostic roles of cyclins in GC patients were investigated by the Kaplan–Meier Plotter database. Then, a comprehensive PubMed literature search was performed to identify expression and prognosis of cyclins in GC. Biological functions of the differentially expressed cyclins were explored through Enrich R platform, and KEGG and transcription factor were analyzed.

**Results:** The expression levels of CCNA2 (cyclin A2), CCNB1 (cyclin B1), CCNB2 (cyclin B2), and CCNE1 (cyclin E1) mRNAs were identified to be significantly higher in GC tissues than in normal tissues in both OncoPrint and The Cancer Genome Atlas datasets. High expressions of CCNA2, CCNB1, and CCNB2 mRNAs were identified to be related with poor overall survival in Kaplan–Meier Plotter dataset. Evidence from clinical studies showed that CCNB1 was related with overall survival in GC patients. Cyclins were associated with several biological pathways, including cell cycle, p53 signaling pathway, FoxO signaling pathway, viral carcinogenesis, and AMPK signaling pathway. Enrichment analysis also showed that cyclins interacted with some certain transcription factors, such as FOXM1, SIN3A, NFYA, and E2F4.

**Conclusion:** Based on our results, high expressions of cyclins were related with poor prognosis in GC patients. The above information might be useful for better understanding the clinical and biological roles of cyclins mRNA and guiding individualized treatments for GC patients.

**Keywords:** gastric cancer, cyclins, expression, prognosis, biological function

## Introduction

Gastric cancer (GC) is estimated to be one of the most leading causes of all deaths worldwide.<sup>1</sup> Every year, nearly one million new cases occur and around 7,00,000 people die of GC.<sup>2</sup> Although there is a decrease in incidence in the recent years, patients with GC still suffer from a poor prognosis, which brought great burden to global health and economy.<sup>3,4</sup> Tumor resection or lymphadenectomy is one of the main treatments for advanced GC.<sup>5</sup> However, surgical treatment alone is associated with high recurrence rates.<sup>6,7</sup> Therefore, identification of the molecular mechanism during GC pathogenesis, as well as identification of potential prognostic biomarkers and drug targets is still needed.

Cyclins, including cyclin A, B, D, and E, act as key controlling elements of the eukaryotic cell cycle.<sup>8</sup> Considerable efforts have been expended in order to understand

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the potential biological mechanism and clinical significance of cyclins in human cancers.<sup>8–11</sup> Many studies demonstrated that the expressions of cyclins were overexpressed in human cancers and correlated with poor clinical outcomes in lung cancer,<sup>12–17</sup> breast cancer,<sup>18–28</sup> and ovarian cancer.<sup>29–34</sup> However, limited numbers of studies evaluated the expressions and prognostic roles of cyclins in GC patients,<sup>35–37</sup> and additional efforts are still needed to explore their biological function.

Oncomine (<http://www.oncomine.org>),<sup>38</sup> a cancer microarray database and web-based data mining platform, could automatically compute differential expression profiles between cancer and normal tissues for a gene of interest. Kaplan–Meier Plotter (KM Plotter; <http://kmplot.com/analysis/>) is capable of assessing the effect of 54,675 genes on survival using 1,065 GC samples.<sup>39</sup> In the present study, we aim to evaluate the expressions and prognostic roles of different cyclins in GC patients through the above two databases. For further biological function analysis of cyclins, enrichment analysis was performed by Enrich platform.<sup>40</sup> In this study, we aim to study the clinical significance and biological mechanism of cyclins during GC pathogenesis through bioinformatics methods.

## Materials and methods

### Oncomine database analysis

Oncomine (<http://www.oncomine.org>), which contains 715 datasets and 86,733 samples, was utilized to examine the cyclins mRNA expressions between GC and normal tissues.<sup>38</sup> The thresholds were restricted to the following: *P*-value: 0.05; fold change: 2.0; gene rank: all; and data type: mRNA. We performed comparisons by cancer vs normal analysis. Fold change, *P*-value, gene rank, and sample sizes were obtained from studies that showed statistically significant differences.

Then, the GSE13861, GSE27342, GSE13911, and GSE19826 datasets were employed from Oncomine database, and the raw data of cyclins expressions were downloaded. After normalization, a meta-analysis was conducted by Review Manager. The standardized mean difference was calculated. *P*-value <0.05 was considered to be statistically significant. Heterogeneity among studies was evaluated by the Cochran's *Q* test and *I*<sup>2</sup> statistic. The *P*-value for Cochran's *Q* test <0.1 or *I*<sup>2</sup>>50% was considered to be heterogeneous. Pooled analysis was applied with a fixed-effects model if no heterogeneity was detected. Random-effects model was applied if any heterogeneity existed.

For validation, the expressions of cyclins for GC patients in The Cancer Genome Atlas (TCGA; [https://cancergenome.](https://cancergenome.nih.gov/)

[nih.gov/](https://cancergenome.nih.gov/)) dataset were analyzed. The TCGA GC data included 32 normal and 375 GC samples.

### KM Plotter database analysis

KM Plotter (<http://kmplot.com/analysis/>) is capable of assessing the effect of 54,675 genes on survival using 10,461 cancer samples.<sup>39</sup> Currently, it contains 5,143 breast, 1,618 ovarian, 2,437 lung, and 1,065 GC patients with a mean follow-up of 69/40/49/33 months, respectively. The prognostic roles of cyclins in GC were assessed by KM Plotter. GC patients were divided into high- and low-expression groups by the median values of mRNA expression. The overall survival (OS) of GC patients was measured by calculating logrank *P*-value and hazard ratio (HR) with 95% CI. Number of cases, HRs with 95% CIs, and *P*-values were extracted from the KM Plotter webpage. In this study, “array quality control” was selected, and “exclude biased arrays” without follow-up. The GSE14210, GSE15459, GSE22377, GSE29272, and GSE51105 datasets were employed for pooled survival analysis from KM Plotter database.

### Literature search of expression and prognosis of cyclins in GC

A comprehensive PubMed literature search was performed to identify expression and prognosis of cyclins in GC. The following keywords were used: “gastric cancer”, “stomach adenocarcinoma”, “gastric adenocarcinoma”, “survival”, “prognosis”, “gene expression”, “CCNA2”, “CCNB1”, “CCNB2”, and “CCNE1”. The prognosis roles of cyclins were reviewed by comprehensive literature search.

### Enrichment analysis of cyclins

In order to obtain additional biological information of the differentially expressed cyclins, a comprehensive functional gene set analysis was performed using the EnrichR platform.<sup>40</sup> EnrichR platform includes 69 gene-set libraries, such as KEGG, WikiPathways, Gene Ontology, and transcription factor (TF). *P*-value and *Z*-score were calculated for enrichment analysis, and protein–protein interaction (PPI) network was constructed. The *P*-value is computed using a standard statistical method used by most enrichment analysis tools – Fisher's exact test or the hypergeometric test. The *Z*-score is computed using a modification to Fisher's exact test in which an *Z*-score was computed for deviation from an expected rank. For multiple hypotheses, Benjamini–Hochberg (BH) procedure was used.

## Results

### Cyclins mRNA expression in GC patients

Using Oncomine analysis, we investigated mRNA levels of cyclins in GC patients. The differentially expressed cyclins in four datasets<sup>41–44</sup> are shown in Table 1. The mRNA expression levels of CCNA2, CCNB1, CCNB2, CCND2, and CCNE1 were significantly higher in GC than in normal tissues in each single dataset. CCNB1 and CCNE1 mRNA expressions were also significantly higher in gastric diffuse adenocarcinoma and gastric intestinal adenocarcinoma, respectively. The pooled analysis, by comparing different studies in Oncomine datasets, showed that CCNA2, CCNB1, CCNB2, and CCNE1 were significantly higher in GC than in normal tissues (Figure 1).

The expressions of cyclins were analyzed in TCGA GC dataset for validation. The mRNA expression levels of CCNA2, CCNB1, CCNB2, and CCNE1 were significantly higher in GC than in normal tissues, while CCND2 did not show the significance (Figure 2). The receiver operating

characteristic (ROC) curves of CCNA2, CCNB1, CCNB2, and CCNE1 were constructed for GC diagnosis (Figure S1). Besides, CCNB1 and CCNE1 mRNA expressions were also significantly higher in diffuse gastric adenocarcinoma and gastric intestinal type adenocarcinoma, respectively (Figure 3). Also, the ROC curves of CCNB1 and CCNE1 were constructed for GC Lauren subtype classification (Figure S2).

In summary, CCNA2, CCNB1, CCNB2, and CCNE1 were identified to be differentially expressed in GC tissues in both Oncomine and TCGA datasets. Furthermore, CCNB1 and CCNE1 expressions were also differentially expressed in GC Lauren subtype, which indicated that CCNB1 and CCNE1 might be helpful to classify GC Lauren subtypes.

### Prognostic role of cyclins mRNA in GC patients

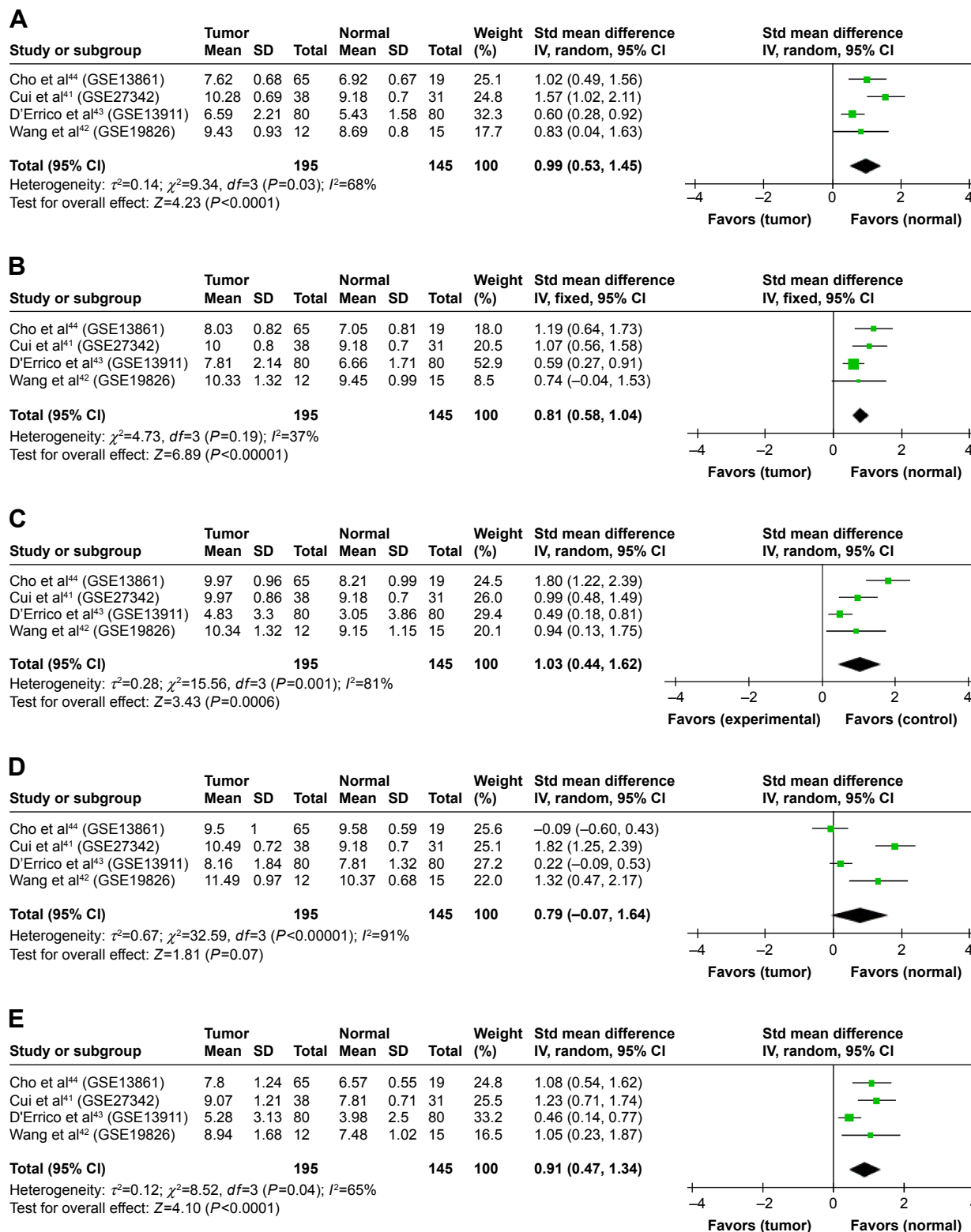
The prognostic roles of differentially expressed cyclins in GC were assessed by KM Plotter. Survival curves were plotted for GC patients. High expressions of CCNA2

**Table 1** The differentially expressed cyclins mRNA in GC

Symbol	Dataset	Sample (tumor/normal)	Fold change	P-value	Gene rank
<b>GC vs normal</b>					
CCNA2	Cui et al <sup>41</sup>	80/80	2.204	3.02E–05	513
CCNB1	Cui et al <sup>41</sup>	80/80	2.006	2.13E–05	484
	Wang et al <sup>42</sup>	12/15	2.251	1.20E–02	3,624
CCNB2	Cui et al <sup>41</sup>	80/80	3.111	6.38E–04	1,009
	Wang et al <sup>42</sup>	12/15	2.555	9.00E–03	3,222
CCND2	Wang et al <sup>42</sup>	12/15	2.449	2.27E–04	489
CCNE1	Cui et al <sup>41</sup>	80/80	2.232	2.00E–03	1,385
	Wang et al <sup>42</sup>	12/15	3.099	7.00E–03	2,918
<b>Gastric intestinal type adenocarcinoma vs normal</b>					
CCNA2	D'Errico et al <sup>43</sup>	26/31	2.898	1.03E–10	445
CCNB1	D'Errico et al <sup>43</sup>	26/31	3.126	1.58E–10	488
CCNB2	D'Errico et al <sup>43</sup>	26/31	3.087	2.66E–10	551
CCND2	D'Errico et al <sup>43</sup>	26/31	4.016	2.17E–05	3,409
CCNE1	D'Errico et al <sup>43</sup>	26/31	3.598	1.71E–08	1,186
	Cho et al <sup>44</sup>	20/19	2.059	7.11E–04	1,013
<b>Diffuse gastric adenocarcinoma vs normal</b>					
CCNB1	D'Errico et al <sup>43</sup>	6/31	2.187	8.00E–03	2,390
CCNE1	Cho et al <sup>44</sup>	31/19	2.594	3.35E–06	421
<b>Gastric mixed adenocarcinoma vs normal</b>					
CCNA2	D'Errico et al <sup>43</sup>	4/31	2.261	6.00E–03	3,126
	Cho et al <sup>44</sup>	10/19	2.059	6.20E–04	920
CCNB1	D'Errico et al <sup>43</sup>	4/31	3.294	8.00E–03	3,492
	Cho et al <sup>44</sup>	10/19	2.406	1.12E–04	414
CCNB2	D'Errico et al <sup>43</sup>	4/31	2.168	1.20E–02	4,049
CCND2	D'Errico et al <sup>43</sup>	4/31	3.300	8.00E–03	3,462
	Cho et al <sup>44</sup>	10/19	2.459	2.00E–03	1,423
CCNE1	D'Errico et al <sup>43</sup>	4/31	2.417	1.40E–02	4,360
<b>Gastric adenocarcinoma vs normal</b>					
CCNB1	Cho et al <sup>44</sup>	4/19	2.516	2.40E–02	1,555

**Note:** Data were extracted from the Oncomine database.

**Abbreviation:** GC, gastric cancer.



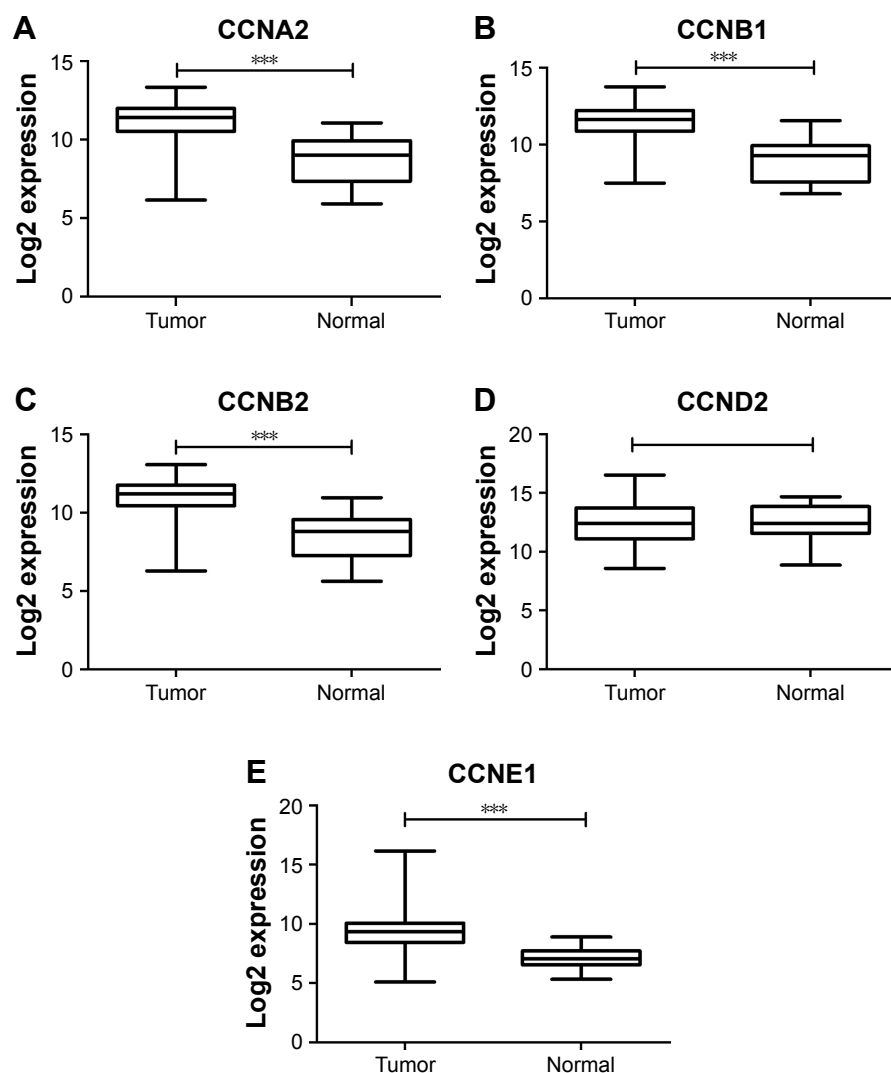
**Figure 1** Cyclins expression in gastric cancer in Oncomine datasets.

**Notes:** (A) CCNA2, (B) CCNB1, (C) CCNB2, (D) CCND2, and (E) CCNE1. Data were analyzed in Oncomine datasets by comparative meta-analysis.

**Abbreviations:** GC, gastric cancer; std, standard.

(HR=1.36, 95% CI: 1.09–1.68,  $P=0.0055$ ), CCNB1 (HR=1.34, 95% CI: 1.10–1.63,  $P=0.0034$ ), CCNB2 (HR=1.47, 95% CI: 1.19–1.83,  $P=0.00038$ ), and CCNE1 (HR=1.40, 95% CI: 1.15–1.71,  $P=0.00093$ ) were related with poor OS (Figure 4).

The prognostic role of differentially expressed cyclins was reviewed in the previously published studies for validation (Table 2). Up to now, there are no data about the prognosis roles of CCNA2 and CCNB2. Three studies



**Figure 2** Cyclins expression in GC in TCGA dataset.

**Notes:** (A) CCNA2, (B) CCNB1, (C) CCNB2, (D) CCND2, and (E) CCNE1. Data were analyzed in TCGA dataset. \*\*\* $P < 0.001$ .

**Abbreviations:** GC, gastric cancer; TCGA, The Cancer Genome Atlas.

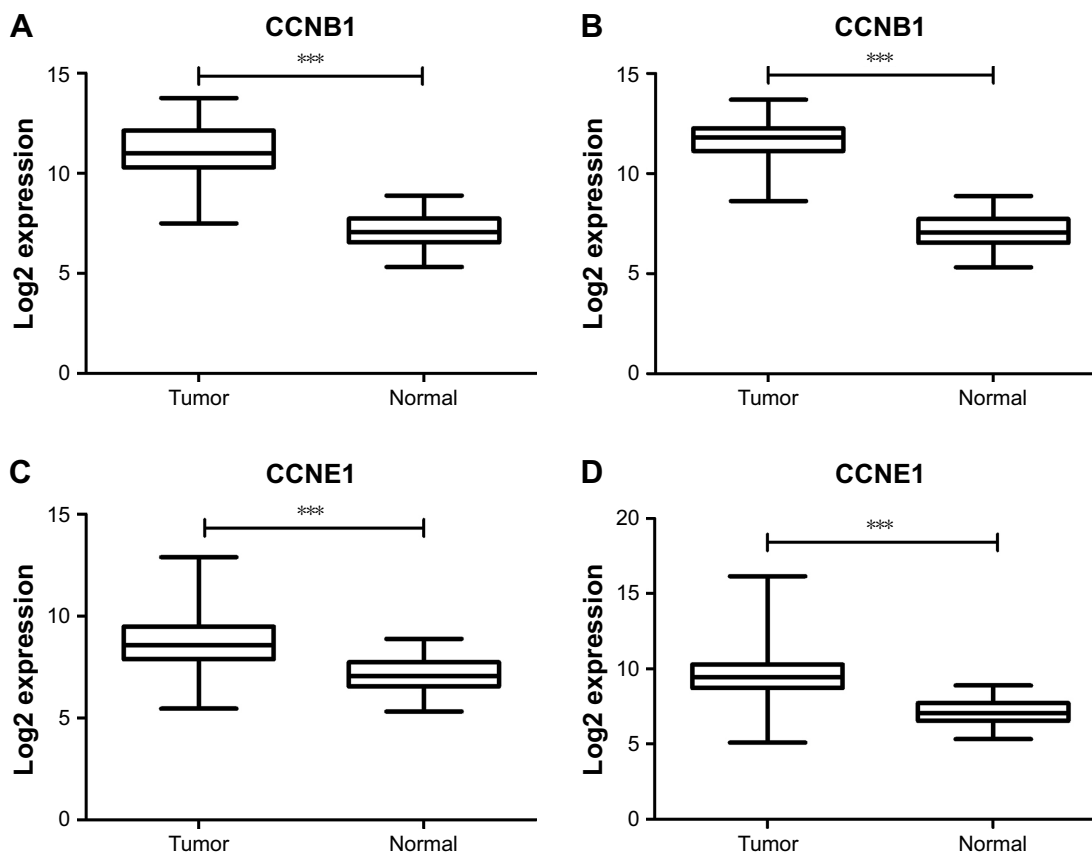
evaluated the relationship between CCNB1 expression and clinicopathological features in GC. Three studies showed that CCNB1 high expression was related with lymph node metastasis. Two studies evaluated the prognosis role of CCNB1. The study from Kim<sup>53</sup> did not indicate poor prognosis for CCNB1 overexpression GC patients. But Kim enrolled only 23 patients, and the results might not be reliable. The study involving 482 GC patients from Begnami et al<sup>55</sup> showed poor 5-year OS for CCNB1 high-expression patients. Only one study evaluated the prognosis role of CCNE1. CCNE1 was associated with Lauren classification, tumor size, lymphatic and vascular invasion, but not with OS.

Then, further efforts were made to investigate the prognosis role of CCNB1 in GC. High expression of CCNB1 (HR =2.63, 95% CI: 1.72–4.00,  $P=3.5E-06$ ) predicted poor

OS in GC patients who received 5-FU-based chemotherapy treatment (Figure 5A). Furthermore, high expression of CCNE1 predicted poor OS in *HER2*-negative GC patients (HR =1.42, 95% CI: 1.08–1.87,  $P=0.0117$ ; Figure 5B). The above results indicated that CCNE1 might be useful for guiding individualized treatments for GC patients.

## Enrichment analysis of cyclins

The PPI network constructed is shown in Figure 6. In the PPI network, CDK1 and CDK2 were identified as PPI hub proteins. Several biological pathways were involved, including cell cycle, p53 signaling pathway, FoxO signaling pathway, viral carcinogenesis, and AMPK signaling pathway. Enrichment analysis also showed that cyclins were related with some TFs, such as FOXM1, SIN3A, NFYA, and E2F4 (Table 3).



**Figure 3** CCNB1 and CCNE1 expressions in GC Lauren subtype.

**Notes:** (A) CCNB1 expression in diffuse gastric adenocarcinoma. (B) CCNB1 expression in gastric intestinal type adenocarcinoma. (C) CCNE1 expression in diffuse gastric adenocarcinoma. (D) CCNE1 expression in gastric intestinal type adenocarcinoma. Data were analyzed in TCGA dataset. \*\*\* $P < 0.001$ .

**Abbreviations:** GC, gastric cancer; TCGA, The Cancer Genome Atlas.

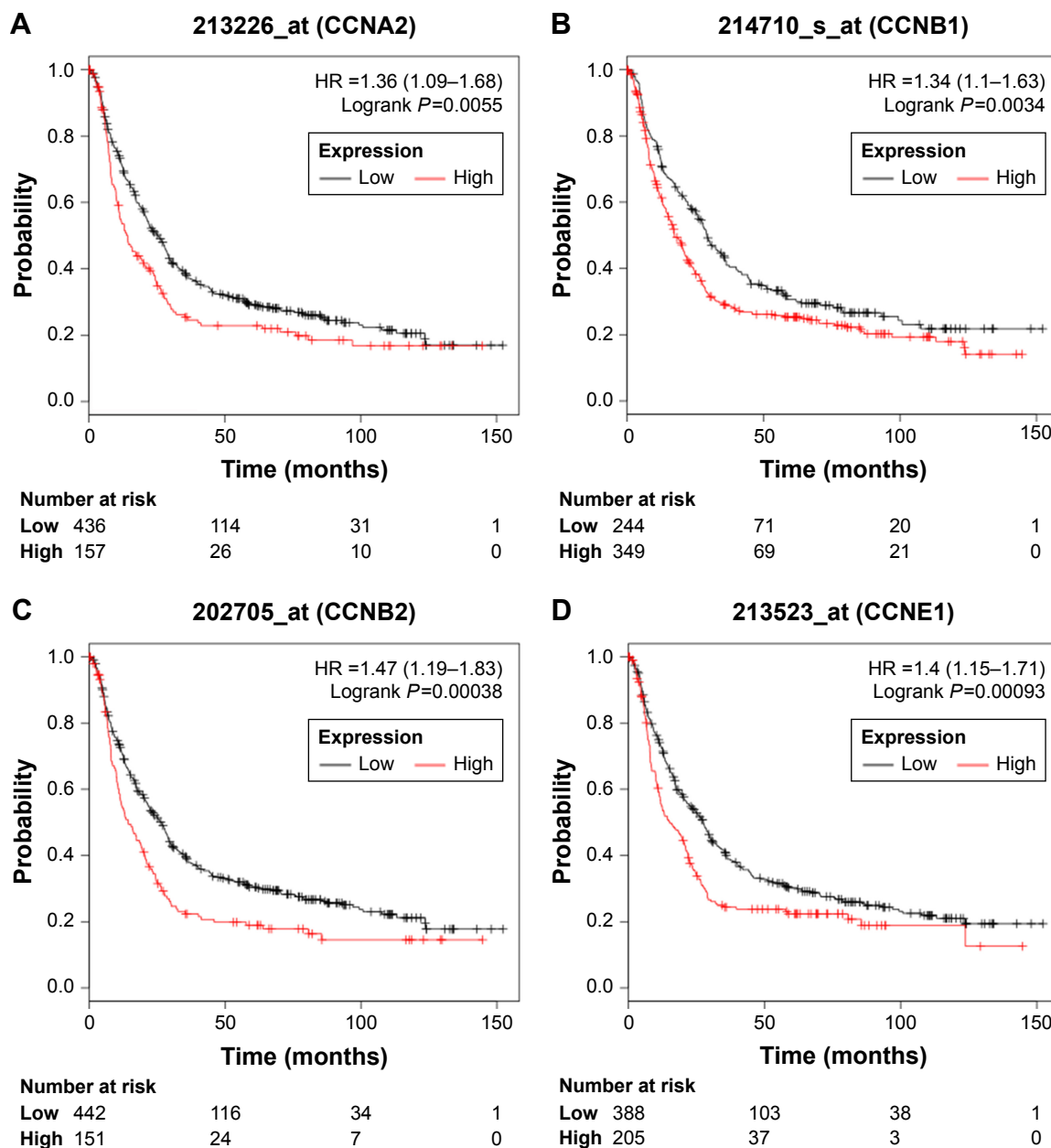
## Discussion

Publicly available microarray data have vast potential to serve as a source of biomarker discovery because there is enormous quantity of existing gene expression data.<sup>45,46</sup> Potential roles for biomarkers include early diagnosis of disease, disease classification, prediction of prognosis model, and response to drug target therapy.<sup>47,48</sup> Identification of genes associated with clinical survival rate and target therapy might provide a novel insight into GC pathogenesis and guide individualized treatments. In our study, the expression and prognosis roles of cyclins expressions were analyzed, and biological function was evaluated by enrichment analysis. Our study showed that high expression of cyclins mRNA was related with GC prognosis.

CCNA2 is overexpressed in several human cancers and correlated with tumor progression and shorter survival in lung, breast, and colorectal cancer.<sup>12,18,49–51</sup> Our study showed that CCNA2 overexpression was an indicator of poor prognosis. Up to now, there is no study evaluating the expression and prognostic role of CCNA2 in GC patients. More efforts

are needed to confirm expression pattern and prognostic role of CCNA2 in GC patients.

CCNB1 and CCNB2, which are key regulators of progression through the G2/M transition during the cell cycle, played significant roles in the progression and development of human cancers.<sup>13–15,19–24,35</sup> In this report, CCNB1 and CCNB2 were upregulated in GC, which was consistent with previous studies.<sup>35,52–54</sup> The CCNB1 mRNA overexpression was also confirmed in different GC Lauren subtypes, which indicated that CCNB1 might be helpful to guide GC classification. Relevant research showed that overexpression of CCNB1 was associated with clinical stage,<sup>54</sup> lymph node metastasis,<sup>35,53,54</sup> and poor survival in GC patients.<sup>35</sup> However, there are no data evaluating the prognostic role of CCNB2 in GC patients. In our study, high expression of CCNB1 and CCNB2 mRNAs predicted poor OS in GC patients through KM Plotter database analysis. ISL1, which could promote cell proliferation, is a novel regulator of the CCNB1 and CCNB2 genes.<sup>55</sup> Downregulation of CCNB1 expression contributes to oridonin-induced cell cycle arrest at G2/M phase and growth inhibition in SGC-7901 GC cells.<sup>56</sup> Inhibition of



**Figure 4** Prognosis roles of differentially expressed cyclins in GC patients in KM Plotter datasets.  
**Notes:** (A) CCNA2, (B) CCNB1, (C) CCNB2, and (D) CCNE1. Data were analyzed in KM Plotter datasets.  
**Abbreviations:** GC, gastric cancer; KM Plotter, Kaplan–Meier Plotter.

**Table 2** The relationship between differentially expressed cyclins and clinicopathological features in previously published studies

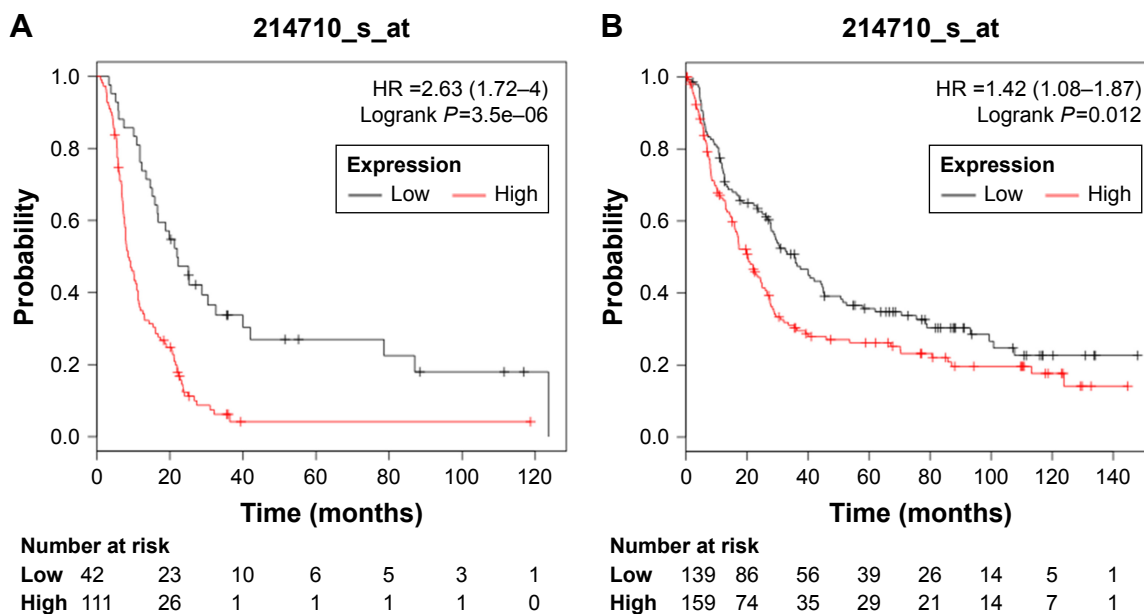
	CCNB1	CCNE1
Differentiation	(54)*	NA
Lauren classification	(35)*	(37)*
Tumor size	NA	(37)*
Stage	(54)*	NA
Lymphatic invasion	(54)*	(37)*
Lymph node metastasis	(35, 53, 54)*	NA
Vascular invasion	NS (54)	(37)*
Overall survival	(35)*, NS (53)	NS (37)

**Note:** \*P < 0.05.

**Abbreviations:** NA, not available; NS, not significant.

GC cell growth by CCNB1 and CCNB2 may explain poor prognosis in GC patients.

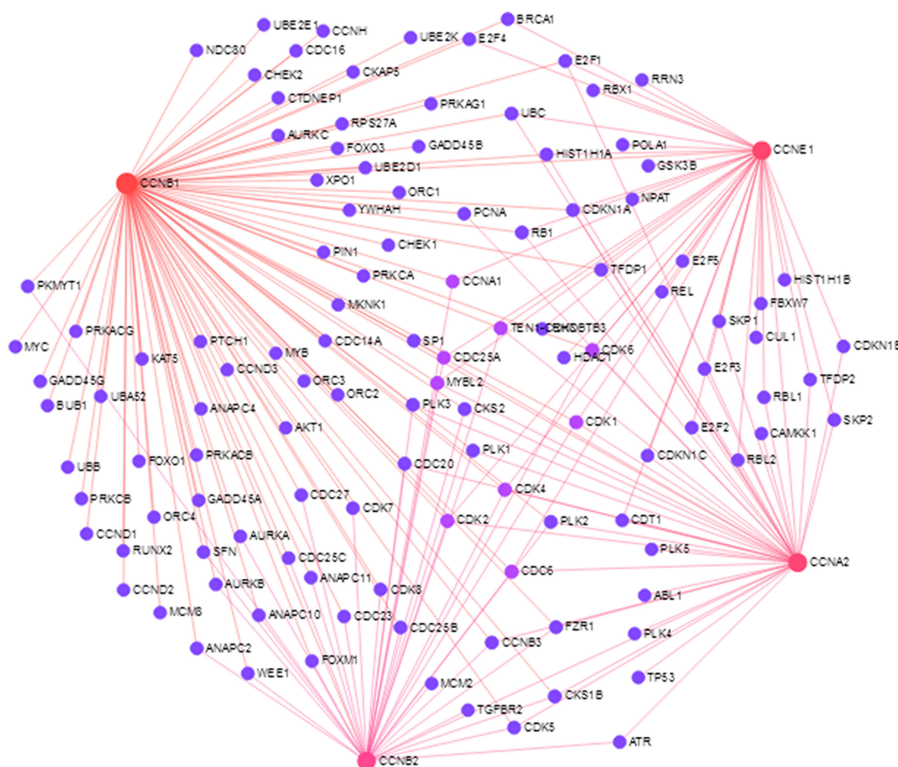
The expression pattern of CCND2 presented conflicting results in GC. Some studies demonstrated that absence of CCND2 expression is associated with promoter hypermethylation in GC,<sup>57</sup> as well as in breast, lung, prostate, and liver cancers.<sup>58–62</sup> But some studies showed that CCND2 mRNA is overexpressed in GC.<sup>36,63–65</sup> Therefore, more studies are needed to evaluate the expression of CCND2 in GC to resolve the conflicting picture. In this report, there was no difference of CCND2 expression between GC and normal tissues.



**Figure 5** Prognosis roles of CCNB1 in different GC patients. **Notes:** (A) Prognostic role of CCNE1 in GC patients who received 5-FU-based chemotherapy treatment. (B) Prognostic role of CCNE1 in GC HER2-negative patients. Data were analyzed in KM Plotter datasets. **Abbreviations:** GC, gastric cancer; KM Plotter, Kaplan–Meier Plotter.

Relevant research showed overexpression of CCND2 was related with better differentiation, lymph node metastasis, and poor survival, which were confirmed in GC by immunohistochemistry analysis.<sup>64,65</sup> Another research showed that

high expression of CCND2 mRNA was related with depth of invasion, lymph node metastasis, distant metastasis, and TNM stage and predicted poor prognosis in GC patients.<sup>36</sup> But the samples involved in the above studies that evaluated



**Figure 6** Protein–protein interaction network of differentially expressed cyclins.



**Table 3** Enrichment analysis of cyclins

	P-value	Z-score	Genes involved
<b>KEGG pathway</b>			
Cell cycle	2.252E-08	-1.733198	CCNA2;CCNB2;CCNB1;CCNE1
p53 signaling pathway	1.255E-06	-1.737387	CCNB2;CCNB1;CCNE1
FoxO signaling pathway	0.0008354	-1.724599	CCNB2;CCNB1
Viral carcinogenesis	0.0014146	-1.829714	CCNA2;CCNE1
AMPK signaling pathway	0.0391648	-1.561928	CCNA2
<b>Transcription factors</b>			
FOXM1	8.277E-06	-1.918853	CCNA2;CCNB2;CCNB1
SIN3A	0.0069103	-1.730395	CCNA2;CCNB2;CCNB1
NFYA	0.0347278	-1.662154	CCNA2;CCNB2;CCNB1
E2F4	0.0359975	-1.660036	CCNA2;CCNE1

the prognostic role of CCND2 were small. Therefore, more studies are needed to evaluate the expression and prognostic role of CCND2 in GC.

CCNE1 is considered to be involved in the oncogenic process.<sup>9,66</sup> CCNE1 overexpressions are significantly correlated with unfavorable prognosis in lung and breast cancers.<sup>25-27,31-34</sup> Relevant research demonstrated that CCNE1 in both mRNA and protein levels was associated with intestinal Lauren classification.<sup>37</sup> In our study, CCNE1 was upregulated and predicted poor prognosis for GC patients. Recent evidence showed that CCNE1 was the target gene of microRNA-25, and cell apoptosis was observed when microRNA-25 and CCNE1 expressions downregulated,<sup>67</sup> which might explain poor prognosis in GC patients with high CCNE1 mRNA expression.

*HER2* is a 185-kDa protein, encoded by a gene located on chromosome 17q21. *HER2* overexpression was detected in 6%–23% of GC patients.<sup>68,69</sup> *HER2*-targeted therapies, using trastuzumab in addition to chemotherapy, showed significant benefit for patients with advanced GC.<sup>70</sup> In this study, high expression of CCNB1 predicted poor OS in *HER2*-negative GC patients, which indicated that CCNB1 might be useful for guiding individualized treatments for GC patients. 5-FU adjuvant therapy of GC significantly improves OS.<sup>71</sup> In this study, we found that high expression of CCNB1 predicted poor OS in GC patients who receive 5-FU-based chemotherapy treatment. Therefore, CCNB1 might be a potential therapy target for GC patients who receive 5-FU-based chemotherapy treatment.

In order to gain further insight into the biological mechanism of cyclins, enrichment analysis was performed. Cell cycle and p53 signaling pathway were identified as the most significant pathways that were related with cyclins. Dysregulated cell cycle progression is an important biological event during cancer pathogenesis. Present evidences

have indicated that cyclins, CDKs, E2Fs, and p53 play an important role in cell cycle regulation.<sup>8</sup> The above cell cycle regulators were the target molecules of most oncogenes. Recent studies showed that several molecules could inhibit cell proliferation and induce cell cycle arrest at G2/M phase in GC cell lines, with downregulation of CDK1 and CCNB1 expressions but upregulation of p21 Waf1/Cip1, p27 Kip1, and p53 expressions.<sup>72-74</sup> Another study showed that SPPH-induced S-phase arrest was mediated by upregulating the expression of E2F1 and downregulating those of CCNE1, CDK2, and CCNA2.<sup>75</sup> FOXM1 was identified as the most significant TF through Enrich R platform. FOXM1 has been shown to regulate transcription of cell cycle genes, including Cdc25B, CDK1, cyclin B, and p27 Kip.<sup>76,77</sup> Knockdown of FOXM1 resulted in enhanced cell growth inhibition in AGS cells.<sup>77</sup> The above results provided a novel insight of cyclins into GC pathogenesis, which might be helpful for better understanding the biological mechanism of cyclins.

While the present study provides important insights into expression and prognostic roles of cyclins in GC patients at the mRNA level, it would be sensible to highlight the strengths and limitations of our study. Firstly, the publicly available databases were employed to analyze the expressions and prognostic roles of cyclins in GC patients. We pooled four datasets to evaluate the expressions of cyclins through Oncomine database. To evaluate the prognosis roles of cyclins, five GEO datasets were pooled to calculate the pooled HR. All the results of expression pattern and prognostic roles of cyclins were not based on a single dataset. Secondly, we analyzed the expressions of cyclins in TCGA dataset for validation. Also, the prognosis roles of cyclins were reviewed by comprehensive literature search. However, we did not perform polymerase chain reaction (PCR) or immunohistochemical to validate our findings. Though we

performed an enrichment analysis, there are no experimental data about the mechanism of how cyclins affect the prognosis of GC patients, and additional efforts are needed to explore the biological roles of cyclins during GC pathogenesis.

## Conclusion

In summary, CCNA2, CCNB1, CCNB2, and CCNE1 mRNA expressions are significantly higher in GC than in normal tissues. Furthermore, CCNB1 and CCNE1 could be helpful to guide GC Lauren classification. High expression of CCNB1 mRNA predicted poor OS in GC patients who received 5-FU-based chemotherapy treatment. Besides, high expression of CCNB1 predicted poor OS in *HER2*-negative GC patients. Enrichment analysis showed that cyclins are related with several biological pathways and interacts with some certain TFs. The above information might be useful for better understanding the clinical and biological roles of cyclins mRNA and guiding individualized treatments for GC patients.

## Disclosure

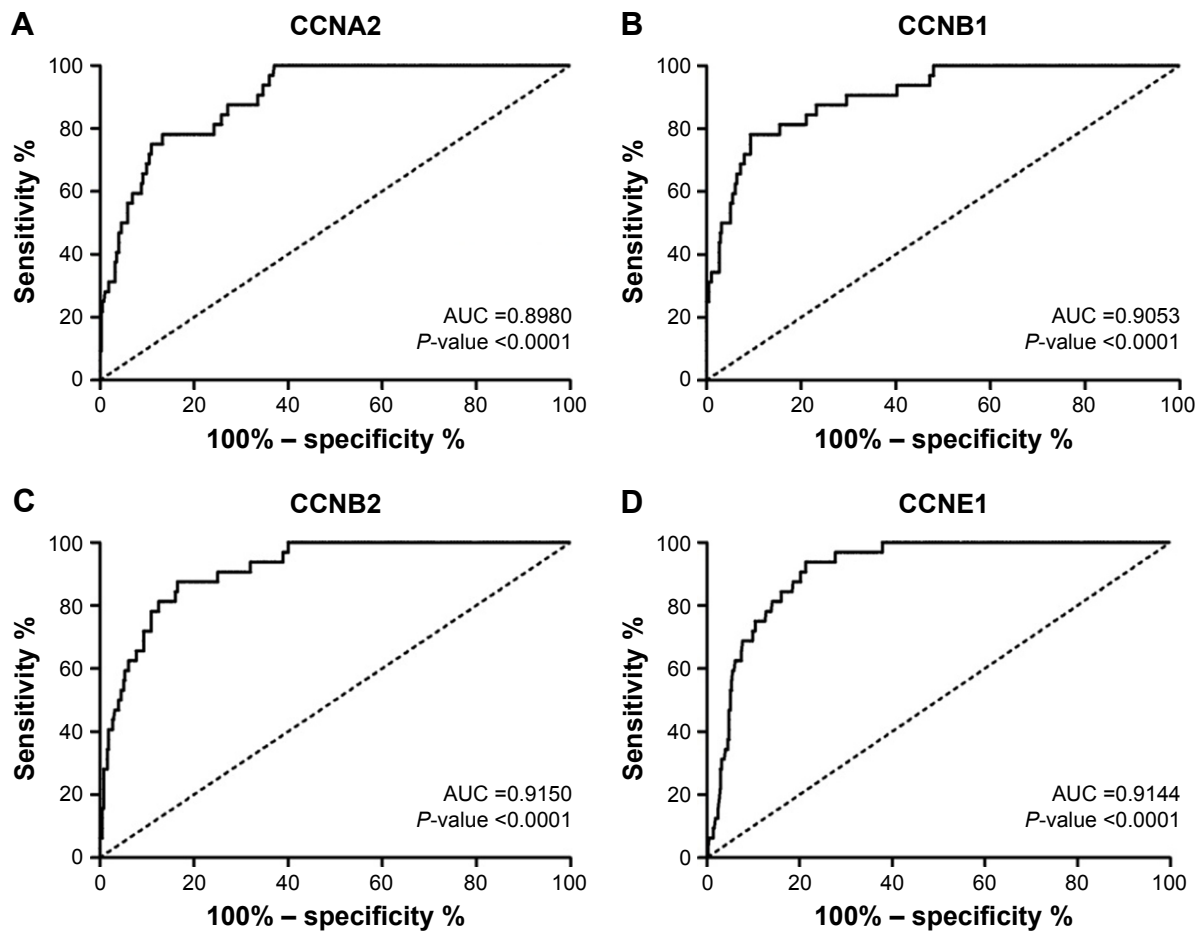
The authors report no conflicts of interest in this work.

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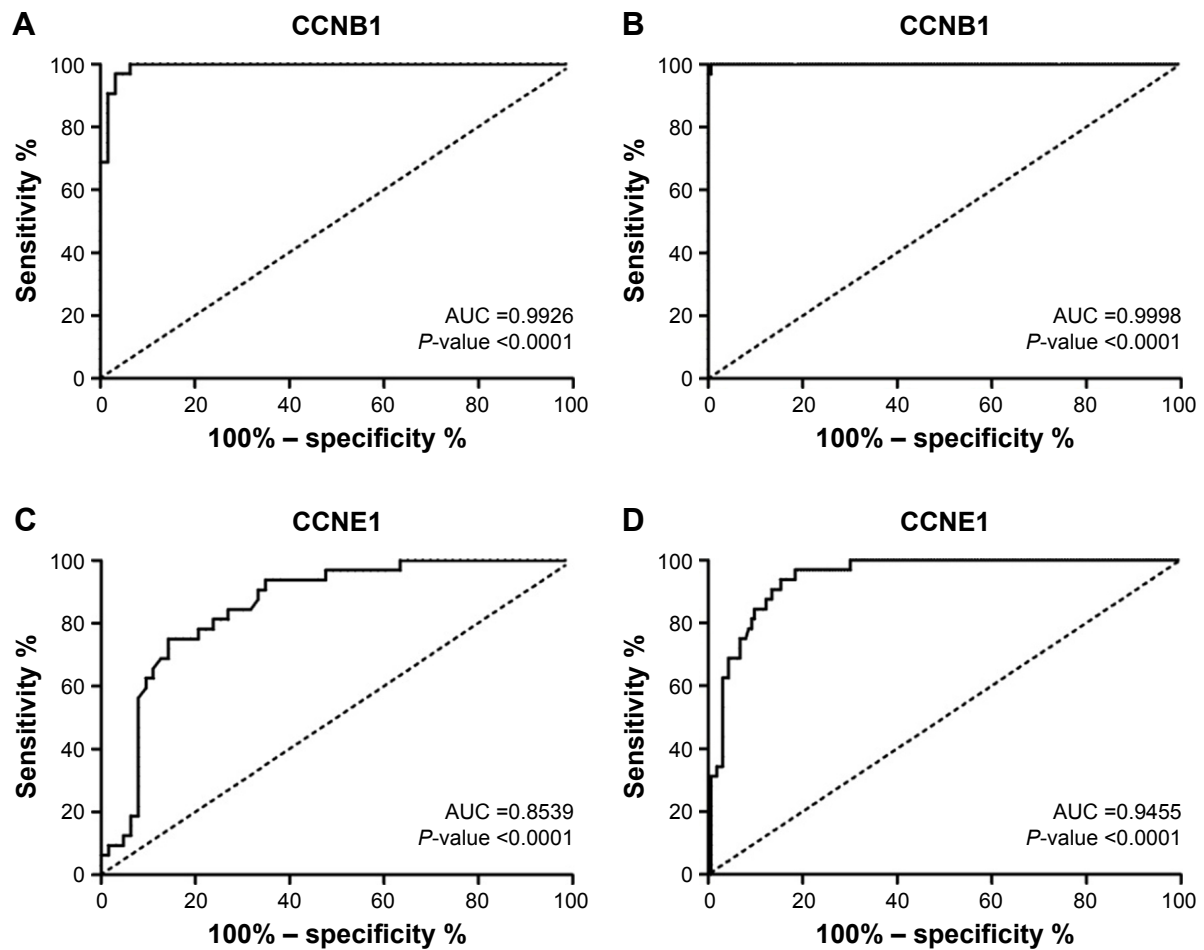
## Supplementary materials



**Figure S1** The ROC curves of CCNA2, CCNB1, CCNB2, and CCNE1 were constructed for GC diagnosis.

**Notes:** (A) CCNA2, (B) CCNB1, (C) CCNB2, and (D) CCNE1.

**Abbreviations:** AUC, area under curve; GC, gastric cancer; ROC, receiver operating characteristic.



**Figure S2** The ROC curves of CCNB1 and CCNE1 for GC Lauren subtype classification.

**Notes:** (A) CCNB1 for diffuse gastric adenocarcinoma. (B) CCNB1 gastric intestinal type adenocarcinoma. (C) CCNE1 for diffuse gastric adenocarcinoma. (D) CCNE1 gastric intestinal type adenocarcinoma.

**Abbreviations:** AUC, area under curve; GC, gastric cancer; ROC, receiver operating characteristic.

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