CDCA1/2/3/5/7/8 as novel prognostic biomarkers and CDCA4/6 as potential targets for gastric cancer

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Background: Increasing evidence had suggested that cell division cycle-associated (CDCA) family proteins play prominent roles in multiple types of cancer. However, the expression pattern and prognostic value of CDCAs in gastric cancer were still poorly understood.

Methods: In this study, bioinformatics was used for the first time to comprehensively discuss the expression changes of the CDCA protein family in gastric cancer. We studied the transcription and survival data of CDCAs in patients with gastric cancer in Oncomine, GEPIA, DAVID, cBioportal, and other databases.

Results: We identified that the CDCA 1/2/3/4/5/6/7/8 were overexpressed gastric cancer than in normal tissues. There was no significant difference in CDCAs expression among different gastric cancer stages. High expression of CDCA4/6 in patients with gastric cancer was closely related to low overall survival (OS), first progression survival (FPS), and post-progression survival (PPS). In contrast, high CDCA1/2/3/5/7/8 expression predicted a better prognosis. The genetic mutation rate of *CDCA2* and *CDCA4* was 4%, ranking first. The main biological process of CDCAs protein family enrichment was cell division, the main cell component involved was centromeres of chromosomes, and the main molecular function involved was protein binding.

Conclusions: The study suggested that CDCA1/2/3/5/7/8 were expected to be new prognostic markers for gastric cancer, and CDCA4/6 might be potential targets for the treatment of gastric cancer.

Keywords: Cell division cycle-associated proteins (CDCA proteins); gastric cancer; bioinformatics; prognosis

Introduction

Gastric cancer was one of the most significant causes of cancer-related morbidity and mortality throughout the world. The incidence of gastric cancer in East Asia, particularly China, increases strikingly, which placed a huge burden on global public health (1). Although treatments such as surgery and chemotherapy had improved the survival and prognosis, the 5-year survival rate remained unsatisfactory. Therefore, the identification of novel prognostic biomarkers and therapeutic targets related to the carcinogenesis of gastric cancer was of great value.

Cell division cycle-associated (CDCA) protein family acted as crucial regulators in the process of cell division and proliferation. Increasing evidence had suggested that CDCAs played prominent roles in multiple types of cancer,

Transl Cancer Res 2021;10(7):3404-3417 | https://dx.doi.org/10.21037/tcr-20-1050

such as breast cancer, lung cancer, colorectal cancer, bladder cancer, oral cancer, and so on (2-6). For instance, the study by Thang *et al.* demonstrated that CDCA1 may represent a new prognostic and therapeutic target for oral cancer (2). The findings of Adams *et al.* had highlighted CDCA3 as a prognostic factor in lung cancer (7). Moreover, some studies had proposed that CDCA3 might serve as an oncogene in gastric cancer (GC) (8). However, the expression pattern of CDCA protein subtypes in gastric cancer was not clear yet. Meanwhile, due to a large number of CDCA protein subtypes, the key subtype had not been preliminarily screened for the study of gastric cancer.

In the present study, we utilized bioinformatics analysis to study the expression differences and prognostic value of CDCA subtypes in gastric cancer for the first time. Initially, we validated the expression of the CDCA protein family in various malignant tumors in several databases. Subsequently, we identified the prognostic value, genetic alterations, and gene ontology analysis of key CDCA members in gastric cancer tissues. We present the following article in accordance with the MDAR checklist (available at https://dx.doi.org/10.21037/tcr-20-1050).

Methods

Oncomine analysis

The Oncomine (http://www.oncomine.org) database was a publicly accessible online database, which integrated numerous published tumor microarray data (9). We used this online database to identify the differences in the expression of major CDCA subtypes between different tumor tissues and normal tissues. Subsequently, we focused on verifying the expression of the main CDCA protein subtypes in gastric cancer datasets. The mRNA expression of CDCA protein between cancer and normal tissues was compared by *t*-test. The P value was set up at 0.05 and the log2(fold change) was defined as 1.5.

GEPIA analysis

Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn), a novel interactive online tool, was applied to conduct various functions using the sequencing expression data of cancer samples from TCGA and GTEx projects, such as tumor/normal differential expression analysis, pathological stage analysis, and so on (10). We further verified major CDCA

subtypes expression level in gastric cancer using GEPIA, and screened out the differentially expressed genes consistent with Oncomine database results. Meanwhile, with the aid of GEPIA, we explored whether there are differences in the expression of these genes in different pathological stages.

Survival analysis

Kaplan-Meier plotter (http://kmplot.com/analysis/), which collected a large amount of gene chip data and clinical data from EGA, GEO, and TCGA databases, could assess the survival impact of more than 50,000 genes on 21 sorts of cancers (11). The prognostic values of the 8 selected genes were assessed by calculating the overall survival (OS), first progression survival (FP), and post-progression survival (PPS).

Genetic alteration analysis

The cBioPortal for Cancer Genomics (https://www.cbioportal.org/) was an open comprehensive platform, which integrated data mining, data integration, and visualization (12). We selected the gastric adenocarcinoma dataset (TCGA, Provisional) in the TCGA database and utilized cBioportal to analyze the genetic variation of hub genes.

Enrichment analysis and protein-protein interaction (PPI) analysis

To clarify potential biological meanings behind the hub genes, we utilized DAVID (the Database for Annotation, Visualization and Integrated Discovery, version 6.8) to carry out the GO (Gene Ontology) enrichment analysis and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis (13). The P value cutoff was set to 0.05 and the result was visualized through the ggplot package of R software (version 3.5.3). Through the enrichment analysis in the DAVID database, we used the KEGG database (https://www.kegg.jp/) to identify the most promising signaling pathway related to the hub genes. The protein-protein interaction network was then constructed using the STRING (Search Tool for the Retrieval of Interacting Genes, https://string-db.org/) database to identify the interaction relationship and adjacent network among genes (14). The study was conducted in accordance with the Declaration of Helsinki

Analysis type by cancer	Cancer vs. Normal CDCA1 (NCF2)		Cancer vs. Normal CDCA2		Cancer vs. Normal CDCA3		Cancer vs. Normal CDCA4		Cancer vs. Normal CDCA5		Cancer vs. Normal CDCA6 (CBX2)		Cancer vs. Normal CDCA7		Cancer vs. Normal CDCA8	
Bladder Cancer	1		1		4		4		2		2		1		4	
Brain and CNS Cancer	9	1	7	1	8	1	2	1	4	1	5	3	9	1	6	1
Breast Cancer	15	1	13	1	24	1	17	1	24	1	11	3	6	3	22	1
Cervical Cancer	2		2		3		1		2			1	2		4	
Colorectal Cancer	21		14		20		19		19		15	1	22		13	
Esophageal Cancer	1		2		3		2	2	2			1	4	1	5	
Gastric Cancer	12		6		8		8		11		7		9		11	
Head and Neck Cancer	6		6		7		3		8		3		3		4	
Kidney Cancer	1		2		2	1	2		1		2	1	2		2	2
Leukemia		5	1	2		8	4	3		2	5	1	5	1	1	7
Liver Cancer	2		1		2		2		2				2		5	
Lung Cancer	8		4		7		5		12		5		9		12	
Lymphoma	5		5	3	13	1	3	1	13		5	1	5	3	5	1
Melanoma	2		2		3				2					1	2	
Myeloma				1			2								2	
Other Cancer	8	11	3	5	7	4	8	1	6	4	6	1	11		6	8
Ovarian Cancer	2		1		2		3		1				5		7	
Pancreatic Cancer	5		1	1	5		2	2	3	1		1	4		2	1
Prostate Cancer	2	1	2		4							2		2		2
Sarcoma					9		11			1	2			1	9	2
Significant Unique Analyses	99	19	73	14	130	16	97	11	112	10	68	16	99	13	121	24
Total Unique Analyses	207		188		273		267		211		273		201		292	

Figure 1 The mRNA expression of CDCA protein family in different cancers (Oncomine database). The figure showed the numbers of datasets with statistically significant mRNA over-expression (red) or down-expression (blue) of related gene. The P value was set up at 0.05. The number in each cell represented the number of analyses that met the threshold. We utilized the percentile of related gene in the top of all genes measured in each research to analyze the gene rank. Cell color was determined by the best gene rank percentile for the analyses within the cell.

(as revised in 2013).

Statistical analysis

The difference of CDCAs mRNA expression between cancer tissues and adjacent normal tissues was compared by *t*-test. The P value was set to 0.05, and log2(fold change) was defined as 1.5. Kaplan-Meier test and Log-rank test were used to compare the difference of survival prognosis among different gene expression levels. P<0.05 was considered statistically significant. The biological processes, cellular components and molecular functions of key genes were analyzed by GO enrichment analysis.

Results

Expression level of CDCAs in gastric cancer

We utilized the Oncomine database to preliminarily analyze the expression of the main subtypes of CDCAs in 20 malignant tumors. The results revealed that the main CDCA subtypes—CDCA1, CDCA2, CDCA3, CDCA4, CDCA5, CDCA6, CDCA7, CDCA8—expressed differently in various types of tumors compared with the corresponding normal tissues (*Figure 1*). We further analyzed the expression of CDCAs in gastric cancer using the Oncomine database. The results showed that the expression of CDCA1, CDCA2, CDCA3, CDCA4, CDCA5, CDCA6,

Table 1 Significant difference in expression of CDCA protein family subtypes between gastric cancer and normal gastric tissues

CDCA subtype	Types of gastric cancer *vs.* normal	Fold change	P value	t value
CDCA1	Gastric cancer *vs.* normal	2.700	4.71E-11	6.943
CDCA2	Gastric cancer *vs.* normal	2.774	1.29E-5	4.336
CDCA3	Gastric cancer *vs.* normal	2.106	2.19E-5	4.575
CDCA4	Gastric intestinal type adenocarcinoma *vs.* normal	2.214	7.44E-12	8.857
CDCA5	Diffuse gastric adenocarcinoma *vs.* normal	2.674	3.71E-9	7.255
CDCA6	Diffuse gastric adenocarcinoma *vs.* normal	2.290	6.01E-9	6.862
CDCA7	Gastric cancer *vs.* normal	2.111	7.94E-8	5.490
CDCA8	Gastric intestinal type adenocarcinoma *vs.* normal	2.028	5.28E-10	7.560

CDCA7, and CDCA8 was up-regulated in gastric cancer tissues with statistical significance. In the meantime, the fold changes of CDCA1, CDCA2, CDCA3, CDCA4, CDCA5, CDCA6, CDCA7 and CDCA8 were 2.700, 2.774, 2.106, 2.214, 2.674, 2.290, 2.111 and 2.028, respectively (*Table 1*).

The relationship between CDCAs expression level and clinical stages in patients with gastric cancer

GEPIA (Gene Expression Profiling Interactive Analysis) was applied to verify the expression level of claudins. A total of 408 gastric cancer samples and 211 normal gastric tissues were included in the study dataset. The expression level of CDCA1, CDCA2, CDCA3, CDCA4, CDCA5, CDCA6, CDCA7, and CDCA8 in gastric cancer tissues was higher than that in normal tissues (*Figure 2*). Also, we found that although CDCA1, CDCA2, CDCA3, CDCA4, CDCA5, CDCA6, CDCA7, and CDCA8 were significantly different between gastric cancer tissues and normal samples, there was no clear difference in various stages of gastric cancer (*Figure 3*).

Increased expression of CDCA1/2/3/5/7/8 and decreased expression of CDCA4/6 are associated with improved prognosis of gastric cancer

We further analyzed the relationship between the hub genes—*CDCA1, CDCA2, CDCA3, CDCA4, CDCA5, CDCA6, CDCA7, CDCA8*—and the prognosis and survival of gastric cancer. Kaplan-Meier plotter was applied to plot the relationship between gene expression level and OS, FPS, and PPS of gastric cancer. The results showed that the higher expression levels of CDCA1, CDCA2, CDCA3,

CDCA5, CDCA7 in gastric cancer patients indicated worse OS, FP, PPS. On the contrary, gastric cancer patients with high expression level of CDCA4, CDCA6 showed better prognosis and survival (*Figures 4,5*). The above data differences had statistical significance. Notably, lower expression of CDCA8 predicted better OS and PPS, but there was no significant difference in FP (*Figure 5*).

Genetic alteration analysis and PPI construction of CDCAs

We utilized cBioportal to analyze the distribution of genetic alterations of the hub genes in gastric cancer samples. The results showed that among the 8 hub genes, the mutation rate of *CDCA2* and *CDCA1* (NUF2) genes was 4%, ranking first. The gene mutation rates of *CDCA3* and *CDCA5* were the lowest, at only 1.5%. The cumulative genetic alteration rate of each subtype of gastric cancer ranged from 10% to 30% (*Figure 6*). We constructed the PPI network and showed the connections among the hub genes, and found some adjacent genes—*BUB1, SPC25, AURKB, NDC80, SPC24*.

GO and KEGG enrichment analysis of hub CDCAs

We used GO and KEGG enrichment analysis to attempt to expound the biological meaning behind hub genes. GO analysis mainly referred to three sections: biological process, cellular component and molecular function. When it came to biological process (BP), they were mainly related to cell division, sister chromatid cohesion, mitotic nuclear division, and so on. As for cellular component, the hub genes primarily participated in nucleus, nucleoplasm, chromosome. Concerning molecular function, they were

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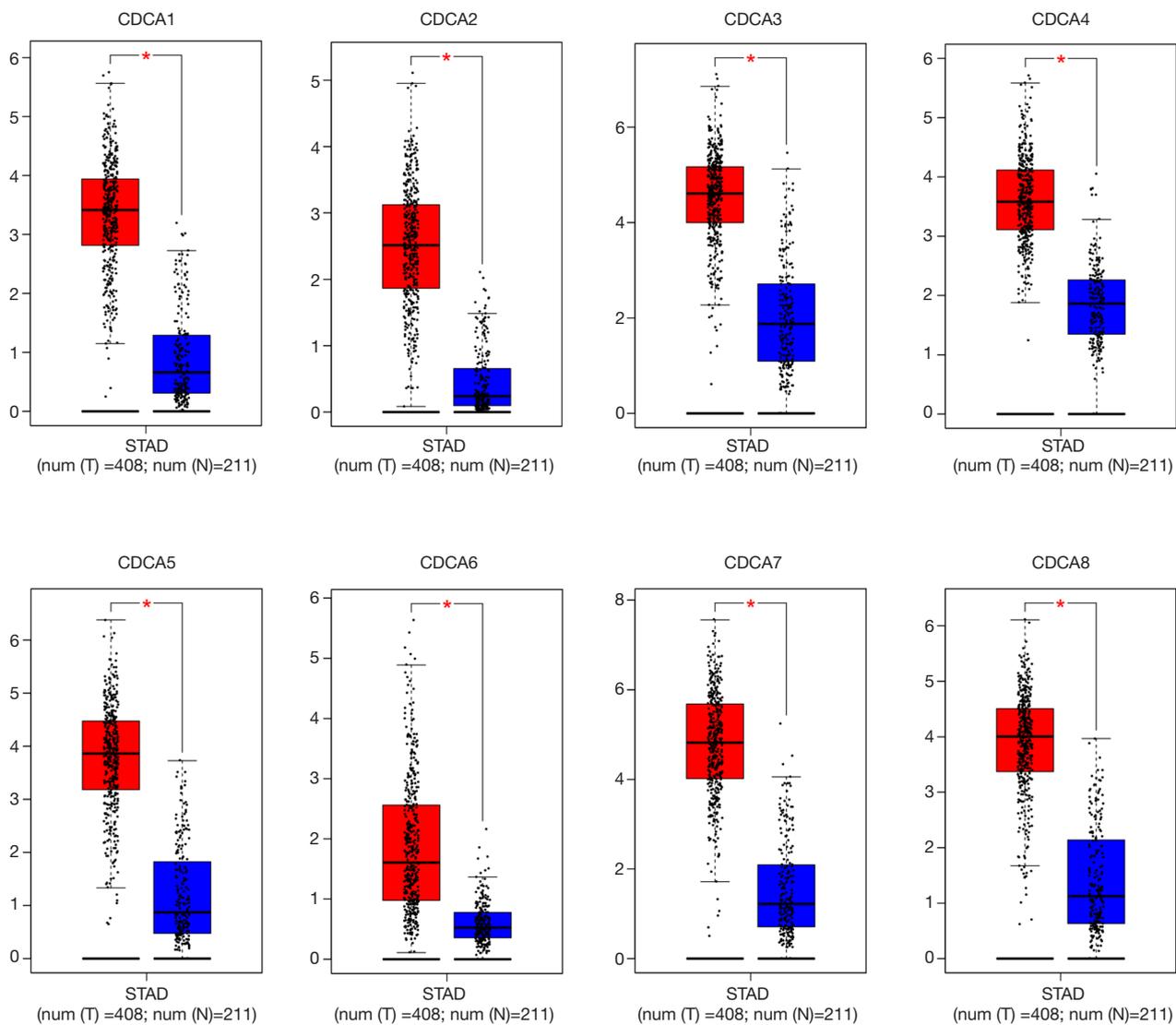
Figure 2 The mRNA expression level of CDCAs in gastric cancer (GEPIA). There were 480 gastric cancer samples, represented by red color. The blue color represented the 211 adjacent normal gastric tissues.

significantly enriched in structural molecule activity, identical protein binding. Besides, variations in KEGG pathway enrichment analysis were closely associated to protein binding (*Figure 7*). KEGG enrichment analysis of these hub genes showed no significant enrichment pathway.

Conclusions

In the present study, we used bioinformatics analysis to preliminarily identify the expression patterns of CDCA subtypes in different tumor samples and corresponding normal tissues for the first time. The results revealed

that there were significant differences in the expression of different protein subtypes in different tumor tissues, suggesting that CDCAs were closely associated with the occurrence and development of malignant tumors. The roles of CDCAs in the tumorigenesis of gastric cancer were largely unknown. In this paper, we determined the prognostic value and expression patterns of key CDCA subtypes through a comprehensive analysis of multiple databases. These pointed out the key direction for further studying the relationship between CDCAs and gastric cancer in the future.

CDCA1, also known as NUF2, coded for a protein that
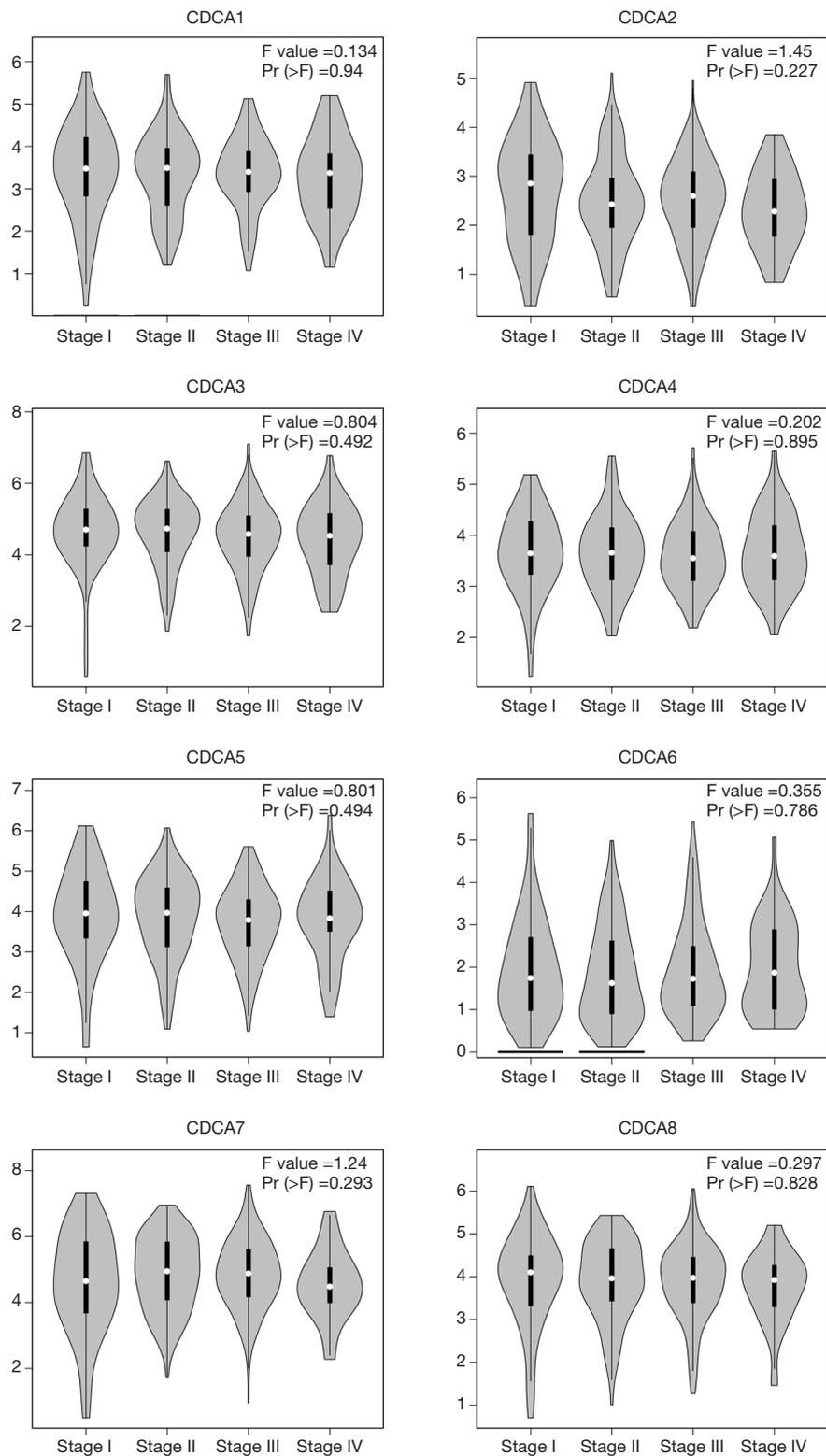
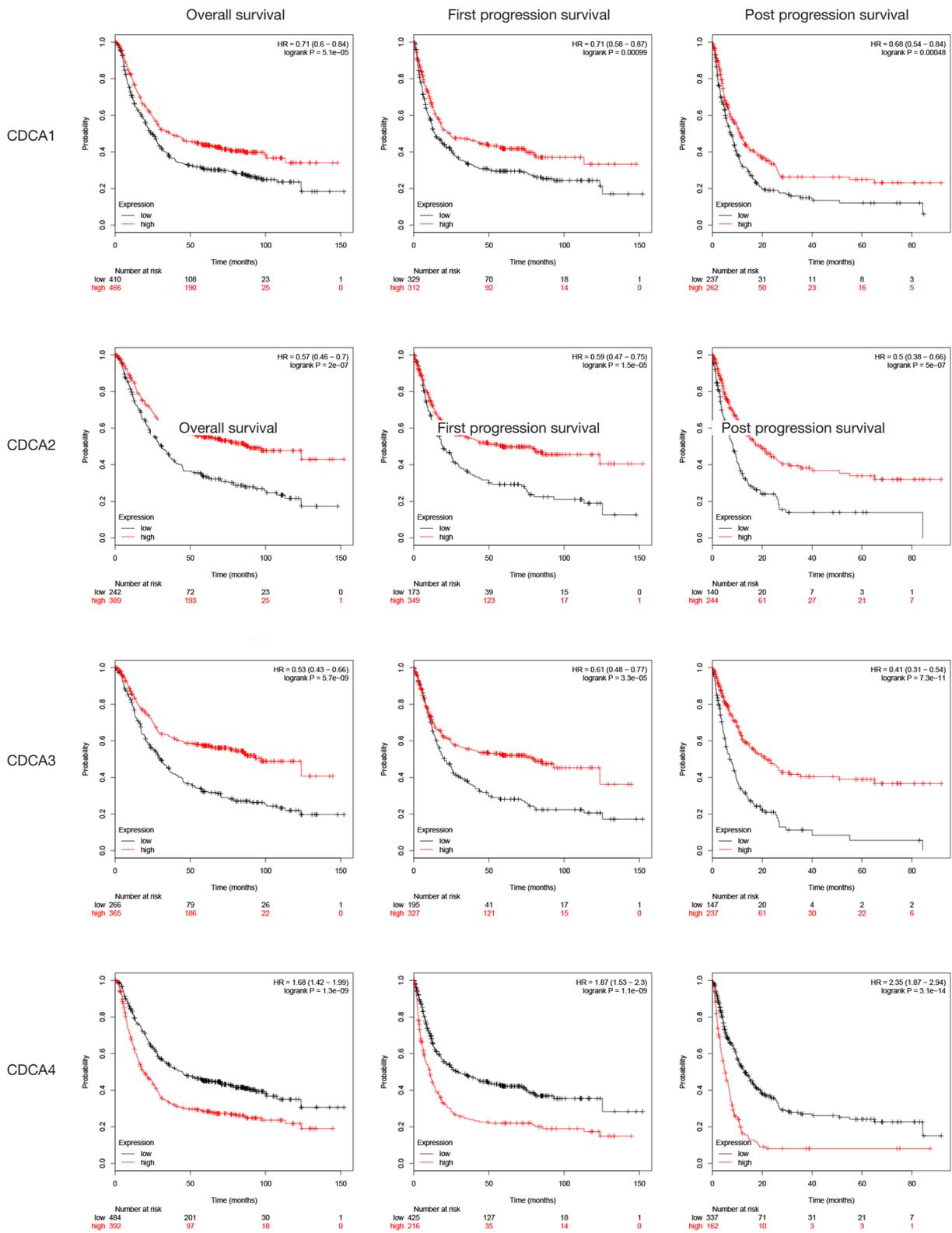**Figure 3** Correlation between CDCAs expression level and tumor stage in gastric cancer patients (GEPIA).

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Figure 4 The prognostic value of CDCA1/2/3/4 in gastric cancer patients (Kaplan-Meier plotter).

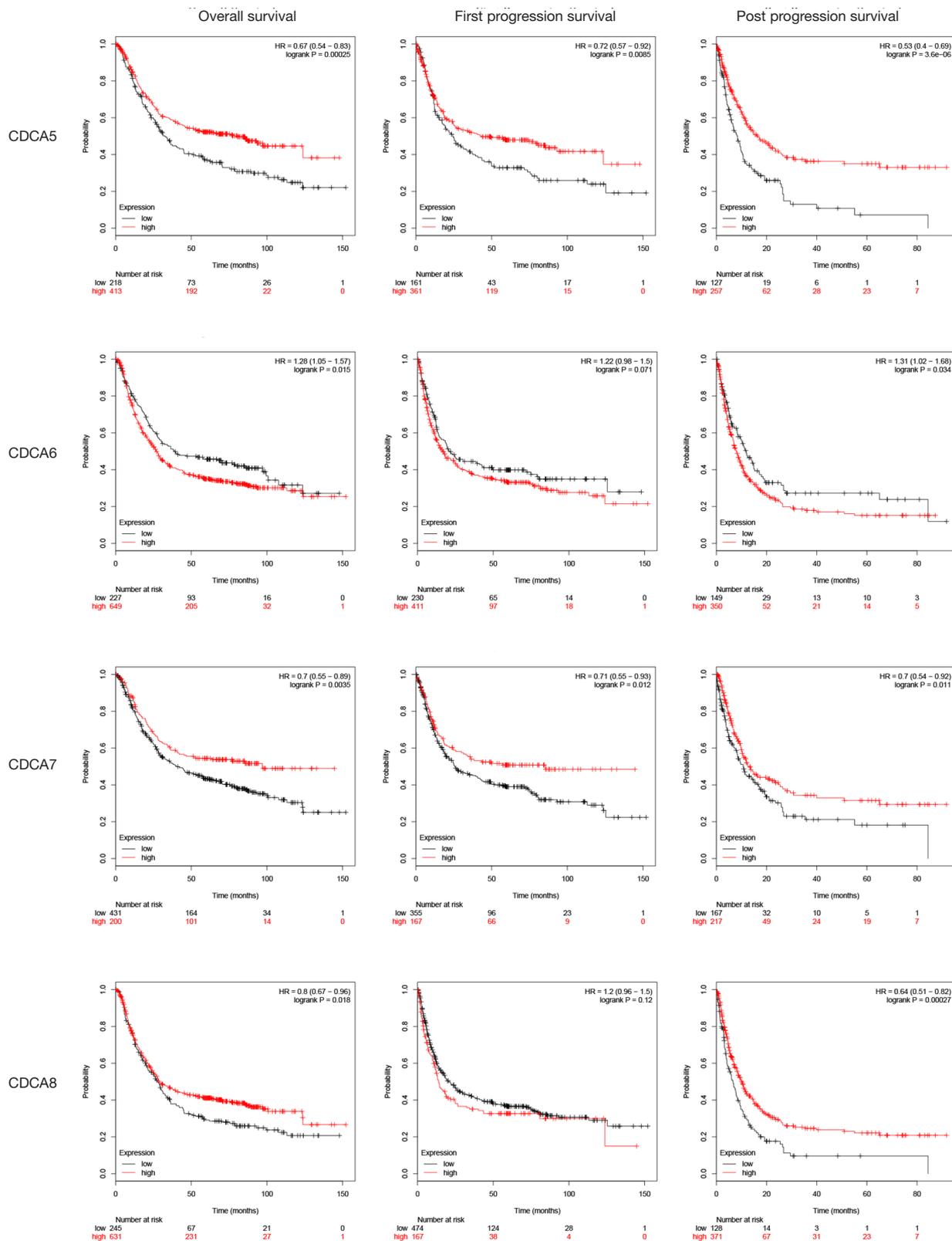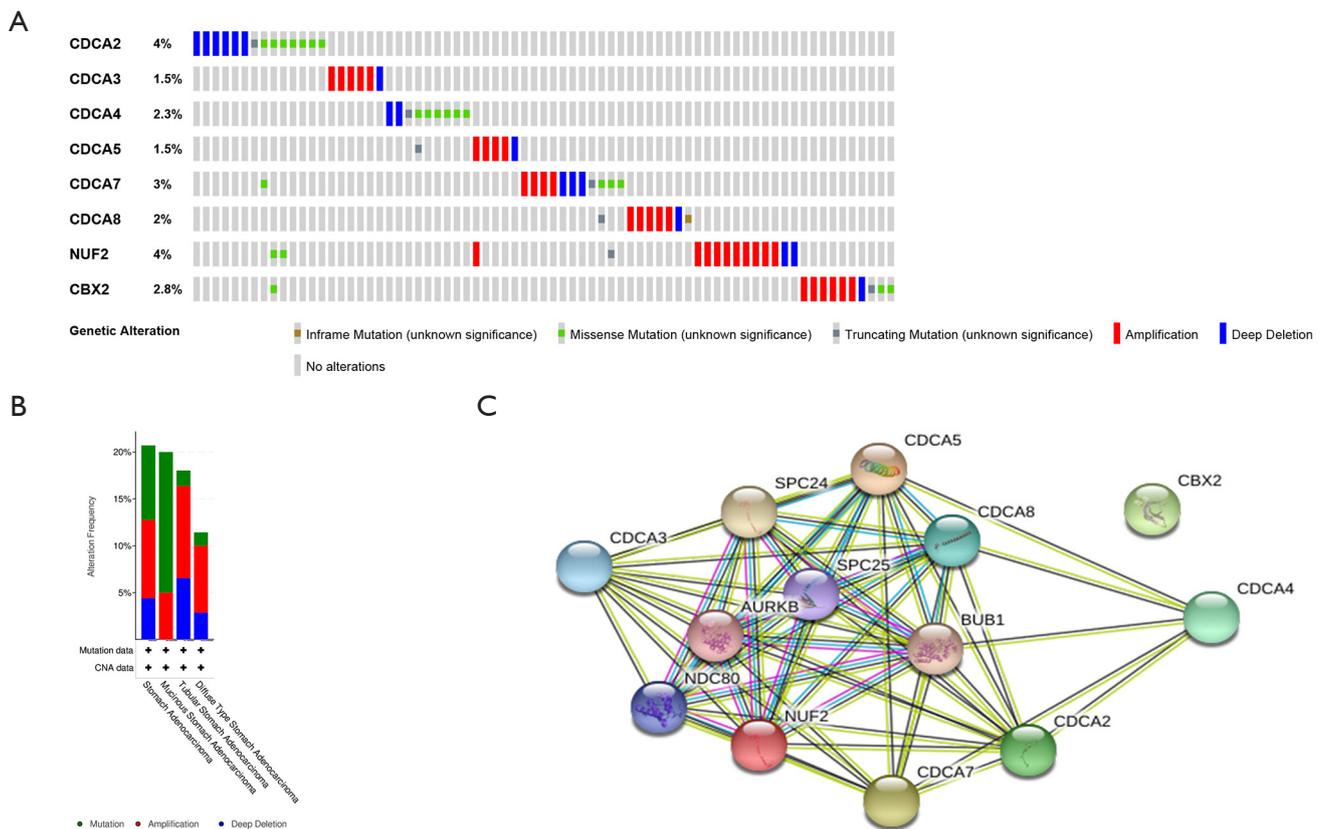**Figure 5** The prognostic value of CDCA5/6/7/8 in gastric cancer patients (Kaplan-Meier plotter).

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Figure 6 The mutation analysis and the protein-protein interaction (PPI) network construction of CDCAs in gastric cancer. (A) The genetic alteration proportion and distribution of CDCAs in gastric cancer (cBioportal). (B) The alteration frequency distribution of CDCAs in gastric cancer subtypes (cBioportal). (C) The PPI network construction of CDCA protein family.

was critical for nuclear division and microtubule stability and played a regulatory role in chromosome separation (15). CDCA1 knockout might result in the death of mitotic cells. CDCA1 had been reported to be upregulated in multiple types of cancer, such as oral cavity carcinoma (OCC), lung cancer (LC), colorectal cancer CRC, prostate cancer (PC), and even gastric cancer (GC) (2-4,16,17). Studies indicated that high CDCA1 expression was associated with poor prognosis in oral cancer patients and colorectal cancer patients, and knockdown of CDCA1 might significantly induce apoptosis of OCC cells, CRC cells, and PC cells (2,4,17). Obara conducted a phase I clinical trial for patients with castration-resistant prostate cancer using a CDCA1 peptide vaccination, which suggested that CDCA1-derived peptide vaccine might effectively induce peptide-specific cytotoxic T lymphocyte (CTL) responses and played a role in cancer inhibition (18). In this study, the data demonstrated that CDCA1 was overexpressed in gastric

cancer compared with normal gastric tissues, which was consistent with Ohnuma's findings (16). Notably, our data showed that high CDCA1 expression was associated with better OS, FP, PPS.

CDCA2 was a nuclear protein, which was considered to participate in the regulation of DNA damage response in the cell cycle (19). Uchida *et al.* demonstrated that CDCA2 expression level was upregulated in oral squamous cell carcinoma (OSCC), and CDCA2 might be related to OSCC progression by preventing cell-cycle arrest and apoptosis (20). The study by Shi *et al.* revealed that CDCA2 was upregulated in lung adenocarcinoma (LAC) compared with normal lung tissues, and the overexpression of CDCA2 might promote the proliferation of LAC cells by upregulating CCNE1. And CCDA2 might act as an independent risk factor for OS in LAC patients (21). Feng *et al.*'s findings showed that overexpression of CDCA2 might promote CRC cell proliferation through activation

A

Enrichment Analysis

B

Enrichment Analysis

C

Enrichment Analysis
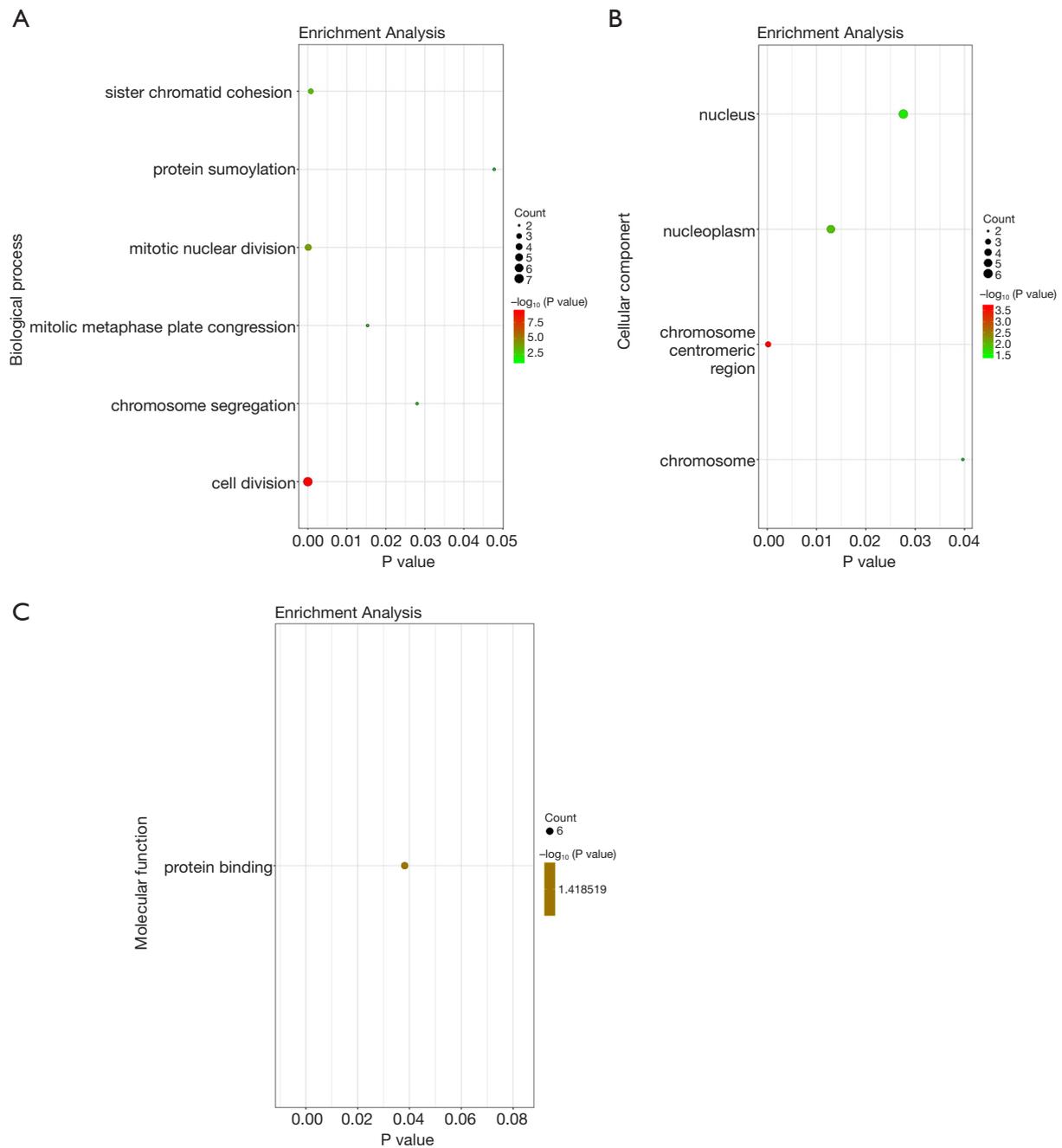
Figure 7 The Gene Ontology (GP) enrichment analysis of CDCAs using DAVID. The GO enrichment analysis mainly contained three terms, including biological process (A), cellular components (B), molecular functions (C).

of the PI3K/AKT pathway (22). Our data showed that the expression of CDCA2 was increased in gastric cancer, and elevated CDCA2 expression predicted better OS, FP, PPS in gastric cancer patients.

CDCA3 was commonly thought to be associated with

night blindness. In recent years, it had been found that the expression level of CDCA3 in breast cancer, non-small cell lung cancer, oral squamous cell carcinoma, gastric cancer, and colorectal cancer was increased compared with the normal adjacent tissues (7,8,23-25). Studies also found that

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its high expression was associated with poor prognosis in patients with breast cancer and non-small cell lung cancer (7,23). Chen *et al.* found that HoxB3 promoted the progress of prostate cancer by up-regulating CDCA3 expression (24). In colorectal cancer, CDCA3 controlled G1/S phase transition by regulating the expression of transcription factor E2F1. Knockdown of CDCA3 expression led to stagnation of G1/S phase transition, which in turn led to inhibit tumor cell growth (26). The mechanism of CDCA3 in gastric cancer had not been thoroughly studied. Our data showed that CDCA3 was highly expressed in gastric cancer, and its high expression was associated with better prognosis—OS, FP, PPS—in patients with gastric cancer.

Cell division cycle-related protein 4 was a member of the TRIP-Br transcription co-factor family and a target gene of transcription factor E2F, which could inhibit the transcriptional activation and cell proliferation dependent on E2F (5,27). CDCA4 was also found to promote the proliferation of breast cancer cells and reduce their apoptosis (28). Other studies had suggested that CDCA4 might be involved in the expression regulation of oncogene JUN (27). There were few studies on CDCA4 in other tumors. This data showed that the expression level of CDCA4 increased in gastric cancer, and it was worth noting that its high expression was usually associated with poor prognosis in patients with gastric cancer.

CDCA5 was a key regulator of sister chromatid aggregation and separation during cell division. CDCA5 played a role in multisystem malignancies and was seen as an indicator of poor prognosis. Studies showed that CDCA5 was upregulated in hepatocellular carcinoma (HCC), and increased expression of CDCA5 was associated with poor prognosis in HCC tissues (29). It was also suggested that the CDCA5 may enhance cell proliferation and inhibit apoptosis through the AKT pathway in HCC (30). In esophageal squamous cell carcinoma, increased CDCA5 expression was associated with advanced TNM staging and reduced overall survival (31). CDCA5 might also promote the progression of colorectal cancer by activating ERK signaling pathway (32). Meanwhile, CDCA5 expression increased in lung cancer, gastric cancer, bladder cancer, breast cancer, and melanoma, and might play a carcinogenic role by promoting cell proliferation and growth (33-37). Our data observed that CDCA5 was overexpressed in gastric cancer tissues compared with normal gastric tissues. *Figure 5* showed that high expression of CDCA5 predicted better OS, FP, PPS in gastric cancer patients.

CDCA6, also known as CBX2 (chromobox2), encoded a component of the polycomb multiprotein complex, which might maintain the transcriptionally repressive state of many genes throughout development. Mechanistically, the overexpression of CBX2 might maintain cancer stem cells in an undifferentiated state and inhibit tumor suppressors, which in turn led to poor survival (38). Studies reported that CBX2 expression was upregulated in breast cancer, prostate cancer, osteosarcoma, hepatocellular carcinoma, and elevated CBX2 expression was significantly and independently associated with poor survival (39-42). In gastric cancer, CDCA6, namely CBX2, was overexpressed in GC tissues compared with adjacent normal samples. High expression of CDCA6 was associated with poor prognosis, namely, lower OS, FP, PPS.

CDCA7 expression was significantly elevated in gastric cancer. Ye *et al.* reported that high expression of CDCA7 was associated with poorer disease-free survival in patients with breast cancer (43). Currently, there were few studies on CDCA7 in gastric cancer. Our data suggested that overexpression of CDCA7 meant better OS, FP, PPS in GC patients.

CDCA8 encoded a crucial component of the chromosomal passenger complex, which acted as a key regulator of mitosis (37). CDCA8 overexpression was detected in various malignant tumors, such as breast cancer, bladder cancer, cutaneous melanoma, lung cancer, renal clear cell carcinoma (6,44-47). Bi *et al.* demonstrated that the high expression level of CDCA8 was correlated with poor clinicopathological features of bladder cancer patients (6). Jiao *et al.* found that the increased expression of CDCA8 was positively associated with FOXM1 expression and shorter overall survival in breast cancer (47). Ci *et al.* discovered that overexpression of CDCA8 promoted the malignant progression of cutaneous melanoma and lead to poor prognosis (46). A study by Hayama *et al.* showed that overexpression of CDCA8 played a significant role in lung carcinogenesis (44). Our data indicated that CDCA8 is upregulated in gastric cancer. Meanwhile, overexpression of CDCA8 was correlated with better prognosis—OS, FP, PPS.

Abnormal expression of the CDCA protein family in a variety of tumors had been recognized. However, the study of CDCA protein subtypes in gastric cancer was still poorly understood. In this study, bioinformatics was used for the first time to study the expression pattern and prognostic value of CDCA family subtypes in gastric cancer. Our data showed that CDCA family members were highly expressed in gastric cancer. The results also suggested that CDCA4/6

might be potential therapeutic targets for gastric cancer. And CDCA1/2/3/5/7/8 were expected to be new prognostic markers for gastric cancer. These results needed to be further confirmed by subsequent experiments. Although the research on CDCA protein family and gastric cancer was still in the early stage, its potential clinical value needed to be further studied.

Footnote

Reporting Checklist: The authors have completed the MDAR checklist. Available at https://dx.doi.org/10.21037/tcr-20-1050

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/tcr-20-1050). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Institutional ethical approval and informed consent were waived.

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