Peixin Lu et al.: CDCA family members in gastric carcinoma

Multidimensional study of CDCA family members in gastric carcinoma with prognostic value

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ABSTRACT

Gastric cancer (GC) represents a widespread malignancy, having a poor prognosis, making it necessary to search for reliable biomarkers. Cell Division Cycle Associated protein (CDCA) family, comprising CDCA1-8, acts as a key in tumor progression. However, CDCAs expression and their impact on prognosis in gastric cancer, especially stomach adenocarcinoma (STAD), have not been clarified. Consequently, we carried out a multifaceted study aimed at exploring the CDCAs expression levels and appraising their potential prognostic values in patients with STAD, using bioinformatic tools. Remarkable upregulation of all 8 CDCAs was identified in STAD tissues, as compared with the healthy tissues. Elevated CDCA4/7/8 mRNA expression predicted a short overall survival (OS), while STAD patients, showing increased transcriptional levels of CDCA7, exhibited a short disease-free survival (DFS). The most frequent alteration was low mRNA expression among all mutations. The function enrichment analysis incorporating Gene Ontology (GO) together with Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway showed that cell cycle, foxO signaling pathway and Epstein-Barr virus were relevant to the main functions of CDCAs. Finally, through the immune infiltration analysis, a remarkable relationship was found between CDCAs expression and the extent of infiltrating levels in six immunocytes. Therefore, differentially expressed CDCAs were assessed as potential biomarkers of the prognosis of STAD patients, aiming at the improved survival of these patients. Furthermore, this study might offer new ideas for the design and development of immunotherapeutic drugs.

KEYWORDS: Gastric cancer; CDCA protein family; bioinformatics analysis; biomarker; prognosis
INTRODUCTION

Gastric cancer represents a widespread malignancy carcinoma with fifth highest incidence and third highest mortality rate worldwide, and over 1 million new patients and nearly 800 thousand deaths occurred in 2018 [1]. The number of GC cases may increase in the future due to aging populations [2]. Various molecular and histological subtypes are featured in GC, mainly subdivided into four groups: microsatellite instability, Epstein-Barr virus, diffuse and intestinal subtypes [3]. Since GC detected at initial diagnosis is often at an advanced stage [4], the options for surgical treatment are often narrowed, which results in a worse outcome. Targeted therapy backed by palliative operation and chemotherapy are envisioned to be an important complementary therapy for GC with the aim of extending living period and increasing the quality of life for advanced GC patients [5]. Moreover, the discovery of prognostic biomarkers for the diagnosis and management of GC is urgently needed.

Cell division has an indispensable contribution to life process. Many studies have confirmed that many abnormalities in the division of cells can trigger the growth for malignant carcinomas [6-9]. Cell Division Cycle Associated (CDCA) protein family is deeply involved with the division of cells, comprising 8 members, CDCA1-8. CDCA1 (also called NUF2), a component in the Ndc80 complex, is responsible for the regulation of mitosis and spindle checkpoint [10]. CDCA2 can promote cancer cell proliferation, as mediated via the HIF-1α pathway [11]. CDCA3 has been described to control the cell cycle by degrading the endogenous cell cycle inhibitor WEE1 G2 checkpoint kinase [12, 13]. Through negatively feedback-modulated the activator E2F, CDCA4 operates as an essential regulatory agent in cell proliferation [14]. CDCA5 can regulate sister chromatid cohesion and separation in cell division [15]. CDCA6 (CBX2) binds mitotic chromosomes, allowing inheritance of repressive locus along cell division [16]. CDCA7 serves as an essential
transcription factor that is governed by c-Myc [17] and CDCA8 is an important regulator of mitosis [18].

In previous reports, the prognostic values of CDCAs are well documented in several cancers, for cases including ovarian cancer, hepatocellular carcinoma and endometrial carcinomas [19-21]. But the functionality and prognostic utility of the CDCA family in GC, especially stomach adenocarcinoma (STAD), remain unknown and elusive. Therefore, we performed an all-sided analysis of the CDCAs in STAD and made an exploration on the potential of them as prognostic biomarkers to offer the doctors added support for the selection of optimal treatment, leading to improved outcomes.

MATERIALS AND METHODS

ONCOMINE

AS an efficient online database, ONCOMINE (www.oncomine.org) provides powerful, genemo-wide expression analysis [22], with which the CDCAs expression in transcriptional extents in various carcinoma types was investigated. We set the \( p \)-value as 0.05, the fold change index as 2 and gene rank as top 10\% for the thresholds. And \( t \)-test was chosen for the exploration.

Ualcan

Ualcan serves as a multi-faced and integrated web source providing analysis of cancers (http://ualcan.path.uab.edu/) [23]. Here the CDCAs expressed degrees was analyzed in the “TGCA Gene analysis” modular and the “Stomach adenocarcinoma” dataset. When a \( p \)-value < 0.05, the differences were deemed to exert a statistical meaning.

GEPIA

GEPIA, an Internet-based database, provides functions including differential expression, correlation and survival analysis on the basis of TCGA and GTEx data (http://gepia.cancer-
The distinguished analysis of mRNA expression of STAD versus normal tissues, pathological stages analysis, correlative prognostic analysis and multiple gene analysis were performed with this tool. The $p$-values were obtained with $t$-test, and Kaplan-Meier curves were chosen to present the findings of the survival analysis.

**Kaplan-Meier Plotter (KM plotter)**

KM Plotter allows users to estimate the impact of the expression of over 50 thousand genes on the survival of patients with GC ([http://kmplot.com/analysis/](http://kmplot.com/analysis/)) [25]. The value of prognosis of CDCAs in STAD patients was investigated. The $p$-value, hazard ratio index, 95% confidence interval and number of people at risk were displayed in the figures. And 0.05 was the set for $p$-value meeting the threshold to be statistically significant.

**cBioPortal**

cBioPortal is an accessible tool for browsing multiple dimensional cancer datasets ([www.cbioportal.org](http://www.cbioportal.org)) [26]. Backed by TCGA database, the changes of CDCAs in STAD samples were derived from the cBioPortal. We also got the mutation type and corresponding protein alteration details (selecting the data with the highest mutation frequency) from it.

**PolyPhen-2 and PROVEAN**

PolyPhen-2 ([http://genetics.bwh.harvard.edu/pph2/](http://genetics.bwh.harvard.edu/pph2/)) helps users explore influences of missense mutation on protein functions [27], and PROVEAN ([http://provean.jcvi.org/index.php](http://provean.jcvi.org/index.php)) also provides predicted function analysis of gene mutation [28]. Supported by these two tools, we measured the effect of CDCAs mutation on protein functions.
STRING

STRING (https://string-db.org/) is a library of predictive associations between protein and proteins, including physical and functional associations [29]. We carried out a protein and protein interaction (PPI) network of CDCAs and got 50 relative genes for further exploration.

GeneMANIA

GeneMANIA acts as an Internet-based resource designed for helping users explore the possible relationships for the genes of interest (http://www.genemania.org) [30]. The predictive value of CDCAs was analyzed through GeneMANIA.

David 6.8 And Hiplot

David 6.8 offers users a thorough set of functionalities commenting tools to elucidate the biofunctions of the presented genes (https://david.ncifcrf.gov/) [31]. We conduct functional enrichment analysis including GO and KEGG for CDCAs and 50 relative genes. Then the results obtained from David 6.8 were visualized using Hiplot, a scientifically based resource for information analysis (https://hiplot.com.cn/). Three parts, which are named biological process (BP), cellular components (CC) and molecular function (MF), are comprised in the GO analysis.

TIMER

TIMER, as a user-friendly tool, provides a systematized evaluation of immunological infiltrating degrees on various kinds of carcinoma (https://cistrome.shinyapps.io/timer/) [32]. The relationships of CDCAs expressed degrees and immunological infiltrating levels in patients with STAD were carried out in Gene module, shown as the scatterplot.
Statistical analysis

T-test was utilized to perform the expression analysis for CDCAs in STAD with ONCOMINE, UALCAN and GEPIA. One-way ANOVA was applied for exploring the expressed extents of CDCAs in different stages of STAD. Survival analysis including OS and DFS was carried out with KM plots and GEPIA using log-rank test. The infiltration association analysis was conducted using spearman correlation test. A p value < 0.05 was set to be statistically significant.

Ethical statement

There were no local ethical recognition or statement officials since the clinical data were obtained in a publicly available manner from TCGA database. Informed consent was not required because this research was conducted on the basis of publicly available data from the TCGA database.

RESULTS

Overexpression of CDCAs mRNAs in patients with STAD

ONCOMINE was selected aimed at analyzing the CDCA gene transcriptional data to investigate the distinct CDCAs expression in STAD patients (Figure 1). In comparison with paired healthy tissues, the transcriptional levels for all CDCAs were markedly elevated in STAD tissues, which is consistent with data in Table 1. For instance, from DEerrico’s dataset the CDCA1 mRNA expressed extents appeared to be up-regulated in diffuse gastric adenocarcinoma, gastric mixed adenocarcinoma and gastric intestinal-type adenocarcinoma, for which the matching fold changes were 2.858, 4.498 and 5.515. And the transcription levels of CDCA2 were also significantly elevated in gastric mixed adenocarcinoma and gastric intestinal type adenocarcinoma, for which respectively corresponding fold changes were 3.287 and 3.851 [33].
Levels of transcription of CDCAs in STAD tumors together with normal tissues were also estimated with UALCAN. The findings indicated that the mRNA levels of CDCA1 ($p < 1E-12$), CDCA2 ($p = 1.62E-12$), CDCA3 ($p = 7.62E-11$), CDCA4 ($p < 1E-12$), CDCA5 ($p < 1E-12$), CDCA6 ($p = 1.62E-12$), CDCA7 ($p = 1.62E-12$), CDCA8 ($p < 1E-12$) were remarkably up-regulated (Figure 2). The mRNA expression of CDCA7 was identified to be the most up-regulated after the comparison of the relevant levels of all CDCAs in STAD tissues (Figure 3).

Subsequently, we carried out an assessment of the connections between differentially expressed CDCAs and different pathologic phases of STAD patients using GEPIA, but they did not change notably during the different phases of STAD (Figure 4).

**Prognostic value of the CDCAs in patients with STAD**

Aimed at gauging the value of varying expression of CDCAs in the development of STAD, an analysis estimating the impacts of CDCAs on the clinical results was performed with GEPIA. Depicted in Figure 5A are the OS curves. Patients belong to the group of elevated CDCA7 expression were observed to have a remarkably shortened OS ($p = 0.022$). As another indicator, the effects of CDCAs expression on the DFS were also investigated (Figure 5B). STAD patients who were highly expressed in CDCA7 presented with notably reduced DFS ($p = 0.0023$). In the exception of CDCA7, other members in CDCA family appeared to exert no obvious consequence on OS as well as DFS.

Then the KM plotter was also used to study the implications of CDCAs on the outcomes for patients with STAD. The findings indicated that high transcriptional degrees of CDCA4 (HR = 1.27, $p = 0.017$) and CDCA8 (HR =1.39, $p = 0.0011$) were remarkably linked to adverse OS in STAD patients (Figure 6).
Mutation, PPI network and predicted protein functions of CDCAs in patients with STAD

The gene variations of CDCAs in STAD cases were analyzed using cBioPortal online tool. As shown in Figure 7A, for CDCA1 (NUF2), CDCA2, CDCA3, CDCA4, CDCA5, CDCA6 (CBX2), CDCA7 and CDCA8, the respective changes constituted 8, 8, 6, 5, 5, 5, 6 and 7% of the STAD samples. And the most frequent variation in the samples was the under-expression of mRNA (Figure 7A). Subsequently, predicted function analysis of proteins was performed to assess the pathogenicity affected by CDCAs gene mutation (PolyPhen-2 and PROVEAN). We found that the missense mutation of CDCA1 (Score: 0.565) and CDCA3 (Score: 0.520) was possibly damaging while the missense mutation of CDCA4 (Score: 0.938) was probably damaging the protein functions (Figure S1A). The nonsense mutation of CDCA8 was predicted to be deleterious to protein functions (Figure S1B).

Then the underlying interconnections of different CDCAs were identified via PPI network charts using STRING (Figure 7B). The functionality of these variously expressed CDCAs was implicated in cell cycle. Besides, the result of GeneMANIA demonstrated that mitosis, nuclear division and organelle were linked to the functions of CDCAs together with their correlated molecules (Figure 7C).

**Predicted functional and pathway enrichment assessment for CDCAs in STAD patients**

CDCAs and relative fifty genes from STRING were analyzed using DAVID 6.8 and Hiplot tool. The items with the highest enrichment in the BP group are revealed in the Figure 8A, cell division, mitotic cell cycle, anaphase-promoting complex-dependent catabolic process, modulation of ubiquitin-protein ligase activity engaged with mitotic cell cycle, protein ubiquitination engaged with ubiquitin-dependent protein catabolic process, sister chromatid
cohesion, protein K11-linked ubiquitination and proteasome-mediated ubiquitin-dependent protein catabolic process. The top 10 projects with the highest enrichment within the CC group were anaphase-promoting complex, cytosol, condensed chromosome kinetochore, kinetochore, nucleoplasm, spindle, midbody, centromeric region chromosome, centrosome and nucleus. In the group of MF, the differentially expressed CDCAs and the relevant genes appeared to be chiefly enriched in protein binding and phosphatase binding, histone kinase activity, protein kinase activity, protein serine/threonine kinase activity, anaphase-promoting complex binding, cyclin-dependent protein serine/threonine kinase activity, ATP binding and microtubule motor activity. As presented in Figure 8B, cell cycle, oocyte meiosis, progesterone-mediated oocyte maturation, ubiquitin mediated proteolysis, HTLV-I infection, foxO signaling pathway, vital carcinogenesis, p53 signaling pathway, small cell lung carcinoma, Epstein-Barr virus infection and hepatitis B were the top 10 KEGG pathways remarkably related to the tumorigenesis and progression of STAD.

**The infiltration analysis of immune cells of CDCAs in patients with STAD**

The levels of immunocytes are linked with the growth and the progress of carcinoma cells. Therefore, through the utilization of the TIMER database we performed exploration on the relevance between CDCAs and immunological infiltration (Figure 9). CDCA1 (NUF2) expression was in a negative relationship with the immunological infiltration of CD8$^+$ T cell (Cor = -0.269, $p = 1.50E$-7), CD4$^+$ T cell (Cor = -0.197, $p = 1.52E$-4), macrophage (Cor =-0.356, $p = 1.61E$-12), neutrophil (Cor = -0.215, $p = 2.86E$-5) and dendritic cell (Cor = -0.303, $p = 2.67E$-9). There existed a reverse trend between CDCA2 expression and infiltrating levels of CD8$^+$ T cell (Cor = -0.157, $p = 2.45E$-3), CD4$^+$ T cell (Cor = -0.162, $p = 1.89E$-3), macrophage (Cor = -0.348, $p = 5.31E$-12) and dendritic cell (Cor = -0.191, $p = 2.12E$-4). CDCA3 expression was negatively related to infiltrating degrees of B cell (Cor = -0.295, $p = 7.81E$-9), CD8$^+$ T cell (Cor = -0.135, $p = 9.17E$-3), CD4$^+$ T cell (Cor
similarly, the expression of CDCA4 was negatively related to infiltrating extents of B cell (Cor = -0.264, p = 2.69E-7), CD8+ T cell (Cor = -0.114, p = 2.78E-2), CD4+ T cell (Cor = -0.192, p = 2.17E-4), macrophage (Cor = -0.326, p = 1.31E-10) and dendritic cell (Cor = -0.121, p = 1.93E-2). The CDCA5 expressed degrees were negatively related to infiltrating degrees of B cell (Cor = -0.296, p = 6.98E-9), CD8+ T cell (Cor = -0.134, p = 9.93E-3), CD4+ T cell (Cor = -0.247, p = 1.72E-6), macrophage (Cor = -0.363, p = 6.04E-13) and dendritic cell (Cor = -0.166, p = 1.30E-3). Negative relationships were observed between the expressed content of CDCA6 (CBX2) and the infiltrating levels of B cell (Cor = -0.124, p = 1.67E-2), CD8+ T cell (Cor = -0.176, p = 6.57E-4), macrophage (Cor = -0.147, p = 4.53E-3), neutrophil (Cor = -0.19, p = 2.27E-4) and dendritic cell (Cor = -0.167, p = 1.23E-3). CDCA7 expression was in a negative association with the infiltrating extents of CD4+ T cell (Cor = -0.199, p = 1.25E-4), macrophage (Cor = -0.277, p = 5.90E-8) and dendritic cell (Cor = -0.147, p = 4.63E-3). With regard to CDCA8, the infiltration of B cell (Cor = -0.207, p = 6.18E-5), CD8+ T cell (Cor = -0.151, p = 3.62E-3), CD4+ T cell (Cor = -0.242, p = 2.87E-6), macrophage (Cor = -0.373, p = 1.15E-13) and dendritic cell (Cor = -0.209, p = 5.14E-5).

**DISCUSSION**

GC exerts the fifth highest incidence and the third highest mortality of carcinomas. Mortality rates of gastric cancer remain to be high, with the number of deaths accounting for 8.2% in all cancer types [1]. The bad outcomes make the exploration for the prognostic biomarkers necessary.

Consisting of CDCA1-8, Cell Division Cycle Associated (CDCA) protein family serves an irreplaceable part in cell division. Studies have reported that members of CDCA family are
involved in carcinoma proliferation, apoptosis, invasion activity and medication tolerance [36-38]. CDCAs also participate in many pathways related to cancers. For example, CDCA2 modulated CCND1 expression as a result of the activation of the PI3K/AKT pathway, thus promoting the development of colorectal carcinoma cell [39]. CDCA5 could disrupt the cellular behavior of liver cell tumors through the AKT pathway [40]. CDCA6 (CBX2) was reported to have a strong relationship with Hippo pathway and YAP in liver cancer cells [41]. However, the distinctive functions of CDCAs in STAD needs to be further explored. Here, the prognostic value as well as bio-functions of CDCAs in STAD was analyzed comprehensively.

Initially, we explored the transcriptional expression profile of CDCAs and the relatedness of them to the pathological phases of STAD. It was observed that all 8 genes were remarkably upregulated in STAD compared with normal tissue. Moreover, STAD patients carrying highly expressed levels of CDCA4, CDCA7 and CDCA8 were notably linked with shorter OS. And increased levels of CDCA7 in STAD patients were in correspondence with poorer DFS. CDCA4 can modulate the proliferation and apoptosis in carcinomas by differentially taking control of the transcriptional activity of E2Fs and p53 [14, 42]. Studies have demonstrated that CDCA7 knockdown has a great limit on the migration of cancer cells through regulation of tubulin and actomyosin cytoskeleton dynamics in lymphoma [37]. CDCA8 knockdown is reported to inhibit the ROCK signaling, resulting in the repression of the proliferation and invasion in cutaneous melanoma cells [43]. These findings led to the suggestion that the variously expressed CDCAs, especially CDCA4, CDCA7 and CDCA8, may have a substantial impact on STAD.

In addition to the various genomic expression in STAD, genomic mutations together with epigenetic changes affect tumor progression [44]. Therefore, the molecular characteristics
of CDCAs in STAD were further explored. Some gene changes of CDCAs in STAD were noticed, in which the decreased levels of mRNA expression were the highest altered. The result suggested underlying roles of CDCAs in STAD. Alterations in protein functions such as the regulation of proteins by upstream stimulatory factor 2 might influence the cancer process [45]. The mutations of CDCA1, CDCA3, CDCA4 and CDCA8 were shown to impair the protein functions, which might be adverse for the prognosis of STAD patients.

PPI network from STRING and GeneMANIA demonstrated that the CDCAs are closely linked with cell cycle, mitosis and nuclear division. The regulation of cell cycle might be targeted to promote the manufacture of drugs to help the treatment for cancers such as breast cancers [46]. Mitosis was reported to be reckoned as a critical period, during which cells under surveillance might provide a strategy to inhibit tumor growth [47]. Abnormal nuclear division caused by chromosome instability when one kind of biogenesis factor named NOP53 was worn out was involved in pathologies and cancers [48].

Then functional enrichment analysis was carried out. Identified in the findings, the functional characteristics of the genes were in close links with pathways like cell cycle, foxO signaling pathway and Epstein-Barr virus infection. There have been some studies showing the cell cycle is intimately involved in the tumorigenesis and progression and influences proliferation and senescence greatly [49-53]. FoxO, a participant of a transcription factor family named forkhead, is involved in cellular differentiation, cellular proliferation, apoptosis, DNA injury and mend and has been a therapeutic target in cancer [54]. Epstein-Barr virus associated gastric malignancy has become a subtype, which reflects its critical role in STAD [55].

There is increased support that immunocyte infiltration is a crucial agent influencing tumor progress and relapse [56]. Here, the levels of CDCAs were identified to be in a notable link
with the immunological infiltrating extents of several immunocytes, B cells, CD8\(^+\) T cells, CD4\(^+\) T cells, macrophages, neutrophils and dendritic cells, which indicated that CDCAs also might reflect the immunological conditions in addition to the prognosis of the tumor.

The reactivity of CD8\(^+\) T cells enhanced by inhibiting CD155 and TIGIT (one kind of T-cell surface molecule) might improve the tumor mice survival in GC [57]. The anti-tumor effects were also observed in CD4\(^+\) T cells and macrophages: the polarization of M1 macrophages could cause the activation of CD4\(^+\) T cells, facilitating the cancer cell elimination in lung carcinoma [58]. B cells could exert anti-tumor effects through different pathways and initiating humoral immunity [59]. The application of dendritic cells vaccine showed a great prospect in cancers such as lung cancer [60]. CDCAs were shown to obviously reduce these immune cells in STAD, constituting a possible cause of poor patient prognosis. These results are helpful for the development of new immunotherapies. The checkpoint inhibitors have been investigated to have a proven effect on the GC [61], which might give us an insight because CDCAs participated in the cell cycle, and blocking them may be effective in improving the prognosis of STAD patients.

There appeared to be several constraints in our research. All of the data under analysis here was based on different online databases, having the potential to cause background heterogeneity. Further cellular experimentations along with clinical researches would be necessary to confirm the results and investigate the underlying mechanisms of the possible contributions of CDCAs in STAD.

**CONCLUSION**

This study suggested that differentially expressed CDCAs were assessed to be potential biomarkers involving the prognosis of STAD patients and might offer new visions for the design of innovative immunotherapies.
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**TABLES AND FIGURES**

**Table 1.** The transcript levels of the CDCAs in various kinds of STAD (ONCOMINE).

| Name    | Type                          | Fold  | P Value     | t-test  | Reference |
|---------|-------------------------------|-------|-------------|---------|-----------|
| CDCA1   | Gastric Intestinal Adenocarcinoma | 5.515 | 2.71E-12    | 8.999   | [33]      |
|         | Gastric Mixed Adenocarcinoma  | 4.498 | 8.34E-5     | 6.201   | [33]      |
|         | Diffuse Gastric Adenocarcinoma | 2.858 | 0.001       | 3.820   | [33]      |
|         | Gastric Mixed Adenocarcinoma  | 2.129 | 2.99E-5     | 5.660   | [34]      |
|         | Gastric Adenocarcinoma        | 2.217 | 0.0017      | 3.402   | [34]      |
|         | Gastric Mixed Adenocarcinoma  | 2.086 | 6.42E-4     | 3.889   | [35]      |
| CDCA2   | Gastric Mixed Adenocarcinoma  | 3.287 | 3.02E-5     | 5.881   | [33]      |
|         | Gastric Intestinal Adenocarcinoma | 3.851 | 2.55E-9     | 6.930   | [33]      |
| CDCA3   | Diffuse Gastric Adenocarcinoma | 2.106 | 2.19E-5     | 4.575   | [34]      |
|         | Gastric Mixed Adenocarcinoma  | 2.301 | 0.001       | 3.552   | [34]      |
|         | Gastric Intestinal Adenocarcinoma | 3.275 | 5.23E-9     | 6.841   | [33]      |
| CDCA4   | Gastric Intestinal Adenocarcinoma | 2.214 | 7.44E-8     | 8.857   | [33]      |
|         | Gastric Mixed Adenocarcinoma  | 2.674 | 3.71E-9     | 7.255   | [34]      |
| Tumor Type                         | CDCA6   | CDCA7   |
|-----------------------------------|---------|---------|
| Gastric Mixed Adenocarcinoma      | 2.521   | 2.514   |
| Gastric Intestinal Adenocarcinoma | 2.134   | 2.077   |
| Gastric Adenocarcinoma            | 2.616   | 2.256   |
| Gastric Mixed Adenocarcinoma      | 2.102   | 2.077   |
| Gastric Intestinal Adenocarcinoma | 2.278   | 4.485   |
| Diffuse Gastric Adenocarcinoma    | 4.977   | 2.290   |
| Gastric Mixed Adenocarcinoma      | 5.711   | 2.077   |
| CDCA6                             | 2.290   | 2.514   |
| CDCA7                             | 2.521   | 2.514   |
| Gastric Intestinal Adenocarcinoma | 2.134   | 2.077   |
| Gastric Adenocarcinoma            | 2.616   | 2.256   |
| Gastric Mixed Adenocarcinoma      | 2.102   | 2.077   |
| Gastric Intestinal Adenocarcinoma | 2.278   | 4.485   |
| Diffuse Gastric Adenocarcinoma    | 4.977   | 2.290   |
| Gastric Mixed Adenocarcinoma      | 5.711   | 2.077   |
| CDCA8   | Gastric Intestinal Adenocarcinoma | 2.028 | 5.28E-10 | 7.560 | [35] |
|---------|-----------------------------------|-------|----------|-------|------|
| Gastric Intestinal Adenocarcinoma | 3.851 | 1.49E-8  | 6.550  | [33] |

**Figure 1.** The expression profile of the CDCAs at mRNA stage in GC tissues (ONCOMINE). The graph exhibits the counts of data sets with remarkable changes in the mRNA expression for CDCAs: elevated (red) and reduced (blue). Information enclosed in the green box described the transcript levels of CDCA1-8 were obviously up-regulated in GC tissues.
Figure 2. Levels of CDCAs expression in STAD (UALCAN). The expressed degrees of CDCA1-8 were notably up-regulated in STAD versus normal tissues ($p < 0.05$).

Figure 3. The relative levels of CDCA2 in STAD (GEPIA). The relevant expression of CDCA7 appeared to be the most up-regulated out of all the CDCAs.
Figure 4. Correlations between various CDCAs and the pathological phases of STAD (GEPIA). They did not change notably during the different phases of STAD.
Figure 5. Prognostic value of CDCAs in STAD (GEPIA). (A) The OS curves of CDCAs. Elevated transcript levels of CDCA7 ($p = 0.022$) tended to be remarkably linked with poorer OS; (B) The DFS curves of CDCAs. STAD patients belong to up-regulated CDCA7 groups ($p = 0.0023$) were notably relevant to shorter DFS. OS: Overall Survival; DFS: Disease Free Survival.
**Figure 6.** Prognostic value of CDCAs in STAD. Increased levels of CDCA4 \((p = 0.0017)\) together with CDCA8 \((p = 0.0011)\) appeared to be remarkably corelated with short OS in STAD patients. OS: Overall Survival

**Figure 7.** Change in genes and PPI network of CDCAs in STAD (cBioPortal, STRING and GeneMANIA). (A) Summarized changes of variously expressed CDCAs in STAD. Low mRNA was the most common change; (B, C) PPI network of CDCAs.
Figure 8. The functionally enriched analysis including GO together with KEGG pathways for various CDCAs and 50 relative genes in STAD (David 6.8 and Hiplot). (A) Bubble charts of BP group, CC group and MF group of the GO; (B) Bubble charts of KEGG pathway.
**Figure 9.** Connections between different CDCAs and the infiltrating extents of immunocytes (TIMER). The levels of CDCAs were identified to be notably linked with the extent of immunological infiltration of immunocytes ($p < 0.05$).

**SUPPLEMENTAL DATA**

**Figure S1.** The effect of gene mutations on protein functions. (A) The missense mutations of CDCA1 (Score = 0.565), CDCA3 (Score = 0.520) and CDCA4 (Score = 0.938) showed disruptive effects on protein functions (PolyPhen-2). The higher the score is, the greater the likelihood of damage is. (B) The nonsense mutation of CDCA8 was predicted to be deleterious for protein functions.