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Original Article

Discovery of CDK signature and CDK5 as potential biomarkers for predicting prognosis and immunotherapeutic response in gastric cancer peritoneal metastases

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Abstract: Gastric cancer peritoneal metastases (GCPM) are a leading cause of death in gastric cancer patients. In this study, we focused on the expression of cyclin-dependent protein kinases (CDK), essential regulators of transcription, metabolism, and cell differentiation, in GCPM. Utilizing the GSE62254 cohort, we established a CDK signature (CDKS) model comprising ten CDK gene family members. Analysis of both the GSE62254 and TCGA cohorts revealed that patients with low CDKS had a worse prognosis compared to those with high CDKS. Furthermore, patients with high CDKS demonstrated positive responses from immunotherapy, as observed in the KIM cohort. We investigated the association between CDKS and the tumor microenvironment, including immune escape mechanisms. Immunohistochemistry analysis revealed a positive correlation between CDK5 and PD-L1 expression in gastric cancer. Furthermore, we found that CDK5 knockdown led to the inhibition of PD-L1 expression in gastric cancer cells. Our findings highlight the potential of CDKS as a prognostic biomarker and an indicator of immunotherapy response in gastric cancer patients. Moreover, our study suggests that targeting CDK5 could provide a new pathway for exploring immunotherapeutic research.

Keywords: Gastric cancer, peritoneal metastases, CDK, CDK5, PD-L1

Introduction

Gastric cancer is a widespread disease in eastern Asia, ranking as the fifth most commonly diagnosed cancer and the third leading cause of cancer-related death [1]. Gastric cancer peritoneal metastasis (GCPM) often recurs in patients with gastric cancer and is associated with notably poor survival outcomes [2]. Conventional imaging techniques may not reliably detect or measure GCPM. Therefore, diagnostic laparoscopy with peritoneal lavage cytology has been used to identify disseminated cancer cells within the peritoneal cavity to improve diagnosis accuracy [3]. Patients who are at high risk for developing GCPM may not experience significant benefits from conventional therapies [4]. Therefore, it is recommended to adopt a combination of systemic therapy and peritoneal-directed treatment strategies, such as intraperitoneal chemotherapy, which have demonstrated an encouraging trend towards improving disease outcomes. While GCPM has been linked to various clinicopatho-

logical characteristics, genetic mutations, and molecular signatures, their usefulness in clinical practice remains limited [5, 6]. Presently, treatment choices for gastric cancer predominantly rely on the disease stage due to the absence of personalized targets. To refine risk stratification accuracy and enhance survival prediction post-curative resection, there's an urgent need to identify precise biomarkers related to peritoneal metastasis recurrence. Such biomarkers can facilitate personalized therapeutic decisions even before surgery, optimizing treatment plans.

The cyclin-dependent protein kinases (CDK) were initially identified as serine/threonine-specific protein kinases that are activated by cyclin partners to regulate the progression of the eukaryotic cell cycle [7-9]. There are now twenty proteins recognized as members of the CDK family. Generally, aside from CDK family members that regulate the cell cycle (CDK1, CDK2, CDK4, and CDK6), an important subset of this family (CDK7, CDK8, CDK9, CDK12, and CDK13) regulates transcription through phosphorylation of the seven-peptide repeat sequence in the C-terminal domain (CTD) of RNA polymerase II [9]. CDK7 has a unique role in indirectly regulating the cell cycle by activating CDK1, CDK2, CDK4, and CDK6 [10, 11]. CDK3 facilitates the transition from the quiescent phase (G0) to G1 phase by phosphorylating the retinoblastoma protein (pRB) [12]. Other CDK family members (CDK5, CDK10, CDK11, CDK14-18, and CDK20) have diverse and often tissue-specific functions [13]. For instance, CDK5 was among the earliest CDK family members to be studied in non-cycling cells [14]. CDK10, in contrast, regulates gene transcription without phosphorylating RNA pol II and is known to phosphorylate substrates like the ETS2 oncogene and protein kinase PKN2 [15, 16]. Research has shown that most CDK family members are highly expressed in gastric cancer tissues [17-20], whereas CDK5, CDK10, and CDK12 have lower expression levels [21, 22]. However, research on CDK family members in gastric cancer peritoneal metastasis remains limited.

This study aims to create a CDK signature (CDKS) to predict gastric cancer peritoneal metastasis, survival, and immunotherapy responses. Our findings demonstrated that CDKS was an independent prognostic indicator and was strongly correlated with gastric cancer immunotherapy response. It is worth noting that CDK5 is positively correlated with PD-L1 expression, and knockdown of CDK5 can significantly inhibit PD-L1 expression in gastric cancer cells.

Methods

Clinical data acquisition and extraction

We collected both RNA-sequencing (RNA-seq) expression data and relevant clinical information for gastric cancer patients from the Tumor Cancer Genome Atlas (TCGA) database. Additionally, we sourced data for 300 gastric cancer patients in the GSE62254 cohort from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). Additionally, we obtained the PD-L1 treatment cohort for 45 gastric cancer patients (KIM cohort) from the TIDE database (http://tide.dfci.harvard.edu).

Genetic alteration and survival prognosis analysis

To analyze somatic mutation data in gastric cancer, we downloaded data from TCGA using the UCSC XENA website. The Kaplan-Meier curves and log-rank test were employed to compare overall survival (OS) and disease-free survival (DFS) rates among patients. The "survival" R package was used to determine optimal cutoff points.

Immune infiltration analysis

The CIBERSORT algorithm was used to estimate immune infiltration in gastric cancer and analyze correlations between CDKS and immune cells. The "ESTIMATE" R software package was used to assess the stromal score of each sample.

Single-cell level analysis

Tumor Immune Single-cell Hub 2 (TISCH2), also known as the Tumor Immune System Atlas, is a scRNA-seq database that specifically focuses on the tumor microenvironment (TME) [23]. This platform offers extensive cell-type annotation at the single-cell level, enabling detailed exploration of the TME across multiple types of cancer.

Patients and specimens

We collected a total of 20 gastric cancer tissues from the Zhangzhou Affiliated Hospital of Fujian Medical University between November 2020 and November 2021. The Research Ethics Committee of Zhangzhou Affiliated Hospital of Fujian Medical University granted approval for this study. All participating patients provided written informed consent after approval by the relevant institutional protocol.

Immunohistochemistry (IHC) staining analysis

Standard immunoperoxidase staining procedures were employed for IHC staining to assess the protein expression of CDK5 and PD-L1 in gastric cancer. Specifically, slides were incubated with an anti-CDK5 antibody (Santa Cruz Biotechnology, diluted 1:200) and an anti-PD-L1 antibody (Proteintech, Wuhan, China, diluted 1:400). Two independent pathologists evaluated the IHC staining scores for CDK5 and PD-L1 to ensure accuracy. Positively stained cells were categorized into four groups based on the percentage present: 1 (0-25%), 2 (26-50%), 3 (51-75%), and 4 (75-100%). Staining intensity scores for IHC ranged from 0-3: 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). The overall score for each case was determined by multiplying the staining intensity score by the percentage of positive tumor cells.

Cells culture and transfection

The MKN74 and HGC-27 gastric cancer cell lines were obtained from the American Type Culture Collection (ATCC) and cultured in DMEM medium containing 1% penicillin/streptomycin and 10% fetal bovine serum sulfate at 37°C and 5% CO_2. The sequence of shRNAs targeting CDK5 was cloned into the pLVX vector. The sequence of shCDK5-1# was 5'-CCGGTCTGAAGTGTAACCCTGTCCACTCGAGTGGACAGGGTTACACTTCAGATTTTT-3'; The sequence of shCDK5-2# was 5'-CCGGTGTCCAGCGTATCTCAGCAGACTCGAGTCTGCTGAGATACGCTGGACATTTTT-3'. The transfection was performed using lipofectamine 2000 according to the manufacturer's guidelines.

Western blotting

Cells were lysed in Radioimmunoprecipitation Assay (RIPA) buffer containing protease inhibitors to extract protein lysates. The protein concentration of the lysates was determined using the Micro BCA Protein Assay Kit. Samples were subsequently separated on 10% SDS-PAGE and transferred to Amersham Protran nitrocellulose membranes. The nitrocellulose membranes were then incubated with primary antibodies against target proteins CDK5 (diluted 1:1000), PD-L1 (diluted 1:1000), c-MYC (diluted 1:2000), IRF-1 (diluted 1:1000), p-STAT1 (diluted 1:1000), STAT1 (diluted 1:2000) and GAPDH (diluted 1:5000) for 2 h; antibodies were purchased from Santa Cruz Biotechnology. Finally, IRDye® 680RD Goat anti-Mouse IgG or IRDye® 800CW Goat Anti-Rabbit IgG were used to detect and quantify proteins via the Odyssey® CLx Infrared Imaging System (LI-COR Biosciences).

Statistical analysis

Statistical analyses were conducted using the Student's t-test. The Kaplan-Meier method was employed to compute overall survival (OS) and disease-free survival (DFS). Differences between groups were assessed using the log-rank test. A p-value of <0.05 was deemed statistically significant.

Results

The landscape of CDK family gene in gastric cancer peritoneal metastases

We first examined the differential expression of CDK family genes in gastric cancer tissues with and without peritoneal metastasis using the GSE62254 cohort. Our analysis revealed that CDK1, CDK2, CDK4, CDK5, CDK6, CDK7, CDK11, CDK12, CDK16, and CDK19 were expressed at significantly lower levels in gastric cancer tissues with peritoneal metastasis compared to those without metastasis. However, no significant differences in the expression of other CDK family genes were observed between gastric cancer tissues with and without peritoneal metastasis. Consequently, subsequent analyses focused on these ten members of the CDK family genes (**Figure 1A**). We computed the average expression of these ten CDK family genes, termed as the CDK Signature (CDKS).

Functional studies have demonstrated that these genes can activate the cell cycle and apoptosis (**Figure 1B**). Our correlation analysis revealed significant associations between most of these genes and CDK1, CDK2, and CDK12 (**Figure 1C**). An examination of the chromosomal locations of these ten CDK family genes

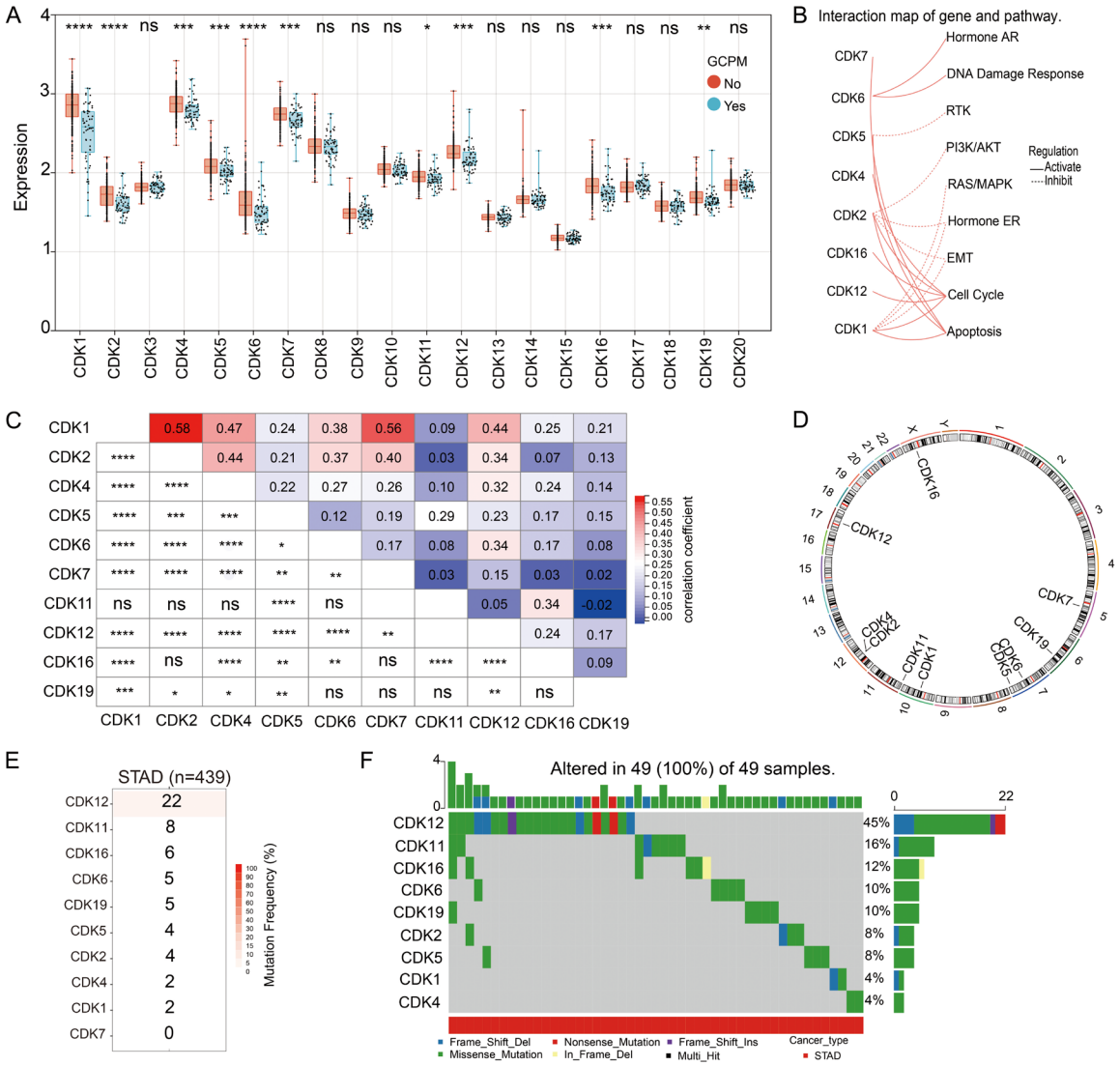

Figure 1. The landscape of genetic variation and correlation of the CDK gene family in gastric cancer peritoneal metastasis. A. Analyzing CDK family gene expression differences in gastric cancer tissues with/without peritoneal metastasis through the GSE62254 cohort; B. Investigating the associations between eight genes and relevant biological pathways; C. Performing Spearman's correlation analysis to assess the relationship among ten genes by utilizing the GSE62254 database; D. Determining the chromosomal locations of the ten genes and analyzing their distribution; E. Identifying the mutation status of ten specific genes in gastric cancer cases by utilizing the TCGA database; F. Displaying the mutation landscape of ten genes in gastric cancer patients from the TCGA database through oncoplots with each vertical column representing an individual sample. ns, $P>0.05$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ****, $P<0.0001$.

showed that all, except CDK16 (located on the X chromosome), were positioned on autosomes (**Figure 1D**). Given the crucial role that copy number variations (CNVs) and gene mutations play in cancer progression, we conducted CNV analysis in the TCGA cohort. Our findings indicated that among the ten genes, CDK12, CDK11, and CDK16 exhibited higher CNV frequencies (**Figure 1E**). The mutation frequency of CDK12 (45%) was the highest, followed by CDK11 (16%), CDK16 (12%), CDK6 (10%), CDK19 (10%), CDK5 (8%), CDK2 (8%), CDK4 (4%), and CDK1 (4%) in 49 patients. CDK7 (0%) had the lowest mutation frequency according to the gene mutation profile (**Figure 1F**).

Analysis of molecular characteristics in CDKS-low and high subtypes

We further investigated the genomic variations between the CDKS-low and CDKS-high sub-

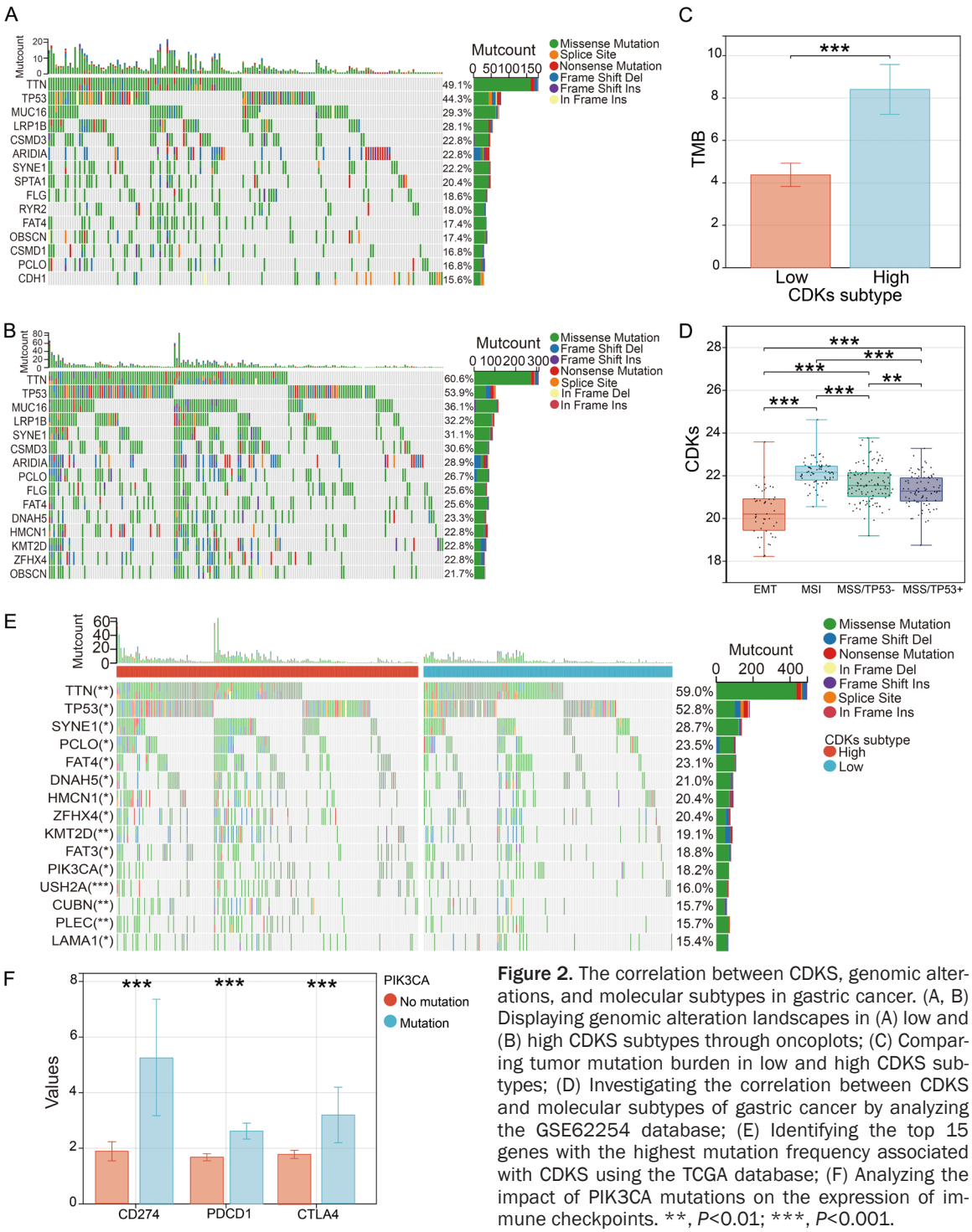

Figure 2. The correlation between CDKS, genomic alterations, and molecular subtypes in gastric cancer. (A, B) Displaying genomic alteration landscapes in (A) low and (B) high CDKS subtypes through oncoplots; (C) Comparing tumor mutation burden in low and high CDKS subtypes; (D) Investigating the correlation between CDKS and molecular subtypes of gastric cancer by analyzing the GSE62254 database; (E) Identifying the top 15 genes with the highest mutation frequency associated with CDKS using the TCGA database; (F) Analyzing the impact of PIK3CA mutations on the expression of immune checkpoints. **, $P<0.01$; ***, $P<0.001$.

types. While the top 15 mutated genes were consistent across both subtypes, the CDKS-high subtype exhibited a higher mutation frequency for most genes compared to the CDKS-low subtype (**Figure 2A**, **2B**). Given the potential of tumor mutational burden (TMB) as a biomarker for immunotherapy, we assessed the TMB variation between the two subtypes. The CDKS-high subtype had a significantly higher TMB than the CDKS-low subtype (**Figure 2C**). Our findings suggest that the two CDKS subtypes possess distinct genomic characteristics, potentially influencing their response to treatments.

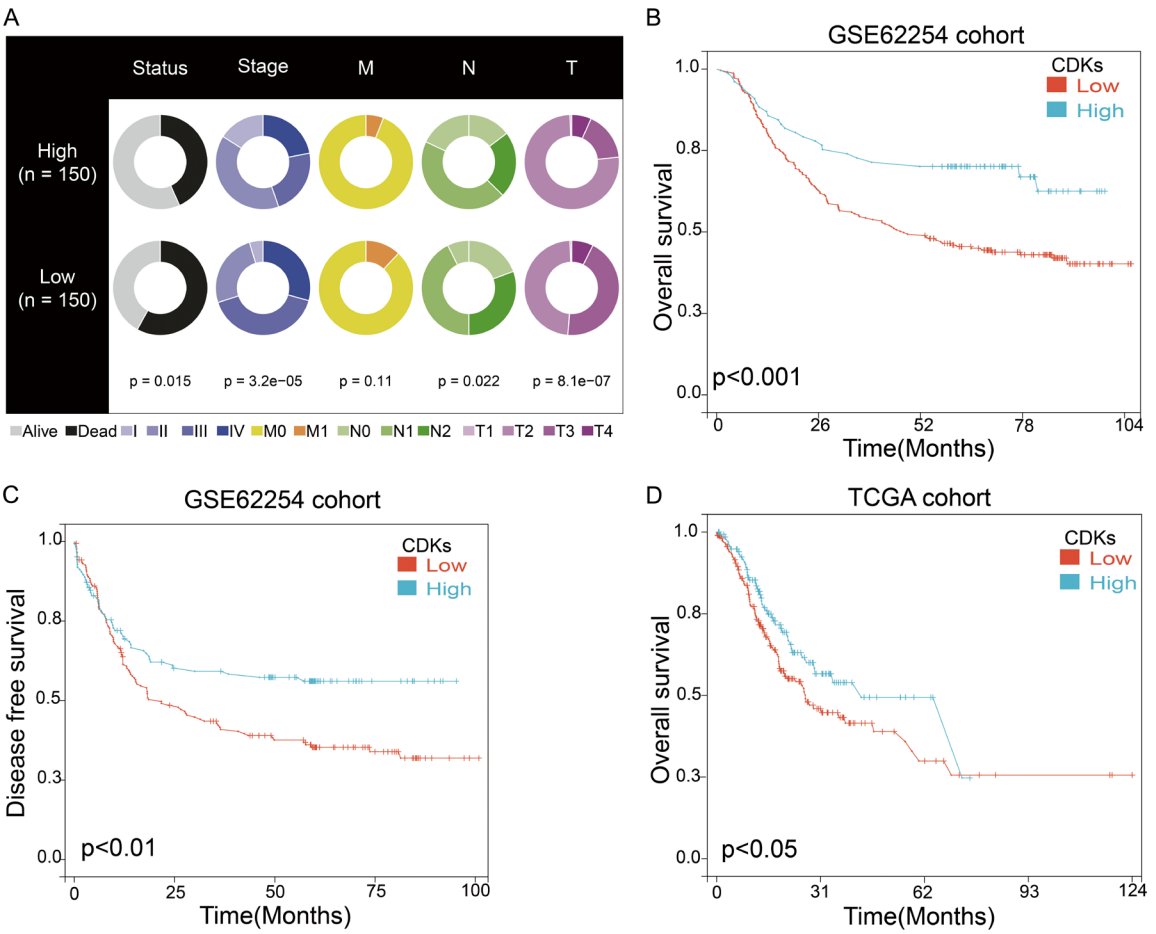

Figure 3. Correlation between CDKS subtypes and clinicopathological features and prognosis in gastric cancer. A. Comparing various clinicopathological features of low CDKS and high CDKS subtypes in the GSE62254 cohort; B-D. Survival analysis that investigates differences in OS and DFS between low CDKS and high CDKS subtypes in both GSE62254 and TCGA cohort.

We classified gastric cancer in the GSE62254 cohort into four molecular subtypes based on distinct molecular features. The epithelial-mesenchymal transition (EMT) subtype had the lowest CDKS value, while the microsatellite instability (MSI) subtype had the highest (**Figure 2D**). We then identified the top 15 mutated genes in both CDKS subtypes (**Figure 2E**). Given the association of elevated immune checkpoint markers with positive immunotherapy responses, we examined the relationship between these mutated genes and immune checkpoint marker expression. Notably, patients with PIK3CA mutations had significantly higher expressions of immune checkpoint markers (CD274, PDCD1, and CTLA4) compared to those with wild-type PIK3CA (**Figure 2F**).

Taken together, our findings suggest that the molecular subtypes of gastric cancer are associated with different CDKS subtypes and genomic characteristics, which may have implications for precision medicine and personalized treatment strategies.

The correlation between CDKS and clinical features and prognosis

Subsequently, we compared the clinical characteristics of the CDKS-low and CDKS-high subtypes in the GSE62254 cohort. Our findings revealed a significantly higher number of stage 3 and 4 patients in the CDKS-low subtype (**Figure 3A**). Regarding clinical outcomes, patients classified under the CDKS-high subtype demonstrated superior prognoses concerning Disease-Free Survival (DFS) and Overall

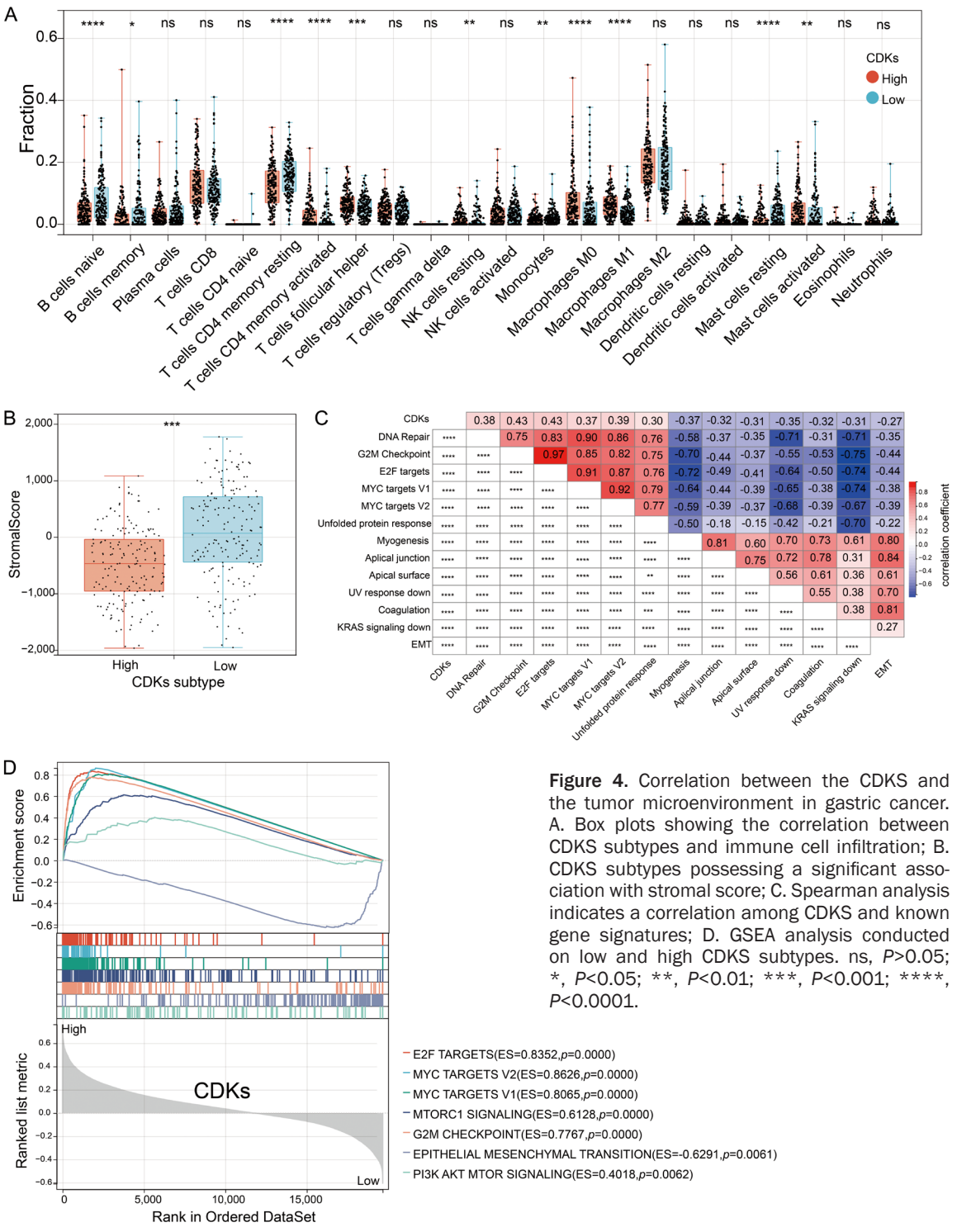

Figure 4. Correlation between the CDKS and the tumor microenvironment in gastric cancer. A. Box plots showing the correlation between CDKS subtypes and immune cell infiltration; B. CDKS subtypes possessing a significant association with stromal score; C. Spearman analysis indicates a correlation among CDKS and known gene signatures; D. GSEA analysis conducted on low and high CDKS subtypes. ns, $P>0.05$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ****, $P<0.0001$.

Survival (OS) when compared to those under the CDKS-low subtype, as highlighted in **Figure 3B-D**. These findings emphasize that CDKS may function as a reliable tool to predict the prognosis of patients with gastric cancer.

CDKS and the tumor microenvironment in gastric cancer

The tumor microenvironment (TME) plays a crucial role in tumor progression and therapeutic response. To understand the relationship

between CDKS subtypes and the TME, we used the CIBERSORT algorithm to evaluate the distribution of 22 immune cell types across the two subtypes. The CDKS-low subtype showed significantly higher infiltration levels of B cells native, B cells memory, Monocytes, T cells CD4 memory resting, and Mast cells resting. Conversely, the CDKS-high subtype showed that T cells follicular helper, T cells CD4 memory activated, NK cells resting, Macrophages M1, Macrophages M0, and Mast cells activated showed significantly higher infiltration levels (**Figure 4A**). Further examination into the relationship between CDKS and stromal scores revealed that the CDKS-low subgroup displayed a significantly higher stromal score (**Figure 4B**). These findings suggest that CDKS subtypes may have distinct immunological characteristics and different interactions with the TME, which may affect their response to immunotherapy and other cancer treatments.

We sought to delineate the functionalities of CDKS by assessing the associations between CDKS and familiar molecular features (**Figure 4C**). Our analysis revealed that G2M checkpoint, DNA repair, MYC targets V2, E2F targets, MYC targets V1, and the unfolded protein response displayed a favorable correlation with CDKS expression, while Myogenesis, apical junction, apical surface, UV response down, coagulation, KRAS signaling down, and EMT were negatively correlated with CDKS expression (**Figure 4D**). Moreover, our GSEA analysis results indicated that E2F targets, MYC targets V1, G2M checkpoint, MYC targets V2, MTORC1 signaling, and PI3K pathways displayed a significant enrichment in the CDKS-high subtype while the EMT pathway exhibited a remarkable enrichment in the CDKS-low subtype. These findings further support the notion that CDKS may indicate the regulation of different molecular pathways and biological processes and have implications for the development of personalized treatment strategies targeting specific CDKS subtypes in gastric cancer.

CDKS predict the response of gastric cancer to immunotherapy

The introduction of immune-based therapies, notably PD1/PDL1 checkpoint inhibitors, represents a pivotal advancement in cancer treatment. Nivolumab, a monoclonal PD-1 blocker, has secured approval in the United States as the primary therapeutic intervention for patients with advanced or metastatic gastric cancer. Given the promising efficacy of immune therapies, primarily PD-1 and PD-L1 checkpoint inhibitors, in treating diverse malignancies, including gastric cancer, our research further evaluated the prognostic function of CDKS in the KIM cohort. This subgroup study comprised advanced gastric cancer patients who underwent PD-L1 blockade therapy. **Figure 5A** displays the CDKS values in patients with distinct treatment responses. Patients who demonstrated stable disease (SD) or disease progression (PD) outcomes exhibited significantly lower CDK expression in comparison to those showing complete response (CR) or partial response (PR) (**Figure 5B**). Importantly, CDKS-low represented the predominant subtype in the PD/SD group (60%), whereas CDKS-high emerged as the predominant subtype in the PR/CR group (75%). These findings further suggest that CDKS may serve as biomarkers of gastric cancer immune response (**Figure 5C**).

Previous studies have highlighted the role of EBV status in predicting immunotherapy outcomes. We observed that EBV-positive patients exhibited higher CDKS values than their EBV-negative counterparts (**Figure 5D**). Our analysis also revealed positive correlations between the expression of CDK2, CDK5, CDK6, CDK7, and CD274, with CDK5 showing the strongest correlation with CD274 expression (**Figure 5E**).

Single-cell RNA sequencing (scRNA-seq) has emerged as a robust technology that characterizes the molecular features of individual cells, offering accurate insights into the tumor microenvironment. In our research efforts to elucidate the functions of CDK5 and CD274 in TME, we delved into the GSE134520 and GSE167297 cohorts through a thorough analysis (**Figure 6A**, **6C**). We found that CDK5 was mainly expressed in dendritic cells (DCs), malignant cells, plasma cells, epithelial cells, and mast cells (**Figure 6B**), while CD274 was mainly expressed in DCs, malignant cells, and mast cells (**Figure 6D**). Our immunohistochemistry data further established a positive correlation between CDK5 and PD-L1 expression, suggesting a potential regulatory role of CDK5 in PD-L1 expression in gastric cancer (**Figure 6E-G**).

Knockdown of CDK5 expression can inhibit PD-L1 expression

To elucidate the regulatory role of CDK5 on PD-L1 expression, we treated HGC-27 and

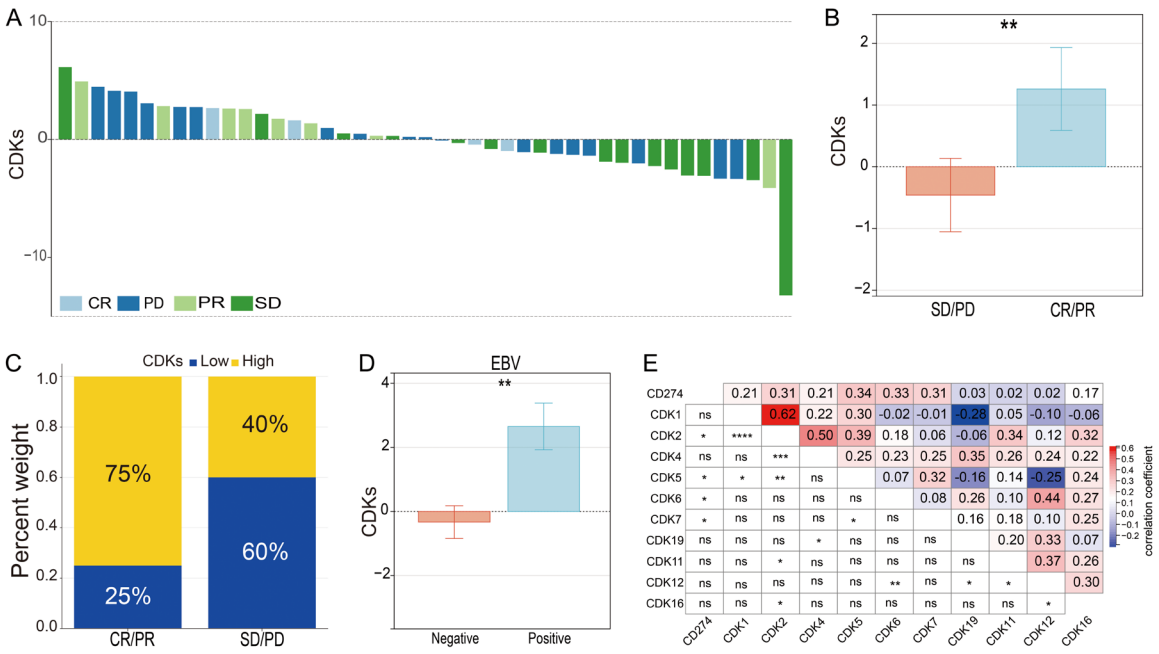

Figure 5. Predicting responses to immune checkpoint blockade treatment. A. Assessing the correlation between CDKS and response to immunotherapy in the KIM cohort. CR (complete response), PD (progressive disease), PR (partial response), SD (stable disease); B. Comparing CDKS levels between PD/SD and PR/CR groups; C. Comparing the proportion of patients with different immunotherapy responses in two CDKS subtypes; D. Comparing CDKS levels between EBV positive and negative status; E. Analyzing the correlation between the expression of ten genes and CD274. ns, $P>0.05$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ****, $P<0.0001$.

MKN74 gastric cancer cells with IFN-γ to upregulate PD-L1 expression. This manipulation allowed for a better observation of the regulatory effect of CDK5 on PD-L1 expression (**Figure 7A**, **7B**). Upon CDK5 knockdown, we observed a significant reduction in the expression of PD-L1, c-MYC, IRF-1, p-STAT1, and STAT1 (**Figure 7C**, **7D**). PD-L1, c-MYC, IRF-1, p-STAT1, and STAT1 play crucial roles in tumor immunity. PD-L1 is regarded as an important molecule for tumor immune evasion and is expressed in both tumor cells and tumor-related immune cells. c-MYC is a transcription factor that controls tumor cell proliferation and metabolism. IRF-1 is a key regulator in immune responses and exerts anti-tumor effects by regulating gene expression. p-STAT1 and STAT1 are two important signaling components closely related to the activation of signaling pathways involved in tumor immunity.

Discussion

Gastric cancer is a common gastrointestinal malignancy, posing a significant global health risk [24]. Unfortunately, patients with advanced gastric cancer often develop peritoneal metastasis after surgical intervention, typically signaling a poor long-term prognosis. Peritoneal metastases are a major contributor to the high mortality rate associated with gastric cancer [25, 26]. However, current treatment options, such as chemotherapy, intraperitoneal infusion, and palliative surgery, tend to have limited efficacy in managing metastases. Early detection of peritoneal metastases remains a challenge, especially when only minimal ascites are present, or metastases are concealed. Conventional detection methods, such as imaging or biopsies, may prove ineffective at improving metastatic detection rates in these cases. This highlights the critical need for identifying novel predictive indicators that can detect early stage peritoneal metastasis, significantly improving the likelihood of successful diagnosis and treatment in individuals with gastric cancer.

Many CDK gene family members display increased expression in gastric cancer. For example, CDK1 and CDK2 expression is significantly associated with poor prognosis [27, 28].

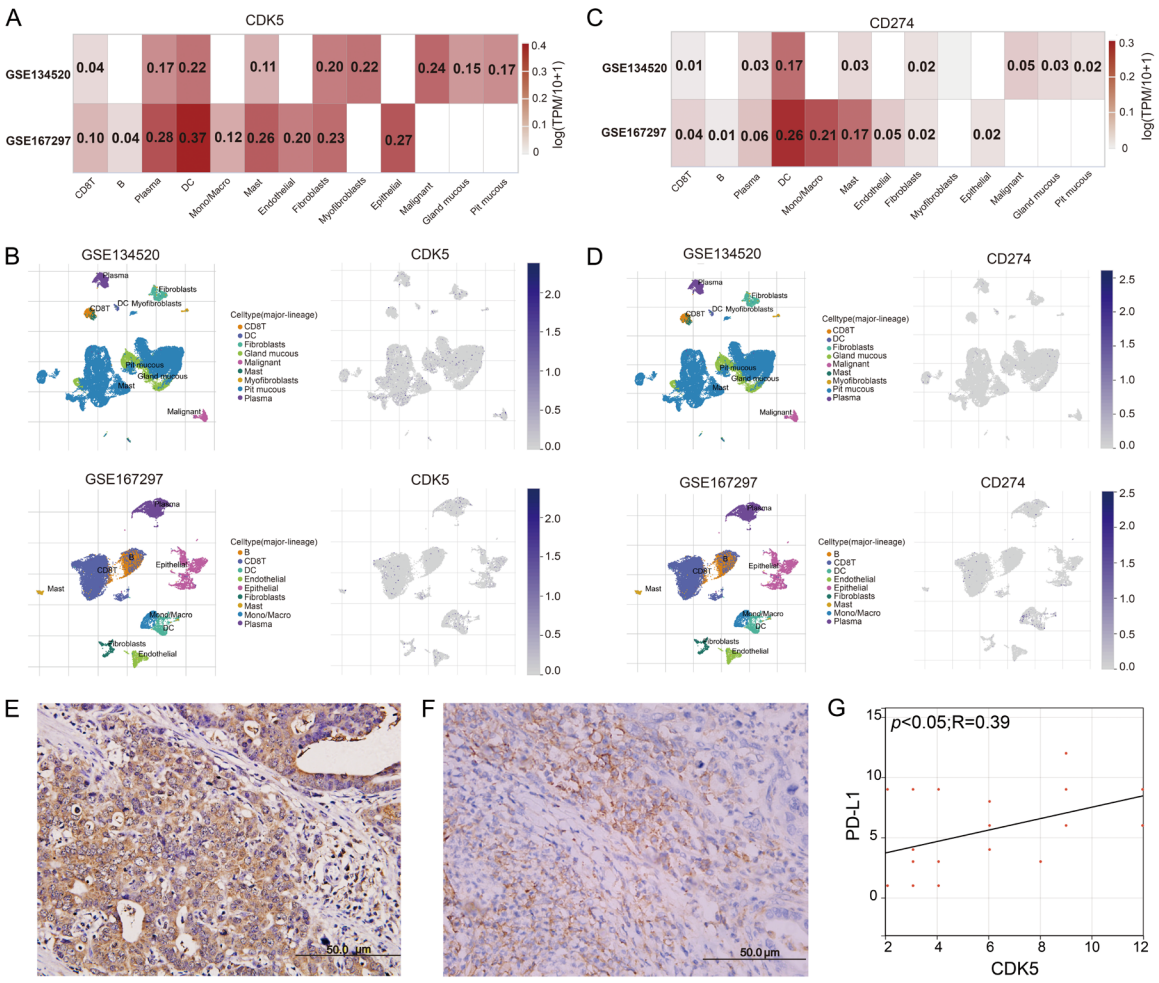

Figure 6. Correlation analysis of CDK5 and PD-L1 expression in gastric cancer. (A) Violin diagram displays the distribution of CDK5 expression in different cells from different databases; (B) Single-cell cluster map of CDK5 in different databases; (C) Violin diagram displays the distribution of PD-L1 expression in different cells from different databases; (D) Single-cell cluster map of PD-L1 in different databases; (E-G) Immunohistochemistry results for (E) CDK5 and (F) PD-L1 and (G) their correlation analysis.

Studies have shown that CDK4 is overexpressed in gastric cancer, potentially driving gastric tissue carcinogenesis [29]. However, three CDK gene family members (CDK5, CDK10, and CDK12) have been reported to have reduced expression in gastric cancer [30]. CDK5 is noticeably downregulated in gastric cancer tissues. This observance correlates with the increased severity of the disease as evidenced in gastric cancer lymph node metastasis [31]. Furthermore, there is a reduction in nuclear accumulation or localization of CDK5 in gastric cancer cells. Treatment with small-molecule inhibitor NS-0011 can inhibit CDK5 and increase its localization in the cell nucleus, thus inhibiting tumor occurrence and proliferation in xenografts. CDK5 can also interact with PP2A to deter gastric cancer cell metastasis [32]. CDK10 expression is significantly lower in gastric cancer tissues than in normal tissues. Overexpression of CDK10 leads to inhibition of invasion, migration, and proliferation of gastric cancer cells, while knockdown of CDK10 exhibits enhanced tumorigenesis [30]. In some cancers, CDK12 is expressed at high levels, while in others, it has a low-level of expression. In gastric cancer, it has been observed to be downregulated and correlated with adverse outcomes, poorly differentiated adenocarcinoma, and advanced stages [22], suggesting CDK12 may act as a tumor suppressor in gastric cancer.

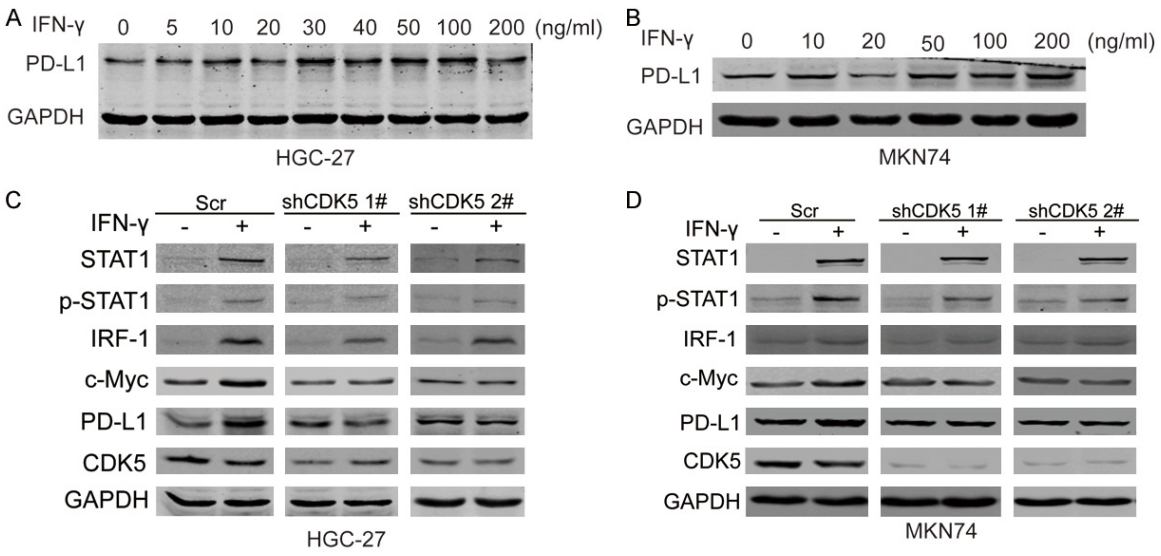

Figure 7. Knockdown of CDK5 expression can inhibit PD-L1 expression. (A) Effects of IFN-γ induction on PD-L1 expression in HGC-27cells; (B) Effects of IFN-γ induction on PD-L1 expression in MKN74 cells; (C, D) The impact of CDK5 knockdown on the expression of PD-L1, c-MYC, IRF-1, p-STAT1, and STAT1 in (C) HCG-27 and (D) MKN74 cells.

In our study, we observed that CDK1, CDK2, CDK4, CDK5, CDK6, CDK7, CDK11, CDK12, CDK16, and CDK19 were expressed at lower levels in gastric cancer tissues with peritoneal metastasis than in those without. Based on the expression profile of the aforementioned genes, we developed a CDK Signature (CDKS) model for the prognosis of gastric cancer metastasis. Survival analysis revealed that patients in the CDKS-high group had better disease-free survival (DFS) and overall survival (OS) outcomes than those in the CDKS-low group.

Research has highlighted a critical link between gene mutations and tumor metastasis [33, 34]. The significant role of TMB in enhancing cancer immunotherapy response is also emphasized by the elevated expression of tumor neoantigens [35]. Our results show that high CDKS subtypes had increased somatic mutation rates and TMBs, while low CDKS subtypes showed the reverse. Numerous studies consistently report that EBV-positive gastric cancer patients respond better to PD1 inhibitors like pembrolizumab [36]. Our research found that the EBV-positive subtype had a notably higher CDKS than the EBV-negative subtype, further supporting CDKS as a reliable classification tool for gastric cancer patients.

Subsequently, our study delved into the potential of CDKS as a prognostic factor in gastric cancer management. Immune checkpoint inhibitors are currently a primary focus in immunotherapy research. Prior studies have shown that using checkpoint inhibitors targeting PD1, PD-L1, and CTLA4 results in better overall survival than traditional chemoradiotherapy [37]. Our data suggests that patients with a CDKS-high subtype respond more positively to immunotherapy, indicating the potential of CDKS as a predictor for effective immunotherapy in gastric cancer.

Our relevance analysis indicates that CDK5 expression was most related to PD-L1. Additionally, CDK5 knockdown in gastric cancer cells significantly suppressed PD-L1 expression. Our earlier study revealed that CDK5 can inhibit gastric cancer cell metastasis [32]. Similarly, Cao et al. reported a correlation between reduced CDK5 expression and gastric cancer severity based on tumor and lymph node metastasis and a 5-year mortality rate. Overexpression of CDK5 also inhibited the proliferation and xenograft implantation of gastric cancer cells [21]. Thus, besides CDK5's previously reported ability to inhibit gastric cancer cell growth and metastasis, CDK5 might also influence gastric cancer progression by modulating PD-L1 expression.

However, it is important to recognize several limitations of this study. Firstly, there is a need for further research to explore the molecular mechanism through which CDK5 regulates the expression of PD-L1. Secondly, although external validation cohorts were utilized, more independent validation studies are required to confirm the prognostic value and clinical utility of the CDKS model. Despite these limitations, our study provides valuable insights into potential targets for diagnosing, prognosing, and treating gastric cancer.

Conclusion

This study analyzed the expression of CDK gene family members in gastric cancer peritoneal metastasis and established a CDK Signature model for predicting the prognosis of patients with gastric cancer. Our findings suggest that CDKS has the potential to stratify patients, enabling identification of those more likely to benefit from immunotherapy. Furthermore, our findings suggest that knockdown CDK5 is capable of suppressing PD-L1 expression, pointing to a new avenue for further exploration into immunotherapeutic research.

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We obtained written consent from all participants involved in the study.

Disclosure of conflict of interest

None.

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