Am J Transl Res 2024;16(2):432-445
www.ajtr.org /ISSN:1943-8141/AJTR0153814

Original Article

CDCA8, a mitosis-related gene, as a prospective pan-cancer biomarker: implications for survival prognosis and oncogenic immunology

Hanjie Hu[1], Muhammad Umair[2], Sikandar Ali Khan[3], Aliya Irshad Sani[4], Sahar Iqbal[5], Fatima Khalid[6], Rizwana Sultan[7], Mostafa A Abdel-Maksoud[8], Ayman Mubarak[8], Turki M Dawoud[8], Abdul Malik[9], Ibrahim A Saleh[10], Abdul Aziz Al Amri[11], Norah Khaled Algarzae[12], Ahmad S Kodous[13,14], Yasir Hameed[15]

[1]Department of Thoracic Surgery, National Cancer Center/National Clinical Research Center for Cancer/ Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China; [2]Department of Physiology, Gomal Medical College, MTI, Dera Ismail Khan, Pakistan; [3]Department of Biochemistry Khyber Girls Medical College, Peshawar, Pakistan; [4]Department of Biochemistry, Ziauddin Medical College, Karachi 74700, Pakistan; [5]Department of Pathology, Azra Naheed Medical College, Lahore 54000, Pakistan; [6]Department of Pathology, Al Aleem Medical College, Lahore, Pakistan; [7]Department of Pathology, Faculty of Veterinary and Animal Sciences, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan; [8]Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia; [9]Department of Pharmaceutics, College of Pharmacy, King Saud University, Saudi Arabia; [10]Faculty of Science, Zarqa University, Zarqa 13110, Jordan; [11]Biochemistry Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia; [12]Department of Physiology, College of Medicine, King Saud University, Riyadh 11149, Saudi Arabia; [13]Radiation Biology Department, National Center for Radiation Research & Technology (NCRRT), Egyptian Atomic-Energy Authority (EAEA), Egypt; [14]Department of Molecular Oncology, Cancer Institute (WIA), 38, Sardar Patel Road, Chennai, P.O. Box 600036, Tamilnadu, India; [15]Department of Biotechnology, Institute of Biochemistry Biotechnology and Bioinformatics, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan

Received October 7, 2023; Accepted January 24, 2024; Epub February 15, 2024; Published February 28, 2024

Abstract: Background: Human cell division cycle-associated protein 8 (CDCA8), a critical regulator of mitosis, has been identified as a prospective prognostic biomarker in several cancer types, including breast, colon, and lung cancers. This study analyzed the diagnostic/prognostic potential and clinical implications of CDCA8 across diverse cancers. Methods: Bioinformatics and molecular experiments. Results: Analyzing TCGA data via TIMER2 and GEPIA2 databases revealed significant up-regulation of CDCA8 in 23 cancer types compared to normal tissues. Prognostically, elevated CDCA8 expression correlated with poorer overall survival in KIRC, LUAD, and SKCM, emphasizing its potential as a prognostic marker. UALCAN analysis demonstrated CDCA8 up-regulation based on clinical variables, such as cancer stage, race, and gender, in these cancers. Epigenetic exploration indicated reduced CDCA8 promoter methylation levels in Kidney Renal Clear Cell Carcinoma (KIRC), Lung Adenocarcinoma (LUAD), and Skin Cutaneous Melanoma (SKCM) tissues compared to normal controls. Promoter methylation and mutational analyses showcased a hypomethylation and low mutation rate for CDCA8 in these cancers. Correlation analysis revealed positive associations between CDCA8 expression and infiltrating immune cells, particularly CD8+ and CD4+ T cells. Protein-protein interaction (PPI) network analysis unveiled key interacting proteins, while gene enrichment analysis highlighted their involvement in crucial cellular processes and pathways. Additionally, exploration of CDCA8-associated drugs through DrugBank presented potential therapeutic options for KIRC, LUAD, and SKCM. In vitro validation using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) confirmed elevated CDCA8 expression in LUAD cell lines (A549 and H1299) compared to control cell lines (Beas-2B and NL-20). Conclusion: This study provides concise insights into CDCA8's multifaceted role in KIRC, LUAD, and SKCM, covering expression patterns, diagnostic and prognostic relevance, epigenetic regulation, mutational landscape, immune infiltration, and therapeutic implications.

Keywords: CDCA8, pan-cancer, biomarker, prognosis, treatment

https://doi.org/10.62347/WSEF7878

Introduction

Cancer, a complex group of diseases characterized by uncontrolled cell growth, poses a significant global health challenge [1-3]. It can affect any part of the body, often leading to the formation of tumors [4, 5]. Understanding its molecular basis, early detection, and targeted treatments are crucial in the ongoing battle against this multifaceted and often devastating condition [6].

The process of tumorigenesis is intricately complex, with genetic alterations playing a pivotal role [7]. In recent years, the elucidation of tumor molecular mechanisms has been significantly advanced through whole-genome sequencing analysis, thereby fostering genome-driven oncology care [8, 9]. Common mechanisms underlying the onset and progression of diverse tumors may exist, making it valuable to conduct a pan-cancer expression analysis of various genes and explore their correlation with clinical characteristics and potential molecular mechanisms. The Cancer Genome Atlas (TCGA) database, housing comprehensive DNA, RNA, protein, and epigenetic datasets, facilitates extensive pan-cancer analyses [10].

The Human cell division cycle associated 8 (CDCA8) gene is responsible for producing the Borealin/Dasra B protein, which is a crucial element of the chromosome passenger complex (CPC) [11]. This complex, composed of INCENP, Survivin, Aurora B, and Borealin/Dasra B, is a dynamic structure essential during cell division [12]. CDCA8 plays a vital role in guiding the CPC to the centromere, rectifying errors in kinetochore binding, and stabilizing bipolar spindles [13, 14]. Earlier research has indicated that the elevated expression of CDCA8 is linked to increased growth of tumor cells, including in colorectal and lung cancers [15, 16]. Furthermore, heightened CDCA8 levels have been associated with an unfavorable prognosis in gastric cancer [17]. Despite these findings, there remains a gap in our understanding of the precise roles CDCA8 plays in the development of other tumors. Therefore, conducting a pan-cancer analysis of CDCA8 holds practical significance, as it allows for the exploration of its potential mechanisms in tumor development and assesses the feasibility of considering it as a novel therapeutic target.

In this investigation, we performed a pan-cancer analysis of CDCA8 utilizing data from the TCGA database along with molecular experiments. The analysis incorporated gene expression, survival status, promoter methylation analysis, mutational analysis, gene enrichment analysis, immune infiltration, and other factors to delve into the potential molecular mechanisms of CDCA8 across various types of cancers.

Methodology

Gene expression analysis

In the current study, we utilized the TIMER2 website (http://timer.cistrome.org/) [18] to examine the expression differences of CDCA8 in various tumors and their corresponding adjacent normal tissues from the TCGA database. For certain tumors lacking normal adjacent tissues in TIMER2, such as adrenocortical carcinoma (ACC), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), and testicular germ cell tumors (TGCT), we employed the GEPIA2 website (http://gepia2.cancer-pku.cn/#analysis) [19]. This allowed us to compare the expression of CDCA8 in tumor tissues versus adjacent normal tissues and generate box plots.

Survival prognosis analysis

GEPIA2, a powerful online tool (http://gepia2.cancer-pku.cn/) [19], facilitates survival analysis in cancer research. Leveraging data from TCGA and GTEx databases, GEPIA2 allows users to assess the impact of specific genes on patient survival across diverse cancer types. In the present study, we used GEPIA2 for the survival prognosis analysis of CDCA8 across patients with different cancers.

CDCA8 expression landscape across different clinical variables

UALCAN (http://ualcan.path.uab.edu/) [20] is an invaluable resource for expression analysis across various clinical variables. Drawing from TCGA data, it enables researchers to explore gene expression patterns in relation to patient demographics, tumor stage, and other clinical parameters. In our study, we used UALCAN resource for the expression analysis of CDCA8

across different clinical variables of the specified cancer types.

Promoter methylation analysis of CDCA8

OncoDB (http://oncodb.org/) [21] is a valuable tool for promoter methylation analysis in cancer research. It provides interactive visualizations, allowing users to explore DNA methylation patterns in specific genes across diverse cancer types. In this investigation, we utilized OncoDB platform to analyze the promoter methylation level of CDCA8 across specified cancer types.

cBioportal database

cBioPortal (https://www.cbioportal.org/) [22] is an essential platform for mutational analysis in cancer. Leveraging large-scale genomics data, it enables researchers to explore and interpret genomic alterations, including mutations, copy number variations, and mRNA expression changes. The user-friendly interface facilitates in-depth investigation of these alterations across various cancer types, contributing to a better understanding of the molecular landscape and potential therapeutic targets. In the current research, we used cBioPortal for mutational analysis of the CDCA8 gene across specified cancers.

Immune cell infiltration analysis

TIMER2 (http://timer.cistrome.org/) [18] is a crucial tool for immune cell infiltration analysis in cancer research. Utilizing TCGA data, it allows researchers to explore the abundance of immune cell subtypes within tumor tissues. In this investigation, we used TIMER2 platform to explore the correlation between CDCA8 and CD+ T immune cell infiltration across specified cancers.

Protein-protein interaction and pathway analysis of CDCA8

STRING (https://string-db.org/) stands as a crucial asset for researchers investigating molecular interactions [23]. It aggregates and predicts protein-protein interactions, facilitating a comprehensive grasp of intricate biological processes and networks. In this study, we employed the STRING database to build the protein-protein interaction (PPI) network for the genes enriched with CDCA8.

DAVID (https://david.ncifcrf.gov/) is a fundamental tool for gene enrichment analysis. Utilizing functional annotation, it identifies biological themes within gene lists, shedding light on their roles and interactions [24]. DAVID's versatility allows researchers to uncover significant biological pathways, aiding in the interpretation of large-scale genomic data and enhancing our understanding of gene functions and their implications in various biological processes. In the current study, we used DAVID to explore CDCA8-associated pathways.

Drug prediction analysis

DrugBank, a comprehensive pharmaceutical database (https://go.drugbank.com/), serves as an essential resource for researchers and clinicians [25]. It provides a wealth of information on drug compounds, including their chemical structures, mechanisms of action, pharmacokinetics, and therapeutic indications. With a vast collection of data on both approved and investigational drugs, DrugBank supports drug discovery, development, and clinical decision-making, fostering advancements in healthcare. In this study, we used DrugBank database for the drug prediction analysis of CDCA8 gene.

In vitro validation of CDCA8 expression

The normal control cell lines derived from human bronchial epithelial cells, such as Beas-2B and NL-20, were cultured in high-glucose DMEM medium (cat. no. 23-10-013-CV; Corning, Inc.). The LUAD cell line A549 was cultured in high-glucose F12K medium (cat. no. 21127022; Thermo Fisher Scientific, Inc.), while the LUAD cell line H1299 was cultured in high-glucose RPMI-1640 medium (cat. no. 10-040-CV; Corning, Inc.). All media included 10% FBS (cat. no. 10091148; Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were routinely incubated in a cell incubator with 5% CO_2 at 37°C.

The cell lines underwent total RNA extraction using isopycnic centrifugation, following a previously described method [22]. Subsequently, the extracted RNA underwent a DNA digestion step through incubation with RNase-free DNase I (Roche, Germany) at 37°C for 15 minutes. The quality of the obtained RNA was assessed using a 2100 Bioanalyzer (Agilent Technologies, Germany).

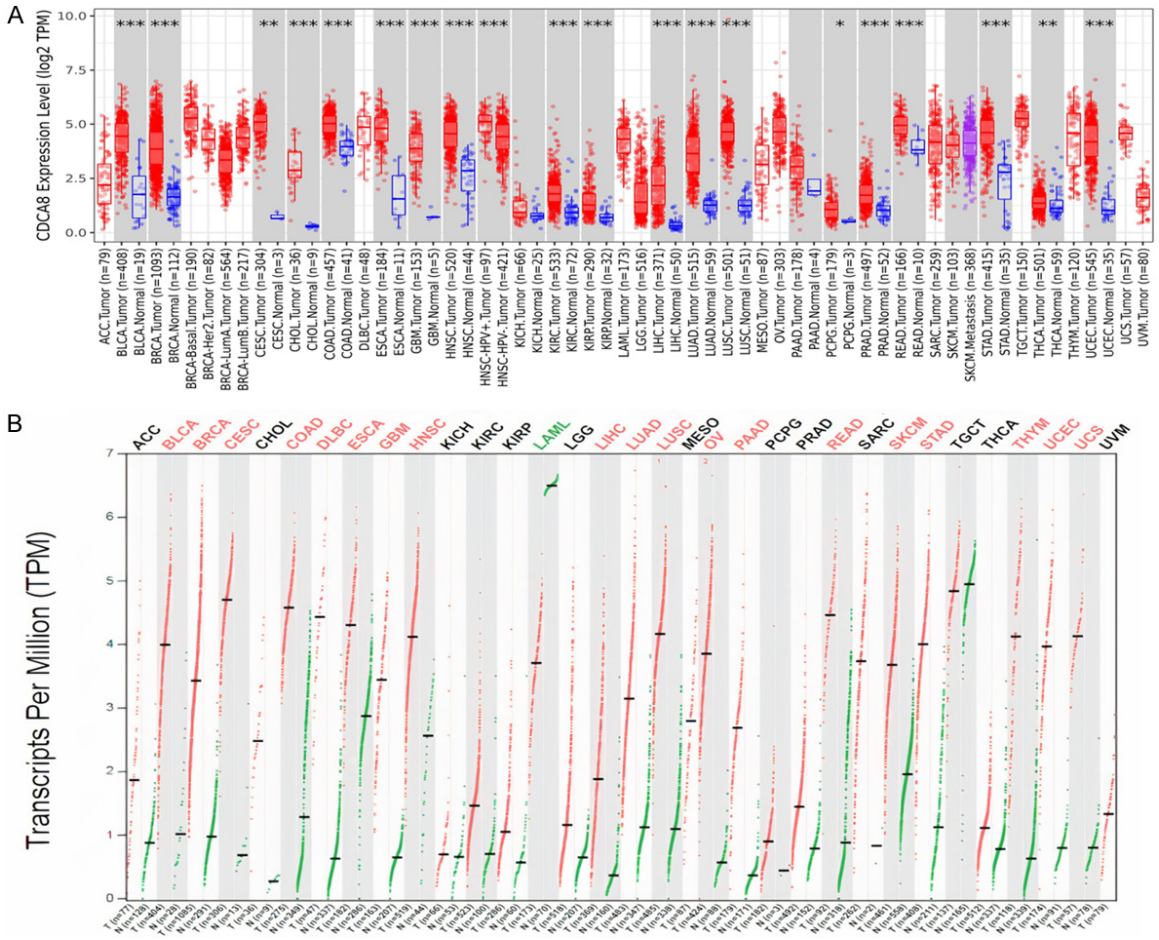

Figure 1. This figure displays a comprehensive pan-cancer expression analysis of CDCA8 across 33 tumor types, utilizing the TIMER2 and GEPIA2 databases. (A) Illustrates the pan-cancer expression analysis through the TIMER2 database, while (B) presents the analysis via the GEPIA database. Significance was determined at a *p*-value < 0.05. CDCA8 = Human cell division cycle-associated protein 8.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis

The specific procedures were as follows: Initially, the PrimeScript™ RT reagent kit (Takara, Japan) facilitated the reverse transcription of extracted RNA from cell lines into complementary DNA (cDNA). Subsequently, RT-qPCR was conducted on an ABI ViiA 7 Real Time PCR System (Thermo Fisher, USA) using SuperReal SYBR Green Premix Plus (Tiangen Biotech, China) as a fluorescent dye. GAPDH served as the internal reference in this study. All experiments were independently triplicated. The primer sequences for CDCA8 and GAPDH are provided below. The 2-ΔΔCt method was employed to assess the relative expression of each hub gene [26].

GAPDH-F 5'-ACCCACTCCTCCACCTTTGAC-3', GAPDH-R 5'-CTGTTGCTGTAGCCAAATTCG-3'; CDCA8-F 5'-CAGTGACTTGCAGAGGCACAGT-3', CDCA8-R 5'-CTCATTTGTGGGTCCGTATGCTG-3'.

Statistical analysis

All other analyses were performed with the use of R software (version 3.6.3). A *p*-value below 0.05 was considered statistical significance. Correlation analysis between two variables was conducted using the Spearman test.

Results

CDCA8 was overexpressed in various cancers

Figure 1 illustrates the expression disparities of CDCA8 between tumor and adjacent normal tissues across various cancers in the TCGA database. According to findings from the TIMER2 and GEPIA2 databases, the CDCA8 gene exhibited significant up-regulation (*p*-value < 0.05) in 23 cancer types compared to

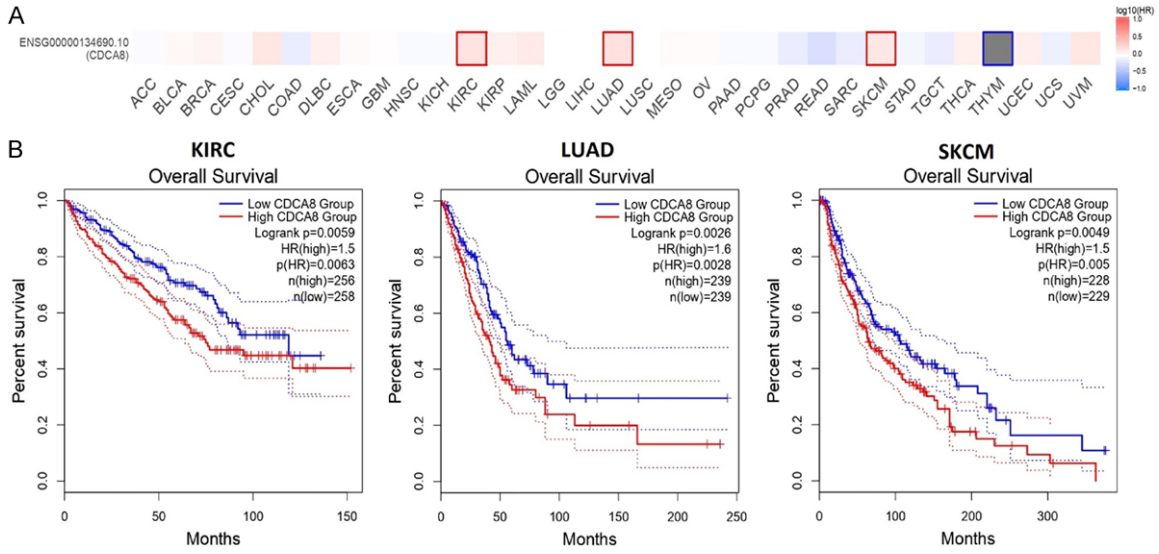

Figure 2. This figure depicts a comprehensive pan-cancer survival analysis of CDCA8 across 33 tumor types, conducted on the GEPIA2 platform. (A) Showcases the survival map of CDCA8, while (B) presents Kaplan-Meier curves specifically for KIRC, LUAD, and SKCM patients. Significance was established at a *p*-value < 0.05. CDCA8 = Human cell division cycle-associated protein 8, KIRC = Kidney Renal Clear Cell Carcinoma, LUAD = Lung Adenocarcinoma, SKCM = Skin Cutaneous Melanoma.

their respective normal tissues. These cancers included adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma (CESC), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), ovarian serous cystadenocarcinoma (OV), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD), thymoma (THYM), uterine corpus endometrial carcinoma (UCEC), and uterine carcinosarcoma (UCS).

The prognostic significance of CDCA8

To assess CDCA8's prognostic significance across various cancers, Kaplan-Meier (KM) curves were generated to analyze the correlation between CDCA8 expression and overall survival (OS) in cancer patients. Results showed that in 3 tumor types; CDCA8 expression was associated with poorer Overall survival (OS), including KIRC, LUAD, and SKCM (**Figure 2**). While in other types of 20 tumors, including ACC, BLCA, BRCA, CESC, CHOL, COAD, DLBC, ESCA, GBM, HNSC, KIRP, LIHC, LUSC, OV, PRAD, READ, STAD, THYM, UCEC, and UCS, overexpressed CDCA8 was not found to be associated with poor OS (**Figure 2**). Overall, these results suggest that elevated expression of CDCA8 may be associated with poorer clinical prognosis in three tumor types.

Association of CDCA8 expression with different clinical variables in patients with KIRC, LUAD, and SKCM

Utilizing UALCAN, we examined the expressions of CDCA8 in cohorts of patients with KIRC, LUAD, and SKCM based on various clinical variables. CDCA8 expression was significantly (*p*-value < 0.05) up-regulated in KIRC, LUAD, and SKCM patients with cancer stage 1-4 as compared to the levels in the normal group (**Figure 3**). Moreover, we found the significant (*p*-value < 0.05) overexpression of CDCA8 in KIRC, LUAD, and SKCM patients of Caucasian, African-American, and Asian populations as compared to the levels in the corresponding normal groups (**Figure 3**). Based on the gender, CDCA8 was significantly (*p*-value < 0.05) overexpressed in both men and women with KIRC, LUAD, and SKCM as compared to the levels in

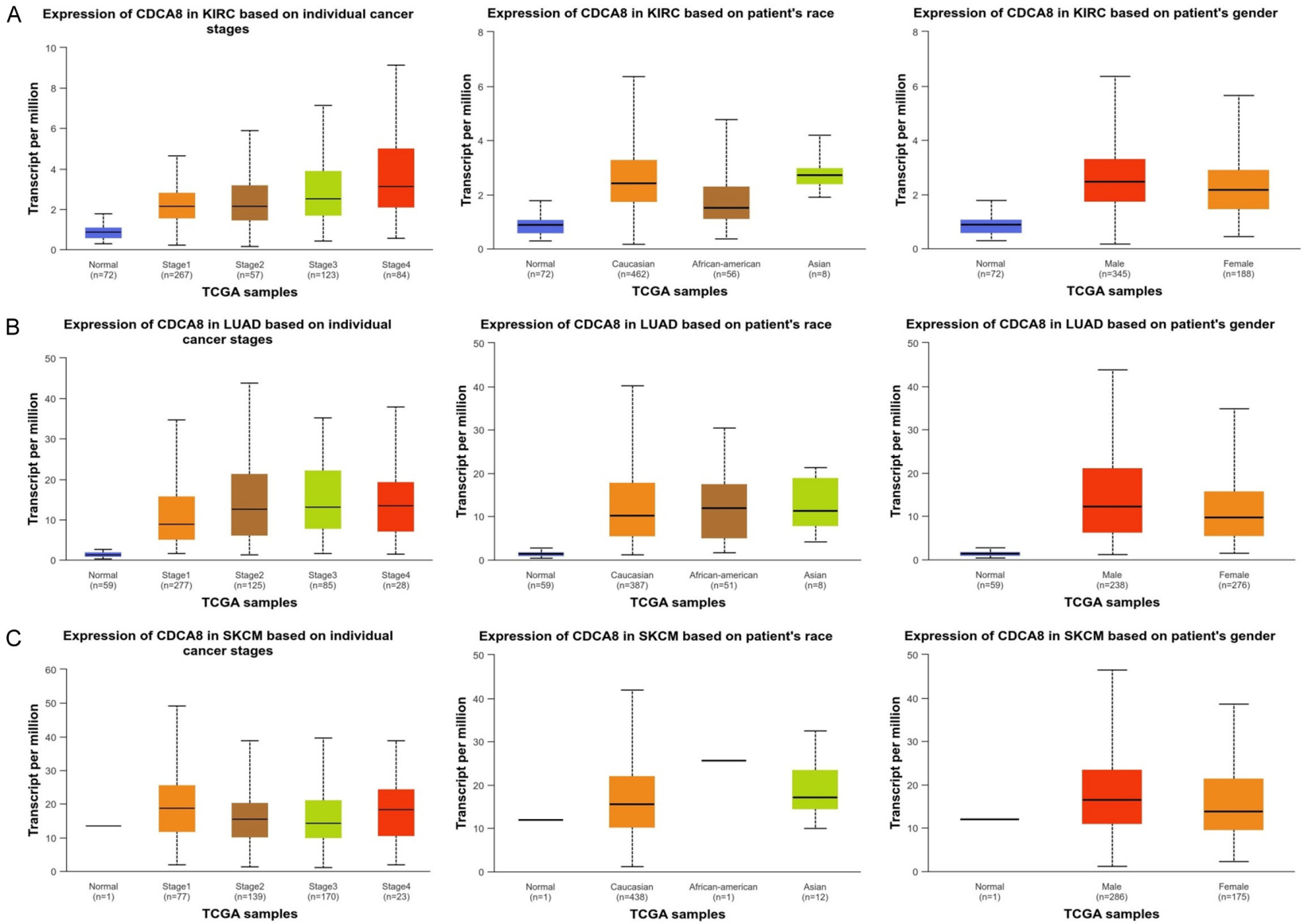

Figure 3. This figure illustrates the expression analysis of CDCA8 among KIRC, LUAD, and SKCM patients, categorized by various clinical variables through UALCAN. A. Displays CDCA8 expression across KIRC patients, considering different clinical variables such as cancer stage, race, and gender. B. Demonstrates CDCA8 expression among LUAD patients based on clinical variables such as cancer stage, race, and gender. C. Depicts CDCA8 expression in SKCM patients across different clinical variables, including cancer stage, race, and gender. Significance was established at a *p*-value < 0.05. CDCA8 = Human cell division cycle-associated protein 8, KIRC = Kidney Renal Clear Cell Carcinoma, LUAD = Lung Adenocarcinoma, SKCM = Skin Cutaneous Melanoma.

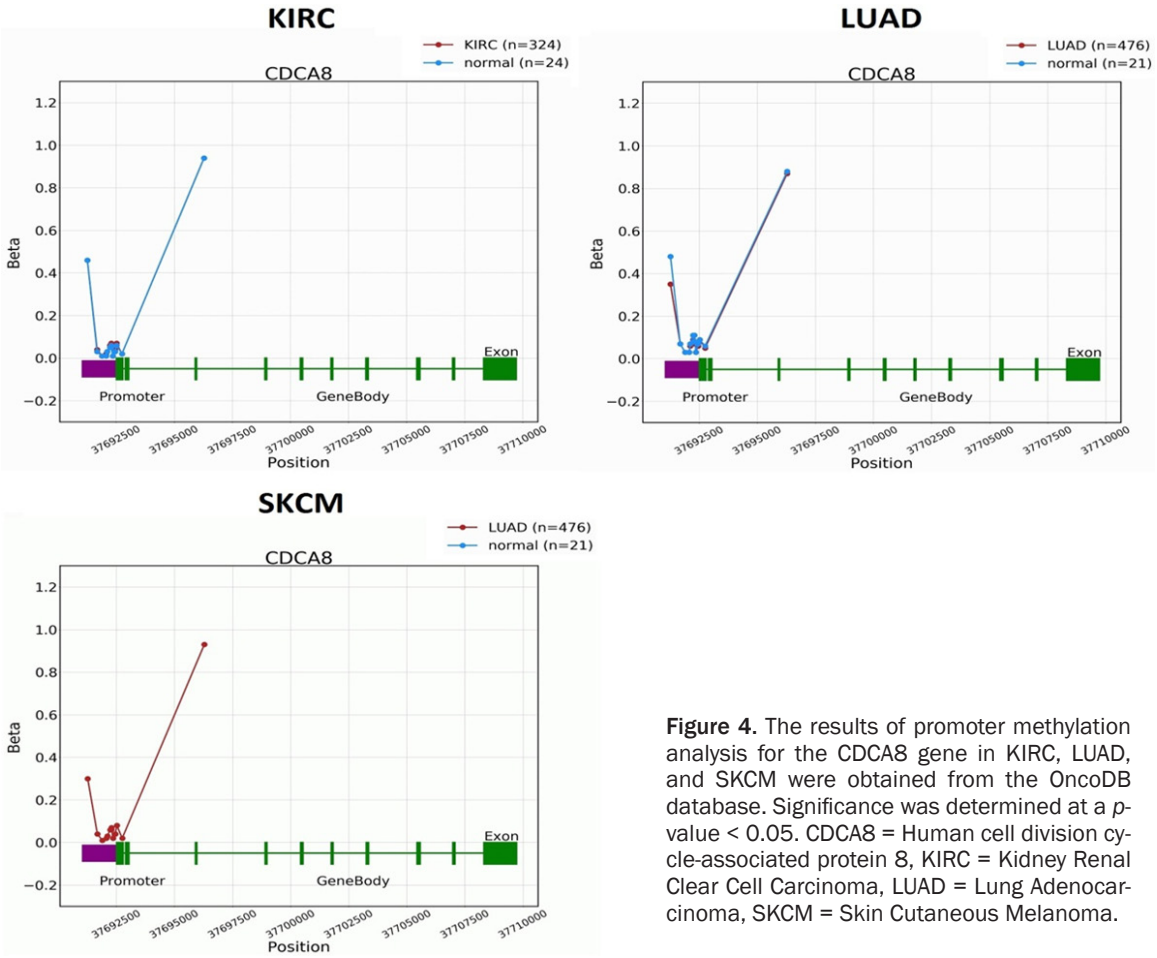

Figure 4. The results of promoter methylation analysis for the CDCA8 gene in KIRC, LUAD, and SKCM were obtained from the OncoDB database. Significance was determined at a *p*-value < 0.05. CDCA8 = Human cell division cycle-associated protein 8, KIRC = Kidney Renal Clear Cell Carcinoma, LUAD = Lung Adenocarcinoma, SKCM = Skin Cutaneous Melanoma.

the corresponding normal groups (**Figure 3**). Above all, these findings demonstrate a strong association between CCDC6 expression and clinical variables in KIRC, LUAD, and SKCM.

Promoter methylation analysis of CDCA8

The widely recognized impact of gene promoter methylation on gene expression, combined with the observed elevated expression of CDCA8 in KIRC, LUAD, and SKCM compared to normal controls, prompted us to investigate into whether the promoter methylation levels of CDCA8 were lower in tissue samples of KIRC, LUAD, and SKCM than in their corresponding adjacent control tissues. Utilizing TCGA data through the OncoDB database, we assessed the CDCA8 promoter methylation levels in KIRC, LUAD, and SKCM tissues and their adjacent controls. As depicted in **Figure 4**, the CDCA8 promoter methylation levels in KIRC, LUAD, and SKCM tissues were significantly (*p*-value < 0.05) reduced compared to normal controls.

Mutational analysis of CDCA8

We assessed the mutational status of CDCA8 in the TCGA cohorts for KIRC, LUAD, and SKCM. **Figure 5** illustrates that CDCA8 exhibited the highest mutation rate (1.28%) among patients with SKCM. Conversely, in patients with KIRC and LUAD, the mutation rates were 0% and 0.53%, respectively (**Figure 5**). In summary, these results indicate that CDCA8 does not undergo frequent mutations in patients with KIRC, LUAD, and SKCM.

Correlation analysis between CCDC6 expression and infiltrating immune cells

Tumor infiltrating CD8+ T cells affect the patients' survival in various tumors. In this study, we explored the associations between CDCA8 expression and two key types of infiltrating immune cells: CD8+ T cells and CD4+ T cells. Notably, both CD8+ T cells and CD4+ T cells exhibited a significant positive correlation

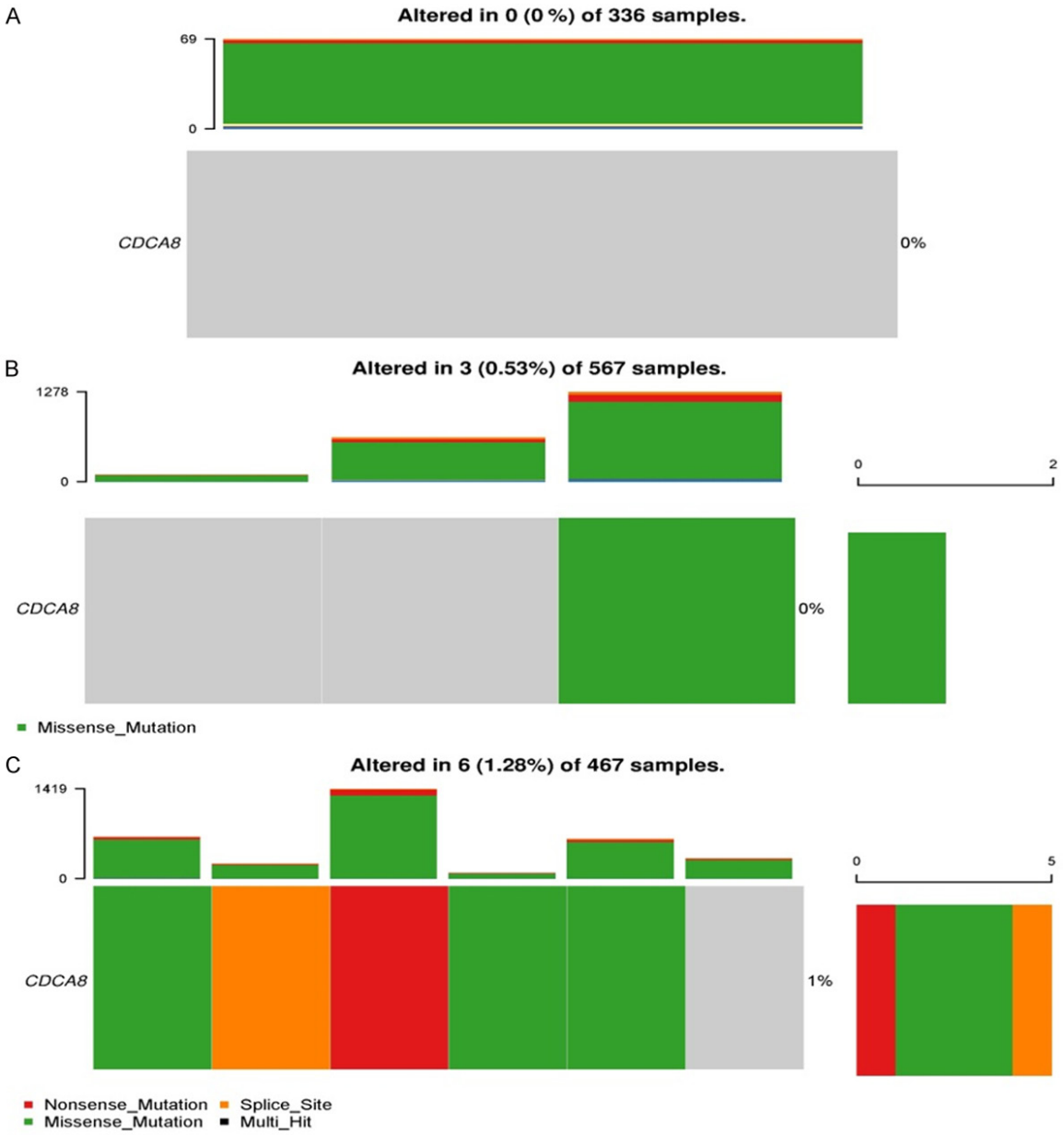

Figure 5. The results of CDCA8 mutational analysis in KIRC, LUAD, and SKCM using the cBioPortal database. A. Mutation analysis results of the CDCA8 in KIRC. B. Mutation analysis results of the CDCA8 in LUAD. C. Mutation analysis results of the CDCA8 in SKCM. Different colored bars show different kind of mutations. CDCA8 = Human cell division cycle-associated protein 8, KIRC = Kidney Renal Clear Cell Carcinoma, LUAD = Lung Adenocarcinoma, SKCM = Skin Cutaneous Melanoma.

with CDCA8 expression levels in patients with KIRC, LUAD, and SKCM (**Figure 6**).

Identification of CDCA8-interacting proteins and gene enrichment analysis

We used STRING database to assess PPI for CDCA8 and other proteins. The main 10 proteins interacting with CDCA8 are shown in **Figure 7A** and include BUB1, CDK1, INCENP, CCNB1, AURKB, BIRC5, CDC20, SGO1, ATP51A, and KIF20A. The outcomes of our gene enrichment analysis suggest that these genes are associated with "Mitotic checkpoint complex, chromosome passenger complex, and outer kinetochore" etc. cellular components (CC) terms (**Figure 7B**), "Histone kinase activity (H3-S28 specific, ubiquitin ligase ac-

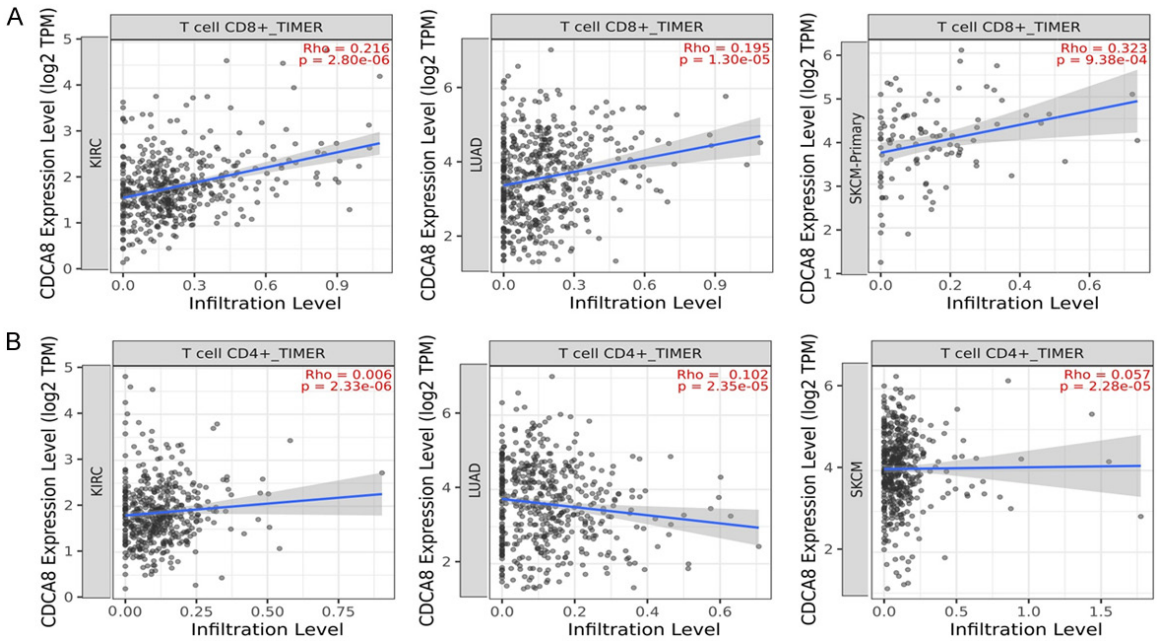

Figure 6. This figure presents a correlation analysis of CDCA8 with immune cell infiltration using the TIMER2 database. A. Illustrates the correlation analysis of CDCA8 with CD8+ T immune cells across KIRC, LUAD, and SKCM. B. Depicts the correlation analysis of CDCA8 with CD4+ T immune cells in the same cancer types. Significance was determined at a *p*-value < 0.05. CDCA8 = Human cell division cycle-associated protein 8, KIRC = Kidney Renal Clear Cell Carcinoma, LUAD = Lung Adenocarcinoma, SKCM = Skin Cutaneous Melanoma.

tivator, activity, and patched binding)" etc. molecular function (MF) terms (**Figure 7C**), "Mitotic spindle assembly checkpoint signaling, spindle checkpoint singling, and negative reg. of mitotic metaphase/anaphase transition" etc. biological processes (BP) terms (**Figure 7D**), and "Cell cycle, oocyte meiosis, progesterone-mediate oocyte maturation, and p53 signaling pathway" etc. Kyoto Encyclopedia of Genes and Genomes (KEGG) terms (**Figure 7E**).

CDCA8-associated drugs

Utilizing the DrugBank database, we investigated six drugs associated with CDCA8, namely Cyclosporine, Cytarabine, Dasatinib, Calcitriol, Metamfetamine, and Palbociclib. These drugs exhibit the potential to decrease CDCA8 expression during the treatment of patients with KIRC, LUAD, and SKCM.

In vitro validation of CDCA8 expression

In this segment of our investigation, we confirmed the expression of the CDCA8 gene in LUAD and control cell lines using the RT-qPCR technique. Initially, two control cell lines (Beas-2B and NL-20) and two LUAD cell lines (A549 and H1299) were procured and cultured in suitable media. Subsequently, RNA extraction and cDNA synthesis were conducted. The RT-qPCR analysis of CDCA8 genes using the synthesized cDNA revealed elevated CDCA8 expression in LUAD cell lines (A549 and H1299) compared to the control cell lines (Beas-2B and NL-20) (**Figure 8**).

Discussion

CDCA8 stands as a pivotal regulatory gene in mitosis [27], exerting a crucial influence in various cancers by promoting cell proliferation and invasion, potentially functioning as an oncogene [28, 29]. Earlier research has documented heightened transcriptional activity of CDCA8 in embryos, embryonic stem cells, and cancer cells [30, 31]. However, a thorough pan-cancer investigation into the status of CDCA8 has not been undertaken until now. Via pan-cancer analysis, our study revealed a significant upregulation of CDCA8 in 23 types of matched tumor samples. Furthermore, heightened CDCA8 expression was associated with poorer overall survival (OS) in patients with KIRC, LUAD, and SKCM, indicating that CDCA8 could

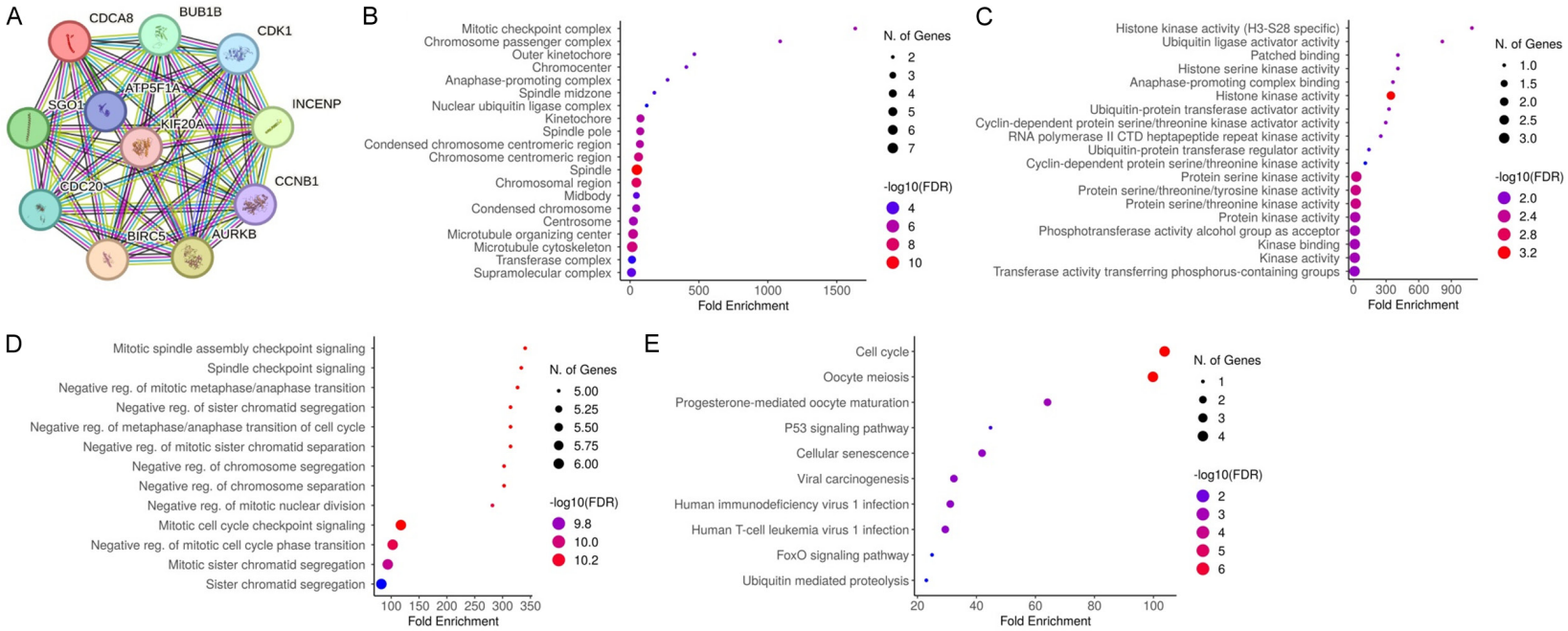

Figure 7. This figure showcases the protein-protein interaction network and gene enrichment analysis of CDCA8. (A) Presents the protein-protein interaction network involving CDCA8 and its binding partners. (B-D) Detail the cellular component (CC), molecular function (MF), and biological process (BP) terms, respectively, of CDCA8 and its binding partners. Additionally, (E) outlines the Kyoto Encyclopedia of Genes and Genomes (KEGG) terms associated with CDCA8 and its interacting proteins. Significance was determined at a *p*-value < 0.05. CDCA8 = Human cell division cycle-associated protein 8.

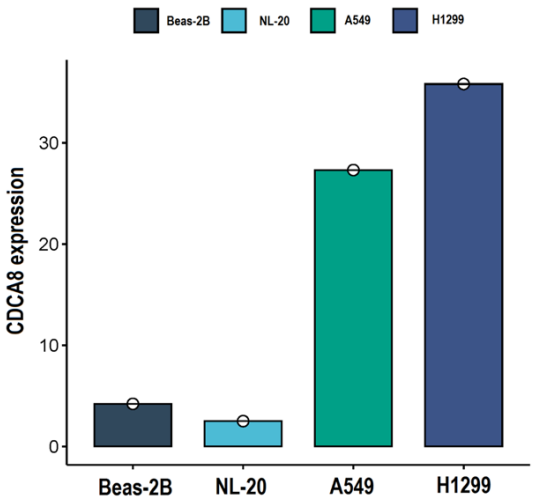

Figure 8. CDCA8 expression analysis across LUAD (A549 and H1299) and normal control cell lines (Beas-2B and NL-20) using RT-qPCR technique. CDCA8 = Human cell division cycle-associated protein 8, LUAD = Lung Adenocarcinoma, RT-qPCR = Reverse transcription-quantitative polymerase chain reaction.

potentially function as a viable prognostic factor in these three specific malignancies (KIRC, LUAD, and SKCM).

Epigenetic alterations play a crucial role in the carcinogenesis, with DNA methylation representing a significant facet of epigenetics [32, 33]. Often characterized as a 'silencing' epigenetic mark, DNA methylation contributes to reinforcing the repression of transcription [34-36]. Imbalances in enzyme activity are implicated in alterations to promoter methylation and histone acetylation levels, ultimately contributing to the progression of tumors [37]. In our study, we observed the hypomethylation of CDCA8 gene across the KIRC, LUAD, and SKCM patients. The reduction in CDCA8 promoter methylation levels resonates with the regulatory role of methylation in CDCA8 expression reported in the literature [38, 39]. This convergence emphasizes the importance of epigenetic regulation (promoter methylation level) in CDCA8 dynamics among KIRC, LUAD, and SKCM patients.

The widely acknowledged understanding is that cancers are initiated by genetic mutations, which confer biological advantages to cancer cells over adjacent normal cells [40, 41]. Presently, advancements in high-throughput technologies and systems biology approaches have generated vast datasets illustrating the mutation heterogeneity within cancer cells [42-44]. In this investigation, we employed the cBioPortal tool to assess the mutational profile of CDCA8 in KIRC, LUAD, and SKCM. Our analysis affirms the observation that CDCA8 does not undergo frequent mutations in these specific cancers.

The tumor microenvironment (TME) of KIRC, LUAD, and SKCM is a complex and spatially structured mixture of hepatic non-parenchymal, tumor, and immune cells [45]. Exploiting the immune cell infiltration within the tumor, immune checkpoint blockade aims to revitalize an effective antitumoral immune response [46]. Guided by this principle, several immunotherapy drugs, such as nivolumab and pembrolizumab, have been employed in treating KIRC, LUAD, and SKCM. The composition of the TME significantly influences the response to immune checkpoint blockade. In our findings, we observe a positive correlation between high CDCA8 expression in KIRC, LUAD, and SKCM and increased infiltration of CD8+ T cells and CD4+ T cells. Best to our knowledge, we are the first to report such kind of correlation between CDCA8 and CD8+ T cells and CD4+ T cells across KIRC, LUAD, and SKCM.

In the present study, it was noted that, along with various binding partners, the CDCA8 gene participates in diverse pathways in KIRC, LUAD, and SKCM, including "Cell cycle, oocyte meiosis, progesterone-mediate oocyte maturation, and p53 signaling pathway etc.". The involvement of these pathways in cancer development is already well studied in previous research [47, 48].

Conclusion

In summary, our study indicates a substantial up-regulation of CDCA8 in KIRC, LUAD, and SKCM, proposing its potential as a significant diagnostic and prognostic biomarker for these malignancies. Additionally, we identify potential dysregulated mechanisms of CDCA8 in these cancers, including a hypomethylated promoter. Nonetheless, experimental validation of these findings is warranted in future research.

Acknowledgements

The authors extend their appreciation to the Researchers Supporting Project number (RSP2024R376), King Saud University, Riyadh, Saud Arabia.

Disclosure of conflict of interest

None.

Address correspondence to: Yasir Hameed, Department of Biotechnology, Institute of Biochemistry Biotechnology and Bioinformatics, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan. E-mail: Yasirhameed2011@gmail.com; Mostafa A Abdel-Maksoud, Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia. E-mail: Mabdmaksoud@ksu.edu.sa

References

[1] Umapathy VR, Natarajan PM and Swamikannu B. Review of the role of nanotechnology in overcoming the challenges faced in oral cancer diagnosis and treatment. Molecules 2023; 28: 5395.

[2] Hameed Y, Usman M, Liang S and Ejaz S. Novel diagnostic and prognostic biomarkers of colorectal cancer: capable to overcome the heterogeneity-specific barrier and valid for global applications. PLoS One 2021; 16: e0256020.

[3] Xu W, Li H, Hameed Y, Abdel-Maksoud MA, Almutairi SM, Mubarak A, Aufy M, Alturaiki W, Alshalani AJ, Mahmoud AM and Li C. Elucidating the clinical and immunological value of m6A regulator-mediated methylation modification patterns in adrenocortical carcinoma. Oncol Res 2023; 31: 819-831.

[4] Eid RA, Alaa Edeen M, Shedid EM, Kamal ASS, Warda MM, Mamdouh F, Khedr SA, Soltan MA, Jeon HW, Zaki MSA and Kim B. Targeting cancer stem cells as the key driver of carcinogenesis and therapeutic resistance. Int J Mol Sci 2023; 24: 1786.

[5] Hameed Y, Ahmad M, Ejaz S and Liang S. Identification of key biomarkers for the future applications in diagnostics and targeted therapy of colorectal cancer. Curr Mol Med 2022; 2022: 207.

[6] Sufyan M, Shokat Z and Ashfaq UA. Artificial intelligence in cancer diagnosis and therapy: current status and future perspective. Comput Biol Med 2023; 165: 107356.

[7] Zhang FL and Li DQ. Targeting chromatin-remodeling factors in cancer cells: promising molecules in cancer therapy. Int J Mol Sci 2022; 23: 12815.

[8] Nakagawa H and Fujita M. Whole genome sequencing analysis for cancer genomics and precision medicine. Cancer Sci 2018; 109: 513-522.

[9] Priestley P, Baber J, Lolkema MP, Steeghs N, de Bruijn E, Shale C, Duyvesteyn K, Haidari S, van Hoeck A, Onstenk W, Roepman P, Voda M, Bloemendal HJ, Tjan-Heijnen VCG, van Herpen CML, Labots M, Witteveen PO, Smit EF, Sleijfer S, Voest EE and Cuppen E. Pan-cancer whole-genome analyses of metastatic solid tumours. Nature 2019; 575: 210-216.

[10] Cancer Genome Atlas Research Network; Weinstein JN, Collisson EA, Mills GB, Shaw KR, Ozenberger BA, Ellrott K, Shmulevich I, Sander C and Stuart JM. The cancer genome atlas pan-cancer analysis project. Nat Genet 2013; 45: 1113-1120.

[11] Zhang C, Zhao L, Leng L, Zhou Q, Zhang S, Gong F, Xie P and Lin G. CDCA8 regulates meiotic spindle assembly and chromosome segregation during human oocyte meiosis. Gene 2020; 741: 144495.

[12] Wang Z, Fan M, Candas D, Zhang TQ, Qin L, Eldridge A, Wachsmann-Hogiu S, Ahmed KM, Chromy BA, Nantajit D, Duru N, He F, Chen M, Finkel T, Weinstein LS and Li JJ. Cyclin B1/Cdk1 coordinates mitochondrial respiration for cell-cycle G2/M progression. Dev Cell 2014; 29: 217-232.

[13] Egloff AM, Vella LA and Finn OJ. Cyclin B1 and other cyclins as tumor antigens in immunosurveillance and immunotherapy of cancer. Cancer Res 2006; 66: 6-9.

[14] Jackman M, Marcozzi C, Barbiero M, Pardo M, Yu L, Tyson AL, Choudhary JS and Pines J. Cyclin B1-Cdk1 facilitates MAD1 release from the nuclear pore to ensure a robust spindle checkpoint. J Cell Biol 2020; 219: e201907082.

[15] Lin S, Luo S, Zhong L, Lai S, Zeng D, Rao X, Huang P and Weng X. Cost-effectiveness of atezolizumab plus chemotherapy for advanced non-small-cell lung cancer. Int J Clin Pharm 2020; 42: 1175-1183.

[16] Chen E, He Y, Jiang J, Yi J, Zou Z, Song Q, Ren Q, Lin Z, Lu Y, Liu J and Zhang J. CDCA8 induced by NF-YA promotes hepatocellular carcinoma progression by regulating the MEK/ERK pathway. Exp Hematol Oncol 2023; 12: 9.

[17] Kanehisa M, Sato Y, Kawashima M, Furumichi M and Tanabe M. KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res 2016; 44: D457-D462.

[18] Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, Li B and Liu XS. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res 2020; 48: W509-W514.

[19] Tang Z, Kang B, Li C, Chen T and Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res 2019; 47: W556-W560.

[20] Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I,

Chakravarthi B and Varambally S. UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia 2017; 19: 649-658.
[21] Tang G, Cho M and Wang X. OncoDB: an interactive online database for analysis of gene expression and viral infection in cancer. Nucleic Acids Res 2022; 50: D1334-D1339.
[22] Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C and Schultz N. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012; 2: 401-404.
[23] Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P, Jensen LJ and Mering Cv. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res 2019; 47: D607-D613.
[24] Sherman BT, Hao M, Qiu J, Jiao X, Baseler MW, Lane HC, Imamichi T and Chang W. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Res 2022; 50: W216-W221.
[25] Liu CJ, Hu FF, Xia MX, Han L, Zhang Q and Guo AY. GSCALite: a web server for gene set cancer analysis. Bioinformatics 2018; 34: 3771-3772.
[26] Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25: 402-408.
[27] Zhao Z, Mu H, Feng S, Liu Y, Zou J and Zhu Y. Identification of biomarkers associated with hepatocellular carcinoma stem cell characteristics based on co-expression network analysis of transcriptome data and stemness index. Crit Rev Eukaryot Gene Expr 2022; 32: 47-60.
[28] Kruppa J and Jung K. Automated multigroup outlier identification in molecular high-throughput data using bagplots and gemplots. BMC Bioinformatics 2017; 18: 232.
[29] Ci C, Tang B, Lyu D, Liu W, Qiang D, Ji X, Qiu X, Chen L and Ding W. Overexpression of CDCA8 promotes the malignant progression of cutaneous melanoma and leads to poor prognosis. Int J Mol Med 2019; 43: 404-412.
[30] Xiang C, Sun WH, Ke Y, Yu X and Wang Y. CDCA8 contributes to the development and progression of thyroid cancer through regulating CDK1. J Cancer 2022; 13: 2322-2335.
[31] Liu Z, Zhou L, Li D, Lu H, Liu L, Mao W, Yu X, Fan Y, Huang Q, Wang F and Wan Y. N6-methyladenosine methyltransferase METTL3 modulates the cell cycle of granulosa cells via CCND1 and AURKB in Haimen goats. FASEB J 2023; 37: e23273.
[32] Zhang L, Sahar AM, Li C, Chaudhary A, Yousaf I, Saeedah MA, Mubarak A, Haris M, Nawaz M, Reem MA, Ramadan FA, Mostafa AAM, Feng W and Hameed Y. A detailed multi-omics analysis of GNB2 gene in human cancers. Braz J Biol 2022; 84: e260169.
[33] Khalil T, Okla MK, Al-Qahtani WH, Ali F, Zahra M, Shakeela Q, Ahmed S, Akhtar N, AbdElgawad H, Asif R, Hameed Y, Adetunji CO, Farid A and Ghazanfar S. Tracing probiotic producing bacterial species from gut of buffalo (Bubalus bubalis), South-East-Asia. Braz J Biol 2022; 84: e259094.
[34] Khan M and Hameed Y. Discovery of novel six genes-based cervical cancer-associated biomarkers that are capable to break the heterogeneity barrier and applicable at the global level. J Cancer Res Ther 2023; 2023: 23.
[35] Usman M, Hameed Y and Ahmad M. Does human papillomavirus cause human colorectal cancer? Applying Bradford Hill criteria postulates. Ecancermedicalscience 2020; 14: 1107.
[36] Ahmad M, Khan M, Asif R, Sial N, Abid U, Shamim T, Hameed Z, Iqbal MJ, Sarfraz U and Saeed H. Expression characteristics and significant diagnostic and prognostic values of ANLN in human cancers. Int J Gen Med 2022; 1957-1972.
[37] Xu X, Peng Q, Jiang X, Tan S, Yang Y, Yang W, Han Y, Chen Y, Oyang L, Lin J, Xia L, Peng M, Wu N, Tang Y, Li J, Liao Q and Zhou Y. Metabolic reprogramming and epigenetic modifications in cancer: from the impacts and mechanisms to the treatment potential. Exp Mol Med 2023; 55: 1357-1370.
[38] Yi Y, Li Y, Li C, Wu L, Zhao D, Li F, Fazli L, Wang R, Wang L, Dong X, Zhao W, Chen K and Cao Q. Methylation-dependent and -independent roles of EZH2 synergize in CDCA8 activation in prostate cancer. Oncogene 2022; 41: 1610-1621.
[39] Wan S, He Y, Zhang B, Yang Z, Du FM, Zhang CP, Fu YQ and Mi J. Overexpression of CDCA8 predicts poor prognosis and promotes tumor cell growth in prostate cancer. Front Oncol 2022; 12: 784183.
[40] Ullah L, Hameed Y, Ejaz S, Raashid A, Iqbal J, Ullah I and Ejaz SA. Detection of novel infiltrating ductal carcinoma-associated BReast CAncer gene 2 mutations which alter the deoxyribonucleic acid-binding ability of BReast CAncer gene 2 protein. J Cancer Res Ther 2020; 16: 1402-1407.
[41] Zhu X, Tang L, Mao J, Hameed Y, Zhang J, Li N, Wu D, Huang Y and Li C. Decoding the mechanism behind the pathogenesis of the focal segmental glomerulosclerosis. Comput Math Methods Med 2022; 2022: 1941038.

[42] Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA Jr, Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE and Kinzler KW. An integrated genomic analysis of human glioblastoma multiforme. Science 2008; 321: 1807-1812.

[43] Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, Mandelker D, Leary RJ, Ptak J, Silliman N, Szabo S, Buckhaults P, Farrell C, Meeh P, Markowitz SD, Willis J, Dawson D, Willson JK, Gazdar AF, Hartigan J, Wu L, Liu C, Parmigiani G, Park BH, Bachman KE, Papadopoulos N, Vogelstein B, Kinzler KW and Velculescu VE. The consensus coding sequences of human breast and colorectal cancers. Science 2006; 314: 268-274.

[44] Leary RJ, Lin JC, Cummins J, Boca S, Wood LD, Parsons DW, Jones S, Sjöblom T, Park BH, Parsons R, Willis J, Dawson D, Willson JK, Nikolskaya T, Nikolsky Y, Kopelovich L, Papadopoulos N, Pennacchio LA, Wang TL, Markowitz SD, Parmigiani G, Kinzler KW, Vogelstein B and Velculescu VE. Integrated analysis of homozygous deletions, focal amplifications, and sequence alterations in breast and colorectal cancers. Proc Natl Acad Sci U S A 2008; 105: 16224-16229.

[45] Sangro B, Sarobe P, Hervás-Stubbs S and Melero I. Advances in immunotherapy for hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 2021; 18: 525-543.

[46] Petitprez F, Meylan M, de Reyniès A, Sautès-Fridman C and Fridman WH. The tumor microenvironment in the response to immune checkpoint blockade therapies. Front Immunol 2020; 11: 784.

[47] Gellersen B, Fernandes M and Brosens JJ. Non-genomic progesterone actions in female reproduction. Hum Reprod Update 2009; 15: 119-138.

[48] Aquila S and De Amicis F. Steroid receptors and their ligands: effects on male gamete functions. Exp Cell Res 2014; 328: 303-313.