Increased Expression of CDCA2 in Glioma Predicts Poor Prognosis as Identified by Population-based Analysis

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Abstract

Background: The cell division cycle-associated protein 2 (CDCA2) was found to be a cell cycle-related protein. CDCA2 was previously reported to be associated with proliferation and migration in multiple cancer. We evaluated the role of CDCA2 in glioma using publicly available data from the Chinese Glioma Genome Atlas (CGGA) and The Cancer Genome Atlas (TCGA).

Methods: The relationships between clinical characteristics and CDCA2 expression were analyzed using the Wilcoxon test or Kruskal-Wallis test. Clinicopathologic characteristics associated with overall survival (OS) were analyzed using Cox regression analysis and Kaplan-Meier method. Gene Set Enrichment Analysis (GSEA) was performed to sort the signaling pathway associated with the expression of CDCA2. Pearson correlation test was used to analyse the expression correlation between CDCA2 and well-known cell cycle-related genes.

Results: Glioma with high CDCA2 expression was more prone to be associated with advanced malignancy clinical pathologic characteristics and worse prognosis than that with low CDCA2 expression in TCGA and CGGA dataset ($P < 0.001$). The multiple analysis revealed that CDCA2 was independently associated with OS (HR:1.396; [CI]:1.236 – 1.577, $P < 0.001$). GSEA showed that cell cycle checkpoint, cell cycle G1/S phase transition, DNA damage checkpoint and regulation of cell cycle arrest were enriched in CDCA2 high expression phenotype. Pearson correlation test revealed that CDCA2 was co-expression with well-known key cell cycle-related genes (CCNA2, CCNB1, CCNB2, CCNE1, CCNE2, CDK1, CDK2, CDK4, CDK6).

Conclusion: High CDCA2 expression may be a potential prognosis molecular marker of poor survival in glioma. Cell cycle regulation pathway may be the key pathway regulated by CDCA2 in glioma.

Background

Glioma is the most common lethal primary intracranial neoplasm[1, 2]. Despite the aggressive surgery with adjuvant radiotherapy and chemotherapy, the median survival time of patients suffering glioma is no more than 15 months[3–5]. Patients with glioma may differ greatly in molecular characteristic, clinical prognosis and treatment response. Traditional and targeted therapies are far from satisfaction[6, 7]. Therefore, revealing the tumor-related molecular marker based on the pathogenesis and development of glioma has become the focus of current research.

Cell division cycle-associated 2 (CDCA2) is a cell cycle-related nuclear protein, which can bind to protein phosphatase 1 γ (PP1 γ) and regulate DNA damage response in cell cycle[8, 9]. The expression of CDCA2 was also found to be correlated with several well-known cell cycle-related genes, such as CDC2, CDC7, CDC23, cyclin, MCAK, MKI67a[10]. Recent reports indicated that CDCA2 was up-regulated in colorectal cancer[11], esophageal squamous cell carcinoma[12], breast cancer[13] and might act as a prognostic factor. Previous literature reported that the expression of CDCA2 mRNA was higher in neuroblastoma than types of glioma[14]. However, the correlation between CDCA2 expression and the prognosis of glioma has not been study.

In the present study, we analyzed the expression level of CDCA2 to investigate its prognostic value in glioma. Furthermore, GSEA was performed to gain insight into the biological pathway in glioma pathogenesis related to CDCA2 regulatory network.

We demonstrated that high expression of CDCA2 might be an independent prognostic indicator associated with poor survival in glioma. GSEA showed that cell cycle regulation pathways were associated with CDCA2 high expression phenotype. CDCA2 expression was also positively correlated with the expressions of well-known key cell cycle regulation genes.

Methods

Source population

The gene expression data was downloaded from TCGA official website for low grade glioma (LGG) and glioblastoma multiforme (GBM) project and corresponding clinical information was downloaded from UCSC Xena (<https://xenabrowser.net/>). In CGGA database, the gene expression data and corresponding clinical information were downloaded from official website (<http://www.cgga.org.cn/>). The gene expression levels displayed in CGGA and TCGA database had been standardized. Patients with unknown information of survival and essential clinical characteristics were excluded in this study. The clinical information and gene

expression profiles of patients with glioma in the CGGA dataset (n = 748) include age, gender, histology, the WHO grade, radiotherapy, chemotherapy, isocitrate dehydrogenase (IDH) mutation status, chromosome 1p/19q co-deletion status, recurrent status and vital status; in the TCGA dataset (n = 513), clinical information includes age, gender, histology, the WHO grade, radiotherapy, recurrent status and vital status, as shown in Table 1.

Table 1
Glioma patients characteristics in CGGA and TCGA

Clinical characteristics	CGGA		TCGA	
	Total(748)	%	Total(513)	%
Age(Median)	43		46	
Younger	397	53.1	262	51.1
Old	351	46.9	251	48.9
Gender				
Male	442	59.1	297	57.9
Female	306	40.9	216	42.1
Histology				
LGG	458	61.2	366	71.3
GBM	290	38.8	147	28.7
WHO grade				
II	218	29.1	192	37.4
III	240	32.1	174	33.9
IV	290	38.8	147	28.7
Radiotherapy				
Yes	625	83.6	337	65.7
No	123	16.4	176	34.3
Chemotherapy				
Yes	520	69.5	/	
No	228	30.5	/	
IDH mutation status				
Mutate type	409	54.7	/	
Wild type	339	45.3	/	
1p/19q co-deletion status				
Co-deletion	155	20.7	/	
Non-co-deletion	593	79.3	/	
PRS_type				
Primary	501	67.0	492	95.9
Recurrent	222	29.7	21	4.1
Secondary recurrent	25	3.3	/	
Vital status				
Alive	272	36.4	360	70.2
Dead	476	63.6	153	29.8

Statistical analysis

The Wilcoxon test or Kruskal-Wallis test was used to analyze the relationship between clinical characteristics and CDCA2 gene expression. Kaplan-Meier survival curve was generated to estimate survival distribution and log-rank test was used to assess statistical significance between CDCA2 low expression and high expression. Univariate and further multivariate COX regression analysis were conducted to assess the prognostic value of CDCA2 gene expression in patients with glioma. The hazard ratio (HR) and 95% Confidence Interval (95% CI) were calculated. Correlation between CDCA2 gene expression and well-known cell cycle-related genes expression was analyzed by Person's correlation test. Receiver operating characteristic (ROC) curve and area under the curve (AUC) were constructed to evaluate the prediction accuracy of CDCA2 expression for 1-year, 3-year and 5-year survival. The goal of GSEA is to utilize a predefined group of genes (mainly by previous biological knowledge, published information about biochemical pathways or co-expression in previous experiments) for ranking the genes in accordance with the extent of differential expression within the 2 types of samples, then verifying that the predefined group of genes tend to occur toward the bottom or top in the sorting table[15]. To explore the differences in pathways as well as biological functions in the low- and high-expression sets of such prognostic CDCA2 gene, GSEA was used to explore potential pathway and GO analysis within the Molecular Signatures Database (MSigDB) and c5 (GO gene sets). The nominal p-value and normalized enrichment score (NES) were used to sort the pathway enriched in each phenotype. All statistical analyses were conducted using R statistical software (version 3.6.2). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics and clinical pathologic features in TCGA and CGGA dataset

As shown in Table 1, 748 cases were downloaded from CGGA dataset and 513 cases were downloaded from TCGA dataset with clinical and gene expression data in December 2019. The median age at diagnosis was 43 years old in CGGA dataset and 46 years old in TCGA dataset. In CGGA study cohort, 61.2% of tumors were LGG, 38.8% were GBM. WHO II grade was found in 218 (29.1%), WHO III grade in 240 (32.1%) and WHO IV grade in 291 (38.8%). The IDH mutation status included 409 mutate type status (54.7%) and 339 wild type status (45.3%). The 1p/19q co-deletion status included 155 co-deletion status (20.7%) and 593 non-deletion status (79.3%). The recurrent status included primary, recurrent and secondary recurrent status. Most cases (67.0%, $n = 501$) were primary type, 29.7% ($n = 222$) were recurrent type and 3.3% ($n = 25$) were secondary recurrent type. In vital status, 272 cases (36.4%) were alive and 476 cases (63.6%) were dead. In TCGA study cohort, 71.3% of tumors were LGG, 28.7% were GBM. WHO II grade was found in 192 (37.4%), WHO III grade in 174 (33.9%) and WHO IV grade in 147 (28.7%). Most cases (95.9%, $n = 492$) were primary type, 4.1% ($n = 21$) were recurrent type. In vital status, 360 cases (70.2%) were alive and 153 cases (29.8%) were dead.

Association with CDCA2 expression and clinicopathologic variables in TCGA and CGGA dataset

A total of 748 cases with CDCA2 expression data and clinicopathologic information were analyzed from CGGA dataset. As shown in Fig. 1 (A-G), increased expression of CDCA2 significantly correlated with the old age, recurrent status, chemotherapy, WHO IV grade, IDH wild type, 1p/19q non-co-deletion status and GBM histology type (all p -value < 0.05). The high expression level of CDCA2 was correlated with old age compared with younger age (Fig. 1A). CDCA2 expression level was higher in recurrent status (Fig. 1B). CDCA2 high expression level was positively correlated with glioma grade (Fig. 1D and G). The expression levels of CDCA2 were higher in the IDH wild-type and 1p/19q non-co-deletion status compared with the corresponding group (Fig. 1E and F). In TCGA dataset, as shown in Fig. 2 (A-D), increased expression of CDCA2 significantly correlated with the old age, WHO grade, GBM histology type and recurrent status (all p -value < 0.05). These results suggested that glioma with high CDCA2 expression was more prone to associated with advanced clinical pathologic characteristics than low CDCA2 expression.

CDCA2 is a predictive marker in patients with glioma in CGGA dataset

In order to further identify the prognostic value of CDCA2 expression in patients with glioma, the Cox proportional hazard model was used in CGGA dataset. The univariate analysis demonstrated that CDCA2 expression, age, histology, recurrent status, WHO grade,

chemotherapy, IDH mutant type and 1p/19q co-deletion status affected the overall survival of patients ($P < 0.001$; Fig. 3A). Subsequently, multivariate Cox proportional hazards analysis of the aforementioned significant factors was conducted. The expression of CDCA2 was demonstrated to be an independent prognostic factor in the survival time of patients with glioma ($P < 0.001$; Fig. 3B). Kaplan-Meier survival analysis was conducted to evaluate the relationship between the CDCA2 expression and the prognostic of patients with glioma. The patients with relatively high CDCA2 expression showed a significantly shorter survival time than those with low CDCA2 expression ($P < 0.001$; Fig. 3C). The area under the receiver operating characteristic (ROC) for CDCA2 expression as a predictor of 1-year survival, 3-year survival and 5-year survival in CGGA database was 0.793, 0.796 and 0.709, respectively ($P < 0.001$; Fig. 3D).

CDCA2 involved in cell cycle regulation signaling pathway

To identify signaling pathway associated with CDCA2 expression that are differentially activated in patients with glioma, we conducted GSEA analysis between low and high CDCA2 expression data sets. GSEA reveals significant differences (false discovery rate (FDR) < 0.05 , NOM p-val < 0.05) in enrichment of MSigDB Collection (C5.all.v.7.1symbols and h.all.v.7.1symbols). We selected the significant enriched signaling pathways based on their NES (Fig. 4 and Table 2). The Fig. 4 showed that CDCA2 high expression phenotype was associated with cell cycle checkpoint, cell cycle G1/S phase transition, DNA damage checkpoint and regulation of cell cycle arrest in GSEA analysis. Furthermore, we performed Pearson's correlation analysis to investigate association between CDCA2 expression and well-known key cell cycle-related genes in the dataset. The results showed that CCNA2, CCNB1, CCNB2, CCNE1, CCNE2, CDK1, CDK2, CDK4, CDK6, which were important cell cycle regulation gene, were positively correlated with CDCA2 expression, all p-value < 0.001 (Fig. 5A-I).

Table 2
Gene set enriched in phenotype high

MSigDB collection	Gene set name	NES	NOM p-val	FDR q-val
C5.all.v.7.1symbols.gmt	GO_CELL_CYCLE_CHECKPOINT	2.026	0	0.0166
h.all.v.7.1symbols.gmt	GO_CELL_CYCLE_G1_S_PHASE_TRANSITION	2.028	0	0.0173
	GO_G1_DNA_DAMAGE_CHECKPOINT	2.022	0	0.0166
	GO_POSITIVE_REGULATION_OF_CELL_CYCLE_ARREST	2.034	0	0.0161
	GO_POSITIVE_REGULATION_OF_CELL_CYCLE_PROCESS	2.060	0	0.0173
	GO_REGULATION_OF_CELL_CYCLE_ARREST	2.021	0	0.0160
	GO_REGULATION_OF_CELL_CYCLE_G1_S_PHASE_TRANSITION	2.019	0	0.0154
	GO_SIGNAL_TRANSDUCTION_INVOLVED_IN_CELL_CYCLE_CHECKPOINT	2.050	0	0.0179
NES: normalized enrichment score; NOM: nominal; FDR: false discovery rate				

Discussion

Glioma is the most common and lethal type of brain tumor, which accounts for 46% of intracranial tumors[16]. With the development of sequencing technology, molecular factors for prognosis of glioma have been well studied, such as IDH mutations, 1p/19q co-deletion, ATRX mutation, TERT promoter mutation and PTEN loss[17–20]. However, traditional therapeutic approaches, including surgery, radiotherapy, chemotherapy and targeted therapy do not achieve satisfactory results. Genome differences in patients with glioma make different prognosis of patients. It is necessary for us to explore more prognostic markers to further understand the mechanism of glioma and predict prognosis of patients with glioma. The researches of cancer development revealed that CDCA2 was related to the occurrence and development in multiple cancers[11, 12, 21, 22]. To our knowledge, the expression of CDCA2 and its potential prognostic impact on glioma has not been explored. The potential role of CDCA2 in glioma is the focus in our present study.

In the present study, bioinformatic analysis using high throughput RNA-sequencing data from TCGA and CGGA revealed that an increased expression of CDCA2 in glioma was associated with advanced clinical pathologic characteristics (old age, recurrent status, high grade and GBM sub-type), shorter survival time and poor prognosis. Further, multivariate Cox proportional hazards analysis showed that increased expression of CDCA2 was demonstrated to be an independent prognostic factor in the survival time of patients with glioma in CGGA dataset. To investigate the function of CDCA2 in glioma, we conducted GSEA using CGGA data. GSEA showed that cell regulation pathways were enriched in CDCA2 high expression phenotype. Pearson's correlation analysis also showed that increased expression of CDCA2 was positively correlated with well-known key cell regulation genes. This suggested that CDCA2 might serve as a potential marker of prognosis and therapeutic target in glioma.

Previous study reported that the expression of CDCA2 mRNA was higher in neuroblastoma than that of lower stage tumor[14]. CDCA2 was also over-expressed in other cancer types and associated with poor prognosis. Recent study demonstrated that CDCA2 promoted colorectal cancer cells proliferation by activating the AKT/CCND1 pathway in vitro and in vivo[11]. More studies were carried out to identify that CDCA could be an signature gene with prognostic value for luminal breast cancer, esophageal squamous cell carcinoma, bladder cancer, melanoma and Synovial sarcoma[12, 13, 21, 23]. In this work, we also demonstrated that strong expression of CDCA2 in glioma was associated with advanced clinical pathologic characteristics and higher expression of CDCA2 was an independent prognostic indicator associated with poor survival in glioma.

CDCA2 was first identified by Walker as a novel cell-cycle associated gene using microarray analyses of co-expression of the well-known cell-cycle genes[10]. Mulcahy and Lamond further revealed that CDCA2 binded to protein phosphatase 1 (PP1), responsible for the targeting of PP1 to chromatin in anaphase. CDCA2/PP1 complex was involved in cell cycle regulation and proliferation[24]. Vagnarelli demonstrated that the CDCA2/PP1 complex was critical component of the chromatin reorganization machinery responsible for chromosome de-condensation at the transition from mitosis to G1[25]. Moreover, the study showed that the complex modulated ATM activation, setting the threshold for checkpoint activation[9, 26, 27]. CDCA2 was released from the chromatin at DNA damage sites, which presumably facilitated DNA damage response (DDR) activation[8]. DDR was activated in pre-cancerous cells as a barrier to suppress cell proliferation, cancer progress and reduced in late-stage cancer cells. It was possible that strong expression of CDCA2 could result in desensitization of cells to DDR[28]. Depletion of CDCA2 re-activates the DDR and drive the cells into apoptotic pathway[29]. In our study, we observed that CDCA2 high expression phenotype was associated with cell cycle checkpoint, cell cycle G1/S phase transition, DNA damage checkpoint and regulation of cell cycle arrest in GSEA analysis. In OSCC cell line, depletion of CDCA2 caused a decrease in cell proliferation due to cell cycle arrest in G1 phase and down-regulation of CDK4, CDK6, Cyclin D1/E[22]. In our study, we also demonstrated that the expression of CDCA2 was positively correlated with expressions of well-known key cell cycle regulation genes (CCNA2, CCNB1, CCNB2, CCNE1, CCNE2, CDK1, CDK2, CDK4, CDK6). Cyclin family plays a pivotal part in cell cycle regulation and is involved in a range of biological processes[30, 31]. CDKs, a family of proteins that are involved in the regulation of the cell cycle, are frequently over-expressed or mutated in cancer, and CDK2/4/6 inhibitors (CDK2/4/6i) have been developed to be relatively safe and effective cancer therapeutics[32, 33]. From the work presented above, it appears that the CDCA2 may be a crucial functional factor in mitosis and a crucial hub for the regulation of chromatin organization and the maintenance of genome stability. Further work is needed to determine what is the molecular role of CDCA2 in glioma cell cycle regulation pathway and can it be a potential target therapy for glioma treatment? However, the prediction expression of protein using mRNA expression was far from perfect. The correlation between CDCA2 mRNA expression and CDCA2 protein expression could not be clearly identified because of the limitation in our study. Further study in patients with glioma is required.

In conclusion, high CDCA2 expression may be used as an independent prognostic molecular indicator of poor survival in patients with glioma. Moreover, the expression of CDCA2 was positively correlated with expressions of well-known key cell cycle regulation genes, and the cell cycle regulation pathway may be the key pathway regulated by CDCA2 in glioma. Further experimental validation required to prove the biological impact of CDCA2 in glioma.

Abbreviations

CDCA2:Cell division cycle-associated protein 2; CGGA:Chinese Glioma Genome Atlas; TCGA:The Cancer Genome Atlas; OS:Overall survival; CI:Confidence interval; LGG:Low grade glioma; GBM:Glioblastoma multiforme; GSEA:Gene set enrichment analysis; IDH:Isocitrate dehydrogenase; PP1 γ :Protein phosphatase 1 γ ; CDK1:Cyclin dependent kinase 1; CDK2:Cyclin dependent kinase 2;

CDK4:Cyclin dependent kinase 4; CDK6:Cyclin dependent kinase 6; CCNA2:Cyclin A2; CCNB1:Cyclin B1; CCNB2:Cyclin B2; CCNE1:Cyclin E1; CCNE2:Cyclin E2; ROC:Receiver operating characteristic

Declarations

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Authors' contributions

YH. L. designed the study. WW. D. analyzed data and wrote the paper. All authors read and approved the final version of the manuscript.

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Availability of data and materials

The datasets generated and/or analysed during the current study are available in the TCGA and CGGA repository.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Ostrom QT, Gittleman H, Liao P, Vecchione-Koval T, Wolinsky Y, Kruchko C, Barnholtz-Sloan JS: **CBTRUS Statistical Report: Primary brain and other central nervous system tumors diagnosed in the United States in 2010-2014.** *Neuro Oncol* 2017, **19**(suppl_5):v1-v88.
2. Lapointe S, Perry A, Butowski NA: **Primary brain tumours in adults.** *LANCET* 2018, **392**(10145):432-446.
3. Hombach-Klonisch S, Mehrpour M, Shojaei S, Harlos C, Pitz M, Hamai A, Siemianowicz K, Likus W, Wiechec E, Toyota BD *et al*: **Glioblastoma and chemoresistance to alkylating agents: Involvement of apoptosis, autophagy, and unfolded protein response.** *Pharmacol Ther* 2018, **184**:13-41.
4. Li XT, Tang W, Xie HJ, Liu S, Song XL, Xiao Y, Wang X, Cheng L, Chen GR: **The efficacy of RGD modified liposomes loaded with vinorelbine plus tetrandrine in treating resistant brain glioma.** *J LIPOSOME RES* 2019, **29**(1):21-34.
5. Wick W, Osswald M, Wick A, Winkler F: **Treatment of glioblastoma in adults.** *Ther Adv Neurol Disord* 2018, **11**:1277012020.
6. Kong Z, Yan C, Zhu R, Wang J, Wang Y, Wang Y, Wang R, Feng F, Ma W: **Imaging biomarkers guided anti-angiogenic therapy for malignant gliomas.** *Neuroimage Clin* 2018, **20**:51-60.

7. Auffinger B, Spencer D, Pytel P, Ahmed AU, Lesniak MS: **The role of glioma stem cells in chemotherapy resistance and glioblastoma multiforme recurrence.** *EXPERT REV NEUROTHER* 2015, **15**(7):741-752.
8. Vagnarelli P: **Repo-man at the intersection of chromatin remodelling, DNA repair, nuclear envelope organization, and cancer progression.** *ADV EXP MED BIOL* 2014, **773**:401-414.
9. Peng A, Lewellyn AL, Schiemann WP, Maller JL: **Repo-man controls a protein phosphatase 1-dependent threshold for DNA damage checkpoint activation.** *CURR BIOL* 2010, **20**(5):387-396.
10. Walker MG: **Drug target discovery by gene expression analysis: cell cycle genes.** *Curr Cancer Drug Targets* 2001, **1**(1):73-83.
11. Feng Y, Qian W, Zhang Y, Peng W, Li J, Gu Q, Ji D, Zhang Z, Wang Q, Zhang D *et al*: **CDCA2 promotes the proliferation of colorectal cancer cells by activating the AKT/CCND1 pathway in vitro and in vivo.** *BMC CANCER* 2019, **19**(1):576.
12. Shi X, Li Y, Sun Y, Zhao X, Sun X, Gong T, Liang Z, Ma Y, Zhang X: **Genome-wide analysis of lncRNAs, miRNAs, and mRNAs forming a prognostic scoring system in esophageal squamous cell carcinoma.** *PEERJ* 2020, **8**:e8368.
13. Xiao B, Chen L, Ke Y, Hang J, Cao L, Zhang R, Zhang W, Liao Y, Gao Y, Chen J *et al*: **Identification of methylation sites and signature genes with prognostic value for luminal breast cancer.** *BMC CANCER* 2018, **18**(1):405.
14. Krasnoselsky AL, Whiteford CC, Wei JS, Bilke S, Westermann F, Chen QR, Khan J: **Altered expression of cell cycle genes distinguishes aggressive neuroblastoma.** *ONCOGENE* 2005, **24**(9):1533-1541.
15. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES *et al*: **Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles.** *Proc Natl Acad Sci U S A* 2005, **102**(43):15545-15550.
16. Louis DN: **Molecular pathology of malignant gliomas.** *Annu Rev Pathol* 2006, **1**:97-117.
17. Ma J, Benitez JA, Li J, Miki S, Ponte DAC, Galatro T, Orellana L, Zanca C, Reed R, Boyer A *et al*: **Inhibition of Nuclear PTEN Tyrosine Phosphorylation Enhances Glioma Radiation Sensitivity through Attenuated DNA Repair.** *CANCER CELL* 2019, **36**(6):690-691.
18. Haase S, Garcia-Fabiani MB, Carney S, Altshuler D, Nunez FJ, Mendez FM, Nunez F, Lowenstein PR, Castro MG: **Mutant ATRX: uncovering a new therapeutic target for glioma.** *Expert Opin Ther Targets* 2018, **22**(7):599-613.
19. Philip B, Yu DX, Silvis MR, Shin CH, Robinson JP, Robinson GL, Welker AE, Angel SN, Tripp SR, Sonnen JA *et al*: **Mutant IDH1 Promotes Glioma Formation In Vivo.** *CELL REP* 2018, **23**(5):1553-1564.
20. Eckel-Passow JE, Lachance DH, Molinaro AM, Walsh KM, Decker PA, Sicotte H, Pekmezci M, Rice T, Kosel ML, Smirnov IV *et al*: **Glioma Groups Based on 1p/19q, IDH, and TERT Promoter Mutations in Tumors.** *N Engl J Med* 2015, **372**(26):2499-2508.
21. Sugawara S, Yamada Y, Arai T, Okato A, Idichi T, Kato M, Koshizuka K, Ichikawa T, Seki N: **Dual strands of the miR-223 duplex (miR-223-5p and miR-223-3p) inhibit cancer cell aggressiveness: targeted genes are involved in bladder cancer pathogenesis.** *J HUM GENET* 2018, **63**(5):657-668.
22. Uchida F, Uzawa K, Kasamatsu A, Takatori H, Sakamoto Y, Ogawara K, Shiiba M, Bukawa H, Tanzawa H: **Overexpression of CDCA2 in human squamous cell carcinoma: correlation with prevention of G1 phase arrest and apoptosis.** *PLOS ONE* 2013, **8**(2):e56381.
23. Lagarde P, Przybyl J, Brulard C, Perot G, Pierron G, Delattre O, Sciort R, Wozniak A, Schoffski P, Terrier P *et al*: **Chromosome instability accounts for reverse metastatic outcomes of pediatric and adult synovial sarcomas.** *J CLIN ONCOL* 2013, **31**(5):608-615.
24. Trinkle-Mulcahy L, Andersen J, Lam YW, Moorhead G, Mann M, Lamond AI: **Repo-Man recruits PP1 gamma to chromatin and is essential for cell viability.** *J CELL BIOL* 2006, **172**(5):679-692.
25. Vagnarelli P, Hudson DF, Ribeiro SA, Trinkle-Mulcahy L, Spence JM, Lai F, Farr CJ, Lamond AI, Earnshaw WC: **Condensin and Repo-Man-PP1 co-operate in the regulation of chromosome architecture during mitosis.** *NAT CELL BIOL* 2006, **8**(10):1133-1142.
26. Bakkenist CJ, Kastan MB: **DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation.** *NATURE* 2003, **421**(6922):499-506.
27. Brew CT, Aronchik I, Hsu JC, Sheen JH, Dickson RB, Bjeldanes LF, Firestone GL: **Indole-3-carbinol activates the ATM signaling pathway independent of DNA damage to stabilize p53 and induce G1 arrest of human mammary epithelial cells.** *INT J CANCER* 2006, **118**(4):857-868.

28. Halazonetis TD, Gorgoulis VG, Bartek J: **An oncogene-induced DNA damage model for cancer development.** *SCIENCE* 2008, **319**(5868):1352-1355.
29. Gutierrez-Martinez P, Hogdal L, Nagai M, Kruta M, Singh R, Sarosiek K, Nussenzweig A, Beerman I, Letai A, Rossi DJ: **Diminished apoptotic priming and ATM signalling confer a survival advantage onto aged haematopoietic stem cells in response to DNA damage.** *NAT CELL BIOL* 2018, **20**(4):413-421.
30. Hirayama M, Wei FY, Chujo T, Oki S, Yakita M, Kobayashi D, Araki N, Takahashi N, Yoshida R, Nakayama H *et al.* **FTO Demethylates Cyclin D1 mRNA and Controls Cell-Cycle Progression.** *CELL REP* 2020, **31**(1):107464.
31. Mu K, Li L, Yang Q, Yun H, Kharaziha P, Ye DW, Auer G, Lagercrantz SB, Zetterberg A: **A standardized method for quantifying proliferation by Ki-67 and cyclin A immunohistochemistry in breast cancer.** *ANN DIAGN PATHOL* 2015, **19**(4):243-248.
32. Vijayaraghavan S, Moulder S, Keyomarsi K, Layman RM: **Inhibiting CDK in Cancer Therapy: Current Evidence and Future Directions.** *TARGET ONCOL* 2018, **13**(1):21-38.
33. Tadesse S, Anshabo AT, Portman N, Lim E, Tilley W, Caldon CE, Wang S: **Targeting CDK2 in cancer: challenges and opportunities for therapy.** *DRUG DISCOV TODAY* 2020, **25**(2):406-413.

Figures

Figure 1

CDCA2 expression patterns in the CGGA dataset. (A) The expression level of CDCA2 was correlated with old age. (B) CDCA2 expression level was high in recurrent status. (C) CDCA2 expression level was higher in chemotherapy status compared with non-chemotherapy. (D) CDCA2 expression level was positively correlated with tumor grade. (E) CDCA2 expression level was higher in IDH wild-type than mutant-type. (F) CDCA2 expression level was higher in 1p/19q non-co-deletion status than 1p/19q co-deletion status. (G) CDCA2 expression was highest in GBM than other glioma subtypes. PRS_type, primary recurrent, secondary recurrent type; A, astrocytoma; AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma; GBM, glioblastoma multiforme; O, oligodendroglioma; rA, recurrent astrocytoma; rAA, recurrent anaplastic astrocytoma; rAO, recurrent anaplastic oligodendroglioma; rGBM, recurrent glioblastoma multiforme; rO, recurrent oligodendroglioma; sGBM, secondary glioblastoma multiforme.

Figure 2

CDCA2 expression patterns in the TCGA dataset. (A) The expression level of CDCA2 was correlated with old age. (B) CDCA2 expression level was positively correlated with tumor grade. (C) CDCA2 expression was higher in GBM than LGG. (D) CDCA2 expression level was higher in recurrent status than primary status. GBM, glioblastoma multiforme; LGG, low grade glioma;

Figure 3

Prognostic efficiency of the expression level of CDCA2 in patients with glioma from CGGA database. (A) Univariate analysis showed that CDCA2 expression affected the overall survival of patients. (B) Multivariate Cox proportional hazards analysis showed that CDCA2 expression was an independent prognostic factor in the survival time of patients with glioma. (C) Comparison of the overall survival time between the CDCA2 high and low expression groups of patients with glioma. (D) The predictive value of CDCA2 expression for 1-year, 3-year and 5-year survival. AUC, area under the curve.

Figure 4

Enrichment plots from gene set enrichment analysis(GSEA) GSEA results show cell regulation pathways are differentially enriched in CDCA2-related glioma.

Figure 5

Positive correlation between the expression of CDCA2 and cell cycle regulation genes. Increased CDCA2 expression level was associated with increased expression levels of CCNA2, CCNB1, CCNB2, CCNE1, CCNE2, CDK1, CDK2, CDK4, CDK6 (A-I).