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ORIGINAL ARTICLE

Prognostic significance of CDC25C in lung adenocarcinoma: An analysis of TCGA data

Zengfei Xia[a,1], Wen Ou-yang[a,1], Ting Hu[b], Ketao Du[c,*]

[a] *The Second Clinical Medical College, Zhujiang Hospital, Southern Medical University, Guangzhou, Guangdong, China;* [b] *Beijing International Travel Healthcare Center, Beijing, China;* [c] *Department of Rehabilitation, Chenzhou NO.1 People's Hospital, Chenzhou, Hunan, China*

Abstract

Objective: Cell division cycle 25C (CDC25C) is involved in the regulation of the G2/M phase transition and is associated with various cancers, including non-small cell lung cancer. We evaluated its prognostic value in lung adenocarcinoma (LUAD) based on data from The Cancer Genome Atlas (TCGA).

Methods: Kruskal–Wallis test, Wilcoxon signed-rank test, and logistic regression were used to evaluate relationships between clinical-pathologic features and CDC25C expression. Cox regression analyses and the Kaplan–Meier method were used to evaluate factors contributing to prognosis. Gene set enrichment analysis (GSEA) was performed.

Results: High CDC25C expression in LUAD was associated with a high tumor extent (odds ratio (OR) = 2.23 (1.52–3.29), $P < 0.001$), regional lymph node invasion (OR = 2.18 (1.48–3.22), $P < 0.001$), OR = advanced stage (OR = 2.47 (1.72–3.59), $P < 0.001$), and poor status (OR = 1.87 (1.19–2.96), $P = 0.007$). A univariate analysis showed that high CDC25C expression is associated with a short overall survival (OS) (HR: 1.873; 95% CI: 1.385–2.535; $P < 0.001$) and poor progression-free survival (HR: 1.503; 95% CI: 1.173–1.926; $P = 0.0012$). In a multivariate analysis, high CDC25C expression was associated with poor OS (HR = 2.193; CI: 1.394–3.452, $P = 0.001$). GSEA showed the enrichment of cell cycle, apoptosis, p53-dependent G1 DNA damage response, S-phase, mitotic M-M G1 phases, and FA-mediated cell death in the CDC25C high-expression phenotype.

Conclusions: CDC25C predicts poor prognosis in LUAD and may function in cell cycle regulation and FAS-mediated apoptosis.

Keywords CDC25C, Lung adenocarcinoma, TCGA.

Introduction

Lung adenocarcinoma (LUAD) is the most common and aggressive type of non-small cell lung cancer (NSCLC), accounting for around 40% of all lung cancers, and is an important cause of respiratory cancer deaths [1]. Lung and bronchus cancers represent 14% and 12% of all cancers in men and women, respectively, making it the second most frequent cancer in both sexes [2, 3]. The 5-year relative survival rate for lung cancer was 18% in 2007–2013 [2]. LUAD is typically diagnosed at an advanced stage, after metastasis [4]. Low-dose computed tomography has improved the diagnosis of lung cancer, thereby reducing mortality by up to 20%; however, scanning for lung cancer is not available to many individuals [5–7]. Thus, there is pressing need to identify the high-risk patients with poor prognosis so that novel and intensive protocols could be initiated earlier to improve survival.

Epidermal growth factor receptor (EGFR) mutations are common in LUAD, and patients with these mutations show good responses to EGFR tyrosine kinase inhibitors, but there is a risk of drug resistance [8]. Fas modulates the effects of EGFR mutations on lung cancer cells and influences the effects of tyrosine kinase inhibitors [9,10]. Combined with Fas

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* Corresponding author.

E-mail address: 15096105210ryan@sina.com

[1] The authors contributed equally to this work.

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ligand (FasL), Fas regulates physiological programmed cell death by triggering apoptosis via the Fas-FADD complex and induces cell cycle arrest [11, 12]. Decoy receptor 3 (DcR3), which binds to FasL to inhibit FasL-induced apoptosis, is involved in the development of lung cancer, suggesting a role of Fas [13].

Activated at the G2/M switch, CDC25C is an important cyclin that induces G2/M phase transition in mitotic and meiotic cells; it subsequently dephosphorylates Cdc2/cyclin B-Cdk1 and is further activated by Cdc2 in the amplification step after the activation of Cdc2, forming a positive feedback that ultimately triggers a G2/M phase transition to promote mitosis [14–16]. Accordingly, CDC25C is of great important in tumor development and has become a focus of research on targeted therapies [17]. A study of FPD/AML (familial platelet disorder/acute myeloid leukemia) has shown that CDC25C mutations could disrupt the G2/M checkpoint and promote malignant transformation [18]. The abnormal activation of CDC25C induced by a variety of factors has been found in many cancers, such as colorectal cancer, oral cancer, and gastric cancer [17,19,20]. Thus, a series of drugs targeting CDC25C aimed at inducing G2/M phase arrest to inhibit cancer growth have been developed, and Hsp90 inhibitors, p90RSK, CDC25C inhibitors, and Acetyl-macrocalin B (A-macB) have been tested in NSCLC cells *in vitro* [21,22]. In particular, Hsp90 inhibitors have an additive effect on cell growth inhibition *in vitro* when combined with irradiation [23]. Although CDC25C plays an important role in the growth of many types of tumors and many studies have evaluated the regulation of CDC25C *in vitro*, it is not clear if CDC25C is a prognostic factor in LUAD. What's more, in NSCLC one of two major lung cancer, the high CDC25C expression had been reported and showed the higher CDC25C level coresponding with the poor suvivial [24]. Therefore, it is expectable that in LUAD, CDC25C expression level may work as a diagnosis marker.

In this study, to assess the prognostic value of CDC25C expression in human LUAD, data were obtained from TCGA. For an in-depth study of the biological pathways involved in LUAD, GSEA of CDC25C was performed. Our results indicated that a high level of CDC25C expression coordinated with the poor survival in LUAD. Several key pathways, including the FAS pathway, ATRBRCA pathway, p53HIPOXIA pathway, ATM pathway, cell cycle, proteasome, MCM pathway, ACTINY pathway, VEGR pathway, and EIF pathway, were related to high CDC25C expression in the GSEA. These findings suggest that high CDC25C expression predicts poor prognosis and may contribute to the assessment of disease progression and the effects of medication in patients with LUAD.

Materials and methods

RNA-sequencing data and bioinformatics analysis

Gene expression data with clinical information from LUAD projects (522 cases, Workflow Type: HTSeq-FPKM) were collected from TCGA. The exclusion criteria were normal LUAD samples and an overall survival of $<$30 days [25]. Then, level 3 HTSeq-FPKM data were transformed into TPM (transcripts per million reads) for the following analyses. The TPM data for 490 patients with LUAD were used for further analyses. Unavailable or unknown clinical features in 490 patients were regarded as missing values and the data are summarized in Supplement Table 1.

Gene set enrichment analysis and network construction

To determine whether an a priori defined set of genes differs significantly between two biological states, a computational method termed GSEA was used [26]. Gene expression data were divided into a high group and a low group according to the median CDC25C expression level, and gene set permutations were performed 1000 times for each analysis. In the whole process, the expression level of CDC25C was regarded as a phenotype. The pathways enriched for each phenotype were classified by the nominal *P*-value and normalized enrichment score (NES).

After the gene enrichment was made, to further investigate the crosstalk between the CDC25C and Fas pathway-associated genes, a regulation network with CDC25C and the genes was modeled and analyzed in the STRING website (https://string-db.org/). The data of the model was then processed by the Cytoscape software to generate a visual image.

Statistical analysis

All statistical analysis and plots were produced using R (v.3.5.1). Wilcoxon rank sum test and Wilcoxon signed rank test were used to analyze the expression of CDC25C in non-paired samples and paired samples, respectively. Kruskal–Wallis test, Wilcoxon signed-rank test, and logistic regression were used to evaluate relationships between clinical-pathologic features and the expression of CDC25C. Cox regression analyses and Kaplan-Meier method were used to evaluate prognostic factors. Other clinical factors, such as TNM stage (TNM stage is a way of staging tumors, in which T represents the range of primary tumors, N represents the presence and extent of regional lymph node metastasis and its scope, M represents the presence or absence of a distant transfer.), diffusing capacity for carbon monoxide (DLCO) predictive percent, bronchodilator FEV1%, packs of cigarettes per year, tobacco smoking history, race, location in lung parenchyma, disease state, age, and gender, may influence survival; accordingly, multivariate Cox analysis was used to compare the impact of CDC25C expression on survival along with other clinical traits; the median CDC25C expression was regarded as the cut-off value.

Results

Patient characteristics

The data (shown in Supplement Table 1) were collected from TCGA in October 2018 and included 490 primary tumors with both clinical and gene expression data. Dividing patients into two groups by age, the percentage of patients younger than 60 years was 27.5, and the older than 60 years was 72.5. According to TNM stage, 162 (33.8%) of 479 cases had regional

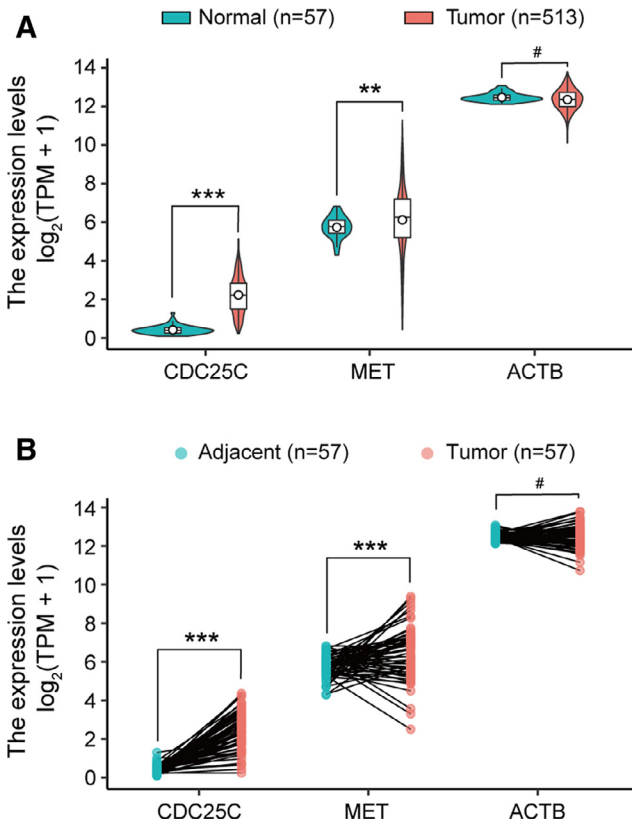

Fig. 1 Expression levels of CDC25C in paired tumor and adjacent samples (A) and non-paired samples (B) were analyzed by Wilcoxon signed rank tests. In each figure, the MET gene expression is presented as the positive control while the ACTB as the negative control. The higher expression level of CDC25C is showed in both paired tumor and adjacent samples. $^{\#}P < 0.05$., $^{**}P < 0.01$, $^{***}P < 0.001$.

lymph node invasion and 24 (6.9%) of 348 cases had distant metastases. With respect to cancer status, 292 (73.4%) cases were tumor-free and 106 (26.6%) had a tumor. With respect to the disease state, 263 (54.6%) were stage I, 115 (23.8%) were stage II, 79 (16.4%) were stage III, and 25 (5.2%) were stage IV. In addition, patient respiratory function data were included. A DLCO predictive percent below accounted for 62.4% of 194 patients with DLCO data. Approximately 43.2% of 229 cases had a poor bronchodilator FEV1% of below 80%.

Associations between CDC25C expression and clinicopathologic variables

As shown in Fig. 1, the expression levels of CDC25C in paired tumor and adjacent samples and non-paired samples were analyzed by Wilcoxon signed rank tests, with MET as positive control and ACTB as a negative control. There was a significant difference in expression between normal and tumor tissues ($P < 0.001$), with higher expression of CDC25C in tumor tissues. The Kruskal–Wallis test and Wilcoxon signed-rank test showed that a higher level of CDC25C is significantly correlated with a higher T stage ($P < 0.001$), N stage ($P < 0.001$), M stage ($P < 0.05$), and disease state ($P < 0.001$) (Fig. 2A–D). The poor cancer status, which was evaluated using the Wilcoxon signed-rank test, was associated with higher CDC25C expression ($P < 0.01$) (Fig. 2E). Poor respiratory function ($P < 0.05$) was associated with high CDC25C expression, based on a bronchodilator FEV1% below 80% (Fig. 2F).

A univariate logistic regression of CDC25C expression (with a median expression value of 3.63) indicated that higher expression levels are significantly correlated with poor prognostic features (Table 1), including LUAD with a greater primary tumor extent (OR = 2.23 (1.52–3.29) for T1 vs. T2/T3/T4, $P < 0.001$), more serious regional lymph node invasion (OR = 2.18 (1.48–3.22) for N0 vs. N1/N2/N3, $P < 0.001$) more advanced stage (OR = 2.47 (1.72–3.59) for Stage I vs. Stage II/Stage III/Stage IV, $P < 0.001$), poor bronchodilator FEV1% (OR = 0.55 (0.32–0.93) for <80 vs. ≥80, $P = 0.026$), and poor cancer status (OR = 1.87 (1.19–2.96) for tumor free vs. with tumor, $P = 0.007$). These results indicated that tumors with high CDC25C expression are associated with poor outcomes.

Univariate and multivariate analyses of survival

As shown in Fig. 2G and H, a Kaplan–Meier survival analysis was performed to evaluate the relationship between CDC25C level and prognosis. High CDC25C expression was more strongly associated with a worse prognosis than low CDC25C expression ($P < 0.001$). A univariate analysis revealed that high CDC25C expression is associated with a short overall survival (OS) (hazard ratio [HR]: 1.873; 95% confidence interval [CI]: 1.385–2.535; $P < 0.001$) and poor progression-free survival (HR: 1.503; 95% CI: 1.173–1.926; $P = 0.0012$) (Table 2a).

To further explore factors associated with survival, a multivariate Cox regression analysis was performed using TNM stage, disease state, and cancer status. High CDC25C expression was still an independent factor associated with poor OS (HR, 2.193; CI: 1.394–3.452, $P = 0.001$) (Table 2b).

GSEA identifies a CDC25C-related signaling pathway

As many pathways contribute to tumor formation, the poor survival associated with high CDC25C expression may be related to a number of signaling pathways activated in LUAD. GSEA of differences between low and high CDC25C expression data sets was performed to identify the key signaling pathways associated with CDC25C. Significant differences (FDR < 0.05, normalized $P < 0.05$) in the enrichment of the MSigDB Collection (c2.cp.v6.2.symbols) of many pathways were observed. The most significantly enriched signaling pathways based on their NES are shown in Table 3 and Fig. 3 In particular, CDC25C was related to the cell cycle, apoptosis, p53-dependent G1 DNA damage response, S-phase, mitotic M-M G1 phases, and FAS-mediated cell death pathways. What's more, a regulation network with CDC25C and the FAS gene (or broader genes) was made (Supplemental Figure 1), the network showed the sophisti-

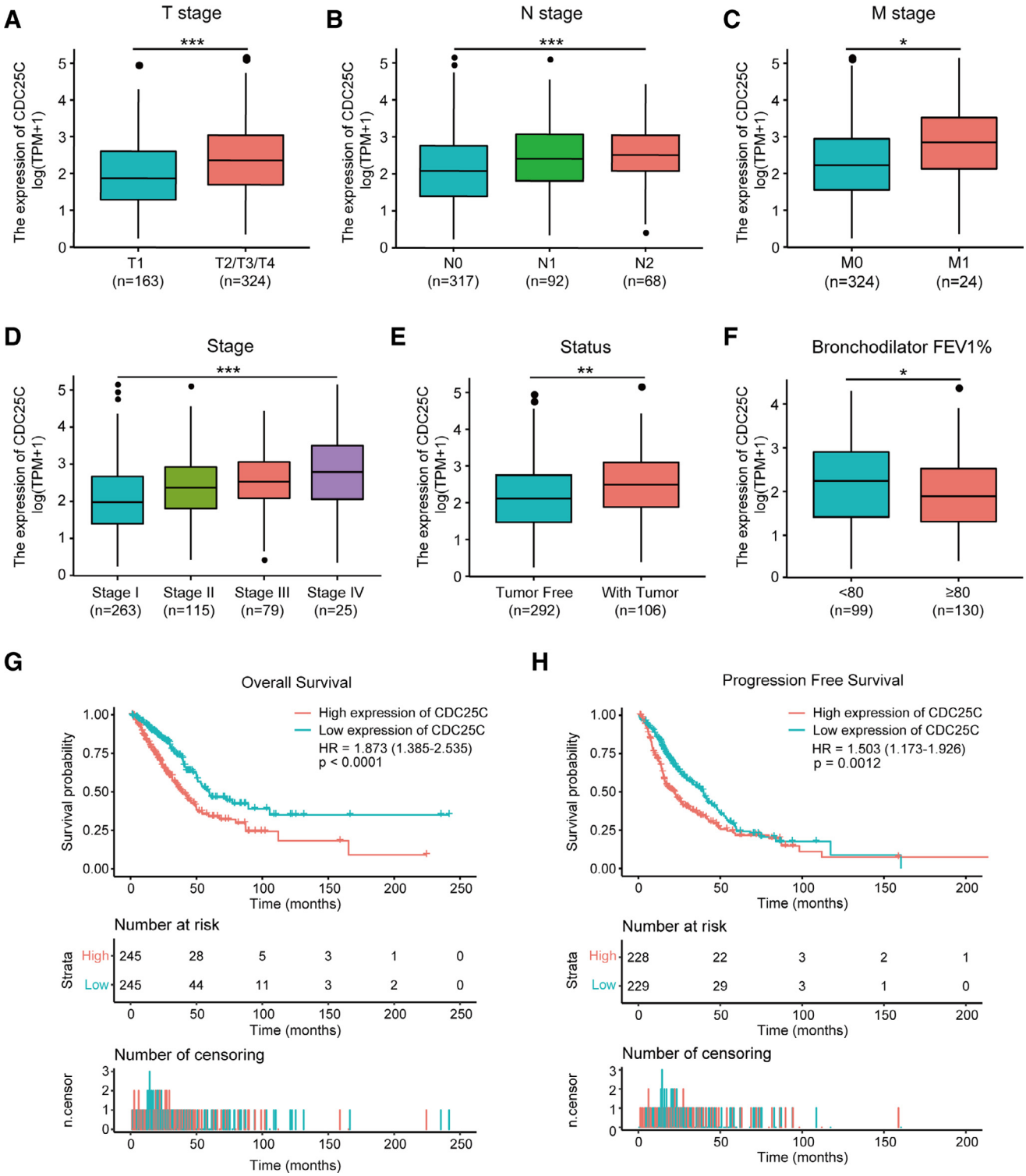

Fig. 2 Association between CDC25C expression and clinicopathologic characteristics, including A–C: TNM stage, D: disease stage, E: cancer status, F: bronchodilator FEV1%. Impact of CDC25C expression on OS (G) and PFS (H) in patients with LUAD in a TCGA cohort. As showed in the Box diagram, the higher expression level of CDC25C is associated with not only the poor pathological staging, but also the poor clinical symptoms, which links to the poor survival outcomes. In the Box charts, the black closed circles in the end of the vertical line means the number beyond the maximum of the vertical axis, one black cycle means one data beyond the maximum of the vertical axis. TCGA, The Cancer Genome Atlas; LUAD, lung adenocarcinoma; OS, overall survival; PFS, progress-free survival. $^{*}P < 0.05$., $^{**}P < 0.01$, $^{***}P < 0.001$.

Table 1 CDC25C expression[a] associated with clinical pathological characteristics (logistic regression).

Clinical characteristics	Total (N)	Odds ratio in CDC25C expression	*p*-value
T (T1 vs. T2/T3/T4)	487	2.23(1.52–3.29)	<0.001
N (N0 vs. N1/N2/N3)	479	2.18(1.48–3.22)	<0.001
M (M0 vs. M1)	348	2.34(0.98–6.19)	0.066
Stage (Stage I vs. Stage II/Stage III /Stage IV)	482	2.47(1.72–3.59)	<0.001
DLCO predictive percent (<80 vs. ≥80)	194	0.97(0.54–1.74)	0.914
Bronchodilator FEV1% (<80 vs. ≥80)	229	0.55(0.32–0.93)	0.026
Location in lung parenchyma (Central Lung vs. Peripheral Lung)	176	1.06(0.57–1.98)	0.854
Person neoplasm cancer status (Tumor free vs. With tumor)	398	1.87(1.19–2.96)	0.007

[a] Categorical dependent variable, greater or less than the median expression level.

Table 2 a. Associations with overall survival and clinicopathologic characteristics in TCGA patients using Cox regression. b. Multivariate survival model after variable selection.

Clinicopathologic variable	HR (95% CI)	*p*-value
a.		
T (T1 vs. T2/T3/T4)	1.711(1.206–2.427)	0.003
N (N0 vs. N1/N2/N3)	2.658(1.969–3.587)	<0.001
M (M0 vs. M1)	2.200(1.283–3.772)	0.004
Stage (StageI vs. Stage II/Stage III/Stage IV)	3.017(2.209–4.120)	<0.001
DLCO predictive percent (<80 vs. ≥80)	0.758(0.417–1.375)	0.362
Bronchodilator FEV1% (<80 vs. ≥80)	0.616(0.375–1.012)	0.056
Packs of cigarettes per year (<40 vs. >=40)	1.042(0.723–1.500)	0.827
Tobacco smoking history (Year) (1/2 vs. 3/4/5)	1.061(0.778–1.446)	0.710
Race (Not White vs. White)	1.397(0.853–2.288)	0.184
Location in lung parenchyma(Central Lung vs. Peripheral Lung)	0.948(0.588–1.527)	0.826
Status (Tumor free vs. With tumor)	5.230(3.702–7.389)	<0.001
Age (<60 vs. >=60)	1.054(0.755–1.470)	0.758
Gender (Female vs. male)	1.071(0.797–1.438)	0.650
CDC25C (Low vs. high)	1.873(1.385–2.535)	<0.001
b.		
T (T1 vs. T2/T3/T4)	1.307(0.752–2.271)	0.342
N (N0 vs. N1/N2/N3)	1.933(0.960–3.890)	0.065
M (M0 vs. M1)	0.720(0.335–1.547)	0.400
Stage (StageI vs. Stage II/Stage III/Stage IV)	0.889(0.408–1.936)	0.767
Status (Tumor free vs. With tumor)	5.932(3.771–9.330)	<0.001
CDC25C (Low vs. high)	2.193(1.394–3.452)	0.001

Table 3 Gene sets enriched in phenotype high.

MSigDB collection	Gene set name	NES	NOM *p*-val	FDR *q*-val
c2.cp.v6.2.symbols.gmt	KEGG_CELL_CYCLE	2.461	<0.001	<0.001
	REACTOME_S_PHASE	2.402	<0.001	0.001
	REACTOME_APOPTOSIS	2.393	<0.001	<0.001
	REACTOME_P53_DEPENDENT_G1_DNA_DAMAGE_RESPONSE	2.384	<0.001	<0.001
	REACTOME_MITOTIC_M_M_G1_PHASES	2.373	<0.001	<0.001
	BIOCARTA_FAS_PATHWAY	1.881	<0.001	0.019

NES: normalized enrichment score; NOM: nominal; FDR: false discovery rate. Gene sets with NOM *p*-val <0.05 and FDR *q*-val <0.25 are considered as significant.

cated crosstalk between the CDC25C and others gene, revealing the important role of CDC25C in LUAD.

Discussion

Previous studies have established the important role of CDC25C phosphatase in cell cycle regulation as a bispecific phosphatase that activates cell cycle progression by eliminating the inhibitory phosphate groups on cyclin-dependent kinases (CDK) [27]. CDC25C primarily triggers G2/M transition in the cell cycle by the dephosphorylation of cyclin B-Cdk1 [28]. Recent studies have also shown that CDC25C phosphatase can determine cell survival by the regulation of apoptosis signaling kinase 1, and that p53 can initiate cell cycle arrest by directly binding to the CDC25C promoter to mediate the DNA damage-induced CDC25C down-regulation, which prevents abnormal cell

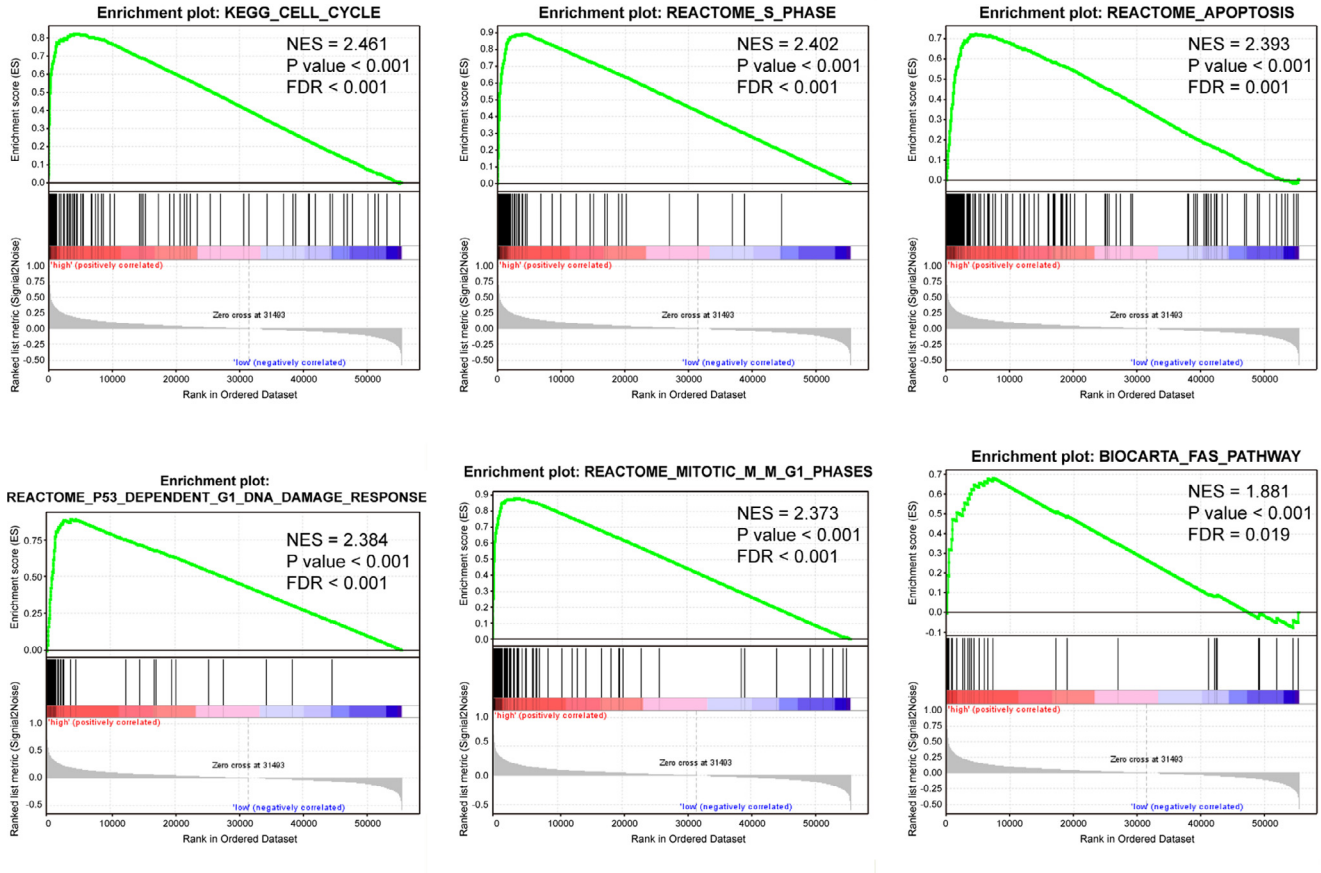

Fig. 3 Enrichment plots from the gene set enrichment analysis (GSEA). Several pathways and biological processes were differentially enriched in CDC25C-related LUAD, including the cell cycle, S phase, apoptosis, p53-dependent G1 DNA damage response, mitotic M-M G1 phase, and FAS_PATHWAY. ES, enrichment score; NES, normalized ES; NOM *P*-val, normalized *P*-value.

division in humans [16,29]. Based on these studies, multiple signaling pathways, including abnormal signaling pathways that are common in tumors, are closely related to CDC25C, and the overexpression of CDC25C phosphatase is also closely related to various cancers [30]. Many *in vitro* studies of colorectal cancer, oral cancer, FPD/AML, gastric cancer, and NSCLC have evaluated the expression and function of CDC25C in tumor development; drugs related to CDC25C, such as CDC25C inhibitors, are promising based on *in vitro* studies of NSCLC [17–23]. However, little is known about the expression of CDC25C and its prognostic value in LUAD.

In this study, a bioinformatics analysis of the prognostic value of CDC25C in LUAD was performed using high-throughput RNA sequencing data obtained from TCGA. Elevated CDC25C expression in LUAD was associated with advanced clinicopathological features (advanced clinical stage and distant metastasis), poor respiratory function, short survival time, and poor prognosis. To further investigate the function of CDC25C in LUAD, in addition to the functions previously reported in other tumors, we used TCGA data for GSEA and found that the S-phase and the M/G1 phase in the cell cycle as well as FAS-mediated apoptosis in LUAD were differentially enriched in the CDC25C high-expression phenotype. This suggests that CDC25C not only serves as a potential prognostic marker, but might also serve as a reference for predicting tumor and respiratory function statuses of patients; it may even be a potential therapeutic target by affecting FAS apoptosis regulation in LUAD.

Fas (also known as Apo-1 or CD95) belongs to the tumor necrosis factor receptor (TNFR) superfamily and contains a death domain. It initiates programmed cell death and induces cell cycle arrest; thus, it is involved in the pathogenesis of various malignancies and immune system diseases [31]. In this study, we found that CDC25C may exhibit crosstalk with the FAS pathway; both play a significant role in tumorigenesis and have important roles in regulating the cell cycle and apoptosis. However, it is not clear whether CDC25C and the FAS pathway have a synergistic effect or a complementary effect, and the detail regulation network with CDC25C and the FAS pathway has not been reported. What's more, as showed in the regulation network, CDC25C has a sophisticated crosstalk with a bulk of other genes, thus, a web lab work should be taken into the next plant. In addition, CDC25C is regulated by ERK-MAP kinases, suggesting that CDC25C may be involved in a more complex regulatory network [14].

It is worth noting that CDC25C may be involved in the broader regulation of the cell cycle, rather than being limited to G2/M transition. In this study, GSEA results showed that the CDC25C high-expression phenotype is significantly associated with the regulation of the S-phase and M/G1 phase in the cell cycle as well as FAS-mediated apoptosis in LUAD. According to previous studies, RNA silencing of S-phase kinase interactive protein 2 can inhibit lung cancer cell

proliferation and centrosome amplification [32]. In addition, during the process of CPT-11-induced apoptosis in NSCLC cells, the expression of ectopic p16 (ink4) can enhance the delay in S phase progress [33,34]. These findings suggest that the S phase has an important impact on the progression of NSCLC. Based on the pathway analysis and the effect of CDC25C on prognosis, CDC25C may participate in the regulation of S phase during the development of LUAD, but the underlying regulatory mechanism requires further clarification.

The correlation between CDC25C mRNA and protein expression should be verified using cell experiments and clinical samples, and using mRNA levels to predict protein expression is far from perfect [35]. Furthermore, due to the limitations of our study design, additional important signaling pathways associated with CDC25C may have been missed, and the relevant pathways should be examined further. To further investigate the mechanism of CDC25C in the LUAD, we had made some plants for some wet lab work in the sooner future.

In conclusion, CDC25C may predict poor prognosis and may have important roles in the regulation of S-phase and M/G1 phase of the cell cycle as well as the FAS-mediated apoptosis in LUAD. Additional experiments are needed to demonstrate the biological impact of CDC25C in LUAD. In addition, further studies should evaluate the relationship between CDC25C expression and clinical features, LUAD stage, and prognosis using additional larger clinical data; this information may facilitate the identification of a new biomarker to effectively evaluate the tumor stage, improve treatment, and aid the drug development.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Author contributions

All authors contributed toward data analysis, drafting and critically revising the paper, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2019.04.001.

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