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Over-Expressed CDCA4 Indicates Poor Clinical Efficacy as a Potential Therapeutic Target for Lung Adenocarcinoma

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ABSTRACT The clinicopathological information and the gene expression data were utilised via The Cancer Genome Atlas (TCGA) databases. The results have shown that CDCA 4 was over-expressed in lung adenocarcinoma (LUAD) than those in normal or surrounding non-carcinoma profiles. The value of the receiver operating characteristic (ROC) curve revealed that CDCA4 can be a novel biomarker with better value for diagnose. Besides, CDCA4 expression levels were correlated with higher grades of clinical pathologic stage, higher age, and the residual tumour. CDCA4 expression was observably correlate with immune cell infiltration including macrophage cells, CD4 + T cells and B cells. Over-expression of CDCA4 in LUAD was related to a poor overall survival and first progression survival time. Additionally, the nomograms indicated that over-expression of CDCA4 might be a potential prognosis biomarker for LUAD.

INTRODUCTION

Non-small cell lung cancer (NSCLC) represents the most prevalent carcinoma type (Herbst et al. 2018). Lung adenocarcinoma (LUAD) accounts for the largest proportion of NSCLC pathological subtype, has been related to more than 1 million cancer-related deaths each year worldwide (Gibson et al. 2019). In spite of various therapies, including surgery, chemotherapy, targeted treatment and immunotherapy having positive effects on the prognosis, the efficacy with long-term survival still remains unsatisfactory. Researchers have demonstrated that LUAD patients have a poor 5-year survival outcome (Gibson et al. 2019; Herbst et al. 2018; Siegel et al. 2021).

This might be due to the fact that early detection of LUAD is limited. In general, most patients of LUAD are initially diagnosed with the progressive disease, resulting in a rapid development. Thus, identifying the effective diagnosis biomarkers of LUAD for early diagnosis is important.

The cell division cycle-associated 1-8 (CDCA 1-8) acts a prominent role in cell proliferation and division regulation. Previous researchers have found that CDCAs acted as crucial regulators in multiple tumors (Kobayashi et al. 2014; Li et al. 2021; Pang et al. 2019; Thang et al. 2016; Wu et al. 2020). Over-expressed genes were related to the poor prognosis in these cancers (Shi et al. 2017; Xiao et al. 2018). For instance, Thang et al. (2016) revealed that CDCA1 might act as a new therapeutic and prognostic marker for oral carcinoma. Other articles (Phan et al. 2018) have shown that CDCA3, CDCA5 and CDCA8 are highly expressed in carcinoma tissue from breast compared with normal tissue, decreasing the breast cancer patients' survival.

CDCA4 can regulate the cell cycle, which can occur when cells run into the G1/S phase and be associated with the cell proliferation (Hayashi et al. 2006). Until now, the value of prognostic of the CDCA4 in cancer was not clear. In a previous article, Xu et al. (2018) pointed out that CDCA4 contributes to regulating breast cancer cell proliferation and biological processes. Other studies have revealed that it increases the level of E2F, resulting in cell proliferation (Hayashi et al. 2006). Also, an article found that obviously upregulation of CDCA4 was detected in hepatocellular carcinoma tissue and had a negative impact on survival. A study on its function and the relation of CDCA4 with cancer has not been shown in LUAD. Ran et al. (2021) has shown that over-expression of CDCA4 was found in patients with NSCLC. While, these were not further studied to advance this field. Lately, Tan et al. (2022) has

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demonstrated that the expression of CDCA4 is elevated in LUAD and potentially acts as a therapeutic target and predictive marker.

Objectives

Consequently, the researchers first used the comprehensive bioinformatic methods and the publicly accessible online databases to identify prognostic significance of CDCA4 in LUAD and to predict the overall survival (OS) outcome of LUAD patients rely on the TCGA data using a nomogram model.

METHODOLOGY

Data Source

The gene profile and paired clinical data were downloaded via the Cancer Genome Atlas (TCGA) database (Rau et al. 2019), including 535 LUAD and 59 paired normal tissues. Then, 57 pairs of LUAD and paired adjacent normal structures were specifically analysed for the data. The researchers further estimated the clinical characteristics and prognostic value obtained from TCGA. According to the median value of gene expression, the researchers grouped the patients as high and low expression.

Gene Expression Profiling Interactive Analysis

The researchers used the Gene Expression Profiling Interactive Analysis 2 (GEPIA2) (Tang et al. 2019) database to estimate the CDCA4 expression analysis in LUAD and took the survival analysis to rely on the expression profile of CDCA4. The database includes the RNA sequencing expression profiles rely on the TCGA and the GTEx projects.

Survival Analysis Related to Gene Expression

The online Kaplan - Meier plotter platform (Nagy et al. 2018) was performed to explore the relation of CDCA4 on LUAD OS and disease-free survival (DFS). The hazard ratios (HRs) and p-values based on log-rank test were presented. The survival curves were automatically evaluated by the website.

Immune Cell Infiltration Analysis

The relationship between CDCA4 expression and immune cells infiltration was evaluated. Using the R package "GSVA", the researchers presented the 24 common immune cells infiltration enrichment to evaluate the relation. The researchers further assessed the link by the published statistical approach, TIMER 2.0 (Li et al. 2020), using the Spearman's method. Besides, with the help of this method, the researchers assessed the immune cells' infiltration levels for high-CDCA4 in comparison with low-CDCA4 expression groups by t-test, Weltch t-test, or nonparametric rank-sum test.

CDCA4-related Survival Analysis by COX Regression

In univariate survival analysis by Cox regression, the differential expression of CDCA4 on LUAD OS across two different cohorts has been performed.

Then, the researchers conducted the multivariate analysis to evaluate the diagnostic potential of CDCA4 with survival in LUAD. $P < 0.05$ represents CDCA4 had a statistically significant impact on survival outcome.

Statistical Analyses

R language (Version 3.6.3) was taken to conduct the analyses, and the differences in expression was utilised with the R package ggplot2. The Mann-Whitney U test and paired t-test were performed for the difference between LUAD tissue and adjacent lung tissue. The 'plotROC' package was conducted to evaluate the diagnostic potential of CDCA4.

The nonparametric tests (including Kruskal-Wallis test, Wilcoxon rank sum test) and logistic regression analysis were used to evaluate the association between patients' characteristics and the expression level of CDCA4.

In order to identify the association between the clinicopathological information and the expression of CDCA4 across two different cohorts, the researchers conducted the chi-square test, or Fisher's exact test instead if there was small number of expected frequencies.

The researchers analyzed the immune cells infiltration by means of the R package "GSVA", TIMER 2.0 and the Spearman's method.

The survival analyses were performed to analyse the prognostic value of CDCA4 by Kaplan-Meier and Cox regression methods.

By the method of the Cox hazard regression, the multivariate Cox hazard regression included the parameters with p value less than 0.1, and p value less than 0.05 in the univariate analysis represent statistically significant.

The nomograms model with its calibration plots was performed by R package "rms". The time-dependent receiver operating characteristic (ROC) curves were adopted to judge the prognostic capacity of models (Foucher et al. 2012; Zheng et al. 2004). A decision curve analysis (DCA) graph evaluated the value of the predictive new model (Steyerberg et al. 2008; Vickers et al. 2006).

RESULTS

LUAD Patients' Characteristics

The patients' characteristics and prognostic profile of 406 LUAD cases were downloaded via TCGA database, including the age of the patients, gender, grade of histology, clinical pathologic stage, lifetime, and survival status. Samples with incomplete clinical information were excluded, 292 cases were extracted for further analysis finally (Table 1).

Expression Pattern of CDCA4 in LUAD

The researchers assessed the CDCA4 expression in LUAD in comparison with normal profiles, revealing that the level of the CDCA4 expression in LUAD were dramatically elevated than normal profiles ($P < 0.001$, Fig.1A). In the meantime, the consistent result was found in comparison of LUAD tissues with paired adjacent non-cancerous profiles ($P < 0.001$, Fig. 1B).

Furthermore, the ROC curve showed the area under the curve (AUC) was 0.913, indicating that CDCA4 might be a novel biomarker with better diagnostic value (Fig. 1C).

Also, the researchers conducted an expression pattern of CDCA4 analysis via GEPIA2. The consistent findings revealed that CDCA4 was highly expressed in tumour profiles than those in healthy cases ($P < 0.05$, Fig. 1D).

Table 1: LUAD patients' characteristics

Index		*Frequency (percentage)#*
Total number		535 (100%)
Age, median (Interquartile Range)		66 (59.72)
Age	<=65	255 (49.4%)
	>65	261 (50.6%)
Sex	Women	286 (53.5%)
	Man	249 (46.5%)
Smoker	No	75 (14.4%)
	Yes	446 (85.6%)
TNM stage	T1	175 (32.9%)
	T2	289 (54.3%)
	T3	49 (9.2%)
	T4	19 (3.6%)
	N0	348 (67.1%)
	N1	95 (18.3%)
	N2	74 (14.3%)
	N3	2 (0.4%)
	M0	361 (93.5%)
	M1	25 (6.5%)
Clinical pathologic stage	Stage I	294 (55.8%)
	Stage II	123 (23.3%)
	Stage III	84 (15.9%)
	Stage IV	26 (4.9%)
Anatomic neoplasm subdivision	Left	205 (39.4%)
	Right	315 (60.6%)
	Central Lung	62 (32.8%)
	Peripheral Lung	127 (67.2%)
Residual tumor, n (%)	R0	355 (95.4%)
	R1	13 (3.5%)
	R2	4 (1.1%)
Primary treatment outcome	Progressive disease	71 (15.9%)
	Stable disease	37 (8.3%)
	Partial response	6 (1.3%)
	Complete response	332 (74.4%)
OS event, n (%)	Alive	343 (64.1%)
	Dead	192 (35.9%)
DSS event, n (%)	Alive	379 (76%)
	Dead	120 (24%)

#: Unless otherwise specified.

Relation between CDCA4 Expression and Clinical Data

Figures 2A-2F have displayed that the CDCA4 expression positively associated with the poorer TNM stage, clinical pathologic stage, older, and the more obvious residual tumour ($P < 0.001$). In the meantime, consistent findings were also shown in Table 2.

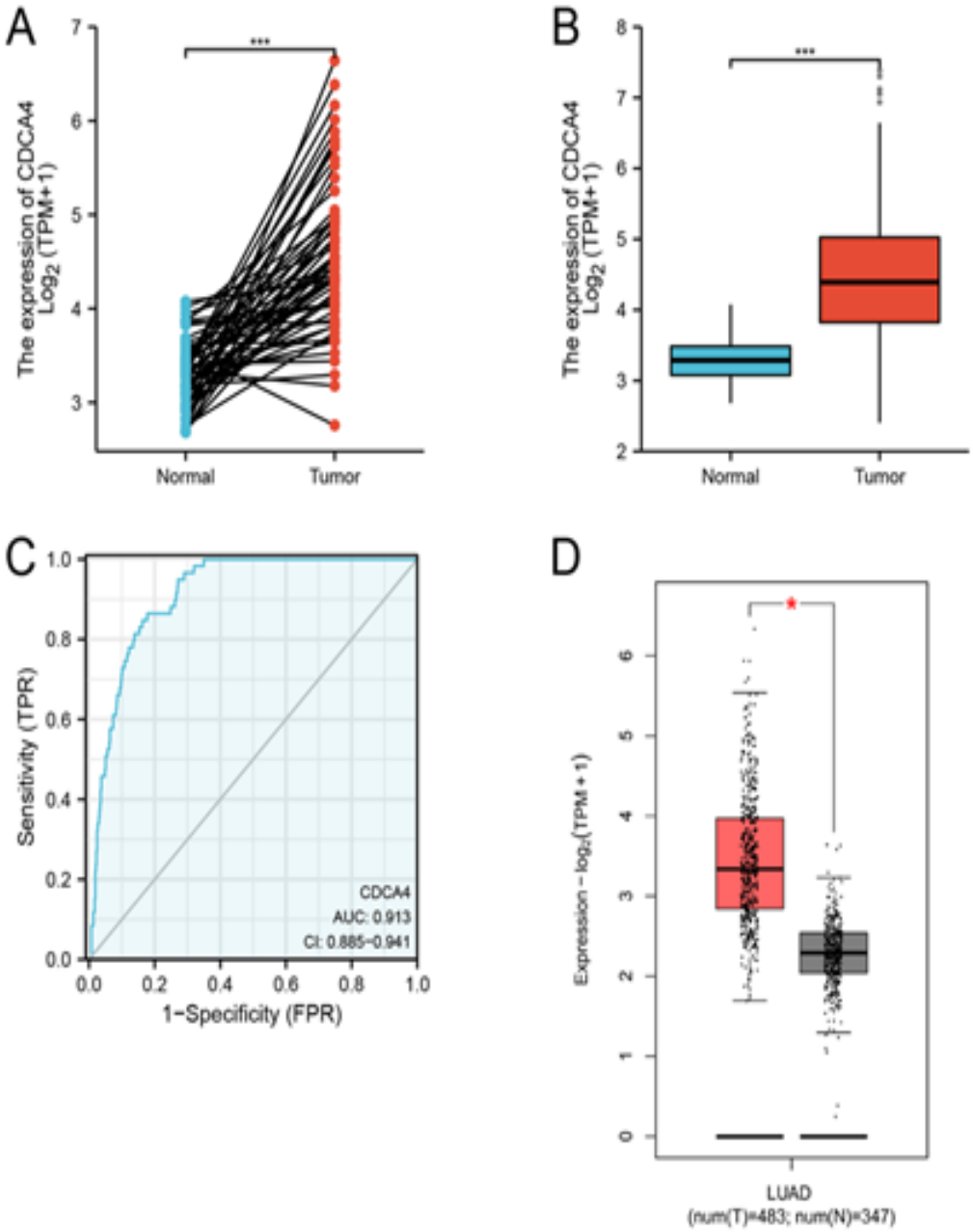

Fig. 1. Association between the CDCA4 expression and lung adenomatous (LUAD). (A) the expression analysis compared tumor tissues with normal tissues via TCGA. (B) the expression analysis compared tumor tissues with para-cancerous tissues via TCGA. (C) ROC curve of CDCA4 expression in LUAD. (D) Analysis of the differential expression compared tumor tissues with normal tissues via GEPIA2

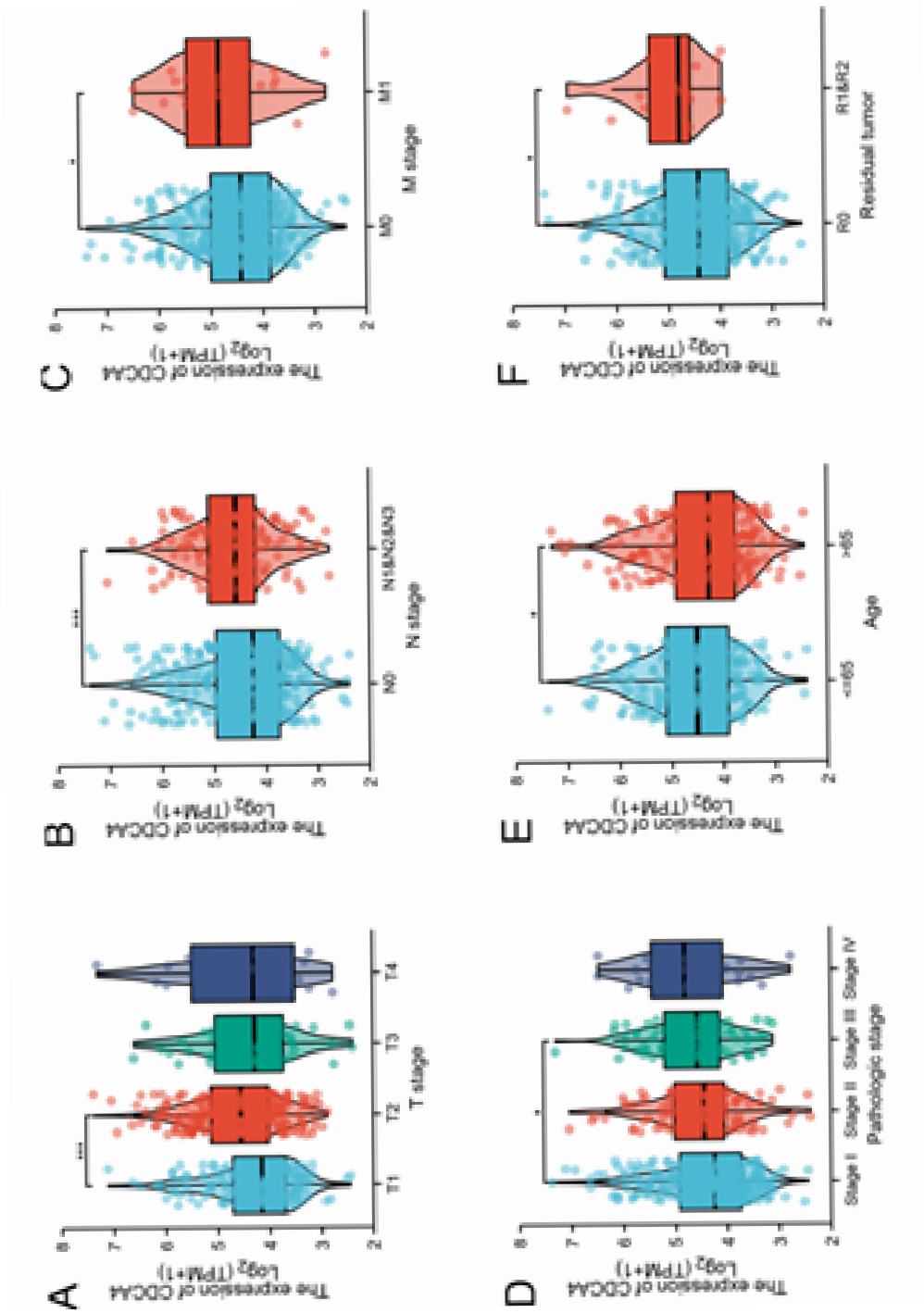

Fig. 2. Relationships between CDCA4 level and patients' characteristics. T stage (A), N stage (B), M stage (C), clinical pathologic stage (D), age (E) and the residual tumour(F).

Table 2: Association between CDCA4 level and LUAD patients' characteristics

Index	*Low expression cohort*	*High expression cohort*	*P value*
n	267	268	
Age, median (Interquartile Range)	67 (60.74)	64 (58.71)	0.005#
Age, n (%)			0.028
<=65	114 (22.1%)	141 (27.3%)	
>65	143 (27.7%)	118 (22.9%)	
Sex, n (%)			0.317
Women	149 (27.9%)	137 (25.6%)	
Man	118 (22.1%)	131 (24.5%)	
T stage, n (%)			< 0.001
T 1	109 (20.5%)	66 (12.4%)	
T 2	119 (22.4%)	170 (32%)	
T 3	28 (5.3%)	21 (3.9%)	
T 4	10 (1.9%)	9 (1.7%)	
N stage, n (%)			< 0.001
N0	193 (37.2%)	155 (29.9%)	
N1	39 (7.5%)	56 (10.8%)	
N2	24 (4.6%)	50 (9.6%)	
N3	0 (0%)	2 (0.4%)	
M stage, n (%)			0.064
M0	178 (46.1%)	183 (47.4%)	
M1	7 (1.8%)	18 (4.7%)	
Clinical pathologic stage, n (%)			< 0.001
Stage I	167 (31.7%)	127 (24.1%)	
Stage II	57 (10.8%)	66 (12.5%)	
Stage III	30 (5.7%)	54 (10.2%)	
Stage IV	8 (1.5%)	18 (3.4%)	
Primary treatment outcome, n (%)			0.197
Progressive disease	28 (6.3%)	43 (9.6%)	
Stable disease	19 (4.3%)	18 (4%)	
Partial response	3 (0.7%)	3 (0.7%)	
Complete response	177 (39.7%)	155 (34.8%)	
Residual tumor, n (%)			0.025
R0	173 (46.5%)	182 (48.9%)	
R1	3 (0.8%)	10 (2.7%)	
R2	0 (0%)	4 (1.1%)	
Anatomic neoplasm subdivision, n (%)			0.590
Left	106 (20.4%)	99 (19%)	
Right	154 (29.6%)	161 (31%)	
Smoker, n (%)			0.327
No	42 (8.1%)	33 (6.3%)	
Yes	219 (42%)	227 (43.6%)	

#: p value based on Wilcoxon rank sum test.

Relationship of CDCA4 and Immune Cells Infiltration Features

As displayed in Figure 3A, Th2 cells, aDc, CD56(dim) natural killer (NK) cells, T helper cells, and Tgd cells were all positively associated with CDCA4 expression. However, most other immune cell types have showed the negative association with CDCA4.

In addition, Figure 3B has shown the comparison of infiltration immune levels between groups (high CDCA4 expression versus low CDCA4 expression). The researchers investigated the infiltration ten most relevant immune cells levels, that is, Th17, Tcm, Th2, iDC, NK, neutrophils, macrophages, aDC, DC, CD8T, B cell, T cell, which demonstrated consistent findings with those in Figure 3A.

Using TIMER, the researchers also shown the relation of CDCA4 expression with immune infiltration levels. The CDCA4 expression was dramatically associated with Th2 cells, aDc, CD56(dim) NK cells, T helper cells, and Tgd cells (Fig. 3C).

Prognostic Capacity of the Expression of CDCA4 in LUAD

Shown as in Figures 4A-4C, high levels of CDCA4 indicated the undesirable OS outcome (HR: 1.87, 95% confidence interval (CI): 1.48, 2.37; $P < 0.001$, Fig. 4A), poor first progression survival (FPS) (HR: 2.1; 95%CI: 1.52, 2.9; $P < 0.001$, Fig. 4B), but no statistical difference was found in post progression survival (PPS) (HR: 1.05; 95% CI: 0.66, 1.67; $P = 0.85$, Fig. 4C).

Additionally, the researchers validated the high level of CDCA4 expression in OS (Fig. 4D) and DFS (disease-free survival) (Fig. 4E) by GEPIA2. Besides, a consistent analysis was performed via GEPIA2. It implied that a high level of CDCA4 expression in LUAD had poor prognosis with OS and DFS.

Cox Proportional Hazards Regression Model of Overall Survival

As displayed in Table 3, in univariate analysis, TNM stage, clinical pathologic stage, primary therapy result, residual tumour and increased CDCA4 expression had poor OS ($P < 0.05$).

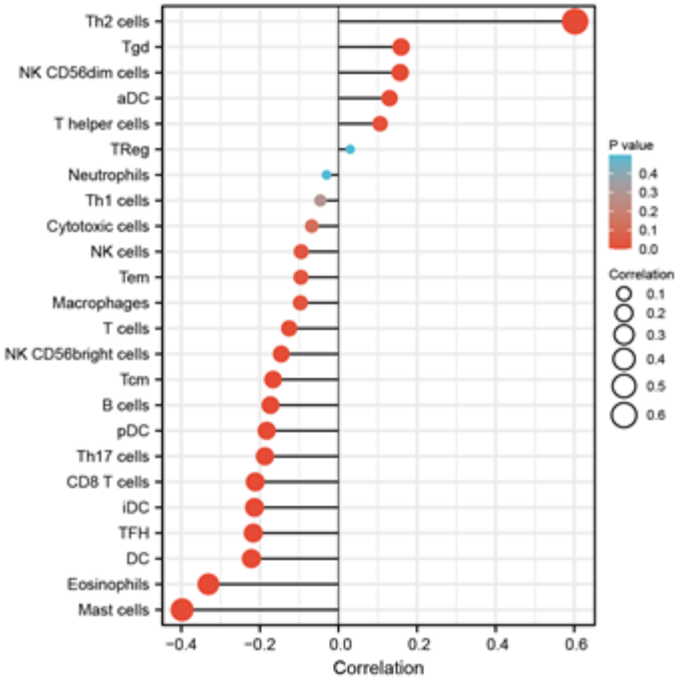

Fig. 3. Association of CDCA4 level and immune cells infiltration features. (A) 24 common immune cells infiltration enrichment. (B) Infiltration immune cell types that significantly different between high and low CDCA4 expressing cohorts (a-l). (C) The verified results based on TIMER 2.0 platform (a-f)

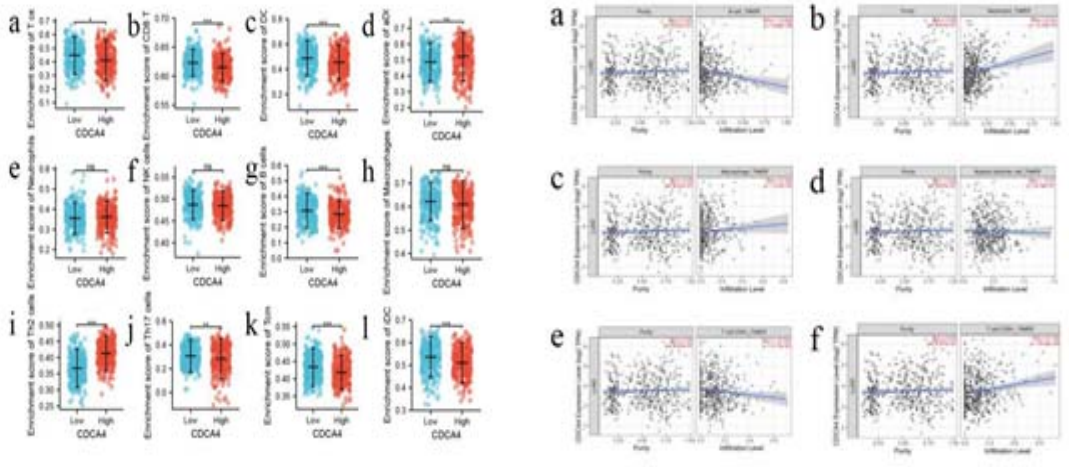

Fig. 4. The Kaplan-Meier curves showed the comparisons between the high and low CDCA4 expression cohorts with the (A) OS and (B) FPS and (C) PPS. Analysis of the high expression of CDCA4 with a poorer (D) OS and (E) DFS via GEPIA2.

Table 3: Univariate and Multivariate Cox regression analyses of CDCA4 expression and LUAD patients' OS outcome

Index	*N*	*Univariate*		*Multivariate*	
		HR with 95% CI	*P value*	*HR with 95% CI*	*P value*
T stage					
T1	175	Reference			
T2	282	1.52 (1.07-2.17)	**0.020**	1.02 (0.58-1.79)	0.952
T3	47	2.94 (1.75-4.94)	**<0.001**	3.09 (1.11-8.63)	**0.031**
T4	19	3.33 (1.75-6.32)	**<0.001**	4.42 (1.28-15.30)	**0.019**
N stage					
N0	343	Reference			
N1	94	2.38 (1.70-3.35)	**<0.001**	2.65 (0.97-7.25)	0.058
N2	71	3.11 (2.14-4.52)	**<0.001**	3.74 (1.18-11.89)	**0.025**
N3	2	0.00 (0.00-Inf)	0.994	0.00 (0.00-Inf)	0.996
M stage					
M0	352	Reference			
M1	25	2.14 (1.25-3.65)	**0.006**	1.09 (0.34-3.53)	0.887
Clinical pathologic stage					
Stage I	290	Reference			
Stage II	121	2.42 (1.69-3.46)	**<0.001**	0.57 (0.21-1.59)	0.284
Stage III	81	3.54 (2.44-5.15)	**<0.001**	0.58 (0.18-2.11)	0.405
Stage IV	26	3.79 (2.19-6.55)	**<0.001**		
Primary therapy outcome					
Progressive disease	71	Reference			
Stable disease	37	0.30 (0.15-0.62)	**0.001**	0.33 (0.11-0.95)	**0.041**
Partial response	5	0.70 (0.17-2.90)	0.625	1.79 (0.39-8.17)	0.452
Complete response	326	0.27 (0.19-0.39)	**<0.001**	0.23 (0.13-0.40)	**<0.001**
Residual tumor					
R0	347	Reference			
R2&R1	16	3.88 (2.17-6.94)	**<0.001**	1.93 (0.67-5.57)	0.223
Anatomic neoplasm subdivision					
Left	200	Reference			
Right	312	1.04 (0.77-1.40)	0.810		
Another anatomic subdivision					
Central Lung	62	Reference			
Peripheral Lung	120	0.91 (0.57-1.46)	0.706		
Age	516				
<=65	255	Reference			
>65	261	1.22 (0.92-1.64)	0.172		
CDCA4					
Low	262	Reference			
High	264	1.55 (1.16-2.07)	**0.003**	1.70 (1.03-2.79)	**0.037**

In addition, multivariate analysis was conducted to indicate increased expression of CDCA4 with high diagnostic performance for OS (HR:1.70; 95%CI: 1.03, 2.79; P =0.037, Table 3).

A Clinical Nomogram on the Value of Expression of CDCA4

According to the survival analyses, the researchers conducted a nomogram to establish the 1, 3, and 5 years survival models that can help clinicians make the most appropriate prognosis of LUAD.

The status of tumour, main treatment result, and the expression of CDCA4 were performed to set as a clinical prognostic risk score for LUAD (Fig. 5A), which were independent clinical variables of LUAD patients that related to the survival outcome. The concordance index (C-index) is 0.722, revealing that the nomogram model provided a favourable predicted worth for OS in adenocarcinoma of lung.

Meanwhile, the precision of the nomogram was analysed via a calibration chart (Fig. 5B). The outcome indicated that the role of CDCA4 ex-

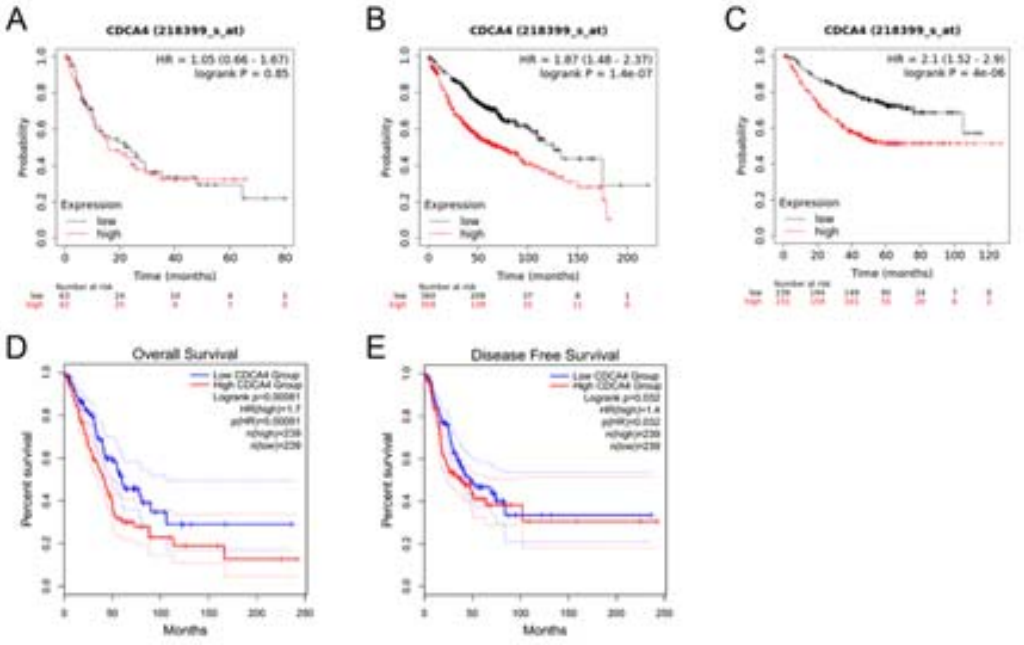

Fig. 5. Nomogram model was established to predict the survival outcomes. Nomogram model (A) and its calibration plot (B) to predict 1-, 3-, and 5-year OS.

pression could be set as a better predict element for the LUAD patients' survival outcome.

The Predictive Accuracy of New Models

The AUC were 0.797, 0.746, 0.704 for 1, 3, 5-year OS outcomes respectively (Figure 6A-6C). Adding to the specificity and sensitivity, DCA curves were commonly performed to evaluate the specific model's clinical utility by investigating the value of benefits compared with harms (Fig. 6D).

DISCUSSION

LUAD has high of recurrence rate and poor survival efficacy. Lately, the extensive research on the prognostic biomarkers of LUAD have remained rare. Thus, it is necessary and meaningful to develop a novel prognostic tool with clinical diagnosis significance and prognostic value for LUAD (Chen et al. 2020).

CDCA4 is a target gene of E2F transcription factors that regulate the cell cycle and proliferation (Pang et al. 2019; Tategu et al. 2008). In Li's research, the result indicated that CDCA4/6 thought to be the potential targets for gastric tumor (Li et al. 2021). Also another study has indicated that CDCA4 might have underlying diagnostic and prognostic worth for liver cancer (Chen et al. 2020). In the latest study, over-expressed CDCA4 was associated with the unfavourable clinicopathological elements and poor prognosis (Tan et al. 2022). Thus, CDCA4 was selected as the study object to assess the essentiality, diagnosis value, and potential mechanism in LUAD based on the TCGA database.

In order to help afford a certain rationale, resulting in making an early diagnosis, prognosis evaluation, and specific therapy for LUAD, firstly, the researchers demonstrated that the CDCA4 expression was over-regulated in LUAD issue versus normal and adjacent normal issue, revealing that CDCA4 acted as an imperative factor in

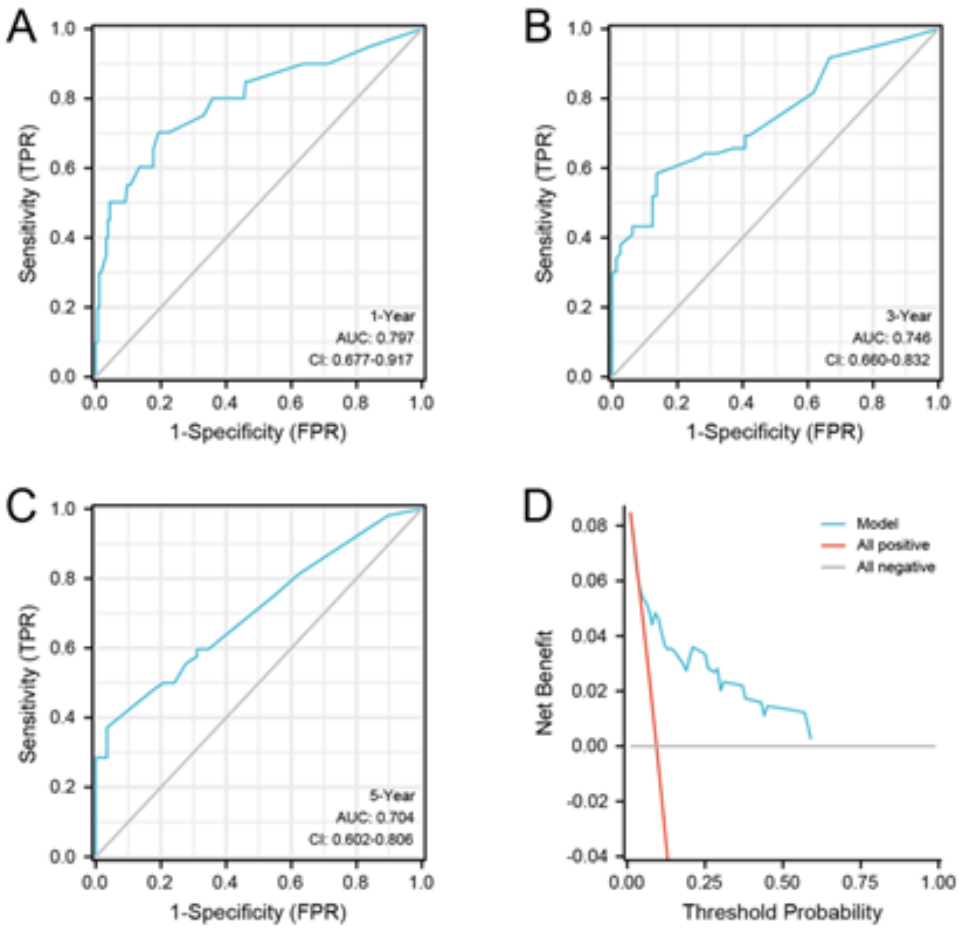

Fig. 6. The ROC curves of the models based on the C-index. Curves of 1-year (A), 3-year (B), and 5-year (C) OS outcomes. (D) The DCA curves indicated the clinical value of the model by the Riskscore.

tumorigenesis and development. Besides, the ROC analysis showed that CDCA4 may be acted as a novel diagnostic marker to differentiate the LUAD and normal cases. The consistent findings have been demonstrated in patients with gastric cancer (Li et al. 2021).

Furthermore, over-expressed CDCA4 was associated with the poorer TNM stage, clinical pathologic stage, older, and the more obvious residual tumour.

According to survival analysis, the overexpression of CDCA4 was correlated with both worse OS and DFS, indicating that CDCA4 may act as a new diagnostic biomarker for prediction of LUAD patients' survival.

Moreover, TNM stage, clinical pathologic stage, primary treatment result, residual tumour and high-level expression of CDCA4 were significant independent elements for OS in univariate Cox analysis, while the result of the multivariate cox analysis shown that the upregulation of CDCA4 was independent risk factor for OS.

Then, the researchers provided a new graphical prognostic nomogram combining CDCA4

expression with clinicopathological data exhibiting the satisfactory potential for clinical application. For example, a R0 excisional (0 points) LUAD patient with AJCC stage T2 (2.5 points) N+(30 points) M0 (0 points) and over-expressed CDCA4 (22.5 points) had 55 points totally. The 3 and 5-year predicted OS rates would be about 65 percent and 30 percent, separately. These estimates might be effective in predicting the prognosis of LUAD.

The time-dependent ROC curves indicated the novel model had a well predictive accuracy. The DCA graph also revealed a well clinical worth for predicting OS with the new model.

Above all, CDCA4 has been revealed to act as a valuable hypothetical biomarker associated with an unfavourable prognosis in LUAD. The mechanism of CDCA4 affecting results in LUAD still awaits systematic investigation in future. CDCA4 has shown to be associated with the regulation of genes determining the hematopoietic stem and progenitor cells' growth and differentiation (Abdullah et al. 2001). Previous study (Xu et al. 2018) has shown that CDCA4 promoted breast carcinoma cells proliferation and decreased apoptosis in vitro.

This research also revealed that there is a marked relation between the CDCA4 expression and some immune cells infiltration, including Th2 cells, CD56 (dim) NK cells, T helper cells etc. Further work will be necessary in order to establish whether CDCA4 can play an essential part in regulating LUAD immune infiltration.

So far, the specific mechanism of CDCA4 in LUAD has not been discussed deeply. The further articles need to be fully investigated.

The research has some advantages. Firstly, the study is the first nomogram combining clinicopathological characteristics and CDCA4 for predicting survival in LUAD patients. Secondly, the researchers conducted the time-dependent ROC curve and DCA curves to analysis the prognostic capacity of the novel model. The findings indicated that combining the CDCA4 expression and patients' characteristics had ideal prognostic capacity for survival outcomes.

There are still some limitations in the study. One is that the novel model relied on only one dataset, and the results have not been verified by experimental verifications or clinical data by the researchers. Thus, further laboratory experiments are essential to evaluate the mechanisms of CDCA4 in LUAD. Another limitation is that the clinical information is limited and does not contain other hypothetical risk parameters. Finally, no external data were used to validate the predictive accuracy of nomograms. Moreover, the prognostic value of CDCA4 in adenocarcinoma of lung needs to be validated in future.

CONCLUSION

In conclusion, the above results indicated the over-expressed CDCA4 in lung adenocarcinoma than normal tissues, which might be linked to prognosis. The over-expressed CDCA4 in LUAD patients was markedly correlated with advanced clinical data. Survival analyses showed that increased the expression of CDCA4 indicated poor survival in LUAD as an independent risk factor. Adding the expression signature of CDCA4 to the clinical nomogram promotes the prognostic value in LUAD, especially for overall survival prediction. The model needs further validation in further external research.

RECOMMENDATIONS

More specific functions of CDCA 4 in the progression of LUAD are expected to be discovered in the near future. Its effect mechanism in lung disorders warrants further study.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

FUNDING SOURCES

None.

AUTHOR CONTRIBUTIONS

The researchers declare that all the listed authors have participated actively in the research and all meet the requirements of the authorship. Dr. SMH designed the study and wrote the paper, Dr. WFZ performed research and contributed important reagents, Dr. LJS managed the literature searches and analyses, and Dr. PJL undertook the statistical analysis.

DATA AVAILABILITY STATEMENT

The datasets used or analysed during the current research are available from the corresponding authors on reasonable request.

REFERENCES

Abdullah JM, Jing X, Spassov DS, Nachtman RG, Jurecic R 2001. Cloning and characterization of Hepp, a novel gene expressed preferentially in hematopoietic progenitors and mature blood cells. *Blood Cells Mol Dis,* 27(3): 667-676. doi: 10.1006/bcmd.2001.0434

Chen C, Chen S, Pang L, Yan H, Luo M, Zhao Q et al. 2020. Analysis of the expression of cell division cycle-associated genes and its prognostic significance in human lung carcinoma: A review of the literature databases. *Biomed Res Int,* 2020: 6412593. doi: 10.1155/2020/6412593

Foucher Y, Danger R 2012. Time dependent ROC curves for the estimation of true prognostic capacity of microarray data. *Stat Appl Genet Mol Biol,* 11(6): Article 1. doi: 10.1515/1544-6115.1815

Gibson AJW, Li H, D'Silva A, Tudor RA, Elegbede AA, Otsuka S et al. 2019. Comparison of clinical characteristics and outcomes in relapsed versus De Novo Metastatic non-small cell lung cancer. *Am J Clin Oncol,* 42(1): 75-81. doi: 10.1097/COC.0000000000000483

Hayashi R, Goto Y, Ikeda R, Yokoyama KK, Yoshida K 2006. CDCA4 is an E2F transcription factor family-induced nuclear factor that regulates E2F-dependent transcriptional activation and cell proliferation. *J Biol Chem,* 281(47): 35633-35648. doi: 10.1074/jbc.M603800200

Herbst RS, Morgensztern D, Boshoff C 2018. The biology and management of non-small cell lung cancer. *Nature,* 553(7689): 446-454. doi: 10.1038/nature25183

Kobayashi Y, Takano A, Miyagi Y, Tsuchiya E, Sonoda H, Shimizu T et al. 2014. Cell division cycle-associated protein 1 overexpression is essential for the malignant potential of colorectal cancers. *Int J Oncol,* 44(1): 69-77. doi: 10.3892/ijo.2013.2177

Li Z, Liu Z, Li C, Liu Q, Tan B, Liu Y et al. 2021. CDCA1/2/3/5/7/8 as novel prognostic biomarkers and CDCA4/6 as potential targets for gastric cancer. *Transl Cancer Res,* 10(7): 3404-3417. doi: 10.21037/tcr-20-1050

Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q et al. 2020. TIMER2.0 for analysis of tumor-infiltrating immune cells. *Nucleic Acids Res,* 48(W1): W509-W514. doi: 10.1093/nar/gkaa407

Nagy A, Lanczky A, Menyhart O, Gyorffy B 2018. Validation of miRNA prognostic power in hepatocellular carcinoma using expression data of independent datasets. *Sci Rep,* 8(1): 9227. doi: 10.1038/s41598-018-27521-y

Pang S, Xu Y, Chen J, Li G, Huang J, Wu X 2019. Knockdown of cell division cycle-associated protein 4 expression inhibits proliferation of triple negative breast cancer MDA-MB-231 cells in vitro and in vivo. *Oncol Lett,* 17(5): 4393-4400. doi: 10.3892/ol.2019.10077

Phan NN, Wang CY, Li KL, Chen CF, Chiao CC, Yu HG et al. 2018. Distinct expression of CDCA3, CDCA5, and CDCA8 leads to shorter relapse free survival in breast cancer patient. *Oncotarget,* 9(6): 6977-6992. doi: 10.18632/oncotarget.24059

Rau A, Flister M, Rui H, Auer PL 2019. Exploring drivers of gene expression in the Cancer Genome Atlas. *Bioinformatics,* 35(1): 62-68. doi: 10.1093/bioinformatics/bty551

Siegel RL, Miller KD, Fuchs HE, Jemal A 2021. Cancer Statistics, 2021. *CA Cancer J Clin,* 71(1): 7-33. doi: 10.3322/caac.21654

Steyerberg EW, Vickers AJ 2008. Decision curve analysis: A discussion. *Med Decis Making,* 28(1): 146-149. doi: 10.1177/0272989X07312725

Shi R, Zhang C, Wu Y, Wang X, Sun Q, Sun J et al. 2017. CDCA2 promotes lung adenocarcinoma cell proliferation and predicts poor survival in lung adenocarcinoma patients. *Oncotarget,* 8(12): 19768-19779. doi: 10.18632/oncotarget.15519

Tan J, Chen F, Ouyang B, Li X, Zhang W, Gao X 2022. CDCA4 as a novel molecular biomarker of poor prognosis in patients with lung adenocarcinoma. *Front Oncol,* 12: 865756. doi: 10.3389/fonc.2022.865756

Tang Z, Kang B, Li C, Chen T, Zhang Z 2019. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic Acids Res,* 47(W1): W556-W560. doi: 10.1093/nar/gkz430

Tategu M, Nakagawa H, Hayashi R, Yoshida K 2008. Transcriptional co-factor CDCA4 participates in the regulation of JUN oncogene expression. *Biochimie,* 90(10): 1515-1522. doi: 10.1016/j.biochi.2008.05.014

Thang PM, Takano A, Yoshitake Y, Shinohara M, Murakami Y, Daigo Y 2016. Cell division cycle associated 1 as a novel prognostic biomarker and therapeutic target for oral cancer. *Int J Oncol,* 49(4): 1385-1393. doi: 10.3892/ijo.2016.3649

Vickers AJ, Elkin EB 2006. Decision curve analysis: A novel method for evaluating prediction models. *Med Decis Making,* 26(6): 565-574. doi: 10.1177/0272989X06295361

Wu B, Huang Y, Luo Y, Ma A, Wu Z, Gan Y et al. 2020. The diagnostic and prognostic value of cell division cycle associated gene family in Hepatocellular Carcinoma. *J Cancer,* 11(19): 5727-5737. doi: 10.7150/jca.46554

Xu Y, Wu X, Li F, Huang D, Zhu W 2018. CDCA4, a downstream gene of the Nrf2 signaling pathway, regulates cell proliferation and apoptosis in the MCF7/ADM human breast cancer cell line. *Mol Med Rep,* 17(1): 1507-1512. doi: 10.3892/mmr.2017.8095

Xiao B, Chen L, Ke Y, Hang J, Cao L, Zhang R et al. 2018. Identification of methylation sites and signature genes with prognostic value for luminal breast cancer. *BMC Cancer,* 18(1): 405. doi: 10.1186/s12885-018-4314-9

Zheng Y, Heagerty PJ 2004. Semiparametric estimation of time-dependent ROC curves for longitudinal marker data. *Biostatistics,* 5(4): 615-632. doi: 10.1093/biostatistics/kxh013

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