DCK is an Unfavorable Prognostic Biomarker and Correlated With Immune Infiltrates in Liver Cancer

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Abstract

Background: The biological function of deoxycytidine kinase in tumor is not yet clear, and there are a few studies relating to the correlation of deoxycytidine kinase gene with the occurrence and development of liver cancer. **Methods:** The messenger RNA expression of deoxycytidine kinase was analyzed with the use of the UALCAN and GEPIA database. Moreover, we assessed the function of deoxycytidine kinase on clinical prognosis with Kaplan-Meier plotter database. The relationship between deoxycytidine kinase and cancer immune infiltrates was investigated via Tumor Immune Estimation Resource site. Furthermore, Tumor Immune Estimation Resource was also used to evaluate the correlations between the expression of deoxycytidine kinase and gene marker sets of immune infiltrates. **Results:** The deoxycytidine kinase messenger RNA level significantly upregulated in patients with liver cancer compared to normal liver samples. Moreover, the increased expression of deoxycytidine kinase messenger RNA was closely associated with reduced overall survival and disease-free survival in all liver cancers. In addition, deoxycytidine kinase expression displayed a strong correlation with infiltrating levels of macrophages, neutrophils, and dendritic cells in liver cancer, and deoxycytidine kinase expression was positively correlated with diverse immune marker sets in liver cancer. **Conclusions:** All the above findings suggested that increased expression of deoxycytidine kinase was significantly related to unfavorable prognosis in patients with liver cancer. And deoxycytidine kinase is correlated with immune infiltrating levels, including those of B cells, macrophages, neutrophils, and dendritic cells in patients with liver cancer. These findings suggest that deoxycytidine kinase can be used as a prognostic biomarker for determining prognosis and immune infiltration in liver cancer. And deoxycytidine kinase is a potential target for liver cancer therapy, and these preliminary findings require further study to determine whether deoxycytidine kinase-targeting reagents might be developed for clinical application in liver cancer.

Keywords

liver cancer, DCK, overall survival (OS), relapse-free survival (RFS), KM plotter, tumor-infiltrating immune cells

Abbreviations

DCK, deoxycytidine kinase; DCs, dendritic cells; GEPIA, Gene Expression Profiling Interactive Analysis; HR, hazard ratio; KM, Kaplan-Meier; mRNA, messenger RNA; NK, natural killer; OS, overall survival; RFS, relapse-free survival; TAMs, tumor-associated macrophages; TCGA, The Cancer Genome Atlas; Tfh, follicular helper T; Th1, T-helper 1; Th17, T-helper 17; Th2, T-helper 2; TIICs, tumor-infiltrating immune cells; TIL, tumor-infiltrating lymphocytes; TIMER, Tumor Immune Estimation Resource; Tregs, regulatory T cells.

Introduction

The new global cancer statistics presented that liver cancer ranks second among the deaths which arise from cancer.[1] Liver cancer affects more than 500 000 people worldwide.[2] The occurrence rate of liver cancer in Asian countries is higher due to the infection of hepatitis B virus and hepatitis C virus.

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The prognosis of liver cancer is poor if it is not detected early. Thus, it's urgent for us to explore mechanisms that result in the incidence of liver cancer and identify some biomarkers that can be used for early detection of liver cancer as well as new approaches to its treatment.

Plenty of studies displayed that immune-related mechanisms play a significant role in the development of liver cancer, and immune therapeutic strategies are considered as a promising direction for the treatment of liver cancer.[3-5] Immune therapy, such as programmed death ligand-1 inhibitors, programmed death-1, showed promising anticancer effects in liver cancer.[6-8] Furthermore, many studies have revealed that the tumor-associated macrophages (TAMs) and tumor-infiltrating lymphocytes (TIL) affect the prognosis and curative efficacy of chemotherapy and immunotherapy.[9-13] Thus, it's vital for us to explore the detail of the immune phenotypes of tumor–immune interactions and identify new immune-related therapeutic targets in liver cancer.

We did transcriptome sequencing between normal liver tissue and hepatocellular carcinoma tissue. According to the sequencing results, we got the data of high expression of deoxycytidine kinase (DCK) in hepatocellular carcinoma tissue. And then we used UALCAN and GEPAI database to verify the messenger RNA (mRNA) expression level of DCK in liver cancer and normal liver sample.

With some bioinformatics web tools (GEPIA, UALCAN, the Human Protein Atlas and Kaplan-Meier [KM] plotter database), we analyzed the relationship between the mRNA expression level of DCK and the prognosis of patients with liver cancer. At the same time, to examine the association between DCK and the tumor immune infiltrating cells, we used Tumor Immune Estimation Resource (TIMER; cistrome.shinyapps.io/timer) to check the relationship between them.

We found that high mRNA expression of DCK was an adverse prognostic factor in patients with liver cancer, and DCK had a close correlation with immune infiltrates including B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells (DCs) in liver cancer. Our findings verified the key role of DCK in liver cancer and provide a potential relationship and an underlying mechanism between DCK and tumor–immune interactions.

Deoxycytidine kinase is a pyrimidine salvage enzyme that plays an important role in the phosphorylation of several deoxynucleoside analogs, which are universally used as anticancer and antiviral agents. The activity of DCK widely varied in different cancer cells and tissues.[14,15] And the deficiency of DCK leads to a resistance to deoxycytidine and deoxyadenosine analogs.[16] While the role of DCK in the development of tumorigenesis had been rarely reported, some reports had pointed out that knockdown of DCK facilitates apoptosis and inhibited proliferation and tumorigenicity *in vivo* in cervical cancer HeLa cells[17] and in pancreatic cancer.

Deoxycytidine kinase negatively regulated the proliferation and metastasis of cancer cells.[18] However, the role of DCK in liver cancer remains poorly understood.

Materials and Methods

Analysis of the DCK Expression Level Between Liver Cancer and Normal Liver Sample

UALCAN (http://ualcan.path.uab.edu) was an online tool whose data come from The Cancer Genome Atlas (TCGA) level RNA-seq and clinical data with 31 cancer types. And it analyzed the relative expression of a particular gene in different cancer subgroups based on individual cancer stages, age, gender, race, or other clinic pathologic characters.[19] With UALCAN, we evaluated the mRNA expression level and methylation level of DCK in liver cancer and normal liver sample. Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia.cancer-pku.cn/index.html) is an interactive web that contains 9736 tumors sample and 8587 normal samples from TCGA and the GTEx projects.[20] Gene Expression Profiling Interactive Analysis was utilized to generate survival curves, including overall survival (OS) and disease-free survival, based on gene expression with the log-rank test and the Mantel-Cox test in 33 different types of cancer. Although the data of the 2 platforms above are all from the TCGA database, we still chose these 2 databases for verification to make our results more reliable because different databases use different encodings.

Survival Analysis of DCK in Liver Cancer

Kaplan-Meier plotter was an online database established with gene expression data and survival information of patients with cancer downloaded from the intergovernmental Group on Earth Observations (GEO). Currently, liver cancer, gastric cancer, ovarian cancer, and lung cancer databases have been provided.[21-25] The database includes many clinical data such as cancer stage, grade, gender, and smoking history, and treatment groups contain surgery, chemotherapy, and radiotherapy. Deoxycytidine kinase was entered into the KM plotter database (http://kmplot.com/analysis/) to obtain survival plots, the high and low expression groups were classified according to the mRNA expression above or below the median. These cohorts were compared with a KM survival plot, and hazard ratio (HR), 95% CI, and log-rank P value were determined and displayed on the web page. A P value $<.05$ was regarded as statistically significant. The Human Protein Atlas (www.proteinatlas.org) measured the RNA level, as well as used antibody profiling to precisely localize the corresponding proteins across 32 human tissues.[26] With the KM plotter, Human Protein Atlas, GEPIA, and UALCAN, we verified the association of DCK expression and prognosis of patients with liver cancer.

Analysis of the Connection of DCK Expression Level and Immune Infiltrates

Tumor Immune Estimation Resource (cistrome.shinyapps.io/timer) is an online database that contains 10 897 samples which cover 32 cancer types from TCGA to evaluate the richness of immune infiltrates and provide a systematic analysis of immune infiltrates across diverse cancer types.[27] Tumor Immune Estimation Resource uses a deconvolution previously

published statistical method to evaluate the abundance of tumor-infiltrating immune cells (TIICs) from gene expression profiles.[28] We analyzed the correlation of DCK expression with the abundance of immune infiltrates including B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and DCs in gene modules. And correlations between the expression of DCK and TIICs gene markers were explored via correlation modules. The gene markers of TIICs included markers of B cells, T cells (general), CD8+ T cells, monocytes, M1 macrophages, M2 macrophages, TAMs, neutrophils, natural killer (NK) cells, DCs, follicular helper T (Tfh) cells, T-helper 1 (Th1) cells, T-helper 2 (Th2) cells, T-helper 17 (Th17) cells, regulatory T cells (Tregs), and exhausted T cells. These gene markers are referenced in previous studies.[29-31] The correlation module which generated the expression scatter plots between a pair of user-defined genes in different type cancer, together with the Spearman correlation and the estimated statistical significance. Deoxycytidine kinase was used for the x-axis with gene symbols, and TIICs-related marker genes are put in the y-axis. The gene expression level was displayed with log2 RSEM.

Statistical Analysis

Survival curves and the results generated by KM plots, UALCAN, the Human Protein Atlas, and GEPIA are shown with HR and P or Cox P values from a log-rank test. Unpaired T test was used for the comparison between 2 mean values. The correlation of gene expression was evaluated by Spearman correlation and statistical significance, and the strength of the correlation was determined using the following guide for the absolute value: 0.00 to 0.19 "very weak," 0.20 to 0.30 "moderate, and "0.30 to 0.50 "strong." Significance was defined at ***P < .001, **P < .01, and *P < .05.

Results

Deoxycytidine Kinase Generally Increased Expressed in Liver Cancer

We used UALCAN (http://ualcan.path.uab.edu/index.html) and GEPIA to check the mRNA expression level of DCK between liver cancer tissue and normal mammary tissue. The result displayed that DCK was significantly upregulated in liver cancer tissue compared to normal liver samples (Figure 1A and B). And Figure 1C displayed that the promoter methylation level of DCK is higher in normal liver tissue than in liver cancer tissue (P < .01).

Overexpression of DCK Was an Unfavorable Prognostic Factor in Liver Cancer

The prognosis value of DCK was analyzed by using 4 different online data sets, KM plots, GEPIA, UALCAN, and the Human Protein Atlas databases.

To investigate the prognostic values of DCK in patients with liver cancer, we used the KM plotter bioinformatics analysis platform (http://kmplot.com/analysis/) to evaluate the association between the mRNA expression level of DCK and the ending of patients with liver cancer. A total of 364 patients were available for the analysis of OS and relapse-free survival (RFS). Moreover, the gender, stage, histone grade, vascular invasion status, alcohol consumption, and hepatitis virus state were collected and assessed. The survival curves revealed that in patients with liver cancer, the high expression level of DCK (RNA-SeqID:1633) indicated worse prognosis as Figure 2A and B showed high expression of DCK resulted in shorter OS (HR = 1.90, 95% CI = 1.32-2.72, P value = .00043) and RFS (HR = 1.53, 95% CI = 1.10-2.13, P value = .011). Furthermore, by performing GEPIA, UALCAN, and the Human Protein Atlas, we explored the association of DCK expression and prognosis of patients with liver cancer. As shown in Figure 2C and E, DCK high expression presented shorter OS (P < .05).

To further confirm DCK's prognosis value in liver cancer, we used the pathology atlas of HPA to analyze the expression and prognosis. As shown in Figure 2F, at best expression cutoff 3.69%, 229 low DCK patients showed better outcomes compared with 136 high DCK patients (P = 6.9e-7).

Prognosis in Patients With mRNA Expression of DCK and Patient Clinicopathological Features

In order to better understand the potential mechanisms and correlation of DCK expression in liver cancer, we investigated the association of the DCK expression and clinical characteristics in patients with liver cancer by performing the KM plotter databases. The clinicopathological features of patients with liver cancer such as the gender, stage, histone grade, vascular invasion status, alcohol consumption, and hepatitis virus were collected and assessed. High DCK expression was an adverse factor resulted in reduced patient OS (HR = 1.85, 95% CI = 1.01-3.36, P value = .042, Table 1) while the RFS (HR = 1.52, 95% CI = 0.84-2.76, P value = .16, Table 1) has no obvious difference in female patients with liver cancer. In male patients with liver cancer, high DCK expression indicated shorter patient OS (HR = 2.37, 95% CI = 1.52-3.69, P value = 9.2e-05, Table 1) and RFS (HR = 1.67, 95% CI = 1.09-2.54, P value = .017, Table 1).

In patients having liver cancer with hepatitis virus, high DCK expression led to reduced patient OS (HR = 2.13, 95% CI = 1.12-4.08, P value = .019, Table 1) and RFS (HR = 1.70, 95% CI = 1.03-2.80, P value = .035, Table 1) .While in patients having liver cancer without hepatitis virus, high DCK expression was also an unfavorable factor that indicated worse OS (HR = 2.08, 95% CI = 1.28-3.37, P value = .0026, Table 1), and the RFS (HR = 1.32, 95% CI = 0.84-2.07, P value = .23, Table 1) had no significant difference.

As for patients with alcohol consumption, high DCK expression was a bad factor because of reduced patient OS (HR = 1.55, 95% CI = 0.77-3.12, P value = 0.21, Table 1) and RFS (HR = 2.16, 95% CI = 1.09-4.29, P value = .024, Table 1). As for patients without alcohol consumption, high DCK expression means shorter OS (HR = 2.08, 95% CI = 1.28-3.37, P value = .0026, Table 1) and RFS (HR = 1.32, 95% CI = 0.84-2.07, P value = 0.23, Table 1).
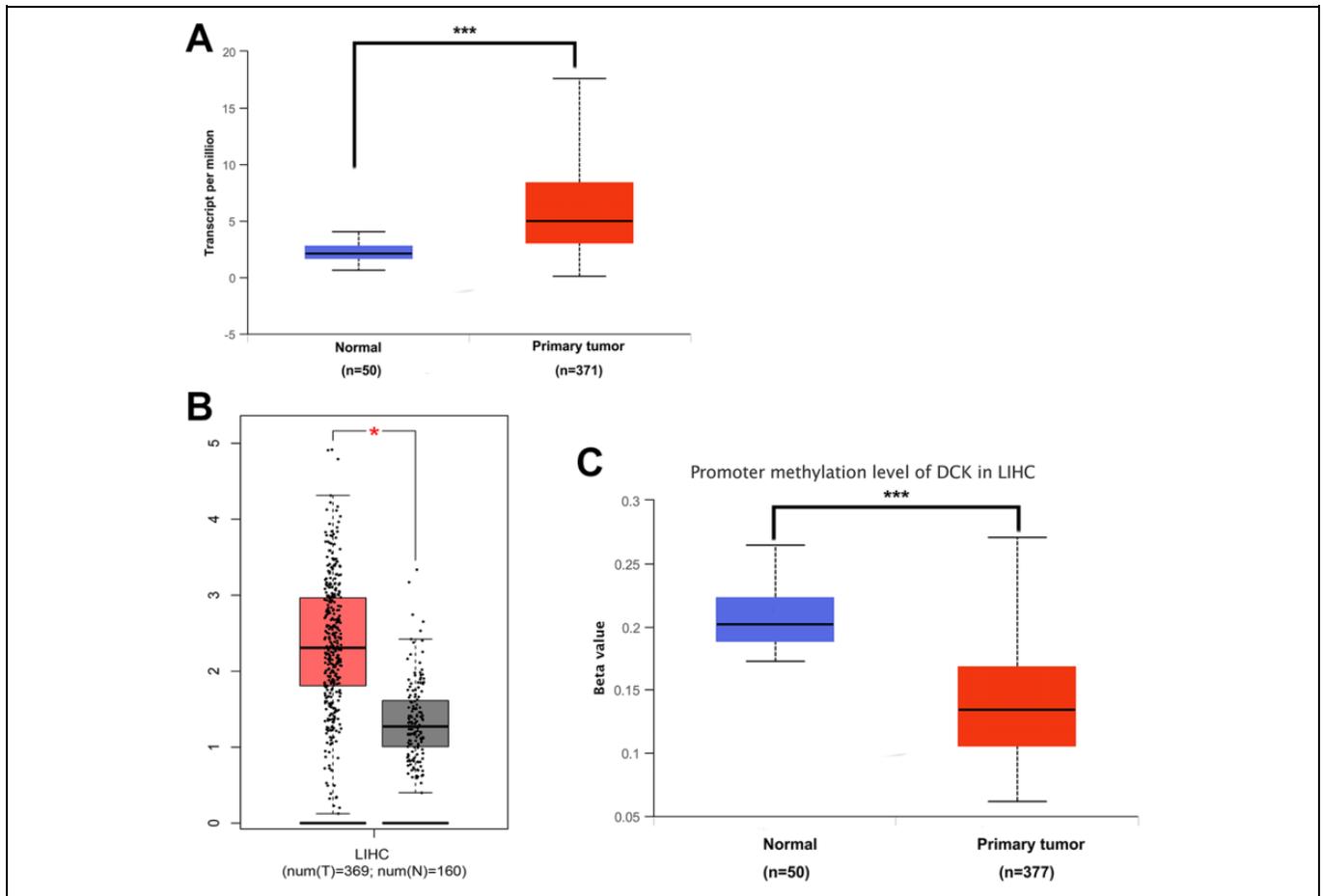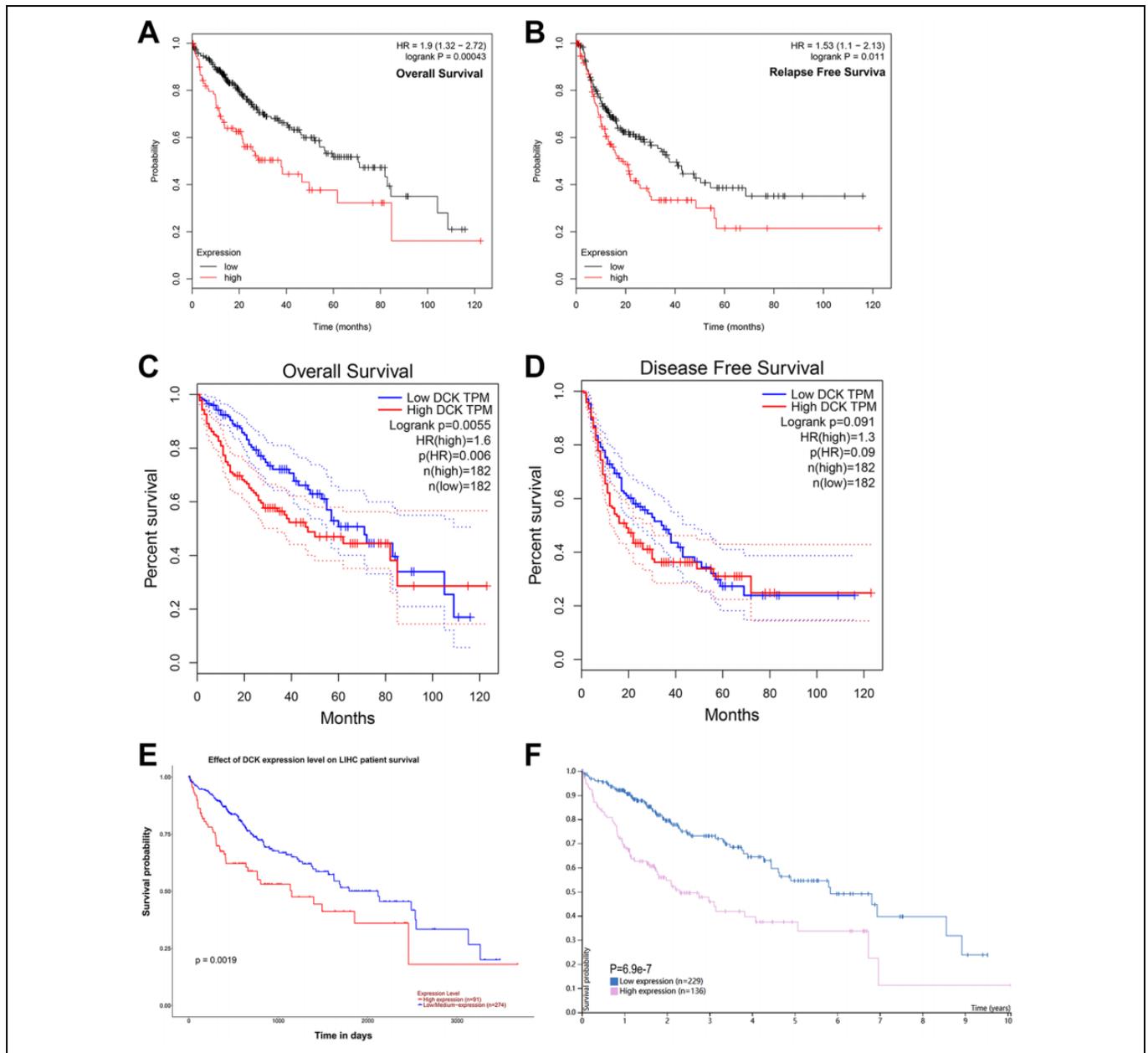
Figure 1. The expression level of DCK in liver cancer and normal breast cancer. A and B, The expression of DCK was higher in liver cancer compared to normal liver tissues. Data derived from UALCAN and GEPIA database. C, The promoter methylation level of DCK is higher in normal liver tissue than in liver cancer tissue. Data derived from UALCAN database. DCK indicates deoxycytidine kinase; GEPIA, Gene Expression Profiling Interactive Analysis.

In patients with vascular invasion, high DCK expression did not have great influences on the OS (HR = 2.1, 95% CI = 0.97-4.53, P value = .054, Table 1) nor RFS (HR = 1.59, 95% CI = 0.84-3.01, P value = .15, Table 1). In patients without vascular invasion, high DCK expression implied reduced patient OS (HR = 2.23, 95% CI = 1.33-3.76, P value = .0019, Table 1) as well as RFS (HR = 2.6, 95% CI = 1.33-5.1, P value = .0038, Table 1).

In patients with grade I liver cancer, higher DCK was an unfavorable prognostic factors as Table 1 presented shorter OS (HR = 5.71, 95% CI = 1.96-16.62, P value = 4e-04) while the RFS (HR = 0.46, 95% CI = 0.13-1.62, P value = .21) in patients with grade I liver cancer had no relationship with the expression level of DCK. In patients with grade II liver cancer, higher DCK means shorter OS (HR = 2.42, 95% CI = 1.42-4.13, P value = .00079) and RFS (HR = 2.04, 95% CI = 1.23-3.38, P value = .0046). In patients with grade III liver cancer, higher DCK complied with shorter OS (HR = 2.21, 95% CI = 1.17-4.19, P value = .012) and RFS (HR = 1.53, 95% CI = 1.1-2.13, P value = .011). While the number of patients in grade IV is too less to calculate the OS and RFS.

As presented in Table 1, in patients with stage I liver cancer, higher DCK means shorter OS (HR = 2.2, 95% CI = 1.19-4.08, P value = .0098) and RFS (HR = 1.88, 95% CI = 1.05-3.35, P value = .03). In patients with stage II liver cancer, higher DCK represented worse OS (HR = 2.21, 95% CI = 1.00-4.87, P value = .044) and RFS (HR = 1.58, 95% CI = 0.81-3.1, P value = 0.18). In patients with grade III liver cancer, higher DCK made no sense with the prognosis of patients with liver cancer as shown in Table 1 (OS: HR = 1.41, 95% CI = 0.78-2.54, P value = .25; and RFS: HR = 0.73, 95% CI = 0.4-1.34, P value = .31). And the patient number in stage IV is too less to calculate the OS and RFS. In order to further assess the prognostic value of DCK in patients with different stage liver cancer, we explored the association of the DCK expression level and tumor stages. In patients having liver cancer with tumor stage I + II, high DCK expression means unfavorable prognosis in patients with liver cancer as shown in Table 1 (OS: HR = 1.8, 95% CI = 1.1-2.95, P value = .017; RFS: HR = 1.69, 95% CI = 1.09-2.61, P value = .017). In patients having liver cancer with tumor stage III + IV, high expression of DCK had no relationship with the outcome of patients with liver cancer as shown in Table 1 (OS: HR

Figure 2. Prognostic value of DCK expression in patients with liver cancer. A and B, The survival curves revealed that in patients with liver cancer, the high expression level of DCK (RNA-SeqID:1633) indicated a worse prognosis. Data derived from Kaplan-Meier (KM) plotter database. C-F, DCK high expression presented worse outcomes in patients with liver cancer. Data derived from GEPIA, UALCAN, and the Human Protein Atlas database. DCK indicates deoxycytidine kinase.

= 1.38, 95% CI = 0.78-2.44, P value = .27; and RFS: HR = 0.73, 95% CI = 0.4-1.34, P value = .31).

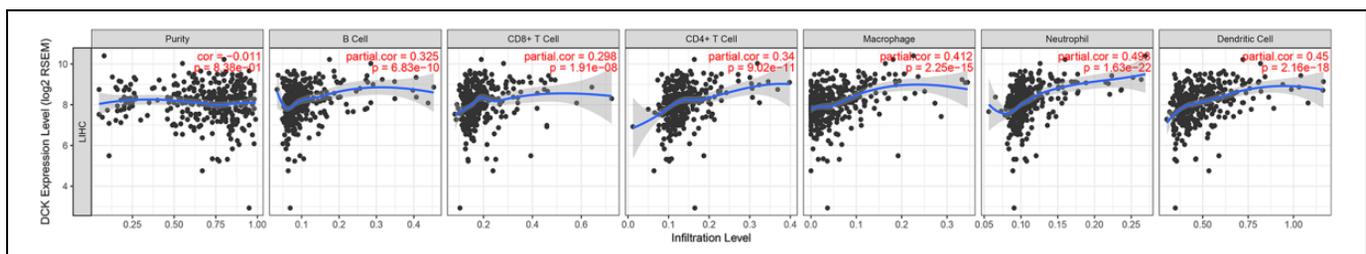## DCK Expression is Correlated With Immune Infiltration Level in Liver Hepatocellular Carcinoma

Many studies had reported that TIL is an independent predictor of lymph node status and survival in cancers.[32,33] Therefore, we assessed the associations of DCK expression with immune infiltration levels in liver hepatocellular carcinoma from the TIMER database. And the results showed that high DCK

expression level has positive correlations with infiltrating levels of B cell (r =0.325, P = 6.83e-10), CD8+ T cells (r = 0.298, P = 1.91e-08), CD4+ T cells (r = 0.34, P = 9.02e-11), macrophages (r = 0.412, P = 2.25e-15), neutrophil (r = 0.498, P = 1.63e-22), and DCs (r =0.45, P = 2.16e-18) in liver hepatocellular carcinoma (Figure 3). While DCK expression has no significant correlations with tumor purity (r = −0.011, P = 8.38e-01). These findings suggest that DCK may play a specific role in immune infiltration in liver hepatocellular carcinoma, especially those of B cell, macrophages, neutrophils, and DCs.

Table 1. Correlation of DCK mRNA Expression and Clinical Prognosis in Liver Cancer With Different Clinicopathological Factors by Kaplan-Meier Plotter.

Name	RNA-Seq ID		OS			RFS		
DCK	1633	Clinicopathological features	HR	95% CI	*P* value	HR	95% CI	*P* value
		Gender						
		Male	2.37	1.52-3.69	9.2e-05	1.67	1.09-2.54	.017
		Female	1.85	1.01-3.36	.042	1.52	0.84-2.76	.16
		Hepatitis virus						
		Yes	2.13	1.12-4.08	.019	1.7	1.03-2.8	.035
		No	2.08	1.28-3.37	.0026	1.32	0.84-2.07	.23
		Alcohol consumption						
		Yes	1.55	0.77-3.12	.21	2.16	1.09-4.29	.024
		No	2.08	1.28-3.37	.0026	1.32	0.84-2.07	.23
		Vascular invasion						
		Yes	2.1	0.97-4.53	.054	1.59	0.84-3.01	.15
		No	2.23	1.33-3.76	.0019	2.6	1.33-5.1	.0038
		Grade						
		I	5.71	1.96-16.62	4e-04	0.46	0.13-1.62	.21
		II	2.42	1.42-4.13	.00079	2.04	1.23-3.38	.0046
		III	2.21	1.17-4.19	.012	1.53	1.1-2.13	.011
		Stage						
		I	2.2	1.19-4.08	.0098	1.88	1.05-3.35	.03
		II	2.21	1-4.87	.044	1.58	0.81-3.1	.18
		III	1.41	0.78-2.54	.25	0.73	0.4-1.34	.31
		Stage						
		I + II	1.8	1.1-2.95	.017	1.69	1.09-2.61	.017
		III + IV	1.38	0.78-2.44	.27	0.73	0.4-1.34	.31

Abbreviations: DCK, deoxycytidine kinase; HR, hazard ratio; mRNA, messenger RNA; OS, overall survival; RFS, relapse-free survival.

Figure 3. Correlation of DCK expression with immune infiltration level in LIHC, high DCK expression level has positive correlations with infiltrating levels of B cell, CD8+ T cells, CD4+ T cells, macrophages, neutrophil, and DCs in liver hepatocellular carcinoma. While DCK expression has no significant correlations with tumor purity. DCs indicates dendritic cells; DCK, deoxycytidine kinase; LIHC, liver hepatocellular carcinoma.

Correlation Exploration Between DCK Expression and Immune Marker Sets

In order to further investigate the relationship between DCK expression and immune infiltrating cells in liver hepatocellular carcinoma, we assessed immune marker sets of various immune cells including T cells, CD8+ T cells, B cells, monocytes, TAMs, M1 and M2 macrophages, neutrophils, NK cells, and DC in liver hepatocellular carcinoma with the TIMER databases. Furthermore, we analyzed the different functional T cells, such as Tfh cells, Th17 cells, Th1 cells, Th2 cells, Tregs, and exhausted T cells, because tumor purity is an important factor that affects the analysis results of immune infiltration in tumor samples by genomic approaches.[34]

With the correlation adjustment by tumor purity, the results displayed that DCK expression level had a close connection with most immune marker sets of various immune cells in liver hepatocellular carcinoma (Table 2). Specifically, we showed Neuropilin 1 (NRP1) of Th1 cells, Integrin Subunit Alpha M (ITGAM) of neutrophils, and CCR8 of Treg presented significantly correlate with DCK expression liver cancer ($P < .001$; correlation value >0.30).

Discussion

Deoxycytidine kinase plays a vital part in catalyzing the process of deoxyribonucleoside salvage, which is significant in maintaining normal DNA metabolism. Deoxycytidine kinase can also

Table 2. Correlation Analysis Between DCK and Related Genes and Markers of Immune Cells in TIMER.

Description	Gene markers	None Cor	None P	Purity Cor	Purity P
CD8+ T cell	CD8A	0.114	.033	0.125	.020
	CD8B	0.023	.672	0.020	.711
T cell (general)	CD3D	0.030	.578	0.028	.603
	CD3E	0.068	.206	0.076	.161
	CD2	0.054	.312	0.058	.286
B cell	CD19	0.153	.004	0.159	.003
	CD79A	0.043	.429	0.043	.427
Monocyte	CD86	0.218	.000	0.259	.000
	CSF1R	0.170	.002	0.200	.000
TAM	CCL2	0.116	.031	0.132	.014
	CD68	0.045	.407	0.045	.404
	IL10	0.147	.006	0.167	.002
M1 macrophage	NOS2	0.070	.194	0.069	.200
	IRF5	0.053	.329	0.052	.334
	PTGS2	0.221	.000	0.256	.000
M2 macrophage	CD163	0.175	.001	0.200	.000
	VSIG4	0.161	.003	0.183	.001
	MS4A4A	0.134	.013	0.154	.004
Neutrophils	CEACAM8	0.046	.397	0.045	.407
	ITGAM	0.345	.000	0.366	.000
	CCR7	0.045	.400	0.048	.376
Natural killer cell	KIR2DL1	0.026	.628	0.026	.637
	KIR2DL3	0.126	.019	0.127	.018
	KIR2DL4	0.138	.010	0.138	.010
	KIR3DL1	0.129	.016	0.129	.017
	KIR3DL2	0.009	.864	0.007	.898
	KIR3DL3	0.083	.123	0.083	.125
	KIR2DS4	0.008	.883	0.007	.890
Dendritic cell	HLA-DPB1	0.096	.074	0.106	.048
	HLA-DQB1	0.050	.358	0.050	.352
	HLA-DRA	0.227	.000	0.260	.000
	HLA-DPA1	0.195	.000	0.223	.000
	CD1C	0.088	.104	0.093	.085
Th1	NRP1	0.399	.000	0.411	.000
	ITGAX	0.194	.000	0.222	.000
	TBX21	0.002	.966	0.003	.955
	STAT1	0.264	.000	0.269	.000
	IFNG	0.122	.023	0.125	.020
	TNF	0.149	.006	0.162	.003
Th2	GATA3	0.156	.004	0.177	.001
	STAT6	0.002	.973	0.002	.965
	STAT5A	0.026	.636	0.023	.668
	IL13	0.058	.285	0.058	.287
Tfh	BCL6	0.106	.049	0.106	.050
	IL21	0.149	.005	0.150	.005
Th17	STAT3	0.119	.027	0.122	.023
	IL17A	0.106	.049	0.106	.050
Treg	FOXP3	0.277	.000	0.284	.000
	CCR8	0.514	.000	0.544	.000
	STAT5B	0.159	.003	0.162	.003
	TGFB1	0.129	.016	0.140	.009
T cell exhausted	PDCD1	0.066	.220	0.069	.204
	CTLA4	0.126	.019	0.134	.013

(continued)

Table 2. (continued)

Description	Gene markers	None Cor	None P	Purity Cor	Purity P
	LAG3	0.043	.420	0.048	.376
	HAVCR2	0.212	.000	0.250	.000
	GZMB	0.047	.388	0.054	.315

Abbreviations: Cor, correlated; DCK, deoxycytidine kinase; LIHC, liver hepatocellular carcinoma; TAM, tumor-associated macrophage; TIMER, Tumor Immune Estimation Resource; Th1, T-helper 1; Th2, T-helper 2; Tfh, follicular helper T; Th17, T-helper 17; Treg, regulatory T cells.

make many antiviral and anticancer nucleoside analogs to activation, such as fludarabine, gemcitabine, cladribine, and zalcitabine. For instance, in pancreatic cancer, DCK activates gemcitabine, and the decreased expression of DCK is thought of as a significant factor that governs gemcitabine resistance by reducing the level of the active gemcitabine form.[18]

Deoxycytidine kinase plays an important role in converting circular deoxynucleotides to deoxynucleotide triphosphates through a salvage deoxynucleotide biosynthetic pathway.[35]

Besides, DCK plays an important role in maintaining the normal physiological function of the deoxyuridine library and activating nucleoside prodrug analogs after phosphorylation, displaying high expressions in tissues.[36] In recent years, the research focus on DCK mainly lies in the relationship between the expression level of DCK and the drug resistance of tumor cells in chemoradiotherapy, and the research results revealed that there is a close correlation between the expression of DCK and drug resistance of tumor cells.[37,38] So far, the biological function of DCK in the process of tumorigenesis and tumor development is unclear, and few articles are studying the relationship between the DCK gene and the occurrence and development of liver cancer. Although the function of DCK in cancer has not been extensively studied, it is known that DCK activity is quite high in lymphoid tissues and upregulated in circulating B and T lymphocytes.[39,40] Here, we report that high mRNA expression level of DCK correlates with a worse prognosis in patients with liver cancer. Furthermore, our analyses show that in liver cancer, immune infiltration levels and diverse immune marker sets are correlated with levels of DCK expression. Thus, our study provides insights into understanding the potential role of DCK in tumor immunology and its use as a cancer biomarker in liver cancer.

In our study, we had shown that DCK was upregulated in patients with liver cancer (Figure 1A and B). In order to explain the reason for DCK higher expression in liver cancer sample, we detected the DNA methylation level of liver cancer and normal liver tissue and find the promoter methylation level of DCK is higher in normal liver tissue than in liver cancer tissue ($P < .01$; Figure 1C). There were many reasons account for genes increased expressed in cancers, such as gene amplification, DNA methylation level, and so on.[41-43] The

overexpression of some gene is a very complicated process, which may result from many factors. At present, we can only explain a possibility of high expression of DCK in liver cancer. And with KM plotter bioinformatics analysis platform (http://kmplot.com/analysis/), we found that DCK was an unfavorable predict factor in patients with liver cancer, especially in male patients (Figure 2 and Table 1), those having liver cancer with hepatitis virus (Table 1) those with alcohol consumption (Table 1) and in patients without vascular invasion (Table 1).

In terms of histone grade, patients who were in grade II and III, high expression of DCK means bad outcomes (Table 1). In addition, a high level of DCK mRNA expression was shown to be correlated with poor prognosis of liver cancer in stage I. And in patients with liver cancer who belong to stage I + II, elevated mRNA expression of DCK level indicated shorter survival time while the expression of DCK has no relationship with the prognosis of liver cancer who were in tumor stage III + IV (Table 1). Together, these findings strongly suggest that DCK is a prognostic biomarker in liver cancer.

To further verify the relationship between the DCK expression level and the prognosis of patients with liver cancer, we performed The Human Protein Atlas (www.proteinatlas.org) to detect the protein expression level of DCK in liver cancer tissue. The Human Protein Atlas showed a map of 32 human tissue protein expression levels.[44] With the Human Protein Atlas (www.proteinatlas.org), we detected the DCK protein expression in liver cancer tissues. And we found there was almost no DCK protein expression in liver cancer tissues. Thus, we thought DCK negatively expressed in liver cancer tissue in protein level, while DCK highly expressed in liver cancer tissue in the mRNA level. The above situation may be caused by the following reasons: gene expression is divided into 2 steps: transcription and translation, and these 2 steps have synthesis and degradation, respectively.[45] Thus, when the level of mRNA increases, it indicates that its synthesis or stability is enhanced, but this process may not necessarily be accompanied by an increase in the level of translation, so it is not uncommon that the level of protein expression may remain the same, and it is not uncommon that protein expression even declined in certain cases, but this seems to be relatively rare. In addition, some proteins can also reduce the translation level by regulating the untranslated region at the 3′-end. Similarly, the decrease of protein stability is also a very common reason, for example, the expression of p53 in acute myeloid leukemia is normal in mRNA, but the protein level is very low, and the increased expression of MDM2 is related to the degradation of p53.[46-48]

Another important discovery of our study is that we found DCK expression is correlated with various immune infiltration levels in liver cancer. Our results reveal that there are positive relationships between DCK expression level and infiltration level of B cell, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and DCs in liver cancer (Figure 3).

Moreover, the association of DCK expression and the marker genes of immune cells as shown in Table 2 implicated that DCK may play an important role in regulating tumor immunology in liver cancer. Also, we found that there is a clear correlation between the DCK expression and the regulation of some markers of neutrophils, T helper cells (Th1), and Treg in liver cancer, which may explain a potential mechanism that DCK regulates T cell and neutrophil functions. These results indicate that in liver cancer, DCK makes a great contribution to the recruitment and regulation of some immune infiltrating cells.

To sum up, to our knowledge, this article is the first report that reveals the relationship between the expression level of DCK and the prognosis of patients with liver cancer. Our results showed that in liver cancer, the higher expression of DCK has a significant correlation with a worse prognosis of patients with liver cancer.

Authors' Note

Shu Fang Hu and Xia Lin contributed equally to this work. There has no animal nor human studies in our study.

Declaration of Conflicting Interests

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