Dickkopf-1 is a Prognostic Biomarker and Correlated With Immune Infiltrates in Head and Neck Squamous Cell Carcinoma

Chaofen Zhao
Guizhou Medical University https://orcid.org/0000-0003-3149-0578
Lina Liu
Guizhou Medical University
Qianyong He
Guizhou Medical University
Yuanyuan Li
Guizhou Medical University
Jieqing Jiang
Guizhou Medical University
Yue Chen
Guilin Medical University
Xiaomei Li
Guizhou Agricultural Sciences and Technology Information: Guizhou Provincial Academy of Agricultural Sciences
Yaxue Tang
Guizhou Medical University
Yuxin Li
Guizhou Medical University
Dingan Zhou
Guizhou Medical University
Feng Jin (jinf8865@yeah.net)
Guizhou Medical University https://orcid.org/0000-0003-0238-9059

Research

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Abstract

Background: Head and neck squamous cell carcinoma(HNSCC) has a high level of immune infiltration, and immunotherapy generates new hope to patients with head and neck tumors. It is currently one of the most promising treatments for HNSCC. Previous studies have shown that Dickkopf-1 (DKK1), an inhibitor of Wnt signaling, is related to the prognosis of a variety of tumors. DKK1 is a key mediator of immune activation and is highly associated with tumor progress and tumor microenvironment. However, the association between DKK1 expression and the prognosis of HNSCC and tumor infiltrating lymphocytes is still unknown.

Objective: We aim to test the relationship between DKK1 and HNSCC prognosis and tumor infiltrating lymphocytes.

Methods: We explored the DKK1 expression differences in multiple tumor tissues and normal tissues via examing TIMER, Oncomine, and UALCAN databases. Then, we searched the Kaplan-Meier database to assess the correlation between DKK1 mRNA levels and clinical outcomes. Subsequently, the TIMER platform and TISIDB website were selected to evaluate the correlation between DKK1 and the level of tumor immune cell infiltration. We used cBioPortal and UCSC Xena database to further explore the reasons for the aberrant expression of DKK1 in tumorigenesis.

Results: We found that DKK1 level was significantly elevated in HNSCC tissues compared with normal tissues. The expression of DKK1 is related to the tumor stage, tumor grade, histology, lymph node metastasis and worse clinical prognosis of HNSCC. In addition, DKK1 is negatively correlated with different tumor-infiltrating immune cells in HNSCC. Moreover, DKK1 is closely related to the genetic markers of a variety of immune cells. Also, the prognosis of HNSCC is worse based on the high DKK1 expression of immune cells. Copy number variation (CNV) and DNA methylation may be the cause of abnormal up-regulation of DKK1 in HNSCC.

Conclusion: DKK1 can be used as a biomarker for predicting the prognosis and immune infiltration of HNSCC. These results provide us with important clues to better understand the role of DKK1 in the immunotherapy of head and neck tumors from the perspective of bioinformatics.This study provides a theoretical basis for clinical research on the treatment of head and neck squamous cell carcinoma with the DKK1 gene as a target. It may play a major role in advancing the immunotherapy of head and neck squamous cell carcinoma and enhance the therapeutic effect.

Introduction

HNSCC is the sixth most common cancer in the world[1]. More than 600,000 cases of HNSCC are diagnosed worldwide each year[2]. HNSCC accounts for 95% of head and neck cancers, occurring in the mouth, oropharynx, throat, or hypopharynx[3]. Despite the aggressive multimodal treatment methods, including surgical resection, chemotherapy, radiotherapy, targeted therapy and immunity therapy, the five year survival rate of HNSCC patients is less than 50%[4], mainly because local recurrence or distant

metastasis occurs in approximately 80–90% of patients with advanced HNSCC[5]. Therefore, in-depth research and exploration of the molecular mechanisms of head and neck squamous cell carcinoma and finding new therapeutic targets or potential biomarkers will provide more strategies for the treatment of HNSCC.

In recent years, an extensive number of studies have indicated that the tumor-infiltrating immune cells were associated with prognosis in HNSCC[6]. In addition, several studies have pointed out the numbers of infiltrating immune cells reflecting antitumor immune response in HNSCC are predictive of overall survival[6]. Deeply understanding the immune activity in the tumor microenvironment will offer clinicians with more precise prognostic information in the HNSCC. Multiple studies demonstrated that tumor infiltrating immune cells were related to tumorigenesis and progression in HNSCC[7]. Tumor-infiltrating lymphocytes, and especially T helper 1 cells, activate interferon-mediated signaling and induce expression of PD-L1 on cells in the tumor environment, which protects tumor cells from tumor-directed immunity in HNSCC[8]. Macrophage infiltration is pivotal for tumor immune evasion, angiogenesis, growth, and metastasis[9]. CD4 + CD25 + FoxP3 + Tregs in circulation have been associated with poor prognosis in HNSCC[10]. For HNSCC, previous studies suggested that high Treg counts are associated with better prognosis[11].These findings demonstrate that tumor infiltration of immune cells may be a useful drug target that improving clinical outcomes in HNSCC.

DKK1, a member of the DKK family, is an inhibitor of Wnt signaling pathway, which regulates diverse cellular and biological processes, including cell proliferation, invasion, migration, and apoptosis, metastasis through both β-catenin-dependent and independent mechanisms, in different tissues and numerous tumors[12]. Dysregulation of DKK1 has recently emerged as a potential biomarker of cancer progression and prognosis for several types of malignancies[13]. A recent study reveals that DKK1 impairs the tumor response to PD-1 blockade by inactivating CD8 + T cells in defcient mismatch repair colorectal cancer[14]. Another study shows that upregulation of DKK1 could modulate anti-tumor immune populations within the tumor microenvironment by decreasing CD45 + leukocyte infiltration and reducing NK and CD8 + T cells[15].To summarize, DKK1 had immunoregulatory effects, including promoting Th2 cells response, reducing the functionality of T cells through MDSC modulation and suppressing the proliferation of CD8 + T cells and NK cells, thus contributing to inflammatory response and cancer immune evasion[16].Thus, further research of DKK1 in oncology has garnered a lot of attention.DKK1 has multifaceted functions in the tumor microenvironment. However, the underlying mechanisms of HNSC progression and tumor-infiltrating lymphocytes remains unclear.

In this study, we used Oncomine, TIMER, UALCAN datasets,and Kaplan–Meier plotter web to analyze DKK1 expression and its association with the prognosis. Furthermore, we used the TIMER web resource and TISIDB database to analyze the correlation between DKK1 and tumor-infiltrated immune cells in the tumor microenvironment. Besides, we further explored the molecular mechanisms of DKK1 dysregulation via cBioPortal and UCSC Xena database, such as analysis of the CNV, DNA methylation, and somatic cell mutations. Our findings underline the vital role of DKK1 in HNSCC prognosis. Also, we provide an

underlying mechanism of DKK1 expression in potentially regulating the infiltration of immune cells, partly affecting the prognosis of HNSCC.

Results

The DKK1 mRNA Expression in Different Cancers

The mRNA expression level of DKK1 in cancers and normal tissues of various tumors were analyzed on Oncomine database. As shown in Figure 1A, higher mRNA expression level of DKK1 was presented in brain and CNS, head and neck, liver, esophageal, lung and pancreatic, while DKK1 had a lower expression in bladder and prostate cancers. The mRNA-seq data of TCGA analyzed by the TIMER database was used to validate the expression levels of DKK1 in cancers. DKK1 with a higher expression level existed in cholangiocarcinoma(CHOL),

colon adenocarcinoma(COAD), esophageal carcinoma(ESCA), head and neck squamous cell carcinoma(HNSC), liver hepatocellular carcinoma(LIHC), lung squamous cell carcinoma

(LUSC), thyroid carcinoma(THCA) and stomach adenocarcinoma(STAD). In contrast, the level of DKK1 was significantly declined in bladder urothelial carcinoma(BLCA), breast invasive carcinoma(BRCA), kidney chromophobe(KICH), kidney renal papillary cell carcinoma(KIRP) and prostate adenocarcinoma(PRAD). These data indicated that DKK1 had different expression levels in different cancers, suggesting that DKK1 exerted diverse functions in various cancers (Figure 1B). Besides, we used the UALCAN database to verify the findings in Oncomine and TIMER website and reported higher expression of DKK1 in HNSC tissues than in normal tissues(Figure 1C). Notably, DKK1 expression was associated with individual cancer stages, tumor grade, HPV status, nodal metastasis status in HNSC(Figures 1D–H).

Prognostic Significance of DKK1 mRNA Expression in Human Cancers

We explored the Kaplan-Meier plotter databases for the prognostic significance of DKK1 expression in human cancers. High level of DKK1 exhibited a poor survival period for patients with HNSC, KIRP, BRCA, LUAD, PAAD, STAD and LIHC(Figures 2A-G).While as for SARC and OV, highly expressed DKK1 has little effect on overall survival(Figures 2H, I). As Kaplan-Meier plotter analyzes only OS value, We evaluated the multiple clinical prognostic indicators of DKK1 in a variety of cancers by R project using “survival” and “ggplot2” packages. Forest plot showed DKK1 as a risk factor of HNSC, STAD, ACC, LUAD and PADD in OS, DSS and PFI.(Figure 3A-C).These findings demonstrated that DKK1 is a hazard for predicting worse prognostic in HNSC.

Correlation of DKK1 Expression With Clinical subtypes of Head and Neck Squamous Cell Cancer

Then, we evaluated the association of DKK1 expression with different clinicopathological factors of HNSC using the Kaplan-Meier Plotter database (Table 1).Upregulated levels of DKK1 was related to worse prognostic outcomes in females (OS: HR=2.49, P=0.0033), males (OS: HR=2.3P=2.1e−06), stage 2 (OS: HR=2.94, P=0.0097), stage 3 (OS: HR=5.52, p=0.00022), stage 4 (OS: HR=1.73,P=0.0026) , grade1(OS: HR=3.81, P=0.0049), grade2(OS: HR=2.02, P=6.8e−05; RFS:HR=3.36, P=0.019) and grade3(OS: HR=2.69, P=0.00027) in HNSC. Similarly, High DKK1 level was correlated with poorer OS and RFS in high mutation burden (OS: HR=2.48, P=1.3e−06; RFS: HR=0.25, p=0.027) and low mutation burden(OS: HR=1.91, P=0.0026). From above mentioned clinicopathological factors, high expression of

DKK1 could be considered to be the worse prognostic indictor for HNSC.

DKK1 Expression is Correlated With Immune Infiltration in Head and Neck Squamous Cell Carcinoma

Although DKK1 was associated with prognosis and immune infiltration of hepatocellular carcinoma, esophageal cancer and pancreatic cancer, the relationship between DKK1 immune infiltration and head and neck squamous cell carcinoma is still unclear. The TIMER database was utilized to analyze the correlation between DKK1 level with immune infiltration levels in different cancer types. The results exhibited that DKK1 expression is significantly negative correlated with B cells (r=-0.198, p=1.37e-05) and CD8+T cells (r=-0.181, p=7.19e-05) in HNSC (Figure 4A). In addition, we investigated prognostic value of DKK1 level and tumor infiltrating immune cells in HNSC, using cox proportional hazard model by TIMER. The result reveal that DKK1 expression ($p<0.001$) was significantly correlated with clinical prognosis in HNSC (Table 2).Then, we utilized TISIDB database to furtherly survey the relationship between DKK1 level and 28 tumor immune infiltrating cell subtypes. These results indicated that DKK1 is associated with eleven immune cell subtypes in HNSC (Figure 4B, Table3). Especially, activated CD8 T cell (r=-0.143, p=0.00105), central memory CD8 T cell (r=0.179, p=4.1e-05), effector memory CD8 T cell (r=-0.137, p=0.00169), type 17 T helper cell (r=-0.115, p=0.00877), activated B cell (r=-0.187, p=1.78e-05), immature B cell(r=-0.156, p=0.000338), and DKK1 are moderately correlated (Figure 4C). These results strongly imply that DKK1 could serve as a major tumor immune infiltration regulator in HNSC.

DKK1 Expression Was Correlated With Markers of Immune Cells

We evaluated the correlation between DKK1 expression and tumor-infiltrating immune cell gene marker in HNSC tissues by checking the TIMER database.Our results showed that the DKK1 level in HNSC tissues negatively correlated with the marker genes of B cells and CD8+T cells. Notably, we found that the DKK1

was correlated with various subtypes of B cells markers, CD79A, CD19, T cells marker levels, including CD8+T markers, CD8A, CD8B, cell T (general) markers, CD3D, CD3E, CD2, T cell exhaustion marker, PDCD1, Dendritic cell markers, NRP1, CD1C, M1 Macrophage markers, PTGS2, IRF5, M2 Macrophage markers, VSIG4, CD163, Monocyte marker, CD86, Natural killer cell marker, KIR3DL1, Neutrophils markers, CCR7, ITGAM, TAM markers, CCL2, CD68, Th1 markers, TBX21, STAT4, IFNG , Th2 markers, GATA3, STAT6, STAT5A, and Treg markers, STAT5B, FOXP3, TGF-b, in HNSC (Table4). These findings indicate that DKK1 is involved in regulating tumor immune infiltration in HNSC.

Prognostic Value of DKK1 Expressions in Head and Neck Carcinoma Based on Immune Cells

This study illustrated that the DKK1 level was associated with the immune infiltration of HNSC. Also, upregulated DKK1 has a worse prognosis in HNSC patients.Therefore, we state a hypothesis that DKK1 may affect the prognosis of HNSC patients partly through immune infiltration.We use Kaplan-Meier plotter to analyze DKK1 expression in HNSC following B cells, CD4+ memory T-cells, CD8+ T-cells, regulatory T-cells, type 1 T-helper cells, type 2 T-helper cells, natural killer T-cells, mesenchymal stem cells and macrophage cells.We found that high DKK1 levels in HNSC in enriched B cells (p=2.7e−06), CD4+memory T cells (p=2e−08), CD8+T cells (1.6e−06), regulatory T cells (p=1.5e−05), type 1 T helper cells (p=3.3e-05), type 2 T-helper cells (p=7.6e-08), natural killer T cells (p= 0.011), mesenchymal stem cells(p=0.001), macrophages (p=1.7e−05) had a worse prognosis (Figures 5A-l). The above analysis suggested that immune infiltration may affect the prognosis of HNSC patients with high DKK1 expression to a certain extent.

Gene expression, Copy Number Variation, and Mutation feature analysis of DKK1 in HNSC

DKK1 expression was significantly rised in HNSC. We assessed the source of elevated DKK1 levels. DNA methylation, gene mutation, copy number variation were evidently related to genetic and epigenetic regulation, and were critically associated with the occurrence and development of tumors.We analyzed the genetic alterations of DKK1 in HNSC patients by using the cBioPortal online tool. We observed the genetic alteration status of DKK1 in different tumor samples in the TCGA cohort, which mainly include “mutation”, “amplification”, and “copy number deletion”. The “amplification” type of CNA was the primary type in the head and neck cancer cases, which show an alteration frequency of ~2.2% (Figure 6A-B). The types of the DKK1 genetic alteration were further presented in Figure 6B. Besides, we confirmed the copy number variation, somatic mutation, DNA methylation levels of the DKK1 in HNSC via the UCSC Xena database. The heatmap shows that the expression of DKK1 mRNA was involved in copy number variation and DNA methylation, but not with a somatic mutation in HNSC(Figure 6C). The cBioPortal results show that DKK1 mRNA is increased in the samples in which DKK1 is amplified(Figure 6D). So, we suggested that CNV and DNA methylation might contribute to the elevated level of DKK1 in HNSC.

Table 1

Correlation of DKK1 mRNA expression and prognosis in HNSC with different clinical subtypes by Kaplan-Meier plotter

Clinical subtypes	HNSC			
	OS		RFS	
	HR	P	HR	P
Gender				
Female (n=4305)	2.49 (1.33-4.67)	0.0033	0.34 (0.11-1.02)	0.043
Male (n=3184)	2.3 (1.61-3.27)	2.1e−06	2.76 (0.96 − 7.99)	0.051
Stage				
1 (n=1790)	6.71 (0.69-65.63)	0.06	0.35 (0.08-1.59)	0.16
2 (n=1707)	2.94 (1.25-6.93)	0.0097	0 (0-Inf)	0.11
3 (n=1269)	5.52(2.03-14.96)	0.00022	2.34 (0.6-9.08)	0.21
4 (n=676)	1.73 (1.2-2.47)	0.0026	-	-
Grade				
1 (n=304)	3.81(1.41-10.29)	0.0049	2.36(0.47-11.94)	0.29
2 (n=1297)	2.02 (1.42-2.87)	6.8e−05	3.36 (1.15-9.87)	0.019
3 (n=1510)	2.69 (1.54-4.67)	0.00027	0.3 (0.06-1.51)	0.12
4 (n=93)	-	-	-	-
Mutation burden				
High(n=3510)	2.48 (1.7-3.63)	1.3e−06	0.25 (0.07-0.94)	0.027
Low(n=3390)	1.91 (1.25-2.94)	0.0026	2.3 (0.91-5.85)	0.071

Table 2

The cox proportional hazard model of DKK1 and six tumor-infiltrating immune cells in HNSC (TIMER)

	HNSC				
	coef	HR	95%CI_l	95%CI_u	p.value
B cell	-1.769	0.170	0.013	2.155	0.172
CD8 T cell	-0.892	0.410	0.064	2.638	0.348
CD4 T cell	-2.990	0.050	0.002	1.136	0.060
Macrophage	2.269	9.668	0.800	116.786	0.074
Neutrophil	-0.976	0.377	0.021	6.831	0.509
Dendritic	0.863	2.371	0.534	10.520	0.256
DKK1	0.133	1.142	1.060	1.230	0.000

Table 3

The correlation between DKK1 expression and tumor lymphocyte infiltration in HNSC (TISIDB)

	HNSC	
	r	p
Activated CD8 T cell (Act _CD8)	-0.143	0.00105
Central memory CD8 T cell (Tcm _CD8)	0.179	4.1e-05
Effector memory CD8 T cell (Tem _CD8)	-0.137	0.00169
Activated CD4 T cell (Act _CD4)	-0.011	0.795
Central memory CD4T cell (Tcm _CD4)	0.1	0.0227
Effector memory CD4 T cell (Tem _CD4)	-0.078	0.0751
T follicular helper cell(Tfh)	-0.073	0.0956
Gamma delta T cell (Tgd)	0.056	0.204
Type 1 T helper cell (Th1)	-0.074	0.0908
Type 17 T helper cell (Th17)	-0.115	0.00877
Type 2 T helper cell (Th2)	0.067	0.126
Regulatory T cell (Treg)	0.019	0.671
Activated B cell (Act _B)	-0.187	1.78e-05
Immature B cell (Imm _B)	-0.156	0.000338
Memory B cell (Mem _B)	-0.026	0.561
natural killer cell (NK)	-0.066	0.129
CD56bright natural killer cell (CD56bright)	0.104	0.017
CD56dim natural killer cell (CD56dim)	-0.068	0.119
Myeloid derived suppressor cell (MDSC)	-0.1	0.0225
Natural killer T cell (NKT)	0.038	0.386
Activated dendtritic cell (Act _DC)	-0.023	0.594
Plasmacytoid dendtritic cell (pDC)	0.045	0.306
Immature dendtritic cell (iDC)	-0.044	0.314
Macrophage (Macrophage)	-0.011	0.807
Eosinophi (Eosinophil)	-0.144	0.001
Mast (Mast)	-0.099	0.0235
Monocyte(Monocyte)	0.005	0.914

Neutrophil (Neutrophil)	0.052	0.239

Table 4

Correlation analysis between DKK1 and related genes and markers of immune cells in TIMER.

Description	Gene markers	HNSC			
		None		Purity	
		cor	p	cor	p
B cell	CD19	-0.162	1.95e-04	-0.171	1.42e-04
	CD79A	-0.15	5.88e-04	-0.155	5.77e-04
CD8+T cell	CD8A	-0.146	8.15e-04	-0.138	2.09e-03
	CD8B	-0.181	3.06e−05	-0.175	9.13e−05
T cell (general)	CD3D	-0.179	4.05e−05	-0.172	1.26e−04
	CD3E	-0.148	6.94e−04	-0.139	2.08e−03
	CD2	-0.153	4.53e−04	-0.145	1.29e−-03
T cell exhaustion	CTLA4	0.065	1.35e−01	-0.06	1.85e−01
	LAG3	-0.08	6.69e−02	-0.071	1.15e−01
	HAVCR2	0.001	8.7e−01	0.031	4.98e−01
	GZMB	-0.082	6.01e−02	-0.074	1.03e−01
	PDCD1	-0.117	7.49e−03	-0.109	1.52e−02
Dendritic cell	ITGAX	-0.01	8.19e−01	0.004	9.30e−01
	NRP1	0.185	2.15e−05	0.208	3.38e−06
	CD1C	-0.126	4.04e−03	-0.108	1.64e−02
	HLA-DPA1	-0.07	1.09e−01	-0.051	2.58e−01
	HLA-DRA	-0.07	1.13e−01	-0.054	2.30e−01
	HLA-DQB1	-0.044	3.21e−01	-0.031	4.95e−01
	HLA-DPB1	-0.107	1.46e−02	-0.095	3.48e−02
M1 Macrophage	PTGS2	0.124	4.66e−03	0.123	6.27e−03
	IRF5	-0.128	3.37e−03	-0.124	6.07e−03
	NOS2	-0.005	9.17e−01	0.003	9.44e−01
M2 Macrophage	MS4A4A	0.07	1.08e−01	0.095	3.49e−02
	VSIG4	0.098	2.56e−02	0.123	6.27e−03
	CD163	0.076	8.43e−02	0.098	3.01e−02
Monocyte	CSF1R	-0.015	7.35e−01	0.006	8.89e−01

	CD86	0.115	8.52e−03	0.145	1.28e−03
Natural killer cell	KIR2DS4	-0.078	7.67e−02	-0.075	9.77e−02
	KIR3DL3	-0.051	2.47e−01	-0.034	4.56e−01
	KIR3DL2	-0.054	2.18e−01	-0.033	4.67e−01
	KIR3DL1	-0.096	2.84e−02	-0.105	1.95e−02
	KIR2DL4	-0.032	4.59e−01	-0.023	6.17e−01
	KIR2DL3	-0.066	1.31e−01	-0.052	2.52e−01
	KIR2DL1	0.028	5.22e−01	0.042	3.49e−01
Neutrophils	CCR7	-0.138	1.59e−03	-0.137	2.33e−03
	ITGAM	0.1	2.22e−02	-0.08	7.48e−02
	CEACAM8	−0.016	7.14e−01	-0.022	6.30e−01
TAM	CCL2	0.075	8.8e−02	0.094	3.76e−02
	IL10	-0.005	9.06e−01	-0.012	7.88e−01
	CD68	0.11	1.16e−02	0.135	2.77e−03
Tfh	BCL6	-0.037	4e−01	-0.035	4.35e−01
	IL21	-0.064	1.44e−01	-0.042	3.51e−01
Th1	TBX21	-0.12	6.22e−03	-0.11	1.42e−02
	STAT4	0.098	2.54e−02	0.126	5.23e−03
	STAT1	0.047	2.82e−01	0.065	1.51e−01
	IFNG	-0.11	1.19e−02	-0.101	2.44e−02
	IL13	-0.067	1.25e−01	-0.049	2.82e−01
Th2	GATA3	-0.073	9.56e−02	-0.076	9.17e−02
	STAT6	0.065	1.39e−01	0.093	4.01e−02
	STAT5A	-0.103	1.86e−02	0.095	2.49e−02
Th17	STAT3	-0.063	1.49e−01	-0.042	3.52e−01
	IL17A	-0.095	3.03e−02	-0.081	7.30e−02
Treg	FOXP3	-0.098	2.54e−02	-0.088	5.16e−02
	CCR8	-0.028	5.28e−01	-0.008	8.52e−01
	STAT5B	0.108	1.37e−02	0.121	7.07e−03

	TGFB1	0.199	4.63e−06	0.209	1.84e−06

Discussion

Dickkopf-1, a 35-kDa protein, belongs to Dickkopf gene family which regulates tumor invasion, proliferation, metastasis and the extracellular microenvironment[16, 24]. In this study, we analyzed DKK1 expression, prognostic value, tumor immune cell infiltration, copy number variation, somatic mutation, and DNA methylation in HNSC for the first time.

In the present study, we found that DKK1 expression was highly elevated in HNSC, compared to normal samples. Moreover, DKK1 mRNA expressions were remarkably related to HNSC patients' individual cancer stages, tumor grade, nodal metastasis status and HPV status. At the same time, high DKK1 levels were associated with lymph node metastasis, high grade, late staging, HPV-ve and high mutation burden(Fig. 1D-H, Table 1). DKK1 mRNA was lower expressed in HPV positive HNSCC compared with HPV negative HNSCC (Fig. 1G,H), which suggests that DKK1 plays a specific role in HPV negative HNSCC. These results manifest that DKK1 plays a vital role in the progression and metastasis of HNSCC. Our research results are consistent with previous reports. DKK1 was upregulated in some tumor tissues and associated with clinicopathological factors, including late N stage, lymph nodal metastasis, high grade, and TNM staging[25–27].

Survival analysis and forest plot showed high DKK1 expression had worse OS, DSS and PFI in head-neck squamous cell carcinoma.Numerous preclinical studies have demonstrated that high expression of DKK1 can promote tumor growth, invasion and metastasis in both in vitro cell models and in vivo animal models, these conclusions of studies are consistent with our results in this study[28, 29]. Shi et al reported that high expression of Dickkopf-related protein 1 is related to lymphatic metastasis and indicates poor prognosis in intrahepatic cholangiocarcinoma patients after surgery[30]. Our results significantly indicate that DKK1 can be used as a potential biomarker for HNSCC.

DKK1 showed the ability to regulate immune cell activities as well as the immunosuppressive tumor microenvironment in previous research[16].On the basis of these prior reports, we tested whether DKK1 correlates with immune evasion in HNSC. We found that DKK1 expression level is associated with several immune infiltrating cells in HNSCC, including B cells, CD8 + T cells and type 17 T helper cell(Table 2–3, Fig. 4). These results indicate that DKK1 is contributed to the regulation of tumor immune cells. Sui et al reported that DKK 1 impairs the tumor response to PD-1 blockade by inactivating CD8 + T cells in colorectal cancer[14]. Another study demonstrated that metastatic castration-resistant prostate cancer (mCRPC) tumors with high DKK1 expression exhibit a CD8 + T-cell-poor tumor microenvironment infiltrated with immature M0 macrophages and M2 macrophages[31]. Our analysis and the results of these study have a mutual verification effect.

Moreover, we further analyzed the immunotype markers in HNSC. After cell purity adjustment, DKK1 was negtively correlated with many immune cell signatures in HNSC(Table 4). Here we provide that DKK1 expression in HNSC correlates with immune infiltration activity. Besides, our results suggest that DKK1 can negatively modulate B cells, CD8 + T cells, CD4 + memory T cells and results in T cell exhaustion. Obviously, overexpression DKK1 was negatively associated with Treg and T cells exhaustion markers, such as PDCD1, FOXP3. FOXP3 is an effective target for identifying Treg in the cancer microenvironment and significantly promote mediated tumor immune escape[32].There was a weighty correlation between DKK1 level and several T-helper cells (Th1, Th2, and Th17) symbol in HNSC. These links may show the hidden mechanisms for DKK1 regulation of T cell function in HNSC. Similarly, DKK1 is negatively correlated with infiltrating levels of most myeloid-derived suppressor cells (MDSCs) in HNSC, such as mesenchymal stem cells, macrophages. Based on these studies, we speculate that DKK1 may be related to the worse prognosis of HNSC by recruiting and regulating immune cells.

In our present study, we also found that the high expression of DKK1 enriched in a variety of immune cell cohorts of HNSCC has a poor prognosis through Kaplan Meier-Plotter database analysis. Tregulatory (Treg) cells can contribute to tumor immune evasion by suppressing anti-tumor T-effector response[33]. Dendritic cells promote tumor metastasis by increasing Treg cells and reducing CD8 + T cytotoxicity cell[34]. MDSCs were defined as the heterogeneous group of immature myeloid cells recruited by tumors, which could induce CD8 + T cell tolerance in tumor[35]. Emerging experimental evidence reveales that DKK1 has been participated in T cell differentiation and induction of cancer evasion of immune surveillance by accumulating MDSCs[16]. These findings can explain that the high DKK1 expression partially affects the prognosis of HNSC patients through immune infiltration.

Genetic and epigenetic changes play a promising role in regulating cancer development and immune tolerance[36]. For example, mutant PD-L1 with structural variations leads to aberrant PD-L1 expression and immunosuppression[37, 38]. These studies have revealed key roles of epigenetic processes in regulating immune cell function and mediating antitumour immunity. The different regulatory mechanisms of DKK1 expression may reflect the varied roles on different cellular localization and cell types. preliminary investigation indicated that DKK1 expression was genetically and epigenetically regulated through CNA and DNA methylation.DNA copy number variation, including gene amplification, deletion, diploid and gain. Copy number variation (CNV) plays a vital role in pathogenesis and carcinogenesis. Zhang et al[39] demonstrated that MT1-deletion can be an independent prognostic factor in hepatocellular carcinoma. David et al found that DNA methylation as a likely mechanism that governs DKK1 expression in metastatic castration-resistant prostate cancer[31]. A further implication of this result is that elevated DKK1 could be a resistance mechanism to therapeutic DNA methyltransferase inhibition, which is currently being tested in clinical trials. Therefore, we speculate that DNA hypomethylation and CNV may be a cause for DKK1 upregulated in HNSC. In the next step, we will conduct experimental verification.

Conclusions And Future Outlooks

The upregulated DKK1 is highly associated with individual clinical characteristics, poor prognosis, and immune filtration. Copy number variation and DNA methylation may attribute to DKK1 upregulated. Furthermore, our study offers a promising sight that DKK1 may affect the prognosis of HNSC through tumor immune infiltration. Thence, this research provides foresights for further studies on tumor immunotherapy of HNSC. The present study is the preliminary part of a larger study, including validation in a prospectively registered study population. Definitely, we will further verify and conduct experiments in the future.This study provides a theoretical basis for clinical research on the treatment of head and neck squamous cell carcinoma with the DKK1 gene as a target. It may play a major role in advancing the immunotherapy of head and neck squamous cell carcinoma and enhance the therapeutic effect.

Methods

Oncomine

The Oncomine database (https://www.oncomine.org) is a web-based data mining platform with a microarray database of most human cancers. It is mainly used for gene expression analysis, enrichment analysis, co-expression analysis and interaction network[17]. We use the Oncomine database to analyze the expression of DKK1 in different types of cancer.

TIMER

TIMER database (https://cistrome.shinyapps.io/timer/) is a website for comprehensive analysis of gene expression and tumor-infiltrating immune cells of different cancer types. This website evaluates the abundances of tumor infiltrating cells (B cells, CD4+T cells, CD8+T cells, neutrophils, dendritic cells and macrophages), using the TIMER[18]. TIMER web also permits the user to investigate gene expression in normal tissues and in cancer tissues. We applied the TIMER website to explore the various expression of DKK1 in tumor and normal tissues in different cancers. We assessed the correlation of DKK1 with six tumor immune infiltrating cells and biomarker of sixteen immune cells. We also used this website to analyze the relationship between immune infiltrating cells and gene expression that affects clinical prognosis in HNSC. The levels of gene expression were presented as log2 RSEM.

UALCAN

UALCAN database (http://ualcan.path.uab.edu/index.html) is available for online analysis of various gene expression in tumor and normal tissue from the TCGA transcriptome sequencing data and clinical data of thirty-one malignancies[19]. In addition, the UALCAN database offers survival prognosis data according to gene expression differences in thirty-one tumor types. Our research used the UALCAN database to validate the analysis results of the Oncomine database, and furtherly determined the correlation between DKK1 gene expression and clinicopathological factors.

Kaplan-Meier Plotter

Kaplan-Meier plotter (http://kmplot.com/analysis/) is a tool for prognostic analysis[20].

Kaplan-Meier plotter database was applied to evaluates the relationship between clinic outcomes and DKK1 expression in different tumors. We carried out a prognostic analysis based on DKK1 expression levels in corresponding immune cell subgroups using this website. We computed hazard ratios (HRs) of 95% confidence intervals (CIs) and the log-rank p-value.

TISIDB

TISIDB database (http://cis.Hku.hk/TISIDB/) is an integrated repository portal for tumor-immune system interactions[21]. We analyze the spearman correlations between DKK1 expression and tumor-infiltrating lymphocytesviathis across TISIDB.

UCSC Xena

UCSC Xena database (http://xena.ucsc.edu/) is a genome-related database[22]. The database is available to analyze copy number variation , somatic mutation, gene expression, and methylation. This website also offers clinical information such as patient treatment and survival.

cBioPortal

The cBioPortal for Cancer Genomics (http://www.cbioportal. org) is a repository of cancer genomics datasets[23]. We surveyed the copy number alterations (CNA) and mutations of DKK1 in HNSC and other cancers.

Statistical Analysis

The DKK1 expression was analyzed across the TIMER, Oncomine and UALCAN database. Survival curves and forest map were maked using the Kaplan-Meier plotter database, R project using“survival”and R ”ggplot2” packages. We used Spearman’s correlation analysis to judge the correlation of DKK1 gene expression in the TIMER. $P<0.05$ were considered statistically significant.

Abbreviations

DKK1, Dickkopf-1; TCGA, The Cancer Genome Atlas; HNSC, Head and Neck squamous cell carcinoma; CHOL, Cholangiocarcinoma; KIRP, Kidney renal papillary cell carcinoma; BRCA, Breast cancer; LUAD, Lung adenocarcinoma; LUSC, Lung squamous cell carcinoma; THCA, Thyroid carcinoma; BLCA, Bladder

urothelial carcinoma; KIRP, Kidney renal papillary cell carcinoma; KICH, Kidney chromophobe; PRAD, Prostate adenocarcinoma; STAD, Stomach adenocarcinoma; PAAD, Pancreatic ductal adenocarcinoma; LIHC, Liver hepatocellular carcinoma; COAD, Colon adenocarcinoma; ESCA, Esophageal carcinoma; SARC, Sarcoma; OV, Ovarian cancer; OS, overall survival; DSS, disease specific survival; PFI, progress free interval; HR, hazard ratio; TILs, Tumor infiltrating lymphocytes; CNV, Copy number variation.

Declarations

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Author Contributions

CFZ conceived the idea and contributed to the drafting of the manuscript. LNL and QYH extracted the data. YYL and JQJ performed the analysis. YC and XML contributed to the material preparation. YXT and YXL helped to edit pictures. DAZ contributed to the study conception and revision of the manuscript, and FJ approved the final version of the submit-ted manuscript. All authors read and approved the final manuscript.

Availability of Data and Materials

The author will provide all the data detailed in this article for free.

Ethics Approval and Consent to Participate

This article does not contain any research conducted by any author on human participants or animals.

Consent for Publication

Everyone's data included in the study was approved for publication.

Competing Interests

The authors report that they have no conflicts of interest. The authors alone were responsible for the content and writing of the article.

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Figures

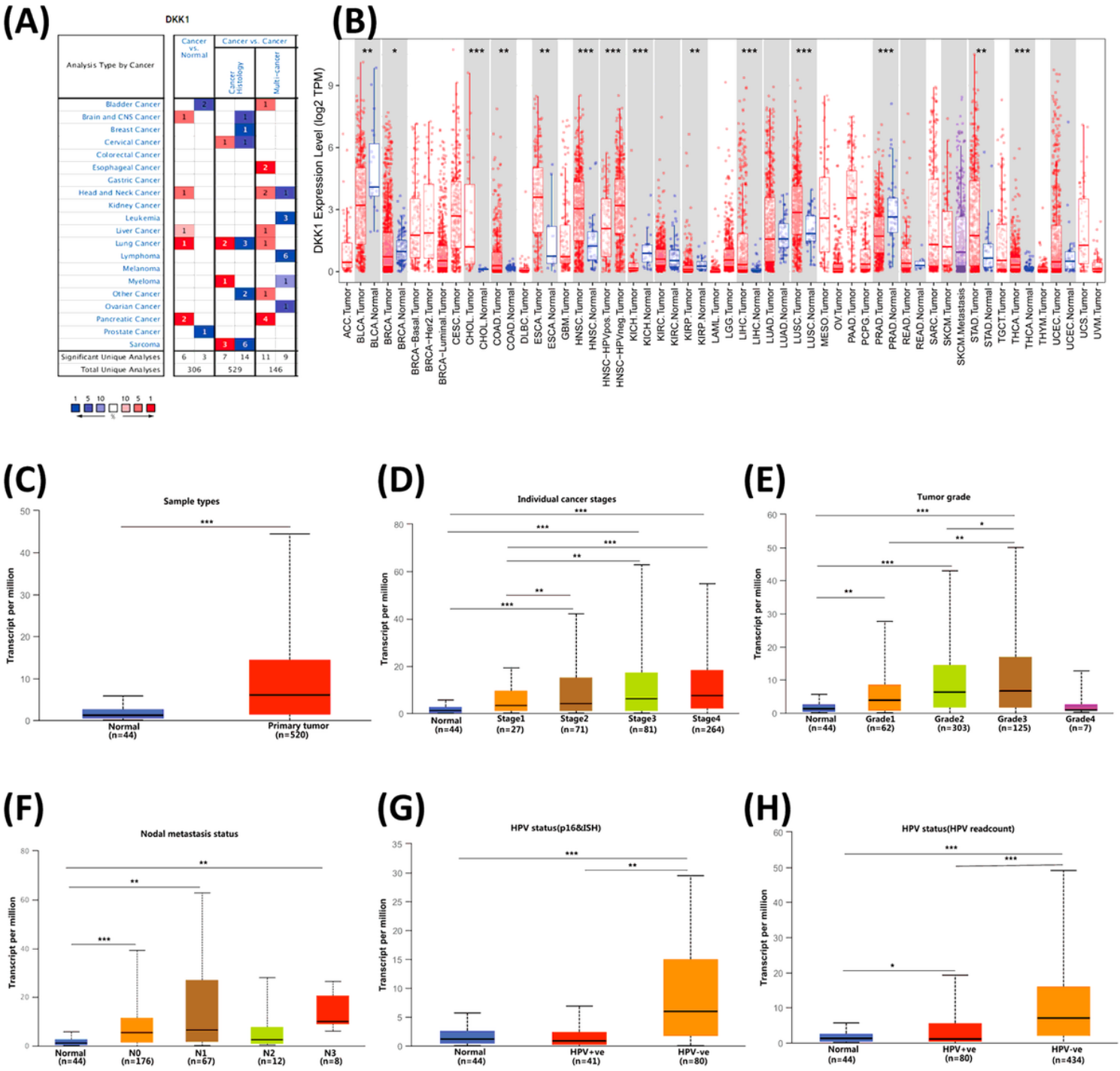

Figure 1

The expression of DKK1 in different human cancers and its relationship with individual clinical characteristics of HNSC. (A) DKK1 mRNA level in different types of cancer tissues compared with normal tissues in the Oncomine database. (B) DKK1 expression levels of different human cancers in the TIMER database. (C) DKK1 expression difference in HNSC sample types. (D-H) DKK1 mRNA expressions were remarkably correlated with HNSC patients' individual cancer stages (D), tumor grade (E), nodal metastasis status(F), HPV status(G, H).(***p<0.001,**p<0.01,*p<0.05). DKK1, Dickkopf-1;HNSC, Head and Neck squamous cell carcinoma.

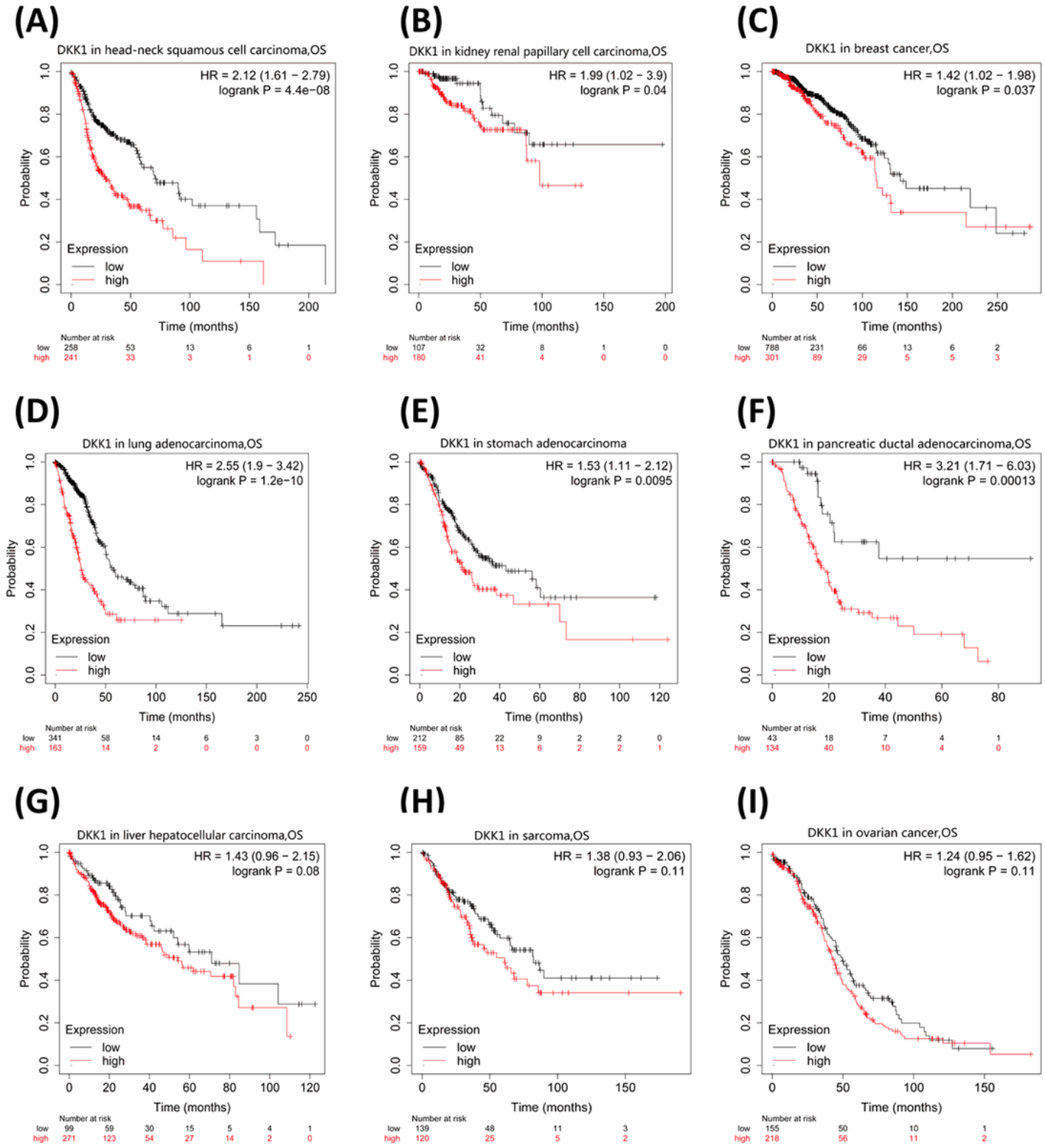

Figure 2

Survival curves of OS in nine human cancers analyzed with mRNA-seq data of TCGA of Kaplan-Meier plotter databases. (A-G) High DKK1 expression had poor OS in HNSC(n=500), KIRP(n=288), BRCA(n=1090), LUAD(n=513), STAD(n=375), PAAD(n=177) and LIHC(n=371). (H, I) Difference in survival among high and low DKK1 levels in SARC(n=259) and OV(n=374).DKK1, Dickkopf-1; TCGA, The Cancer Genome Atlas; OS, overall survival; HNSC, Head and Neck squamous cell carcinoma; KIRP, Kidney renal papillary cell carcinoma; BRCA, Breast cancer; LUAD, Lung adenocarcinoma; STAD, Stomach adenocarcinoma; PAAD, Pancreatic ductal adenocarcinoma; LIHC, Liver hepatocellular carcinoma; SARC, Sarcoma; OV, Ovarian cancer.

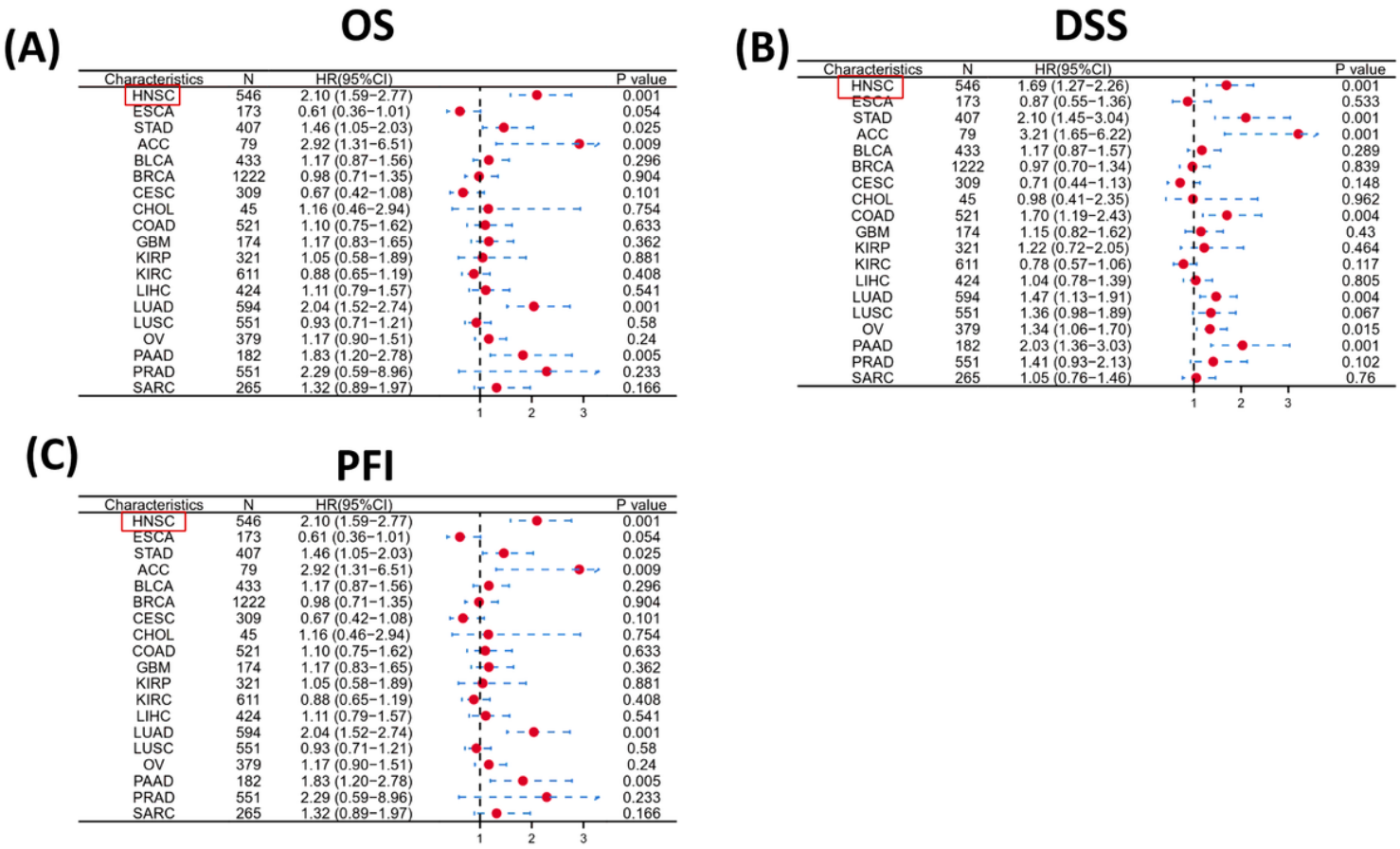

Figure 3

Forest plot of the prognostic significances in different cancer subgroups of DKK1. (A–C) Prognostic HR of DKK1 in various cancers for OS (A), DSS (B), PFI (C). OS, overall survival; DSS, disease specific survival; PFI, progress free interval; HR, hazard ratio.

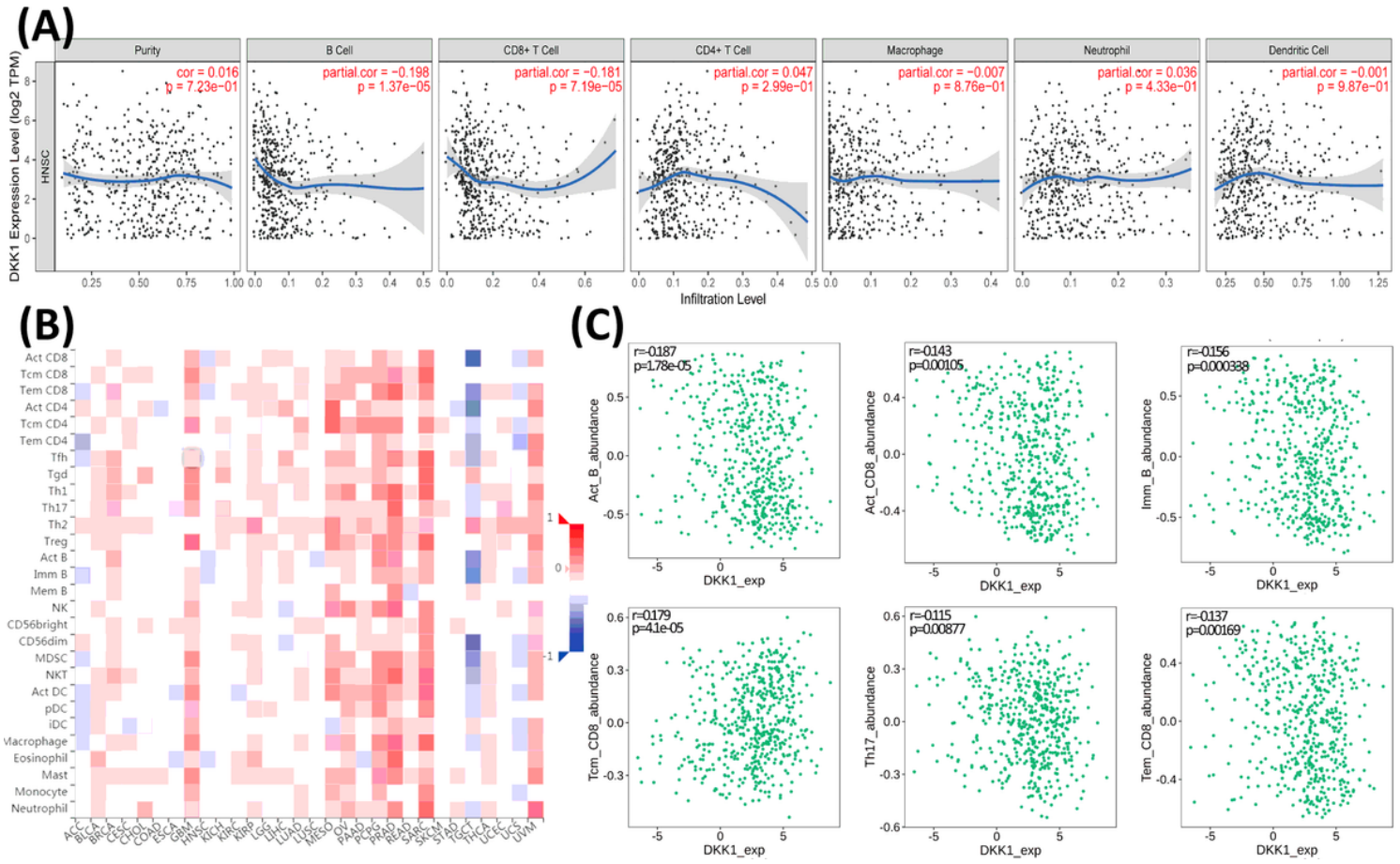

Figure 4

Correlation of DKK1 expression with immune filtration level in human cancers using the TIMER and TISIDB database.(A)The expression of DKK1 in HNSC tissue is negatively correlated with the level of tumor immune infiltration of B cells and CD8+ T cells. (B) Relations between expression of DKK1 and 28 types of TILs in human heterogeneous cancers. (C) Top 6 TILs were displaying significant differences in Spearman's correlation with DKK1 expression in HNSC. TILs, Tumor infiltrating lymphocytes.

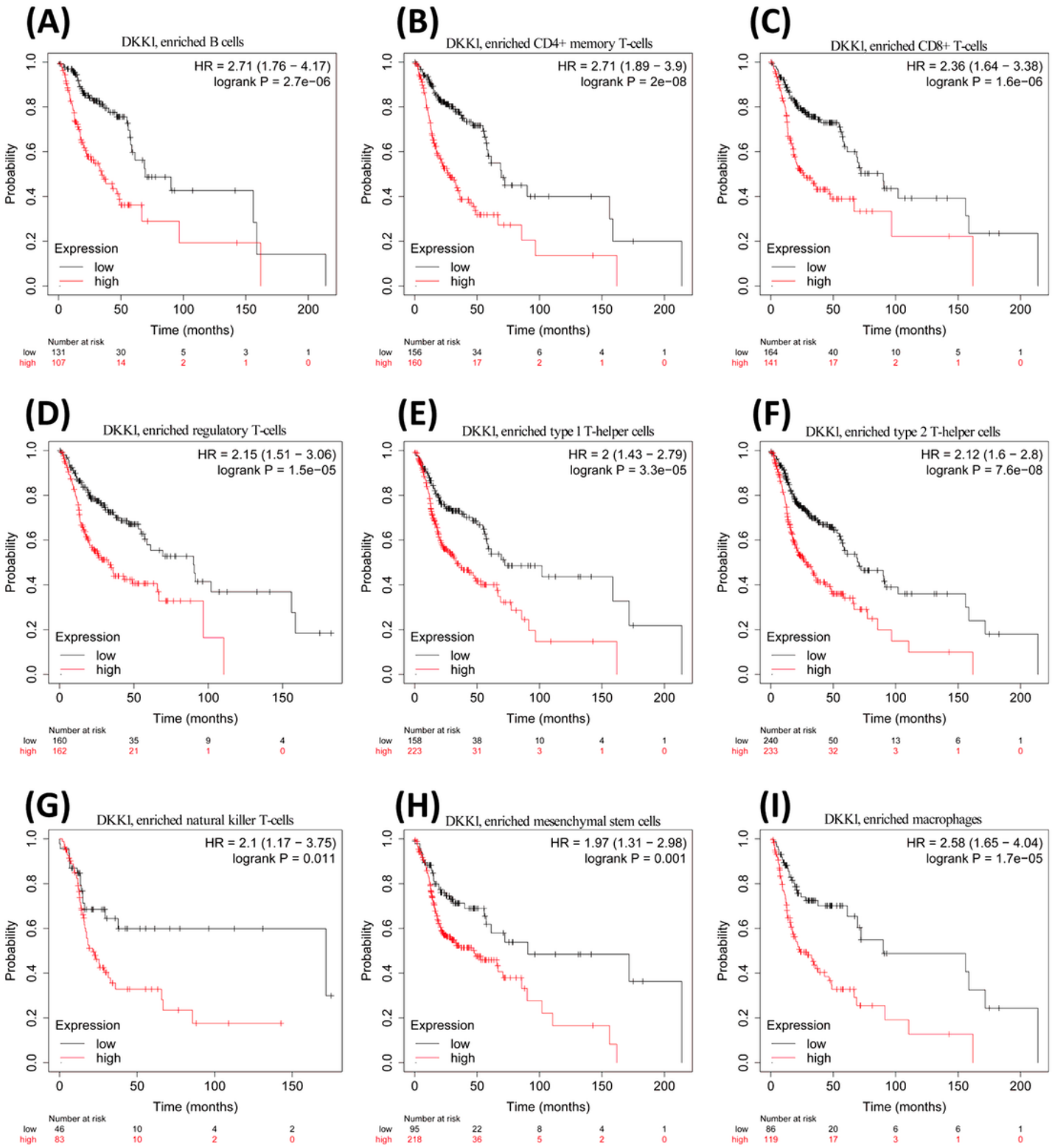

Figure 5

Comparison of Kaplan-Meier survival curves of the low and high expression of DKK1 in HNSC based on immune subtypes. (A–I) High DKK1 level enriched in B cells, CD4+ memory T-cells, CD8+ T-cells, regulatory T-cells, type 1 T-helper cells, type 2 T-helper cells, natural killer T-cells, mesenchymal stem cells and macrophage cells had worse OS in HNSC.

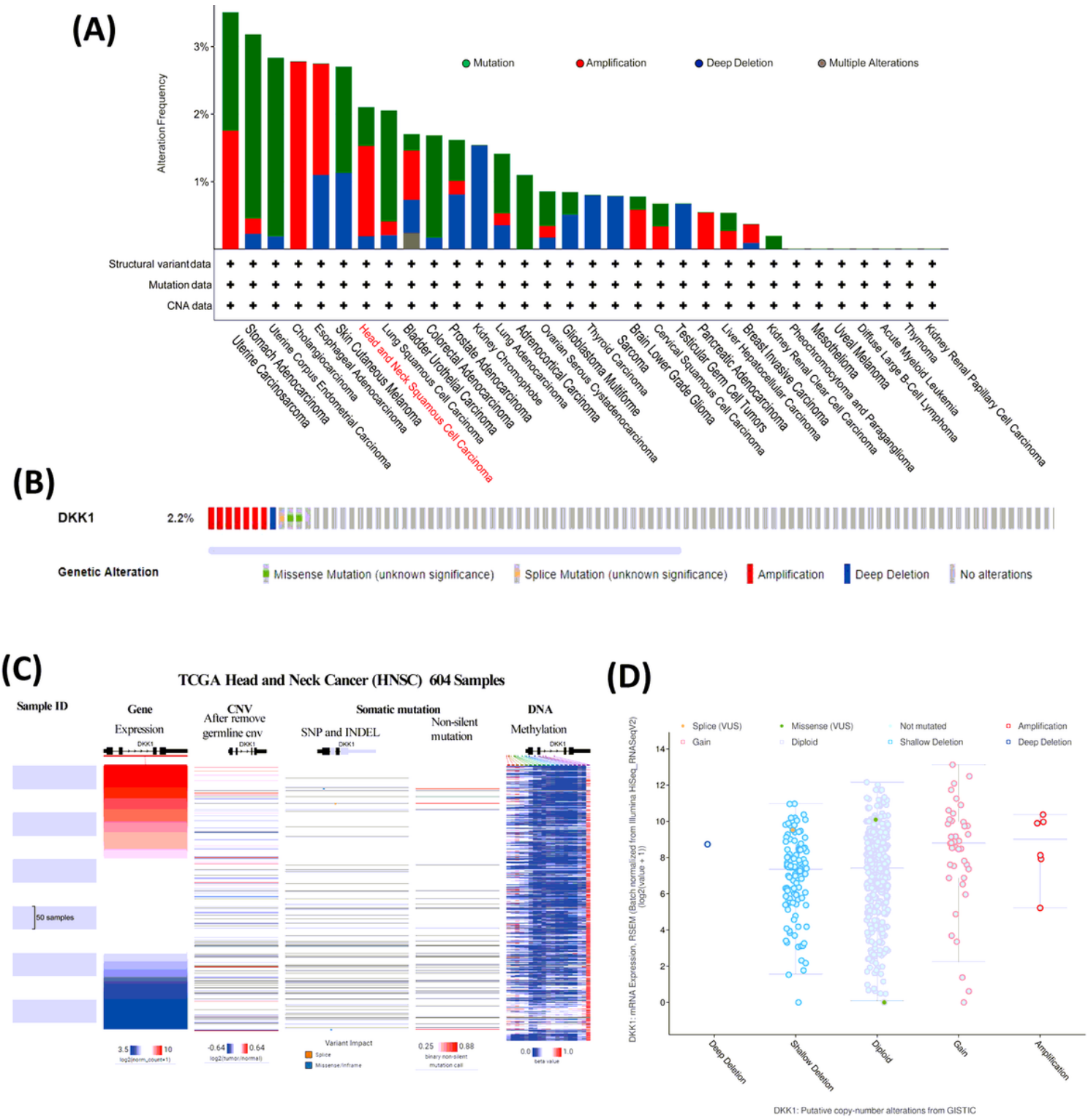

Figure 6

Gene expression, CNV and mutation feature analysis of DKK1. (A) CNA and mutation frequency data of DKK1 in different tumors studies were accessed from cBioPortal. (B) The alteration frequency with mutation site are displayed in HNSC using the cBioPortal tool. (C) Heatmap showing the correlations between DKK1 mRNA and CNV, somatic mutation, and DNA methylation in HNSC via UCSC Xena

database. (D) A plot showing the relationship between DKK1 mRNA abundance and CNA in the DKK1 gene in tumors from the HNSC using the cBioPortal tool. CNV, Copy number variation.