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Prognostic value and immunological role of Kruppel-like transcription factor 9 gene in pan-carcinoma

Weichao Cai, MM[a], Yecheng Li, MD[b,*], Weihong Cao, MD[a,*]

Abstract

Objective: To investigate the correlation between the expression of Kruppel-like transcription factor 9 (KLF9) and the prognostic value of tumors as well as its relationship with tumor immune invasion.

Methods: A series of bioinformatics methods were used to analyze the relationship between KLF9 and tumor prognosis, tumor mutation burden, microsatellite instability (MSI), and immune cell infiltration in multiple carcinomas.

Results: In multiple tumor tissues, the expression of KLF9 was lower compared with paracancerous tissues. Therefore, KLF9 can serve as a protective factor to improve the prognosis of carcinoma patients with certain tumor types. KLF9 was closely related to the clinical staging of various carcinomas. The expression of KLF9 was not only associated with tumor mutation burden and MSI in some tumor types, but also positively correlated with immune and stromal cells in multiple tumors. Further studies have found that, the level of immune cell infiltration was significantly related to the expression of KLF9.

Conclusion: KLF9 can affect the prognosis of pan-carcinoma, which is related to immune invasion. Therefore, KLF9 can be used as a potential biomarker for the prognosis of pan-carcinoma.

Abbreviations: BLCA = bladder urothelial carcinoma, BRCA = breast invasive carcinoma, CESC = cervical squamous cell carcinoma and endovervical adenocarcinoma, CHOL = cholangiocarcinoma, COAD = colon adenocarcinoma, DFI = disease-free interval, DSS = disease-specific survival, ESCA = esophageal carcinoma, HNSC = head and neck squamous cell carcinoma, KIRC = kidney renal clear cell carcinoma, KIRP = kidney renal papillary cell carcinoma, KLF9 = Kruppel-like transcription factor 9, LAML = acute myeloid leukemia, LIHC = liver hepatocellular carcinoma, LUAD = lung adenocarcinoma, LUSC = lung squamous cell carcinoma, MESO = mesothelioma, MSI = microsatellite instability, OS = overall survival, OV = ovarian serous cystadenocarcinoma, PAAD = pancreatic adenocarcinoma, PFI = progression-free interval, PRAD = proatate adenocarcinoma, READ = rectum adenocarcinoma, SARC = sarcoma, SKCM = skin cutaneous meianoma, STAD = Stomach adenocarcinoma, TCGA = The Cancer Genome Atlas, TGCT = teaticular germ cell tumors, THCA = thyroid carcinoma, THYM = thymoma, TMB = tumor mutation burden, TME = tumor microenvironment, UCEC = uterine corpus endometrial carcinom, UCS = uterine carcinosarcoma.

Keywords: immune cell infiltration, Kruppel-like transcription factor 9, pan-carcinoma, prognostic value

1. Introduction

Globally, carcinoma has become a major disease due to its high morbidity and mortality, making it threatening to human health and burdensome to society.[1] In view of the previous treatment experience, traditional therapeutic methods, including surgery, chemotherapy, radiotherapy, and existing immune checkpoint blocking therapy, have failed to achieve satisfactory effect.[2] Based on the complex occurrence and progression of tumors, it is important to analyze pan-oncogene expression, as well as its relationship with clinical prognosis and tumor microenvironment.

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Research data supporting this publication are available from The Cancer Genome Atlas (TCGA) repository at www.portal.gdc.cancer.gov.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee, as well as the 1964 Helsinki Declaration and its subsequent amendments or comparable ethical standards. The data in this manuscript are from the public database, and there are no ethical issues.

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Kruppel-like transcription factor (KLF), containing 17 different members, and plays various biological roles in tumor progression.[3] As an important member of the KLF family,[4] KLF9 is widely expressed and highly conserved in many tissues.[5] Recent years have witnessed an increasing attention paid on the role of KLF9 in tumors. According to several studies, KLF9 was involved in tumor cell proliferation[6] and immune response.[7] Additionally, KLF was closely related to the occurrence and progression of multiple tumors.[8] Notably, many studies have shown that KLF functioned in a conditional manner.[9]

There are many types of cells in the tumor microenvironment (TME). These cells are complicated, mainly consists of tumor cells, blood vessels, extracellular matrix, and other nonmalignant cells, among which invasive immune cells account for a large proportion.[10] Increasing evidence have shown that invasive immune cells figure prominently in various malignant tumors with high prognostic value.[11,12] Many researchers believed that tumor mutational burden (TMB) can serve as a predictive biomarker for the prognosis of tumor immune response due to its association with microsatellite instability (MSI).[13,14]

In this study, The Cancer Genome Atlas (TCGA) related pan-carcinoma database and Kaplan–Meier plotter were used to evaluate the expression and prognostic value of KLF9 in pan-carcinoma analysis. The potential relationship between KLF9 expression and the number of immune-infiltrating cells was further analyzed. The possible mechanism was explored based on bioinformatics.

2. Materials and methods

2.1. Identification of differential expression of KLF9 gene in human tumors

A retrospective study was conducted using a comprehensive analysis of publicly available data. RNA sequences, somatic mutations (Single Nucleotide Polymorphism and small Insertion and Deletion), and clinicopathological and survival data of 33 carcinomas were collected from UCSC Xena (http://xena.ucsc.edu/). (See Table 1, Supplemental Digital Content, the corresponding abbreviations for the 33 tumors are listed). The expression of KLF9 in various tumor and paracancerous tissues was extracted and evaluated (our data included a total of 11,058 specimens). Wilcox test was used to analyze the differences in KLF9 expression in carcinoma tissues and paracancerous tissues of distinct tumor types. P value < .05 was considered statistically significant. The boxplot of KLF9 differential expression was plotted using the R package "ggpubr."

2.2. Correlation between the survival rate and clinicopathology based on the expression of KLF9 in human tumors

Survival information of distinct tumor types was collected from TCGA database. Relationship between KLF9 expression and overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI) were further analyzed. Kaplan–Meier method and log-rank test were used for survival analysis (P value < .05).[15] Furthermore, Cox analysis was performed to explore the relationship between KLF9 expression and pan-carcinoma prognosis (P value < .05). The result was displayed in a forest map, which was plotted using the Rpackage "survival" and "forestplot." R-package "limma" and "ggpubr" were used to analyze the correlation between KLF9 expression and tumor clinicopathology. The number of mutations in each tumor sample was further calculated using somatic mutation data. According to the Perl script, the correction number of mutation bases for every 1 million bases was also calculated. MSI score was obtained from TCGA database. Spearman method was used to analyze the correlation between tumor gene expression and TMB or MSI. The visualization of 2 indexes was realized using the radar map generated by R-package "fmsb."

2.3. Correlation of KLF9 expression with tumor immune microenvironment and immune cell infiltration in pan-carcinoma

R-package "estimate" and "limma" were used to calculate immune and stromal cell scores of each sample.[16] Immune infiltration of each tumor type was scored using CIBERSORT.[17] R-package "ggplot2," "ggpubr" and "ggextra" were used to analyze the correlation between KLF9 expression and tumor immune microenvironment and immune cell infiltration (P value < .001 as cutoff value).

2.4. Analysis of co-expression of immune related genes and KLF9 pathway in tumor

R-package "limma" was used for co-expression analysis. R-package "reshape2" and "rcolorbrewer" were used to visualize the results. Kyoto Encyclopedia of Genes and Genomes gene sets were downloaded from the gene set enrichment analysis website (https://www.gsea-msigdb.org/gsea/downloads.jsp). R-package "limma," "org.hs.e.g..db," "clusterprofiler" and "enrichment plot" were applied for enrichment analysis.[18] All statistical tests were performed using R software (version 3.4.4, www.r-project.org). The expression of KLF9 was down-regulated in endometrial carcinoma. Overexpression of KLF9 could inhibit downstream molecules of actin skeletal protein

Table 1

The corresponding abbreviations for 33 tumors.

Abbreviations	Full name
ACC	Adrenocortial carcinoma
BLCA	Bladder urothelial carcinoma
BRCA	Breast invasive carcinoma
CESC	Cervical squamous cell carcinoma and endovervical adenocarcinoma
CHOL	Cholangiocarcinoma
COAD	Colon adenocarcinoma
DLBC	Lymphoid neoplasm diffuse large B-cell lymphoma
ESCA	Esophageal carcinoma
GBM	Glioblastoma multiforme
HNSC	Head and neck squamous cell carcinoma
KICH	Kidney chromophobe
KIRC	Kidney renal clear cell carcinoma
KIRP	Kidney renal papillary cell carcinoma
LAML	Acute myeloid leukemia
LGG	Brain lower grade glioma
LIHC	Liver hepatocellular carcinoma
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
MESO	Mesothelioma
OV	Ovarian serous cystadenocarcinoma
PAAD	Pancreatic adenocarcinoma
PCPG	Pheochromocytoma and paraganglioma
PRAD	Proatate adenocarcinoma
READ	Rectum adenocarcinoma
SARC	Sarcoma
SKCM	Skin cutaneous meianoma
STAD	Stomach adenocarcinoma
TGCT	Teaticular germ cell tumors
THCA	Thyroid carcinoma
THYM	Thymoma
UCEC	Uterine corpus endometrial carcinom
UCS	Uterine carcinosarcoma
UVM	Uveal Melanoma

regulator.[19] *P < .05, **P < .01, and *** P < .001 were considered statistically significant.

3. Results

3.1. Pan-carcinoma analysis of KLF9 mRNA expression levels

To evaluate the expression of KLF9 in different carcinomas, we examined the expression level of KLF9 mRNA in all 33 carcinomas in TCGA pan-carcinoma database. (See Table 1, Supplemental Digital Content, the corresponding abbreviations for the 33 tumors are listed). The differential expression of KLF9 in tumor and paracancerous tissues is shown in Figure 1. The expression of KLF9 was reduced compared with paracancerous tissues in multiple tumor tissues, including Bladder urothelial carcinoma (BLCA), Breast invasive carcinoma (BRCA), Cervical squamous cell carcinoma and endovervical adenocarcinoma (CESC), Cholangiocarcinoma (CHOL), Colon adenocarcinoma (COAD), Esophageal carcinoma (ESCA), Head and neck squamous cell carcinoma (HNSC), Kidney chromophobe, Kidney renal clear cell carcinoma (KIRC), Kidney renal papillary cell carcinoma (KIRP), Liver hepatocellular carcinoma (LIHC), Lung adenocarcinoma (LUAD), Lung squamous cell carcinoma (LUSC), Proatate adenocarcinoma (PRAD), Rectum adenocarcinoma (READ), Stomach adenocarcinoma (STAD), Thyroid carcinoma (THCA), and Uterine corpus endometrial carcinom (UCEC). The absence of this phenomenon in a few other tumor types may be due to insufficient sample size.

3.2. Multifaceted prognostic value of KLF9 in pan-carcinomas

We hoped to further explore the prognostic value of KLF9 in different tumors through pan-carcinoma analysis of different databases. We further analyzed the relationship between KLF9 expression and OS, DSS, DFI, and PFI. The Kaplan–Meier method and log-rank test were used for survival analysis (Fig. 2). KLF9 was found to be a protective factor to improve the prognosis of carcinoma patients with certain tumor types, especially KIRC, Mesothelioma (MESO), and Skin cutaneous meianoma (SKCM). KIRC (OS, P value < .001), Acute myeloid leukemia (LAML) (OS, P value = .042), MESO (OS, P value = .015), STAD (OS, P value = .020), SKCM (OS, P value < .001), KIRC (DSS, P value < .001), MESO (DSS, P value = .033), SKCM (DSS, P value < .001), KIRC (PFI, P value < .001), MESO (PFI, P value = .011), SKCM (PFI, P value = .004), and Uterine carcinosarcoma (UCS) (PFI, P value = .049). Notably, there was no significant association between KLF9 expression and DFI across all tumor types. A univariate Cox proportional hazards regression model was used to analyze the relationship between KLF9 expression and OS, DDS, DFI, and DPI in various carcinomas (Fig. 3), so as to observe the relationship between KLF9 expression and tumor prognosis. Results of OS, DDS and DFI analyses showed the protective role of KLF9 in KIRC, MESO, SKCM. However, the risky role of KLF9 was confirmed in LAML and STAD by OS analysis, and in UCS by PFI analysis.

3.3. Correlation between KLF9 expression and clinicopathology of pan-carcinoma

We analyzed the correlation between KLF9 expression and clinicopathological features of carcinoma. The results showed that the expression of KLF9 was closely related to the clinical stages of various carcinomas (Fig. 4). KLF9 was highly expressed in stage III patients, but less expressed in stage II patients affected by BLCA, BRCA, ESCA, and LUSC. However, Teaticular germ cell tumors (TGCT) showed the opposite trend. In KIRC, KLF9 was highly expressed in stage I patients, but less expressed in

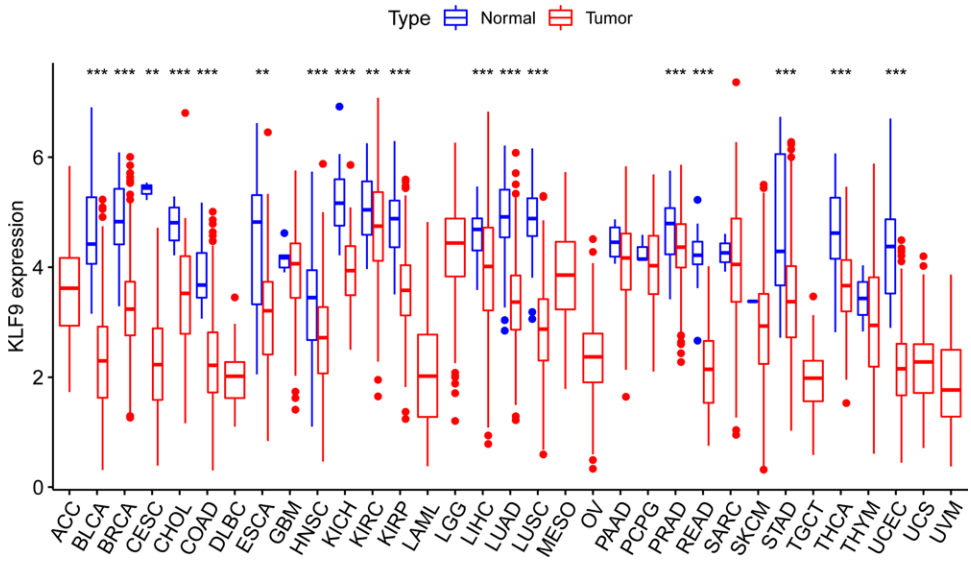

Figure 1. Pan-carcinoma analysis on the expression levels of KLF9 mRNA. The expression of KLF9 was reduced relative to paracancerous tissues in multiple tumors, such as BLCA, BRCA, CESC, CHOL, COAD, ESCA, HNSC, KICH, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, READ, STAD, THCA, and UCEC. Red fusiformis represents tumor tissue and blue fusiformis represents normal tissue. ACC = adrenocortial carcinoma, BLCA = bladder urothelial carcinoma, BRCA = breast invasive carcinoma, CESC = cervical squamous cell carcinoma and endovervical adenocarcinoma, CHOL = cholangiocarcinoma, COAD = colon adenocarcinoma, DLBC = lymphoid neoplasm diffuse large B-cell lymphoma, ESCA = esophageal carcinoma, GBM = glioblastoma multiforme, GSEA = the gene set enrichment analysis, HNSC = head and neck squamous cell carcinoma, KICH = kidney chromophobe, KIRC = kidney renal clear cell carcinoma, KIRP = kidney renal papillary cell carcinoma, KLF9 = Kruppel-like transcription factor 9, LAML = acute myeloid leukemia, LGG = brain lower grade glioma, LIHC = liver hepatocellular carcinoma, LUAD = lung adenocarcinoma, LUSC = lung squamous cell carcinoma, MESO = mesothelioma, OV = ovarian serous cystadenocarcinoma, PAAD = pancreatic adenocarcinoma, PCPG = pheochromocytoma and paraganglioma, PRAD = proatate adenocarcinoma, READ = rectum adenocarcinoma, SARC = sarcoma, SKCM = skin cutaneous meianoma, STAD = stomach adenocarcinoma, TGCT = teaticular germ cell tumors, THCA = thyroid carcinoma, THYM = thymoma, UCEC = uterine corpus endometrial carcinom, UCS = uterine carcinosarcoma, UVM = uveal melanoma.

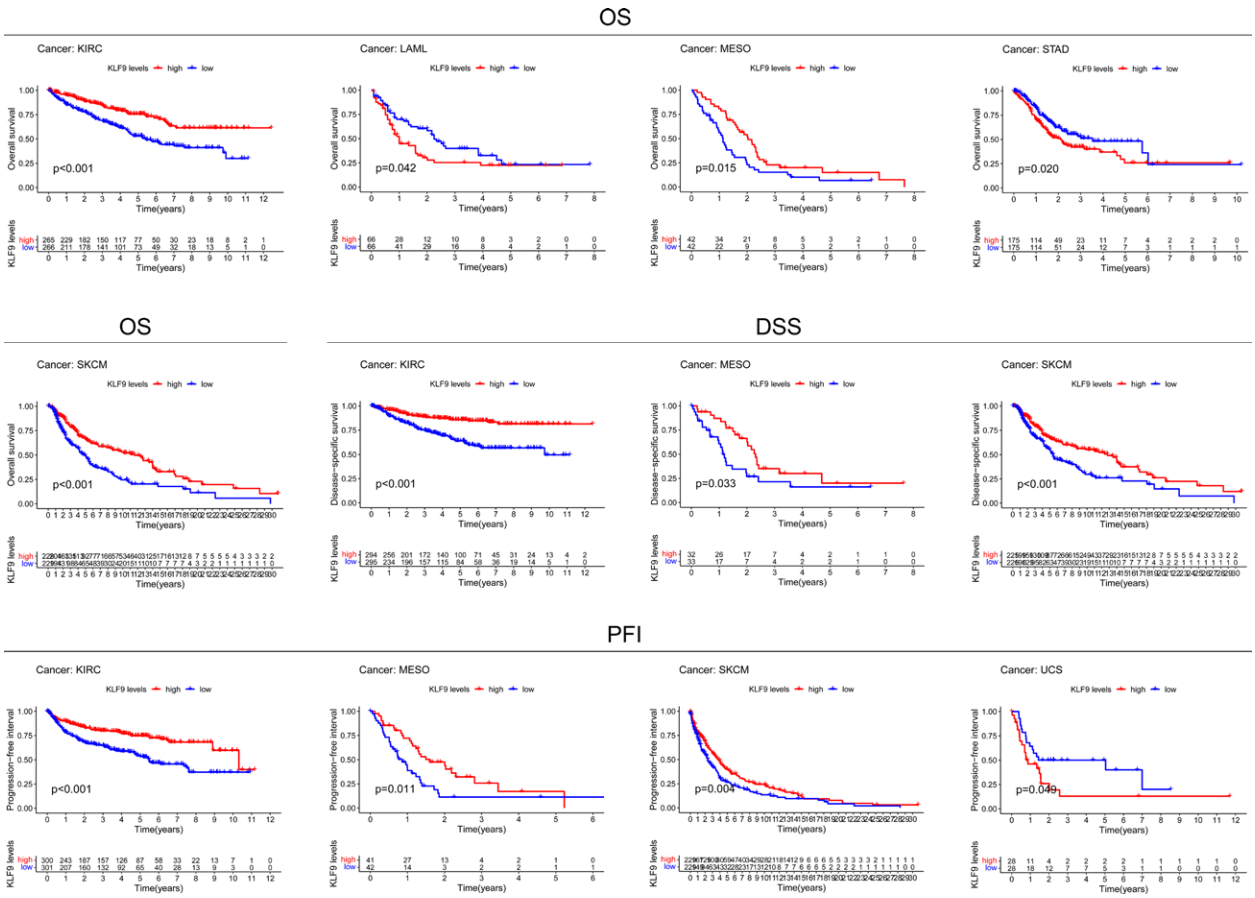

Figure 2. Comparison of Kaplan–Meier survival curves between high and low expressions of KLF9 gene in different types of tumors. We analyzed the relationship between KLF9 expression and overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI). KLF9 can act as a protective factor to improve the prognosis of carcinoma patients in some tumor types, especially in KIRC, MESO, and SKCM. KIRC (OS, *P* value < .001), LAML (OS, *P* value = .042), MESO (OS, *P* value = .015), STAD (OS, *P* value = .020), SKCM (OS, *P* value < .001), KIRC (DSS, *P* value < .001), MESO (DSS, *P* value = .033), SKCM (DSS, *P* value < .001), KIRC (PFI, *P* value < .001), MESO (PFI, *P* value = .011), SKCM (PFI, *P* value = .004), UCS (PFI, *P* value = .049). Notably, no significant association was found between KLF9 expression and DFI across all tumor types. KIRC = kidney renal clear cell carcinoma, KLF9 = Kruppel-like transcription factor 9, LAML = acute myeloid leukemia, MESO = mesothelioma, SKCM = skin cutaneous meianoma, STAD = Stomach adenocarcinoma, UCS = uterine carcinosarcoma.

stage IV patients. In other tumor types, no association was observed between KLF9 expression and clinicopathological features. Statistical analysis was carried out on the expression of KLF9 with the patient's age, gender and tumor grade, and the results showed no significant difference. We failed to conduct statistical analysis due to the lack of obvious indicators and statistical information about medication experience in the database, which will be further verified through subsequent experiments.

3.4. Association of KLF9 expression with TMB and MSI in pan-carcinoma

High TMB is a new biomarker related to the sensitivity of immunesuppressants at immuno checkpoints.[20] Therefore, we investigated the relationship between the expression of TMB and KLF9 in different carcinoma types. The results suggested that KLF9 expression was associated with TMB in some tumor types (n = 13, Fig. 5A). Thereinto, the expression of KLF9 was positively correlated with TMB only in COAD and Thymoma (THYM), but negatively correlated with other tumor types, including BLCA, THCA, TGCT, STAD, PRAD, Pancreatic adenocarcinoma (PAAD), LUSC, LUAD, Brain lower grade glioma, KIRC, and ERCA.

MSI has been detected in multiple tumors,[21] and has been reported as a marker of PD-1 blockade.[22] Therefore, we investigated the relationship between the expression of MSI and KLF9 in different types of carcinomas. The results suggested that KLF9 expression was associated with MSI in some tumor types (n = 11, Fig. 5B). Thereinto, the expression of KLF9 was positively correlated with MSI only in COAD and READ, but negatively correlated with other tumor types, including CESC, lymphoid neoplasm diffuse large B-cell lymphoma, HNSC, KIRP, LUAD, PRAD, STAD, TGCT, and UCS.

3.5. Correlation between KLF9 expression and TME in pan-carcinoma

Immunotherapy, a novel therapeutic approach for tumors, can activate the immune system of the body, thereby producing a strong anti-tumor immune response.[23] Increasing evidence has showed the role of invasive immune cells in various malignant tumors with high prognostic value.[12] Previously, KLF9 was found to be associated with tumor prognosis. On this basis, we continued to explore whether KLF9 is associated with TME. Estimation algorithm was used to calculate the immune and stromal cell scores of the carcinoma types. The expression of KLF9 was found positively correlated with immune cells in multiple tumors (Fig. 6A) (BLCA: $R = 0.49$, P value < 2.2e-16, BRCA: $R = 0.13$, P value = 1.2e-05, COAD, $R = 0.34$, P value = 4.6e-14, ESCA: $R = 0.37$, P value = 1.6e-6, LUAD:

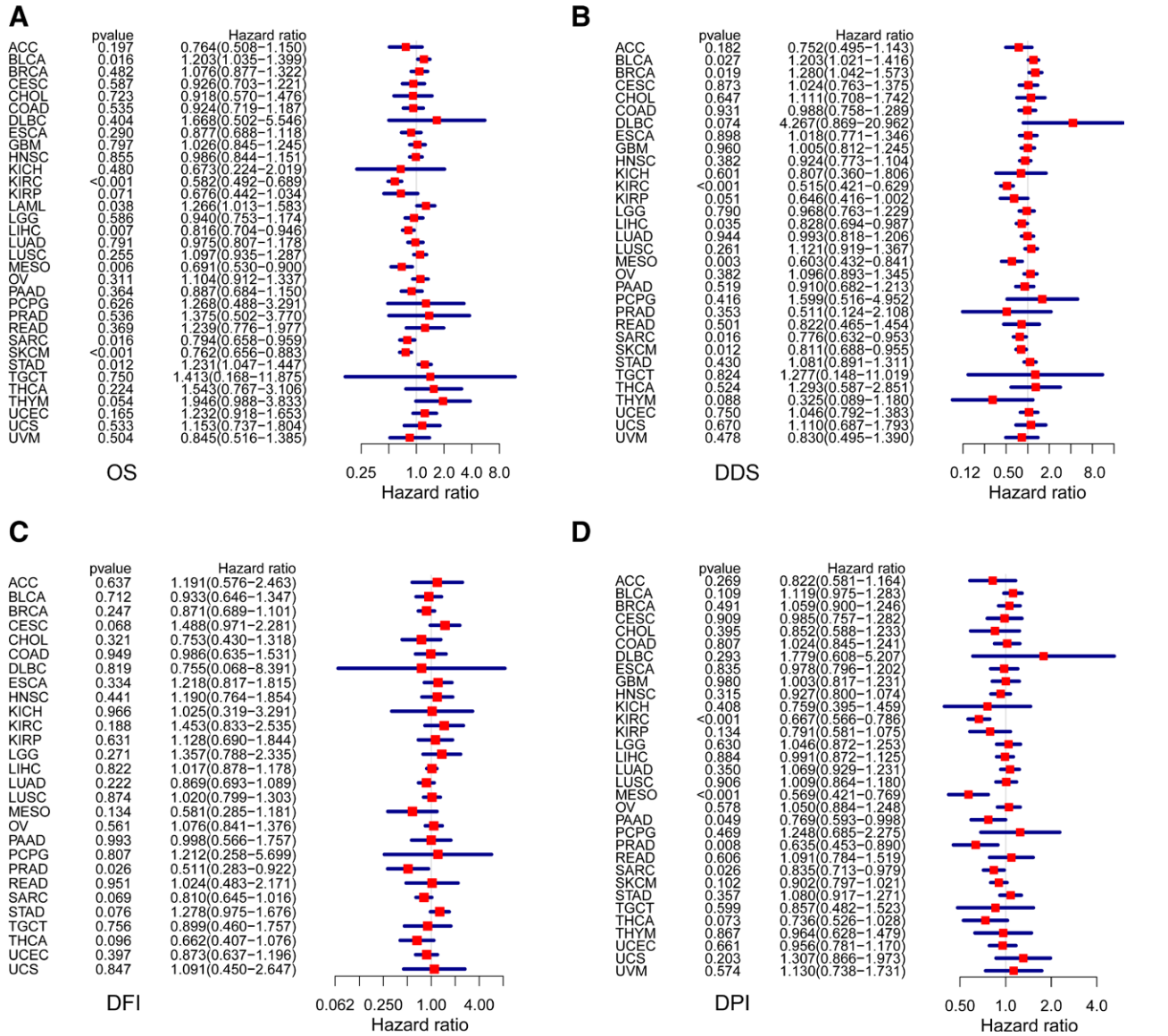

Figure 3. Correlation analysis was used to analyze the correlation between the expression and survival of KLF9 mRNA in different types of TCGA tumors. Univariate Cox proportional hazards regression model was used to analyze the relationship between KLF9 expression and OS (A), DDS (B), DFI (C) and DPI (D) in various carcinomas, so as to observe the relationship between KLF9 expression and tumor prognosis. ACC = adrenocortial carcinoma, BLCA = bladder urothelial carcinoma, BRCA = breast invasive carcinoma, CESC = cervical squamous cell carcinoma and endovervical adenocarcinoma, CHOL = cholangiocarcinoma, COAD = colon adenocarcinoma, DFI = disease-free interval, DLBC = lymphoid neoplasm diffuse large B-cell lymphoma, DSS = disease-specific survival, ESCA = esophageal carcinoma, HNSC = head and neck squamous cell carcinoma, KICH = kidney chromophobe, KIRC = kidney renal clear cell carcinoma, KIRP = kidney renal papillary cell carcinoma, KLF9 = Kruppel-like transcription factor 9, LGG = brain lower grade glioma, LIHC = liver hepatocellular carcinoma, LUAD = lung adenocarcinoma, LUSC = lung squamous cell carcinoma, MESO = mesothelioma, OS = overall survival, OV = ovarian serous cystadenocarcinoma, PAAD = pancreatic adenocarcinoma, PCPG = pheochromocytoma and paraganglioma, PRAD = proatate adenocarcinoma, READ = rectum adenocarcinoma, SARC = sarcoma, SKCM = skin cutaneous meianoma, STAD = stomach adenocarcinoma, TCGA = The Cancer Genome Atlas, TGCT = teaticular germ cell tumors, THCA = thyroid carcinoma, THYM = thymoma, UCEC = uterine corpus endometrial carcinom, UCS = uterine carcinosarcoma, UVM = uveal melanoma.

R = 0.16, P value = .00029, LUSC: R = 0.36, P value < 2.2e-16, Ovarian serous cystadenocarcinoma [OV]: R = 0.31, P value = 6.8e-10, PAAD: R = 0.41, P value = 2.3e-08, PRAD: R = 0.15, P value = 8e-04, STAD: R = 0.27, P value = 8.7e-08). KLF9 expression was also positively correlated with stromal cell expression (Fig. 6B) (BLCA: R = 0.7, P value < 2.2e-16, BRCA: R = 0.44, P value = 2.2e-16, COAD, R = 0.46, P value < 2.2e-16, ESCA: R = 0.54, P value < 2.2e-16, LUAD: R = 0.33, P value = 1.7e-14, LUSC: R = 0.48, P value < 2.2e-16, OV: R = 0.34, P value = 1.1e-11, PAAD: R = 0.53, P value < 2.2e-16, PRAD: R = 0.33, P value = 5.8e-14, STAD: R = 0.61, P value < 2.2e-16).

3.6. Relationship between KLF9 expression and immune cell infiltration in pan-carcinoma

To explore the potential correlation between KLF9 expression and tumor immune cell infiltration, malignant tumors was divided into low-risk and high-risk groups. CIBERSORT algorithm was used to study the relationship between KLF9 expression and infiltration level of 22 immune-related cells.[24] (See Table S1, Supplemental Digital Content, http://links.lww.com/MD/I9, which illustrates a significant correlation between the level of immune cell infiltration and the expression of KLF9). The expression of KLF9 was negatively correlated with B cell memory in multiple tumors, including BLCA, BRCA, CESC,

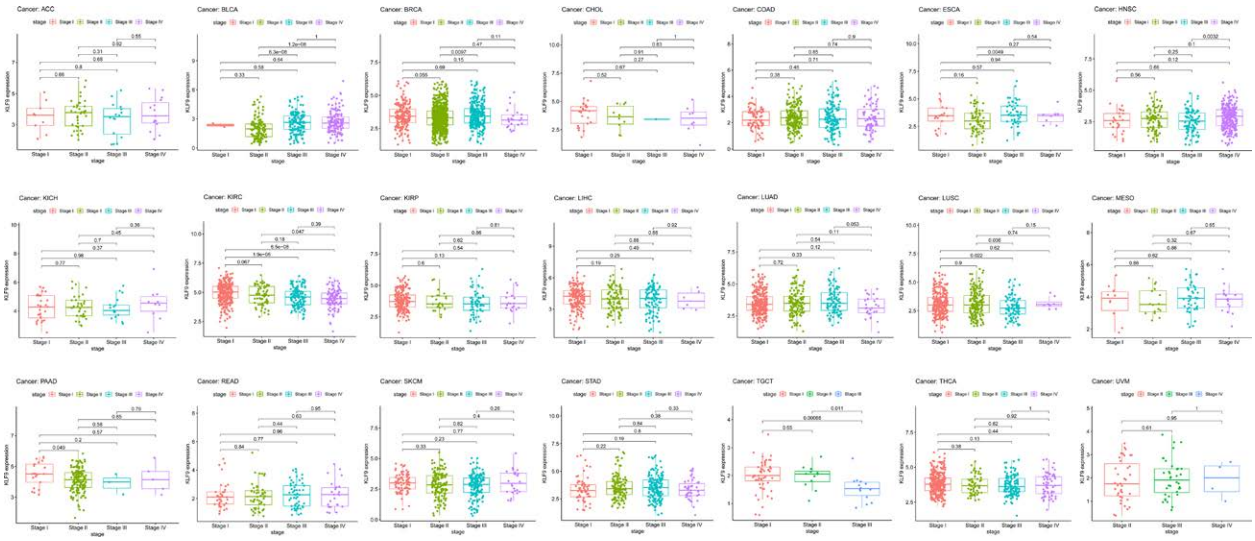

Figure 4. Correlation between KLF9 expression and clinicopathology of pan-carcinoma. KLF9 was closely related to the clinical stages of various carcinomas. KLF9 was highly expressed in stage III patients but less expressed in stage II patients affected by BLCA, BRCA, ESCA, and LUSC. However, the expression of KLF9 in TGCT showed the opposite result. In KIRC, KLF9 was highly expressed in stage I patients but less expressed in stage IV patients. In other tumor types, no association was found between KLF9 expression and clinicopathological features. ACC = adrenocortial carcinoma, BLCA = bladder urothelial carcinoma, BRCA = breast invasive carcinoma, CHOL = cholangiocarcinoma, COAD = colon adenocarcinoma, ESCA = esophageal carcinoma, HNSC = head and neck squamous cell carcinoma, KICH = kidney chromophobe, KIRC = kidney renal clear cell carcinoma, KIRP = kidney renal papillary cell carcinoma, KLF9 = Kruppel-like transcription factor 9, LIHC = liver hepatocellular carcinoma, LUAD = lung adenocarcinoma, LUSC = lung squamous cell carcinoma, MESO = mesothelioma, PAAD = pancreatic adenocarcinoma, READ = rectum adenocarcinoma, SKCM = skin cutaneous meianoma, STAD = stomach adenocarcinoma, TGCT = teaticular germ cell tumors, THCA = thyroid carcinoma, UVM = uveal melanoma.

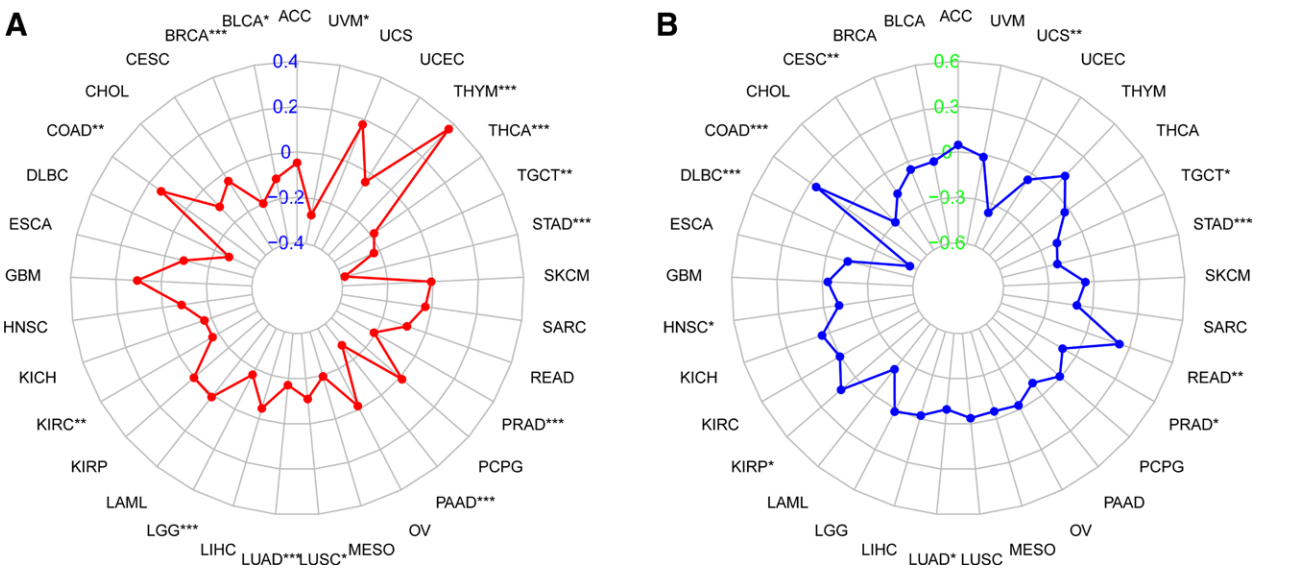

Figure 5. Association of KLF9 expression with TMB and MSI in pan-carcinoma. (A) The expression of KLF9 was associated with TMB in some tumor types (n = 13). Thereinto, the expression of KLF9 was positively correlated with TMB only in COAD and THYM, but negatively correlated with other tumor types, including BLCA, THCA, TGCT, STAD, PRAD, PAAD, LUSC, LUAD, LGG, KIRC and ERCA. (B) The expression of KLF9 was associated with MSI in some tumor types (n = 11). Thereinto, the expression of KLF9 was positively correlated with MSI only in COAD and READ, but negatively correlated with other tumor types, including CESC, Lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), HNSC, KIRP, LUAD, PRAD, STAD, TGCT and UCS. ACC = adrenocortial carcinoma, BLCA = bladder urothelial carcinoma, BRCA = breast invasive carcinoma, CESC = cervical squamous cell carcinoma and endovervical adenocarcinoma, CHOL = cholangiocarcinoma, COAD = colon adenocarcinoma, ESCA = esophageal carcinoma, GBM = glioblastoma multiforme, HNSC = head and neck squamous cell carcinoma, KICH = kidney chromophobe, KIRC = kidney renal clear cell carcinoma, KIRP = kidney renal papillary cell carcinoma, KLF9 = Kruppel-like transcription factor 9, LAML = acute myeloid leukemia, LGG = brain lower grade glioma, LIHC = liver hepatocellular carcinoma, LUAD = lung adenocarcinoma, LUSC = lung squamous cell carcinoma, MESO = mesothelioma, MSI = microsatellite instability, OV = ovarian serous cystadenocarcinoma, PAAD = pancreatic adenocarcinoma, PCPG = pheochromocytoma and paraganglioma, PRAD = proatate adenocarcinoma, READ = rectum adenocarcinoma, SARC = sarcoma, SKCM = skin cutaneous meianoma, STAD = stomach adenocarcinoma, TGCT = teaticular germ cell tumors, THCA = thyroid carcinoma, THYM = thymoma, UCEC = uterine corpus endometrial carcinom, UCS = uterine carcinosarcoma, UVM = uveal melanoma.

COAD, KIRC, PAAD, and PRAD (Fig. 7A), but negatively correlated with dendritic cells activated in BLCA and ESCA (Fig. 7B) and with T cells CD4 naïve in THYM (Fig. 7C). B cell naive was positively correlated with KLF9 expression in BLCA, BRCA, CESC, ESCA, HNSC, KIRC, MESO, PRAD, STAD, and TGCT (Fig. 7D). The expression of KLF9 was positively

correlated with CD4+T cells in BLCA, BRCA, HNSC, KIRC, LIHC, LUAD, LUSC, PRAD, SKCM, and UCEC (Fig. 7E), but negatively correlated with follicular helper T cells in the BLCA, BRCA, KIRC, KIRP, LIHC, LUAD, PRAD, and STAD groups (Fig. 7F). KLF9 was positively associated with the levels of γδ T cells in BRCA but negatively correlated with LAML (Fig. 7G). The expression of KLF9 was negatively correlated with Macrophages M0 in BRCA, KIRC, LIHC, LUAD, PAAD, PRAD, Sarcoma (SARC), and STAD (Fig. 7H). The expression of KLF9 was positively associated with the levels of resting dendritic cells in BRCA (Fig. 7I), but negatively related to the levels of neutrophils in HNSC (Fig. 7J). The expression of KLF9 was negatively correlated with NK cell activation in BRCA, KIRC, LUAD, LUSC, OV, PRAD, THCA, and UCEC (Fig. 7K). The expression of KLF9 was positively associated with the number of resting NK cells in KIRC (Fig. 7L), but negatively correlated with the number of plasma cells in KIRC (Fig. 7M). Resting mast cell levels were positively associated with KLF9 expression in BLCA, BRCA, KIRC, LUAD, and STAD (Fig. 7N), and monocyte levels were positively associated with KLF9 expression in OV, PAAD, SARC, and STAD (Fig. 7O). The expression of KLF9 was negatively correlated with macrophages M2 in TGCT (Fig. 7P). The association between KLF9 expression and macrophages M1 was positive in KIRC, LIHC, SKCM, THCA, and THYM, but negative in LUAD (Fig. 7Q). The expression of KLF9 was negatively correlated with T cells regulatory (Tregs) in BRCA, KIRC, SKCM, and THCA (Fig. 7R). The association between KLF9 expression and the levels of T cells CD4 memory activated was positive in LUSC and PAAD, but negative in COAD and STAD (Fig. 7S). The expression of KLF9 was negatively correlated with T cells CD8 in Glioblastoma multiforme, KIRC, Brain lower grade glioma, and SKCM (Fig. 7T). Our results demonstrated that KLF9 expression was largely associated with the infiltration of immune cells in different ways, which elucidate the difference in patient survival. In most tumor types, elevated KLF9 significantly correlated with Immune cells, prompting a general decline in immune infiltration level.

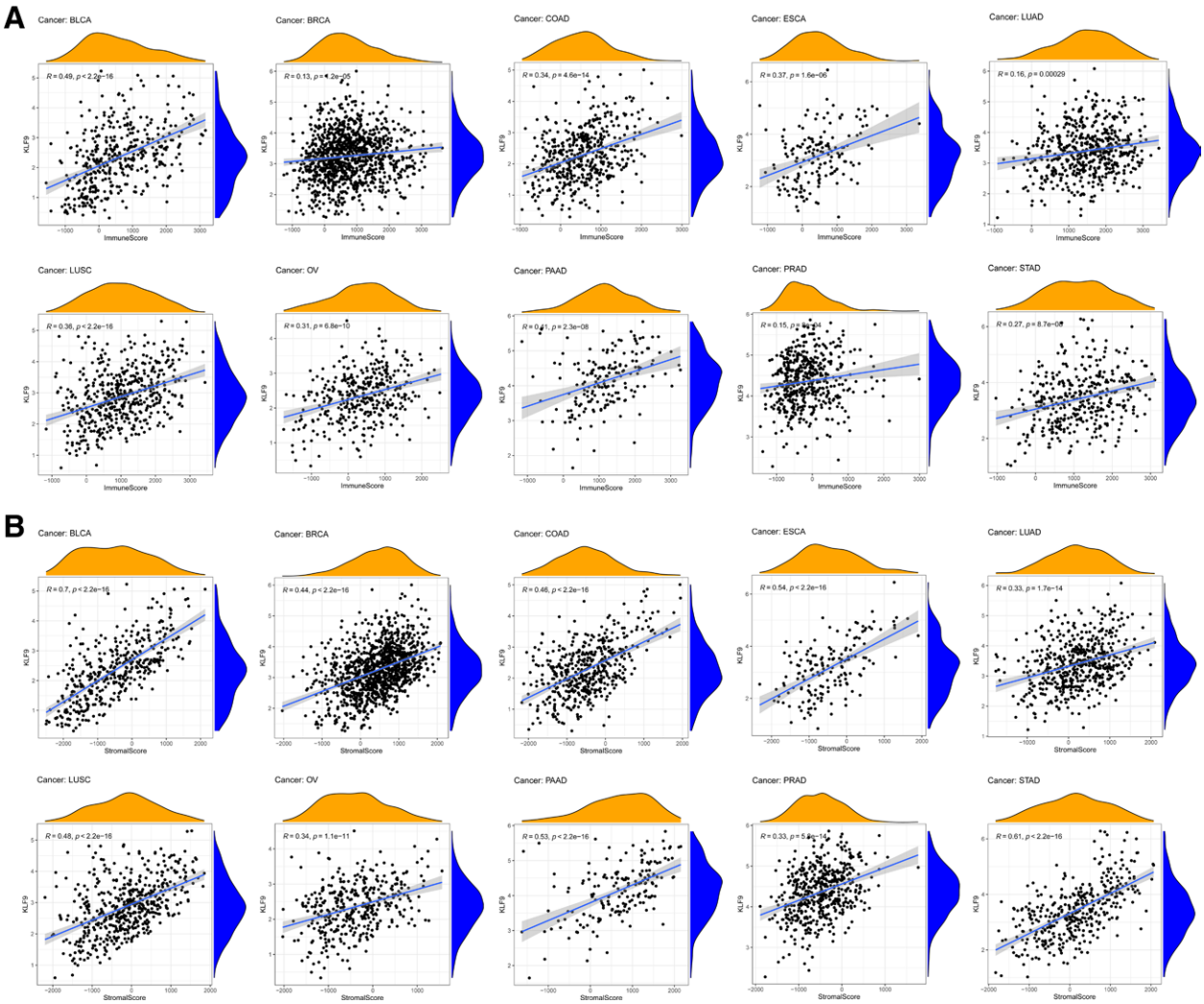

Figure 6. Correlation between KLF9 expression and TME in pan-carcinoma. (A) KLF9 was positively correlated with the immune cell in multiple tumors. BLCA: $R = 0.49$, P value < 2.2e-16, BRCA: $R = 0.13$, P value = 1.2e-05, COAD, $R = 0.34$, P value = 4.6e-14, ESCA: $R = 0.7$, P value = 1.6e-6, LUAD: $R = 0.16$, P value = 0.00029, LUSC: $R = 0.36$, P value < 2.2e-16, OV: $R = 0.31$, P value = 6.8e-10, PAAD: $R = 0.41$, P value = 2.3e-08, PRAD: $R = 0.15$, P value = 8e-04, STAD: $R = 027$, P value = 87e-08. (B) The expression of KLF9 was positively correlated with the expression of stromal cells. BLCA: $R = 0.7$, P value < 2.2e-16, BRCA: $R = 0.44$, P value = 2.2e-16, COAD, $R = 0.46$, P value < 2.2e-16, ESCA: $R = 0.54$, P value < 2.2e-16, LUAD: $R = 0.33$, P value = 1.7e-14, LUSC: $R = 0.48$, P value < 2.2e-16, OV: $R = 0.34$, P value = 1.1e-11, PAAD: $R = 0.53$, P value < 2.2e-16, PRAD: $R = 0.33$, P value = 5.8e-14, STAD: $R = 061$, P value < 2.2e-16. BLCA = bladder urothelial carcinoma, BRCA = breast invasive carcinoma, COAD = colon adenocarcinoma, ESCA = esophageal carcinoma, KLF9 = Kruppel-like transcription factor 9, LUAD = lung adenocarcinoma, LUSC = lung squamous cell carcinoma, OV = ovarian serous cystadenocarcinoma, PAAD = pancreatic adenocarcinoma, PRAD = proatate adenocarcinoma, STAD = Stomach adenocarcinoma, TME = tumor microenvironment.

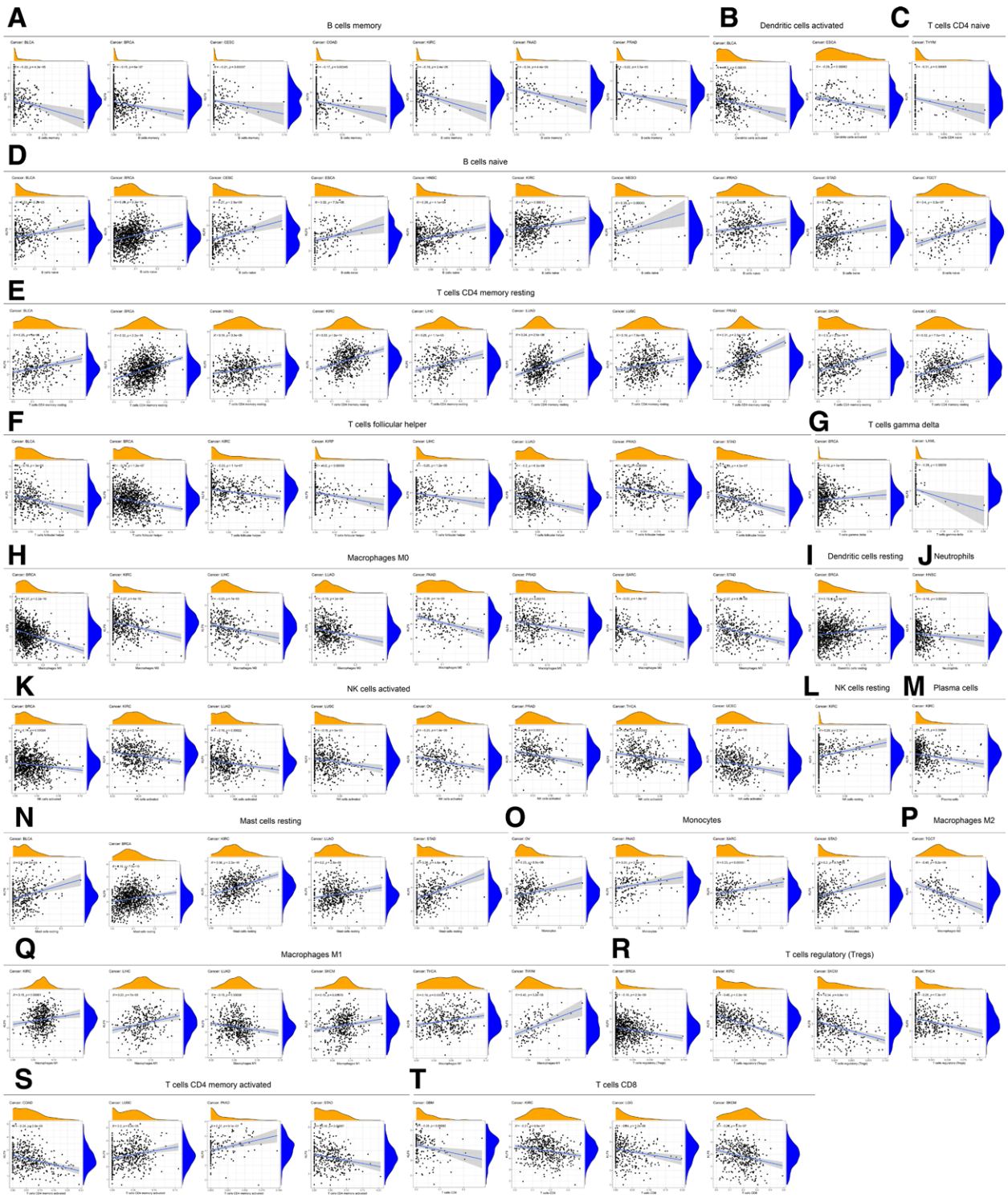

Figure 7. Relationship between KLF9 expression and immune cell infiltration in Pan-carcinoma. (A) The expression of KLF9 was negatively correlated with B cell memory in multiple tumors, including BLCA (R = –0.22, P value < 4.3e-05), BRCA (R = –0.15, P value < 6e-07), CESC (R = –0.21, P value = .00037), COAD (R = –0.17, P value = .00045), KIRC (R = –0.18, P value < 2.4e-05), PAAD (R = –0.34, P value = 4.4e-06) and PRAD (R = –0.22, P value < 3.5e-05). (B) The expression of KLF9 was negatively correlated with dendritic cells activated in BLCA (R = –0.2, P value = .00015) and ESCA (R = –0.28, P value = .00062), and the same was true for T cells CD4 naïve in THYM (R = –0.31, P value = .00065) (C). (D) B cell naive was positively correlated with KLF9 expression in BLCA (R = 0.23, P value = 2.2e-05), BRCA (R = –0.25, P value < 2.2e-16), CESC (R = –0.27, P value < 2.5e-06), ESCA (R = –0.32, P value = 7.3e-05), HNSC (R = –0.26, P value = 4.1e-06), KIRC (R = –0.17, P value = .00012), MESO (R = –0.36, P value = 0.00083), PRAD (R = –0.19, P value = .00035), STAD (R = –0.19, P value = 3e-04) and TGCT (R = –0.4, P value = 3.7e-07). (E) T cells CD4 memory resting was also positively correlated with KLF9 expression in BLCA (R = 0.25, P value = 3e-06), BRCA (R = 0.32, P value < 2.2e-16), HNSC (R = 0.18, P value = 3.3e-05), KIRC (R = 0.33, P value = 2e-14), LIHC (R = 0.25, P value < 1.1e-05), LUAD (R = 0.24, P value < 2.5e-08), LUSC (R = 0.18, P value = 7.9e-05), PRAD (R = 0.31, P value = 2.5e-09), SKCM (R = 0.3, P value = 2.5e-10) and UCEC (R = 0.32, P value = 7.5e-13). (F) The expression of KLF9 was negatively correlated with T cells follicular helper in BLCA (R = –0.19, P value = 3e-04), BRCA (R = –0.16, P value = 1.2e-07), KIRC (R = –0.23, P value = 1.1e-07), KIRP (R = –0.2, P value = .00095), LIHC (R = –0.25, P value = 1.2e-05), LUAD (R = –0.2, P value = 6.3e-06), PRAD (R = –0.17, P value = .00089) and STAD (R = –0.26, P value = 4.2e-07). (G) The expression of KLF9 was positively associated with the levels of T cells gamma delta in BRCA (R = 0.12, P value = 5e-05), but negatively related in LAML (R = –0.28, P value = .00039). (H) The expression of KLF9 was negatively correlated with Macrophages M0 in BRCA (R = –0.27, P value < 2.2e-16), KIRC (R = –0.27, P value = 4e-10), LIHC

(*R* = –0.23, *P* value = 7e-05), LUAD (*R* = –0.16, *P* value = 2e-04), PAAD (*R* = –0.36, *P* value = 1e-06), PRAD (*R* = –0.2, *P* value = .00016), SARC (*R* = –0.33, *P* value = 1.8e-07) and STAD (*R* = –0.27, *P* value = 8.8e-08). (I) The expression of KLF9 was positively associated with the levels of dendritic cells resting in BRCA (*R* = 0.16, *P* value = 2.4e-07), but negatively associated with the levels of neutrophils HNSC (*R* = –0.16, *P* value = .00026) (J). (K) The expression of KLF9 was negatively correlated with NK cells activated in BRCA (*R* = –0.11, *P* value = .00024), KIRC (*R* = –0.21, *P* value = 2.1e-06), LUAD (*R* = –0.16, *P* value = .00022), LUSC (*R* = –0.18, *P* value = 6e-05), OV (*R* = –0.25, *P* value = 1.4e-05), PRAD (*R* = –0.2, *P* value = .00019), THCA (*R* = –0.17, *P* value = .00083) and UCEC (*R* = –0.21, *P* value = 4.4e-06). (L) The expression of KLF9 was positively associated with the levels of NK cells resting in KIRC (*R* = 0.29, *P* value = 2.3e-11), but negatively associated with the levels of plasma cells in KIRC (*R* = –0.15, *P* value = .00046) (M). (N) The levels of mast cells resting were positively associated with KLF9 expression in BLCA (*R* = 0.2, *P* value = 2e-04), BRCA (*R* = 0.19, *P* value = 7.5e-10), KIRC (*R* = 0.36, *P* value = 2.2e-16), LUAD (*R* = 0.2, *P* value = 2.8e-06) and STAD (*R* = 0.35, *P* value = 4.8e-12), and the levels of monocytes were positively associated with KLF9 expression in OV (*R* = 0.23, *P* value = 5.5e-05), PAAD (*R* = 0.31, *P* value = 2.8e-05), SARC (*R* = 0.23, *P* value = .00031) and STAD (*R* = 0.2, *P* value = 9.7e-05) (O). (P) The expression of KLF9 was negatively correlated with macrophages M2 in TGCT (*R* = –0.45, *P* value = 5.2e-09). (Q) The expression of KLF9 was positively associated with the levels of Macrophages M1 in KIRC (*R* = 0.15, *P* value = .00061), LIHC (*R* = 0.23, *P* value = 7e-05), SKCM (*R* = 0.16, *P* value = .00078), THCA (*R* = 0.18, *P* value = .00033) and THYM (*R* = 0.42, *P* value = 3.2e-06), but negatively in LUAD (*R* = –0.15, *P* value = .00038). (R) KLF9 expression was negatively correlated with T cells regulatory (Tregs) in BRCA (*R* = –0.18, *P* value = 2.3e-09), KIRC (*R* = –0.45, *P* value < 2.2e-16), SKCM (*R* = –0.34, *P* value = 6.8e-13) and THCA (*R* = –0.25, *P* value = 7.3e-07). (S) The expression of KLF9 was positively associated with the levels of T cells CD4 memory activated in LUSC (*R* = 0.2, *P* value = 5.2e-06) and PAAD (*R* = 0.37, *P* value = 9.1e-07), but negatively in COAD (*R* = –0.28, *P* value = 2.9e-09) and STAD (*R* = –0.18, *P* value = .00057). (T) The expression of KLF9 was negatively correlated with T cells CD8 in GBM (*R* = –0.26, *P* value = .00082), KIRC (*R* = –0.21, *P* value = 8.5e-07), LGG (*R* = –0.24, *P* value = 3.2e-06) and SKCM (*R* = –0.24, *P* value = 5.3e-07). BLCA = bladder urothelial carcinoma, BRCA = breast invasive carcinoma, CESC = cervical squamous cell carcinoma and endocervical adenocarcinoma, COAD = colon adenocarcinoma, ESCA = esophageal carcinoma, GBM = glioblastoma multiforme, HNSC = head and neck squamous cell carcinoma, KIRC = kidney renal clear cell carcinoma, KIRP = kidney renal papillary cell carcinoma, KLF9 = Kruppel-like transcription factor 9, LAML = acute myeloid leukemia, LGG = brain lower grade glioma, LIHC = liver hepatocellular carcinoma, LUAD = lung adenocarcinoma, LUSC = lung squamous cell carcinoma, MESO = mesothelioma, OV = ovarian serous cystadenocarcinoma, PAAD = pancreatic adenocarcinoma, PRAD = proatate adenocarcinoma, SARC = sarcoma, SKCM = skin cutaneous meianoma, STAD = Stomach adenocarcinoma, TGCT = teaticular germ cell tumors, THCA = thyroid carcinoma, THYM = thymoma, UCEC = uterine corpus endometrial carcinoma.

3.7. Co-expression of immune related genes and KLF9 and analysis of related pathways

To explore the relationship between KLF9 expression and immune-related genes in carcinomas, gene co-expression analysis was performed (Fig. 8). The results showed that, the correlation between KLF9 expression and immune cells varied with different tumor types. (See Table S2, Supplemental Digital Content, http://links.lww.com/MD/I10 and Table S3, Supplemental Digital Content, http://links.lww.com/MD/I11, which illustrates that several immune genes are closely related to the expression of KLF9 in multiple tumors, such as CD44, CD86, CD274, CD40, TFSF18, and PDCD1LG2). We analyzed the correlation between the expression of KLF9 in various tumor tissues and most of the immunostimulatory factors and suppressors. However, how it conducts immune regulation and how to play its biological function remains to be further proved.

We then analyzed the Kyoto Encyclopedia of Genes and Genomes pathway of KLF9 in different types of carcinomas. Different color curves indicated the different regulatory role of KLF9 on different functions or pathways in different carcinomas (Fig. 9). Upward curve peaks indicated positive regulation, and downward curve peaks indicated negative regulation. We found that KLF9 can regulate its downstream pathway positively or negatively in Adrenocortial carcinoma, BLCA, BRCA, CESC, CHOL, HNSC, KIRC, LIHC, LUSC, OV, Pheochromocytoma and paraganglioma, READ, SARC, STAD, and TGCT.

4. Discussion

Transcription factors have been shown to promote the occurrence and progression of various carcinomas.[25] Kruppel-like transcription factor is a DNA-binding transcription regulator[26] that controls basic cellular processes, such as proliferation, differentiation, migration, and maintenance of pluripotency.[26] Previously, KLF9 has been reported to be a basic transcription element-binding protein, because it can specifically bind with the transcription element GC box of the gene promoter region.[26] As an important member of KLF family,[4] KLF9 is involved in the pathogenesis of various carcinomas.[27] Some researchers found that KLF9 was less expressed in the esophagus. Additionally, KLF9 could bind with Tcf4 to inhibit the expression of KLF9β, thereby activating the catenin/TCF signaling pathway.[28] In colorectal carcinoma, the transcription and protein levels of KLF9 were significantly lower than those in normal tissues.[28] In pancreatic carcinoma, KLF9 was considered as a prognostic marker and therapeutic target.[29]

Despite the important role in survival and immunity, the regulation mode and action pathway of KLF9 varied with different tumors. It has been confirmed that KLF9 figures prominently in tumor immune regulation and biological function targets in different tumors which is closely related to survival and immunity. However, how KLF9 exerts its biological effects in different tumors requires further experimental analysis, so as to determine its different mechanisms and pathways.

In the current study, we studied the expression of KLF9 in tumor and paracancerous tissues using a comprehensive analysis of publicly available data. Compared with paracancerous tissues, the expression of KLF9 was reduced in multiple tumor tissues, such as BLCA, BRCA, CESC, CHOL, COAD, ESCA, HNSC, kidney chromophobe, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, READ, STAD, THCA, and UCEC. The absence of this phenomenon in a few other tumor types may be due to insufficient sample size.

Survival information of distinct tumor types was further collected from TCGA database. We analyzed the relationship between KLF9 expression and OS, DSS, DFI, and PFI, and found that KLF9 can act as a protective factor to improve the prognosis of tumor patients with certain tumor types. Next, we analyzed the correlation between KLF9 expression and clinicopathological features of carcinoma. The results showed that the expression of KLF9 was closely related to the clinical stages of various carcinomas.

High TMB is a new biomarker related to the sensitivity of immunosuppressants at immuno checkpoints.[20] Increasing evidence has shown the role of invasive immune cells in various malignant tumors with high prognostic value.[12] MSI has been detected in various tumors[21] and has been reported as a marker of PD-1 blocksade.[22] Our results suggested that KLF9 expression was associated with TMB and MSI in some tumor types. Estimation algorithm was used to calculate the immune and stromal cell scores of the carcinoma types. The expression of KLF9 was found positively correlated with immune cells and stromal cells in multiple tumors. Further studies have found that, the expression of KLF9 was significantly correlated with the infiltration of immune cell, and their correlation varied with different tumor types. Furthermore, KLF9 could positively or negatively regulate its downstream pathways, suggesting that KLF9 may affect tumor prognosis through carcinoma immunity.

Despite the integrated public information, this study still has some limitations.

The cellular level analysis of immune cell markers may be biased, since the majority of microarray and sequencing data was collected through tumor tissue information analysis. This problem could be addressed through single-cell RNA sequencing and other experimental methods in the future.[30] Meanwhile, more data are needed to clarify the relationship between KLF9 and clinical characteristics of tumor patients. Notably, we have explored the relationship between the expression of KLF9 and the infiltration of tumor immune cells, which still need to be further investigated through functional experiments.

It is worth mentioning that we are intrigued by the relationship between COVID-19 and tumors, and we hope to carry out further research in the future.

5. Conclusions

In summary, the prognosis of pan-carcinomas, which is related to immune invasion, can be affected by KLF9, thereby making it a potential biomarker during this process. In this study, the results may provide an enlightening insight to the strategies for clinical

Author contributions

Conceptualization: Weichao Cai, Yecheng Li, Weihong Cao.
Data curation: Weichao Cai, Yecheng Li, Weihong Cao.
Formal analysis: Weichao Cai, Yecheng Li, Weihong Cao.
Writing – original draft: Weichao Cai.
Writing – review & editing: Weichao Cai, Yecheng Li, Weihong Cao.

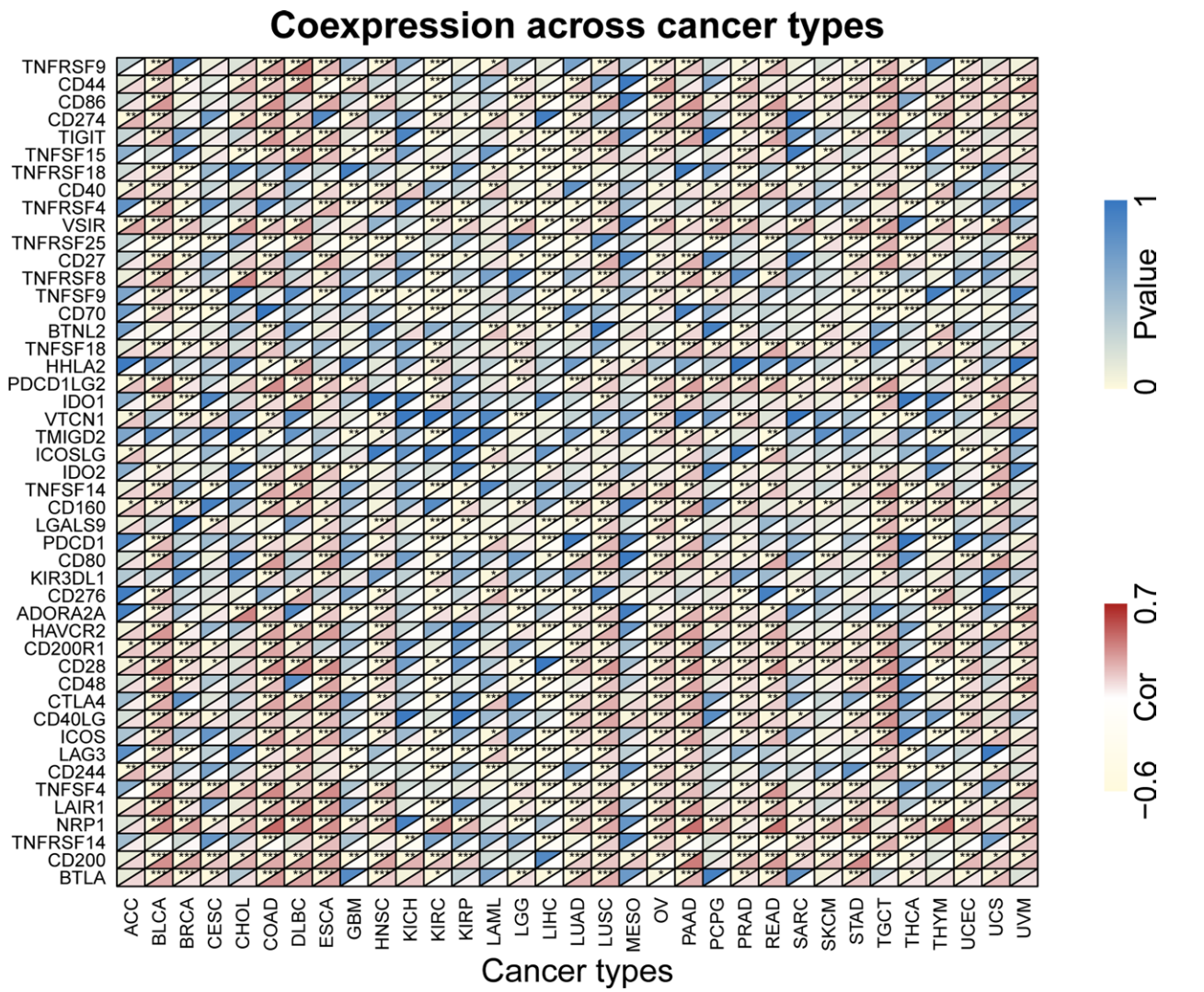

Figure 8. Co-expression analysis of immune gene and KLF9 gene. The correlation between KLF9 expression and immune cells varied with different tumor types. The horizontal axis represents the type of carcinoma, the vertical axis represents the immune gene, and each small rectangular module represents the co-expression of immune gene and KLF9 gene in carcinomas. ACC = adrenocortial carcinoma, BLCA = bladder urothelial carcinoma, BRCA = breast invasive carcinoma, CESC = cervical squamous cell carcinoma and endovervical adenocarcinoma, CHOL = cholangiocarcinoma, COAD = colon adenocarcinoma, DLBC = lymphoid neoplasm diffuse large B-cell lymphoma, ESCA = esophageal carcinoma, GBM = glioblastoma multiforme, HNSC = head and neck squamous cell carcinoma, KICH = kidney chromophobe, KIRC = kidney renal clear cell carcinoma, KIRP = kidney renal papillary cell carcinoma, KLF9 = Kruppel-like transcription factor 9, LAML = acute myeloid leukemia, LGG = brain lower grade glioma, LIHC = liver hepatocellular carcinoma, LUAD = lung adenocarcinoma, LUSC = lung squamous cell carcinoma, MESO = mesothelioma, OV = ovarian serous cystadenocarcinoma, PAAD = pancreatic adenocarcinoma, PCPG = pheochromocytoma and paraganglioma, PRAD = proatate adenocarcinoma, READ = rectum adenocarcinoma, SARC = sarcoma, SKCM = skin cutaneous meianoma, STAD = Stomach adenocarcinoma, TGCT = teaticular germ cell tumors, THCA = thyroid carcinoma, THYM = thymoma, UCEC = uterine corpus endometrial carcinom, UCS = uterine carcinosarcoma, UVM = uveal melanoma.

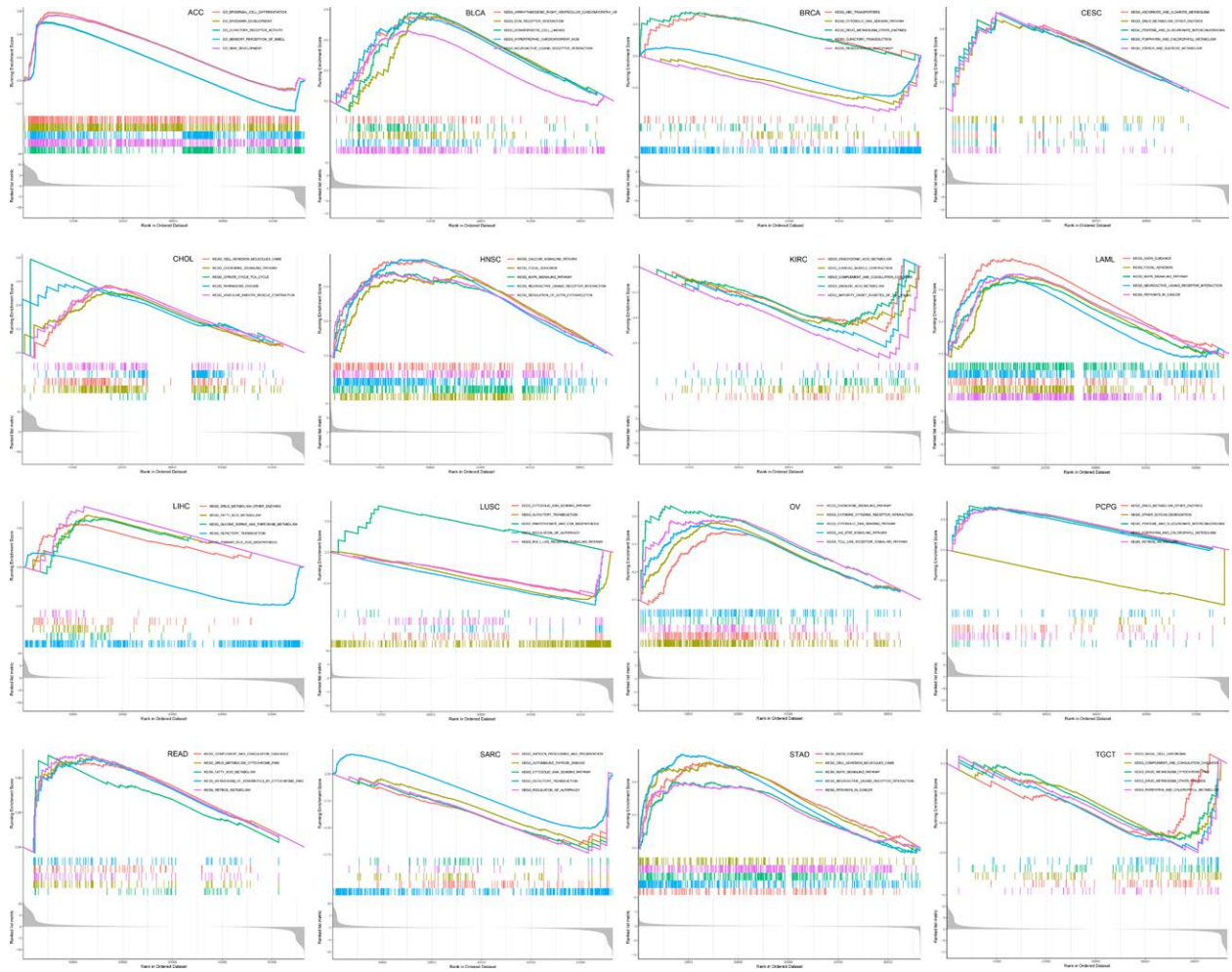

Figure 9. Analysis of KLF9 pathway in different tumors. The KEGG pathway of KLF9 was analyzed in different types of carcinoma. Different color curves indicate the regulatory role of KLF9 gene on different functions or pathways in different carcinomas. Upward curve peaks indicate positive regulation, and downward curve peaks indicate negative regulation. ACC = adrenocortial carcinoma, BLCA = bladder urothelial carcinoma, BRCA = breast invasive carcinoma, CESC = cervical squamous cell carcinoma and endovervical adenocarcinoma, CHOL = cholangiocarcinoma, HNSC = head and neck squamous cell carcinoma, KEGG = Kyoto Encyclopedia of Genes and Genomes, KIRC = kidney renal clear cell carcinoma, KLF9 = Kruppel-like transcription factor 9, LAML = acute myeloid leukemia, LIHC = liver hepatocellular carcinoma, LUSC = lung squamous cell carcinoma, OV = ovarian serous cystadenocarcinoma, PCPG = pheochromocytoma and paraganglioma, READ = rectum adenocarcinoma, SARC = sarcoma, STAD = Stomach adenocarcinoma, TGCT = teaticular germ cell tumors.

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