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Comprehensive Pan-Cancer Study Identifies Hepatic Leukemia Factor as a Crucial Biomarker for Immunity and Prognostic Accuracy

Kang Wen
Tianjin Medical University Cancer Institute and Hospital
Gulijiayina Nuerhashi
Tianjin Medical University Cancer Institute and Hospital
Ziyi Chen
Tianjin Medical University Cancer Institute and Hospital
Jianxi Zhou
Tianjin Medical University Cancer Institute and Hospital
Jingyao Gu
Second Affiliated Hospital of Nanjing Medical University
Peng Chen
chenpeng@tjmuch.com

Tianjin Medical University Cancer Institute and Hospital

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Abstract

Background

The circadian gene hepatic leukemia factor (HLF), a proline and acidic amino acid-rich basic leucine zipper (PAR bZIP) transcription factor, is under-explored in terms of its prognostic and immunotherapeutic roles across various cancers.

Methods

Utilizing databases like UCSC Xena, TIMER2.0, and TCGA, this study assessed HLF's expression variability across numerous cancer forms. The research further assessed the survival outcomes, clinical attributes, and genetic alterations associated with HLF. Additionally, the impact of HLF on immunotherapy outcomes was analyzed through methodologies such as Gene Set Enrichment Analysis, evaluation of the tumor microenvironment, and immune cell infiltration studies.

Results

Findings indicate a notable reduction in HLF's transcription and protein levels in most cancers, highlighting its prognostic relevance for patient survival in specific cancers like CESC, HNSC, KIRC, KIRP, LGG, LUAD, MESO, PAAD, and READ. Furthermore, in certain cancers, a significant correlation between HLF expression and tumor mutation burden (TMB), microsatellite instability (MSI), and clinical features was observed. Gene Set Enrichment Analysis revealed significant links between HLF and immune-related pathways. The study also confirmed a strong association between HLF expression and the infiltration of immune cells, as well as its correlation with chemotherapy resistance-related genes, genes related to immune microenvironment reprogramming, and genes related to carbohydrate metabolism. The biological function of HLF was verified in common lung cancer cell lines. Knockdown of HLF enhanced the proliferation and migration abilities of tumor cells, while overexpression inhibited these abilities.

Conclusions

This comprehensive investigation underscores the potential of HLF as a valuable prognostic and immunotherapeutic biomarker in pan-cancer, offering novel insights and evidence for enhancing cancer treatment strategies.

Introduction

Undoubtedly, the substantial burden of cancer poses a significant challenge to public health, impeding advancements in lifespan extension[1, 2]. The realm of cancer treatment faces critical challenges as tumors, noted for their intricate biology, engage in various processes—such as cell proliferation, evading

growth suppressors, resisting cell death, initiating angiogenesis, promoting invasion, and facilitating metastasis[3]. Additionally, the complex interaction between tumor invasion and the host's immune response plays a crucial role in cancer progression. The immune system, a key regulator in tumor dynamics, enhances the efficacy of immunotherapy by leveraging existing adaptive immune responses within the tumor, including the use of checkpoint inhibitors[4]. Given the pervasive nature of tumors and the complex pathways of oncogenesis, researching the expression levels of genes related to pan-cancer offers substantial promise for breakthroughs in clinical treatments and prognostic predictions.

As suggested by their designation, circadian rhythm-associated molecules, including a spectrum of clock genes and proteins, are pivotal in regulating sleep-wake cycles[5]. Recent research has demonstrated that imbalances in these circadian regulators are implicated in the initiation and spread of cancer through various pathways[6–8]. There has been a notable emphasis on the function of circadian clock genes in oncology. For example, in lung adenocarcinoma, the increased expression of CRY2, BMAL1, and RORA, alongside reduced expression of TIMELESS and NPAS2, has been associated with better outcomes. Conversely, elevated levels of DEC1 and TIMELESS are indicative of poorer overall survival in squamous cell non-small cell lung cancer (NSCLC)[9]. Furthermore, reduced expression of several Period (PER) protein family members, particularly PER1-3, in NSCLC has been linked to adverse clinicopathological characteristics and shorter patient survival. The detrimental influence of PER2 on NSCLC stems from its role in augmenting the expression of tumor suppressor genes such as BAX, TP53, and TP21, thereby obstructing the PI3K/AKT/mTOR signaling pathway[10, 11].

Undoubtedly, the circadian gene hepatic leukemia factor (HLF), part of the proline and acidic amino acid-rich basic leucine zipper (PAR bZIP) transcription factor family, displayed significant decreases in NSCLC tissues that experienced early relapse[12, 13]. This reduction in HLF levels was closely linked with early disease progression and distant metastasis in NSCLC patients. Experimental work revealed that elevating HLF levels hindered, whereas suppressing HLF facilitated, the spread of NSCLC cells to the lungs, bones, liver, and brain in live models. Further studies indicated that reduced expression of HLF promoted anaerobic metabolism, supporting the growth of NSCLC cells in nutrient-scarce environments by activating the NF-κB/p65 pathway through the disruption of PPARα and PPARγ translocation. Additional research pointed out that both genetic alterations and methylation processes were responsible for the decreased expression of HLF in NSCLC tissues[14].

In our research, we investigated HLF expression across various cancer types, focusing on analyses of differentially expressed genes (DEGs), prognostic significance, and enrichment within different tumor classifications. We further examined the correlation between HLF expression, immune cell infiltration, and immunoregulatory factors. Our study also included experimental validation of HLF at the cellular level. These results suggest that HLF is a potentially reliable prognostic biomarker, intimately connected with tumor immunomodulatory processes, and underscore its prospective value as a predictor of immunotherapy outcomes in a pan-cancer context.

Methods

Data collection

All data used in this study were sourced from the UCSC Xena database (https://xena.ucsc.edu/public/). We downloaded gene expression data, mutation profiles, clinical information, and overall survival statistics from the GDC hub (https://portal.gdc.cancer.gov/). Additionally, other survival data were acquired from the Pan-Cancer Atlas Hub[15].

HLF differential expression analysis

We utilized the TIMER2.0 database (Timer.cistrome.org) to analyze the differential expression levels of HLF mRNA across various cancer types. Additionally, we compared cancerous and adjacent non-cancerous tissue samples using data from the TCGA database (https://portal.gdc.cancer.gov/).

Immunofluorescence and Immunohistochemistry

The distribution of HLF in cells and its expression in cancer were assessed using the Human Protein Atlas (HPA) (https://www.proteinatlas.org/). We investigated HLF distribution within cells via immunofluorescence techniques and analyzed protein expression levels through immunohistochemistry[16, 17].

Survival analysis

We evaluated the prognostic significance of HLF using Kaplan-Meier analysis to compare high and low expression groups. Univariate Cox regression analysis was conducted to explore the association between HLF expression and various survival metrics, including overall survival (OS), disease-related survival (DSS), and disease-free interval (DFI), while adjusting for age and tumor stage. For each cancer type, we determined P-values and hazard ratios (HR) with 95% confidence intervals (CI).[18, 19].

Assessment of clinical correlations

We analyzed the clinical correlation between HLF expression and pan-cancer characteristics using R software, focusing on tumor stage (across four stages), race, and age (with a cutoff at 65 years). Statistical significance was established at a p-value less than 0.05[20].

Correlation analysis of TMB and MSI with HLF

Mutation data for HLF were sourced from the UCSC Xena database. We assessed the association of HLF with "Tumor Mutation Burden" (TMB) and "Microsatellite Instability" (MSI) for each cancer type using the Spearman correlation method, and the results were displayed in radar plots[21].

Tumor microenvironment and immune infiltrate analysis

To investigate the infiltration levels of immune and stromal cells across various cancers, we employed the ESTIMATE method to evaluate the correlation between HLF expression and scores for immune and stromal cells. Additionally, we analyzed the relationship between HLF expression levels and various immune cells, such as CD8 T cells and monocytes, using the CIBERSORT tool (available at http://cibersort.stanford.edu/) and data from the TIMER2.0 database (http://timer.cistrome.org/)[22–24].

Co-expression analysis of the HLF gene

We assessed the correlation between HLF and various gene groups, specifically those involved in Cuproptosis, Ferroptosis, lactate metabolism, chemotherapy resistance-related genes, genes related to immune microenvironment reprogramming, genes related to carbohydrate metabolism, and immune chemokines. The outcomes of these co-expression analyses were visually represented using heatmaps.

Statistical analysis

All statistical analyses were conducted using R software (available at https://www.r-project.org/). We considered a p-value of less than 0.05 as statistically significant.

Cell culture

A549, PC9, H1975, and HCC827 cell lines were acquired from the CAS Shanghai Cell Bank. These cells were cultured in DMEM medium (Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco) and antibiotics. The cells were maintained in a 37°C incubator equipped with a 5% CO_2 atmosphere.

Real-time PCR

Total RNA was isolated and extracted using RNAiso Plus (Takara, Dalian, China). The reverse transcription of RNA into cDNA was conducted using the HiScript III 1st Strand cDNA Synthesis Kit (gDNA wiper) (Nanjing Nazyme Bio-technology Co.). The qPCR assay was performed on a LightCycler® 480 Instrument II real-time PCR system, with GAPDH serving as an internal control. The primers used in the procedure were as follows:

GAPDH-F: GGTGAAGGTCGGTGTGAACG,

GAPDH-R: CTCGCTCCTGGAAGATGGTG;

HLF-F: GCAAGGCCGCAGAAAAGAACAA,

HLF-R: ATTTGGCCCAAGGCTCCTTCCTC.

Comparative expression results were calculated using the $2^{-\triangle\triangle Ct}$ methodology.

Result

Analysis of HLF expression in pan-cancers

The differential analysis of HLF expression, utilizing cancer and pan-cancer tissue samples from the TCGA and TIMER2.0 databases, revealed higher HLF expression in normal tissues compared to cancer tissues in various types such as BLCA, BRCA, CESC, CHOL, COAD, GBM, HNSC, KICH, KIRC, LIHC, LUAD, LUSC, PRAD, READ, STAD, THCA, and UCEC (Fig. 1A). Additionally, data from the Human Protein Atlas (HPA) indicated that HLF is predominantly located in the nucleoplasm. The cellular localization of HLF was confirmed in Hep-G2 and PC-3 cells using antibodies HPA071210 and HPA068156 (Fig. 1B ~ C). We also predicted the protein structure of HLF and its distribution within cells (Fig. 1D ~ E). In addition, we obtained information from HPA on the expression of HLF in different databases and different organizations (Supplement 1A ~ D).

Prognostic value of HLF in cancer patients

The potential prognostic value of HLF was assessed using Cox proportional hazards model and Kaplan Meier analysis. The results of the Cox model showed that the expression level of HLF was positively associated with the prognosis of CESC ($p = 0.002$), HNSC ($p = 0.005$), KIRC ($p < 0.001$), KIRP ($p = 0.003$), LGG ($p < 0.001$), LUAD ($p = 0.004$), MESO ($p < 0.001$), and PAAD ($p = 0.002$), as well as negatively in READ ($p = 0.032$). Kaplan-Meier analysis showed that high expression of HLF predicted better OS in LGG ($p < 0.001$), MESO ($p < 0.001$), KIRC ($p < 0.001$), COAD ($p = 0.003$), LUAD ($p = 0.002$), and SARC ($p = 0.003$) (Fig. 2A ~ G).

For DSS, high expression of HLF was a negative factor in PCPG ($p = 0.021$) patients, but a positive factor in CESC patients ($p = 0.008$), COAD patients ($p = 0.05$), HNSC patients ($p = 0.003$), KIRC patients ($p < 0.001$), KIRP patients ($p = 0.002$), LGG patients ($p = 0.002$), LUAD patients ($p = 0.002$), LUSC patients ($p = 0.002$), MESO patients ($p = 0.002$), and UVM ($p = 0.03$). Consistent with the results of the Cox proportional hazards model of DSS, the K-M curve indicated that a high level of HLF was positively correlated with good survival outcomes in HNSC ($p = 0.002$), KIRC ($p < 0.001$), LGG ($p < 0.001$), LUAD ($p < 0.001$), LUSC ($p < 0.001$) and MESO ($p < 0.001$) (Fig. 2H ~ N).

Correlation between HLF expression and OS (A) DSS, P-values less than 0.05 are highlighted in red. (B-G) High expression of HLF predicted better OS than low expression. (H) by utilizing Cox. HR (hazard ratio) > 1 indicates that HLF may be an adverse factor in the occurrence and development of cancer; $0 < HR < 1$ indicates that HLF may be a protective factor in cancer. Kaplan-Meier analysis in patients with high and low HLF expression. (I-N) High expression of HLF predicted better DSS than low expression.

The forest plot showed that high expression of HLF predicted poor DFI in UCEC ($p = 0.002$) and better DFI in BRCA ($p = 0.031$), PAAD ($p = 0.028$), PRAD ($p = 0.013$), and THCA ($p = 0.028$). However, Kaplan-Meier analysis found that UCEC is not statistically significant. In addition, the K-M curve of MESO ($p = 0.032$), PAAD ($p = 0.037$), and SARC ($p = 0.002$) showed that high expression of HLF indicated a better prognosis (Fig. 3A ~ D). Furthermore, in the PFI-related Cox proportional hazards model, HLF also

exhibited significant prognostic value in CESC (p = 0.007), HNSC (p = 0.011), KIRC (p < 0.001), KIRP (p = 0.005), LGG (p < 0.001), LUAD (p < 0.001), LUSC (p = 0.006), MESO (p = 0.006), PAAD (p < 0.001), PRAD (p < 0.006), and UCEC (p = 0.023). Patients with high expression of HLF had prolonged PFI in HNSC (p = 0.004), KIRC (p < 0.001), KIRP (p = 0.008), LGG (p < 0.001), LUAD (p = 0.001), LUSC (p = 0.002), MESO (p = 0.012), PAAD (p < 0.024), and SARC (p < 0.016) (Fig. 3E ~ N).

Prognostic value of HLF in cancer patients

The potential prognostic value of HLF was assessed using Cox proportional hazards model and Kaplan Meier analysis. The results of the Cox model showed that the expression level of HLF was positively associated with the prognosis of CESC (p = 0.002), HNSC (p = 0.005), KIRC (p < 0.001), KIRP (p = 0.003), LGG (p < 0.001), LUAD (p = 0.004), MESO (p < 0.001), and PAAD (p = 0.002), as well as negatively in READ (p = 0.032). Kaplan-Meier analysis showed that high expression of HLF predicted better OS in LGG (p < 0.001), MESO (p < 0.001), KIRC (p < 0.001), COAD (p = 0.003), LUAD (p = 0.002), and SARC (p = 0.003) (Fig. 2A ~ G).

For DSS, high expression of HLF was a negative factor in PCPG (p = 0.021) patients, but a positive factor in CESC patients (p = 0.008), COAD patients (p = 0.05), HNSC patients (p = 0.003), KIRC patients (p < 0.001), KIRP patients (p = 0.002), LGG patients (p = 0.002), LUAD patients (p = 0.002), LUSC patients (p = 0.002), MESO patients (p = 0.002), and UVM (p = 0.03). Consistent with the results of the Cox proportional hazards model of DSS, the K-M curve indicated that a high level of HLF was positively correlated with good survival outcomes in HNSC (p = 0.002), KIRC (p < 0.001), LGG (p < 0.001), LUAD (p < 0.001), LUSC (p < 0.001) and MESO (p < 0.001) (Fig. 2H ~ N).

Correlation between HLF expression and OS (A) DSS, P-values less than 0.05 are highlighted in red. (B-G) High expression of HLF predicted better OS than low expression. (H) by utilizing Cox. HR (hazard ratio) > 1 indicates that HLF may be an adverse factor in the occurrence and development of cancer; 0 < HR < 1 indicates that HLF may be a protective factor in cancer. Kaplan-Meier analysis in patients with high and low HLF expression. (I-N) High expression of HLF predicted better DSS than low expression.

The forest plot showed that high expression of HLF predicted poor DFI in UCEC (p = 0.002) and better DFI in BRCA (p = 0.031), PAAD (p = 0.028), PRAD (p = 0.013), and THCA (p = 0.028). However, Kaplan-Meier analysis found that UCEC is not statistically significant. In addition, the K-M curve of MESO (p = 0.032), PAAD (p = 0.037), and SARC (p = 0.002) showed that high expression of HLF indicated a better prognosis (Fig. 3A ~ D). Furthermore, in the PFI-related Cox proportional hazards model, HLF also exhibited significant prognostic value in CESC (p = 0.007), HNSC (p = 0.011), KIRC (p < 0.001), KIRP (p = 0.005), LGG (p < 0.001), LUAD (p < 0.001), LUSC (p = 0.006), MESO (p = 0.006), PAAD (p < 0.001), PRAD (p < 0.006), and UCEC (p = 0.023). Patients with high expression of HLF had prolonged PFI in HNSC (p = 0.004), KIRC (p < 0.001), KIRP (p = 0.008), LGG (p < 0.001), LUAD (p = 0.001), LUSC (p = 0.002), MESO (p = 0.012), PAAD (p < 0.024), and SARC (p < 0.016) (Fig. 3E ~ N).

The relationship between HLF and clinical information

In the early stages (stage I and II) of cancers such as KICH, KIRC, KIRP, TGCT, and THCA, HLF expression was significantly higher compared to later stages. Specifically, in KIRC, HLF levels were the highest in stage I, showing a distinct difference from stages III and IV. This pattern may be associated with the aggressive proliferation, poor prognosis, and enhanced invasion capabilities of stage IV cancer cells, along with inhibition of cell death. For CHOL, KICH, KIRC, KIRP, and LIHC, HLF expression was also higher in stages I through III compared to stage IV. However, the smaller sample size for stage IV LIHC limited the ability to draw definitive conclusions. Additionally, HLF expression was notably higher in stage I of KICH, LUAD, and TGCT (Fig. 4A ~ I). For patients under 65 years, HLF expression was significantly higher in BRCA, LUAD, SARC, SKCM, and TGCT, whereas in CESC, it was significantly higher in those over 65 years (Fig. 4J ~ O). We continued to investigate the expression of HLF in different race groups and found that it was higher in whites in BLCA and BRCA, higher in blacks in ESCA and KIRP, and higher in yellows in KIRC (Fig. 4P ~ T).

Relationship of HLF with TMB and MSI

Increasing evidence suggests that Tumor Mutation Burden (TMB) and Microsatellite Instability (MSI) can serve as independent biomarkers for evaluating the therapeutic effects of immune checkpoint inhibitors and the prognosis of various cancers [25, 26]. Consequently, our research further examined the correlation between HLF expression and these biomarkers within a pan-cancer analysis. We observed a positive correlation between HLF expression and TMB in cancers such as THYM, LIHC, LAML, KIRP, and CHOL. In contrast, a negative correlation was found in THCA, TGCT, STAD, SARC, PRAD, PAAD, LUAD, LGG, KICH, ESCA, DLBC, and BRCA. Regarding MSI, HLF expression showed a positive relationship in THYM, READ, LGG, LAML, KIRP, GBM, and CHOL, while a negative correlation was noted in ACC, STAD, SARC, PRAD, PAAD, KICH, ESCA, DLBC, and CESC (Fig. 5).

GSEA of HLF in HALLMARK pathways

Single-gene Gene Set Enrichment Analysis (GSEA) was employed to uncover pathways influenced by HLF expression across various cancers. The analysis revealed that HLF positively correlates with immune-related pathways in cancers such as BLCA, BRCA, ESCA, COAD, GBM, LAML, LUSC, and PAAD, including pathways like immune response, detection of chemical stimulus, defense response to gram-negative bacteria, and transporter complex. Additionally, HLF was found to be positively enriched in processes related to fatty acid metabolism, ion transport, and trans-synaptic signaling in SARC, SKCM, STAD, and UVM. Conversely, pathways such as immunoglobulin production, production of molecular mediators of the immune response, and cell activation showed negative enrichment in CHOL, LGG, LIHC, KIRP, KICH, and KIRC. These findings indicate that HLF is generally associated with several critical pathways involved in cancer development (Fig. 6).

Correlation between HLF expression and immune infiltrating level in pan-cancers

According to GSEA, we observed a potential association between HLF and immune-related factors. Therefore, we further performed tumor microenvironment and immune infiltrate analysis. The results showed that HLF had a positive correlation with the immune score in BLCA (R = 0.33), DLBC (R = 0.43), LUAD (R = 0.18), PAAD (R = 0.27), PRAD (R = 0.3), and TGCT (R = 0.31), while HLF was a negative correlation with the immune score in GBM (R = − 0.23), KIRC (R = − 0.19), LGG (R = − 0.53), LIHC (R = − 0.31), OV (R = − 0.16), PCPG (R = − 0.36), and SARC (R = − 0.38). For stromal scores, positive correction with HLF was identified in BLCA (R = 0.45), BRCA (R = 0.22), COAD (R = 0.19), LUAD (R = 0.16), PAAD (R = 0.29), PRAD (R = 0.52), and STAD (R = 0.32). HLF was negatively correlated with the stromal score of HNSC (R = − 0.13), KIRC (R = − 0.14), LGG (R = − 0.35), LIHC (R = − 0.23), PCPG (R = − 0.3), and SARC (R = − 0.19). In BLCA (immune scores: R = 0.33, stromal score: R = 0.45), LUAD (immune scores: R = 0.18, stromal score: R = 0.16), PAAD (immune scores: R = 0.27, stromal score: R = 0.29), PRAD (immune scores: R = 0.3, stromal score: R = 0.52), KIRC (immune scores: R = − 0.19, stromal score: R = − 0.14), LGG (immune scores: R = − 0.53, stromal score: R = − 0.35), LIHC (immune scores: R = − 0.31, stromal score: R = − 0.23), PCPG (immune scores: R = − 0.36, stromal score: R = − 0.3), and SARC (immune scores: R = − 0.38, stromal score: R = − 0.19), HLF transcript levels were consistently negatively correlated with immune and stromal scores (Fig. 7). Fig. 7. Association of HLF with the TME Composition. Immune Score (A) BLCA (B) DLBC (C) GBM (D) KIRC (E) LGG (F) LIHC (G) LUAD (H) OV (I) PAAD (J) PCPG (K) PRAD (L) SARC (M) TGCT; Association of HLF with the TME Composition. StromalScore (N) BLCA (O) BRCA (P) COAD (Q) HNSC (R) KIRC (S) LGG (T) LIHC (U) LUAD (V) PAAD (W) PCPG (X) PRAD (Y) SARC (Z) STAD.

Immune-related cell infiltration is the main mechanism affecting the tumor microenvironment. Therefore, we further studied the relationship between HLF expression and immune infiltration analysis in pan-cancer. We found that HLF was positively correlated with B cells in BLCA, BRCA, and HNSC, while was negatively correlated with KIRP. On the other hand, HLF showed a correlation with T cells in BRCA, HNSC, KIRC, LIHC, and LUAD. We can see that the expression of HLF in BLCA is closely related to B and T immune cells. In addition, HLF is negatively correlated with Neutrophils in BLCA and BRCA. HLF is negatively correlated with Macrophages in BRCA and CESC (Fig. 8).

HLF correlated with the majority of Cuproptosis-related genes, Ferroptosis-related genes, lactate metabolism-related genes, immune chemokines genes, pyroptosis-related genes, and anoikis-related genes

In conclusion, given the significant relationship between HLF and tumor immune regulation, we delved deeper into HLF's role at the genetic level. Co-expression profiling of HLF with ten chemotherapy resistance genes across 33 tumour types reveals concordant up-regulation of drug-efflux transporters ABCB1 and ABCG2, RORA, and metabolic enzyme ALDH1A1, whereas inverse correlation with DUSP4 suggests pathway antagonism. These findings nominate HLF as a chemoresistance master regulator

(Fig. 9A). HLF positively aligns with immune evasion ligand CD47, matricellular modulator CCN1, circadian regulator ARNTL, lipid-metabolic enzyme CPT1A, and epigenetic repressor SETDB1. These data implicate HLF as a master orchestrator of immune-niche reprogramming (Fig. 9B). HLF expression co-varies with central glycolytic enzymes HK1, PFKL, PGK1, and PKM, pentose-phosphate regulators G6PD, PGD and TALDO1, and gluconeogenic transporters G6PC and SLC37A4, whereas inverse association with aldehyde-metabolism gene ALDOB suggests metabolic branchpoint control, carbohydrate-fuelled oncogenic plasticity and therapeutic vulnerability (Fig. 9C). Then we conducted an extensive co-expression analysis involving Cuproptosis-related genes, Ferroptosis-related genes, lactate metabolism-related genes, immune chemokines genes, pyroptosis-related genes, and anoikis-related genes. The analysis particularly focused on 47 immune checkpoint genes, revealing that most genes were significantly positively correlated with HLF in cancers like BLCA, HNSC, PRAD, and TGCT. Conversely, a negative correlation was observed in BRCA, KIRC, LGG, LIHC, SARC, and THCA. This suggests that HLF may play a diverse role in modulating the immune landscape across different cancer types (Supplement 3A). The results showed that Ferroptosis-related genes such as GPX4, HSF1, MUC1, NQO1, and HSPB1 were significantly negatively correlated with HLF in pan-cancer, while positively correlated with NFE2L2, GCLC, AKR1C2, and AKR1C2 (Supplement 3B). In Cuproptosis-related genes, HLF was positively correlated with DBT, ATP7A, ATP7B, and NFE2L2 of pan-cancer, while negatively correlated with CDKN2A and HLF (Supplement 3C). SLC25A12, in lactate metabolism-related genes, was significantly positively correlated with the expression of HLF in pan-cancer. Similarly, we can see that most of the lactate metabolism-related genes in TRMU, SLC16A3, RARS1, PUS1, PIF1, PDSS1, NAXE, DNA2, and IRAK1 are closely related to the expression of HLF (Supplement 3D). In pyroptosis-related genes, HLF was negatively correlated with IL6, IL18, GSDMD, GSDMA, GPX4, GASP6, GASP5, GASP 4, GASP3, and AIM2. We also can find that HLF expression in KIRC, LGG, LIHC, and THCA was negatively correlated with pyroptosis-related genes (Supplement 3E). In anoikis-related genes, HLF was positively correlated with PRKCA and KL of pan-cancer, while negatively correlated with ITGA5, PLAU, SPINK1, CYCS, PRDX4, CD63, SDCBP, BAK1, PLAUR, and BCAR1 (Supplement 3F).

The heatmaps present the correlations of HLF expression with genes related to (A) chemotherapy resistance genes (B) genes related to immune microenvironment reprogramming (C) genes related to carbohydrate metabolism. The upper left corner of each square represents the p-value, * represents $p < 0.05$, ** represents $p < 0.01$, and *** represents $p < 0.001$; the lower half circle represents the correlation between HLF and other genes, red represents positive correlation, and yellow represents negative correlation.

Biological functions of HLF in lung adenocarcinoma cells

Firstly, we found that the mRNA expression level of HLF was significantly higher in 16HBE cells than in A549, PC9, H1975, and HCC827 cells. Then we studied the biological function of HLF in lung adenocarcinoma cells (Fig. 10A). Then, we constructed siRNA as well as overexpression plasmid, verified the knockdown efficiency and overexpression in lung adenocarcinoma cells, and obtained

siRNAs with high knockdown efficiency and plasmids with high overexpression efficiency, respectively (Fig. 10B ~ C). Subsequently, we performed biological function experiments in A549, PC9, HCC827, and H1975 cells. The results showed that the proliferation ability of cells was enhanced after the knockdown of HLF and weakened after overexpression of HLF in CCK8 and clone formation experiments (Fig. 10D ~ G). Similarly, in the Transwell assay, the migration ability of the cells was enhanced with the knockdown of HLF and weakened with the overexpression of HLF. All of these results indicate that HLF as an oncogene can effectively inhibit the proliferation and metastatic ability of lung adenocarcinoma cells, and is expected to be a target for inhibiting tumor progression. (Fig. 11).

Discussion

Our comprehensive investigation highlighted the prognostic and potential immunotherapeutic significance of HLF within a pan-cancer context. We observed elevated gene expression levels of HLF in the majority of tumors, with some cancers showing specific prognostic implications. Moreover, HLF expression demonstrated a strong association with immune and inflammatory pathways, immune cell infiltration, and a wide array of immune-related genes. Consequently, HLF emerges as a potential prognostic biomarker and a predictor of immunotherapy outcomes, underscoring its importance in cancer biology and treatment strategies

HLF, as a member of the proline and acid-rich basic leucine zipper (PAR bZIP) transcription factor family[12, 13], plays a critical role in nervous system development and apoptosis in fibroblasts[27–29]. Aberrant expression of HLF has been linked to the development and progression of various human cancers. It is characterized by downregulation in hematological malignancies and gliomas, while it promotes proliferation, metastasis, and resistance to therapy in cancer cells[30–33]. Conversely, overexpression of HLF stimulates anchorage-independent growth in human basal cell carcinoma and enhances sorafenib resistance in hepatocellular carcinomas by upregulating OCT4 and SOX2 within a positive feedback loop [34, 35]. These findings suggest that HLF's role in cancer can vary dramatically depending on the type of tumor.

The role of circadian rhythm regulators as prognostic markers in cancer is increasingly recognized. For instance, Climent et al. found that the deletion-induced downregulation of PER3 predicted early recurrence and poor prognosis in breast cancer patients, especially those positive for the estrogen receptor (ER). Additionally, Papagiannakopoulos et al. reported that disruptions in circadian genes PER2 and BMAL1 increased lung tumor growth by boosting c-MYC expression and indicated poor progression-free survival in NSCLC patients. These studies underscore the complex but significant impact of circadian rhythm disruptions in cancer prognosis [36].

In our examination of HLF mRNA levels across 33 human tumors from various databases, we documented a significant reduction in expression for most tumors. When comparing transcription levels, we found substantial differences in cancers such as BLCA, BRCA, CESC, CHOL, COAD, GBM, HNSC, KICH, KIRC, LIHC, LUAD, LUSC, PRAD, READ, STAD, THCA, and UCEC. Generally, HLF expression did not show

significant variations based on clinical stage, age, or gender across most cancers. However, in specific cancer types like CESC, HNSC, KIRC, KIRP, LGG, LUAD, MESO, PAAD, and READ, HLF expression emerged as an independent prognostic factor and holds potential as a prognostic marker.

Our GSEA enrichment analysis highlighted a robust association between HLF and various critical biological processes, including immune response, chemical stimulus detection, defense response to gram-positive bacteria, and transporter complexes. These associations underscore HLF's involvement in mechanisms commonly observed in tumors. Our study focused on a spectrum of immune pathways, inflammatory responses, T-cell activities, and macrophage signaling pathways, all of which are fundamentally linked to the process of tumorigenesis.

To delve deeper into the potential significance of HLF, we investigated its relationship with the tumor microenvironment (TME) and immune cell infiltration. Our findings indicated a significant correlation between HLF and the infiltration of diverse immune cell types, such as CD4 + T cells, macrophages, and mechanisms regulating B cells, across various tumor TMEs. Consequently, we hypothesize that HLF may influence the immune landscape within the tumor microenvironment through multiple pathways, potentially impacting a broad array of immune cells rather than targeting specific types. This broad modulation suggests that HLF could be a pivotal factor in the immune dynamics of cancer, offering new avenues for therapeutic intervention.

The immune system utilizes immune cell infiltration to detect and eradicate tumor cells within the tumor microenvironment (TME). This infiltration and the initiation of anti-tumor immune responses are heavily influenced by a wide array of chemokines, chemokine receptors, cytokines, and immune checkpoints. Considering these factors, we assessed the relationship between HLF and immune checkpoint genes. Remarkably, HLF exhibited significant associations with the majority of genes in cancers such as BLCA, DLBC, LUAD, PAAD, PRAD, and TGCT. This alignment with our previous GSEA enrichment results suggests that HLF could serve as a potential regulatory target in the immunotherapy of these cancers, highlighting its crucial role in promoting the recruitment of immune cells[37–39].

The tumor immune microenvironment (TIME) is a critical factor in tumor progression, prognosis, and the response to immunotherapy[40, 41]. In cancers such as BLCA, LUAD, PAAD, and PRAD, we observed a strong positive correlation between the immune score and the extent of immune cell infiltration. Conversely, in KIRC, LGG, LIHC, PCPG, and SARC, a negative correlation was noted. It's important to recognize that different immune cell types have varying impacts on cancer outcomes; for example, CD8 + T cells generally are associated with a favorable prognosis, whereas regulatory T cells (Tregs) often indicate a poorer outcome.Our analysis of immune cell infiltration showed a significant positive relationship between HLF and Treg infiltration. This suggests that patients with HNSC, a rare but highly malignant tumor type with limited treatment options for advanced cases, might have better prognoses when HLF expression is elevated. This aligns with our analytical findings and underscores the potential of immunotherapy, such as checkpoint inhibitors and monoclonal antibodies, as viable treatments for these patients[42]. Significantly, HLF was found to positively correlate with the expression of numerous

immune checkpoint genes in HNSC, including BTLA, CD200, TNFRSF14, TNFSF4, CD244, CD40LG, CD28, CD200R1, ADORA2A, LGALS9, CD160, ICOSLG, CD27, CD40, TNFRSF18, TNFSF15, TIGIT, and TNFRSF9. These genes are key targets for immunotherapy, indicating that HLF may play a role in the immune escape mechanisms observed in BLCA and PRAD tumors. This connection suggests that targeting HLF could enhance the effectiveness of immunotherapeutic strategies, potentially altering the course of treatment for these cancer types.

In our cellular assays, we observed that decreased HLF expression was associated with increased cell proliferation and migration, while increased HLF expression led to a reduction in these processes. This trend was consistent at the mRNA level.

In summary, our research has identified HLF as a pivotal biomarker across a diverse range of malignancies. We have established that HLF has a significant association with prognosis, mutations, and immune responses in pan-cancer contexts. These findings suggest that HLF holds promise as a novel therapeutic target for various types of cancer, potentially revolutionizing treatment approaches and outcomes.

Abbreviations

HLF hepatic leukemia factor

ACC Adrenocortical carcinoma

KIRC Kidney renal clear cell carcinoma

HNSC Head and Neck squamous cell carcinoma

THCA Thyroid carcinoma

LGG Brain Lower Grade Glioma

TMB Tumor Mutation Burden

MSI Microsatellite Instability

DEG differentially expressed gene

OS overall survival

DSS disease-related survival

DFI disease-free interval

PFI progression-free interval

HR hazard ratios

CI confidence intervals

BRCA Breast invasive carcinoma

CHOL Cholangiocarcinoma

COAD Colon adenocarcinoma

KICH Kidney Chromophobe

KIRP Kidney renal papillary cell carcinoma

LIHC Liver hepatocellular carcinoma

LUAD Lung adenocarcinoma

LUSC Lung squamous cell carcinoma

PCPG Pheohromocytoma and Paraganglioma

READ Rectum adenocarcinoma

SARC Sarcoma

PAAD Pancreatic adenocarcinoma

UCEC Uterine Corpus Endometrial Carcinoma

OV Ovarian serous cystadenocarcinoma

CESC Cervial squamous cell carcinoma and endocervical adenocarcinoma

SKCM Skin Cutaneous Melanoma

MESO Mesothelioma

ESCA Esophageal carcinoma

TGCT Testicular Germ Cell Tumors

THYM Thymoma

PRAD Prostate adenocarcinoma

BLCA Bladder Urothelial Carcinoma

STAD Stomach adenocarcinoma

DLBC Lymphoid Neoplasm Diffuse Large B-cell Lymphoma

UVM Uveal Melanoma

Declarations

Data availability statement

All summary statistics based on association data are available free of charge. UCSC Xena database (https://xena.ucsc.edu/public/); GDC hub (https://portal.gdc.cancer.gov/); TCGA database (https://portal.gdc.cancer.gov/); TIMER2.0 database (Timer.cistrome.org); Human Protein Atlas (HPA) (https://www.proteinatlas.org/).

Author contributions

Kang Wen, Gulijiayina Nuerhashi, and Ziyi Chen designed the study, conducted data collection, data processing, statistical analysis, and wrote the manuscript. Jianxi Zhou revised the manuscript. Peng Chen and Jingyao Gu provided overall guidance for this work. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Consent for publication

Not applicable

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Figures

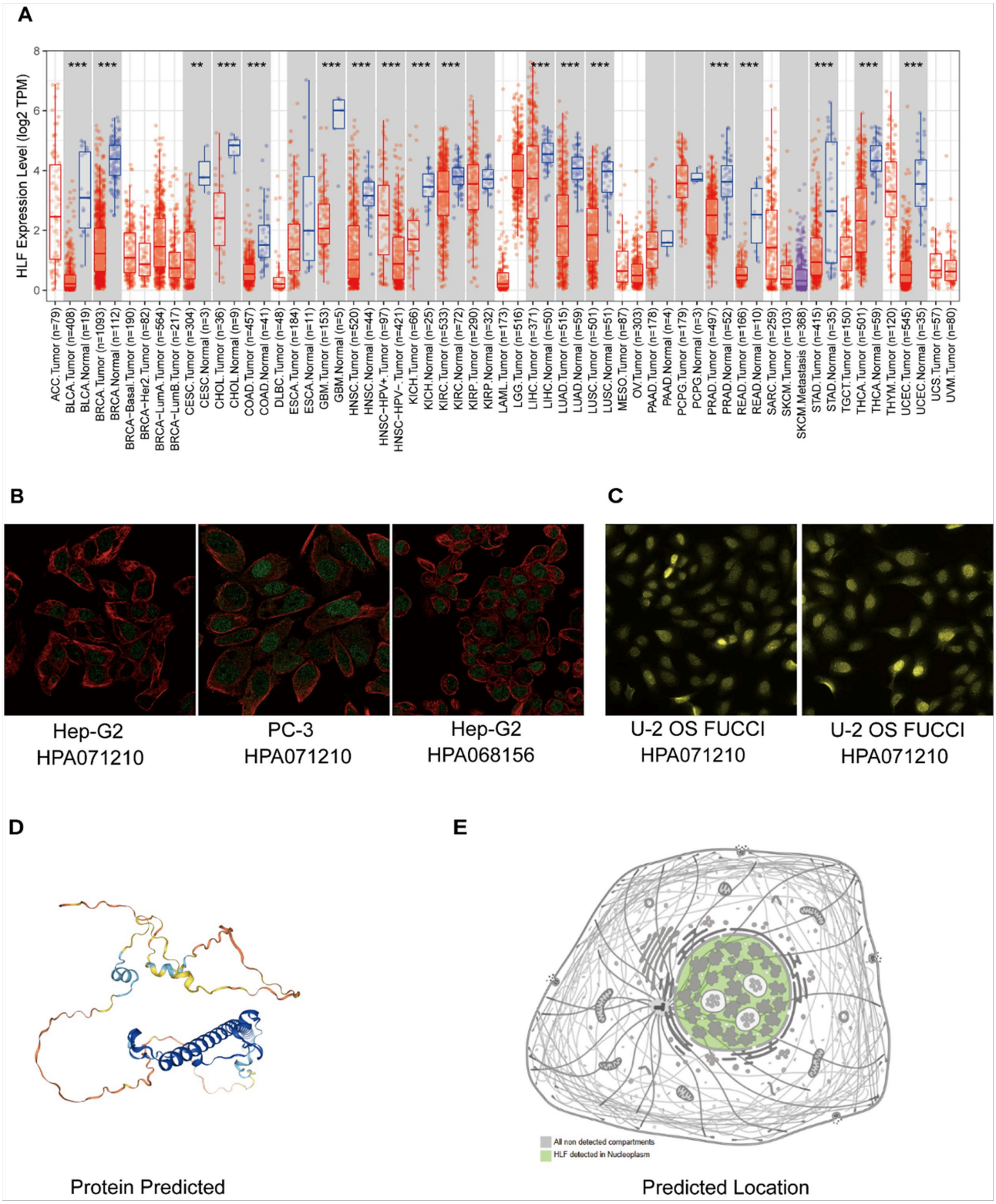


Figure 1

Differences in HLF expression between normal and tumor tissue samples (A) expression of HLF in pan-cancer and corresponding normal samples in TIMER2.0 (B, C) HLF cell location, green indicates the distribution of HLF (D) protein predicted of HLF (E) HLF localization prediction.

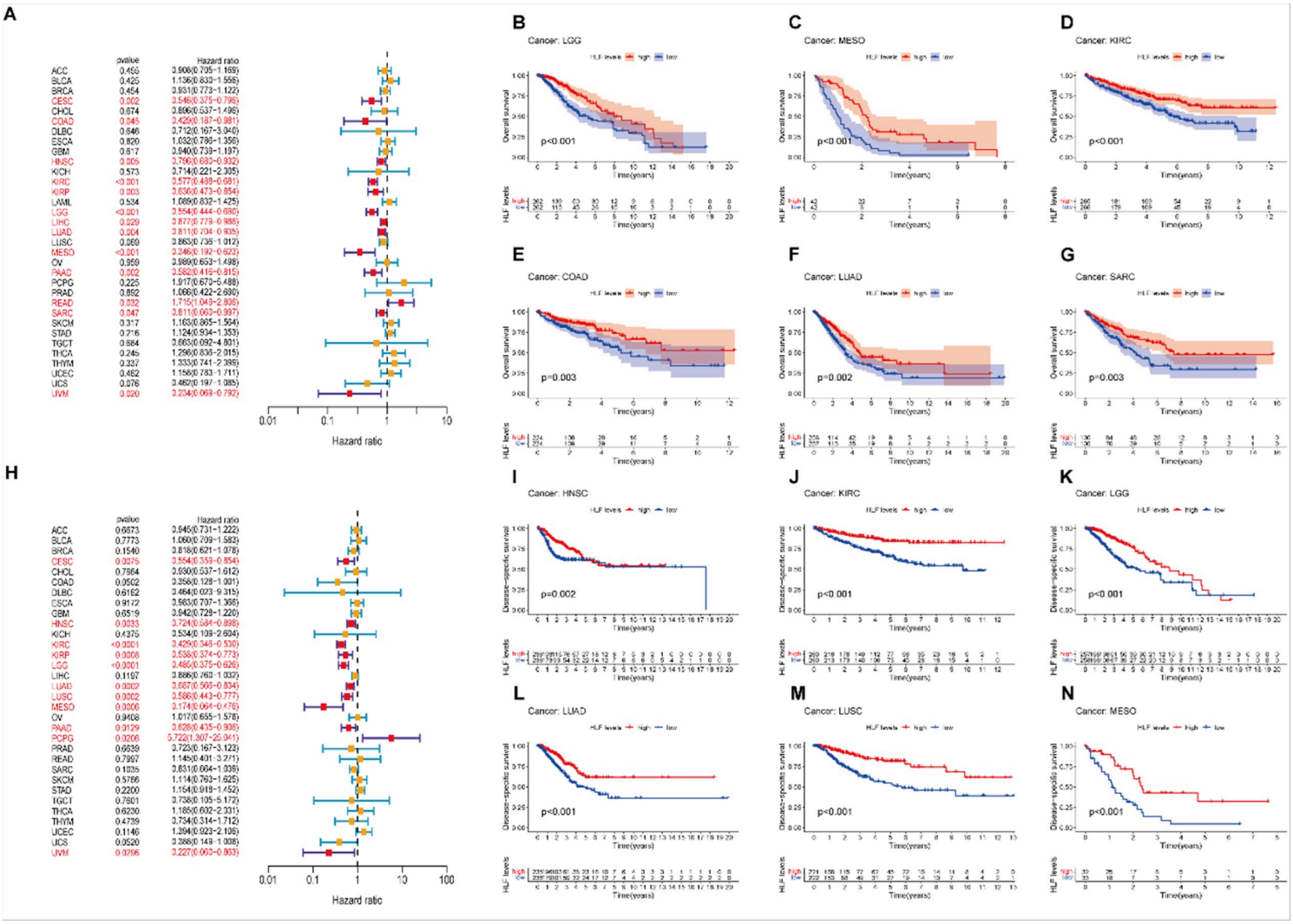


Figure 2

Prognostic assessment of HLF expression in OS and DSS.

Correlation between HLF expression and OS (A) DSS, P-values less than 0.05 are highlighted in red. (B-G) High expression of HLF predicted better OS than low expression. (H) by utilizing Cox. HR (hazard ratio) > 1 indicates that HLF may be an adverse factor in the occurrence and development of cancer; 0 < HR < 1 indicates that HLF may be a protective factor in cancer. Kaplan-Meier analysis in patients with high and low HLF expression. (I-N) High expression of HLF predicted better DSS than low expression.

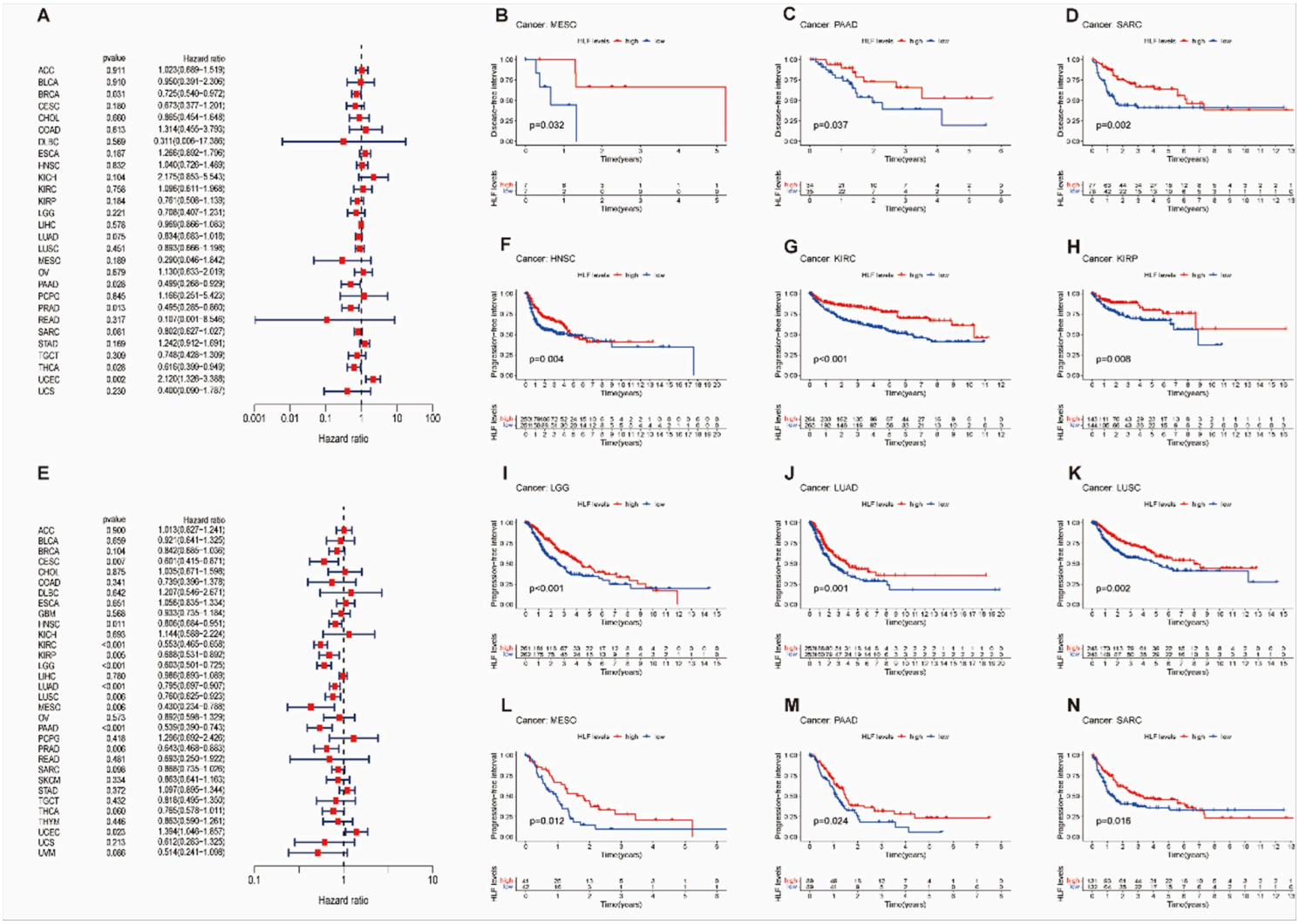


Figure 3

Prognostic assessment of HLF expression in DFI and PFI. Correlation between HLF expression and DFI (A) PFI. (B-D) high expression of HLF predicted better DFI than low expression. (E) by utilizing Cox. HR (hazard ratio) > 1 indicates that HLF may be an adverse factor in the occurrence and development of cancer; 0 < HR < 1 indicates that HLF may be a protective factor in cancer. Kaplan-Meier analysis in patients with high and low HLF expression. (F-N) high expression of HLF predicted better PFI than low expression.

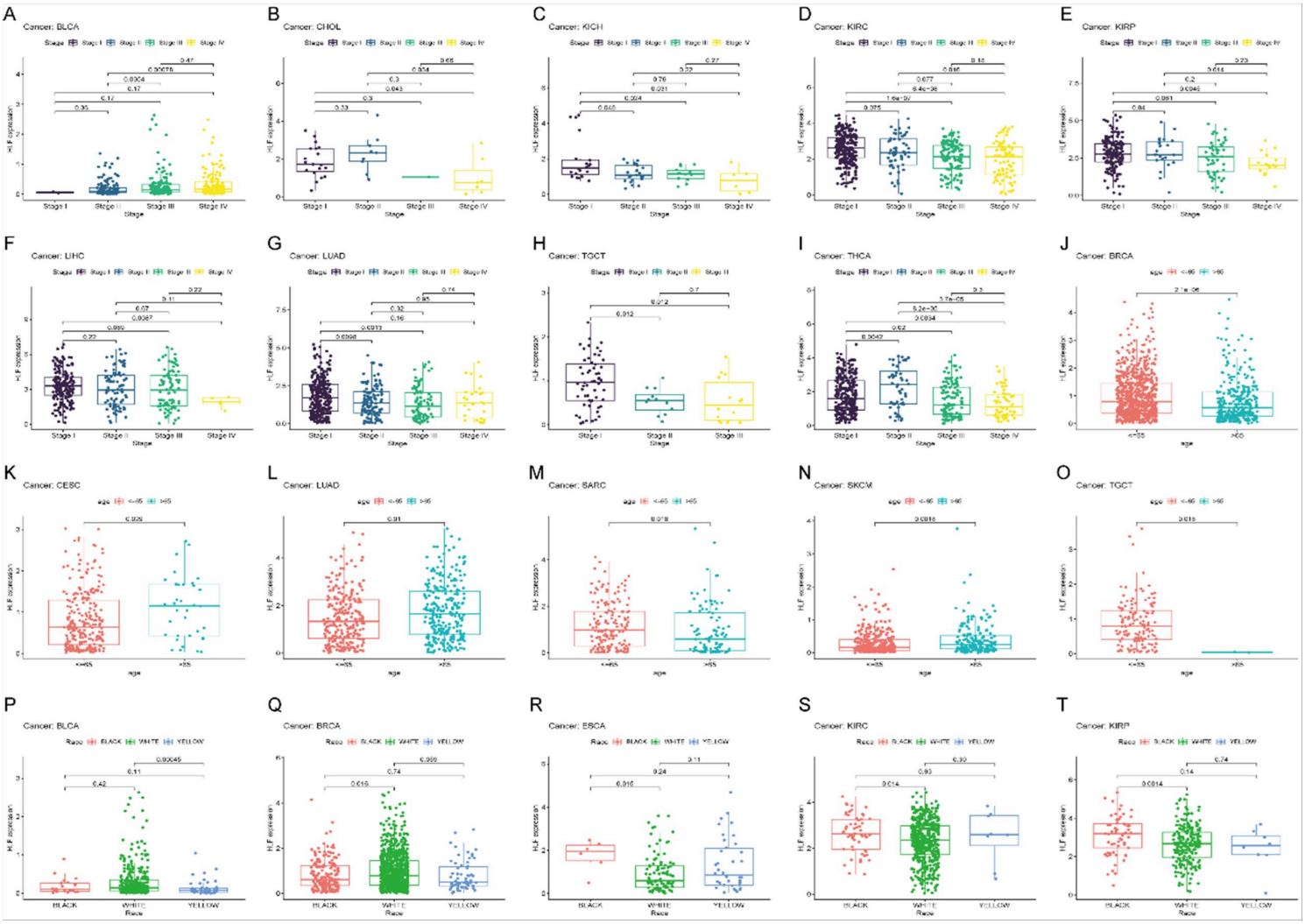


Figure 4

Analysis of clinic correlation of HLF expression. Correlation analysis of HLF expression and tumor clinical stage (A) BLCA (B) CHOL (C) KICH (D) KIRC (E) KIRP (F) LIHC (G) LUAD (H) TGCT (I) THCA; Correlation analysis of HLF expression and age (with 65 years as the cut-off) (J) BRCA (K) CESC (L) LUAD (M) SARC (N) SKCM (O) TGCT; Correlation analysis of HLF expression and race (P) BLCA (Q) BRCA (R) ESCA (S) KIRC (T) KIRP.

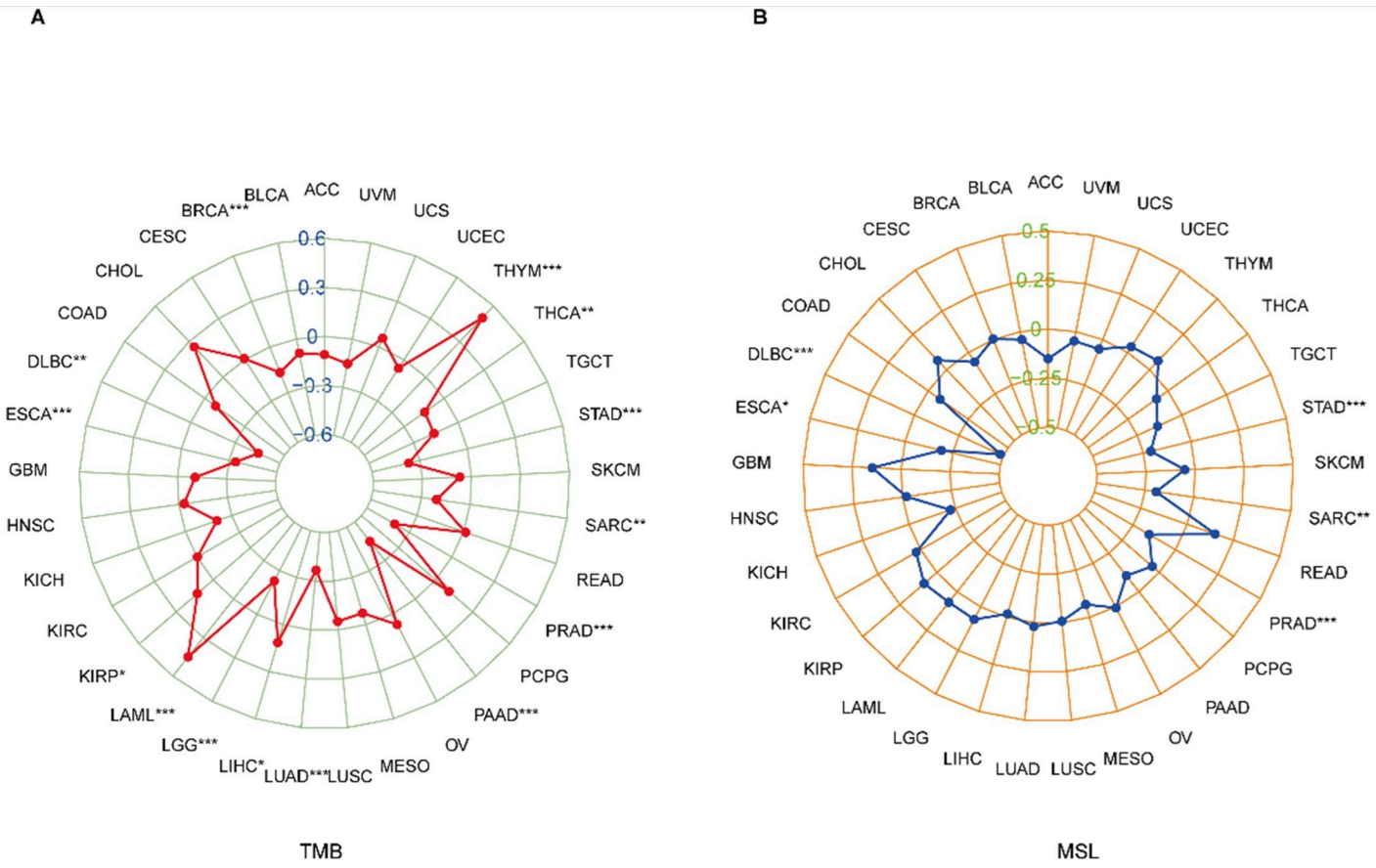


Figure 5

Relationship of HLF expression with TMB (A) and MSI (B).

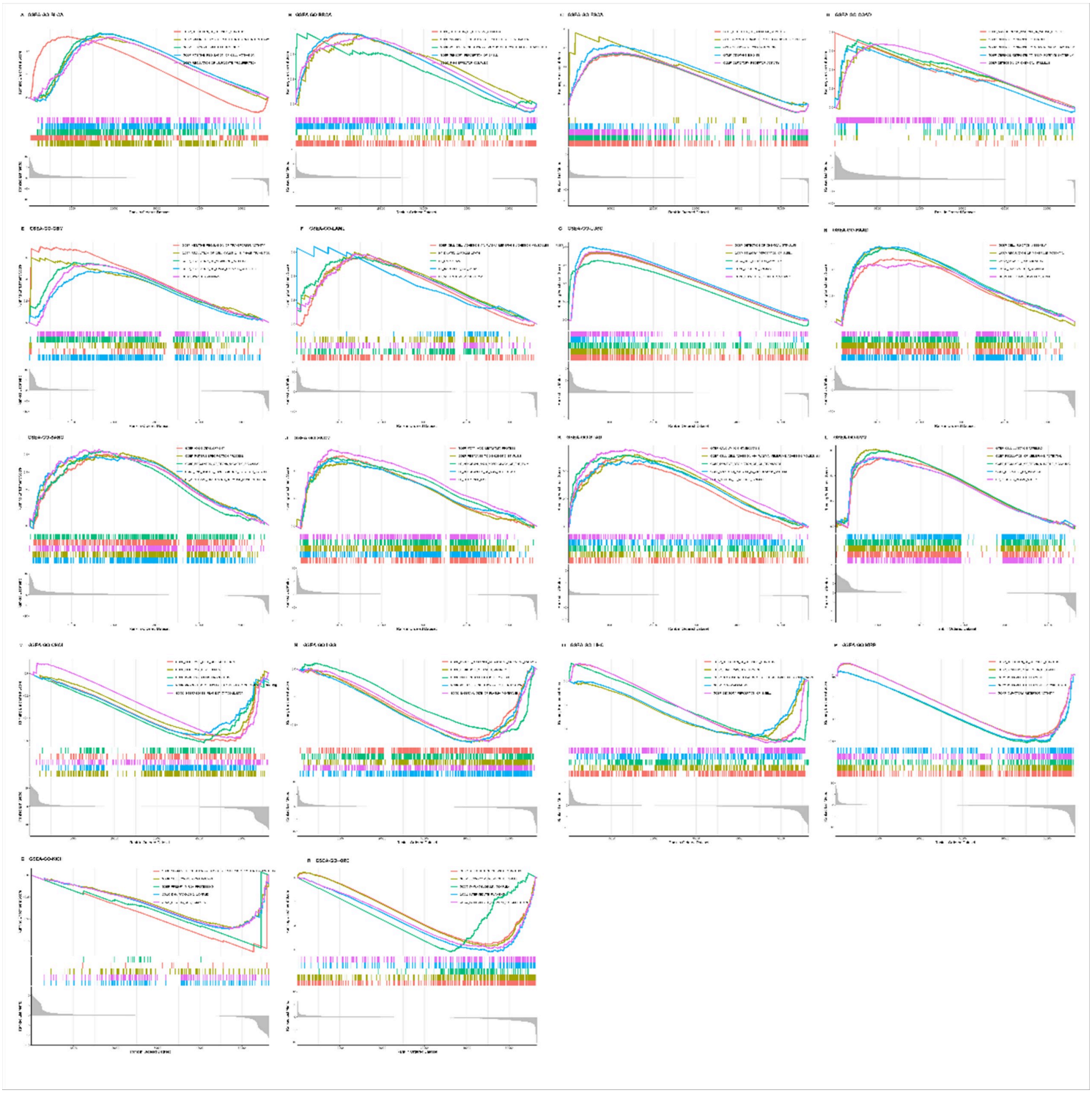


Figure 6

Gene set enrichment analysis of HLF in Pan-cancer. (A) BLCA (B) BRCA (C) ESCA (D) COAD (E) GBM (F) LAML (G) LUSC (H) PAAD (I) SARC (J) SKCM (K) STAD (L) UVM (M) CHOL (N) LGG (O) LIHC (P) KIRP (Q) KICH (R) KIRC.

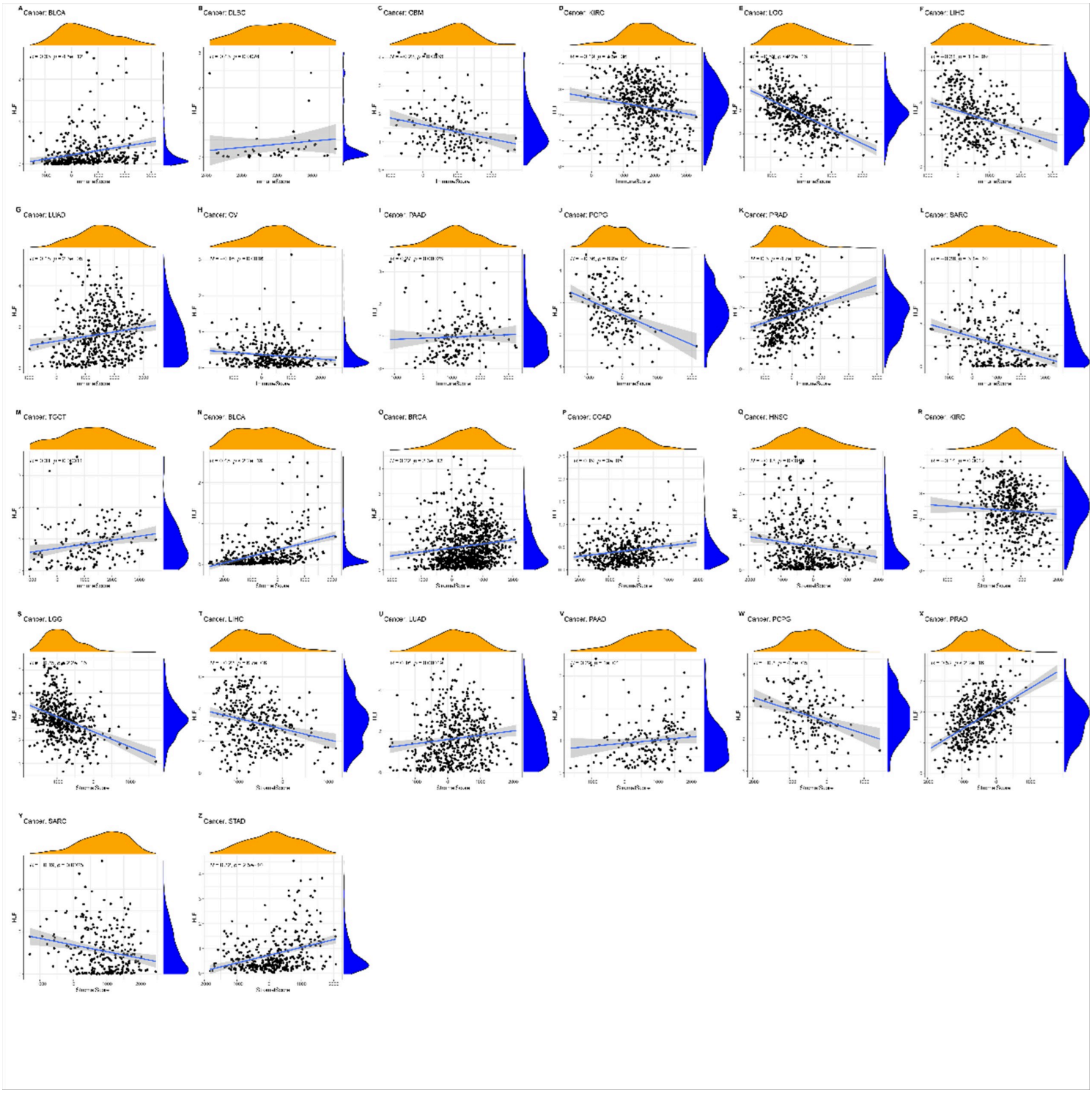


Figure 7

Association of HLF with the TME Composition. Immune Score (A) BLCA (B) DLBC (C) GBM (D) KIRC (E) LGG (F) LIHC (G) LUAD (H) OV (I) PAAD (J) PCPG (K) PRAD (L) SARC (M) TGCT; Association of HLF with the TME Composition. StromalScore (N) BLCA (O) BRCA (P) COAD (Q) HNSC (R) KIRC (S) LGG (T) LIHC (U) LUAD (V) PAAD (W) PCPG (X) PRAD (Y) SARC (Z) STAD.

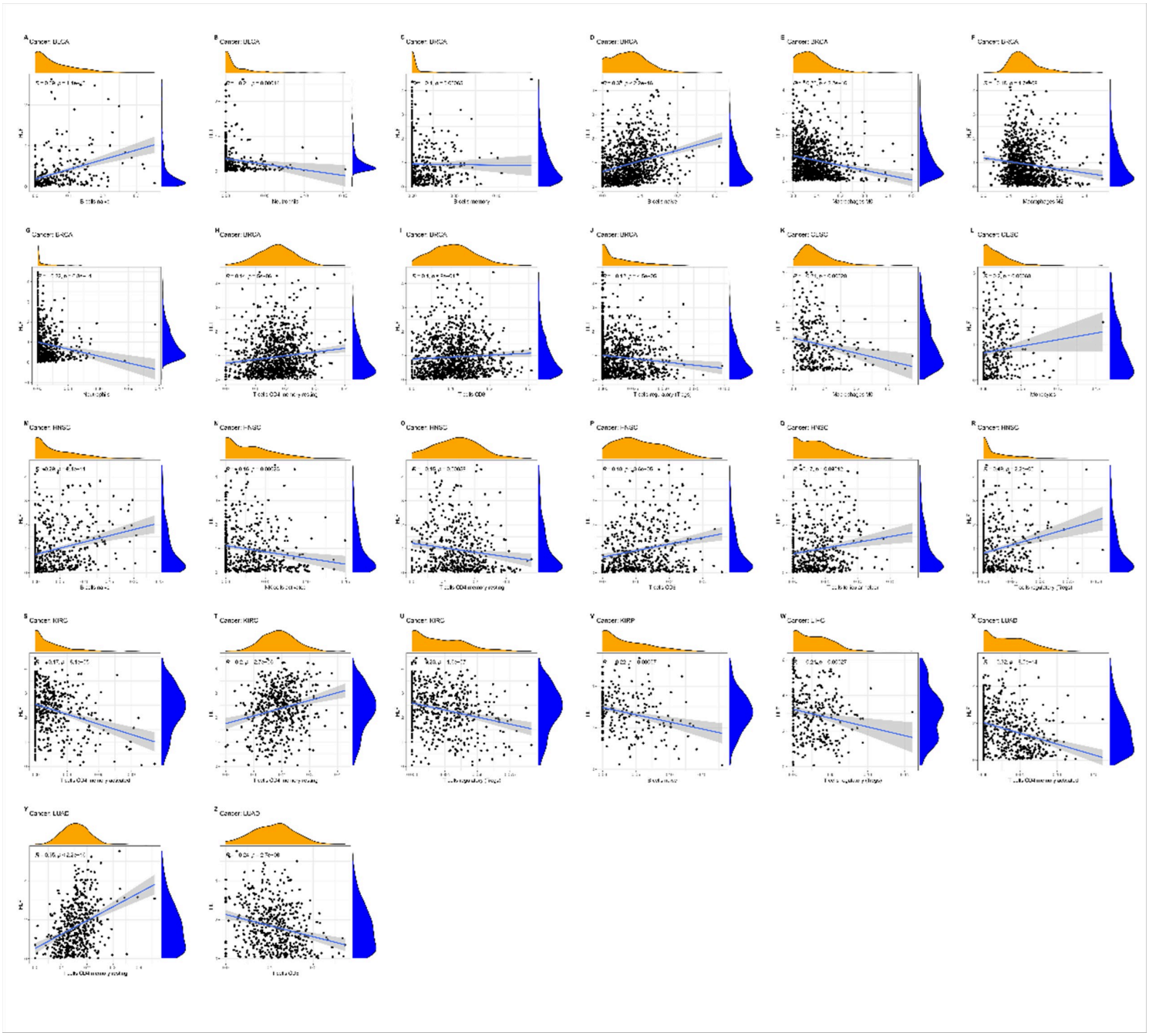


Figure 8

The correlation between HLF expression and immune cell infiltration (A-Z) Scatter plots showing that tumor-infiltrating immune cells were significantly correlated with HLF expression.

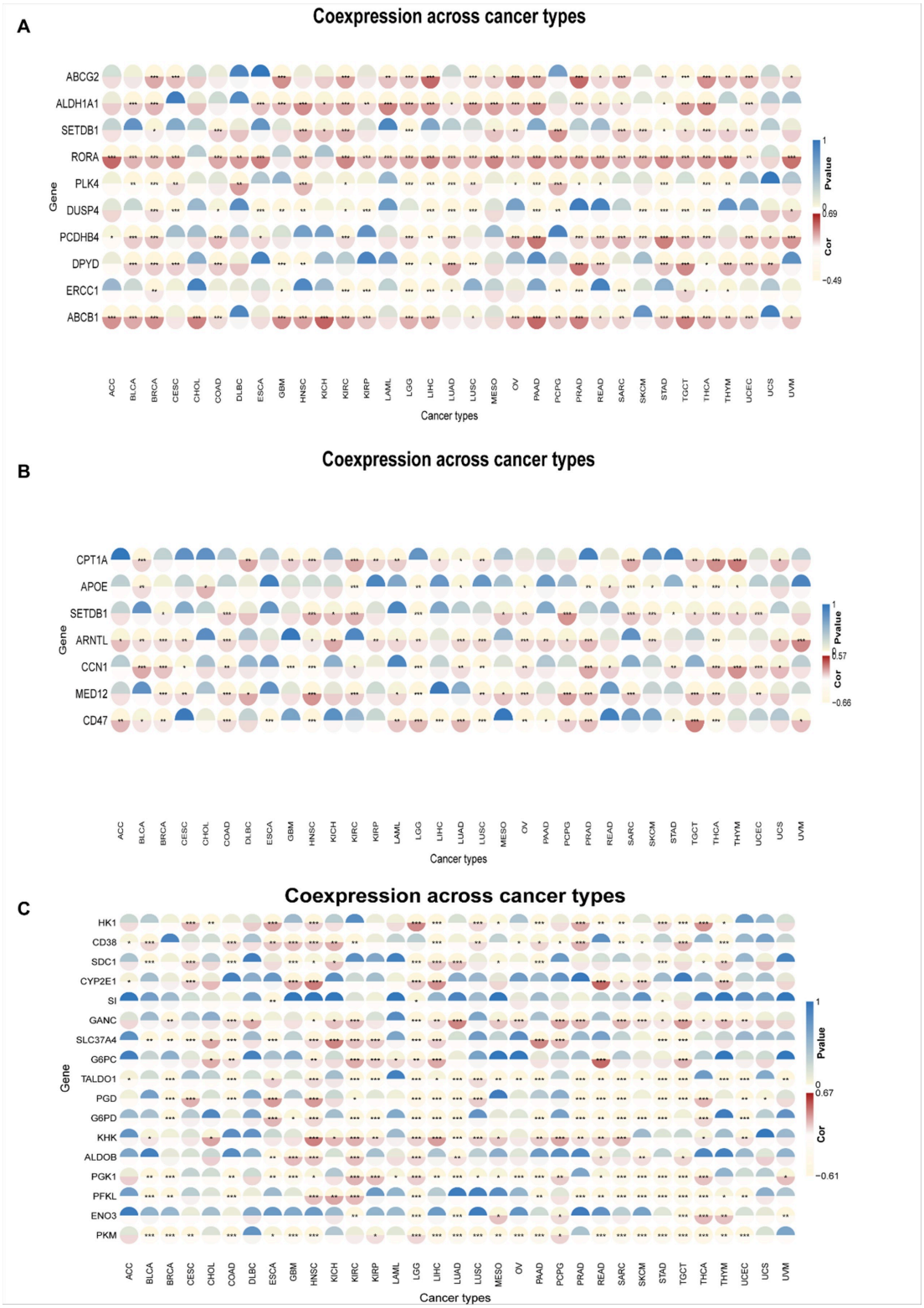


Figure 9

Gene co-expression analysis of HLF in pan-cancer.

The heatmaps present the correlations of HLF expression with genes related to (A) chemotherapy resistance genes (B) genes related to immune microenvironment reprogramming (C) genes related to carbohydrate metabolism. The upper left corner of each square represents the p-value, * represents p <

0.05, ** represents p < 0.01, and *** represents p < 0.001; the lower half circle represents the correlation between HLF and other genes, red represents positive correlation, and yellow represents negative correlation.

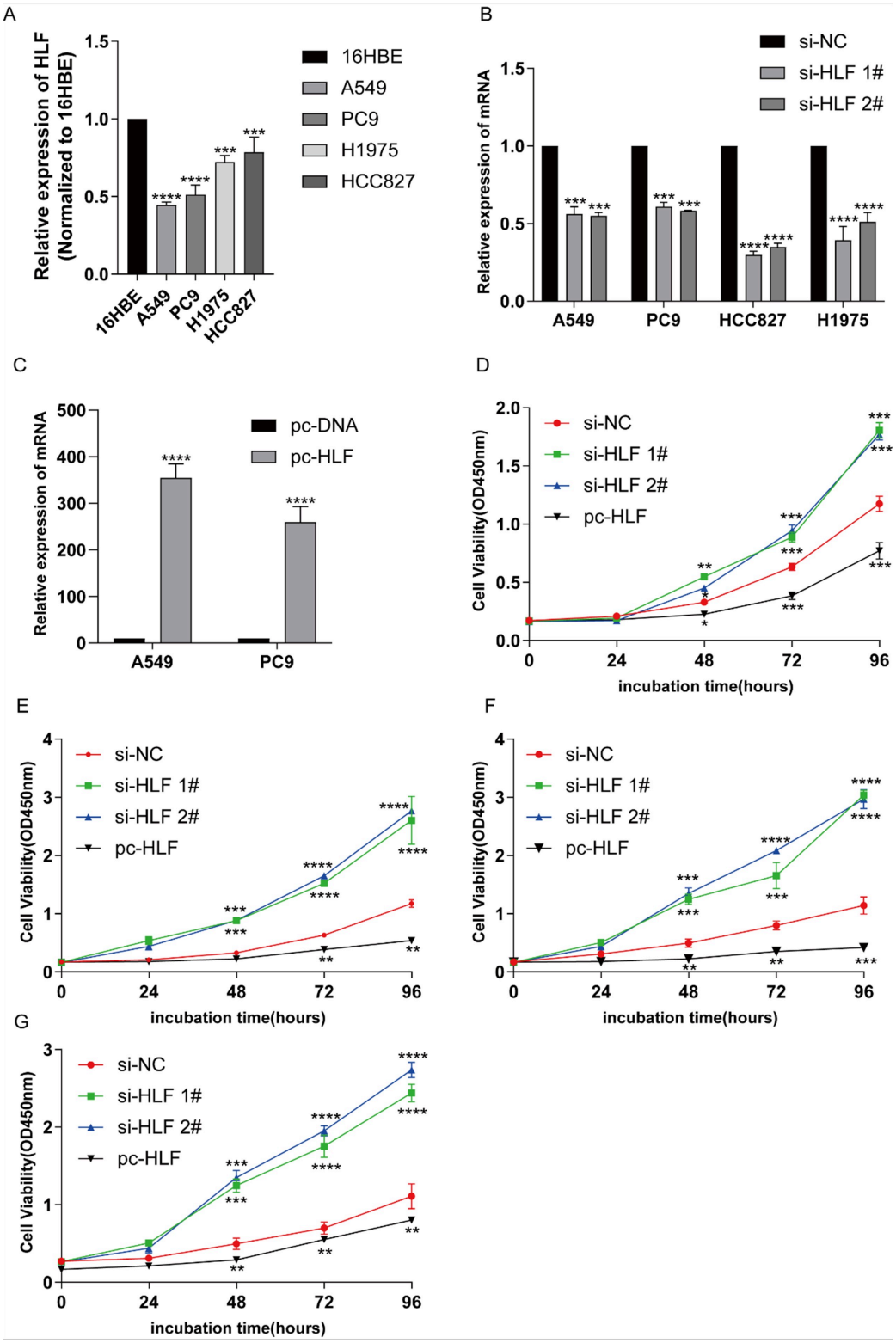


Figure 10

Expression of HLF in lung adenocarcinoma cells and biological function experiments (A) Expression of HLF in lung adenocarcinoma cells (B) HLF knockdown efficiency (C) HLF overexpression efficiency (D)

CCK8 experiments in A549 cells (E) CCK8 experiments in PC9 cell (F) CCK8 experiments in H1975 cell (G) CCK8 experiments in HCC827 cell. The upper left corner of each square represents the p-value, * represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$, and **** represents $p < 0.0001$.

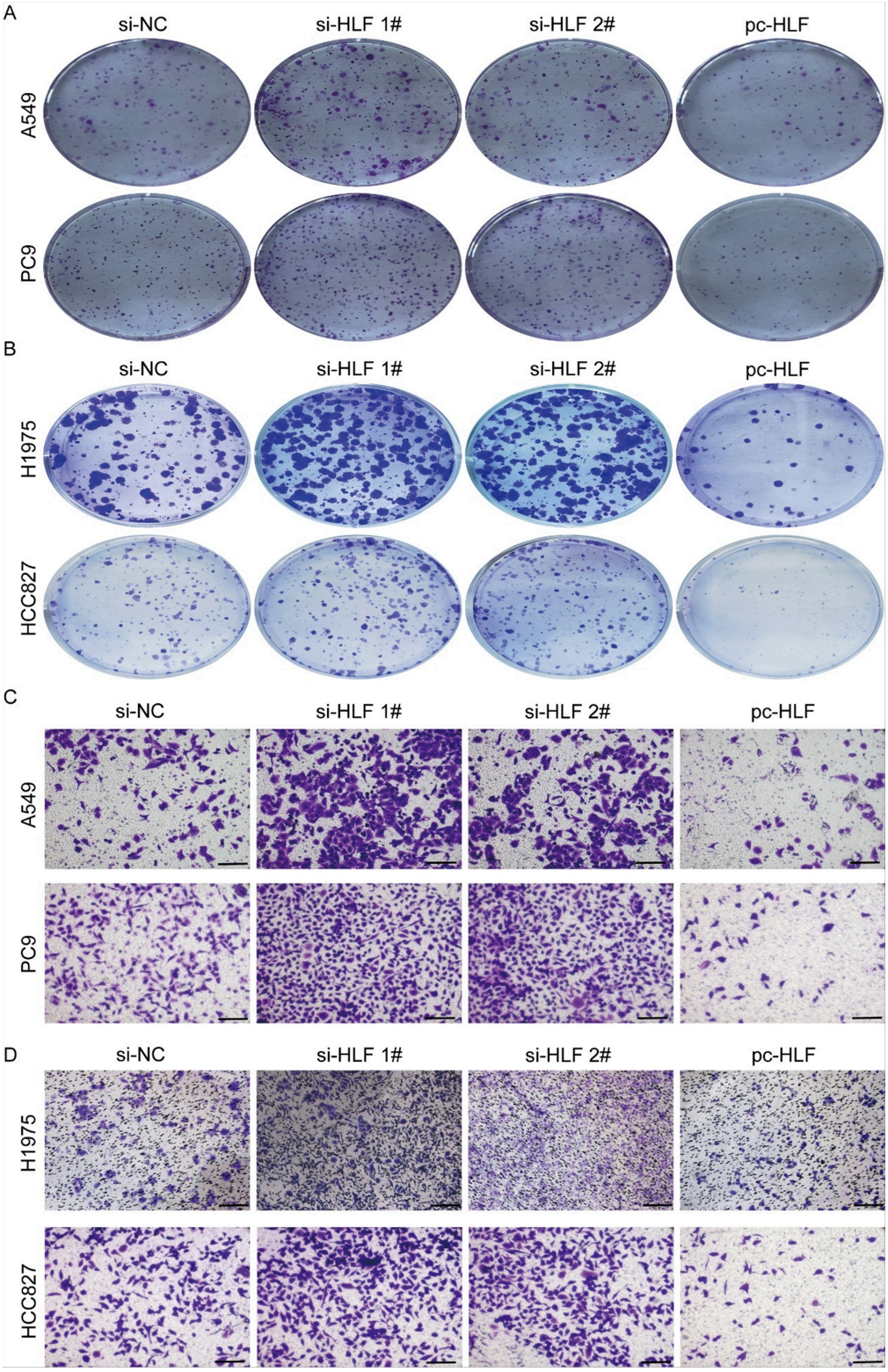


Figure 11

Clone formation experiments and Transwell assay (A) Clone formation experiments with A549 and PC9 (B) Clone formation experiments with H1975 and HCC827 (C) Transwell assay with A549 and PC9 (D)

Transwell assay with H1975 and HCC827 (magnification ×200).

Supplementary Files

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