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Research article

Kinesin Family Member C1: Function in liver hepatocellular carcinoma and potential target for chemotherapeutic

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ABSTRACT

MiR-105 exerts inhibitory effects on the development and progression of various cancers, including breast cancer, lung cancer, and gastric cancer. Through GEO data analysis, we observed decreased expression of miR-105 in liver cancer tissues compared to adjacent tissues. Furthermore, miR-105 downregulates KIFC1 expression levels by targeting its 3' UTR. KIFC1 (Kinesin Family Member C1), a Protein Coding gene, may play a role in mitotic metaphase plate polymerization and mitotic spindle assembly. However, our findings suggest that this gene could serve as a potential chemotherapeutic target for Liver hepatocellular carcinoma (LIHC). We obtained the LIHC dataset from the TCGA database and genotype Tissue Expression Project (GTEx) normal tissue data for differential analysis. Additionally, we utilized the cBioPortal database, tumor immune single-cell center (TISCH) database, gene set enrichment analysis (GSEA), and R software to investigate the possible functions and mechanisms of KIFC1. These findings were further validated through experiments such as immunohistochemistry and wound healing assays. Our results indicate that KIFC1 might be involved in DNA repair and cell cycle regulation in LIHC cells which subsequently impacts tumor cell proliferation; moreover, miR-105 influences hepatoma cell line proliferation via its interaction with KIFC1. Collectively, these results highlight the potential therapeutic significance of targeting KIFC1 for chemotherapy treatment in LIHC patients.

1. Introduction

Liver cancer ranks as the sixth most prevalent cancer worldwide, with China experiencing the fifth highest incidence rate and second highest mortality rate [1]. Hepatocellular carcinoma (LIHC) accounts for over 90 % of liver cancer cases, primarily attributed to hepatitis B or hepatitis C virus infections [2]. The life expectancy of liver cancer patients depends on the clinical stage and tumor location [3]. Current treatment options for liver cancer encompass surgery, ablation, embolization, radiotherapy, targeted therapy, chemotherapy, and immunotherapy [4,5]. Despite advancements in treatment approaches, late-stage diagnosis often hampers effective intervention due to occult disease progression. Consequently, existing treatments frequently fail to reverse disease

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deterioration resulting in a low five-year survival rate among liver cancer patients [6]. To enhance treatment efficacy, it is imperative to investigate the molecular mechanisms underlying hepatocellular carcinoma and identify potential therapeutic targets.

MicroRNAs (miRNAs), a class of non-coding RNAs ranging from 18 to 25 nucleotides in length can effectively regulate various physiological processes such as cell proliferation, migration differentiation and apoptosis by targeting specific genes' 3'UTR regions [7]. miR-122 miR-199 and miR-21 have been established as closely associated with liver cancer occurrence and development [8]. In this study conducted through GEO data analysis we observed decreased expression levels of miR-105 in liver cancer tissues compared to adjacent tissues. Furthermore,mir-105 was found to downregulate KIFC1 expression levels by targeting its 3 'UTR.

Normal mitosis is essential to ensure the normal proliferation of cells. An outstanding characteristic of cancer is the abnormal proliferation of cells [9]. Microtubules, which form spindles and other structures, are a major component of the eukaryotic cytoskeleton. Microtubules and motor proteins play a crucial role in proper chromosome segregation within the mitotic spindle [10]. Currently, targeting mitotic spindles has been clinically verified as a treatment for cancer, and these targeted drugs are being used in clinics [9,11]. In many cancers, there are numerous alterations in kinesin [12]. Kinesin is a type of motor protein that plays an important role in intracellular transport and cell division [8]. KIFC1 belongs to the kinesin-14 family. In tumor cells, Kinesin-14 KIFC1 regulates spindle assembly and centrosome aggregation during cell mitosis, promoting the formation of pseudo-bipolar spindles and chromosome separation to prevent death caused by multipolar spindle formation [13,14]. Knocking down KIFC1 reveals disrupted multistage spindle formation and chromosome segregation disorder in cancer cells, leading to cell death. Studies have found high expression levels of KIFC1 in various cancers such as gastric cancer, breast cancer, prostate cancer, etc. [15,16]. These findings suggest that KIFC1 may serve as a potential therapeutic target for cancer; however, no relevant research has been conducted on liver cancer.

We conducted bioinformatics analysis on RNA sequencing data to investigate the potential function and mechanism of KIFC1 in LIHC. Additionally, we performed tissue and cell experiments to validate our findings. Our study focused on elucidating the impact of KIFC1 expression on hepatocellular carcinoma (HCC) cell proliferation, migration, and invasion. The results suggest that KIFC1 may play a role in DNA repair and cell cycle regulation in LIHC cells, thereby influencing tumor cell proliferation. Furthermore, our proliferation experiments confirmed that miR-105 modulates HCC cell line proliferation through its interaction with KIFC1. These findings indicate that targeting KIFC1 could be a promising therapeutic strategy for chemotherapy in patients with LIHC.\

2. Materials and methods

2.1. Gene expression analysis

The gene expression profile data of Liver hepatocellular carcinoma (LIHC) was obtained from the TCGA database using the R package TCGAbiolinks [17]. The TPM data format was selected for analysis. A total of 374 tumor samples (Tumor) and 50 normal samples (Normal) were included in the dataset. Relevant phenotype information for the TCGA-LIHC samples was downloaded and acquired. Differential analysis was performed using DESeq2 [18] to compare tumor and para-carcinoma groups. Genes with a logFC >2 and adjPvalue <0.05 were classified as up-regulated genes, while genes with a logFC ≤ 2 and adjPvalue <0.05 were classified as down-regulated genes, resulting in a final list of 332 up-regulated genes and 46 down-regulated genes.

We conducted bioinformatics analysis related to miR-105 using the miRNA sequencing data GSE6857, which includes 241 liver cancer sample data and 241 non-cancer sample data, along with the provision of relevant clinical information for analysis. Since this dataset did not provide relevant clinical information, we utilized the TCGA-LIHC dataset for a clinically relevant analysis of miR-105.

2.2. UALCAN

UALCAN is a comprehensive, user-friendly, and interactive web resource for the analysis of cancer OMICS data [20]. It is developed using PERL-CGI and incorporates high-quality graphics through javascript and CSS [19]. UALCAN aims to: a) Facilitate easy access to publicly available cancer OMICS data (TCGA, MET500, CPTAC, and CBTTC). b) Enable users to identify biomarkers or perform in silico validation of potential genes of interest. c) Provide graphical representations and plots illustrating expression profiles and patient survival information for protein-coding, miRNA-coding, and lincRNA-coding genes. d) Evaluate epigenetic regulation of gene expression through promoter methylation analysis. e) Conduct pan-cancer gene expression analysis. f) Offer additional information about selected genes/targets by linking to HPRD, GeneCards, Pubmed, TargetScan, The Human Protein Atlas DRUGBANK Open Targets,and GTEx. These resources provide researchers with valuable insights into their genes/targets of interest. g) Perform data analysis on clinical proteomic consortium including total/phospho-proteins. h) Conduct gene expression and protein expression analyses specifically focused on pediatric brain tumors.

2.3. cBioPortal

The cBio Cancer Genomics Portal (cBioPortal, http://www.cbioportal.org/) provides comprehensive data on over 5,000 tumor samples derived from 20 cancer studies [21,22]. These genomic profiles encompass mutations, putative copy number alterations identified through the genomic identification of significant targets in cancer (GISTIC), and mRNA expression z-scores relative to all samples (log RNA Seq V2 RSEM) with a threshold of ± 2.0. In this study, we conducted an analysis of KIFC1 mutations within the TCGA-LIHC dataset (TCGA PanCancer Atlas) available in the cBioPortal database [23]. Subsequently, these mutations were categorized based on their condition and corresponding survival curves were generated.

2.4. GSEA (Gene set enrichment analysis)

To investigate the disparities in biological processes among different groups, we conducted a gene set enrichment analysis (GSEA) [24] using the gene expression profile datasets of TCGA-LIHC patients. GSEA is a computational approach commonly employed to assess statistical differences in pathway and biological process activity between two biological states based on expression datasets. For GSEA, we obtained the c2. cp.kegg.v6.2.-symbols gene set from the MSigDB database [25], and significance was determined at a false discovery rate (FDR) < 0.25.

2.5. TISCH (Tumor immune single-cell Hub)

The Tumor Immune Single-cell Hub (TISCH, http://tisch.comp-genomics.org/) is a scRNA-seq database that offers comprehensive cell-type annotation at the single-cell level for investigating the tumor microenvironment across diverse cancer types. TISCH integrates high-quality single-cell transcriptomic profiles from 76 tumor datasets encompassing 27 cancer types, comprising nearly 2 million cells [26]. We examined KIFC expression in various immune cells at the single-cell level using four LIHC datasets available in TISCH.

2.6. Immune infiltration

Tumor microenvironment (TME) encompasses tumor tissue, surrounding immune and inflammatory cells, tumor-associated fibroblasts, stromal tissue, as well as various cytokines and chemokines, constituting a comprehensive loading system. The analysis of immune cell infiltration in cancer tissues plays a crucial role in disease research and treatment prognosis prediction. CIBERSORTx is an algorithm that utilizes RNA-Seq data to deconvolve the expression matrix of immune cell subtypes based on linear support vector regression principles [27]. In this study, we employed the CIBERSORTx algorithm to investigate the differences in immune infiltration between tumor tissue and normal tissue. We identified differentially enriched immune cells between these two types of tissues using two datasets. Furthermore, we calculated the Pearson correlation coefficient between KIFC1 expression levels and immune cells while

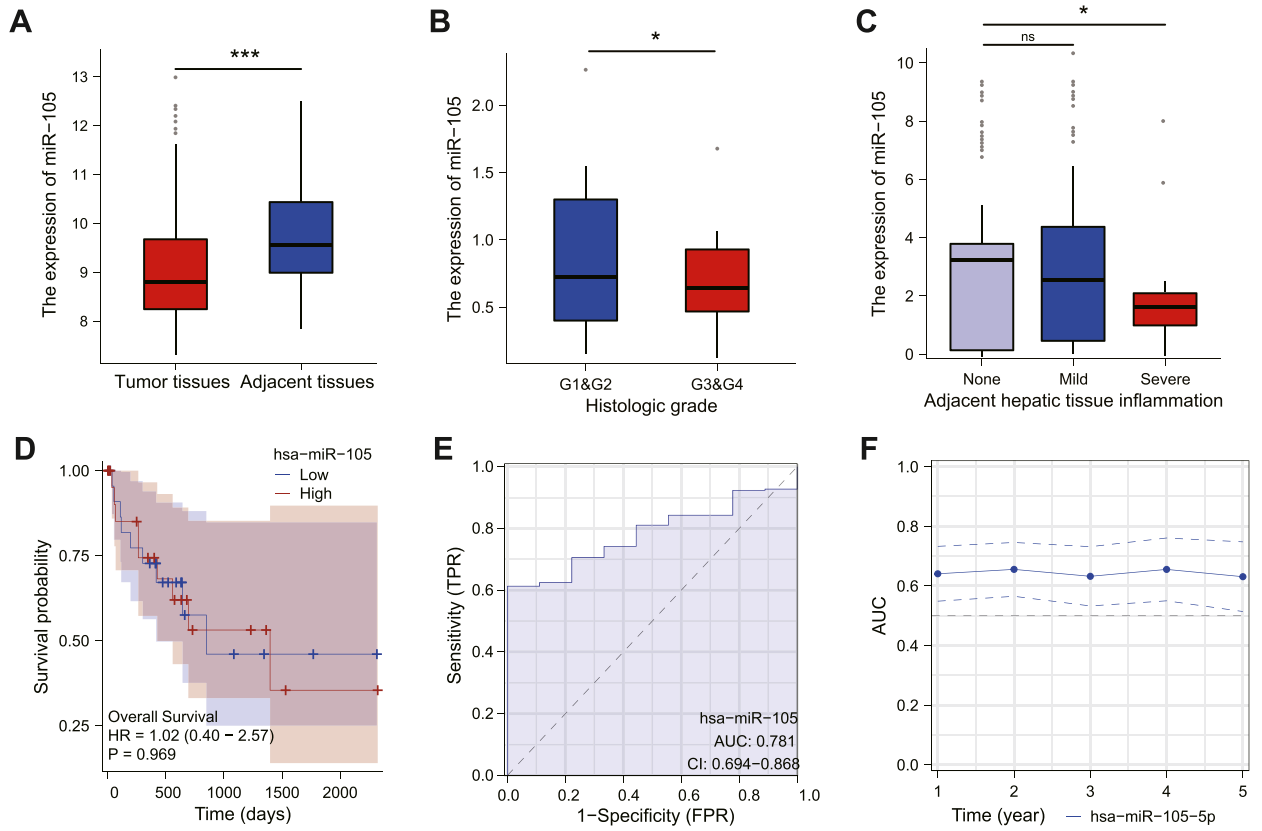

Fig. 1. Clinical Significance Analysis of miR-105.(A) Analyzing the difference in miR-105 expression between liver tumor tissues and adjacent normal tissues using publicly available data.(B) Analyzing the differences in miR-105 among different tumor grades based on public data.(C) Analyzing the differences in miR-105 among tumors with different levels of Inflammation based on public data.(D) Comparing the prognosis of patients with high miR-105 expression and low expression.(E) Receiver operating characteristic (ROC) curve of miR-105 for predictive accuracy.(F) Displaying the area under the curve (AUC) of miR-105 at different time points using line graphs.

evaluating the relationship between KIFC1 gene expression and the level of immune cell infiltration.

2.7. Prognostic analysis

The correlation between KIFC1 expression and clinical features, such as tumor stage, was analyzed and visualized using the ggplot2 package [28]. Prognostic correlation analysis of TCGA-LIHC data was conducted using the rms package [29] and the survival package [30], incorporating variables including T stage, N stage, M stage, tumor status, age, and KIFC1 for overall survival analysis. Nomogram plots and calibration plots were generated at 1, 3, and 5 years. Finally, the relationship between KIFC1 expression and patient prognosis was visualized using the survminer package [31], considering clinical variables such as age, TNM stage, and BIM.

2.8. Cell culture

HepG2 cells were maintained in DMEM supplemented with 10 % FBS, 1 % sodium pyruvate and 1 % penicillin/streptomycin. Cells were cultured in a humidified incubator at 37 °C and 5 % CO2.

2.9. Statistical analysis

All data calculations and statistical analysis were performed using R programming (https://www.r-projec t.org/, version 4.0.2). The Spearman Correlation test was used to assess the correlation between KIFC1 expression and targets of interest, including immune cell infiltration scores (as described in the previous section for six immune cell types). The comparison of KIFC1 expression levels between groups, or between tumor and normal tissues, was performed with paired t-tests or the *t*-test, depending on whether the samples are paired or not. The receiver operating characteristic (ROC) curve was drawn using the timeROC package [32] of R, and the area under the curve (AUC) was calculated to assess the accuracy of the risk score estimates. Results are considered statistically significant at a P-value <0.05.

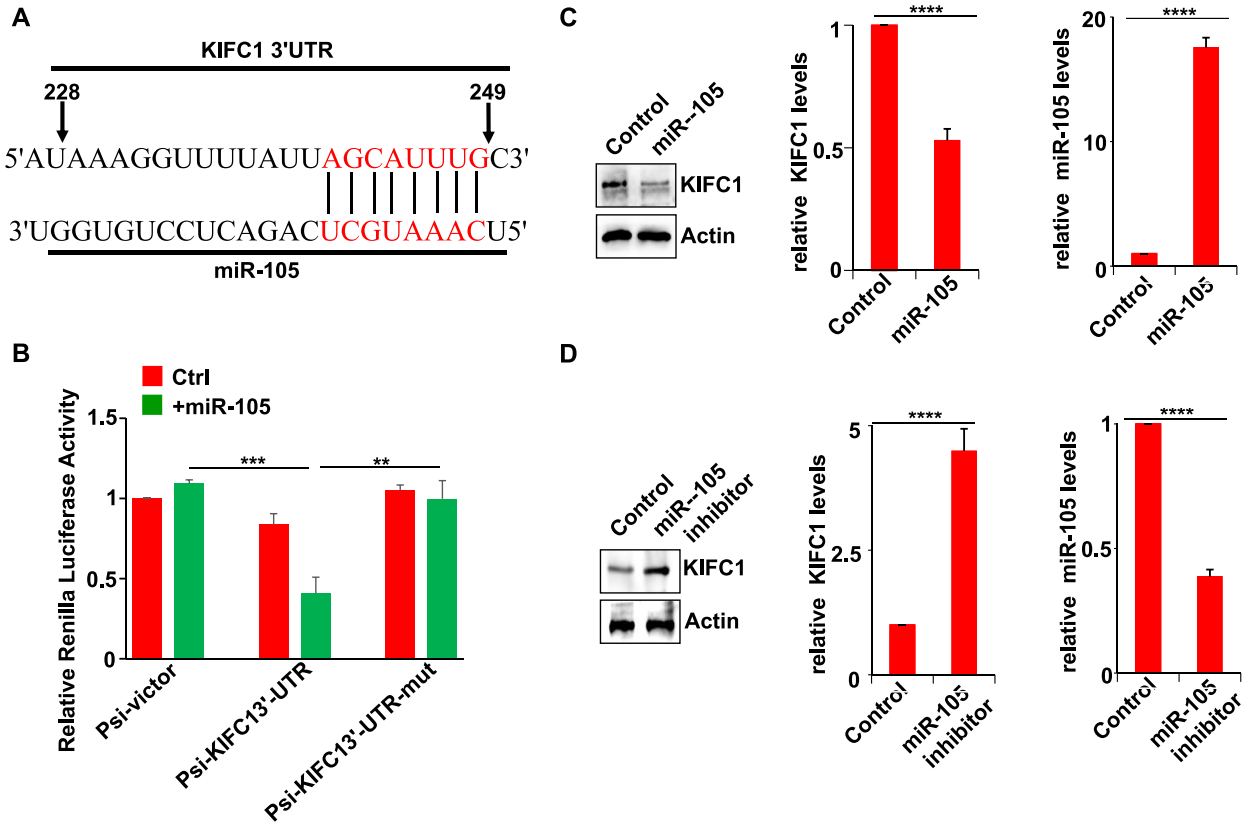

Fig. 2. miR-105 regulates the expression of KIFC1.(A)Schematic illustration of base pairing between miR-105 and the 3'-UTR of KIFC1. (B) HepG2 cells were co-transfected with the reporter construct indicated and Renilla luciferase plasmids. Twenty-four hours later, reporter activity was measured by a luciferase assay.(C)HepG2 cells were transfected with scrambled or miR-105 mimics. Thirty-six hours later, whole cell lysateswere subjected to qPCR and Western blot analysis.(D)HepG2 cells were transfected with scrambled or miR-105 inhibitor. Thirty-six hours later, whole cell lysateswere subjected to qPCR and Western blot analysis.

3. Results

3.1. Clinical Significance Analysis of miR-105

We initially assessed the expression levels of miR-105 in liver tumor tissues and adjacent normal tissues with GSE6857 data, revealing higher expression of miR-105 in the adjacent normal tissues (Fig. 1A). Subsequently, we utilized the TCGA-LIHC database to investigate the clinical significance of miR-105. Initially, we analyzed the expression differences across various tumor grades and observed a gradual decrease in the expression of miR-105 with increasing malignancy (Fig. 1B). In inflamed infiltrated tissues, we noted an inverse correlation between the degree of inflammation and miR-105 expression (Fig. 1C). Furthermore, our analysis on the prognostic impact of miR-105 revealed no significant association with survival outcomes among liver tumor patients (Fig. 1D). However, Receiver Operating Characteristic (ROC) curves demonstrated that miR-105 exhibited high diagnostic efficiency for

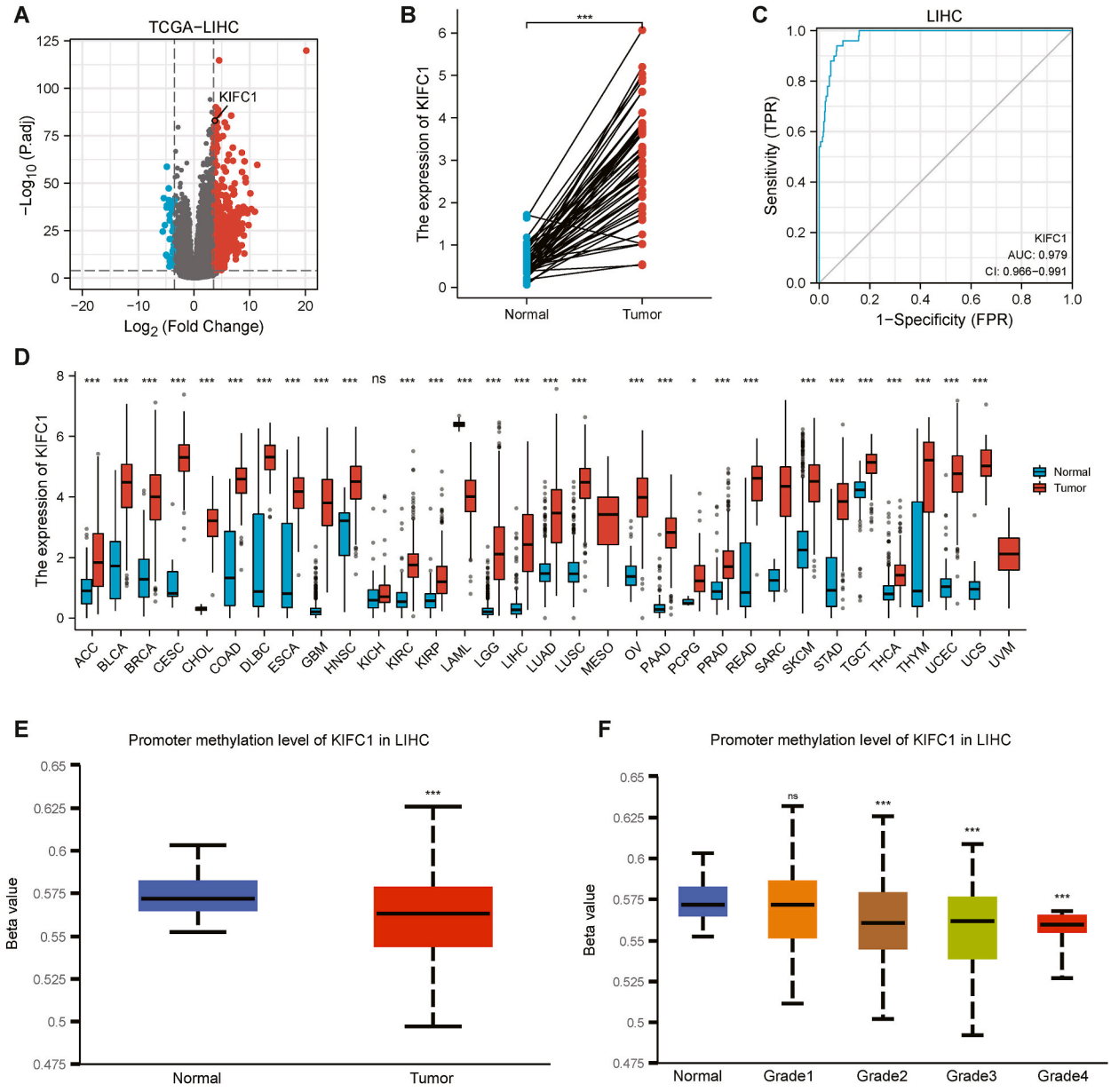

Fig. 3. Differential expression of KIFC1 in LIHC. (A) Volcano plot of differential genes in TCGA-LIHC data; (B) Differences in expression of KIFC1 in LIHC tissues (Tumor) and their paired normal tissues (Normal); (C) ROC curves of KIFC1 in LIHC; (D) Differences in expression of *KIFC1* in various tumor tissues and their corresponding normal tissues; (E) Methylation levels of KIFC1 in LIHC tumor tissues and normal tissues; (F) Methylation levels of KIFC1 in different stage tumor tissues and normal tissues.

identifying liver tumor patients (Fig. 1E), displaying favorable discriminative ability for both early-stage and late-stage tumors (Fig. 1F).

3.2. miR-105 regulates the expression of KIFC1

Using bioinformatics methods, targetscan7.2 predicted that miR-105 potentially targets the 3'-UTR of KIFC1 at positions 194 to 201 bases (Fig. 2A). The luciferase assay confirmed the targeting of miR-105 on the KIFC1 3'-UTR at positions 194 to 201 bases (Fig. 2B). Transfection of miR-105 mimics in HepG2 cell line resulted in down-regulation of both mRNA and protein expression levels of KIFC1 (Fig. 2C). Conversely, inhibition of miR-105 using an inhibitor in HepG2 cell lines increased the expression of KIFC1 mRNA (Fig. 2D), indicating that miR-105 regulates KIFC1 expression by targeting its hacel region within the 3'-UTR.

3.3. Differential expression of KIFC1 in LIHC

The LIHC data were obtained from the TCGA official website, and the compiled dataset consisted of 374 tumor samples (Tumor) and 50 adjacent cancer samples (Normal). Differential analysis was performed based on these groups to identify differentially expressed genes. Subsequently, a volcano plot (Fig. 3A) was generated, revealing 332 up-regulated genes and 46 down-regulated genes. Notably, KIFC1 expression was found to be significantly upregulated in LIHC tissues. To further investigate this finding, we extracted 50 paired samples specifically for plotting the differences in KIFC1 expression alone (Fig. 3B), as well as constructing an ROC curve to evaluate its diagnostic efficacy in hepatocellular carcinoma tissues (Fig. 3C). Remarkably, our results demonstrated that KIFC1 expression was markedly higher in tumor tissues compared to adjacent normal tissues with an AUC value of 0.979, indicating excellent diagnostic potential. Additionally, we conducted pan-cancer analysis of KIFC1 expression (Fig. 3D), which revealed its generally high

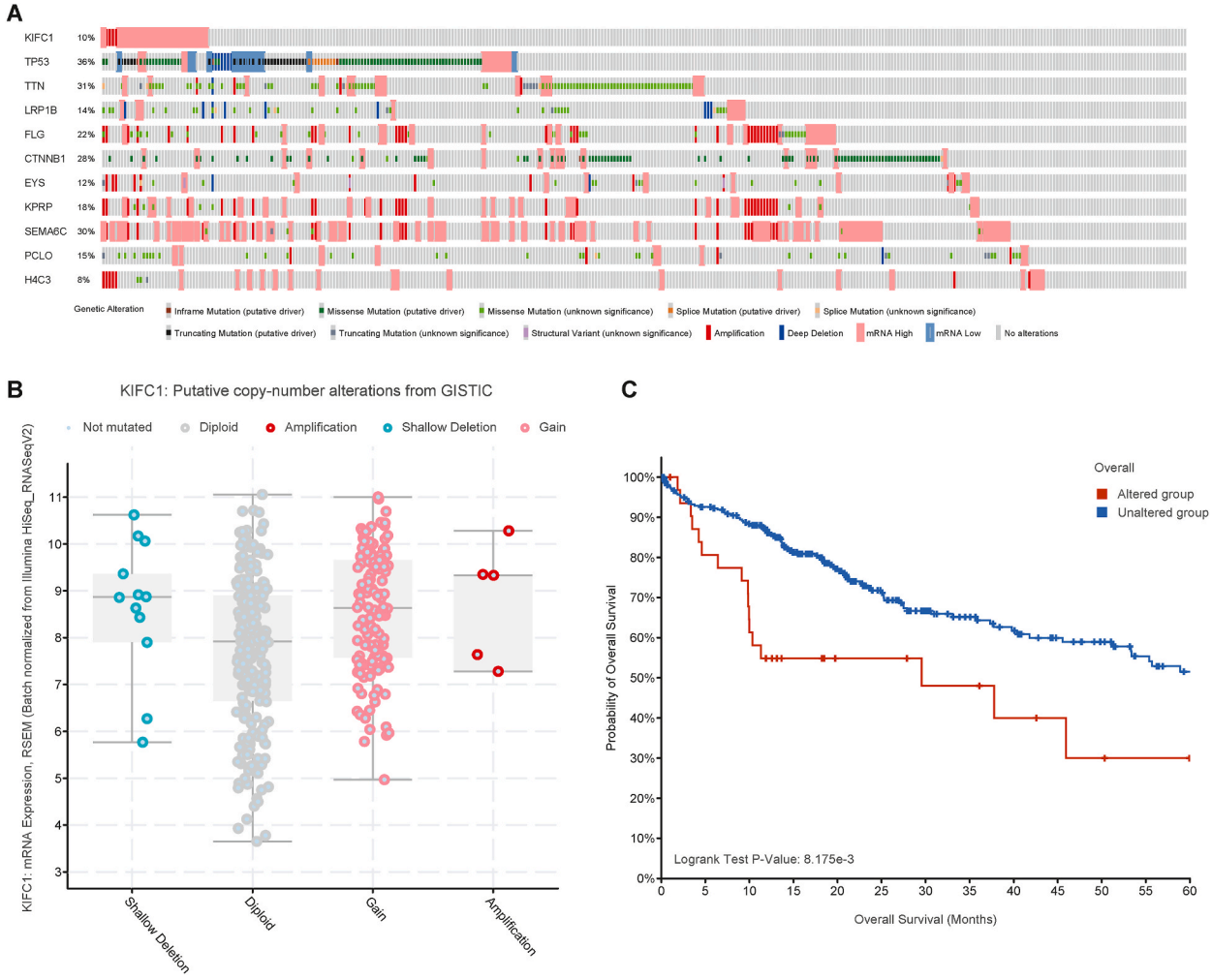

Fig. 4. Mutation of KIFC1 in LIHC. (A) KIFC1 mutation frequency in LIHC; (B) Possible types of KIFC1 mutations in LIHC; (C) Effect of altered group and unaltered group on overall survival of LIHC patients.

expression across various tumors. Furthermore, methylation analysis of the KIFC1 promoter region indicated a decreased methylation level in tumor tissues (Fig. 3E), particularly at different stages compared to normal tissues (Fig. 3F). These findings suggest that increased gene expression may be attributed to reduced methylation levels within the KIFC1 promoter region during tumor development and progression.

3.4. Mutation of KIFC1 in LIHC

We conducted a comprehensive analysis of KIFC1 mutations in the TCGA-LIHC samples (Fig. 4A). The results revealed a mutation rate of 10 % for KIFC1, with various types of mutations observed including diploid alterations, amplifications, shallow deletions, and gains (Fig. 4B). Furthermore, we investigated the impact of these mutations on patient survival rates and found that KIFC1 mutations significantly influenced overall survival outcomes (Fig. 4C). Our data analysis demonstrated statistically significant differences in patient survival associated with potential KIFC1 mutations.

3.5. Functions that KIFC1 plays in LIHC

To investigate the impact of KIFC1 expression levels on LIHC, we conducted separate analyses to explore the associations between gene expression and the biological processes, cellular components, and molecular functions involved in both adjacent normal tissue and tumor tissue data. The results revealed that differentially expressed genes associated with prognosis primarily influenced key functions and pathways such as SIGNALING_BY_RHO_GTPASES, M_PHASE, DNA_REPAIR, RHO_GTPASE_EFFECTORS, and CELL_CYCLE_CHECKPOINTS (Fig. 5A). Furthermore, a visual analysis of the most prominent factors demonstrated a positive correlation with RHO GTPASES-related pathways (Fig. 5B) as well as a significant association with M PHASE (Fig. 5C), all of which were supported by statistically significant corrected p-values. These results are consistent with the mutation results, suggesting that KIFC1 may influence the progression of tumors through pathways such as DNA repair and cell proliferation.

3.6. Single-cell analysis of KIFC1 expression in immune cells

We examined the expression of KIFC1 in diverse immune cell populations using single-cell sequencing data from the TISCH database (Fig. 6A). The findings revealed a significantly elevated expression of KIFC1 in proliferating T cells, while its expression was relatively low in Treg cells and $CD8^+$ T cells, suggesting that KIFC1 may exert a substantial influence on T cell proliferation without

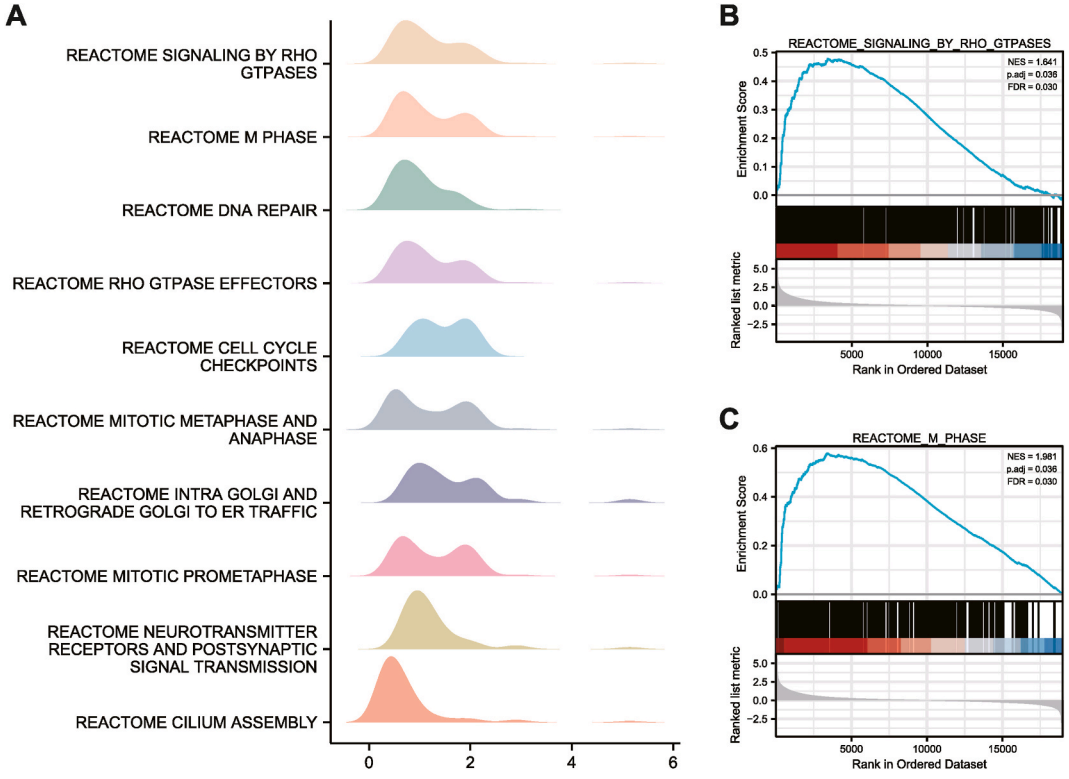

Fig. 5. Functions that KIFC1 plays in LIHC. (A) Mountain map of GSEA results; (B) REACTOME_SIGNALING_BY_RHO_GTPASES; (C) REACTOME_M_PHASE.

affecting their differentiation and functionality. Additionally, we performed comprehensive annotation and visualization of four datasets. Notably, GSE125449 exhibited lower levels of KIFC1 expression across various immune cell types (Fig. 6B), whereas both GSE140228_10X (Fig. 6C) and GSE140228_Smartseq2 (Fig. 6D) demonstrated significantly higher KIFC1 expression specifically in proliferating T cells. Similarly, GSE98628 also displayed elevated KIFC1 expression in these cells (Fig. 6E). The results from single-cell analysis indicate that KIFC1 plays an important role in the tumor immune microenvironment. Therefore, we will proceed with an analysis of the immune infiltration related to KIFC1 to further elucidate the role of this molecule in tumor immunity.

3.7. *Effects of KIFC1 on immune infiltration*

We further conducted an in-depth analysis on the correlation between KIFC1 expression and immune infiltration in hepatocellular carcinoma (Fig. 7A). The findings revealed a weak association between the expression of this gene and the extent of immune infiltration, such as T Reg cells and $CD8^+$ T cells. However, a significant positive correlation was observed with Th2 and T helper cells (Fig. 7B and C), as well as neutrophils and DCs (Fig. 7D and E). These results suggest that while KIFC1 may not directly impact immune cell infiltration in hepatocellular carcinoma, it likely plays a crucial role in regulating immunity.

3.8. *Correlation between KIFC1 expression and the clinical characteristics of LIHC*

We subsequently examined the correlation between KIFC1 expression and clinical characteristics of LIHC. In terms of tumor status,

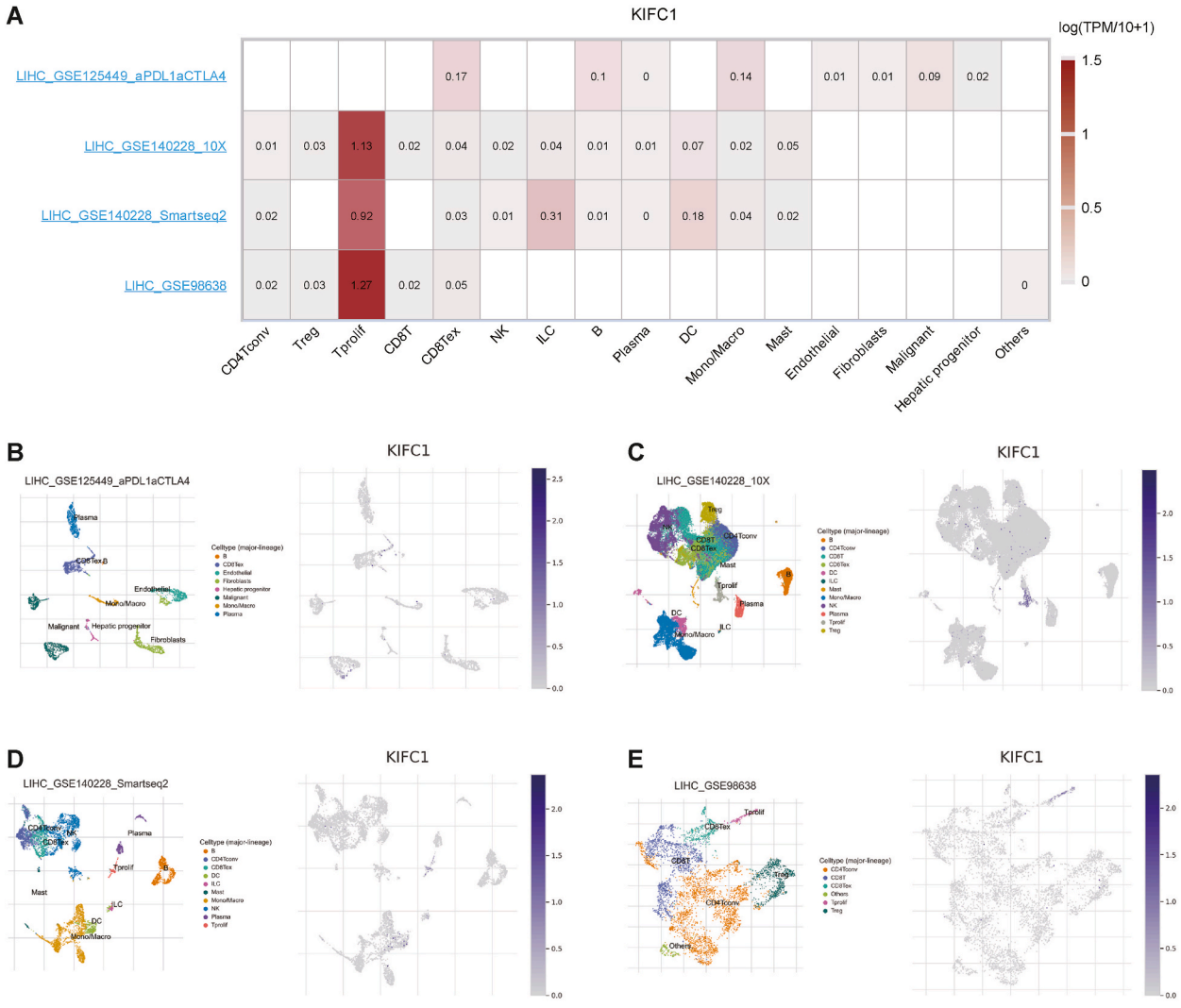

Fig. 6. Single-cell analysis of KIFC1 expression in immune cells. (A) Expression of KIFC1 in immune cells in different LIHC single-cell datasets; (B) Expression of KIFC1 in LIHC_GSE125449 immune cells; (C) Expression of KIFC1 in LIHC_GSE140228_10 × immune cells; (D) Expression of KIFC1 in LIHC_GSE140228_Smartseq2 immune cells; (E) Expression of KIFC1 in LIHC_GSE98638 immune cells.

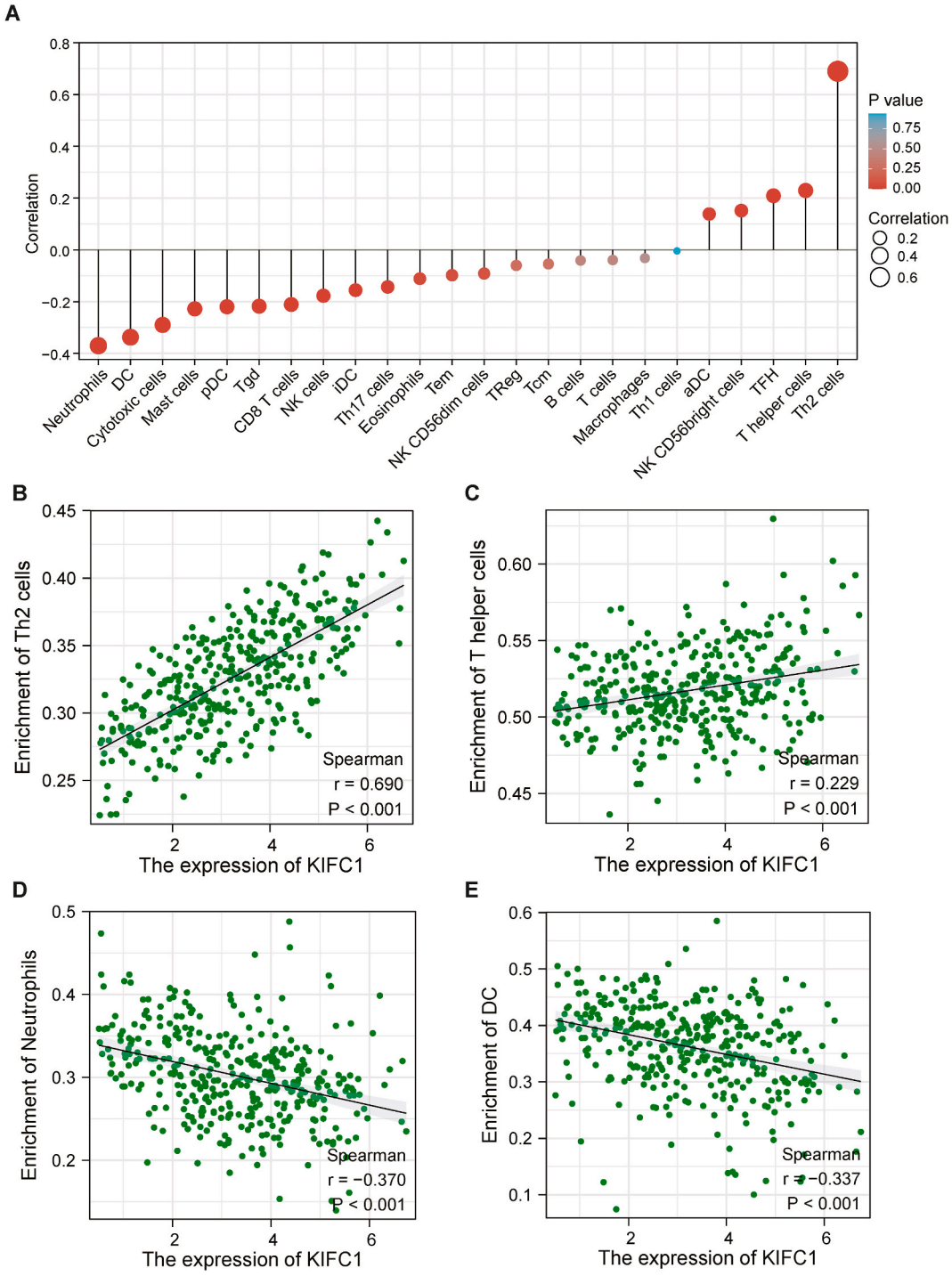

Fig. 7. Effects of KIFC1 on immune infiltration. (A) Immunoinfiltration of KIFC1 in LIHC; (B) Correlation between KIFC1 and Th2 cells; (C) Correlation between KIFC1 and T helper cells; (D) Correlation between KIFC1 and Neutrophils; (E) Correlation between KIFC1 and DC.

we observed significantly higher levels of KIFC1 expression in tumors compared to adjacent normal tissues (Fig. 8A). Similarly, in relation to TNM stage, the expression of KIFC1 was elevated relative to adjacent normal tissues (Fig. 8B–D), suggesting a pivotal role for KIFC1 across all stages of hepatocellular carcinoma. Furthermore, through comprehensive analysis considering multiple clinical factors and the expression of KIFC1, we constructed a Nomogram plot (Fig. 8E) which revealed that high levels of KIFC1 expression profoundly impacted the one-year, three-year, and five-year survival rates while exerting substantial influence on the prognosis of LIHC patients. To validate our predictions from the Nomogram plot accurately, we generated a calibration diagram (Fig. 8F) which

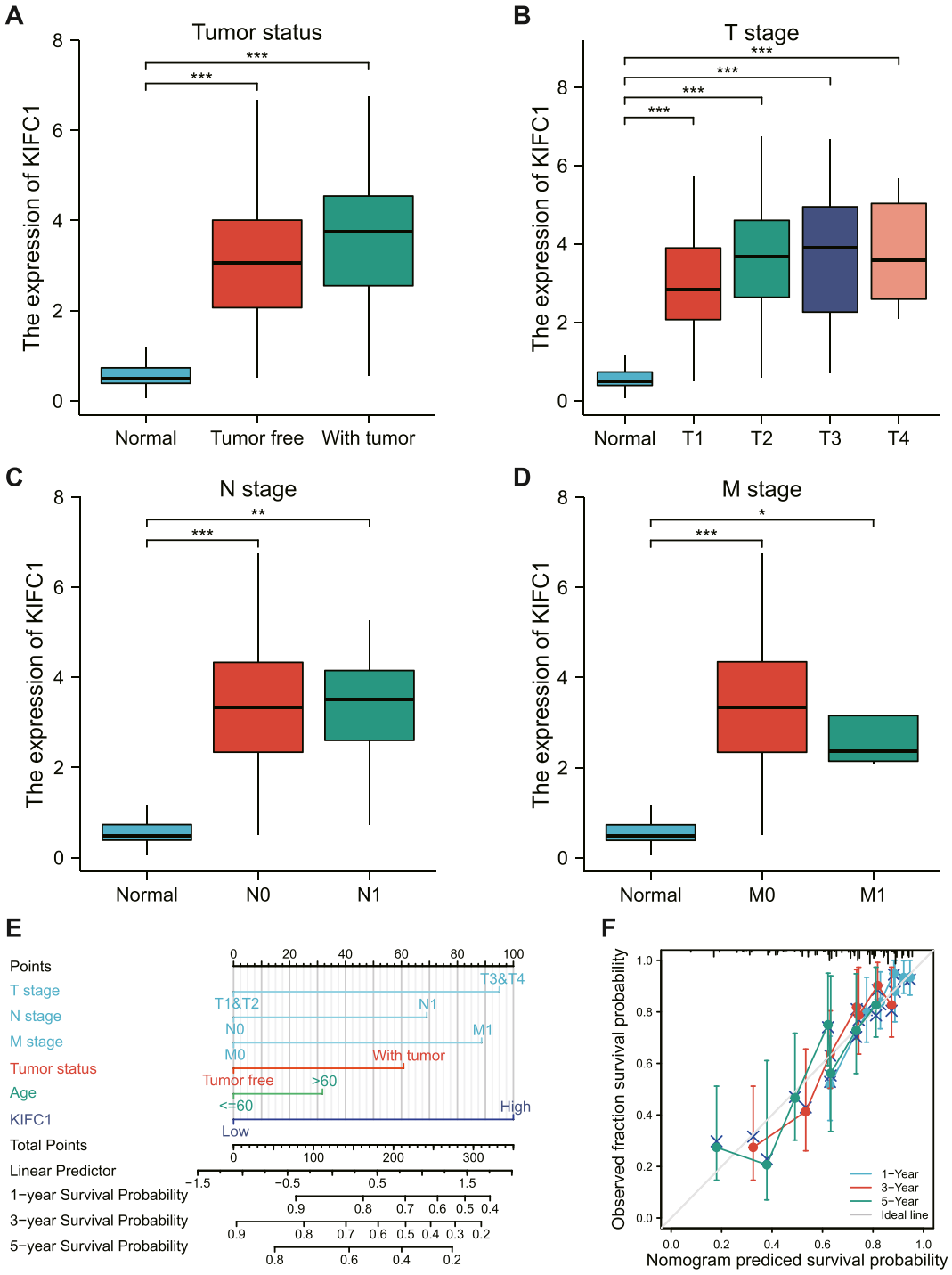

Fig. 8. Correlation between KIFC1 expression and the clinical characteristics of LIHC. (A) Expression of KIFC1 in different states (tumor free and with tumor); (B–D) KIFC1 expression in different TNM stages; (E) Nomogram of TNM stage and KIFC1 expression; (F) Calibration.

demonstrated excellent fit with our predicted outcomes.

3.9. Expression of KIFC1 and the prognosis of LIHC patients

We conducted an analysis to investigate the association between KIFC1 expression and overall survival as well as disease-specific survival in patients with liver hepatocellular carcinoma (LIHC) (Fig. 9A and B). Our findings consistently demonstrated that high

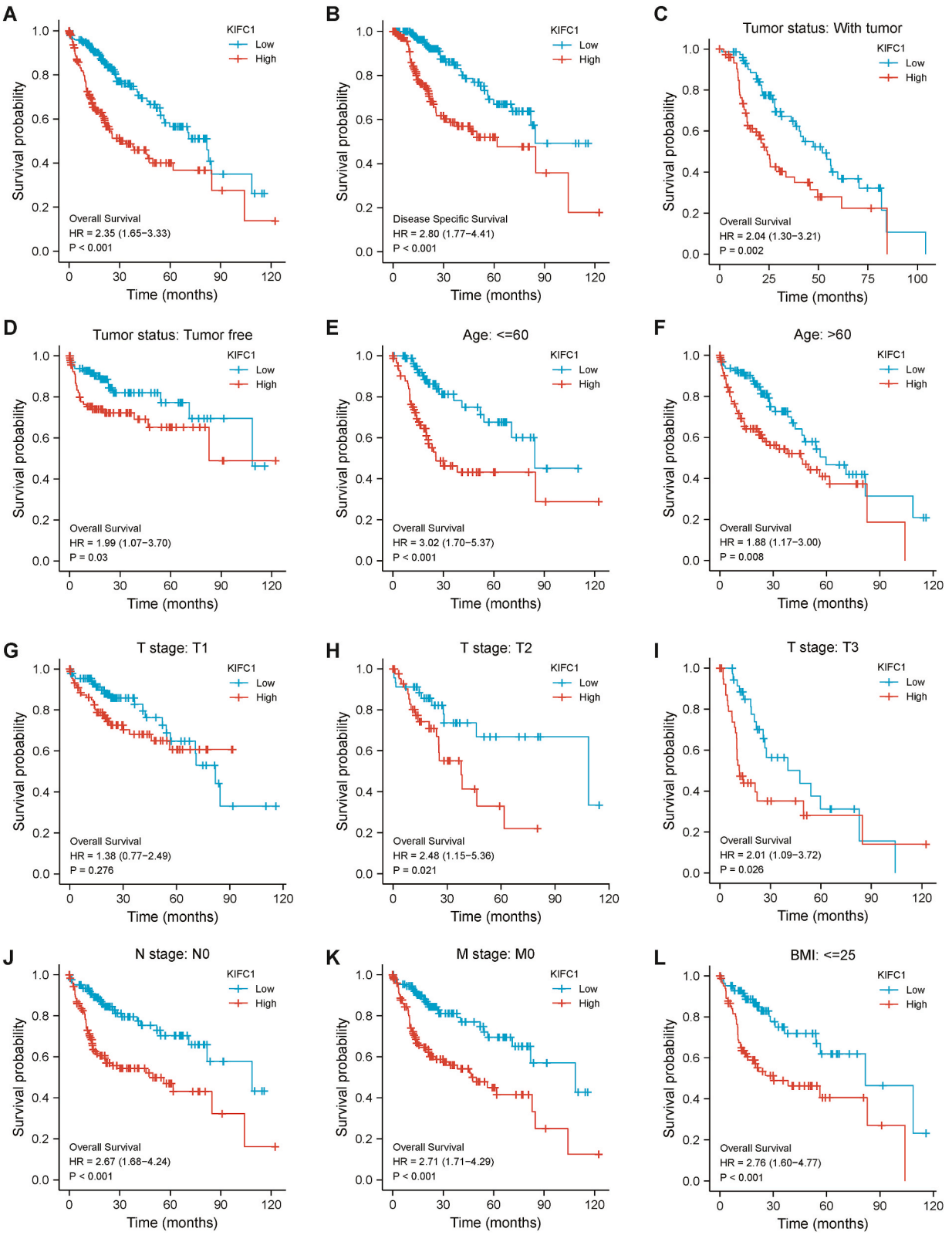

Fig. 9. Expression of KIFC1 and the prognosis of LIHC patients. (A) Overall survival curve; (B) Disease-specific survival curve; (C–D) Tumor status related overall survival curve; (E–F) Age related overall survival curve; (G–K) TNM related overall survival curve; (L) BMI related overall survival curve.

KIFC1 expression was significantly associated with poor prognosis, indicating its potential role as a prognostic risk factor in LIHC. To further validate this observation and minimize confounding factors, we performed subgroup analyses based on tumor status (Fig. 9C and D), age (Fig. 9E–F), T stage (Fig. 9G–I), N stage (Fig. 9J), M stage (Fig. 9K), and BMI (Fig. 9L). In each subgroup, elevated KIFC1 expression remained a significant risk factor for unfavorable patient outcomes, suggesting that targeting KIFC1 may hold promise as a molecular therapeutic strategy to improve the prognosis of LIHC patients.

3.10. Verification of KIFC1 expression difference in LIHC

To validate the expression of KIFC1 in LIHC and adjacent normal tissues, three pairs of LIHC tissues and corresponding adjacent normal tissues were collected for immunohistochemical analysis. The results demonstrated a significantly higher expression level of KIFC1 in LIHC compared to the adjacent normal tissues (Fig. 10), which was consistent with the findings obtained from the aforementioned database analysis.

3.11. miR-105 affects HepG2 proliferation through KIFC1

The expression of miR-105 is downregulated in liver cancer, whereas KIFC1 exhibits upregulation. What is the impact of miR-105 and KIFC1 on liver cancer proliferation? Our observations reveal that miR-105 exerts inhibitory effects on cell proliferation, while KIFC1 promotes cell proliferation. Notably, overexpression of KIFC1 significantly counteracts the inhibitory effect of miR-105 mimics on HepG2 cell proliferation (Fig. 11A). Conversely, silencing of KIFC1 abolishes the promotion of HepG2 cell proliferation induced by miR-105 inhibitor (Fig. 11B).

4. Discussion

MiRNAs play a crucial role in tumorigenesis and tumor progression by modulating the expression of target genes. MiRNA-105, a non-coding RNA molecule, exerts its regulatory function by binding to target mRNAs. In recent years, mounting evidence has demonstrated the significant involvement of miRNA-105 in tumorigenesis and tumor development. Aberrant expression of miR-105

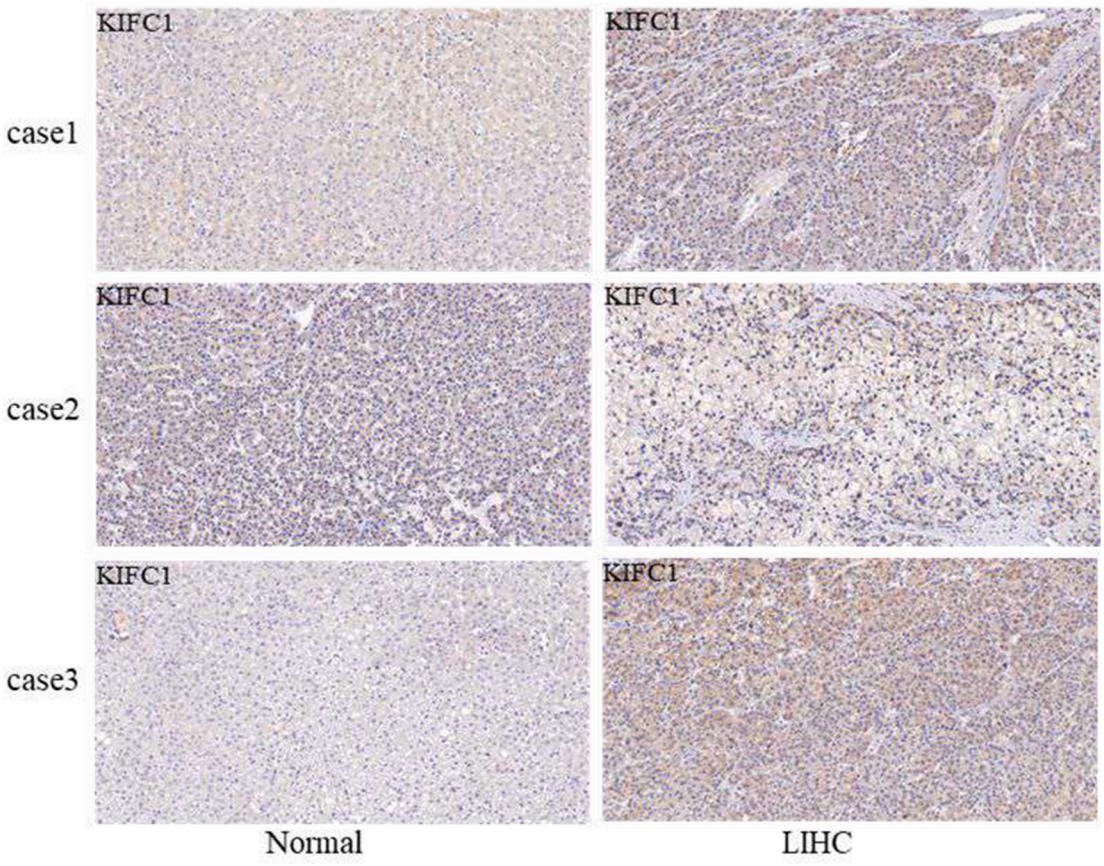

Fig. 10. Verification of KIFC1 expression difference in LIHC. Immunohistochemical staining of KIFC1 in 3 pairs of LIHC tissues and their adjacent normal tissues.

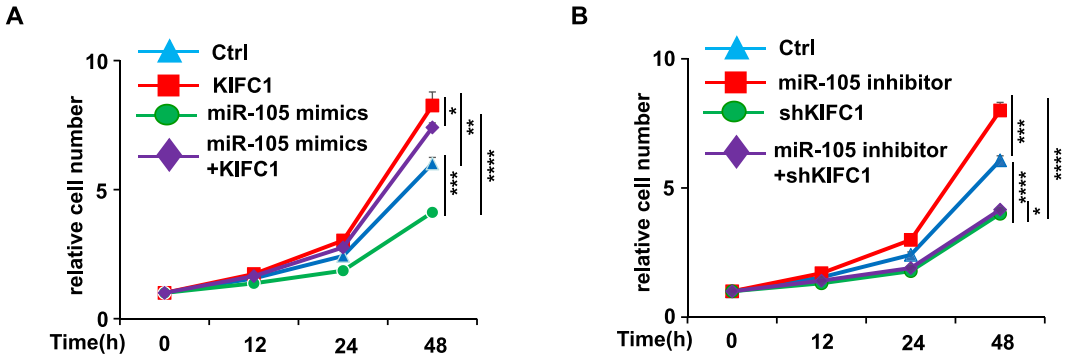

Fig. 11. miR-105 affects HepG2 proliferation through KIFC1.(A,B)HepG2 cells were transfected with the gene indicated and then the Cell proliferation detected by MTT at the time indicated.

has been observed in various tumors and is closely associated with malignancy degree, prognosis, and therapeutic response. Notably, miRNA-105 exhibits inhibitory effects on breast cancer, lung cancer, gastric cancer, among others [33–36]. The mechanism underlying the impact of miR-105 on cancer encompasses multiple aspects and can influence pivotal cellular signaling pathways involved in tumor cell proliferation, invasion, and metastasis inhibition. For instance, Gang Shen et al. [37] revealed that miR-105 suppresses the PI3K/AKT signaling pathway to regulate hepatocellular carcinoma occurrence and progression. The relationship between miRNA-105 and cancer represents a complex yet diverse field of study. Although our understanding regarding the precise mechanisms through which miRNA-105 operates in tumors remains incomplete; an increasing body of research suggests its indispensable role in tumorigenesis and tumor development. This study identified downregulation of miR-105 in liver cancer tissues while highlighting KIFC1 as its targeted regulator.

The gene KIFC1 emerges as a promising therapeutic target, with significantly higher expression in pan-cancer compared to adjacent normal tissues. Laurie G. Kostecka et al. [38] reported an association between elevated KIFC1 expression and poor prognosis in prostate cancer patients. Our analysis reveals reduced methylation levels, increased mutation frequency, and worse prognosis in LIHC patients belonging to the mutant group, suggesting a close relationship between KIFC1 expression and tumorigenesis/development. Experimental findings further demonstrate heightened KIFC1 expression in tumor tissues of LIHC patients. Knockdown of KIFC1 in hepatocellular cancer cell lines inhibits cell proliferation and migration activities, highlighting its potential as a target for suppressing growth and metastasis of hepatocyte tumor cells. Bioinformatics analysis also suggests that KIFC1 may impede tumor cell proliferation by influencing DNA repair and cell cycle processes; however, this mechanism requires further investigation.

We further investigated the potential impact of KIFC1 on the tumor immune microenvironment. Hao Wu et al. [39] previously reported a potential involvement of KIFC1 in pan-carcinoma, but did not elucidate its relationship with immune infiltration in LIHC. In this study, we conducted a comprehensive analysis of single-cell datasets to explore the correlation between KIFC1 and immune cell populations in LIHC for the first time. Our findings revealed a significant association between KIFC1 expression and T cell infiltration, particularly Th2-type cells. Th2 cells primarily function as B cell helpers by promoting B cell proliferation, differentiation, and antibody production, thereby influencing humoral immunity [40]. Notably, there was limited correlation observed between KIFC1 expression and $CD8^+$ T cell infiltration, suggesting that KIFC1 predominantly exerts an immunoregulatory role within the hepatocellular tumor microenvironment rather than directly impacting anti-tumor processes mediated by immune cells [41].

KIFC1 not only influences tumorigenesis and immune cell infiltration in the tumor microenvironment but also significantly impacts patient prognosis. We conducted subgroup analyses, including age and TNM stage, to investigate the effect of KIFC1 on the prognostic survival of patients with LIHC under different conditions. Our findings demonstrate that KIFC1 plays a significant role in determining patient prognosis across various subgroups. Moreover, considering its limited presence in specific cell types (germ cells and fibroblasts) while being redundant in most somatic cells, as well as its crucial involvement in cancer cell mitosis without affecting normal somatic cell processes, targeting KIFC1 holds great potential for chemotherapy.

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Data availability statement

Raw data are available at the TCGA database (https://portal.gdc.cancer.gov/) (accessed on 28 September 2022) and the GEO database: GSE6857 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE6857), GSE125449 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE125449),GSE140228 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE140228) and GSE98628 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE98628).

CRediT authorship contribution statement

Lei Liu: Writing – original draft, Validation, Investigation, Formal analysis. **Fengyang Jing:** Writing – original draft, Visualization, Formal analysis, Conceptualization. **Jia Li:** Writing – original draft, Visualization, Formal analysis. **Pangjun Gong:** Writing – original draft, Visualization, Formal analysis. **Baoqing Shi:** Writing – original draft, Visualization, Formal analysis. **Youming Zhu:** Writing – review & editing, Formal analysis, Conceptualization. **Hongzhu Yu:** Writing – review & editing, Validation, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37832.

References

[1] L. Herszényi, Z. Tulassay, Epidemiology of gastrointestinal and liver tumors, Eur. Rev. Med. Pharmacol. Sci. 14 (4) (2010) 249–258.
[2] M. Ringelhan, J.A. McKeating, U. Protzer, Viral hepatitis and liver cancer, Philos. Trans. R. Soc. Lond. B Biol. Sci. 372 (1732) (2017) 20160274.
[3] H. Chaudhary, H. Aboushi, J. Minkowitz, J.A. Edwards, D. Beltre, P. Parmar, I. Breitman, C. Luhrs, S.I. McFarlane, Liver granulocytic sarcoma with megakaryocytic differentiation: a rare extra medullary involvement that warrants liver biopsy for prompt diagnosis, Cureus 13 (7) (2021) e16366.
[4] D. Anwanwan, S.K. Singh, S. Singh, V. Saikam, R. Singh, Challenges in liver cancer and possible treatment approaches, Biochim. Biophys. Acta Rev. Canc 1873 (1) (2020) 188314.
[5] C.Y. Liu, K.F. Chen, P.J. Chen, Treatment of liver cancer, Cold Spring Harb Perspect Med. 5 (9) (2015) a021535.
[6] L. Kulik, J.K. Heimbach, F. Zaiem, J. Almasri, L.J. Prokop, Z. Wang, M.H. Murad, K. Mohammed, Therapies for patients with hepatocellular carcinoma awaiting liver transplantation: a systematic review and meta-analysis, Hepatology 67 (1) (2018) 381–400.
[7] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[8] E. Callegari, L. Gramantieri, M. Domenicali, L. D'Abundo, S. Sabbioni, M. Negrini, MicroRNAs in liver cancer: a model for investigating pathogenesis and novel therapeutic approaches, Cell Death Differ. 22 (1) (2015) 46–57.
[9] G.H. Williams, K. Stoeber, The cell cycle and cancer, J. Pathol. 226 (2) (2012) 352–364.
[10] N.J. Quintyne, J.E. Reing, D.R. Hoffelder, S.M. Gollin, W.S. Saunders, Spindle multipolarity is prevented by centrosomal clustering, Science. 307 (5706) (2005) 127–129.
[11] C. Dominguez-Brauer, K.L. Thu, J.M. Mason, H. Blaser, M.R. Bray, T.W. Mak, Targeting mitosis in cancer: emerging strategies, Mol Cell 60 (4) (2015) 524–536.
[12] N. Hirokawa, Y. Noda, Y. Tanaka, S. Niwa, Kinesin superfamily motor proteins and intracellular transport, Nat. Rev. Mol. Cell Biol. 10 (10) (2009 Oct) 682–696.
[13] J. Jung, H. Jeong, J.W. Choi, H.S. Kim, H.E. Oh, E.S. Lee, Y.S. Kim, J.H. Lee, Increased expression levels of AURKA and KIFC1 are promising predictors of progression and poor survival associated with gastric cancer, Pathol. Res. Pract. 224 (2021) 153524.
[14] E. Maurizio, J.R. Wiśniewski, Y. Ciani, A. Amato, L. Arnoldo, C. Penzo, S. Pegoraro, V. Giancotti, A. Zambelli, S. Piazza, G. Manfioletti, R. Sgarra, Translating proteomic into functional data: an high mobility group A1 (HMGA1) proteomic signature has prognostic value in breast cancer, Mol. Cell. Proteomics 15 (1) (2016) 109–123.
[15] A. Parvin, S.L. Hao, F.Q. Tan, W.X. Yang, Inhibition of kinesin motor protein KIFC1 by AZ82 induces multipolar mitosis and apoptosis in prostate cancer cell, Gene 760 (2020) 144989.
[16] Y. Sekino, N. Oue, Y. Shigematsu, A. Ishikawa, N. Sakamoto, K. Sentani, J. Teishima, A. Matsubara, W. Yasui, KIFC1 induces resistance to docetaxel and is associated with survival of patients with prostate cancer, Urol. Oncol. 35 (1) (2017) 31.
[17] A. Colaprico, T.C. Silva, C. Olsen, L. Garofano, C. Cava, D. Garolini, T.S. Sabedot, T.M. Malta, S.M. Pagnotta, I. Castiglioni, et al., TCGAbiolinks: an R/Bioconductor package for integrative analysis of TCGA data, Nucleic Acids Res. 44 (8) (2016) e71.
[18] M.I. Love, W. Huber, SJGb Anders, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2 15 (12) (2014) 1–21.
[19] D.S. Chandrashekar, B. Bashel, S.A.H. Balasubramanya, C.J. Creighton, I.P. Rodriguez, B.V.S.K. Chakravarthi, Varambally S. Ualcan, A portal for facilitating tumor subgroup gene expression and survival analyses, Neoplasia 19 (8) (2017) 649–658.
[20] D.S. Chandrashekar, S.K. Karthikeyan, P.K. Korla, H. Patel, A.R. Shovon, M. Athar, G.J. Netto, Z.S. Qin, S. Kumar, U. Manne, C.J. Creighton, S. Varambally, UALCAN: an update to the integrated cancer data analysis platform, Neoplasia 25 (2022) 18–27.
[21] J. Gao, B.A. Aksoy, U. Dogrusoz, G. Dresdner, B. Gross, S.O. Sumer, Y. Sun, A. Jacobsen, R. Sinha, E. Larsson, et al., Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal, Sci. Signal. 6 (2013) pl1.
[22] E. Cerami, J. Gao, U. Dogrusoz, B.E. Gross, S.O. Sumer, B.A. Aksoy, A. Jacobsen, C.J. Byrne, M.L. Heuer, E. Larsson, et al., The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data, Cancer Discov. 2 (2012) 401–404.
[23] K.A. Hoadley, C. Yau, T. Hinoue, D.M. Wolf, A.J. Lazar, E. Drill, R. Shen, A.M. Taylor, A.D. Cherniack, V. Thorsson, R. Akbani, R. Bowlby, C.K. Wong, M. Wiznerowicz, F. Sanchez-Vega, A.G. Robertson, B.G. Schneider, M.S. Lawrence, H. Noushmehr, T.M. Malta, J.M. Stuart, C.C. Benz, P.W. Laird, Cell-of-Origin patterns dominate the molecular classification of 10,000 tumors from 33 types of cancer, Cell 173 (2) (2018) 291–304.
[24] A. Subramanian, P. Tamayo, V.K. Mootha, S. Mukherjee, B.L. Ebert, M.A. Gillette, A. Paulovich, S.L. Pomeroy, T.R. Golub, E.S. Lander, et al., Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles, Proc. Natl. Acad. Sci. U.S.A. 102 (43) (2005) 15545–15550.
[25] A. Liberzon, C. Birger, H. Thorvaldsdóttir, M. Ghandi, J.P. Mesirov, P. Tamayo, The Molecular Signatures Database (MSigDB) hallmark gene set collection, Cell systems 1 (6) (2015) 417–425.
[26] D. Sun, J. Wang, Y. Han, X. Dong, J. Ge, R. Zheng, X. Shi, B. Wang, Z. Li, P. Ren, TISCH: a comprehensive web resource enabling interactive single-cell transcriptome visualization of tumor microenvironment, Nucleic Acids Res. 49 (2020) D1420–D1430.
[27] A.M. Newman, et al., Determining cell type abundance and expression from bulk tissues with digital cytometry, Nat. Biotechnol. 37 (7) (2019) p773–p782.

[28] E.K. Gustavsson, D. Zhang, R.H. Reynolds, S. Garcia-Ruiz, M. Ryten, ggtranscript: an R package for the visualization and interpretation of transcript isoforms using ggplot2, Bioinformatics 38 (15) (2022) 3844–3846.
[29] R. Pajouheshnia, W.R. Pestman, S. Teerenstra, R.H. Groenwold, A computational approach to compare regression modelling strategies in prediction research, BMC Med. Res. Methodol. 16 (1) (2016) 107.
[30] P. Kragh Andersen, M. Pohar Perme, H.C. van Houwelingen, R.J. Cook, P. Joly, T. Martinussen, J.M.G. Taylor, M. Abrahamowicz, T.M. Therneau, Analysis of time-to-event for observational studies: guidance to the use of intensity models, Stat. Med. 40 (1) (2021) 185–211.
[31] K. Ito, D. Murphy, Application of ggplot2 to pharmacometric graphics, CPT Pharmacometrics Syst. Pharmacol. 2 (10) (2013) e79.
[32] P. Blanche, J.F. Dartigues, H. Jacqmin-Gadda, Estimating and comparing time-dependent areas under receiver operating characteristic curves for censored event times with competing risks, Stat. Med. 32 (30) (2013) 5381–5397.
[33] Y. Du, N. Wei, R. Ma, S. Jiang, D. Song, A miR-210-3p regulon that controls the Warburg effect by modulating HIF-1α and p53 activity in triple-negative breast cancer, Cell Death Dis. 11 (9) (2020) 731.
[34] D. Qian, Q. Zheng, D. Wu, B. Ye, Y. Qian, T. Zhou, J. Qiu, X. Meng, Integrated analysis of ceRNA network reveals prognostic and metastasis associated biomarkers in breast cancer, Front. Oncol. 11 (2021) 670138.
[35] J. Gao, T. Pan, H. Wang, S. Wang, J. Chai, C. Jin, LncRNA FAM138B inhibits the progression of non-small cell lung cancer through miR-105-5p, Cell Cycle 22 (7) (2023) 808–817.
[36] J.C. Shang, G.Z. Yu, Z.W. Ji, X.Q. Wang, L. Xia, MiR-105 inhibits gastric cancer cells metastasis, epithelial-mesenchymal transition by targeting SOX9, Eur. Rev. Med. Pharmacol. Sci. 23 (14) (2019) 6160–6169.
[37] G. Shen, X. Rong, J. Zhao, et al., MicroRNA-105 suppresses cell proliferation and inhibits PI3K/AKT signaling in human hepatocellular carcinoma[J], Carcinogenesis 35 (12) (2014) 2748–2755.
[38] L.G. Kostecka, A. Olseen, K. Kang, G. Torga, K.J. Pienta, S.R. Amend, High KIFC1 expression is associated with poor prognosis in prostate cancer, Med. Oncol. 38 (5) (2021) 47.
[39] H. Wu, Y. Duan, S. Gong, Q. Zhu, X. Liu, Z. Liu, An integrative pan-cancer analysis of Kinesin Family Member C1 (KIFC1) in human tumors, Biomedicines 10 (3) (2022) 637.
[40] A. Budhu, X.W. Wang, The role of cytokines in hepatocellular carcinoma, J. Leukoc. Biol. 80 (2006) 1197–1213.
[41] Y.X. Xiao, W.X. Yang, KIFC1: a promising chemotherapy target for cancer treatment? Oncotarget 7 (30) (2016) 48656–48670.