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Exploring the Expression Patterns and Clinical Relevance of MED8 in Pan-cancer and Liver Hepatocellular Carcinoma: Understanding Tumor Biology and Prognostic Significance

(Meneroka Corak Ekspresi dan Perkaitan Klinikal MED8 dalam Pan-kanser dan Karsinoma Hepatosel Hati: Memahami Biologi Tumor dan Kepentingan Prognostik)

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ABSTRACT

This study examined the expression profiles and medical relevance of MED8 in various types of cancer specifically in liver hepatocellular carcinoma (LIHC). MED8, a subunit of the Mediator complex, plays a crucial role in gene expression regulation and has been implicated in various cellular processes and disease pathogenesis, particularly cancer. Through comprehensive analysis of multidimensional genomic and transcriptomic data from large-scale cancer databases, we examined the expression patterns of MED8 across different tissue types and cancer entities. Survival analysis showed the prognostic significance of MED8 expression in multiple cancer types. Additionally, we investigated the connections between MED8 levels and factors related to tumors, including microsatellite instability (MSI), tumor mutational burden (TMB), and cancer stem cell characteristics. Through gene set enrichment analysis (GSEA), enriched pathways and biological processes linked to MED8 expression were identified, providing insight into its functional significance in tumor biology. In the context of LIHC, we conducted a detailed analysis using the Xiantao platform, confirming the clinical significance of MED8 in LIHC and evaluating its diagnostic potential and utility as an independent prognostic marker. This research offers a comprehensive understanding of the diverse functions of MED8 in the field of cancer research, underscoring its significance as both a predictor of disease progression and a potential target for treatment in different types of cancer, particularly focusing on LIHC.

Keywords: LIHC; MED8; pan-cancer; tumor biology

ABSTRAK

Penyelidikan ini mengkaji profil ekspresi dan kaitan perubatan MED8 dalam pelbagai jenis kanser khususnya dalam karsinoma hepatosel hati (LIHC). MED8, subunit kompleks Mediator, memainkan peranan penting dalam peraturan ekspresi gen dan telah terlibat dalam pelbagai proses sel dan patogenesis penyakit, terutamanya kanser. Melalui analisis komprehensif data genomik dan transkriptik multidimensi daripada pangkalan data kanser berskala besar, kami meneliti corak ekspresi MED8 merentas jenis tisu dan entiti kanser yang berbeza. Analisis kelangsungan hidup menunjukkan kepentingan prognostik ekspresi MED8 dalam pelbagai jenis kanser. Selain itu, kami mengkaji hubungan antara tahap MED8 dan faktor yang berkaitan dengan tumor, termasuk ketidakstabilan mikrosatelit (MSI), beban mutasi tumor (TMB) dan ciri sel stem kanser. Melalui analisis pengayaan set gen (GSEA), laluan diperkaya dan proses biologi yang dikaitkan dengan ekspresi MED8 telah dikenal pasti, memberikan gambaran tentang kepentingan fungsinya dalam biologi tumor. Dalam konteks LIHC, kami menjalankan analisis terperinci menggunakan platform Xiantao, mengesahkan kepentingan klinikal MED8 dalam LIHC dan menilai potensi diagnostik dan utilitinya sebagai penanda prognostik bebas. Penyelidikan ini menawarkan pemahaman menyeluruh tentang kepelbagaian fungsi MED8 dalam bidang penyelidikan kanser, menekankan kepentingannya sebagai peramal perkembangan penyakit dan sasaran yang berpotensi untuk rawatan dalam pelbagai jenis kanser, terutamanya yang memfokuskan pada LIHC.

Kata kunci: Biologi tumor; LIHC; MED8; pan-kanser

INTRODUCTION

The Mediator complex, an essential component of the transcriptional machinery, plays a pivotal role in bridging gene-specific transcriptional regulators with the core RNA polymerase II (Pol II) enzyme (Harper & Taatjes 2018; O'Connor-Moneley et al. 2023; Richter et al. 2022). This macromolecular complex orchestrates the intricate interplay between various transcriptional activators and repressors, thereby facilitating the precise regulation of gene expression programs (Zhao et al. 2022). Among the numerous subunits constituting the Mediator complex, MED8 (Mediator complex subunit 8) has garnered significant attention due to its multifaceted roles in cellular processes and disease pathogenesis, particularly cancer (Soutourina 2018).

Accumulating evidence suggests that dysregulation of MED8 expression can profoundly impact various aspects of tumor biology, including cell proliferation, survival, and metastasis (Xue et al. 2023). Moreover, the complex interaction between MED8 and epigenetic regulatory processes, such as DNA methylation and histone modifications (D'Aquila et al. 2020; Thorsen et al. 2012), has been suggested as a possible cause of abnormal gene expression in cancerous cells. Despite the growing recognition of MED8's significance in cancer biology, a comprehensive understanding of its expression patterns, prognostic implications, and functional roles across diverse cancer types remains elusive. Furthermore, the importance of MED8 in certain cancer types, like liver hepatocellular carcinoma (LIHC), requires more research to determine its usefulness as a biomarker for diagnosis and prognosis.

This study aims to conduct a thorough analysis of MED8 expression patterns in different tissue types and cancer types. By leveraging multidimensional genomic and transcriptomic data from large-scale cancer databases, we aim to elucidate the prognostic significance of MED8 expression in diverse malignancies. Additionally, we explore the complex connections between MED8 levels and factors related to tumors, such as microsatellite instability (MSI), tumor mutational burden (TMB), and cancer stemness, to understand how it may impact the tumor microenvironment and immune responses (Choo 2011; Ritterhouse 2019; Subramanian et al. 2005). We use gene set enrichment analysis (GSEA) to uncover the functional consequences of MED8 dysregulation by identifying pathways and biological processes that are enriched with MED8 expression. This method offers a thorough insight into the possible ways in which MED8 plays a role in the start, advancement, and resistance to treatment of tumors. Additionally, we focus on liver hepatocellular carcinoma (LIHC) as a specific cancer type of interest, given the established association between MED8 expression and poor prognosis in this malignancy. Our goal was to confirm the clinical importance of MED8 in LIHC by analyzing it thoroughly on the Xiantao platform. We will assess its diagnostic capabilities, expression variations in different tumor grades, and its effectiveness as a standalone prognostic indicator. By conducting this comprehensive examination, we aim to uncover the diverse functions of MED8 in the field of cancer research, opening up possibilities for its use as a predictive indicator and treatment focus in different types of cancers, with a specific focus on liver hepatocellular carcinoma.

METHODS

COMPREHENSIVE ANALYSIS OF GENE EXPRESSION AND PROTEIN LEVELS

The Genotype-Tissue Expression (GTEx) database was utilized to examine the tissue-specific expression profile of MED8 in various normal tissues (Lonsdale et al. 2013). Data from The Cancer Genome Atlas (TCGA) and the UALCAN web resource were used to analyze the varying levels of MED8 expression in tumor and normal samples (Chandrashekar et al. 2022; Danaher et al. 2018). Immunohistochemistry images from the Human Protein Atlas (HPA) database were used to evaluate the expression of MED8 protein in various cancer types and their respective normal tissues (Colwill & Gräslund 2011).

SINGLE-CELL TRANSCRIPTOMIC ANALYSIS

Single-cell transcriptomic data from the TISCH database (http//tisch.comp-genomics.org/) was used to analyze the levels of MED8 expression in different cell types, such as tumor cells and cells found in the tumor microenvironment (Sun et al. 2021).

SURVIVAL ANALYSIS

The predictive importance of MED8 levels was assessed in 33 different types of cancer from the TCGA database. Cox regression was used to evaluate the relationship between MED8 levels and survival outcomes including overall survival, disease-specific survival, disease-free interval, and progression-free interval. Survival outcomes were compared between patients with high and low MED8 expression levels using Kaplan-Meier survival curves and log-rank tests, with stratification based on the optimal cutoff value determined by the survminer R package.

MSI, TMB, AND STEMNESS ANALYSIS

Data from the TCGA database was used to explore the connections between MED8 levels and factors related to tumors, such as microsatellite instability (MSI), tumor mutational burden (TMB), and stem cell characteristics.

COPY NUMBER ALTERATION AND DNA METHYLATION ANALYSIS

We used the cBioPortal for Cancer Genomics website to examine the copy number changes of MED8 in various

types of cancer. The cBioPortal provides access to multidimensional cancer genomics data and enables the visualization and analysis of genetic alterations (Gao et al. 2013). The UALCAN web resource (http//ualcan.path.uab.edu/) was used to examine the methylation status of the MED8 DNA promoter (Chandrashekar et al. 2022). The UALCAN database allows for the analysis of DNA methylation levels across different cancer types and the comparison of methylation levels between tumor and normal samples.

CO-EXPRESSION AND INTERACTION ANALYSIS

The co-expression analysis of MED8 across different cancer types was performed using the Gene Expression Profiling Interactive Analysis (GEPIA) database (http://gepia.cancer-pku.cn/)(Tang et al. 2017). The database provided the top 50 genes that were co-expressed with MED8 across all types of cancer. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) online tool (https//string-db.org/) was used to explore the possible protein-protein interactions involving MED8 (Szklarczyk et al. 2023). The interactions between MED8 and its binding partners were analyzed and visualized using the STRING database. Furthermore, the GeneMANIA prediction server (http://genemania.org/) was employed to explore the potential interplay between MED8 and other genes or proteins. The GeneMANIA database integrates various sources of data, including protein-protein interactions, gene co-expression, and functional annotations, to generate a comprehensive network of interactions (Franz et al. 2018).

GENE SET ENRICHMENT ANALYSIS (GSEA)

To explore the possible roles of MED8 in human cancer, an analysis of gene set enrichment was conducted with the GSEA tool available at https//www.gsea-msigdb.org/gsea/index.jsp (Subramanian et al. 2005). The GSEA study investigated the genes with varying expression levels in patients with high and low levels of MED8 in different types of cancer from The Cancer Genome Atlas (TCGA) database. The analysis aimed to identify gene sets and pathways enriched in the MED8-high or MED8-low groups, providing insights into the potential biological processes and pathways influenced by MED8 expression.

CLINICAL SIGNIFICANCE ANALYSIS IN LIVER HEPATOCELLULAR CARCINOMA (LIHC)

The clinical significance of MED8 in liver hepatocellular carcinoma (LIHC) was further investigated using the Xiantao platform (https://xiantaozi.com/). The subsequent evaluations were conducted. 1) The expression levels of MED8 were compared between LIHC tumor samples and matched normal samples, 2) The expression levels of MED8 were compared between high-grade and low-grade LIHC tumor samples, 3) ROC curves were created to evaluate the diagnostic capability of MED8 in differentiating LIHC tumor tissues from normal tissues, and 4) Univariate and multivariate Cox regression were performed to assess the correlation of MED8 levels with survival outcomes in patients with LIHC, as well as to ascertain if MED8 serves as a standalone prognostic indicator.

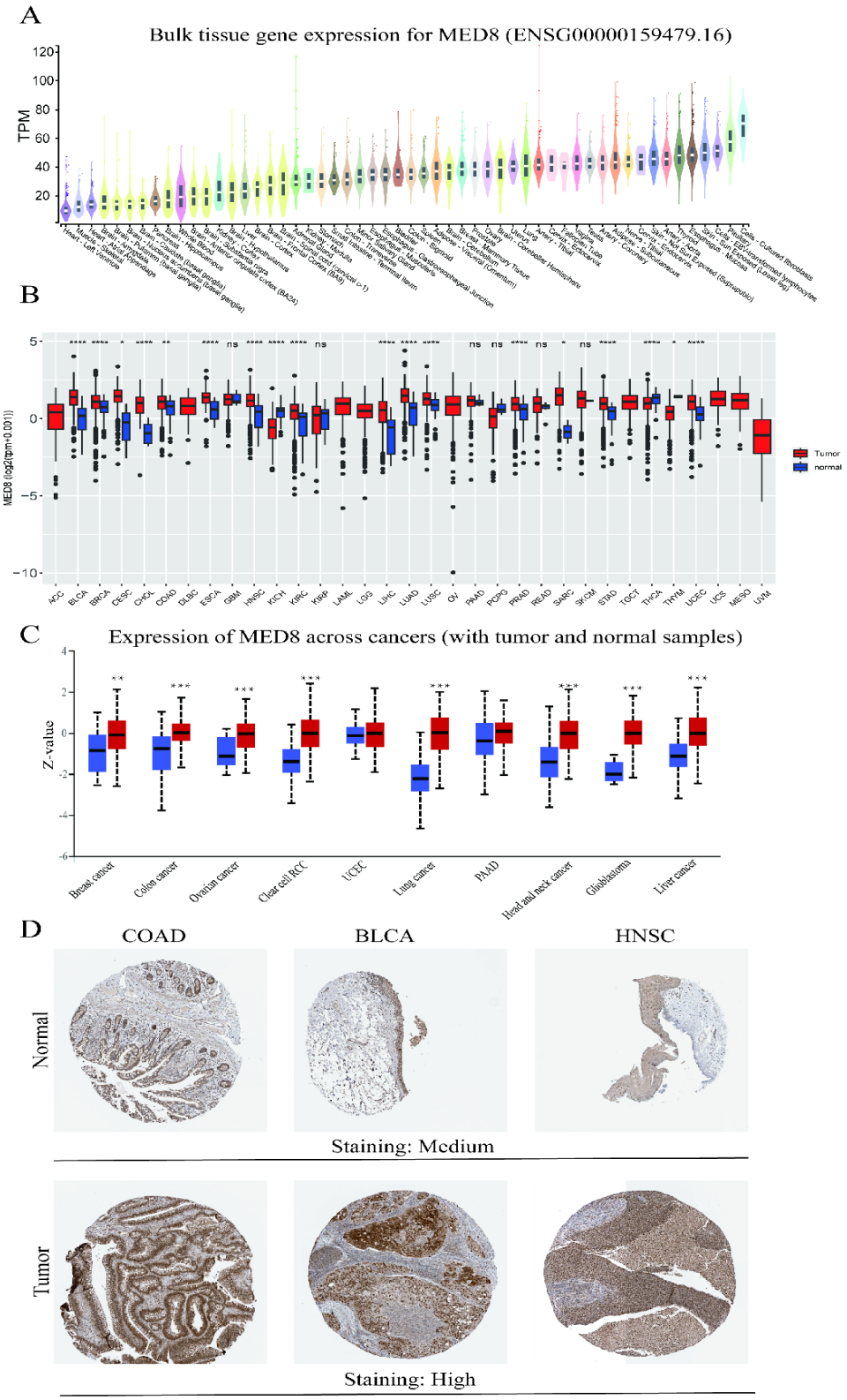
RESULTS

TISSUE-SPECIFIC AND CANCER-ASSOCIATED EXPRESSION PATTERNS OF MED8

The examination of the GTEx dataset unveiled a variegated transcriptional profile of the MED8 gene across an assortment of bodily tissues. Notably, attenuated expression levels were observed within the heart, muscle, and brain, while a significant augmentation manifested in the thyroid, esophagus, and skin, as graphically depicted in Figure 1(A). This tissue-specific expression pattern intimated that MED8 may harbor divergent physiological functions across disparate anatomical domains. The scrutiny of the TCGA database disclosed a differential expression of MED8 in several malignancies and their corresponding non-neoplastic counterparts, as illustrated in Figure 1(B). The results showed a significant increase in MED8 gene expression in 15 different types of tumors, including BLCA, BRCA, CESC, CHOL, COAD, ESCA, HNSC, KIRC, LIHC, LUAD, LUSC, PRAD, SARC, STAD, and UCEC, compared to their normal tissues. Conversely, a significant upregulation of MED8 expression was evident in KICH, THCA, and THYM relative to normal tissues. Moreover, examination of the UALCAN database indicated increased levels of the MED8 protein in eight different types of cancers, including breast, colon, ovarian, clear cell renal cell, lung, head and neck squamous cell, glioblastoma, and liver cancers, compared to their normal tissues (Figure 1(C)). The examination of representative immunohistochemical images from the HPA database unveiled high staining levels of MED8 in COAD, BLCA, and HNSC tumor samples, as evidenced by the intense staining intensity, while the corresponding normal tissues exhibited medium staining levels (Figure 1(D)). These observations lend credence to a potential oncogenic role for MED8 in certain malignancies, while simultaneously intimating a tumor-suppressive function in others, underscoring the intricate and context-dependent nature of its involvement in the intricate tapestry of cancer biology.

MED8 EXHIBITS ELEVATED EXPRESSION IN TUMOR CELLS AND TUMOR MICROENVIRONMENT BY SINGLE-CELL ANALYSIS

Analyzing the single-cell transcriptomic data from the TISCH database (Figure 2), we noticed increased MED8 expression in different tumor cells when compared to normal cells. Interestingly, MED8 also exhibited relatively high expression in tumor microenvironment cells, such



MED8 mRNA expression across different human tissues in the GTEX database;
Comparison of MED8 expression between tumor and normal samples;
Protein expression analysis of MED8 based on the UALCAN platform;
Protein expression of MED8 in COAD, BLCA, HNSC patients in HPA.
*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. ns, not statistically significant

FIGURE 1. Comparative analysis of MED8 expression in human tissues and cancer patients

as fibroblasts, monocytes/macrophages, and endothelial cells. Collectively, these findings suggest that MED8 may play crucial roles in tumor initiation and progression, potentially influencing the crosstalk between tumor cells and their microenvironment.

MED8 EXPRESSION EXHIBITS COMPLEX ASSOCIATIONS WITH SURVIVAL OUTCOMES IN CANCER

We conducted Cox regression analyses on 33 types of cancer from TCGA to assess the prognostic importance of MED8 expression, analyzing OS, DSS, DFI, and PFI. As depicted in Figure 3(A)-3(D), MED8 exhibited intricate associations with these survival metrics, underscoring its context-dependent roles in cancer progression. Elevated MED8 expression conferred a significantly increased risk of mortality, as evidenced by poor OS in THCA, SARC, LIHC, LGG, LAML, KIRP, KICH, and ACC. Similarly, high MED8 levels were associated with inferior DSS in several malignancies, including THCA, SARC, LIHC, LGG, KIRP, KICH, and ACC. Additionally, the transcriptional upregulation of MED8 exhibits an intricate association with unfavorable DFI across a panoply of malignancies, encompassing LIHC, KIRP, and ACC. Furthermore, the overexpression of MED8 manifests a compelling correlation with attenuated PFI in SARC, PRAD, LIHC, LGG, KIRP, KICH, and ACC. Together, these results emphasize the diverse and situation-dependent functions of MED8 in the advancement of cancer and outcomes in medical settings, indicating its promise as a predictive marker and treatment focus in certain cancer scenarios. Furthermore, the study included Kaplan-Meier survival analysis and log-rank tests to categorize patients based on their MED8 expression levels using the most suitable threshold. In GBM, BLCA, LIHC, LAML, ACC, SARC, LGG, UVM, MESO, THCA, KICH, and UCEC, higher MED8 expression was associated with poorer overall survival (Figure 3(E)). Similarly, elevated MED8 levels were associated with reduced DSS in GBM, BLCA, LIHC, SARC, LGG, UVM, KICH, MESO, THCA, UCEC, and ACC (Figure S1). Furthermore, elevated MED8 levels tended to be associated with reduced DFI in COAD, KIRP, ACC, LIHC, and SARC (Figure S2). Moreover, increased levels of MED8 expression were linked to poor progression-free interval in liver hepatocellular carcinoma, sarcoma, low-grade glioma, uveal melanoma, pancreatic adenocarcinoma, thymoma, kidney chromophobe, kidney renal papillary cell carcinoma, and adrenocortical carcinoma (Figure S3). The results suggest that MED8 could serve as a promising prognostic biomarker in certain tumor environments.

CORRELATIONS OF MED8 LEVELS WITH MICROSATELLITE INSTABILITY, TUMOR MUTATIONAL BURDEN, AND STEM CELL CHARACTERISTICS

The TCGA database was used to analyze the relationships between MED8 levels and various tumor-related factors like stemness, MSI, and TMB in the tumor microenvironment. The strong connections found between MED8 levels and MSI in UCEC, SKCM, LUA, and BRCA (Figure 4(A)), along with TMB in ACC, COAD, KICH, LIHC, LUAD, SARC, SKCM, and UCEC (Figure 4(B)), indicate possible impacts on immune response against tumors. Moreover, the positive correlation between MED8 expression and stemness in ACC, BLCA, BRCA, ESCA, LIHC, LUAD, LUSC, PRAD, SARC, STAD, THYM, and UCEC (Figure 4(C)) further highlights the potential role of MED8 in cancer stem cell characteristics.

GENETIC AND EPIGENETIC CHANGES OF MED8 ACROSS DIFFERENT HUMAN CANCERS

Genetic and epigenetic alterations exert a pivotal influence on cancer progression and immune evasion. Analysis of the cBioPortal database showed that ‘amplification’ was the predominant form of genetic aberration across most of the examined human tumor types (Figure 4(D)). The progression of tumors can be altered by DNA methylation, which is a type of epigenetic process. We examined the variance in promoter DNA methylation levels of MED8 between tumors and nearby normal tissues using UALCAN to study the relationship between promoter DNA methylation levels and MED8 expression (Figure 4(E)). Our results showed that MED8 had decreased levels of DNA methylation in various types of cancers, suggesting that reduced methylation of the MED8 promoter could lead to increased expression in these tumors.

THE CO-EXPRESSION AND INTERACTION ANALYSES OF MED8 IN PAN-CANCER

Co-expression analysis of MED8 in pan-cancer was performed using the GEPIA database. We obtained the top 50 co-expressed genes in pan-cancer (Figure 5(A)). To investigate the functional roles of MED8, the protein-protein interactions of MED8 with other partners were analyzed using STRING online tools. The results showed that MED8 interacted with MED1, MED10, MED11, MED12, MED17, MED18, MED28, STN1, TRIP4, and ZC3H13 (Figure 5(B)). Additionally, the results in GeneMANIA indicated that MED8 may interplay with CDK8, MED12, MED13, MED19, MED11, MED20, MED30, MED14, MED29, H1-4, MED18, MED16, MED4, MED10, MED27, MED7, MED24, CDK19, MED26, and MED15 (Figure 5(C)). The interplay between MED8 and various components of the Mediator complex, as well as other interacting partners, highlights its involvement in transcriptional regulation and cellular processes that may contribute to tumor biology.

EXPLORING THE POSSIBLE ROLES OF MED8 IN HUMAN CANCER THROUGH GSEA ANALYSIS

A comprehensive Gene Set Enrichment Analysis (GSEA) was performed to better understand how MED8 affects the prognosis of cancer patients across different types of tumors. In each type of cancer, this study analyzed genes that were

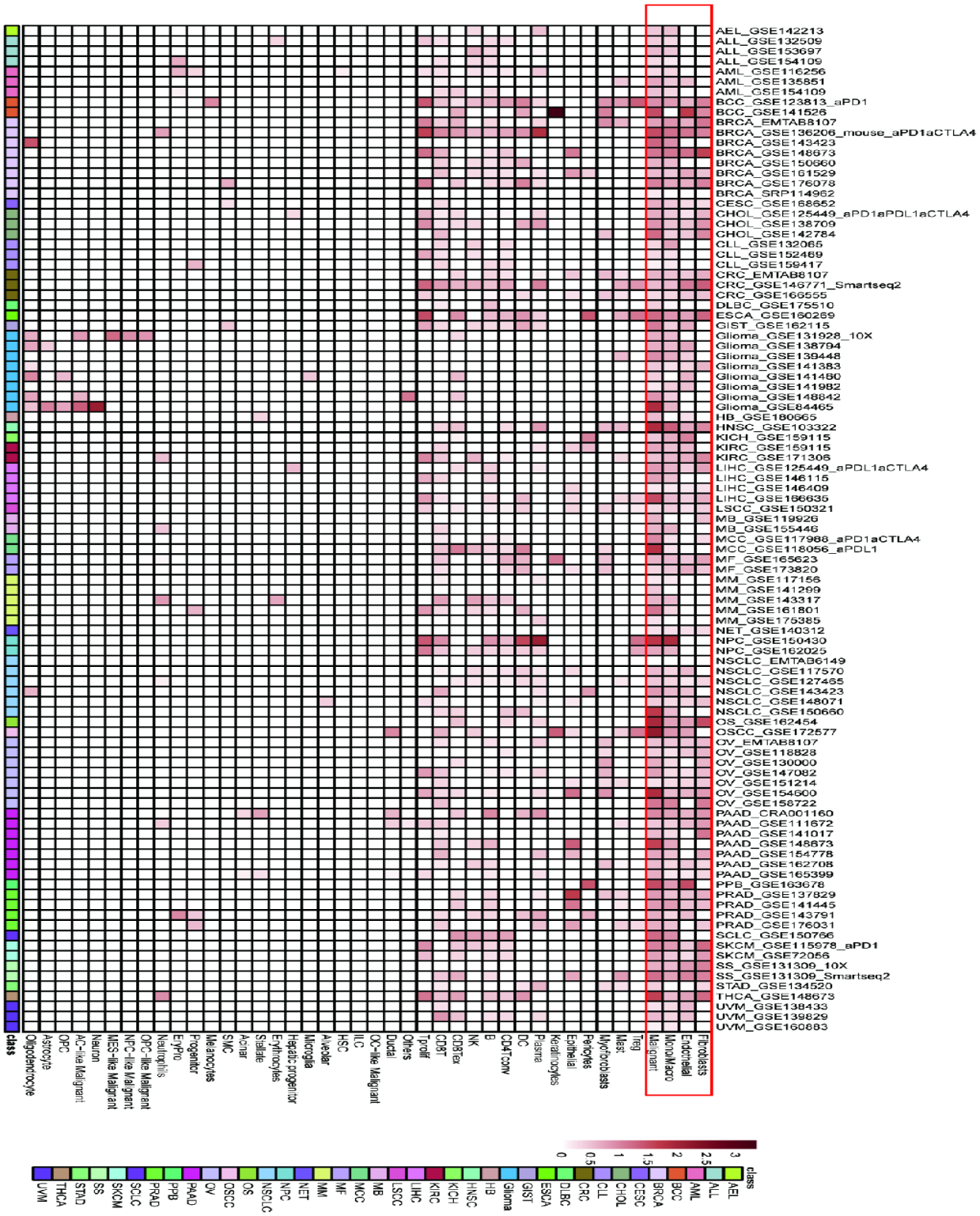
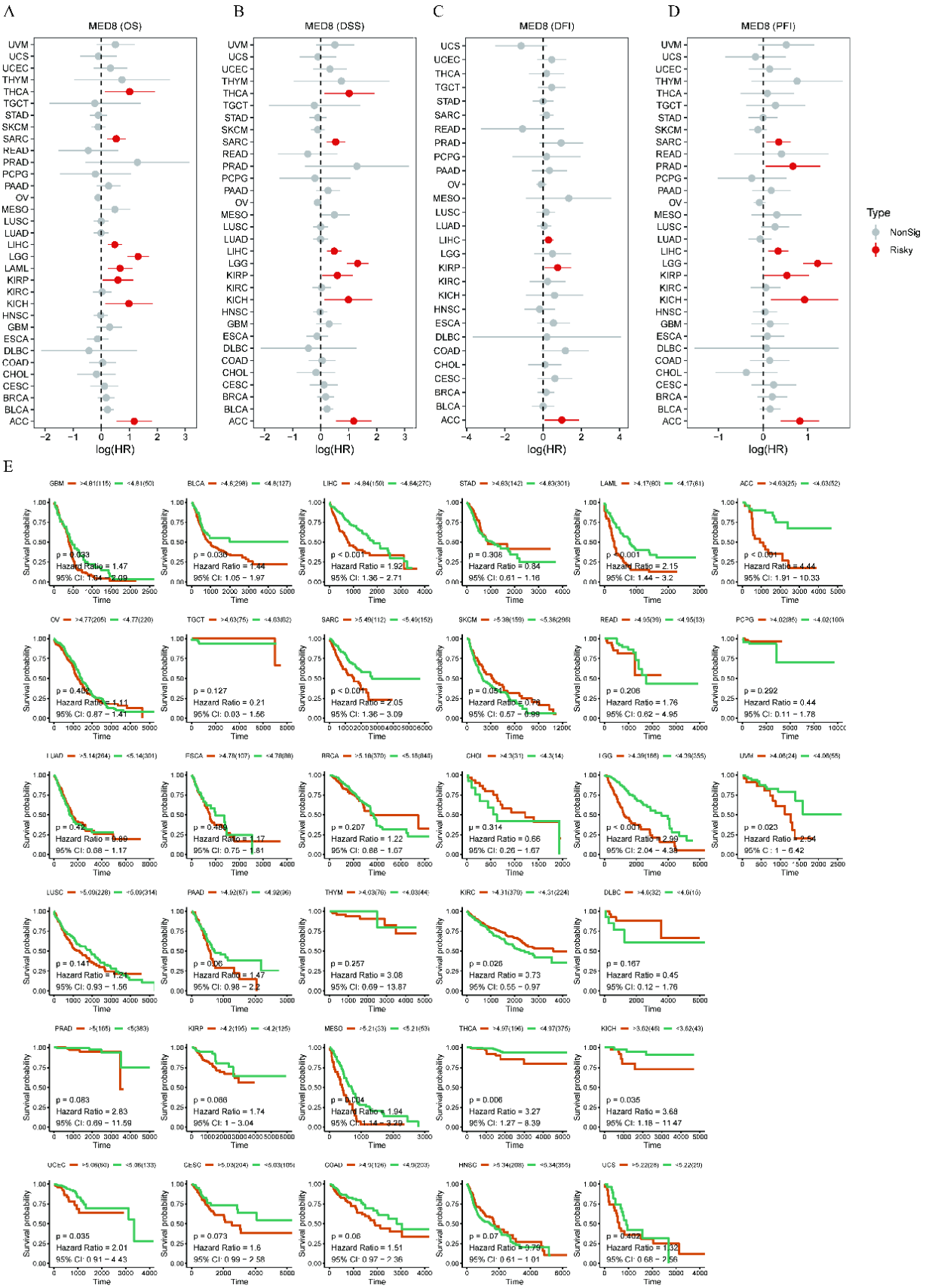
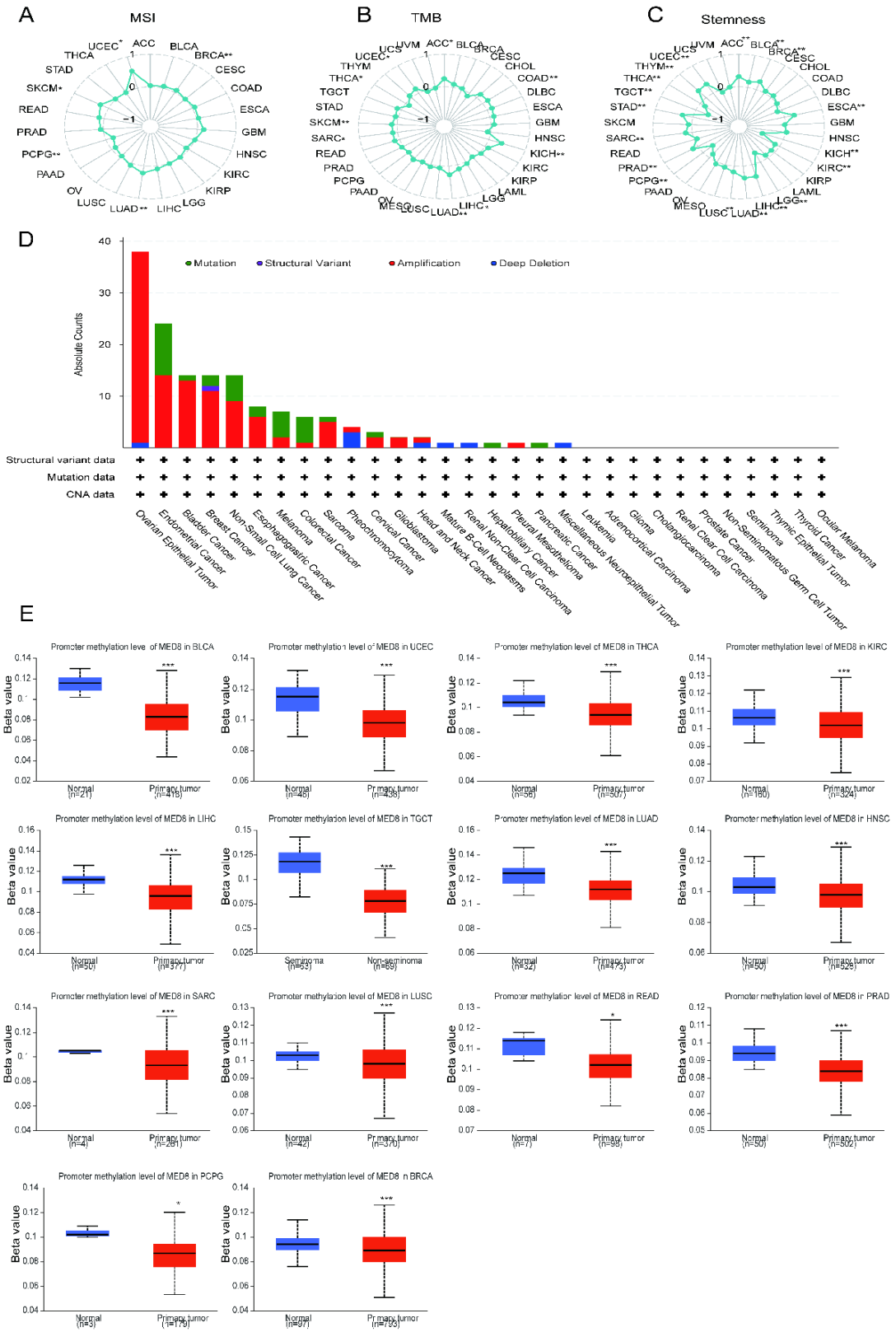


FIGURE 2. Single-cell analysis for MED8 based on Tumor Immune Single-cell Hub (TISCH) database



(A-D) Cox regression analysis of MED8 on overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), progression-free interval (PFI) in pan-cancer described by the forest plot;(E) Kaplan-Meier survival curves for OS

FIGURE 3. Survival analysis of MED8 in pan-cancer



(A-C) Analysis of MSI, TMB, Stemness of MED8 expression in pan-cancer;
(D) MED8 mutation analysis in pan-cancer using cBioPortal;
(E) The methylation level of MED8 was evaluated by UALCAN.
*p < 0.05, ***p < 0.001

FIGURE 4. Comprehensive evaluation of MED8 in pan-cancer: expression, mutation, and epigenetic regulation

expressed differently in patients with high and low levels of MED8. The heatmap shown in Figure 5(D) showed strong and consistent enrichment of various pathways in nearly every type of cancer. Notably, the MYC pathway, which is associated with cell growth and proliferation, showed prominent enrichment in MED8-high patients. This indicates that MED8 might be involved in enhancing cell growth and proliferation by controlling the MYC pathway. Furthermore, patients with high levels of MED8 showed significant enrichment in the mTORC1 pathway, which plays a role in cellular metabolism and nutrient sensing. This finding implies that MED8 may contribute to metabolic reprogramming and nutrient utilization in tumor cells, potentially promoting their survival and growth. The activation of oxidative phosphorylation and DNA repair pathways in patients with high levels of MED8 indicates that MED8 may play a role in controlling cellular energy generation and mechanisms for repairing DNA damage. These pathways are crucial for maintaining cell viability and genomic stability, and their dysregulation can contribute to tumor progression. Interestingly, the interferon response pathway, which plays a critical role in immune regulation and antitumor immune responses, was also significantly enriched in MED8-high patients across multiple cancer types. This finding indicates a potential link between MED8 expression and the modulation of the immune microenvironment, suggesting that MED8 may influence immune responses within the tumor and impact patient prognosis.

CLINICAL SIGNIFICANCE OF MED8 IN LIHC

Previous studies have established a strong association between MED8 expression and poor prognosis in LIHC. Consequently, we conducted a comprehensive analysis to further explore the clinical significance of MED8 in LIHC using the Xiantao platform. In our analysis, we confirmed a significant increase in MED8 expression in LIHC tissues compared to matched samples (Figure 6(A)). Additionally, high-grade samples exhibited even higher levels of MED8 expression than low-grade samples (Figure 6(B)). Diagnostic ROC curves further demonstrated the remarkable ability of MED8 to differentiate tumor tissues from normal tissues (Figure 6(C)). Finally, our comprehensive univariate and multivariate Cox regression analyses solidified MED8 as an independent prognostic marker for LIHC (Figure 6(D)). Collectively, these studies strongly suggest that MED8 may play a pivotal role in promoting the progression of LIHC.

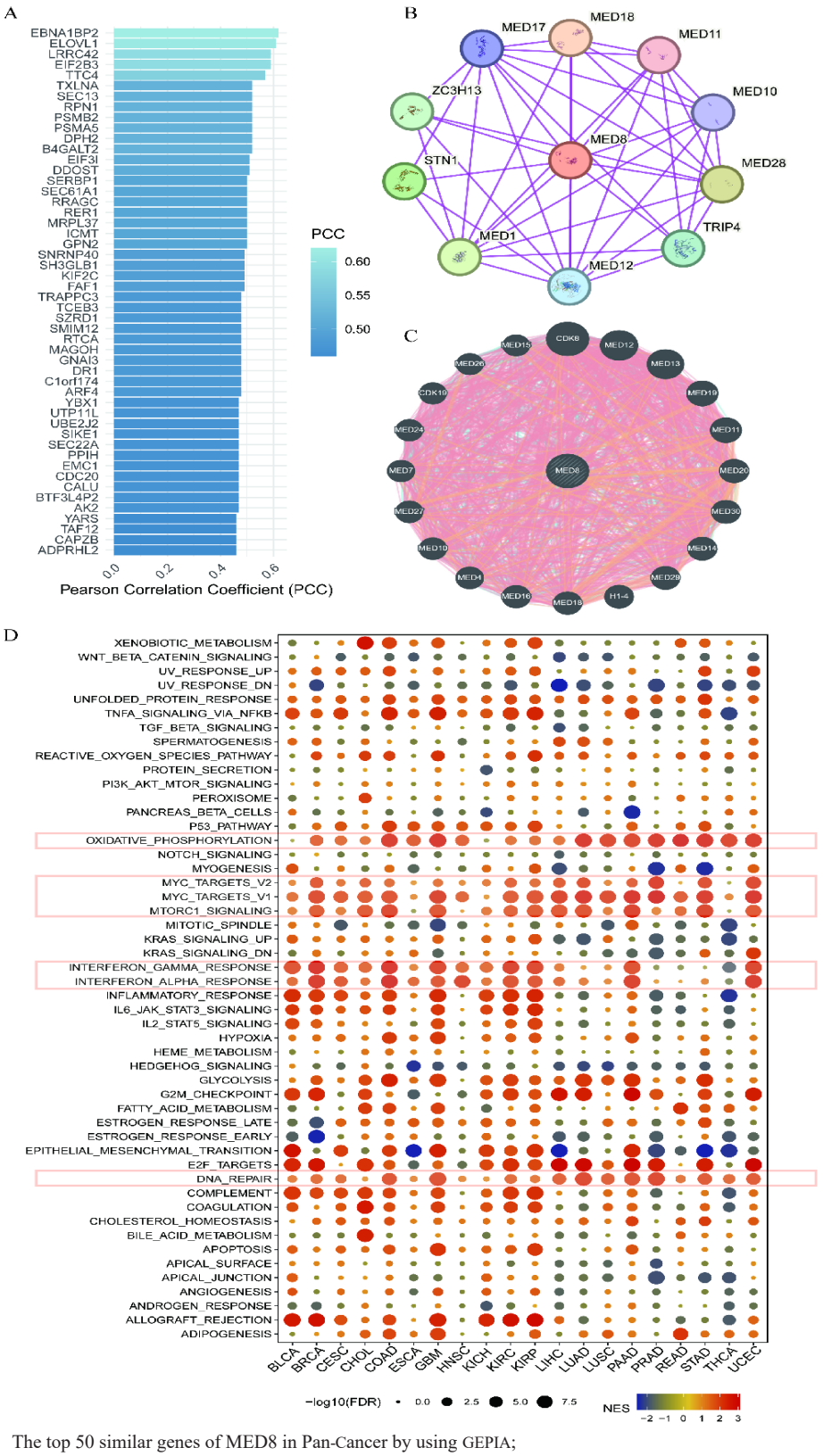
DISCUSSION

The Mediator complex, a critical component of the transcriptional machinery, has emerged as a pivotal player in cancer biology, with its subunits exhibiting intricate and context-dependent roles in tumor initiation and progression. This study thoroughly examines MED8, an essential component of the Mediator complex, in various types of cancer, showing its diverse expression patterns, prognostic significance, and connections to different types of malignancies.

Our findings show a strikingly heterogeneous expression profile of MED8 across various tissue types, suggesting its involvement in diverse physiological processes. The significant increase in MED8 expression in various cancer types, such as bladder, breast, cervical, cholangiocarcinoma, colorectal, esophageal, head and neck, kidney, liver, lung, prostate, sarcoma, stomach, and uterine cancers, indicates a possible oncogenic function in these tumors. Conversely, the elevated expression observed in kidney chromophobe and thyroid cancers intimates a potential tumor-suppressive function, underscoring the context-dependent nature of MED8's impact on cancer biology. Intriguingly, our single-cell transcriptomic analysis showed elevated MED8 expression not only in tumor cells but also in various components of the tumor microenvironment, such as fibroblasts, monocytes/macrophages, and endothelial cells. This finding suggests that MED8 may play crucial roles in shaping the intricate crosstalk between tumor cells and their surrounding microenvironment, potentially influencing processes such as angiogenesis, immune modulation, and metastasis.

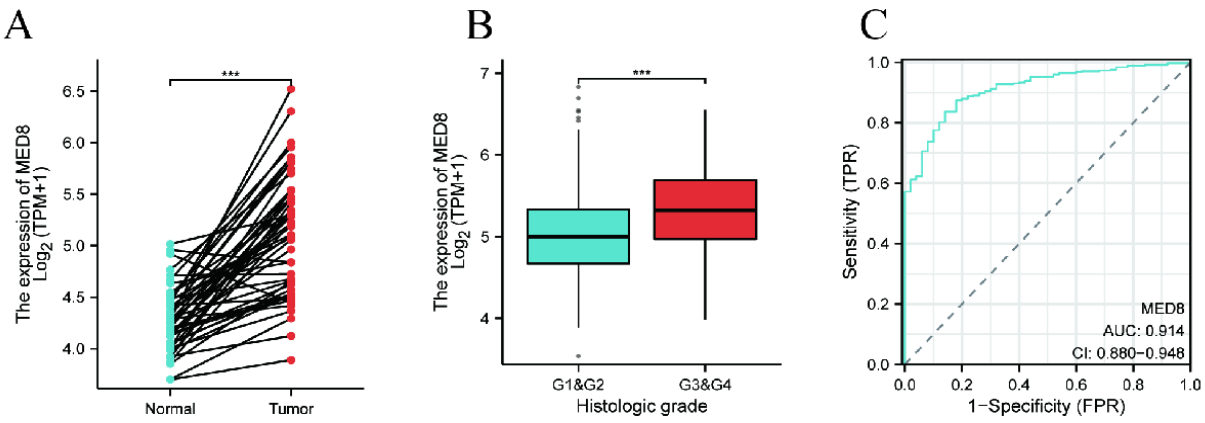
In our study, the importance of MED8 expression as a predictor became a key focus, showing intricate connections among various types of cancer and different measures of survival, such as overall survival, disease-specific survival, disease-free interval, and progression-free interval. High levels of MED8 were associated with a higher risk of death and cancer recurrence in various types of tumors, including thyroid, sarcoma, liver, low-grade glioma, acute myeloid leukemia, kidney renal papillary cell carcinoma, kidney chromophobe, and adrenocortical carcinoma, but the specific prognostic implications seemed to vary depending on the situation. This underscores the need for a nuanced understanding of MED8's roles within specific tumor microenvironments and molecular subtypes.

Exploring the connections between MED8 levels and factors related to tumors, like microsatellite instability (MSI), tumor mutational burden (TMB), and stem cell characteristics, showed fascinating findings. The strong associations found between MED8 levels and MSI in various cancer forms, such as uterine corpus endometrial carcinoma, skin cutaneous melanoma, lung adenocarcinoma, and breast invasive carcinoma, indicate a possible connection between MED8 irregularities and impaired DNA mismatch repair processes. Likewise, the strong connection between MED8 levels and TMB in various cancers like adrenocortical carcinoma, colon adenocarcinoma, kidney chromophobe, liver hepatocellular carcinoma, lung adenocarcinoma, sarcoma, skin cutaneous melanoma, and uterine corpus endometrial carcinoma suggests a possible involvement in influencing the genetic makeup of tumors and immune responses against cancer.



The top 50 similar genes of MED8 in Pan-Cancer by using GEPIA;
PPI network constructed using STRING;
Gene-gene interactions identified by GENAMANIA;
Gene set enrichment analysis (GSEA) of MED8 in pan-cancer.

FIGURE 5. Integrative analysis of MED8 in pan-cancer: Network and functional insights



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Variable	HR	HR.95L	HR.95H	pvalue	Method
Gender	0.8006008	0.5618397	1.140826	2.183982e−01	UniCox
Age	1.0121044	0.9983411	1.026057	8.501469e−02	
T	**1.6830160**	**1.4035949**	**2.018063**	**1.908506e−08**	
Stage	**1.6698854**	**1.3613654**	**2.048324**	**8.656878e−07**	
Grade	1.1137027	0.8808318	1.408139	3.682381e−01	
Platelet count	1.0000005	0.9999979	1.000003	6.877156e−01	
Prothrombin time	1.0142557	0.9763065	1.053680	4.669017e−01	
Radiation therapy	0.9546012	0.3030565	3.006909	9.367411e−01	
Relative family cancer history	1.1918170	0.8256201	1.720437	3.488142e−01	
Weight	0.9933851	0.9835391	1.003330	1.915899e−01	
Residual tumor	1.7181204	0.9133939	3.231834	9.316157e−02	
MED8	**2.0531469**	**1.4073872**	**2.995204**	**1.887730e−04**	
Gender	0.6704278	0.3671648	1.224174	1.930700e−01	MultiCox
Age	1.0160182	0.9924038	1.040195	1.853552e−01	
T	1.5742855	0.5276570	4.696944	4.158322e−01	
Stage	0.9060350	0.3102142	2.646234	8.568029e−01	
Grade	1.5039565	1.0251851	2.206319	3.687232e−02	
Platelet count	0.9999957	0.9999897	1.000002	1.642278e−01	
Prothrombin time	1.0142811	0.9487369	1.084353	6.773880e−01	
Radiation therapy	1.1965734	0.3560377	4.021450	7.716858e−01	
Relative family cancer history	1.6258627	0.8880220	2.976761	1.152327e−01	
Weight	1.0093940	0.9941517	1.024870	2.284276e−01	
Residual tumor	1.0355467	0.2326563	4.609189	9.634293e−01	
MED8	**2.6273772**	**1.5167316**	**4.551307**	**5.690736e−04**	

MED8 expression was upregulated in LIHC tissues compared with that in paired normal tissues;
MED8 was overexpressed in high-grade LIHC;
ROC curves demonstrating the diagnostic value of MED8 in LIHC;
Univariate Cox regression and multivariate Cox regression demonstrated that MED8 was an independent prognostic factor in LIHC.
***p < 0.001

FIGURE 6. Clinical significance of MED8 in hepatocellular carcinoma (LIHC) based on TCGA database

The strong connection between MED8 levels and stem cell characteristics in different types of cancer, such as adrenocortical carcinoma, bladder urothelial carcinoma, breast invasive carcinoma, esophageal carcinoma, liver hepatocellular carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, prostate adenocarcinoma, sarcoma, stomach adenocarcinoma, thymoma, and uterine corpus endometrial carcinoma, indicates that MED8 may play a role in controlling cancer stem cell properties. These findings have profound implications for understanding tumor heterogeneity, therapeutic resistance, and metastatic potential.

Our analysis of genetic and epigenetic alterations showed that amplification was the predominant form of genetic aberration across most cancer types, highlighting the potential impact of MED8 copy number alterations on its dysregulated expression. Moreover, the decreased DNA methylation levels in the MED8 promoter region in various types of cancer, such as bladder urothelial carcinoma, uterine corpus endometrial carcinoma, thyroid carcinoma, kidney renal clear cell carcinoma, liver hepatocellular carcinoma, testicular germ cell tumors, lung adenocarcinoma, head and neck squamous cell carcinoma, sarcoma, lung squamous cell carcinoma, rectum adenocarcinoma, prostate adenocarcinoma, pheochromocytoma and paraganglioma, and breast invasive carcinoma, indicate that epigenetic processes like promoter hypomethylation might play a role in the increased expression of MED8 in these malignancies.

The co-expression and interaction analyses of MED8 unveiled intricate associations with various components of the Mediator complex, as well as other transcriptional regulators and cellular pathways. GSEA offered valuable insights into the potential functional roles of MED8 in cancer biology. The consistent and significant enrichment of the MYC pathway in MED8-high patients across multiple cancer types suggests that MED8 may promote cellular growth and proliferation through the regulation of this pivotal oncogenic pathway. Given the well-established roles of MYC in driving various aspects of tumor biology, including cell cycle progression, metabolism, and stemness, the interplay between MED8 and MYC signaling may represent a critical nexus in cancer pathogenesis.

Furthermore, the enrichment of the mTORC1 pathway in MED8-high patients intimates a potential role for MED8 in modulating cellular metabolism and nutrient sensing, processes that are frequently dysregulated in cancer cells to support their heightened metabolic demands. The observed enrichment of oxidative phosphorylation and DNA repair signaling pathways suggests that MED8 may contribute to the regulation of cellular energy production and genomic integrity, respectively, processes that are crucial for tumor cell survival and proliferation.

Intriguingly, the enrichment of the interferon response pathway in MED8-high patients across multiple cancer types unveils a potential link between MED8 expression and the modulation of the immune microenvironment. This finding raises the intriguing possibility that MED8 may influence antitumor immune responses, either by shaping the immunogenicity of tumor cells or by modulating the recruitment and activity of immune cells within the tumor microenvironment. Elucidating the precise mechanisms underlying this association could pave the way for novel immunotherapeutic strategies targeting MED8 or its associated pathways.

Our comprehensive analysis of liver hepatocellular carcinoma (LIHC) further reinforced the clinical significance of MED8 in this malignancy. The notable increase in MED8 levels in LIHC tissues in comparison to normal samples, along with its heightened presence in high-grade tumors, highlights its promise as a biomarker for diagnosis and prognosis. The remarkable ability of MED8 to differentiate tumor tissues from normal tissues, as demonstrated by the diagnostic ROC curves, highlights its potential utility as a companion diagnostic tool for LIHC.

Given the high expression of MED8 in various cancers and its potential role in cell cycle regulation, targeting MED8 or its associated pathways could emerge as a novel therapeutic strategy. This could involve the development of drugs that specifically inhibit MED8 activity, or that target the signaling pathways in which MED8 is involved, potentially leading to more effective cancer treatments. Understanding the precise mechanisms of MED8's action could lead to the design of targeted therapies that reduce the expression or activity of MED8 in cancer cells, thereby inhibiting tumor growth and progression.

Crucially, our univariate and multivariate Cox regression analyses solidified MED8 as an independent prognostic marker for LIHC, suggesting that its dysregulation may contribute to the aggressive nature and poor clinical outcomes associated with this malignancy. Given the dismal prognosis and limited therapeutic options for LIHC, the identification of MED8 as a key player in its pathogenesis opens up avenues for the development of targeted therapeutic strategies.

LIMITATIONS

Despite providing profound insights, the complexity of single-cell transcriptomic analysis and the challenges associated with data analysis may lead to difficulties in interpretation and potential biases. Our study may be subject to sample selection bias, particularly if the sample does not adequately represent a broader demographic or disease stage range. As a retrospective study, our data collection could be constrained by inconsistencies in recording practices or gaps in information, potentially affecting the accuracy of our findings. Although we have analyzed genetic and epigenetic changes, integrating this data to achieve a comprehensive understanding of MED8's role remains a challenge. Our research is primarily based on correlational analysis, lacking functional experiments to directly demonstrate MED8's impact on cancer progression.

In summary, our comprehensive pan-cancer analysis has unveiled the multifaceted roles of MED8 in tumor biology, elucidating its complex expression patterns, prognostic implications, and functional associations across diverse malignancies. The intricate interplay between MED8 and various oncogenic pathways, tumor microenvironment factors, and epigenetic regulatory mechanisms highlights the potential for targeting MED8 or its associated networks as a therapeutic strategy. Our research in liver hepatocellular carcinoma confirms the importance of MED8 as a biomarker for diagnosis and prognosis and as a possible target for treatment in this aggressive cancer. Further research should concentrate on elucidating the specific molecular pathways through which MED8 impacts the development of LIHC, leading to the creation of targeted therapies for MED8 and individualized treatment strategies.

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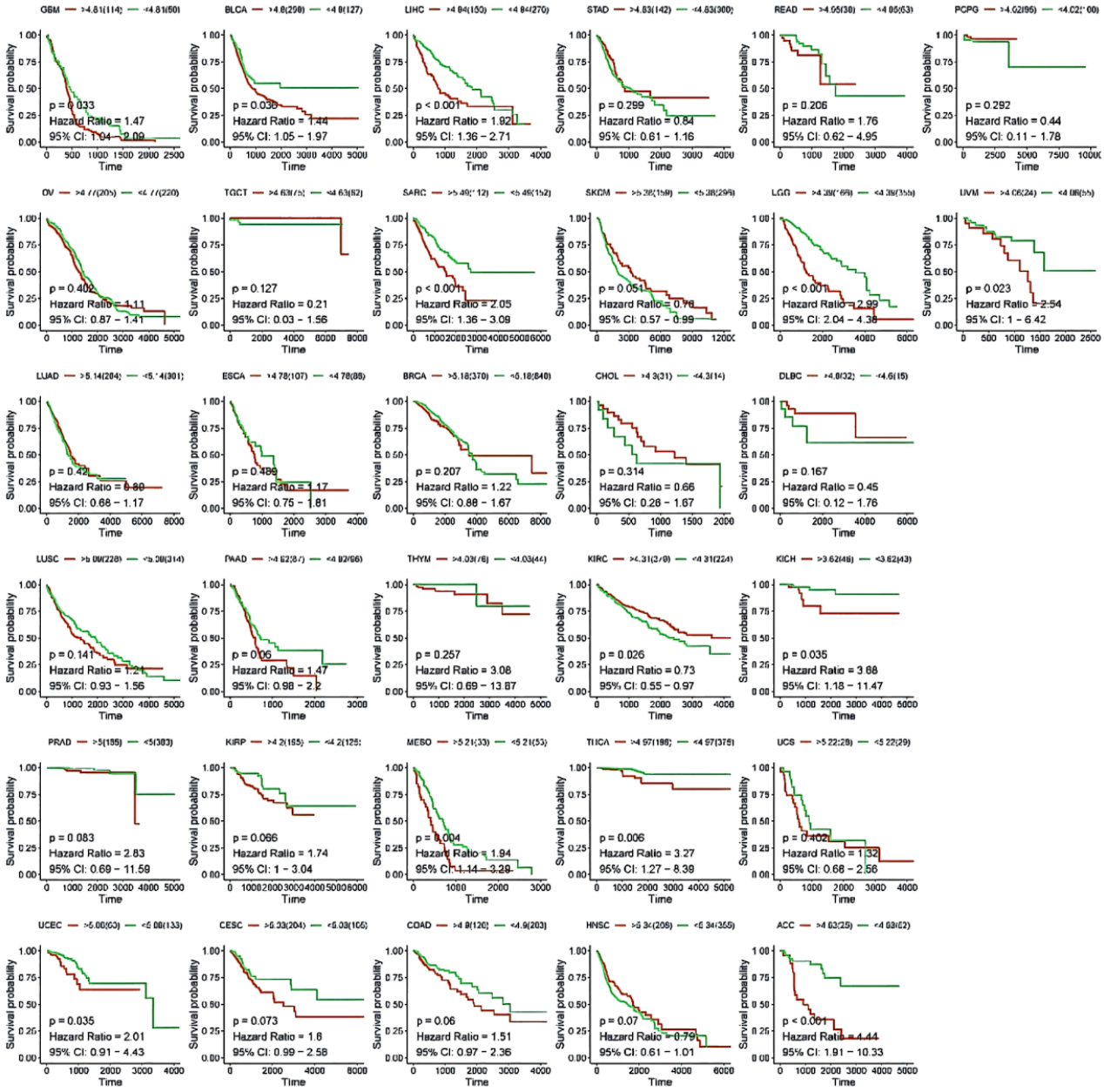

FIGURE S1. Kaplan-Meier survival curves for disease-specific survival (DSS)

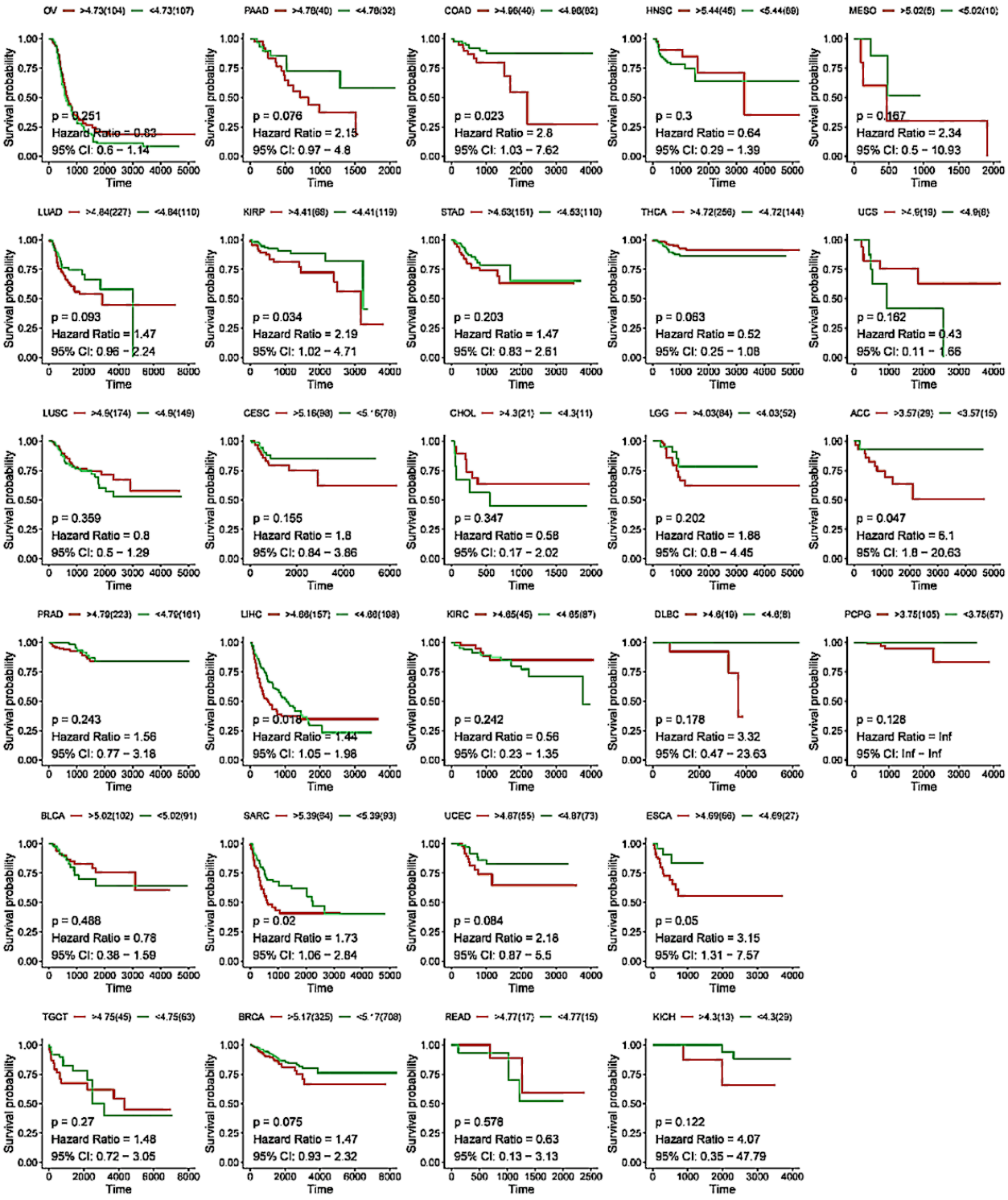

FIGURE S2. Kaplan-Meier survival curves for disease-free interval (DFI)

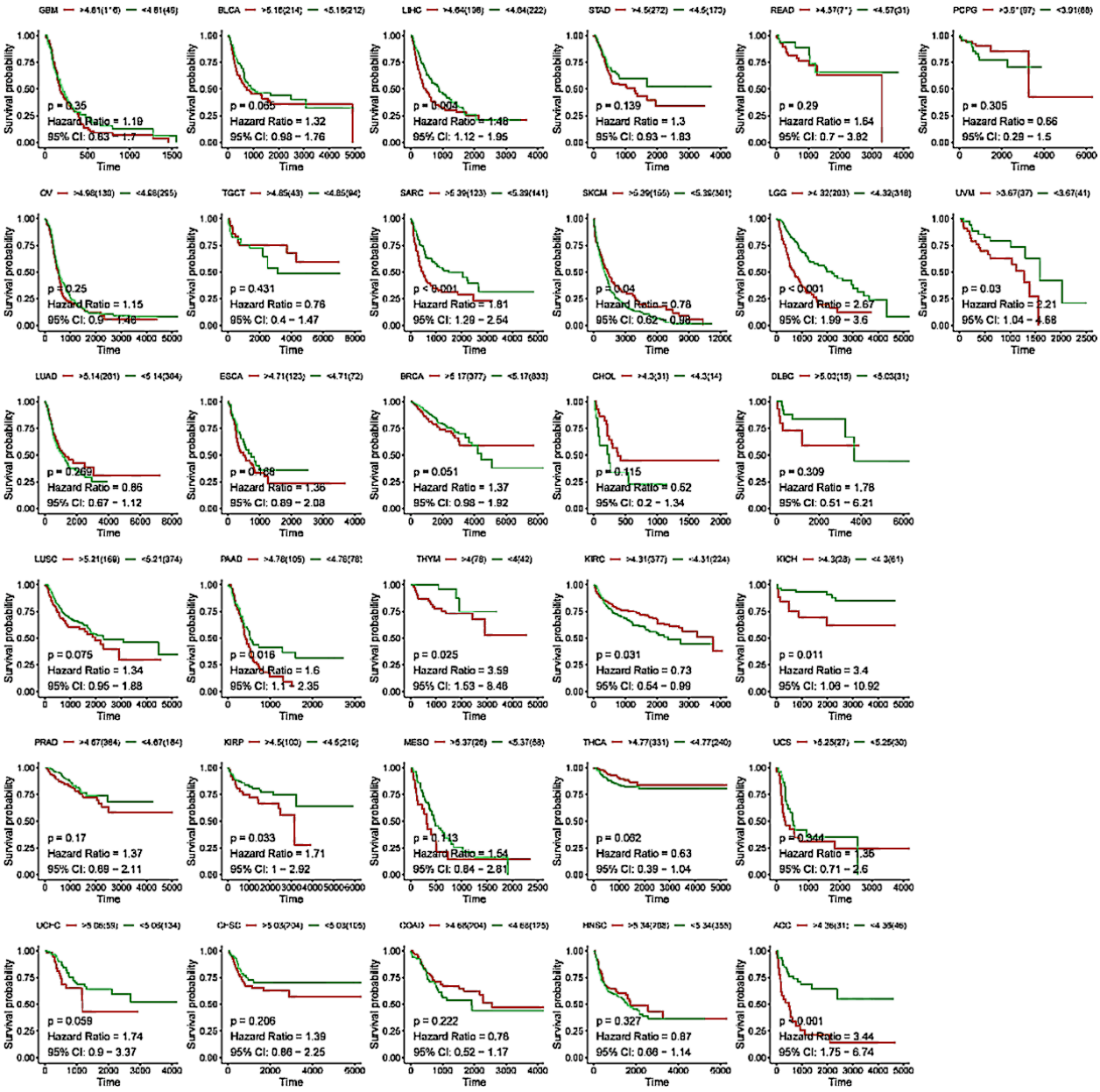

FIGURE S3. Kaplan-Meier survival curves for progression-free interval (PFI)