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Original Article

Mediator complex subunit 8 is a prognostic biomarker in hepatocellular carcinoma

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Abstract: Background: Mediator complex subunit 8 (*MED8*) is known for its role in encoding a subunit of the mediator complex (*MED*), that is critical for transcription. MED8 is significantly expressed in various tumors and has been correlated with an unfavorable prognosis. Nevertheless, no relationships have been found between MED8 and the clinical characteristics of hepatocellular carcinoma (HCC). Methods: To conduct an evaluation of correlations between clinicopathologic characteristics and MED8 expression, the logistic regression, Wilcoxon signed-rank test, and Kruskal-Wallis test were used. To perform analysis of factors contributing to prognosis, the Kaplan-Meier approach and the Cox regression analyses were used. A nomogram on the basis of a Cox multivariate analysis was employed to anticipate the influence of MED8 on patient prognosis. The receiver operating characteristic (ROC) curves were plotted and the areas under the curve (AUC) were calculated to assess the prognostic value of MED8. Both immune infiltration analysis and Gene Set Enrichment Analysis (GSEA) were applied to reveal significant enrichment differences among TCGA data. Quantitative RT-PCR (qRT-PCR) and western blotting were used to verify the difference in the expression of MED8 in normal and hepatocellular carcinoma cells. The immunohistochemical method was used to validate the MED8 expression in tumor and adjoining tissues of HCC patients. Results: A univariate analysis showed that high MED8 expression predicts poor disease-specific survival (DSS) (HR: 2.57; 95% confidence interval (CI) 1.62, 4.07; P<0.001). Multivariate regression analysis showed that high MED8 (adjusted HR: 3.032 (1.817, 5.060); P<0.001) expression and M stage (adjusted HR=4.075 (1.179-14.091) for M1 vs. M0, P=0.026) served as prognostic indicators of unfavorable overall survival in an independent manner in patients with HCC. The C-index for the nomogram was 0.732 (95% CI: 0.698, 0.766) and the AUC of MED8 was 0.817 (95% CI: 0.778, 0.857). Functional analysis showed that the cell cycle checkpoints, p53 dependent G1-DNA damage response, mitotic G1-G1-S phases, and mitotic G2-G2-M phases, were significantly enriched in DEGs associated with MED8 expression. Th2 cells were positively correlated with MED8 expression. Conclusions: MED8 predicts poor prognosis in HCC, possibly through modulating the cell cycle and Th2 cells.

Keywords: Mediator complex subunit 8, mediator, hepatocellular carcinoma, prognosis, diagnostic biomarker

Introduction

Hepatocellular carcinoma (HCC) has been recognized as the sixth most prevalent malignancy and the fourth major contributor to cancer fatalities globally [1]. The molecular mechanisms underlying HCC are not completely understood [2]. Owing to the absence of effective early diagnostic and targeted therapies, the 5-year survival rate is only 11% [3]. Mainstream detection techniques include magnetic resonance imaging (MRI), glycoprotein biomarkers (e.g., AFP), and computed tomography (CT). However, these methods have various limitations. CT and MRI are expensive and difficult to implement at a broad scale, while glycoprotein biomarkers are limited by a lack of specificity to tumor areas [2, 4]. In view of the importance of early tumor detection in optimizing the survival of patients with HCC, it is critical to investigate novel markers for the disease [5].

Mediator is an evolutionarily conserved polyprotein complex composed of 33 subunits in

humans and is an indispensable regulator of transcription [6, 7]. It is divided into four distinct submodules: head, middle, tail, and kinase. Conformational changes by strong interactions with RNA polymerase II (POLII) affect transcription initiation and other important steps in protein expression [8]. Several research reports have demonstrated that the expression levels of certain subunits are altered in various human diseases, especially cancer [6, 7, 9-11]. Mediator complex subunit 8 (*MED8*) is mutated in colorectal cancer cell lines. Furthermore, the expression of MED8 in renal clear cell carcinoma is correlated with a shortened survival duration and high TNM stage, and the expression of MED8 in metastatic tumors is higher than that of primary tumors [12]. However, the association between MED8 and HCC remains largely unclear.

The expression of MED8 in HCC, as well as its prognostic significance, was investigated in the present research using data from the TCGA database. Furthermore, a multi-dimensional analysis was used to evaluate the MED8 and functional networks associated with MED8 in HCC and to examine its function in tumor immunity. We confirmed that MED8 expression was higher in liver HCC samples and was correlated with worse overall survival (OS). We further determined that M stage (M1 vs. M0) and MED8 independently served as prognostic indicators for overall survival (OS). The present research offers a novel diagnostic and prognostic marker for HCC.

Materials and methods

Patients and samples

RNA-seq data (level 3 HTSeq-FPKM format) for 424 patients diagnosed with HCC and corresponding clinical information were downloaded from the TCGA database [13, 14]. RNA-seq data that did not contain clinical information and cases with survival <30 days were not included in the present research. Data in level 3 HTSeq-FPKM format were converted into TPM (transcript per million reads) format, and RNA-seq data for 371 cases containing clinical information were finally obtained for subsequent analyses. A detailed clinicopathologic characteristics of the patients is listed in **Table 1**. Setting the median MED8 expression level as the threshold value, HCC tumor samples were classified into a low-expression group and a high-expression group.

Identification of DEGs

The DESeq2 package was utilized to analyze differentially expressed genes (DEGs) according to HTSeq-count files between groups exhibiting low and high expression in HCC samples. Log fold change (logFC) >2 and adjusted P<0.01 were set as the thresholds for DEGs. Volcano [14] and heat maps were used to visualize the results.

Metascape analysis

We employed the Metascape database to evaluate enrichment for MED8 Ontology (GO) terms in the three broad categories, cellular components (CCs), molecular functions (MFs), and biological processes (BPs) among DEGs between the groups with high and low MED8 expression. Parameter settings were as follows: enrichment factor >1.5, minimum count >3, adjusted P<0.05.

MED8 gene set enrichment analysis (GSEA)

A GSEA was implemented in the R package clusterProfiler (version 3.6.0) [13] to analyze and visualize signaling pathways that might be associated with DEGs between the groups having low and high expression. FDR q<0.2 and adjusted P<0.05 were defined as the statistical significance level.

Immune infiltration analysis by ssGSEA

To analyze the infiltration status of 24 distinct kinds of immune cells in tumor tissues, the ssGSEA method in the GSVA package was applied. The relationships between MED8 and the relative abundances of these 24 cells were described utilizing Spearman correlation coefficients. Subsequently, the rank-sum test was employed for the purpose of evaluating the correlation between elevated MED8 expression and immune cell infiltration.

Statistical analyses

Statistical analyses were conducted utilizing R (version: 6.2). For the purpose of comparing the MED8 expression in HCC tissues and normal controls, the Wilcoxon rank-sum test was applied. Spearman correlation coefficients, as

Table 1. TCGA hepatocellular carcinoma patients' characteristics

Value	Level	Low expression of MED8	High expression of MED8	p	test
n		186	185		chi-square[1]
T stage (%)	T1	106 (57.6%)	75 (40.8%)	0.006	Exact[2]
	T2	35 (19.0%)	59 (32.1%)		
	T3	38 (20.7%)	42 (22.8%)		
	T4	5 (2.7%)	8 (4.3%)		
N stage (%)	N0	128 (99.2%)	124 (97.6%)	0.368	exact
	N1	1 (0.8%)	3 (2.4%)		
M stage (%)	M0	127 (96.9%)	139 (100.0%)	0.054	exact
	M1	4 (3.1%)	0 (0.0%)		
Pathologic stage (%)	Stage I	101 (58.0%)	70 (40.5%)	<0.001	exact
	Stage II	32 (18.4%)	54 (31.2%)		
	Stage III	36 (20.7%)	49 (28.3%)		
	Stage IV	5 (2.9%)	0 (0.0%)		
Gender (%)	Female	58 (31.2%)	63 (34.1%)	0.632	
	Male	128 (68.8%)	122 (65.9%)		
Race (%)	Asian	71 (39.2%)	87 (48.9%)	0.017	exact
	Black or African American	5 (2.8%)	12 (6.7%)		
	White	105 (58.0%)	79 (44.4%)		
Residual tumor (%)	R0	170 (96.6%)	154 (92.8%)	0.081	exact
	R1	5 (2.8%)	12 (7.2%)		
	R2	1 (0.6%)	0 (0.0%)		
Histologic grade (%)	G1	33 (18.0%)	22 (12.0%)	0.001	exact
	G2	100 (54.6%)	77 (42.1%)		
	G3	48 (26.2%)	74 (40.4%)		
	G4	2 (1.1%)	10 (5.5%)		
Adjacent hepatic tissue inflammation (%)	Mild	54 (41.9%)	45 (42.9%)	0.868	
	None	64 (49.6%)	53 (50.5%)		
	Severe	11 (8.5%)	7 (6.7%)		
Child-Pugh grade (%)	A	123 (93.9%)	94 (87.0%)	0.064	exact
	B	7 (5.3%)	14 (13.0%)		
	C	1 (0.8%)	0 (0.0%)		
Vascular invasion (%)	No	114 (69.1%)	92 (61.3%)	0.185	
	Yes	51 (30.9%)	58 (38.7%)		
TP53 status (%)	Mut	20 (11.1%)	82 (46.1%)	<0.001	
	WT	160 (88.9%)	96 (53.9%)		
Age (median [IQR])		62.00 [54.00, 69.00]	60.00 [51.00, 68.00]	0.177	Nonnorm[3]
Height (median [IQR])		168.00 [162.00, 174.00]	167.00 [160.00, 173.00]	0.309	nonnorm
Weight (median [IQR])		73.00 [62.00, 86.00]	66.50 [58.00, 77.00]	0.002	nonnorm
AFP (ng/ml) (median [IQR])		7.00 [3.50, 77.00]	28.00 [7.00, 1099.50]	<0.001	nonnorm
BMI (median [IQR])		25.18 [22.41, 30.08]	24.13 [20.97, 27.47]	0.012	nonnorm
Total bilirubin (mg/dl) (median [IQR])		0.70 [0.50, 1.00]	0.70 [0.50, 1.00]	0.677	nonnorm
Albumin (g/dl) (median [IQR])		4.00 [3.40, 4.30]	4.00 [3.50, 4.30]	0.325	nonnorm
Prothrombin time (median [IQR])		1.10 [1.00, 9.50]	1.10 [1.00, 8.70]	0.104	nonnorm

[1]The default classification variables using the chi-square test. [2]"Exact" indicates that Fisher's Exact Test was used as the statistical approach. [3]The term "nonnorm" was defined as a non-normal distribution, and the Wilcoxon rank-sum test was employed to conduct statistical tests.

well as Fisher's exact test, were utilized to analyze relationships between the level of MED8 expression and clinico-pathologic characteristics. Multivariate Cox regression analysis was used to compare the influence of MED8 expression on survival along with other clinical characteristics. All hypothesis tests were two-tailed and significant if $P<0.05$.

Construction and evaluation of a prognostic model

To screen out independent prognostic factors related to survival, both multivariate and univariate Cox regression analyses [15] were performed by combining MED8 expression data with clinicopathologic factors. The Rms pack-

age was utilized to create a nomogram and MED8 rated calibration plot. Risk scores (RS) were calculated from the multi-factor Cox model. A risk factor association graph showing MED8 expression, patient survival time, survival status, and the distribution of risk scores was MED8 rated. The TCGA-HCC cohort was classified into low- and high-risk groups according to the median MED8 expression level, and a survival curve was created utilizing Kaplan-Meier programming in the Survminer Package. The prognostic model was evaluated by the c-index in the ROC analysis. A threshold of P<0.05 was set as significant.

Expression analysis by qRT-PCR, western blot, and immunohistochemistry (IHC)

The protein and mRNA levels of MED8 were determined by QRT-PCR and western blot in normal liver tissue cell line L-02, hepatoma cell line HepG2, and SMMC-7721, respectively. Immunohistochemical images of Human protein analysis (THPA) were used to determine the distribution and subcellular localization of MED8, as well as protein expression between different tumor samples and matched normal tissues. All three groups of samples were collected from patients from China who were diagnosed with liver cancer for the first time. Two women were diagnosed with hepatitis B virus-related liver cancer and one man was diagnosed with alcoholic liver cancer. Prior to sampling, none of the three patients underwent radiation or chemotherapy.

Results

Demographic characteristics

Basic patient information is listed in **Table 1**. A sum of 371 HCC cases with clinical information was obtained from TCGA, among which 186 cases had low MED8 expression and 185 cases had high MED8 expression using the median value as the threshold. A total of 121 females and 250 males were recruited for the study and the average age was 61 years old. Fisher's exact test or Chi-squared test illustrated a substantial correlation between MED8 levels and the TP53 status (P<0.001), histologic grade (P=0.001), race (P=0.017), pathologic stage (P<0.001), and T stage (P=0.006). The Wilcoxon rank-sum test or *t*-test illustrated a substantial correlation between the MED8 levels and weight (P=0.002), AFP (ng/ml) (P< 0.001), and BMI (P=0.012).

Identification of differentially expressed genes in HCC

The Wilcoxon signed-rank test was used for the purpose of performing the comparison of MED8 data from TCGA between 50 HCC samples and paired adjacent samples as well as between 50 normal samples and 371 HCC samples. The MED8 levels were substantially elevated in HCC samples as opposed to the control samples (P<0.001) (**Figure 1A**, **1B**). To investigate whether MED8 performs an instrumental function in the development of HCC, we used DESeq2 to analyze expression differences based on HTseq-counts between the groups exhibiting low and high MED8 expression. Under the threshold values of |logFC| >2 and *P*.adj <0.05, 582 DEGs were obtained, of which 536 genes were found to be up-modulated and 46 genes were down-modulated. The volcano and heat maps are shown in **Figure 1C** and **1D**, respectively.

Functional enrichment analyses of MED8

Using the clusterProfiler package, DEGs associated with MED8 were evaluated by a MED8 Ontology (GO) analysis, identifying enrichment for 47 terms, including 14 terms in the biological process (BP) category, 16 in the cellular components (CC) category, and 17 in the molecular function (MF) category. The results are presented in **Figure 2A-C**.

MED8-related signaling pathways on the basis of GSEA

A GSEA was performed to determine meaningful signaling pathways in the MED8 data sets for the comparison between the groups exhibiting low and high MED8 expression. With regards to the MSigDB (c2.cp.v62. symbols) dataset, we identified many substantial differences (FDR<0.05, normalized P<0.05). The pathways that were most significantly enriched were obtained based on their NES values, including cell cycle checkpoints, transcriptional modulation by TP53, mitotic G2-G2-M phases, and modulation of TP53 activity, as shown in **Figure 2D-G**.

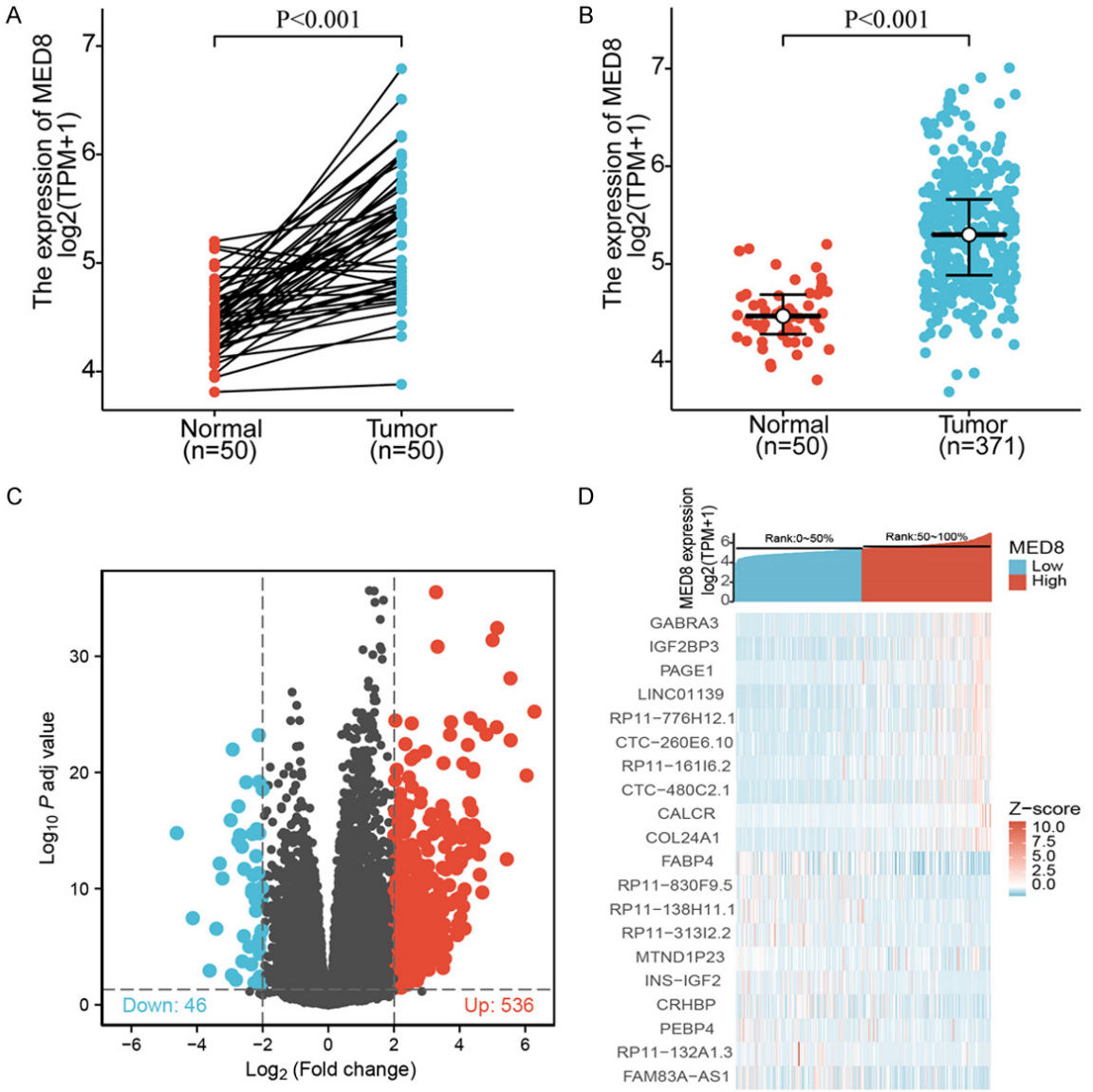

Figure 1. Evaluation of genes with differential expression in the TCGA dataset. (A) Wilcoxon signed-rank tests were utilized to determine the levels of expression of MED8 in paired tumor and surrounding samples and (B) non-paired samples. (C) Differentially expressed genes (DEGs) visualized using a volcano plot. (D) Heat map of the top 50 DEGs among the groups with low and high MED8 expression.

Association between MED8 expression and infiltration of immune cell

The correlation between MED8 expression and 24 different kinds of infiltrating immune cells in the tumor microenvironment (TME) was investigated utilizing Spearman correlation coefficients. MED8 expression was shown to have a positive association with various cells, including Th2 cells, aDCs, T helper cells, and TFH, and a negative association with pDCs, eosinophils, TH17 cells, and neutrophils (**Figure 3A**, P<0.05). Notably, MED8 showed a very strong positive correlation with the abundance of Th2 cells (**Figure 3B**, P<0.001).

The role of MED8 in patient prognosis

As determined by the Kruskal-Wallis rank-sum test, high levels of MED8 were substantially related to the pathologic stage, histologic grade, and T stage in liver HCC based on TCGA data (P<0.05), as depicted by **Figure 4A-C**. Kaplan-Meier plots were charted utilizing the Survminer package to assess the MED8 prognostic significance for disease-specific survival

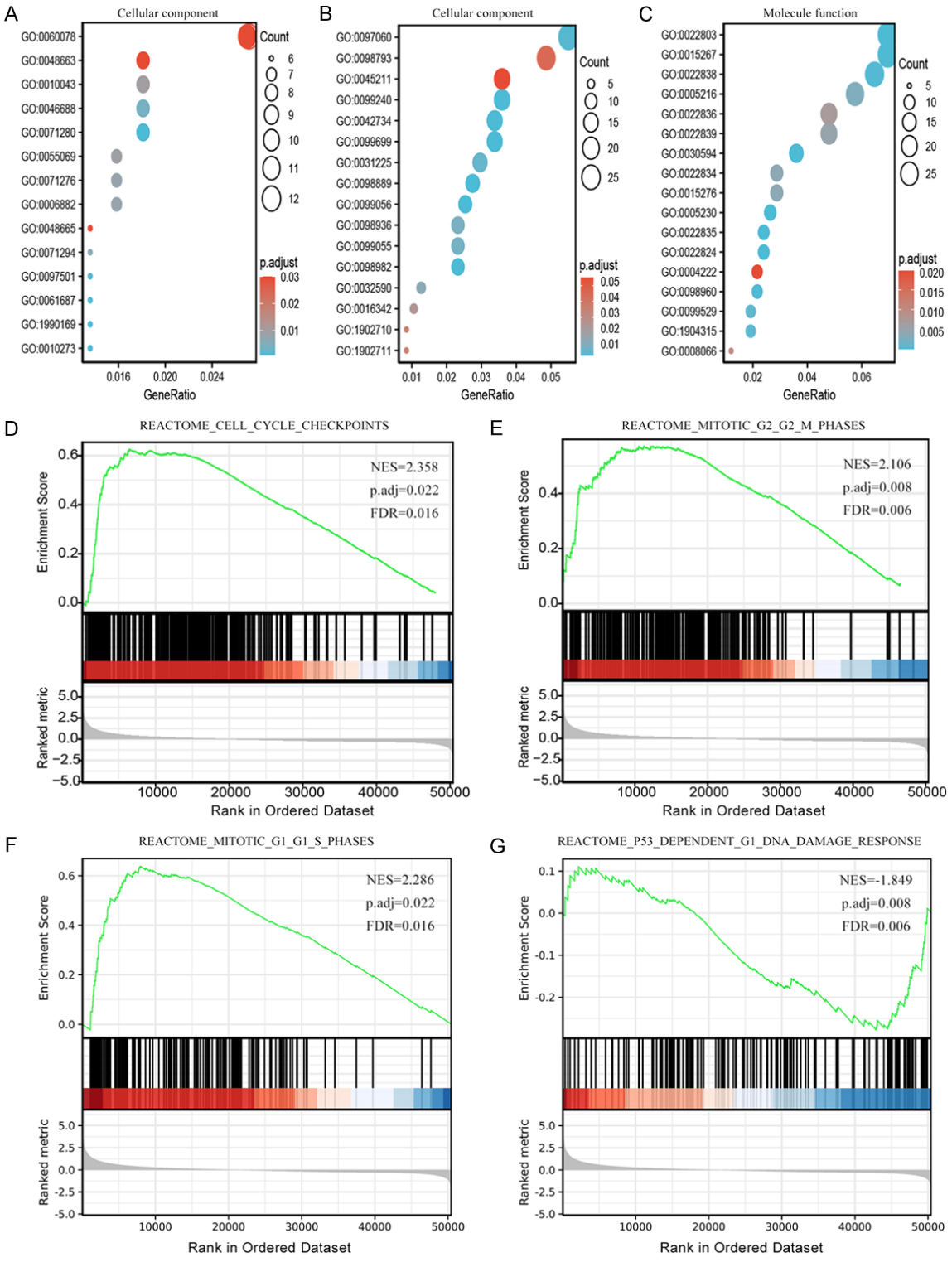

Figure 2. The enrichment analysis of MED8 and neighboring genes. A. Enrichment of biologic processes associated with MED8-related genes. B. Enrichment of cellular components associated with MED8-related genes. C. Enrichment of molecular functions associated with MED8-related genes. D. GSEA results of cell cycle checkpoints. E. GSEA results of mitotic G2-G2-M phases. F. GSEA results in mitotic G1-G1-S phases. G. GSEA results of p53 dependent G1 DNA damage response. ES, enrichment score; NES, normalized ES.

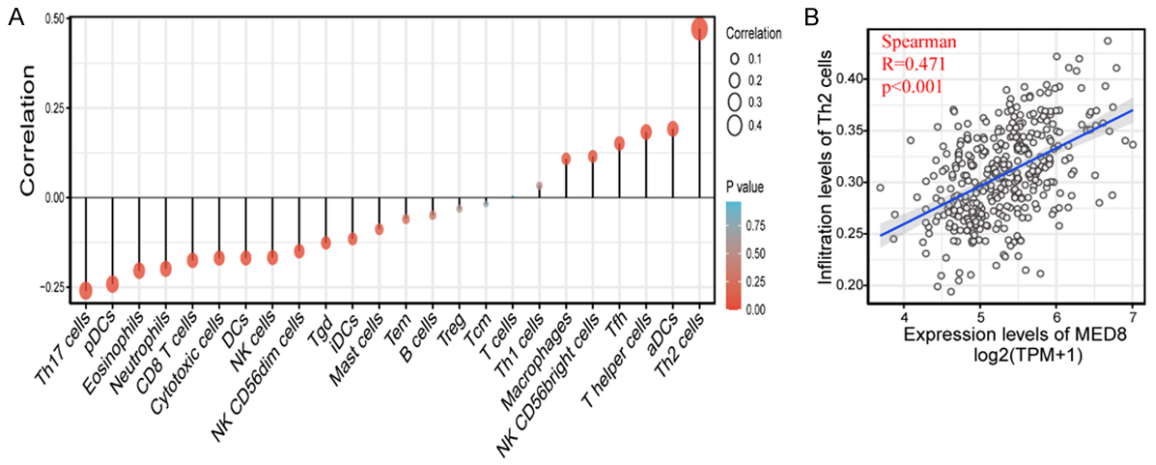

Figure 3. Relationship between MED8 expression and the levels of immune infiltration in patients with HCC. A. Relationship between the relative abundances of 24 distinct immune cell types and the levels of MED8 expression. B. Relationship between the relative enrichment score of Th2 cells and the expression level of MED8.

(DSS). A correlation between high MED8 expression and a worse disease-specific survival was detected (HR=2.57 (1.62-4.07), P<0.001), as shown in **Figure 4D**. Correlations between clinicopathological features and the MED8 expression were analyzed by logistic regression. Levels of MED8 were considerably related to the T stage (OR=1.98 (1.31-3.00), P=0.001), pathologic stage (OR=2.04 (1.33-3.13), P=0.001), histologic grade (OR=2.26 (1.46-3.15), P<0.001), AFP (ng/ml) (OR=2.15 (1.22-3.83), P=0.009), and TP53 status (OR=6.83 (4.01-12.11), P<0.001), as summarized in **Table 2**. High expression of MED8 was associated with poorer disease-specific Survival in G1&G2& G3&G4 subgroups of histologic grade (HR=2.64 (1.64-4.23), P<0.001) (**Figure 4E**). A forest plot was used to demonstrate the prognostic significance of MED8 for DSS in different subgroups of liver HCC based on TCGA data. MED8 was significantly related to the T stage subgroups (T1&T2) (HR=3.096 (1.605-5.971), P<0.001), the pathological stage II (HR=5.058 (1.111-23.028), P=0.036), and stage III (HR=2.393 (1.114-5.144), P=0.025), as shown in **Figure 4F**.

Construction of a prognostic model on the basis of MED8 and clinico-pathological values

Both multivariate and univariate Cox regression analyses were employed to determine whether MED8 independently served as a prognostic indicator for HCC. A univariate Cox regression analysis was performed to combine variables with *P* values less than 0.1 in a single-factor Cox regression, including pathologic stage (P<0.001), T stage (P<0.001), M stage (P=0.018), and MED8 (P<0.001). Further, multivariable Cox regression demonstrated that M stage (P=0.026) and MED8 (P< 0.001) independently served as prognostic indicators for overall survival (P<0.05), as illustrated in **Table 3**. A nomogram was used to evaluate the prognostic model, as shown in **Figure 5A**, including M stage, pathologic stage, and MED8 (c-index: 0.732 (0.698-0.766)). A calibration curve was employed to verify the models' performance including M stage, pathologic stage, and MED8, as shown in **Figure 5B**. We analyzed the diagnostic effectiveness of MED8 in HCC by a ROC analysis, and the area under the curve (AUC) was 0.817, suggesting that MED8 is a possible diagnostic marker, as shown in **Figure 5C**.

MED8 had high expression in hepatocellular carcinoma cells and pathological tissues

To thoroughly validate the difference in the expression of MED8 in normal liver cells and hepatocellular carcinoma cells, *in vivo* and *in vitro* experiments were carried out respectively. As demonstrated in **Figure 6**, the mRNA expression of MED8 (**Figure 6A**) and protein expression of MED8 (**Figure 6B**) in hepatocarcinoma cell lines (SMMC-7721 and HepG2) were elevated as opposed to that in a normal liver cell line (L-02). Analysis of MED8 protein patterns using IHC revealed higher expression in HCC compared to normal tissues (**Figure 6C**).

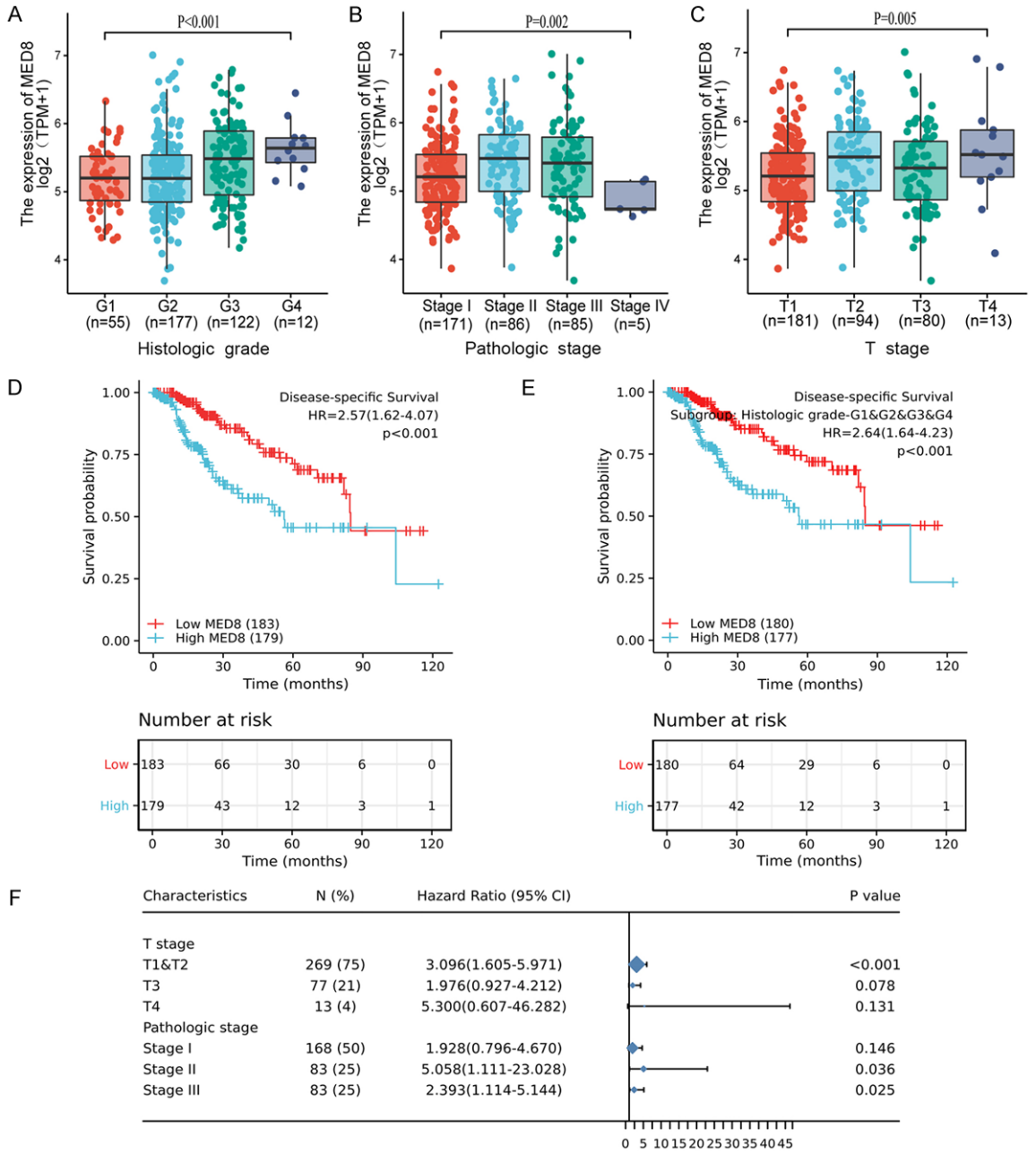

Figure 4. Correlation analysis between MED8 expression levels and clinical values in HCC. The correlation between MED8 expression and clinico-pathologic values, including (A) Histologic grade, (B) Pathologic stage, (C) T stage, the impact of MED8 expression on DSS, and (D) MED8 expression in the pathologic stage on DSS (E) in patients with HCC in the TCGA dataset. (F) A forest plot demonstrates the prognostic significance of MED8 for DSS in distinct subgroups. Spearman correlation coefficients, as well as Fisher's exact test, were utilized to analyze relationships between the level of MED8 expression and clinico-pathologic characteristics. All hypothesis tests were two-tailed and statistically significant if P<0.05. (A-C) compared to normal group and (D-F) compared to low MED8 group.

Discussion

In eukaryotes, mRNA transcription is dependent on RNA polymerase II (POLII). Although many factors are involved in the regulation of POLII activity, the majority of POLII transcripts require the expression of MED. The head and intermediate modules of MED [6] can directly interact with POLII and act as a bridge between transcription factors and the mechanism

Table 2. Association between clinicopathologic features and the categorization of MED8 expression into low and high groups was analyzed by logistic regression

Characteristic	Odds Ratio in MED8 expression	Odds Ratio (OR)	*P*-value
T stage (T4&T2&T3 vs. T1)	368	1.98 (1.31-3.00)	0.001
N stage (N1 vs. N0)	256	3.10 (0.39-63.07)	0.330
Pathologic stage (Stage II & Stage III & Stage IV vs. Stage I)	347	2.04 (1.33-3.13)	0.001
Residual tumor (R1&R2 vs. R0)	342	2.21 (0.84-6.47)	0.122
Histologic grade (G3&G4 vs. G1&G2)	366	2.26 (1.46-3.51)	<0.001
Vascular invasion (Yes vs. No)	315	1.41 (0.89-2.25)	0.149
AFP (ng/ml) (>400 vs. ≤400)	278	2.15 (1.22-3.83)	0.009
Albumin (g/dl) (≥3.5 vs. <3.5)	297	1.41 (0.82-2.46)	0.214
Total bilirubin (mg/dl) (≥2 vs. <2)	301	1.69 (0.63-4.78)	0.299
Prothrombin time (>4 vs. ≤4)	294	0.72 (0.43-1.19)	0.202
Child-Pugh grade (B&C vs. A)	239	2.29 (0.94-5.94)	0.074
TP53 status (Mut vs. WT)	358	6.83 (4.01-12.11)	<0.001

Table 3. Cox regression analyses on the relationships between overall survival and clinical and pathological variables in TCGA patients

Characteristic	Total (N)	HR (95% CI) Univariate analysis	*P*-value Univariate analysis	HR (95% CI) Multivariate analysis	*P*-value Multivariate analysis
T stage (T3&T4 vs. T1&T2)	367	2.540 (1.785-3.613)	<0.001	2.429 (0.326-18.123)	0.387
N stage (N1 vs. N0)	256	2.004 (0.491-8.181)	0.333		
M stage (M1 vs. M0)	270	4.032 (1.267-12.831)	0.018	4.075 (1.179-14.091)	0.026
Pathologic stage (Stage III & Stage IV vs. Stage I & Stage II)	346	2.449 (1.689-3.549)	<0.001	1.123 (0.151-8.352)	0.910
Residual tumor (R1&R2 vs. R0)	341	1.571 (0.795-3.104)	0.194		
Histologic grade (G3&G4 vs. G1&G2)	365	1.120 (0.781-1.606)	0.539		
AFP (ng/ml) (>400 vs. ≤400)	277	1.056 (0.646-1.727)	0.827		
Albumin (g/dl) (≥3.5 vs. <3.5)	296	0.921 (0.565-1.503)	0.743		
Total bilirubin (mg/dl) (≥2 vs. <2)	300	1.166 (0.472-2.879)	0.740		
Prothrombin time (>4 vs. ≤4)	293	1.330 (0.877-2.015)	0.179		
Child-Pugh grade (B&C vs. A)	238	1.616 (0.797-3.275)	0.183		
Vascular invasion (Yes vs. No)	314	1.348 (0.890-2.042)	0.159		
Gender (Male vs. Female)	370	0.816 (0.573-1.163)	0.260		
Age (>60 vs. ≤60)	370	1.248 (0.880-1.768)	0.214		
Weight (>70 vs. ≤70)	343	0.916 (0.640-1.312)	0.634		
Height (≥170 vs. <170)	338	1.208 (0.833-1.753)	0.319		
TP53 status (Mut vs. WT)	357	1.434 (0.972-2.115)	0.069	1.027 (0.616-1.712)	0.919
MED8 (High vs. Low)	370	2.495 (1.740-3.578)	<0.001	3.032 (1.817-5.060)	<0.001

underlying the binding of upstream regulatory elements [8]. The MED head module is mainly composed of proteins encoded by the SRB. MED8, MED18, and MED20 represent submodules of the mediation head domain. MED8 is considered a multi-domain protein comprising a c-terminal helix, a flexible ligand, and an n-terminal helical domain interacting with Med18 (25) [12, 16]. MED8 performs an integral function in the transcription of all eukaryotic organisms, and changes in its function and/or composition may have important functional consequences, contributing to various diseases, including cancer [8, 9, 17]. At present, only few research reports have examined the correlation between MED8 and some cancers. High levels of MED8 in renal clear cell carcinoma have been detected by immunohistochemistry; however, the proliferation and motor capacity of renal clear cell carcinoma cells are significantly reduced after MED8 is silenced with siRNA [12]. Other studies have

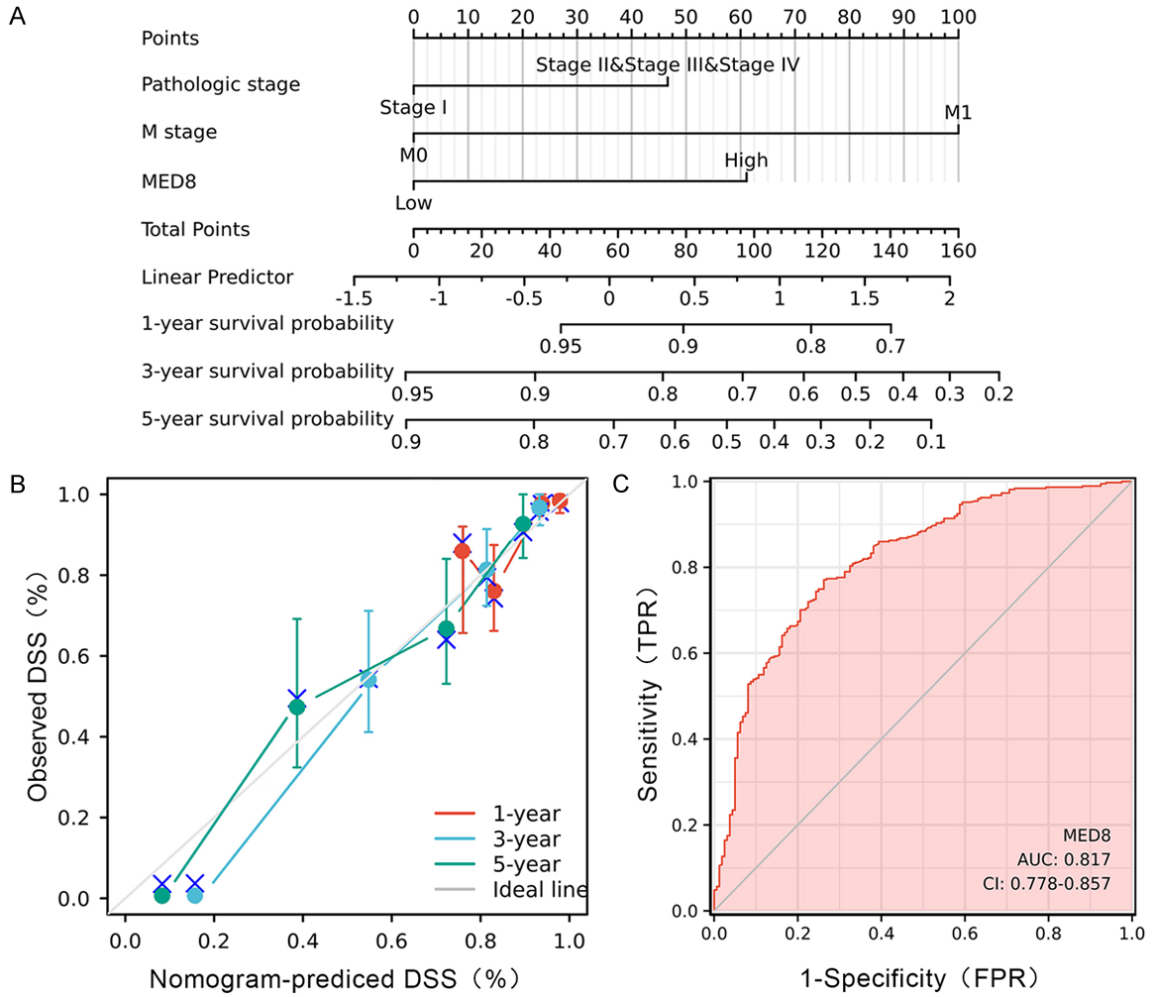

Figure 5. Relationship between the MED8 and other clinical factors. A. Nomogram for anticipating the OS rates over one, three, and five-year in patients with HCC. B. The calibration curve regarding the nomogram in the TCGA dataset. C. ROC was created by plotting the true positive rate against the false-positive rate at various threshold settings with the corresponding AUC labeled around the curve.

shown that MED8 is mutated in colon cancer [18]. Nevertheless, there has been no research into the correlation between MED8 expression and the progression of HCC or patient prognosis.

To characterize the role of MED8 in HCC, we analyzed data for 371 patients with HCC with complete clinical information from TCGA. In the case of comparing HCC tissues to normal samples, the expression levels of MED8 were shown to be elevated in the former. Moreover, high MED8 expression was closely correlated with pathologic parameters, such as the TP53 status, histologic grade, pathologic stage, and T stage, suggesting that the high expression of MED8 participates in the invasion as well as the metastasis of HCC. Similarly, patients with HCC in the group with highly expressed MED8 had a worse overall survival rate in contrast with those in the group with low MED8 expression, illustrating that MED8 might function as a novel diagnostic and prognostic biomarker for HCC.

To further explore the mechanism by which MED8 contributes to the development of HCC, we used data from TCGA for a GSEA. Based on this analysis, genes related to high MED8 expression are enriched for cell cycle checkpoints, mitotic G1-G1-S phases, and mitotic G2-G2-M phases, while genes correlated with low MED8 expression are enriched for p53 dependent G1-DNA damage response. As a

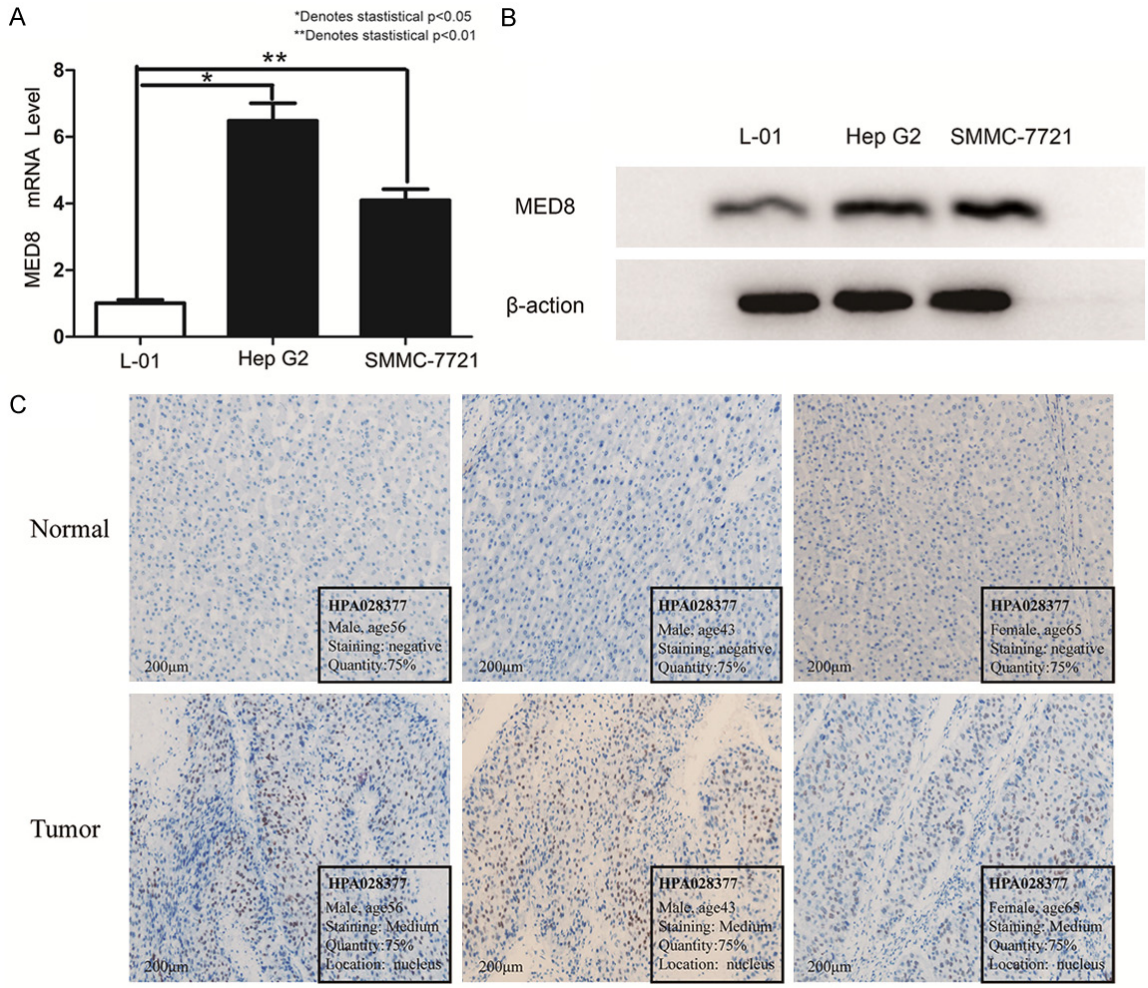

Figure 6. Expression analysis of MED8 in HCC. A. The findings from qRT-PCR of MED8 expression levels in L-01, SMMC-7721, and HepG2 cells. B. The results of western blot of MED8 expression levels in L-01, HepG2, and SMMC-7721 cells. C. Representative immunohistochemical landscapes and thorough information of MED8 in HCC tissues and corresponding normal tissues. All results are expressed as mean ± SD. Compared with control group, *P<0.05, **P<0.01.

tumor suppressor gene, p53 encoded by TP53 performs an integral function in cell cycle modulation by three pathways: 1) P53 can bind to p21 and activate its transcription, thereby inhibiting CDK activity, preventing cells from entering the S phase from the G1 phase, and making cells stop at G1 phase; 2) P53 can induce the synthesis of GADD45, thus inhibiting entry to the S phase; 3) Bax is induced and co-regulates apoptosis with Bcl-2 [19-21]. Our results indicated that genes correlated with high MED8 expression are significantly enriched in cell cycle regulation, while genes associated with low MED8 expression are highly expressed in p53 dependent G1-DNA damage. Therefore, we hypothesized that (1) low MED8 expression enhances the apoptosis of HCC cells by promoting the process of p53-dependent G1-DNA damage, but (2) high MED8 expression enables cells to pass through the S/M phase rapidly by cell cycle regulation, thus facilitating cell proliferation. The two effects clearly elucidate the positive function of MED8 expression in the occurrence and progression of HCC.

The expression level of MED8 is associated with immune cell infiltration, which is another highlight of the present research. The level of MED8 expression was found to be favorably associated with Th2 cells, aDCs, T helper cells, and TFH. It should be noted that MED8 showed a very strong positive correlation with the abundance of Th2 cells. The tumor microenvi-

ronment in the state of chronic inflammation makes infiltrated immune cells differentiate along the direction of tumor growth, invasion, and metastasis, accelerating tumor development and the process of immune escape [22]. Th2 cells are primarily involved in the production of cytokines such as IL-4, IL-5, IL-10, IL-13, and other cytokines that are implicated in humoral immunity. It is confirmed that Th2 cytokines can inhibit the differentiation of CD4+ T cells into T1 cells, weaken the antitumor immune response, and thus promote tumor development. Some studies have shown that the levels of T1 cytokines (IL-2, IL-12, tumor necrosis factor-lep, and interferon-c) in HCC show a decreasing trend, while the levels of Th2 cytokines show an increasing trend, consistent with our analysis results [23]. Th2 cytokines are associated with more aggressive and metastatic HCC phenotypes, and our analysis demonstrated that high MED8 expression is significantly associated with the histologic grade, pathologic stage, and T stage of patients with HCC. This suggests the following corollary: MED8 may regulate Th2 cytokine levels, thereby promoting the metastasis and invasion of HCC. Other studies have shown that adjuvant T/Th2 cells, immature dendritic cells, and macrophages are inversely linked to OS in cancer patients [24], which may effectively explain the poorer prognosis in patients with high MED8 expression.

Although the present research enhances our understanding of the association between MED8 and HCC, there were several drawbacks. First, our results were not confirmed by cytological experiments. Second, owing to the limitations of the database, the sample size included was not sufficiently large. Finally, our study did not comprehensively account for all clinical factors associated with HCC. In follow-up experiments, we will verify the functional mechanism by which MED8 promotes HCC by cellular and zoological experiments and perform more detailed stratification and subgroup analyses.

Conclusions

High MED8 expression anticipates unfavorable survival rates and is correlated with clinicopathological parameters in HCC. Furthermore, MED8 may perform a crucial function in cell cycle regulation and the Th2-mediated tumor immune microenvironment. Our results indicate that MED8 may be an effective biomarker for diagnosis and prognosis in HCC.

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Disclosure of conflict of interest

None.

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