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ORIGINAL RESEARCH

High Midkine Expression Correlates with Poor Prognosis and Immune Cell Infiltration in Hepatocellular Carcinoma

Lili Yan[1], Ji Lv[2], Meimei Xu[1], Hongyu Jia[1], Shanshan Li[1]

[1]Department of Gastroenterology, First Hospital of Qinhuangdao, Qinhuangdao, 066005, People's Republic of China; [2]Department of Breast Surgery, First Hospital of Qinhuangdao, Qinhuangdao, 066005, People's Republic of China

Correspondence: Meimei Xu, Department of Gastroenterology, First Hospital of Qinhuangdao, 258 Wenhua Road, Haigang District, Qinhuangdao, 066005, People's Republic of China, Email gdsd0513@126.com

Objective: This study investigated the role of MDK (Midkine) in hepatocellular carcinoma (HCC) through bioinformatics analysis and experimental validation, focusing on its relationship with tumor immune microenvironment and patient prognosis.

Methods: We employed the GEPIA database to analyze MDK expression patterns across cancer types and specifically in HCC versus normal tissues. MDK expression was validated through immunohistochemistry (IHC) in 100 paired HCC and adjacent tissue samples. Survival analyses were conducted using Kaplan-Meier and Cox regression methods. The relationship between MDK expression and immune cell infiltration was investigated using TIMER 2.0 database and verified through IHC staining of immune cell markers.

Results: MDK expression was significantly elevated in HCC tissues compared to adjacent normal tissues. High MDK expression strongly correlated with tumor number, vascular invasion, advanced clinical stage and poor prognosis, serving as an independent prognostic factor. Notably, elevated MDK expression predicted poor outcomes in patients receiving immunotherapy. Database analysis and IHC analysis revealed that MDK expression positively correlated with regulatory T (Treg) cell infiltration while negatively correlating with natural killer (NK) cell presence, suggesting its role in shaping the tumor immune microenvironment.

Conclusion: High MDK expression in HCC correlates with unfavorable patient outcomes and impacts immune cell infiltration. MDK may serve as a novel prognostic biomarker and potential therapeutic target in HCC treatment.

Keywords: hepatocellular carcinoma, MDK, prognosis, immune microenvironment, precision therapy

Introduction

Hepatocellular carcinoma (HCC), the predominant form of liver cancer, represents one of the most lethal malignancies worldwide, ranking second among cancer-related mortalities.[1] According to projections from the International Agency for Research on Cancer (IARC), an estimated 412.1 million new HCC cases and 293.8 million deaths are expected worldwide in 2023.[1] The disease burden is particularly pronounced in China, which accounts for approximately 55% of global liver cancer cases, highlighting a significant public health challenge. Contemporary therapeutic approaches, encompassing surgical interventions, targeted therapies, organ transplantation, ablative techniques, and chemoembolization, have yielded incremental improvements. However, long-term clinical outcomes remain suboptimal, as evidenced by a remarkably high 5-year recurrence rate exceeding 70%.[2]

Cancer progression is fundamentally influenced by the tumor microenvironment (TME).[3] This complex biological framework comprises neoplastic cells within a sophisticated network of blood vessels, immune components, stromal cells, and extracellular matrix, which collectively modulate tumor growth and metastasis.[4] The dynamic interactions within this ecosystem critically determine tumor evolution, immune evasion, and treatment resistance.[5] The TME harbors diverse immune cell populations, including T cells, natural killer (NK) cells, and regulatory T cells, alongside non-immune elements such as cancer-associated fibroblasts.[6] The delicate balance between anti-tumor and pro-tumor immune responses within this microenvironment critically influences HCC progression and treatment outcomes. HCC is characterized by an immunosuppressive microenvironment,

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marked by elevated regulatory T cell numbers, diminished NK cell function, and activated cancer-associated fibroblasts.[7–9] These conditions foster tumor growth and therapeutic resistance. Understanding the factors that orchestrate this complex microenvironment is essential for developing effective treatment strategies.

In recent decades, immune-checkpoint inhibitors (ICIs) have revolutionized cancer therapeutics.[10] The therapeutic success of ICIs derives from their capacity to sustain anti-tumor immune responses,[11] while offering a favorable safety profile, improved recurrence rates, and the potential to achieve complete remission in select advanced cases.[10] Nevertheless, HCC's inherent heterogeneity restricts the therapeutic efficacy of ICIs to a subset of patients.[12] Consequently, the identification of novel HCC-associated molecular markers and the development of predictive biomarkers for immunotherapy response have become imperative for enhancing patient stratification and therapeutic outcomes.

The Midkine (MDK) gene encodes a member of a distinct family of secreted growth factors, distinguished by its heparin-binding and retinoic acid interaction properties.[13] This multifunctional protein regulates cell proliferation, motility, and neovascularization, thereby contributing significantly to oncogenesis.[14] The diverse oncogenic functions of MDK have established it as a compelling therapeutic target across multiple malignancies.[15] Recent evidence has suggested high MDK expression in HCC, with significant correlations to malignant phenotype and prognosis.[16] Notably, MDK functions in remodeling the immune landscape in melanoma toward a tolerogenic state, suggesting its involvement in tumor immune evasion.[14] However, whether MDK expression affects the immune landscape in HCC has not yet been explored. To address this knowledge gap, we employed a bioinformatics approach to systematically analyze MDK expression patterns in HCC. Specifically, we first examined MDK expression levels and their association with patient survival. We then investigated MDK's impact on immunotherapy outcomes and its relationship with immune cell infiltration in the tumor microenvironment. Our findings demonstrate that high MDK expression in HCC correlates with unfavorable patient outcomes and impacts immune cell infiltration, suggesting that MDK may serve as a novel prognostic biomarker and potential therapeutic target in HCC treatment.

Materials and Methods

Analysis of MDK Gene Expression Across Cancer Types

MDK expression patterns were analyzed using the GEPIA database (gepia.cancer-pku.cn, version 1.0) with the following parameters: $P<0.05$ for statistical significance, log2 fold change >1.5 for differential expression analysis, and transcripts per million (TPM) normalization method for gene expression quantification. The analysis included data from both tumor samples and matched normal tissues across multiple cancer types.

Patient Cohort and Tissue Specimens

We retrospectively analyzed tumor and adjacent tissues from 100 hCC patients who underwent surgical resection between August 2019 and August 2020 at the First Hospital of Qinhuangdao. The study protocol was approved by the institutional Ethics Committee (2019078). Patient selection included those with primary HCC diagnosis confirmed by pathology, age 18–60 years, no prior cancer treatment, and complete clinical and follow-up data. Patients were excluded if they had a history of other malignancies, incomplete clinical records, or previous radio/chemo/immunotherapy. Collected tissues were stored in liquid nitrogen until further analysis.

RNA Extraction and Quantitative Real-Time PCR

Total RNA was extracted from fresh-frozen HCC tissues and matched adjacent non-cancerous tissues using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. RNA quality and quantity were assessed using NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). One microgram of total RNA was reverse transcribed to cDNA using the PrimeScript RT reagent Kit (Takara). Quantitative real-time PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems) on an ABI 7500 Real-Time PCR system. β-actin served as the internal control. The relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method, uing the β-actin gene as an internal reference. The primer sequences were as follows: MDK forward, 5'-CGCGGTCGCCAAAAAGAAAG-3'; MDK reverse, 5'-TACTTGCAGTCGGCTCCAAAC-3'; β-actin forward, 5'-CATGTACGTTGCTATCCAGGC-3'; β-actin reverse, 5'-CTCCTTAATGTCACGCACGAT-3'.

Immunohistochemistry Analysis

Tissue specimens were fixed in 10% formalin, embedded in paraffin, and sectioned at 5-μm thickness. For immunohistochemical staining, sections underwent deparaffinization in xylene, rehydration through graded alcohols, antigen retrieval in citrate buffer (pH 6.0, 95°C, 20 minutes), endogenous peroxidase blocking (3% H_2O_2, 10 minutes), and serum blocking (10% normal goat serum, 30 minutes). Primary antibodies including MDK (Abcam, Ab52637, 1:1000), Foxp3 (Abcam, ab20034, 1:1000), CD56 (Proteintech, 14255-1-AP, 1:2000), and α-SMA (Abcam, Ab5694, 1:1000) were applied and incubated overnight at 4°C, followed by secondary antibody (Abcam, Ab205718, 1:1000) incubation for 1 hour at room temperature. Visualization was achieved using SABC (Beijing Solepol, SA0025) and hematoxylin counterstaining. Staining quantification was performed using ImageJ software on five random fields (×200 magnification) per section.

Clinical Follow-Up and Data Collection

Patients were monitored through regular outpatient visits and telephone follow-ups for 40 months. Clinical parameters collected included demographic data, tumor characteristics, and survival outcomes (OS and PFS).

Survival Analysis

Patient survival analysis was performed using two independent platforms, GEPIA (Gene Expression Profiling Interactive Analysis) and KM Plotter (https://kmplot.com/analysis/), to evaluate the prognostic role of MDK expression in various cancers. In the GEPIA analysis, overall survival (OS) and disease-free survival (DFS) were analyzed using RNA-seq data from tumor and normal tissues. Survival curves were generated using the Kaplan-Meier method, with the Log rank test assessing statistical significance. Hazard ratios (HR) and 95% confidence intervals (CIs) were calculated to compare the relative risk of death or recurrence between high and low MDK expression groups, with patients stratified based on the median expression value. For the KM Plotter analysis, specifically focusing on hepatocellular carcinoma (HCC), patient groups were stratified by immunotherapy status (anti-CTLA4 and anti-PD-1). The impact of MDK expression on OS and progression-free survival (PFS) was assessed using the same median-based grouping method, and survival differences were evaluated using the Log rank test. Kaplan-Meier survival curves were plotted for both platforms, with statistical significance determined by a Log rank test ($p < 0.05$). Hazard ratios with 95% CIs were calculated to quantify the relationship between MDK expression and patient survival. All analyses were conducted using the default parameters of the respective platforms. These analyses aim to explore MDK's prognostic significance in cancer and its potential modulation of immunotherapy response, particularly in HCC.

Immune Cell Infiltration Analysis

The correlation between MDK (Midkine) expression and immune cell infiltration was assessed using TIMER 2.0, an online tool for comprehensive analysis of immune cell infiltrates in various cancer types (http://timer.cistrome.org/). TIMER utilizes a deconvolution-based algorithm to estimate the abundance of immune cells within the tumor microenvironment from gene expression data. The analysis was conducted to evaluate the association between MDK expression and the infiltration levels of several major immune cell populations, including regulatory T cells (Tregs), CD8+ T cells, CD4+ T cells, and natural killer (NK) cells, across multiple cancer types. The correlation between MDK expression and immune cell infiltration was calculated by determining the Spearman correlation coefficient, which quantifies the strength and direction of the relationship between these variables. The correlation analysis was adjusted for tumor purity to reduce the potential confounding effect of non-tumor cells on the immune cell infiltration estimates. The tumor purity-adjusted method was employed to ensure that the observed correlations are independent of the overall tumor cell content, which can influence immune cell detection and infiltration profiles. The results were visualized in a series of scatter plots and presented alongside the corresponding p-values to assess statistical significance.

Statistical Analysis

All statistical analyses were performed using SPSS 21.0 software. Categorical variables were analyzed using Chi-Square tests, while continuous variables, presented as mean ± standard deviation, were compared using *t*-tests. Correlations were assessed using Pearson correlation analysis. Survival analysis was performed using Kaplan-Meier method and Cox regression analysis. Statistical significance was defined as $P<0.05$.

Results

MDK Expression Patterns in Cancer and HCC

We initially characterized MDK expression patterns across cancer types and specifically in HCC tissues. Analysis of the GEPIA database revealed widespread MDK overexpression across multiple cancer types, including BLCA, BRCA, COAD, DLBC, ESCA, HNSC, KICH, KIRC, LAML, LGG, LIHC, LUSC, LUAD, PAAD, READ, SKCM, STAD, TCHT, THCA, UCEC, and UCS (Figure 1A) (all P<0.05). Notably, differential expression analysis demonstrated significantly elevated MDK levels in HCC tissues compared to adjacent non-cancerous tissues (Figure 1B). To validate these bioinformatic findings, we collected 100 pairs of HCC and adjacent non-cancerous tissue samples. qPCR analysis showed a significant increase in MDK mRNA expression in HCC tissues compared to adjacent normal tissues (Figure 1C). Subsequent immunohistochemical analysis confirmed the heightened MDK expression in HCC tissues (Figure 1D). These findings collectively suggest a potential oncogenic role of MDK in HCC pathogenesis.

MDK Expression Correlates with Clinical Features and Patient Outcomes

To investigate the clinical significance of MDK expression in HCC, we stratified 100 hCC patients into high (n=50) and low (n=50) MDK expression groups based on median value of immunohistochemical staining intensity. Clinical correlation analysis revealed that high MDK expression was significantly associated with tumor number, vascular invasion, and advanced clinical stage (P<0.05), while no significant correlations were observed with demographic and behavioral characteristics including age, gender, and drinking history (Table 1). Notably, Kaplan-Meier survival analysis demonstrated that patients with high MDK expression exhibited significantly worse overall survival compared to those with low expression (P<0.05, Figure 2). These findings suggest that MDK expression levels may serve as a potential prognostic indicator in HCC.

MDK Expression Is an Independent Prognostic Factor in HCC

To identify prognostic factors in HCC, we performed univariate analysis of clinicopathological parameters (Table 2). The analysis revealed that PS score, Child-Pugh score, tumor number, tumor size, vascular invasion, metastasis, clinical stage, and MDK expression were significantly associated with patient survival (all P<0.05). Subsequent multivariate Cox regression analysis identified PS score, clinical stage, and MDK expression as independent prognostic factors (P<0.05). Most notably, MDK expression demonstrated a hazard ratio of 2.690 (95% CI: 1.584–4.568), indicating that patients with high MDK expression faced a two-fold increased risk of mortality compared to those with low expression.

MDK Expression Associates with Immunotherapy Response

Patients from the TCGA-LIHC dataset were stratified into high and low MDK expression groups based on median mRNA expression levels. Kaplan-Meier analysis demonstrated that MDK expression levels significantly correlated with overall survival in HCC patients, with high MDK expression predicting inferior survival outcomes (P=0.041, Figure 3A). Given the emerging role of immunotherapy in HCC treatment, we further investigated the relationship between MDK expression and immunotherapeutic efficacy. Stratified survival analysis revealed that patients with high MDK expression exhibited significantly poorer responses to immunotherapy (Figure 3B). This adverse prognostic effect was consistently observed in subgroup analyses of patients receiving either anti-CTLA4 or anti-PD-1 treatment (Figures 3C and D).

MDK Expression Correlates with Immune Cell Infiltration Patterns

To elucidate the mechanism underlying MDK's influence on immunotherapy efficacy, we investigated its relationship with immune cell infiltration in HCC. Bioinformatic analysis using the TIMER2.0 database revealed significant correlations between MDK expression and various immune cell populations. Specifically, MDK expression showed positive correlation with Treg cell infiltration (correlation=0.237, P<0.001, Figure 4A), negative correlation with NK cell infiltration (correlation=−0.123, P<0.001, Figure 4B), and positive correlation with cancer-associated fibroblasts

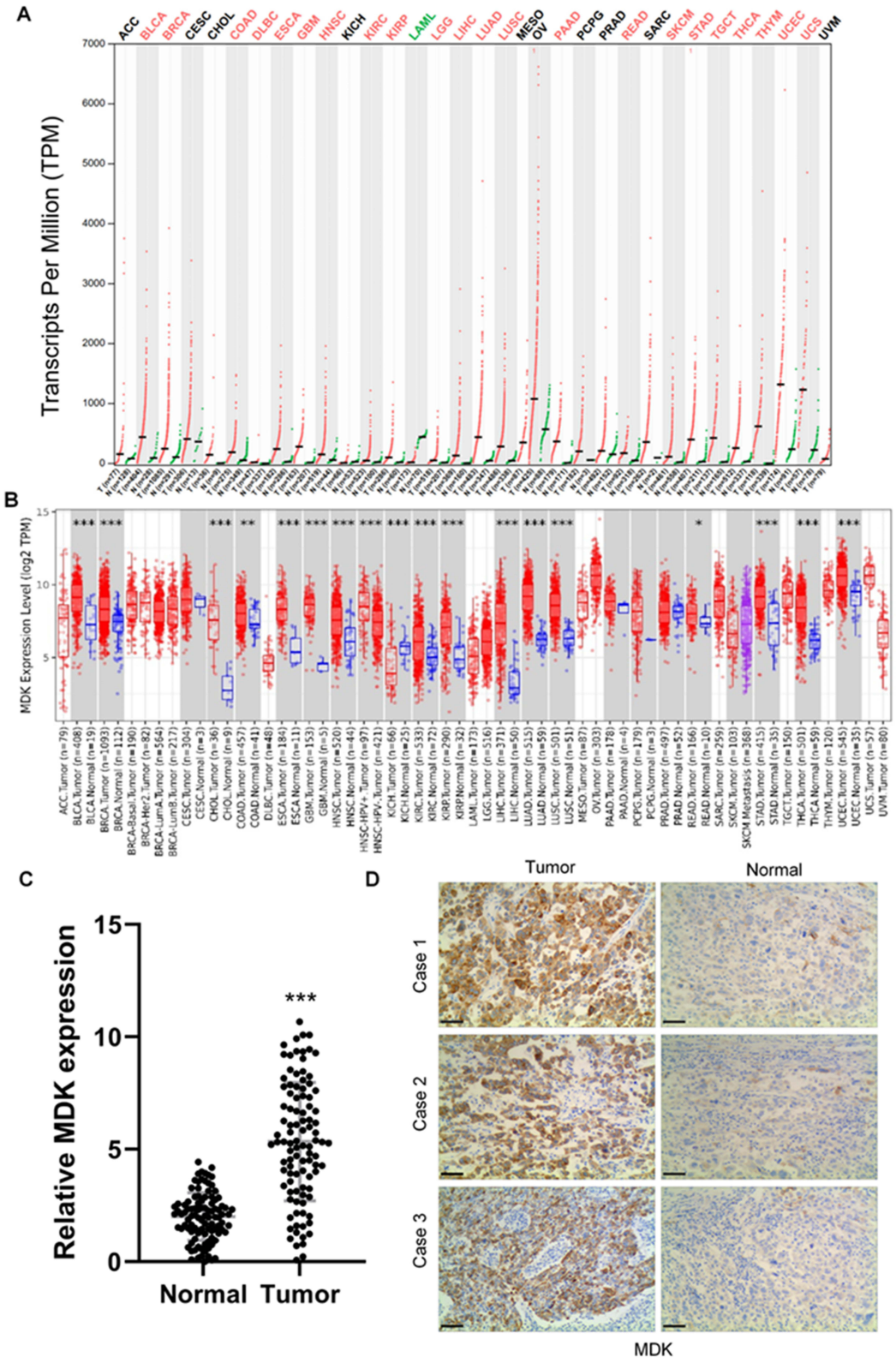

Figure 1 MDK expression analysis across cancer types and in HCC. (**A**) Pan-cancer analysis of MDK expression using GEPIA database. (**B**) Differential expression of MDK between HCC and adjacent normal tissues from GEPIA database. (**C**) qPCR analysis of MDK mRNA expression in 100 pairs of HCC and adjacent normal tissues. (**D**) Representative immunohistochemical staining showing MDK expression patterns in HCC and adjacent normal tissues. Scale bar = 100 μm. *P<0.05; **P<0.01; ***P<0.001.

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Table 1 Correlation Between the MDK Level and Clinicopathological Features in 100 LIHC Patients

Character	MDK Expression		χ^2	P value
	Low Expression	High Expression		
Age				
<60	16	13	0.437	0.509
≥60	34	37		
Gender				
Female	19	17	0.174	0.677
Male	31	33		
Drinking History				
No	18	20	0.17	0.68
Yes	32	30		
HBV infection				
No	24	17	2.026	0.155
Yes	26	33		
PS score				
0–2	43	36	2.954	0.086
3–4	7	14		
Child-Pugh score				
A-B	42	39	0.585	0.444
C	8	11		
Tumor size				
≤3cm	30	22	2.564	0.109
>3cm	20	28		
Tumor number				
≤4	34	24	4.105	0.043
>4	16	26		
Vascular invasion				
No	28	18	4.026	0.045
Yes	22	32		
Extrahepatic metastasis				
No	36	29	2.154	0.142
Yes	14	21		
Stage				
I–II	25	9	11.41	0.001
III–IV	25	41		

(correlation=0.123, P<0.01, Figure 4C). To validate these bioinformatic findings, we performed immunohistochemical analysis on HCC tissue samples. The results confirmed the positive correlation between MDK expression and Treg cell infiltration, as revealed by FoxP3 staining (P<0.05, Figure 5A). Furthermore, immunohistochemical analysis using CD56 as a marker for NK cells showed a negative correlation with MDK expression, while α-SMA staining for cancer-associated fibroblasts demonstrated a positive correlation with MDK expression (P<0.05, Figures 5B and C).

These findings demonstrate that MDK expression significantly influences the immune microenvironment in HCC through modulation of immune cell infiltration patterns. The correlation between MDK expression, tumor prognosis, and immunotherapy response may be attributed to these alterations in the immune landscape, suggesting that MDK could serve as a potential therapeutic target for improving immunotherapy outcomes in HCC.

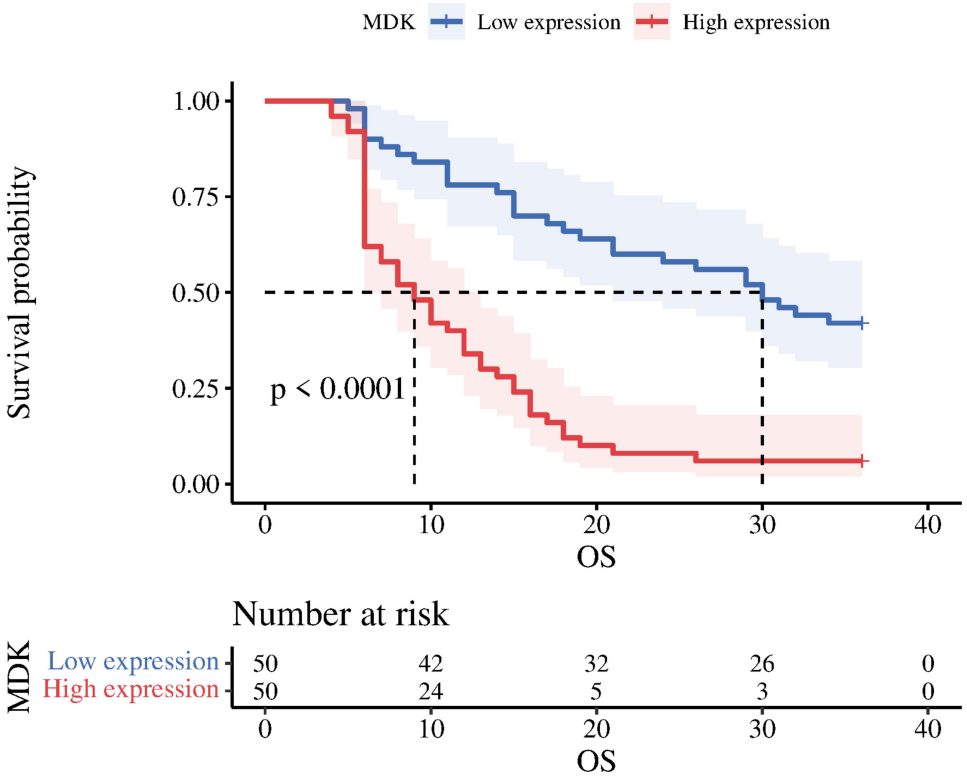

Figure 2 Kaplan-Meier survival analysis of HCC patients stratified by MDK expression levels. To investigate the clinical significance of MDK expression in HCC, we stratified 100 hCC patients into high (n=50) and low (n=50) MDK expression groups based on median value of immunohistochemical staining intensity. High MDK expression correlates with significantly worse overall survival compared to low MDK expression (P<0.0001).

Discussion

In this study, we characterized MDK expression in HCC and its clinical implications. We demonstrated that MDK is significantly upregulated in HCC tissues and its elevated expression strongly correlates with tumor number, vascular invasion, advanced clinical stage and poor patient survival. Notably, we identified MDK as an independent prognostic factor and revealed its predictive value for immunotherapy response. Through both bioinformatic and experimental approaches, we uncovered distinct associations between MDK expression and immune cell infiltration patterns in the tumor microenvironment. These findings not

Table 2 Univariable and Multivariable Cox Analysis of Clinicopathological Features

	Univariate Analysis			Multivariate Analysis		
	OR	95% CI	*P*	OR	95% CI	*P*
Age (>60 vs ≤60)	1.064	0.651–1.738	0.805			
Gender (Male vs Female)	1.414	0.879–2.274	0.153			
Smoking History (Yes vs No)	1.277	0.800–2.037	0.305			
HBV infection (Yes vs No)	1.035	0.656–1.633	0.882			
PS score (3–4 vs 0–2)	5.319	2.995–9.447	<0.001	2.488	1.232–5.024	0.011
Child-Pugh score (C vs A-B)	4.534	2.544–8.082	<0.001	1.482	0.755–2.912	0.253
Tumor number (>4 vs ≤4)	2.154	1.362–3.409	0.001	1.113	0.685–1.809	0.664
Tumor size (>3cm vs ≤3cm)	1.617	1.024–2.551	0.039	1.611	0.919–2.825	0.096
Vascular invasion (Yes vs No)	2.003	1.255–3.197	0.004	0.529	0.239–1.17	0.116
Extrahepatic metastasis (Yes vs No)	2.504	1.564–4.011	<0.001	1.647	0.9–3.014	0.106
Stage (III–IV vs I–II)	3.674	2.151–6.277	<0.001	2.944	0.998–8.681	0.050
MDK (High vs Low)	3.764	2.303–6.154	<0.001	2.690	1.584–4.568	<0.001

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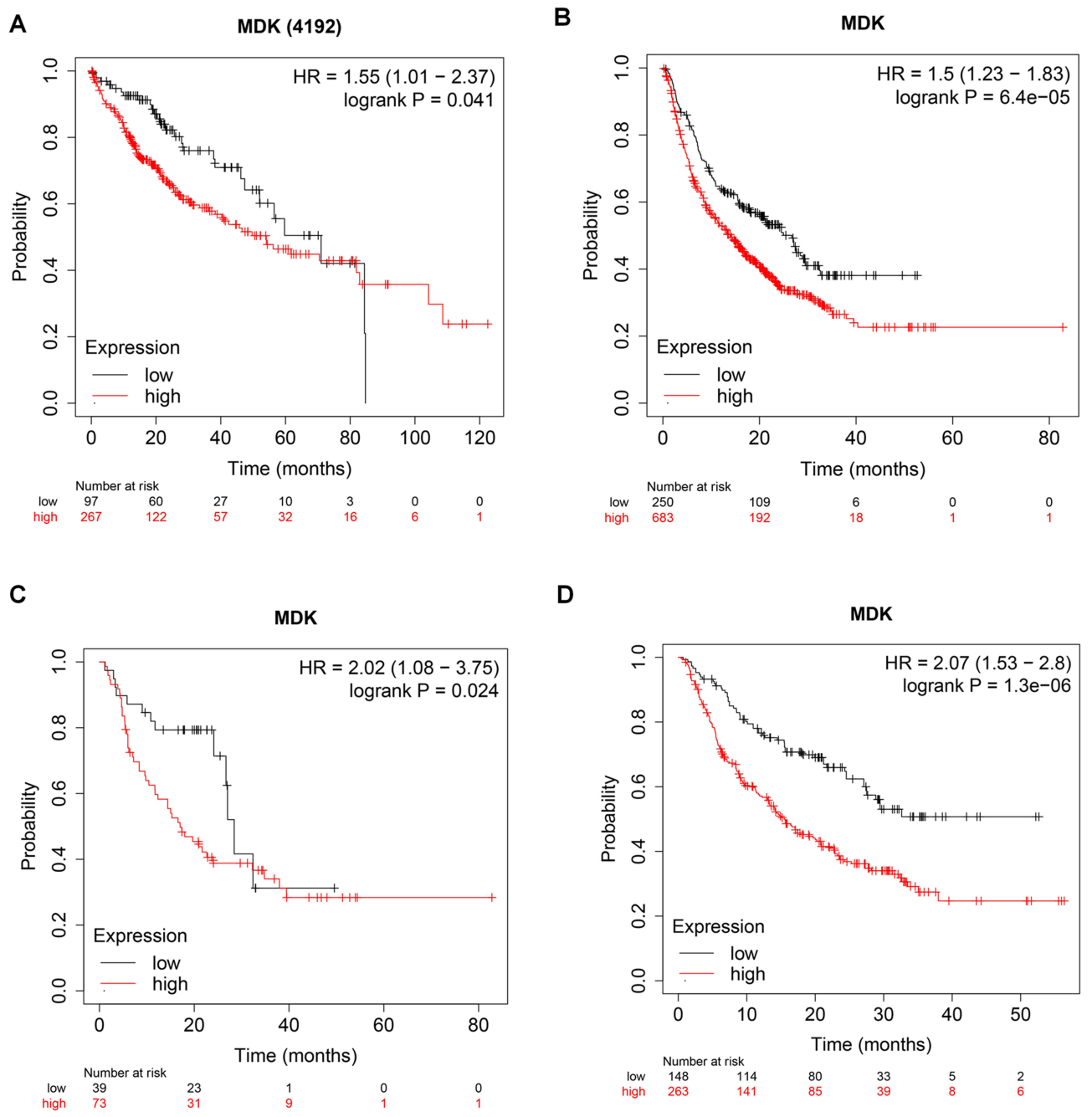

Figure 3 MDK expression predicts immunotherapy response in HCC patients. (**A**) Kaplan-Meier analysis showing correlation between MDK expression and overall survival (P=0.041) (Kaplan-Meier Plotter). (**B**) Impact of MDK expression on immunotherapy response in all treated patients (P<0.0001) (Kaplan-Meier Plotter). (**C**) Survival analysis in anti-CTLA4-treated subgroup stratified by MDK expression (P=0.024) (Kaplan-Meier Plotter). (**D**) Survival analysis in anti-PD-1-treated subgroup stratified by MDK expression (P<0.0001) (Kaplan-Meier Plotter).

only establish MDK as a crucial prognostic biomarker in HCC but also highlight its potential role in modulating immunotherapy outcomes, suggesting MDK as a promising therapeutic target for HCC treatment.

HCC represents a major global health burden, with increasing incidence and a dismal 5-year survival rate below 20%.[17] As the most common primary liver cancer, HCC is characterized by a complex tumor microenvironment,[18,19] featuring extensive stromal components and diverse immune cell populations that critically influence disease progression and clinical outcomes.[20] The distribution and composition of tumor-infiltrating immune cells, particularly the balance between CD163+ macrophages and CD8+ T cells, has emerged as a crucial determinant of patient prognosis.[18,21] This understanding has positioned the tumor

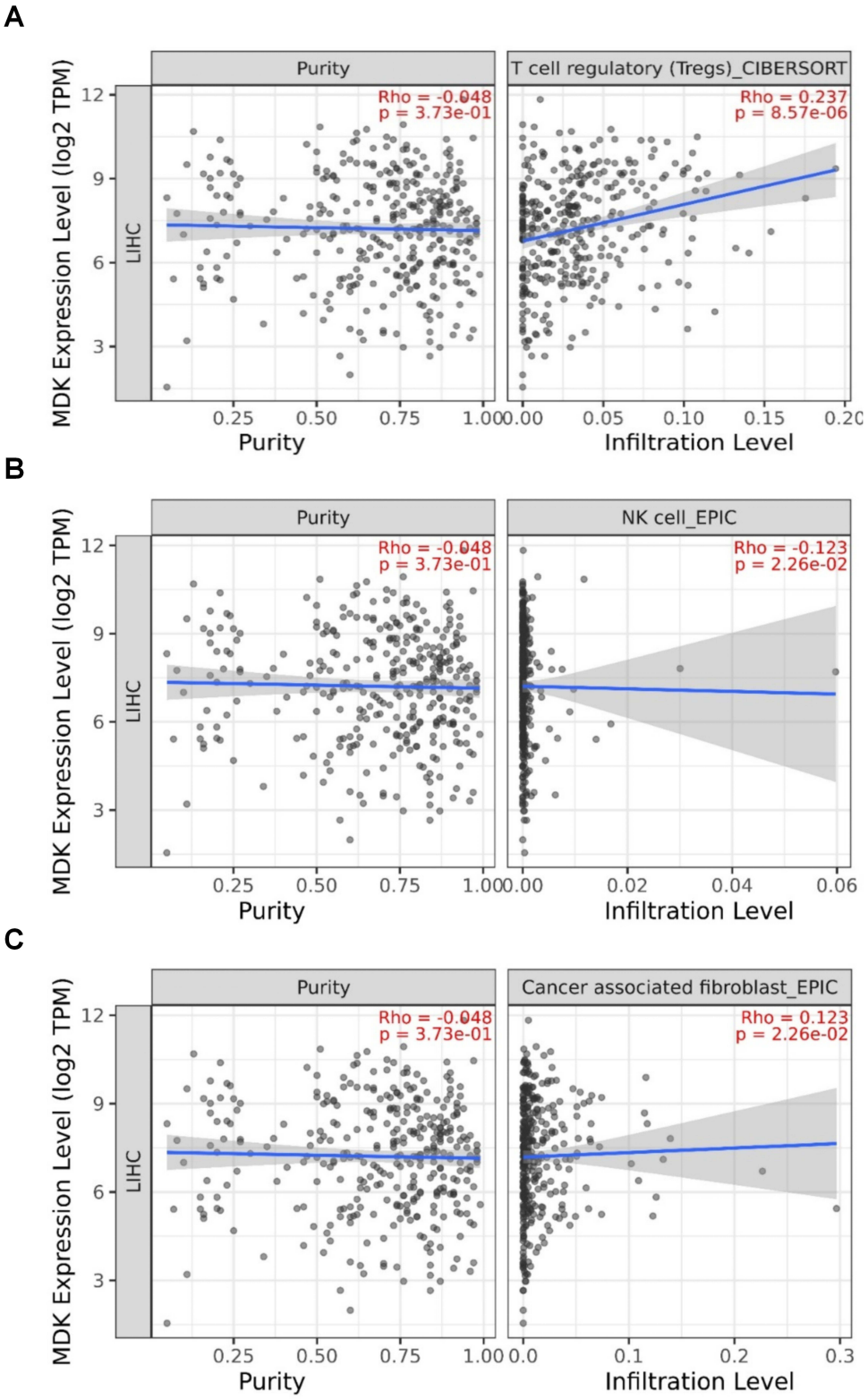

Figure 4 Correlation analysis of MDK expression with immune cell infiltration in HCC using TIMER2.0 database. (**A**) Positive correlation between MDK expression and Treg cell infiltration (correlation=0.237, P<0.001) (TIMER2.0). (**B**) Negative correlation between MDK expression and NK cell infiltration (correlation=−0.123, P<0.01) (TIMER2.0). (**C**) Positive correlation between MDK expression and cancer-associated fibroblasts (correlation=0.123, P<0.01) (TIMER2.0).

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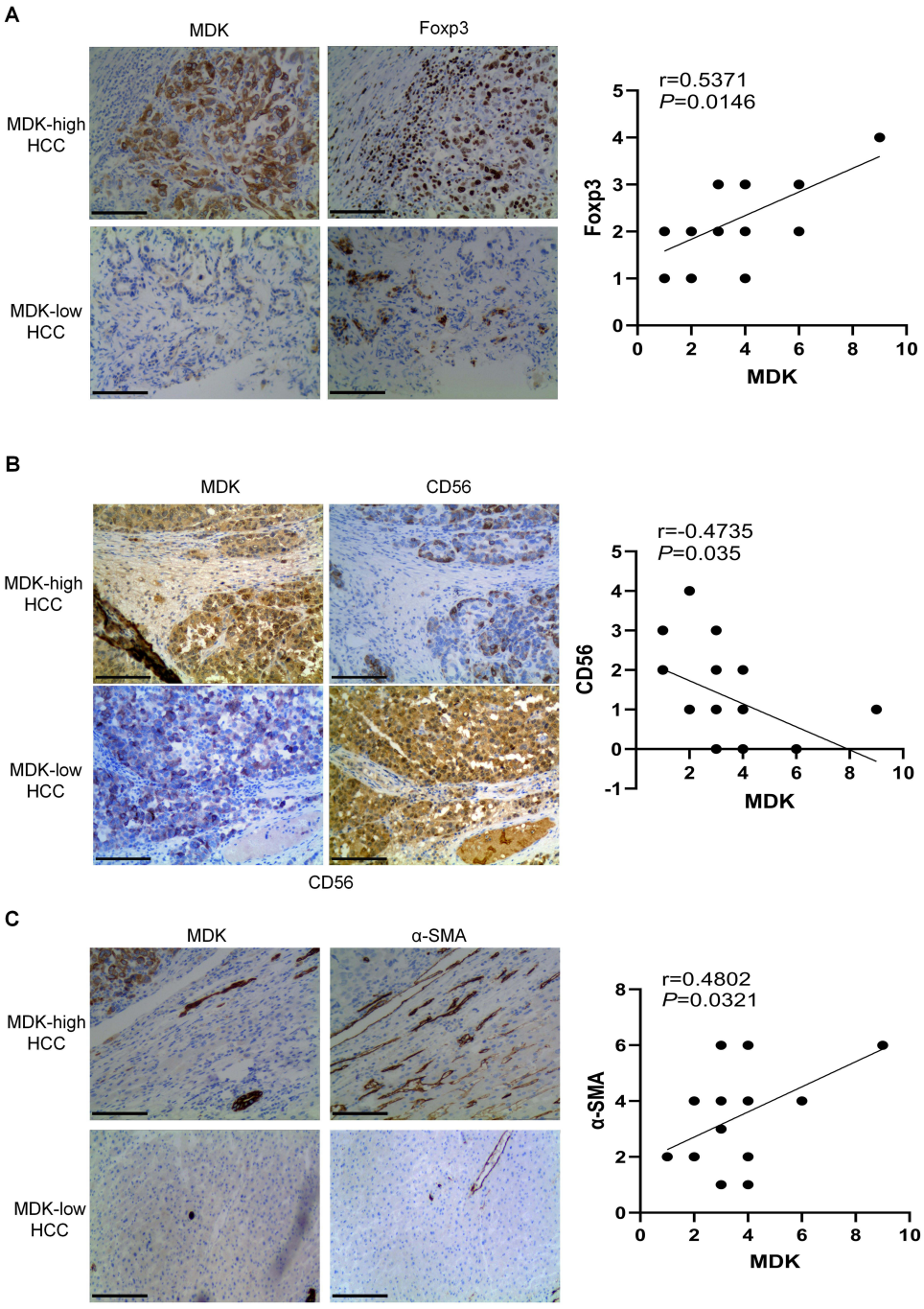

Figure 5 Immunohistochemical validation of MDK's association with immune cell populations in HCC tissues. (**A**) Correlation between MDK expression and Treg cell infiltration (FoxP3 staining, correlation=0.537, P=0.0146). (**B**) MDK expression correlation with NK cell infiltration (CD56 staining, correlation=−0.474, P=0.035). (**C**) MDK expression correlation with cancer-associated fibroblasts (α-SMA staining, correlation=0.480, P=0.032). n=12; Upper panel: MDK-high expression sample; Lower panel: MDK-low expression sample; Scale bar = 200 μm.

immune microenvironment as a key therapeutic target.[22] However, despite advances in immunotherapy, including immune checkpoint inhibitors designed to enhance anti-tumor immunity, response rates in HCC remain suboptimal compared to other cancer types.[23] Our findings regarding MDK's correlation with immune cell infiltration patterns and immunotherapy outcomes align with these observations and provide new insights into potential mechanisms of immunotherapy resistance. The identification of MDK as both a prognostic marker and a modulator of the immune microenvironment addresses the critical need for novel biomarkers and therapeutic targets in HCC.

MDK, initially identified as a heparin-binding growth factor during embryonic development, represents a unique member of the heparin-binding growth factor family along with PTN.[24] This versatile secreted protein is frequently upregulated in various pathological conditions, particularly in cancer, establishing its potential as a biomarker.[25] In cancer biology, MDK regulates multiple oncogenic processes, including cell proliferation, transformation, epithelial-mesenchymal transition (EMT), angiogenesis, mitosis, anti-apoptosis, and immune evasion.[26] A previous study has demonstrated elevated MDK mRNA and serum protein levels in HCC compared to non-tumor tissues.[27] Importantly, MDK overexpression has been linked to advanced tumor stage and poor clinical outcomes in HCC patients.[27] Of particular clinical relevance, MDK has shown enhanced diagnostic capability for alpha-fetoprotein (AFP)-negative HCC, suggesting its utility in early disease detection.[28] Our current findings not only validate MDK's overexpression in HCC and its correlation with advanced disease stage and poor survival but also extend these observations by revealing its relationship with immune cell infiltration patterns, further establishing MDK as a clinically significant prognostic biomarker.

The liver serves as a crucial immunological organ, hosting diverse immune cell populations essential for maintaining homeostasis and defense against pathogens.[29] Chronic liver injury from viral infections or toxins triggers complex modifications in the hepatic immune microenvironment,[30] leading to sustained inflammation, progressive fibrosis, and ultimately HCC development.[31] In the context of cirrhosis, dysregulated inflammatory responses create an immunocompromised state.[32] Our findings provide mechanistic insights into this immune dysregulation, demonstrating that MDK expression significantly correlates with increased infiltration of immunosuppressive Regulatory T cells and cancer-associated fibroblasts, while negatively impacting NK cell presence. This immune cell distribution pattern aligns with the established paradigm of compromised anti-tumor immunity in chronic liver disease, where enhanced immunosuppressive cell populations and diminished cytotoxic immune responses create a permissive environment for tumor progression. The correlation between MDK expression and these specific immune cell populations suggests its potential role in orchestrating the immunosuppressive microenvironment characteristic of HCC.

The tumor microenvironment has emerged as a critical determinant of immunotherapy efficacy, with tumor-infiltrating immune cells (TIICs) serving as key predictors of treatment response and patient survival.[33,34] As a pleiotropic protein, MDK exerts multifaceted effects on tumor progression through modulation of both tumor cells and immune populations.[15] Previous studies in melanoma have demonstrated that MDK activates NF-κB and interferon-related pathways, promoting immunosuppressive macrophage phenotypes and inhibiting CD8+ T cell function.[14] Our current findings extend these observations in HCC, revealing that MDK expression correlates with an immunosuppressive microenvironment characterized by increased Regulatory T cells and cancer-associated fibroblasts, while decreasing NK cell infiltration. This immune cell profile suggests that MDK may contribute to immunotherapy resistance through multiple mechanisms, including the promotion of immunosuppressive cell populations and the inhibition of cytotoxic immune responses.

While our study demonstrates significant correlations between MDK expression and immune cell populations in HCC, several limitations warrant consideration. The precise molecular mechanisms by which MDK modulates Regulatory T cells, NK cells, and cancer-associated fibroblasts remain to be fully elucidated. Future studies should investigate the direct signaling pathways through which MDK influences immune cell recruitment, differentiation, and function. Additionally, experimental validation using MDK knockout or overexpression models would help establish causality. Further investigation of MDK's interaction with specific immune checkpoint molecules and its impact on immunotherapy resistance mechanisms could provide valuable insights for developing targeted combination therapies. These mechanistic studies, combined with larger clinical cohorts, would strengthen MDK's potential as a therapeutic target in HCC.

In conclusion, we demonstrated that MDK overexpression correlates with poor prognosis in HCC and significantly influences the tumor immune microenvironment through modulation of Regulatory T cells, NK cells, and cancer-associated fibroblasts. These findings establish MDK as both a prognostic marker and potential therapeutic target. Future studies should focus on elucidating the molecular mechanisms underlying MDK's immunomodulatory effects to develop more effective therapeutic strategies for HCC.

Data Sharing Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of First Hospital of Qinhuangdao. All methods were performed in accordance with the Declarations of Helsinki. Written informed consents were signed by all participants before the study.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declared that there was no conflict of interest associated with the paper.

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