

# MMP1 is a Promiseful Prognostic Biomarker and Correlating with Immune Infiltrates in Hepatocellular Carcinoma

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## Research Article

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## Abstract

**Background:** Recombinase-aided amplification(RAA) is a new, simple, and ultrafast isothermal molecular diagnostic technique performed within 30min at 39°C–42°C. In this study, we evaluated the clinical performance of four duplex RAA kits for hepatitis B virus(HBV), human adenovirus 3(HAdV3), human adenovirus 7(HAdV7), and *Bordetella pertussis* and one duplex reverse-transcription RAA (RT-RAA) kit for respiratory syncytial virus (RSV).

**Methods:** A total of 392 sera and 374 respiratory tract samples were collected from five institutions in four China regions. Each RAA kit's sensitivity and specificity were compared with those of real-time quantitative polymerase chain reaction(qPCR),real-time quantitative reverse-transcription polymerase chain reaction(qRT-PCR), or sequencing.

**Results:** Compared with qPCR or qRT-PCR, the sensitivities of HBV RAA,RSV RT-RAA, and *B.pertussis* RAA were 97.55%,96.67%, and 100%,respectively, and all of the specificities were 100%.The total coincidence rates were 97.78%(383/392,95%CI:95.63%–98.85%),97.70%(212/217, 95%CI:94.57%–99.16%), and 100% (60/60,95%CI:92.80%–100%),respectively. The Kappa values were 0.977,0.947, and 1,respectively( $P<0.05$ ).Regarding the sequencing, the sensitivities of HAdV3 RAA and HAdV7 RAA were 100% and 97.37%, respectively, and all specificities were 100%.The total coincidence rates were 100% (97/97,95%CI:91.58%–100%) and 98.97%(96/97,95%CI:94.39%–99.82%),and the Kappa values were 1 and 0.978 ( $P<0.05$ ),respectively.

**Conclusions:** With comparable clinical performance, these RAA kits are suitable assays for rapidly detecting pathogens in resource-limited laboratories.

## Introduction

Hepatocellular carcinoma (HCC), remains the third leading cause of tumor-related death worldwide<sup>[1]</sup> and a significant health burden across the board. Chronic viral hepatitis B induced cirrhosis leading to HCC is the most common progress pattern in liver cancer. The top three risk factors for HCC are alcohol consumption, chronic viral hepatitis C and non-alcoholic fatty liver disease<sup>[2]</sup>. Although great strides have been made in advancing early diagnosis, surgical technology<sup>[3]</sup>, targeted treatment<sup>[4, 5]</sup> and immune therapy<sup>[6, 7]</sup>, high rate of recurrence and mortality remain a challenge due to most patients presenting with unresectable lesions or distant metastasis at time diagnosis, leading to a poor prognosis: a 5-year overall survival rate of only 10–18%<sup>[8–10]</sup>.

Matrix metalloproteinases (MMPs), a family of zinc-dependent endoproteases, is significantly related with extracellular matrix degradation by destroying diverse structure proteins, which plays a vital role in apoptosis, angiogenesis, and immune response<sup>[11–13]</sup> of the tumor microenvironment. Notably, MMP-2, alongside MMP-9, are well known as the most common progression markers correlated with various tumor invasion and metastasis, especially in HCC<sup>[14, 15]</sup>. Although MMP1 has been more commonly reported to be expressed in normal liver tissues<sup>[16]</sup>, it has also demonstrated an elevated capacity of invasion and migration in HCC by extracellular matrix (ECM) degradation in the epithelial-mesenchymal transition (EMT)<sup>[17]</sup>. MMP1 can be expressed in a wide range of cells including stromal fibroblasts, macrophages, endothelial cells and epithelial cells, having a low positive rate under normal conditions. However, it's expression can be elevated in malignant tumors with poor prognosis in various cancers (ovarian, liver, lung, gastric, colorectal, and prostate)<sup>[18–23]</sup>. While some studies have reported the interrelation between MMP1 and HCC, its distinct role in prognosis and the associated tumor-immunity are still unclear. Additionally, tumor infiltration immune cells (TIICs) and tumor-associated immune microenvironment are key areas of interest of current research<sup>[24, 25]</sup>. Immune cells may respond to the tumor progression and metastasis through a multitude of pathways and interactions in HCC. The suppression of HCC immune microenvironment facilitates immune tolerance and escape by variant mechanisms<sup>[26]</sup>. MMPs play an important role in bladder cancer metastasis promotion through the B cell induced signaling pathway<sup>[27]</sup> and their upregulation by tumor-associated macrophages contributes to tumor infiltration and metastasis in various carcinomas<sup>[28–30]</sup>, indicating their potential functions in tumor-immunity microenvironment. However, what and how MMP1 influences the immune cells and tumor-associated microenvironment still needs exploration.

This research was carried out to investigate the prognostic potential of MMP1 and its relationship with TIICs' biomarkers in HCC. Several online bio-information analysis tools of public databases were used to examine the MMP1 expression levels (Oncomine and Tumor Immune Estimation Resource (TIMER) databases) and assess its prognostic potential (Kaplan-Meier plotter and Gene Expression Profiling Interactive Analysis (GEPIA) databases), along with the correlation between MMP1 and TIIC biomarkers (TIMER). We demonstrated that MMP1 may be a major independent predictor of prognosis of HCC and revealed the latent interrelationship and mechanism between MMP1 and TIIC biomarkers in patients with HCC.

## Materials And Methods

### Oncomine Database Based Analysis

Oncomine (<https://www.oncomine.org>) is currently the world's largest database of oncogene chip and features an integrated data mining platform, designed to mine cancer gene information. Oncomine, which integrates RNA and DNA-seq data from GEO, TCGA and published literature sources, has the most comprehensive profile of cancer mutations, gene expression data and related clinical information that can be used to identify new biomarkers or therapeutic targets<sup>[31]</sup>. In our study, the MMP1 gene was selected and its expression levels were analyzed in tumor and normal tissues using the Oncomine database. The difference in results was considered statistically significant for p-value < 0.05, with fold change > 1.5. The threshold value of gene rank was set to "top 10%" and data type to "all".

### Kaplan-Meier Plotter Database Analysis

The Kaplan-Meier Plotter database (<http://kmplot.com/analysis>) was constructed based on gene microarray and RNA-seq data from GEO, EGA, and TCGA public databases, and assessed the effect of 54, 675 genes on survival rate in 21 cancer types<sup>[32]</sup>. In order to investigate the relationship between MMP1

expression and the prognosis of patients with liver, breast, ovarian, lung and gastric cancers, Kaplan-Meier Plotter database was utilized to do the Cox regression analysis and hazard ratios (HRs) and p-values from a log-rank test. The parameters were as follow: Affy id/Gene symbol: MMP1; patients split by auto-select best cutoff; HR: yes; 95% confidence interval (CI): yes; with the remaining settings set to their default values.

## GEPIA Database Analysis

GEPIA database (<http://gepia.cancer-pku.cn/index.html>), a public web server for tumor and normal gene expression profiling and interactive analysis based on the data from Genotype-Tissue Expression (GTEx) and TCGA database<sup>[33]</sup>, was utilized in this study to analyze the association between MMP1 expression and patient prognostic potential in a variety of cancers. As a complement to the analysis by the Kaplan-Meier Plotter database, we charted survival curves for overall survival (OS) and disease-free survival (DFS) via GEPIA using the log-rank and Mantel-Cox tests. The Spearman's rank correlation coefficient was calculated with default parameters.

## TIMER Database Analysis

The TIMER 2.0 database (<http://timer.comp-genomics.org>), incorporating 10,009 samples across 23 cancer types from TCGA, is a comprehensive web resource for the systematical evaluation of the clinical relevance of tumor-immune infiltrates and differential gene correlation analysis<sup>[34]</sup>. We selected MMP1 under the gene expression module with the default parameters to obtain heatmaps visualizing the correlation of its expression with different immune infiltrate levels in different cancer types by the purity-adjusted spearman's test. Of the various TIICs, B cells, CD4 + T cells, CD8 + T cells, macrophages, neutrophils and dendritic cells<sup>[35, 36]</sup> were selected for investigation in this study to assess the relationship between MMP1 and tumor-immunity in HCC based on insight from previous studies. Furthermore, correlation analysis was conducted to investigate the relationship between MMP1 expression and different biomarker genes of TIICs as reported in other papers<sup>[37, 38]</sup>, including B cells, CD8 + T cells, Monocytes, tumor-associated macrophages (TAMs), M1 and M2 macrophages, neutrophils, dendritic cells, Tregs and exhausted T cells. Result delineation was attained through Spearman's rho and p-value adjusted by purity.

## Sangerbox database analysis

Sangerbox database (<http://www.sangerbox.com>) is a tool to facilitate a variety of biological information analysis, visualization mapping and convenient data download functions, based on TCGA and GEO databases<sup>[39]</sup>. We investigated the correlation between MMP1 expression levels and the tumor mutational burden (TMB) and microsatellite instability (MSI) in pan-carcinomas using pearson's method.

## Statistical analysis

All statistical analysis and graphing was performed with R (version 4.0.3) and STATA software (College Station, TX: Stata Corp LP, USA). Survival curves were created by the GEPIA and Kaplan-Meier plots database based on R. Forest plots were generated by meta-analysis in metan module of STATA. The relationship between MMP1 expression and TMB and MSI in pan-cancers was evaluated by Pearson's correlation, while the spearman's correlation was used to assess the relationship between MMP1 expression and TIICs, as well as biomarkers. A *p* value < 0.05 was considered statistically significant. The correlations were defined as follows: 0.00–0.19 (none), 0.20–0.39 (low), 0.40–0.59 (moderate), 0.60–0.79 (high), ≥ 0.80 (very high)<sup>[40]</sup>.

## Results

### MMP1 expression in HCC and other carcinomas

MMP1 expression inhibition would ultimately contribute to prevention of HCC progression, according to a previous study<sup>[41]</sup>. In order to confirm the carcinogenesis of MMP1 in HCC, we utilized the Oncomine database to evaluate the levels of MMP1 expression in both tumor and normal tissues of various cancers. Except kidney cancer as well as brain and central nervous system cancer which have a reduced expression of MMP1, together with leukemia, liver cancer and myeloma with censored data, the expression levels of MMP1 gene were upregulated significantly in other types of tumor tissues compared to their respective adjacent normal tissues; including bladder, breast, cervical, colorectal, esophageal, gastric, head and neck, lung, lymphoma, melanoma, ovarian, pancreatic, prostate, and sarcoma cancer tissues (Fig. 1A). Although no discernible relationship between MMP1 expression and HCC was observed using Oncomine, further assessment of MMP1 RNA-seq expression in HCC using TIMER 2.0 based on the TCGA database, indicated that a high expression of MMP1 in 22 types of cancers was detected, including HCC, *p*-value < 0.001 (Fig. 1B). These results strongly suggested that the overexpression of MMP1 might play a positive role in the progression of different neoplasms, especially in HCC.

### Evaluation of MMP1 expression and HCC prognosis

The Kaplan-Meier plotter and GEPIA database were used to evaluate the prognostic potential of MMP1 transcriptional levels in HCC tissues. Interestingly, we found that higher expression of MMP1 was associated with poorer prognosis in patients with HCC (Fig. 2A, disease-specific survival (DSS): HR = 2.41, 95% CI = 1.55–3.76, *p* = 6.1e-05, n = 357; Fig. 2B, overall survival (OS): HR = 2.1, 95% CI = 1.49–2.98, *p* = 1.7e-05, n = 364; Fig. 2C, progression free survival (PFS): HR = 1.96, 95% CI = 1.46–2.63, *p* = 5.9e-06, n = 366; Fig. 2D, relapse free survival (RFS): HR = 1.79, 95% CI = 1.27–2.5, *p* = 0.00065, n = 313). Additionally, we got consistent results with statistical significance in the GEPIA database (Fig. 2E, disease free survival (DFS): HR = 1.4, *p* = 0.018; Fig. 2F, OS: HR = 2, *p* = 0.00023).

Next, we investigated the relationship between the levels of MMP1 expression and the related prognostic in other cancers through the Kaplan-Meier plotter analysis. Similarly, the survival curves showed that high expression of MMP1 in tumor tissues was relevant to poor prognosis in different cancers. For example, poor OS, RFS, post progression survival (PPS) and distant metastasis-free survival (DMFS) in breast cancer were observed to be highly correlated with strong expression of MMP1 (Fig. 3A, OS: HR = 1.54, 95% CI = 1.27–1.86, *p* = 7.6e-06; PPS: HR = 1.37, 95% CI = 1.08–1.73, *p* = 0.0084; RFS: HR = 1.7, 95% CI = 1.54–1.88, *p* < 1e-16; DMFS: HR = 1.67, 95% CI = 1.43–1.95, *p* = 1.1e-10). On the other hand, only poor OS in gastric cancer (Fig. 3B, HR = 0.75, 95% CI = 0.63–

0.9,  $p = 0.0021$ ), ovarian cancer (Fig. 3C, HR = 1.15, 95% CI = 1.01–1.31,  $p = 0.04$ ) and lung cancer (Fig. 3D, HR = 1.16, 95% CI = 1–1.35,  $p = 0.047$ ) respectively, was related to high expression of MMP1.

As a supplement, we assessed the relationship between MMP1 transcriptional level and the other tumor tissues via GEPIA database. The high expression of MMP1 was associated with poor OS in head and neck squamous cell carcinoma (HNSC), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), pancreatic adenocarcinoma (PAAD) (Fig. 4B, E, F), poor DFS in glioblastoma multiforme (GBM) (Fig. 4A), and both in kidney chromophobe (KICH) and kidney renal papillary cell carcinoma (KIRP) (Fig. 4C, D).

## Correlation analysis between MMP1 expression and clinical characteristics of patients with HCC

Furthermore, we evaluated the correlation between MMP1 expression levels and clinicopathological factors in HCC using STATA based on the data of Kaplan-Meier plotter database (Table.1). The characteristics were classified into several subgroups based on stage, grade, ACJJ-T, gender, vascular invasion, race, alcohol consumption, and hepatitis virus. Almost all were independent risk factors for the prognosis of HCC patients with high expression of MMP1 (Fig. 5A-D). In particular, the higher expression of MMP1 was closely associated with worse OS, PFS, RFS and DSS rates in relation to the following factors: grades II (OS: HR = 1.98, 95% CI = 1.18–3.33,  $p = 0.0082$ ; PFS: HR = 2.57, 95% CI = 1.66–3.98,  $p = 1.2\text{e-}05$ ; RFS: HR = 2.39, 95% CI = 1.4–4.06,  $p = 9.3\text{e-}04$ ; DSS: HR = 3.14, 95% CI = 1.54–6.39,  $p = 8.9\text{e-}04$ ), male (OS: HR = 2.9, 95% CI = 1.86–4.52,  $p = 8.3\text{e-}07$ ; PFS: HR = 1.86, 95% CI = 1.29–2.67,  $p = 6.8\text{e-}04$ ; RFS: HR = 1.75, 95% CI = 1.17–2.61,  $p = 0.0057$ ; DSS: HR = 3.4, 95% CI = 1.92–6.02,  $p = 7.9\text{e-}06$ ), Asian (OS: HR = 3.88, 95% CI = 2.14–7.06,  $p = 1.7\text{e-}06$ ; PFS: HR = 2.28, 95% CI = 1.41–3.68,  $p = 5.4\text{e-}04$ ; RFS: HR = 2.11, 95% CI = 1.26–3.52,  $p = 0.0038$ ; DSS: HR = 4.27, 95% CI = 1.93–9.46,  $p = 1\text{e-}04$ ), non-vascular invasion (OS: HR = 2.09, 95% CI = 1.24–3.55,  $p = 0.005$ ; PFS: HR = 1.77, 95% CI = 1.13–2.77,  $p = 0.011$ ; RFS: HR = 1.84, 95% CI = 1.08–3.15,  $p = 0.023$ ; DSS: HR = 2.16, 95% CI = 1.04–4.5,  $p = 0.035$ ), non-alcoholics (OS: HR = 2.59, 95% CI = 1.47–4.58,  $p = 6.7\text{e-}04$ ; PFS: HR = 1.93, 95% CI = 1.28–2.9,  $p = 0.0013$ ; RFS: HR = 1.91, 95% CI = 1.14–3.21,  $p = 0.013$ ; DSS: HR = 4.1, 95% CI = 1.72–9.78,  $p = 5.8\text{e-}04$ ), patients with hepatitis virus infection (OS: HR = 3.33, 95% CI = 1.73–6.39,  $p = 1.3\text{e-}04$ ; PFS: HR = 2.02, 95% CI = 1.27–3.22,  $p = 0.0026$ ; RFS: HR = 1.75, 95% CI = 1.06–2.87,  $p = 0.026$ ; DSS: HR = 3.54, 95% CI = 1.55–8.08,  $p = 0.0014$ ) and those without it (OS: HR = 1.76, 95% CI = 1.1–2.8,  $p = 0.017$ ; PFS: HR = 2.19, 95% CI = 1.41–3.39,  $p = 3.3\text{e-}04$ ; RFS: HR = 1.99, 95% CI = 1.2–3.31,  $p = 0.0066$ ; DSS: HR = 2.41, 95% CI = 1.31–4.44,  $p = 0.0037$ ). These findings indicated that high expression of MMP1 might be one of the contributing factors to poor prognosis in HCC patients with different clinical characteristics.

## MMP1 expression levels and TIIICs in HCC

Tumor infiltrating immunocytes have been shown to influence the progression of patients with different cancers. Thus, it's necessary for us to investigate any relationship between MMP1 expression and TIIICs in HCC via TIMER database. Consistent with previous survival results, a high expression level of MMP1 was positively related with a number of TIIICs and notable outcomes (Fig. 6). It was associated with high infiltration levels of B cells ( $\text{Rho} = 0.268, p = 4.31\text{e-}07$ ), dendritic cells ( $\text{Rho} = 0.432, p = 4.01\text{e-}17$ ), macrophages ( $\text{Rho} = 0.263, p = 7.39\text{e-}07$ ), neutrophils ( $\text{Rho} = 0.268, p = 4.52\text{e-}07$ ), CD4 + T cells ( $\text{Rho} = 0.116, p = 3.07\text{e-}02$ ), and CD8 + T cells ( $\text{Rho} = 0.108, p = 4.57\text{e-}02$ ) (Fig. 6). On the contrary, MMP1 expression was not correlated with tumor purity ( $\text{Rho} = 0.056, p = 3.00\text{e-}01$ ), indicating that perhaps it originates from the cells in the tumor-immunity microenvironment.

## Gene analysis for MMP1 and Immune cell subset biomarkers

Besides verifying the positive correlation between MMP1 expression and several TIIICs, we proceeded to test the correlation of MMP1 expression with levels of specific biomarker genes in the select TIIICs or their subsets in HCC tissues via TIMER database and explore MMP1-involved carcinogenic mechanisms and filtrate promising therapeutic targets in the future. In detail, the correlation analysis was conducted with/without adjustment by purity, indicating significantly positive correlation of co-expression of MMP1 and biomarker genes of TIIICs or subsets of them (Fig. 7). What we found was that almost all the biomarker genes we investigated were positively related to MMP1 expression, including CD19 (B cell biomarker,  $R = 0.147, p = 6.35\text{e-}03$ ), CD79A (B cell biomarker,  $R = 0.155, p = 3.8\text{e-}03$ ) (Fig. 7A), CD8A (CD8 + T cell biomarker,  $R = 0.209, p = 9.55\text{e-}05$ ), CD8B (CD8 + T cell biomarker,  $R = 0.239, p = 7.09\text{e-}06$ ) (Fig. 7B), CD86 (Monocyte biomarker,  $R = 0.377, p = 4.03\text{e-}13$ ), CSF1R (Monocyte biomarker,  $R = 0.29, p = 4.06\text{e-}08$ ) (Fig. 7C), IRF5 (M1 Macrophage biomarker,  $R = 0.168, p = 1.69\text{e-}03$ ), PTGS2 (M1 Macrophage biomarker,  $R = 0.201, p = 1.65\text{e-}04$ ) (Fig. 7D), CD163 (M2 Macrophage biomarker,  $R = 0.158, p = 3.18\text{e-}03$ ), VSIG4 (M2 Macrophage biomarker,  $R = 0.173, p = 1.25\text{e-}03$ ), MS4A4A (M2 Macrophage biomarker,  $R = 0.196, p = 2.51\text{e-}04$ ) (Fig. 7E), ITGAM (Neutrophils biomarker,  $R = 0.364, p = 2.84\text{e-}12$ ) (Fig. 7F), CCL2 (tumor-associated macrophage (TAM) biomarker,  $R = 0.189, p = 4.15\text{e-}04$ ), CD68 (TAM biomarker,  $R = 0.325, p = 6.6\text{e-}10$ ), IL10 (TAM biomarker,  $R = 0.326, p = 5.7\text{e-}10$ ) (Fig. 7H), HLA-DPB1 (Dendritic cell biomarker,  $R = 0.227, p = 2.11\text{e-}05$ ), HLA-DQB1 (Dendritic cell biomarker,  $R = 0.237, p = 8.36\text{e-}06$ ), HLA-DRA (Dendritic cell biomarker,  $R = 0.251, p = 2.37\text{e-}06$ ), HLA-DPA1 (Dendritic cell biomarker,  $R = 0.219, p = 4.02\text{e-}05$ ), CD1c (Dendritic cell biomarker,  $R = 0.12, p = 2.57\text{e-}02$ ), NRP1 (Dendritic cell biomarker,  $R = 0.218, p = 4.29\text{e-}05$ ), and ITGAX (Dendritic cell biomarker,  $R = 0.419, p = 4.38\text{e-}16$ ) (Fig. 7I), as well as the biomarker genes of subsets of T cells, such as FOXP3 (Tregs biomarker,  $R = 0.172, p = 1.33\text{e-}03$ ), CCR8 (Tregs biomarker,  $R = 0.311, p = 3.53\text{e-}09$ ), TGFB1 (Tregs biomarker,  $R = 0.277, p = 1.67\text{e-}07$ ), IL2RA (Tregs biomarker,  $R = 0.356, p = 1.02\text{e-}11$ ), CD4 (Tregs biomarker,  $R = 0.247, p = 3.34\text{e-}06$ ) (Fig. 7G), PDCD-1 (T cell exhaustion biomarker,  $R = 0.268, p = 4.55\text{e-}07$ ), CTLA4 (T cell exhaustion biomarker,  $R = 0.386, p = 1.02\text{e-}13$ ), LAG3 (T cell exhaustion biomarker,  $R = 0.2, p = 1.86\text{e-}04$ ), HAVCR2 (T cell exhaustion biomarker,  $R = 0.436, p = 1.8\text{e-}17$ ), BTLA (T cell exhaustion biomarker,  $R = 0.121, p = 2.49\text{e-}02$ ), TIGIT (T cell exhaustion biomarker,  $R = 0.348, p = 3.14\text{e-}11$ ), and GZMB (T cell exhaustion biomarker,  $R = 0.206, p = 1.12\text{e-}04$ ) (Fig. 7J).

## Expression levels of MMP1 related to TMB and MSI in HCC

Studies have increasingly reported that TMB<sup>[42]</sup> and MSI<sup>[43]</sup> could be used as predictive biomarkers for cancer immunotherapy, which might be the one of most popular methods to predict the therapeutic efficiency of immunotherapy on carcinomas. Therefore, we investigated the correlation between MMP1 expression and TMB/MSI in 32 types cancers via SangerBox. Contrary to what would be expected, there was no significant correlation between MMP1 expression and

TMB/MSI in HCC patients. It was only positively related to TMB in lung adenocarcinoma (LUAD) ( $p=0.0024$ ), prostate adenocarcinoma (PRAD) ( $p=0.046$ ), sarcoma (SARC) ( $p=0.013$ ), breast invasive carcinoma (BRCA) ( $p=7.3e-05$ ), colon adenocarcinoma (COAD) ( $p=0.0017$ ) (Fig. 8A) and MSI in testicular germ cell tumors (TGCT) ( $p=0.00095$ ), kidney renal clear cell carcinoma (KIRC) ( $p=6e-04$ ), and COAD ( $p=3.2d-07$ ) (Fig. 8B). In addition, negative correlation was only observed between MMP1 expression and TMB in head and neck squamous cell carcinoma (HNSC) ( $p=-0.0079$ ) (Fig. 8A) and MSI in pancreatic adenocarcinoma (PAAD) ( $p=-0.023$ ). This might indicate that HCC patients with neither high nor low expression of MMP1 could equally benefit from TMB/MSI targeted immunotherapy.

## Discussion

Although the incidence and mortality of HCC have decreased in South-Eastern Asia due to hepatitis vaccination progress, it is still one of the leading causes of cancer related deaths world wide<sup>[1]</sup>. Lack of efficient prognostic factors leads to delayed diagnosis and intervention, which in turn considerably contribute to poor HCC patient survival. In this study, we demonstrated that MMP1 expression was elevated in HCC and was significantly correlated with worse prognosis utilizing a bioinformatic analysis method based on public databases resources. The MMP1 expression had a positive relationship with differential TIIICs and various immune-related gene biomarkers in HCC. All the findings suggested an underlying mechanism of MMP1 expression in remodulating the tumor-immunity microenvironment and immune escape. To our knowledge, our study is the first one to comprehensively reveal the prognostic value of MMP1 and its relationship with immune infiltration in HCC. MMP1, as a member of MMPs, participated in the EMT which was identified as a strict programmed shift playing a crucial role in tumor invasion and metastasis<sup>[44]</sup>. MMPs could auto-activate and lead to a cascade of interactional activation between each other to enhance their influence in the EMT<sup>[19]</sup>. For invasive HCC, overexpression of MMP1 has been confirmed to correlate with an elevated capacity of invasion and migratory in HCC cells, by the most likely mechanism of ECM degradation promoting the transmembrane migration of tumor cells<sup>[17]</sup>. This speculation explained the outcomes of high expression of MMP1 in tumor tissues and poor prognosis in HCC patients to a certain extent. On the other hand, MMP1-mediated HCC progression could be suppressed by circularDLC1<sup>[41]</sup>, miR-526b<sup>[45]</sup>, ETV4-MMP1 axis<sup>[46]</sup> and ERK/MMP1 signaling pathway<sup>[47]</sup>, indicating multiple pathological pathways of MMP1 carcinogenesis on HCC.

Two databases were utilized for mutual confirmation of the positive relationship between HCC prognosis and MMP1 expression, including OS, PFS, DSS, RFS, and DFS. High expression of MMP1 is closely related to poor survival in various of tumors, which is consistent with our finding thus highlighting the significance of monitoring MMP1 expression level to in attempts to detect and prevent early recurrence. However, MMP1 expression was not highly correlated with all clinicopathological characteristics, subgroups indicating some unknown factors might influence the prognosis in conjunction with these subgroups.

According to resent studies, the integration of clinicopathologic characteristics and TIIICs can be a clinical predictive model for the efficiency of immunotherapy<sup>[48, 49]</sup>. The genesis and development of tumors can involve large numbers of immune infiltrating cells and inflammatory mediators. Although MMP1 is involved in the tumor-immune-related progression of some carcinomas, there is barely any studies regarding the interaction between MMP1 expression and TIIICs in HCC proliferation and migration. Our study, presents a positive interaction between MMP1 expression and TIIICs, signifying the probable utility of MMP1-based prediction in HCC patients associated with immune infiltration. However, the absence of an apparent correlation between MMP1 and tumor purity indicated that MMP1 was expressed from cells in the tumor microenvironment, most likely from TIIICs. Although the function of TIIICs in carcinogenesis is still controversial, a cluster of studies have reported that MMP1 alongside TIIICs plays a vital role in tumor progression<sup>[50-52]</sup>. For further investigation of the interrelationship between MMP1 expression and immune cells infiltration in HCC, we analyzed the data via cox regression. B cells, CD8+/CD4+ T cells and dendritic cells are specific immune cells playing major roles in activating the body's immune response with antitumor effects<sup>[53, 54]</sup>. On the contrary, reports on TAMs have been focused on their function of modulating the tumor microenvironment and promoting tumor proliferation and angiogenesis<sup>[55]</sup>. In our study, all of the immune cells showed a high degree of infiltration in HCC, with almost opposite pro- and anti-tumor activity. The results suggest that high expression of MMP1 may overall promote HCC carcinogenesis via increasing infiltration of TAMs, partially counteracting the antitumor activity of other immune cells. This discovery might contribute to the development of innovative immunotherapeutic agents for HCC patients who do not respond to the current immunosuppressive checkpoint inhibitors. Moreover, the results of TMB/MSI evaluation suggested the patients with HCC might not readily benefit from the treatment of PD-1, necessitating the exploration of inhibitors targeted to new immunosuppressive site.

Coupled with the rapid development of immunotherapy in the origination and progression of cancer, the function and mechanism of tumor-immunity microenvironment have been the frontier area for research to screen out related genes that can serve as innovative biomarkers for diagnosis and prognosis or therapeutic targets<sup>[56]</sup>. We interestingly found that almost all the expression levels of related gene biomarkers in TIIICs were positively correlated with MMP1 expression, as well positive correlation between TIIICs and MMP1 expression. Furthermore, in spite of the anti-tumor activity of gene biomarkers in B cells, CD8+ T cells, dendritic cells and M1 Macrophages, large numbers of biomarkers in Tregs and exhausted T cells which exerted pro-tumor activity of immune-escaping and angiogenesis were observed. This consequence can explain how the MMP1 expression influence the TIIICs and related gene biomarkers to promote the progression of HCC to a certain extent, indicating the complex mechanisms of interaction and tumorigenesis in tumor microenvironment.

There were several notable limitations to our study. First, the data in our study being collected and analyzed based on the big data from authoritative databases as comprehensively as possible, it can only provide a primary theoretical basis and needs further verification by animal experiments and clinical trials. Second, different statistics methods used in data analysis might lead to differentials in results. Furthermore, the correlation coefficients between MMP1 and TIIICs or their biomarker genes were not very strong, suggesting the important direction and terra incognita for further research.

In conclusion, our study revealed that the high expression of MMP1 was closely related with poor prognosis in HCC patients, so is the positive correlation with tumor-immune cell infiltration. Although some mechanisms of interactive network are unknown, we still have reason to believe that MMP1 is a prospective prognostic biomarker in HCC.

## Declarations

### Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the research ethics committee of Lihuili hospital affiliated to Ningbo University at which the studies were conducted (Approval no. KY2021PJ045) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent Since this was an observational but not prospective intervention study, the Ethics Committee provided a waiver of informed consent.

### Conflict of interest

The authors have declared that no conflict of interest exists.

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Not applicable.

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### Authors' contributions

Conception and writing, L.D; Charting and writing, J.M; data analysis, X.C.C and S.Q.M; reference acquisition, T.Y.Z and H.H.S; comments and suggestions, C.D.L; manuscript revision, C.J.L. All the authors approved the final manuscript to be submitted and published.

### Consent to publish

All the authors approved the final manuscript to be submitted and published.

### Availability of Data and Materials

All the data and materials can be obtained in open bioinformation databases mentioned above in our manuscript.

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## Tables

Table 1

Correlation of MMP1 expression and prognosis in hepatocellular carcinoma patients with different clinicopathological factors via Kaplan-Meier plotter.

Clinicopathological factors	Subgroup	Overall survival (n = 364)			Progression-free survival (n = 366)			Relapse-free survival (n = 313)			Disease specific survival (n = 357)		
		No.	Hazard ratio	P value	No.	Hazard ratio	P value	No.	Hazard ratio	P value	No.	Hazard ratio	P value
		Stage	Stage 1	170	2.25(1.21–4.19)	<b>0.0084</b>	170	2.04(1.24–3.35)	<b>0.0043</b>	153	2.08(1.2–3.6)	<b>0.0073</b>	167
		Stage 2	83	1.76(0.81–3.81)	0.15	84	1.56(0.86–2.84)	0.14	74	0.61(0.26–1.43)	0.25	82	3.4e08(NA-NA)
		Stage 3	83	2.39(1.30–4.38)	<b>0.0037</b>	83	1.77(0.98–3.2)	0.055	68	1.83(0.96–3.5)	0.063	81	3.35(1.62–6.93)
		Stage 4	4	NA	NA	5	NA	NA	0	NA	NA	3	NA
	Grade	Grade 1	55	3.2(1.15–8.91)	<b>0.02</b>	55	3.53(1.54–8.08)	<b>0.0017</b>	45	7.81(1.03–59.34)	<b>0.019</b>	55	3.18(0.89–11.32)
		Grade 2	174	1.98(1.18–3.33)	<b>0.0082</b>	175	2.57(1.66–3.98)	<b>1.2e-05</b>	147	2.39(1.4–4.06)	<b>9.3e-04</b>	169	3.14(1.54–6.39)
		Grade 3	118	2.41(1.32–4.39)	<b>0.003</b>	119	1.58(0.95–2.63)	0.073	106	0.65(0.38–1.11)	0.11	116	2.55(1.2–5.43)
		Grade 4	12	NA	NA	12	NA	NA	11	NA	NA	12	NA
AJCC_T	T1	180	2.09(1.16–3.76)	<b>0.012</b>	180	1.99(1.23–3.23)	<b>0.0045</b>	160	2.06(1.21–3.51)	<b>0.0068</b>	177	2.06(0.7–6.04)	
	T2	90	1.6(0.78–3.28)	0.2	92	1.36(0.78–2.37)	0.27	79	0.54(0.23–1.24)	0.14	89	3.81(0.87–16.7)	
	T3	78	3.05(1.64–5.69)	<b>2.3e-04</b>	78	1.8(1-3.24)	<b>0.048</b>	65	1.76(0.9–3.44)	0.097	75	3.82(1.79–8.13)	
	T4	13	NA	NA	13	NA	NA	6	NA	NA	13	NA	
Gender	Female	118	1.71(0.86–3.43)	0.12	120	2.25(1.34–3.77)	<b>0.0016</b>	105	3.02(1.45–6.28)	<b>0.0019</b>	116	2.99(1.04–8.57)	
	Male	246	2.9(1.86–4.52)	<b>8.3e-07</b>	246	1.86(1.29–2.67)	<b>6.8e-04</b>	208	1.75(1.17–2.61)	<b>0.0057</b>	241	3.4(1.92–6.02)	
Vascular invasion	None	203	2.09(1.24–3.55)	<b>0.005</b>	204	1.77(1.13–2.77)	<b>0.011</b>	175	1.84(1.08–3.15)	<b>0.023</b>	200	2.16(1.04–4.5)	
	micro	90	1.92(0.87–4.22)	0.098	91	1.93(1.09–3.4)	<b>0.022</b>	81	1.69(0.9–3.17)	0.1	88	4.03(1.35–11.99)	
	macro	16	NA	NA	16	NA	NA	14	NA	NA	14	NA	
Race	White	181	1.51(0.93–2.45)	0.091	183	1.8(1.21–2.67)	<b>0.0033</b>	147	1.82(1.12–2.97)	<b>0.015</b>	177	2.86(1.21–6.73)	
	Asian	155	3.88(2.14–7.06)	<b>1.7e-06</b>	155	2.28(1.41–3.68)	<b>5.4e-04</b>	143	2.11(1.26–3.52)	<b>0.0038</b>	152	4.27(1.93–9.46)	
Sorafenib treatment	treated	29	5.22(1.09–25.13)	<b>0.026</b>	30	1.54(0.67–3.58)	0.31	22	1.56(0.61–3.99)	0.35	29	5.22(1.09–25.13)	
Alcohol consumption	Yes	115	1.56(0.82–3)	0.17	115	2.32(1.38–3.89)	<b>0.0011</b>	98	2.38(1.31–4.3)	<b>0.0032</b>	115	1.9(0.91–3.94)	
	none	202	2.59(1.47–4.58)	<b>6.7e-04</b>	204	1.93(1.28–2.9)	<b>0.0013</b>	182	1.91(1.14–3.21)	<b>0.013</b>	197	4.1(1.72–9.78)	
Hepatitis virus	Yes	150	3.33(1.73–6.39)	<b>1.3e-04</b>	152	2.02(1.27–3.22)	<b>0.0026</b>	138	1.75(1.06–2.87)	<b>0.026</b>	149	3.54(1.55–8.08)	
	none	167	1.76(1.1–2.8)	<b>0.017</b>	167	2.19(1.41–3.39)	<b>3.3e-04</b>	142	1.99(1.2–3.31)	<b>0.0066</b>	163	2.41(1.31–4.44)	

AJCC, American Joint Committee on Cancer; NA, not available data; P value less than 0.05 is shown in bold.

Table 2

Correlation analysis between *MMP1* and related genes and markers of immune cells in hepatocellular carcinoma via TIMER.

Description	Gene markers	None		Purity	
		correlation	p	correlation	p
B cell	<i>CD19</i>	<b>0.129</b>	1.31e-02	<b>0.147</b>	6.35e-03
	<i>CD79A</i>	<b>0.114</b>	2.81e-02	<b>0.155</b>	3.8e-03
CD8 + T cell	<i>CD8A</i>	<b>0.159</b>	2.19e-03	<b>0.209</b>	9.55e-05
	<i>CD8B</i>	<b>0.198</b>	1.28e-04	<b>0.239</b>	7.09e-06
Monocyte	<i>CD86</i>	<b>0.285</b>	<b>2.18e-08</b>	<b>0.377</b>	<b>4.03e-13</b>
	<i>CD115(CSF1R)</i>	<b>0.209</b>	<b>4.83e-05</b>	<b>0.29</b>	<b>4.06e-08</b>
TAM	<i>CCL2</i>	<b>0.154</b>	<b>2.87e-03</b>	<b>0.189</b>	<b>4.15e-04</b>
	<i>CD68</i>	<b>0.267</b>	<b>1.69e-07</b>	<b>0.325</b>	<b>6.6e-10</b>
	<i>IL10</i>	<b>0.248</b>	<b>1.38e-06</b>	<b>0.326</b>	<b>5.7e-10</b>
M1 Macrophage	<i>IRF5</i>	<b>0.162</b>	<b>1.77e-03</b>	<b>0.168</b>	<b>1.69e-03</b>
	<i>INOS(NOS2)</i>	0.013	8.02e-01	0.025	6.46e-01
	<i>COX2(PTGS2)</i>	<b>0.15</b>	<b>3.84e-03</b>	<b>0.201</b>	<b>1.65e-04</b>
M2 Macrophage	<i>CD163</i>	0.092	7.75e-02	<b>0.158</b>	<b>3.18e-03</b>
	<i>VSIG4</i>	<b>0.125</b>	<b>1.63e-02</b>	<b>0.173</b>	<b>1.25e-03</b>
	<i>MS4A4A</i>	<b>0.123</b>	<b>1.74e-02</b>	<b>0.196</b>	<b>2.51e-04</b>
Neutrophils	<i>CD66b(CEACAM8)</i>	0.068	1.92e-01	0.086	1.1e-01
	<i>CD11b(ITGAM)</i>	<b>0.317</b>	<b>4.31e-10</b>	<b>0.364</b>	<b>2.84e-12</b>
	<i>CCR7</i>	0.019	7.12e-01	0.065	2.25e-01
Dendritic cell	<i>HLA-DPB1</i>	<b>0.173</b>	<b>8.09e-04</b>	<b>0.227</b>	<b>2.11e-05</b>
	<i>HLA-DQB1</i>	<b>0.184</b>	<b>3.8e-04</b>	<b>0.237</b>	<b>8.36e-06</b>
	<i>HLA-DRA</i>	<b>0.191</b>	<b>2.15e-04</b>	<b>0.251</b>	<b>2.37e-06</b>
	<i>HLA-DPA1</i>	<b>0.16</b>	<b>2.02e-03</b>	<b>0.219</b>	<b>4.02e-05</b>
	<i>CD1c(BDCA-1)</i>	0.087	9.4e-02	<b>0.12</b>	<b>2.57e-02</b>
	<i>NRP1</i>	<b>0.218</b>	<b>2.26e-05</b>	<b>0.218</b>	<b>4.29e-05</b>
	<i>ITGAX</i>	<b>0.321</b>	<b>2.38e-10</b>	<b>0.419</b>	<b>4.38e-16</b>
Tregs	<i>FOXP3</i>	<b>0.123</b>	<b>1.82e-02</b>	<b>0.172</b>	<b>1.33e-03</b>
	<i>CCR8</i>	<b>0.254</b>	<b>7.07e-07</b>	<b>0.311</b>	<b>3.53e-09</b>
	<i>STAT5B</i>	0.094	7.2e-02	0.099	6.53e-02
	<i>TGFB1</i>	<b>0.24</b>	<b>2.81e-06</b>	<b>0.277</b>	<b>1.67e-07</b>
	<i>CD25(IL2RA)</i>	<b>0.27</b>	<b>1.26e-07</b>	<b>0.356</b>	<b>1.02e-11</b>
	<i>CD4</i>	<b>0.186</b>	<b>3.21e-04</b>	<b>0.247</b>	<b>3.34e-06</b>
T cell exhaustion	<i>PD-1(PDCD1)</i>	<b>0.219</b>	<b>2.17e-05</b>	<b>0.268</b>	<b>4.55e-07</b>
	<i>CTLA4</i>	<b>0.326</b>	<b>1.26e-10</b>	<b>0.386</b>	<b>1.02e-13</b>
	<i>LAG3</i>	<b>0.179</b>	<b>5.32e-04</b>	<b>0.2</b>	<b>1.86e-04</b>
	<i>TIM-3(HAVCR2)</i>	<b>0.33</b>	<b>6.72e-11</b>	<b>0.436</b>	<b>1.8e-17</b>
	<i>BTLA</i>	0.065	2.12e-01	<b>0.121</b>	<b>2.49e-02</b>
	<i>TIGIT</i>	<b>0.283</b>	<b>2.86e-08</b>	<b>0.348</b>	<b>3.14e-11</b>

TAM, tumor-associated macrophage; Cor, R value of Spearman's correlation; None, correlation without adjustment. Purity, correlation adjusted by purity. Bold values indicate  $p < 0.05$ .

Description	Gene markers	None		Purity	
		correlation	p	correlation	p
	GZMB	0.178	5.75e-04	0.206	1.12e-04

TAM, tumor-associated macrophage; Cor, R value of Spearman's correlation; None, correlation without adjustment. Purity, correlation adjusted by purity. Bold values indicate  $p < 0.05$ ;

## Figures

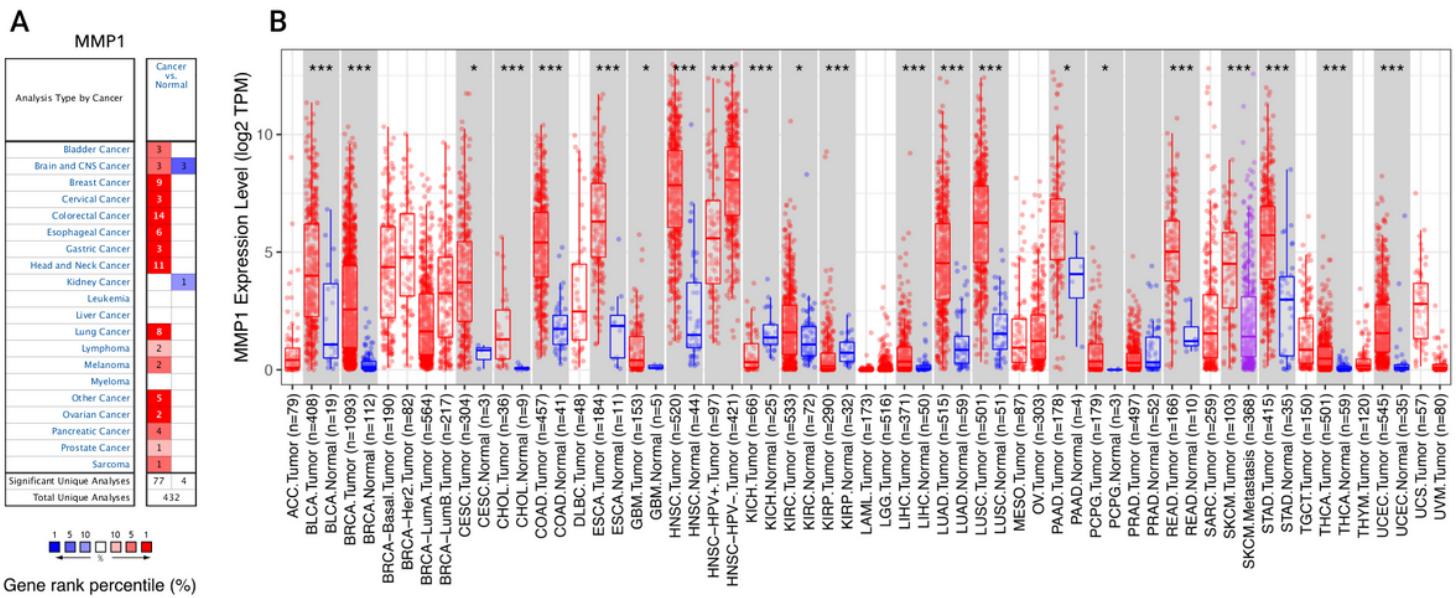
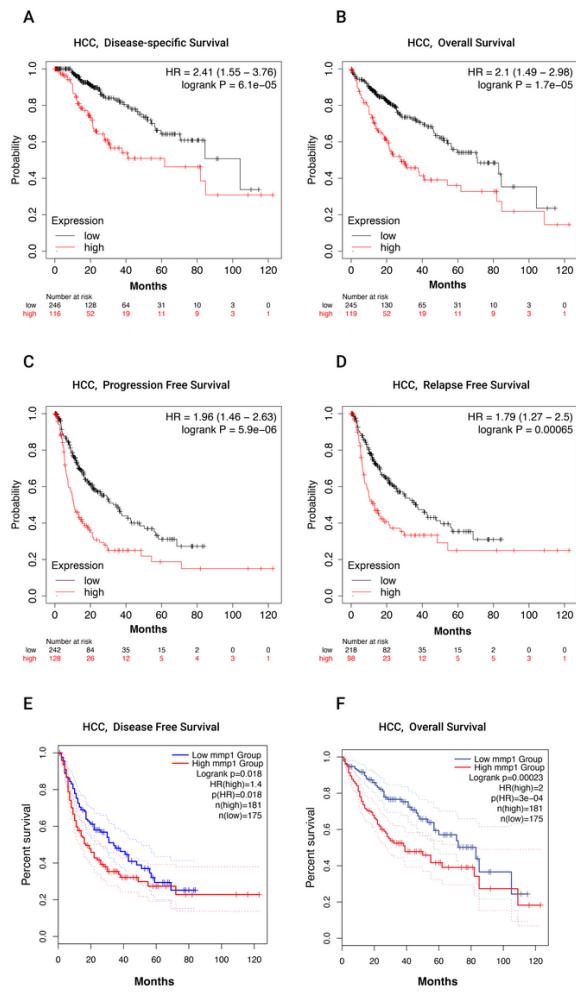


Figure 1

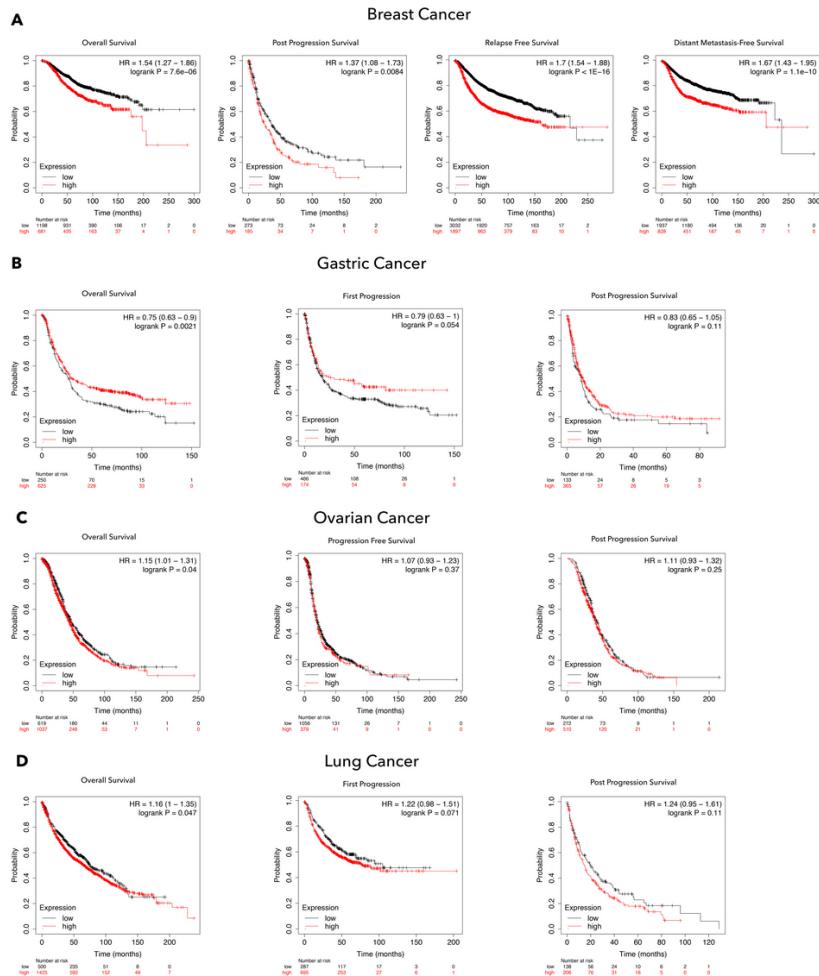
MMP1 expression levels in tumor and normal tissues of various cancers. (A) High expression levels of MMP1 in various cancer tissues compared with adjacent normal tissue using Oncomine analysis. (B) Comparison of MMP1 expression levels in different tumor and normal tissues via TIMER database.

\* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ .



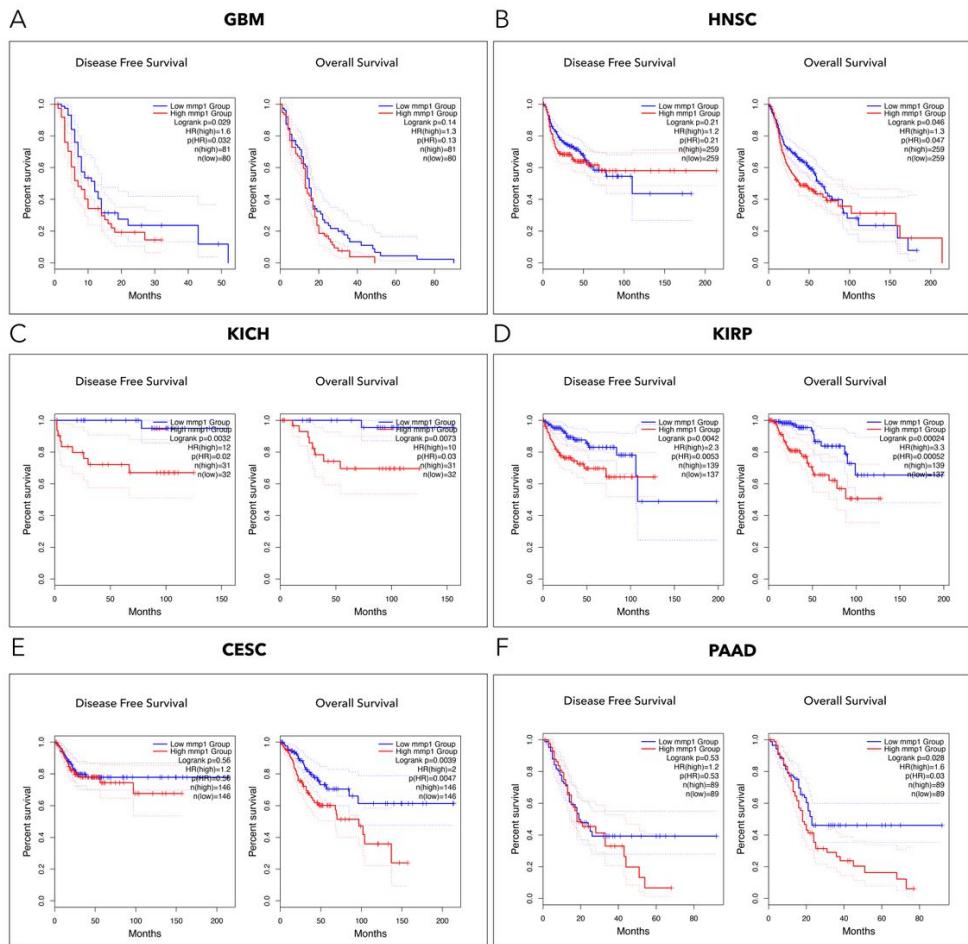
**Figure 2**

Prognostic potential of MMP1 expression in HCC patients via Kaplan-Meier plotter analysis (A-D) and GEPIA database (E-F). (A) Disease-specific survival (DSS), n=357; (B) Overall survival (OS), n=364; (C) Progression Free Survival (PFS), n=366. (D) Relapse free survival, n=313; (E) Disease-free survival (DFS) of HCC cohorts verified with GEPIA database, n=356; (F) OS of HCC cohorts verified with GEPIA database, n=356.



**Figure 3**

Prognostic potential of MMP1 expression in HCC patients via Kaplan-Meier plotter analysis (A-D) and GEPIA database (E-F). (A) Disease-specific survival (DSS), n=357; (B) Overall survival (OS), n=364; (C) Progression Free Survival (PFS), n=366. (D) Relapse free survival, n=313; (E) Disease-free survival (DFS) of HCC cohorts verified with GEPIA database, n=356; (F) OS of HCC cohorts verified with GEPIA database, n=356.



**Figure 4**

Interrelationship evaluation between MMP1 expression and prognostic potential in various carcinomas via GEPIA database. (A) DFS (n=161) and OS (n=161) survival curves in glioblastoma multiforme (GBM). (B) DFS (n=518) and OS (n=518) survival curves in head and neck squamous cell carcinoma (HNSC). (C) DFS (n=63) and OS (n=63) survival curves in kidney chromophobe (KICH). (D) DFS (n=276) and OS (n=276) survival curves in kidney renal papillary cell carcinoma (KIRP). (E) DFS (n=292) and OS (n=292) survival curves in cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC). (F) DFS (n=178) and OS (n=178) survival curves in pancreatic adenocarcinoma (PAAD). DFS, disease free survival; OS, overall survival.

A

B

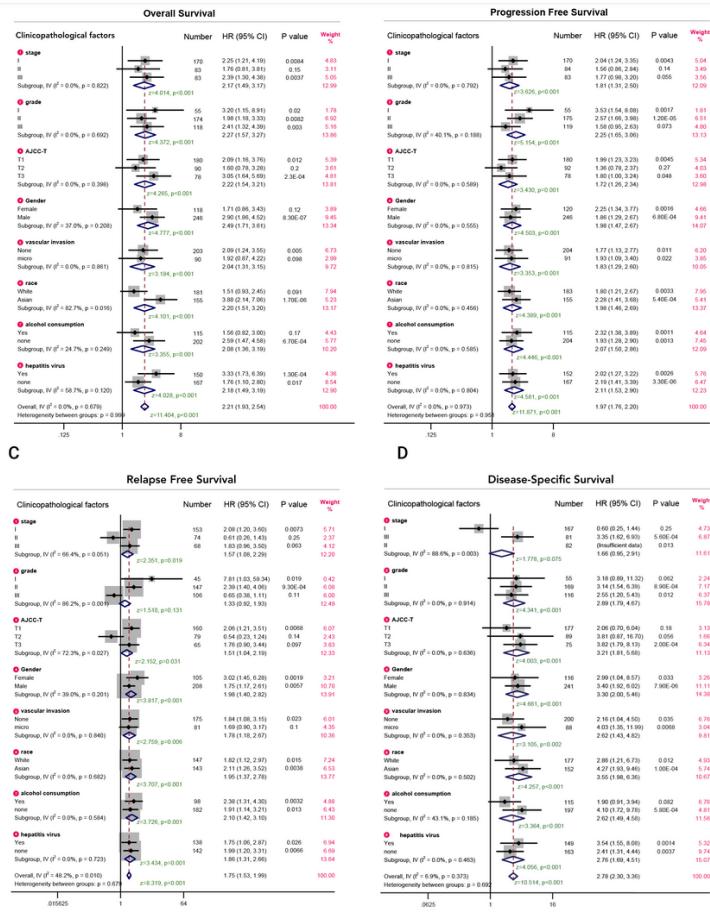


Figure 5

Correlation analysis between MMP1 expression and different clinicopathological characteristics in HCC patients. (A) cox regression analysis of OS in HCC patients. (B) cox regression analysis of PFS in HCC patients. (C) cox regression analysis of RFS in HCC patients. (D) cox regression analysis of DSS in HCC patients. OS, overall survival; PFS, progression free survival; RFS, relapse free survival; DSS, disease specific survival; HR, hazard ratio; CI, confidence interval.

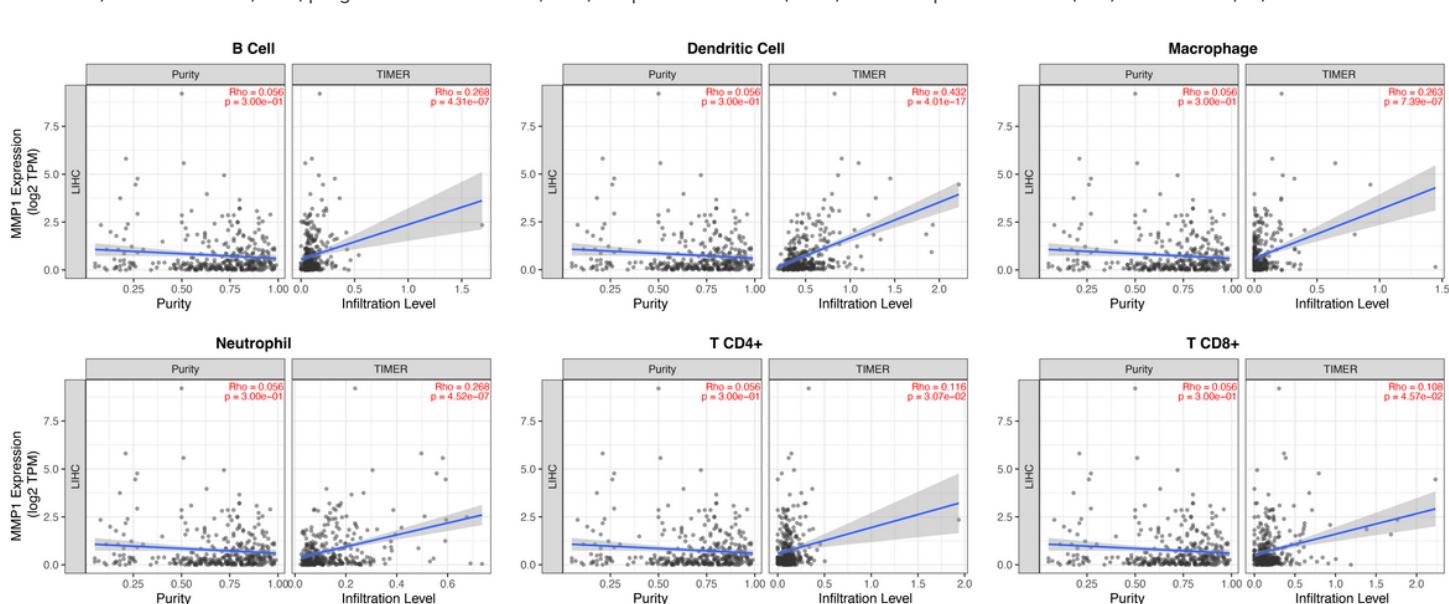
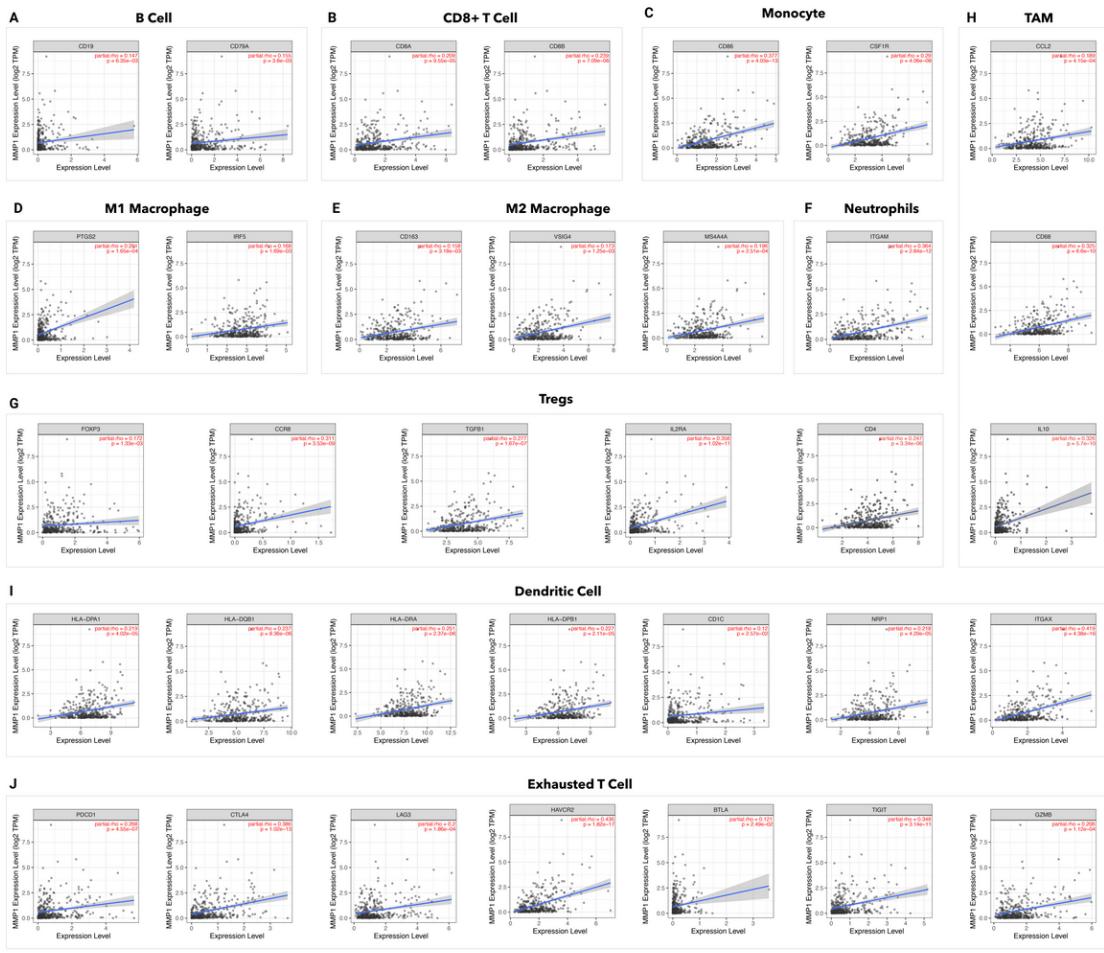


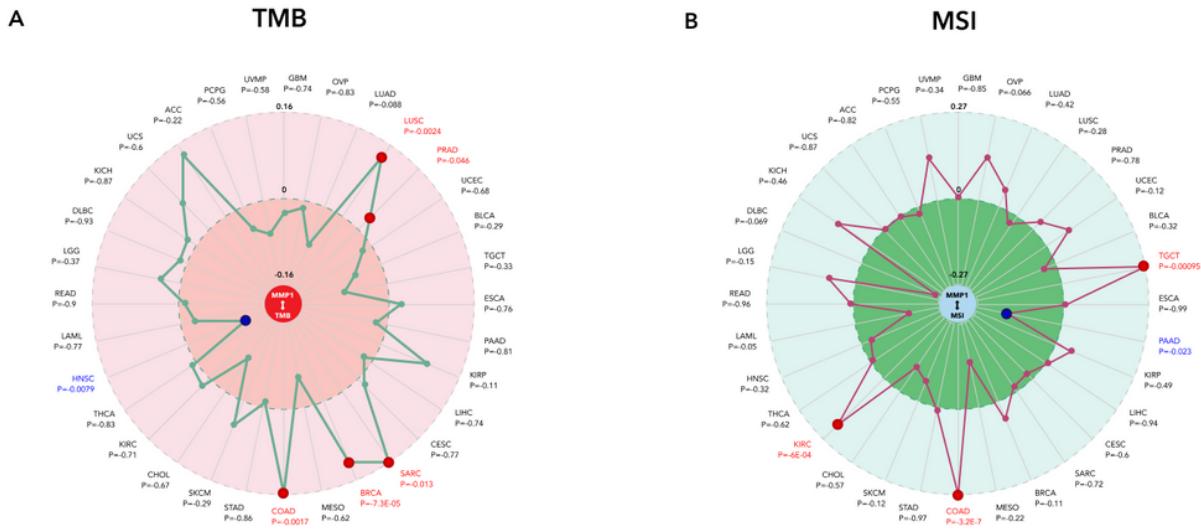
Figure 6

Interrelation evaluation between MMP1 expression and the levels of tumor infiltration immune cells in HCC via TIMER database.



**Figure 7**

Analysis of the correlation between MMP1 and various TIICs' biomarker gene expression in HCC patients using the TIMER 2.0 database. Biomarkers for: (A) B Cells; (B) CD8+ T Cells; (C) monocytes; (D) M1 Macrophages; (E) M2 Macrophages; (F) Neutrophils; (G) Tregs; (H) tumor-associated macrophages (TAMs); (I) dendritic cells and (J) exhausted T Cells.



**Figure 8**

Correlation between MMP1 expression and tumor mutational burden (TMB) and microsatellite instability (MSI). (A) Based on the 32 types of cancers of TCGA, the relationship between MMP1 expression and TMB were evaluated. The partial correlation (cor) values of +0.16 and -0.16 were marked and p-values recorded. (B) Based on the same cancer types as in (A), the relationship between MMP1 expression and MSI was evaluated. Partial correlation (cor) values of +0.27 and -0.27 as well as p-values were recorded.