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ORIGINAL ARTICLE

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MCAM is a prognostic biomarker in patients with liver cirrhosis and HCC

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Abstract

Background: Despite the rising prevalence of liver cirrhosis and HCC worldwide, reliable prognostic blood biomarkers are lacking. Melanoma cell adhesion molecule (MCAM) is a cell adhesion protein, and its cleavage by metalloproteinases, known to be enriched in fibrotic and malignant diseases, results in the release of a soluble form into the blood. The aim of this study was to characterize MCAM expression in patients with chronic liver disease and to evaluate soluble MCAM (sMCAM) as a prognostic blood biomarker in patients with liver cirrhosis and HCC.

Abbreviations: AFP, alpha-fetoprotein; BCLC, Barcelona Clinical Liver Cancer; F0–F4, stages of chronic liver disease; GSEA, gene set enrichment analysis; HA, hyaluronic acid; LI-RADS, Liver Imaging Reporting and Data System; MCAM, melanoma cell adhesion molecule; mRECIST, modified Response Evaluation Criteria in Solid Tumors; sMCAM, soluble melanoma cell adhesion molecule blood level; UMAP, Uniform Manifold Approximation and Projection for Dimension Reduction.

Preliminary data of this study have been presented at the International Liver Congress 2022, the GASL Annual Meeting 2023, and the Experimental Hepatology Days of the FALK Foundation 2023 as poster presentations.

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Methods: Expression of MCAM in liver tissue was assessed using transcriptomic data sets as well as by immunohistochemical analyses in patients with liver cirrhosis and HCC. Moreover, sMCAM blood levels were determined in plasma samples from healthy controls (n = 8), patients with chronic liver disease (n = 66), liver cirrhosis (n = 236), and HCC (n = 72).

Results: Single-cell RNA sequencing and immunohistochemistry indicated MCAM to be highly expressed by liver endothelial cells and fibroblasts. Expression was upregulated in liver tissue of patients with liver fibrosis and especially HCC independent of the underlying etiology ($p < 0.05$, respectively). Blood levels of sMCAM increased with fibrosis stage and peaked in patients with concomitant HCC, showing a comparable diagnostic performance as the fibrosis markers hyaluronic acid (HA) and TIMP1 for diagnosis of liver cirrhosis ($AUROC_{sMCAM} = 0.84$, $AUROC_{HA} = 0.89$, $AUROC_{TIMP1} = 0.87$) and as alpha-fetoprotein (AFP) for diagnosis of HCC ($AUROC_{sMCAM} = 0.72$, $AUROC_{AFP} = 0.72$). Finally, high sMCAM levels predicted worse survival in HCC ($p < 0.001$).

Conclusions: Collectively, our study suggests sMCAM as a blood biomarker of a liver microenvironment that drives the progression of liver disease in patients with liver cirrhosis and HCC.

Keywords: CD146, liver cancer, prediction, hepatocellular carcinoma, tumor microenvironment

INTRODUCTION

Liver cirrhosis is a major public health burden with high morbidity and mortality worldwide.[1] Patients with liver cirrhosis are at high risk of developing HCC, which represents one of the leading causes of death in these patients.[2] Considering the rising numbers of patients with liver cirrhosis and HCC worldwide, stable and easily accessible biomarkers to diagnose and predict patients' prognosis are urgently needed. Transient elastography represents the current clinical gold standard for non-invasive assessment of liver fibrosis; however, several blood markers of liver fibrosis, such as hyaluronic acid (HA) and TIMP1, have found their way into clinical diagnostics.[3,4] In patients with liver cirrhosis, the occurrence and number of previous decompensation events are the strongest prognostic factors.[1] In addition, clinical composite scores, such as the MELD score and transient elastography, have been shown to moderately correlate with survival in patients with compensated cirrhosis.[5,6] In patients with HCC, tumor characteristics such as tumor size and parameters of liver function are the most important determinants of prognosis.[5] Moreover, a high alpha-fetoprotein (AFP) level has been identified as an indicator of poor prognosis.[6] However, a high proportion (57.92%) of HCCs are AFP-low or -negative,[7] and blood biomarkers with better prognostic and predictive discrimination in these patients are yet unavailable. Furthermore, despite its close pathophysiological association, common blood biomarkers that combine risk prediction in patients with both liver cirrhosis and those with concomitant HCC are absent.

Melanoma cell adhesion molecule (MCAM) is a cell surface adhesion molecule functionally involved in cell-cell cohesion and cell differentiation.[8] MCAM has been reported to be overexpressed and associated with migration and invasion in numerous solid cancers, including HCC.[9,10] Interestingly, MCAM cleavage by metalloproteinases, known to be enriched in fibrotic and malignant diseases of the liver, including liver fibrosis and HCC, results in the release of a soluble form of MCAM (sMCAM) that can be detected in patients' blood.[10,11] The aim of this study was to thoroughly characterize MCAM overexpressing cirrhotic and tumorous liver tissue and to evaluate sMCAM as a blood biomarker in patients with chronic liver disease, liver cirrhosis, and patients with HCC.

METHODS

Clinical cohorts retrieved from database repositories

Microarray data sets of clinical cohorts with and without chronic liver disease (GSE33814,[12] n = 25 patients; GSE63067,[13] n = 16 patients; GSE103580,[14]

n = 73 patients; GSE84044,[15] n = 124 patients; GSE49541,[16] n = 72 patients; GSE76427,[17] n = 167 patients; and GSE112790,[18] n = 198 patients) were retrieved from Gene Expression Omnibus (Geo database[19]) and used for unbiased gene expression studies. Single-cell RNA sequencing data sets GSE124395[20] and GSE151530[21] were retrieved from the Geo database and analyzed.

Ethics

All patients provided written informed consent before inclusion. The study was conducted according to federal guidelines and local ethics committee regulations (Albert-Ludwigs-Universität, Freiburg, Germany; vote: EK 474/14 and 20-1066) and in accordance with both the Declarations of Helsinki and Istanbul. Analysis of blood and tissue samples was performed in a retrospective manner.

Human subjects recruited in this study

HCC tumor tissue and adjacent nontumor liver tissue samples were recruited through the Department of Pathology, University Medical Center Freiburg, and used for immunohistochemical imaging studies (n = 9 patients, clinical characteristics shown in Supplemental Table S1, http://links.lww.com/HC9/B47). To investigate the potential of sMCAM as a noninvasive biomarker, sMCAM blood levels were determined in 2 independent cohorts of patients with liver disease and healthy controls. Plasma samples were collected between 2004 and 2024 as part of routine blood samplings and prospectively enrolled in a blood biobank with immediate storage at −20°C. All samples of this biobank were then retrospectively screened for eligibility to this study. Cohort 1 included samples from 8 healthy controls and 66 patients with chronic liver disease and available transient elastography data at the time of blood sampling (Supplemental Table S2, http://links.lww.com/HC9/B47). Cohort 2 included 236 patients with liver cirrhosis and 72 patients with HCC with available clinical follow-up data (Supplemental Table S3, http://links.lww.com/HC9/B47). Given the potential impact of invasive diagnostic or therapeutic procedures on sMCAM bioavailability, patients with TIPS implantation, transarterial chemoembolization, selective internal radiotherapy, liver biopsies, or liver operations at the time of blood withdrawal were excluded. Diagnosis of liver cirrhosis was based on histology or a combination of medical history, characteristic laboratory changes, and diagnostic criteria in ultrasound examination or radiological imaging. The diagnosis of HCC was based on radiological imaging, and the diagnostic criteria were met for category 5 of Liver Imaging Reporting and Data System (LI-RADS based on multiphasic CT or MRI[22]). The stage of liver fibrosis was determined according to liver stiffness measurement by transient elastography and etiology-specific cutoff values.[23] To evaluate the diagnostic value in terms of longitudinal disease monitoring in individual patients (subcohort for longitudinal analyses, Supplemental Table S4, http://links.lww.com/HC9/B47), sMCAM blood levels were further determined at different time points of disease course. In this context, sMCAM levels were measured just before and after curative resection, as well as before receiving systemic therapy with atezolizumab/bevacizumab. Tumor response was assessed by CT or MRI and classified as complete response, partial response, stable disease, or progressive disease using modified Response Evaluation Criteria in Solid Tumors (mRECIST).[24] In all patients, routine clinical diagnostic parameters taken every 6–12 months were assessed retrospectively. The mean time of follow-up was 36.7 months (±29.1).

Immunohistochemistry

From a total number of n = 9 patients who underwent liver resection for treatment of HCC, adjacent nontumorous and corresponding tumorous HCC liver tissue was recruited and immediately fixed in formalin, followed by embedment in paraffin. Paraffin-embedded tissue blocks were sectioned for immunohistochemical staining leading to n = 3–8 technical replicates per donor. Following heat-induced antigen retrieval and peroxidase blocking, the sections were incubated with recombinant anti-CD146 antibody (ab75769, Abcam, RRID: AB_2143375). Sections were washed 3 times in sterile PBS and incubated with horseradish peroxidase–conjugated antibody against rabbit immunoglobulin G (EnVision Detection System, K5007, Dako, Agilent Technologies). After color development using the DAB substrate kit (EnVision Detection System, K5007, Dako, Agilent Technologies), sections were counterstained with Mayer's hematoxylin (Sigma Aldrich). Representative microscopic images were taken using Axio Observer (Zeiss Microscopy, 40×/0.75, room temperature) and AxioCam MRC (Zeiss Microscopy). The acquisition software used was Zen 2 lite. CD146-positive staining was quantified using ImageJ (software version 1.53k).

ELISA

Patients' blood samples were de-frozen and diluted at 1:300 in PBS. Human MCAM/CD146 Duoset ELISA (DY932-05, R&D Systems), Human TIMP-1 Duoset ELISA (DY970, R&D Systems), and Hyaluronan ELISA Kit—Quantitine (DHYAL0, R&D Systems) were

performed according to the manufacturer's instructions. Absorbance at 450 nM was read on a plate reader (TECAN). Each sample was detected in duplicate.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 9.0 (GraphPad Software) software. Gene set enrichment analysis (GSEA) was performed using GSEA software v4.3.2 using the Hallmark collection of gene sets from the Molecular Signature Database (MSigDB v. 7.4)[25] or previously described gene sets of response to atezolizumab/bevacizumab therapy.[26] Cell type enrichment analysis in liver and HCC tissue with high (>median) or low (<median) MCAM expression was performed using xCell, as described.[27] MCAM gene and protein expression in patients' tissues and sMCAM blood levels did not show normal distribution as assessed by the Shapiro-Wilk normality test. Therefore, nonparametric tests were applied for comparison of median expression and blood levels. MCAM expression in patients' tumorous tissues was normalized to mean expression in adjacent liver tissue and compared using the Mann-Whitney *U* test (*U* test). The single-cell RNA sequencing data sets were analyzed using R (v4.3.2) and Seurat (v5.0.1). The data sets were obtained from the GEO database and normalized, scaled, and dimensionally reduced using the default settings of Seurat. Subsequently, UMAP embeddings were calculated using the RunUMAP function, with dimensions set to 1:30 for the GSE124395 data set and 1:10 for the GSE151530 data set. Cell annotations were added based on information provided by the original authors. In the case of the GSE151530 data set, manual annotation of cell types "Hepatocytes" and "Cholangiocytes" was performed. This involved conducting a differential gene expression analysis using the FindAllMarkers function, thereby identifying distinctive marker genes associated with the aforementioned cell types.

sMCAM blood levels in different patient cohorts were analyzed using *U* test. Wilcoxon matched pairs test was used to compare paired samples. The chi-square test was applied for the analysis of categorical variables. The diagnostic test performance of sMCAM blood levels was evaluated by drawing the receiver operating characteristic curve and determining the AUC. The cutoff value for allocation into patients with high or low sMCAM was determined using the Youden Index for differentiation of patients with liver cirrhosis and HCC and detectable sMCAM levels. Kaplan-Meier and log-rank analysis were used to evaluate differences in survival between subgroups of patients with sMCAM concentrations above or below this cutoff value. Univariable and multivariable Cox proportional hazard regression was performed to identify independent prognostic factors in patients with HCC. Parameters with a p value <0.05 in the univariable model entered the multivariable model with no further variable selection. The results are presented as the mean ± SEM unless stated otherwise. Differences in all statistical analyses were considered significant when $*p < 0.05$. $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$. Results from GSEA were adjusted for the false discovery rate using the Kolmogorov-Smirnov test. A false discovery rate <0.05 was considered as statistically significant.

RESULTS

MCAM expression is upregulated in advanced liver fibrosis and HCC independent of the underlying etiology

To investigate the role of MCAM as a biomarker in patients with chronic liver disease, we first analyzed its gene expression in the liver tissue of patients with liver disease of different stages and patients with HCC. Assessment of 2 independent cohorts indicated MCAM gene expression levels to be similar in patients with metabolic dysfunction–associated steatohepatitis and healthy controls (GSE33814[12] and GSE63067[13]; Supplemental Figures S1A, B, http://links.lww.com/HC9/B47). However, MCAM expression significantly increased with fibrotic disease progression, independent of the underlying etiology of chronic liver disease. In fact, MCAM was significantly upregulated in patients with metabolic dysfunction–associated steatohepatitis with advanced fibrosis (fibrosis stage 3–4) compared to those with mild fibrosis (fibrosis stage 0–1) (GSE49541,[16] $p = 0.003$, *U* test, Figure 1A). In line, patients with alcoholic cirrhosis showed significant upregulation of hepatic MCAM gene expression compared to alcoholic steatosis (GSE103580,[14] $p = 0.03$, *U* test, Figure 1B). Further corroborating an etiology-independent association of MCAM with fibrosis, patients with chronic HBV infection and moderate or advanced fibrosis (fibrosis stage 3–4) showed higher expression compared to patients with HBV with mild or absent fibrosis (fibrosis stage 0–2) (GSE84044,[15] $p = 0.01$, *U* test, Figure 1C). Interestingly, MCAM was found to be highly overexpressed in tumorous HCC tissue, as compared to adjacent cirrhotic tissue (GSE76427,[17] $p < 0.0001$, *U* test, Figure 1D). Collectively, these data indicate an etiology-independent increasing expression of MCAM along the progression of chronic liver disease to liver cirrhosis and, finally HCC.

MCAM is most highly expressed by endothelial cells and fibroblasts in both liver fibrosis and HCC

Next, we aim to elucidate the cellular origin and molecular characteristics of MCAM overexpression in liver cirrhosis and HCC. In line with the transcriptomic

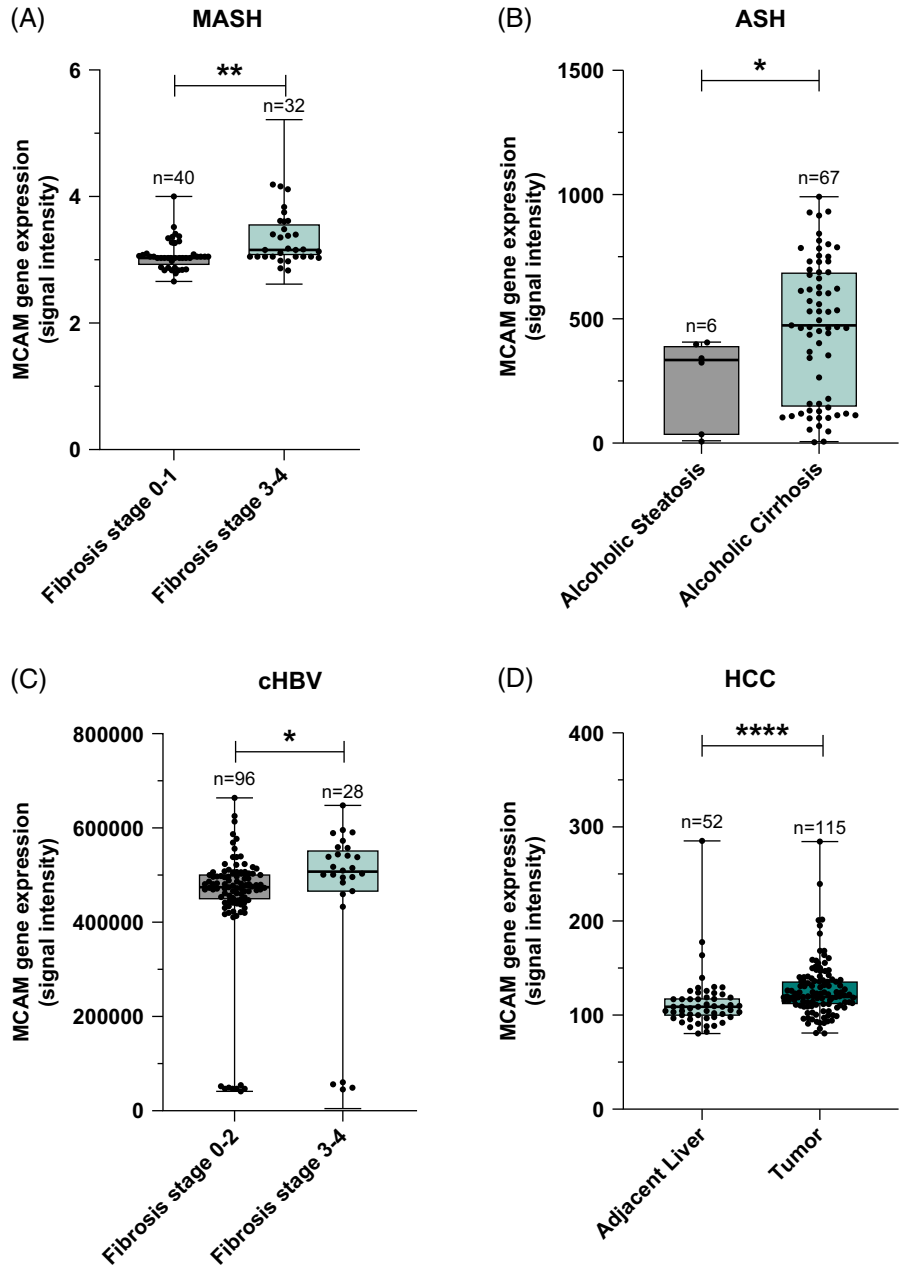

FIGURE 1 MCAM is overexpressed in the livers of patients with liver fibrosis and HCC. (A) MCAM gene expression in the livers of patients with MASH with mild fibrosis (F0–1, n = 40) versus advanced fibrosis (F3–F4, n = 32) is shown as signal intensity (GSE49541,[16] $p = 0.003$, *U* test). All patients of this cohort were diagnosed with previous diagnosis criteria for nonalcoholic steatohepatitis. (B) MCAM gene expression in the livers of patients with alcoholic steatosis (n = 6) versus alcoholic cirrhosis (n = 67) is shown as signal intensity (GSE103580,[14] $p = 0.03$, *U* test). (C) MCAM gene expression in the livers of patients with chronic HBV infection and mild fibrosis (fibrosis stage 0–2, n = 96) versus advanced fibrosis (fibrosis stage 3–4, n = 28) is shown as signal intensity (GSE84044,[15] $p = 0.01$, *U* test). (D) MCAM gene expression in HCC tumorous (n = 115) versus nontumor adjacent liver tissue (n = 52) is shown as signal intensity (GSE76427,[17] $p < 0.0001$, *U* test). Shown values correspond to measured signal intensities in the respective microarrays and are not comparable between different data sets and cohorts. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. Abbreviations: ASH, alcoholic steatohepatitis; cHBV, chronic Hepatitis B virus infection; MASH, metabolic dysfunction–associated steatohepatitis; MCAM, melanoma cell adhesion molecule.

data, immunohistochemistry of MCAM in HCC tumorous and adjacent liver tissue validated MCAM overexpression in HCC also at the protein level ($p < 0.0001$, *U* test, Figures 2A, B). Interestingly, immunohistochemistry further depicted the highest MCAM expression in stromal cells of the liver microenvironment. Indeed, analysis of 2 independent single-cell RNA sequencing data sets[20,21] confirmed highest expression of MCAM in liver endothelial and stellate cells in healthy liver (GSE151530,[21] Figure 2C, Supplemental Figure S2, http://links.lww.com/HC9/B47) as well as in tumor-associated endothelial cells and cancer-associated fibroblasts in HCC (GSE151530,[21] Figure 2D, Supplemental Figure S3, http://links.lww.com/HC9/

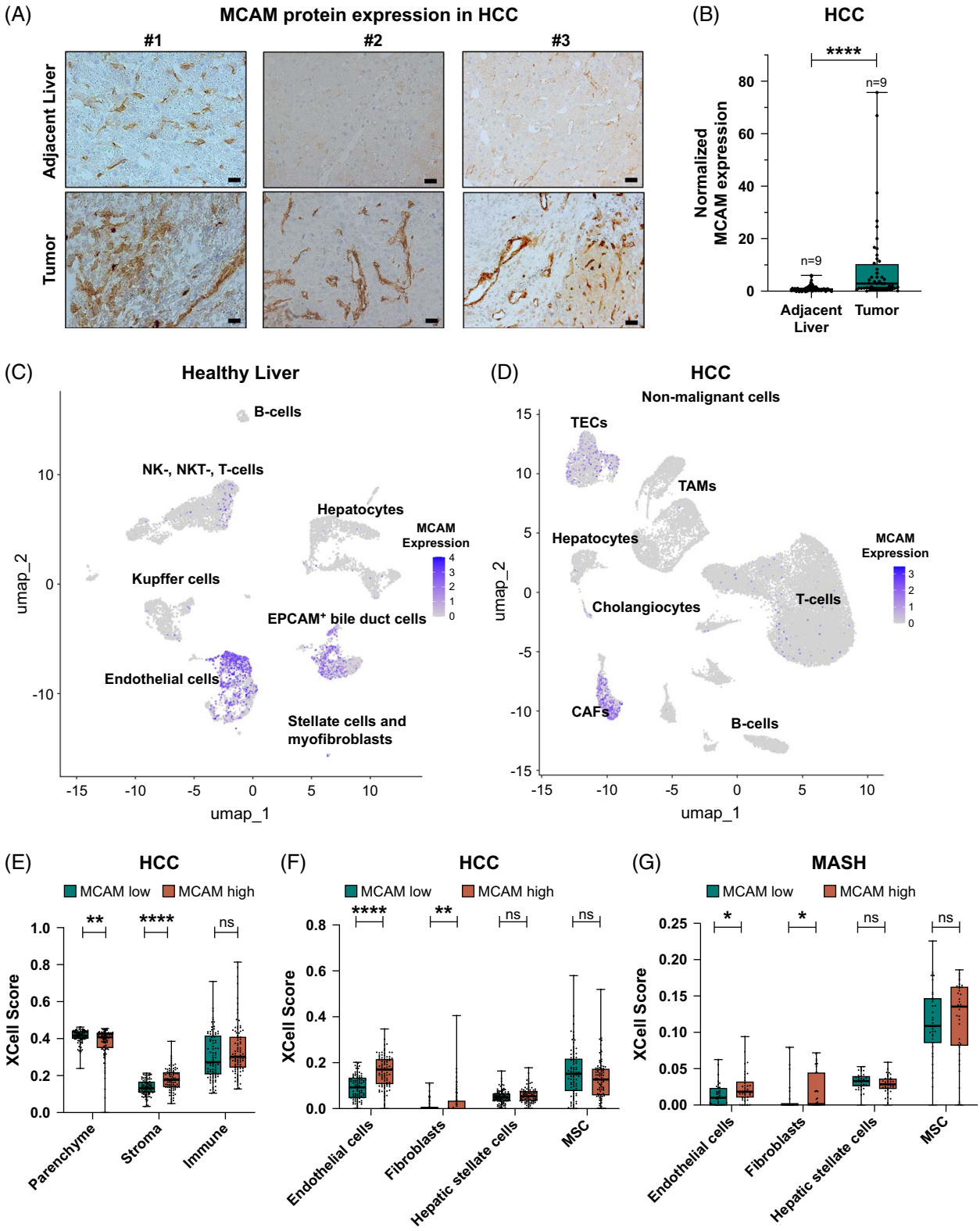

FIGURE 2 Endothelial cells and fibroblasts show the highest expression of MCAM in healthy and diseased liver. (A) Representative microscopic images showing MCAM staining by immunohistochemistry in HCC liver tissue and its corresponding adjacent nontumorous liver tissue from different patients with HCC (n = 3 representative out of 9 in total). Size bars indicate 20 μm. (B) MCAM-positive area (%) by IHC in HCC tumorous and adjacent liver tissue was quantified and is shown as normalized MCAM expression (n = 9 donors, n = 3–8 slices/biological replicates per donor, $p < 0.0001$, *U* test). (C) MCAM gene expression on a single-cell level in different liver resident cell types derived from healthy liver is shown as gene UMAP (GSE124395[20]). (D) MCAM gene expression on a single-cell level in different nonmalignant cells derived from HCC

is shown as gene UMAP (GSE151530[21]). (E) Cell type enrichment analysis in MCAM high or low expressing HCC tumors (GSE112790[18]). Shown are xCell scores (dots represent individual tumor samples) for Hepatocytes as "Parenchyme" and the composite scores for the stromal and immune microenvironment ($p = 0.008$, $p < 0.0001$, and $p = 0.11$, U test, respectively). (F) Stromal cell type enrichment analysis in MCAM high versus low expressing HCC tissue (GSE112790[18]). Box plots indicate xCell scores for the corresponding stromal cell types in MCAM high versus low expressing HCCs ($p < 0.0001$, $p = 0.008$, $p = 0.15$, $p = 0.06$ U test). xCell scores for pericytes are shown as HSCs, the liver-specific pericyte compartment. (G) Stromal cell type enrichment analysis in MCAM high or low expressing MASH tissue (GSE49541,[16] $p = 0.02$, $p = 0.02$, $p = 0.16$, $p = 0.56$, U test). xCell scores for pericytes are shown as HSCs, the liver-specific pericyte compartment. All patients of this cohort were diagnosed with previous diagnosis criteria for NASH. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; *****$p < 0.0001$; Abbreviations: CAFs, cancer-associated fibroblasts; EPCAM, epithelial cell adhesion molecule; FDR, false discovery rate; MASH, metabolic dysfunction–associated steatohepatitis; MCAM, melanoma cell adhesion molecule; MSC, mesenchymal stem cell; NES, normalized enrichment score; NK, natural killer cell; NKT, natural killer T cell; TAMs, tumor-associated macrophages; TECs, tumor endothelial cells; Tregs, regulatory T cell; UMAP, Uniform Manifold Approximation and Projection for Dimension Reduction.

B47). In contrast, malignant tumor cells showed very low or absent expression (GSE151530,[21] Supplemental Figure S4, http://links.lww.com/HC9/B47).

Corroborating stromal cells as the cellular source of increased MCAM expression in liver cirrhosis and HCC, deconvolution of liver bulk transcriptomic data using cell type enrichment analysis revealed significant enrichment of the stromal microenvironment in tumor tissue with high MCAM expression (GSE112790,[18] $p < 0.0001$, Figure 2E). As shown in Figures 2F, G, particularly endothelial cells and fibroblasts were strongly enriched in MCAM overexpressing liver tissue (GSE112790,[18] GSE49541,[16] $p < 0.05$, U test, respectively, Figures 2F, G). While the composite enrichment score for the stromal microenvironment was highly increased in HCCs with high MCAM expression, the tumor immune microenvironment did not differ between tumors with high or low expression ($p = 0.11$, Figure 2E). As expected, given its endothelial cell and fibroblast-dominated microenvironment, both liver and HCC tumor tissues with high MCAM expression showed enrichment of genes related to epithelial-mesenchymal transition and angiogenesis, hallmarks of both liver fibrogenesis and carcinogenesis (Supplemental Figures S5A, B, http://links.lww.com/HC9/B47). Taken together, these data suggest MCAM overexpression in liver cirrhosis and HCC as a consequence of a stromal cell type–enriched liver microenvironment that is associated with liver disease progression, particularly with epithelial-mesenchymal transition and angiogenesis.

sMCAM is a biomarker of chronic liver disease and liver cirrhosis

Cleavage of membranous MCAM by metalloproteinases has previously been described to result in the release of a soluble form of MCAM (sMCAM) that can be detected in patients' blood.[11] Our transcriptomic analysis of MCAM tissue expression indicates MCAM to be already overexpressed in tissue with advanced fibrosis and cirrhosis; thus, we first aimed to evaluate the diagnostic value of sMCAM for diagnosis of liver cirrhosis among patients with chronic liver disease (cohort 1: n = 8 healthy controls and n = 66 patients with chronic liver disease of various etiologies,

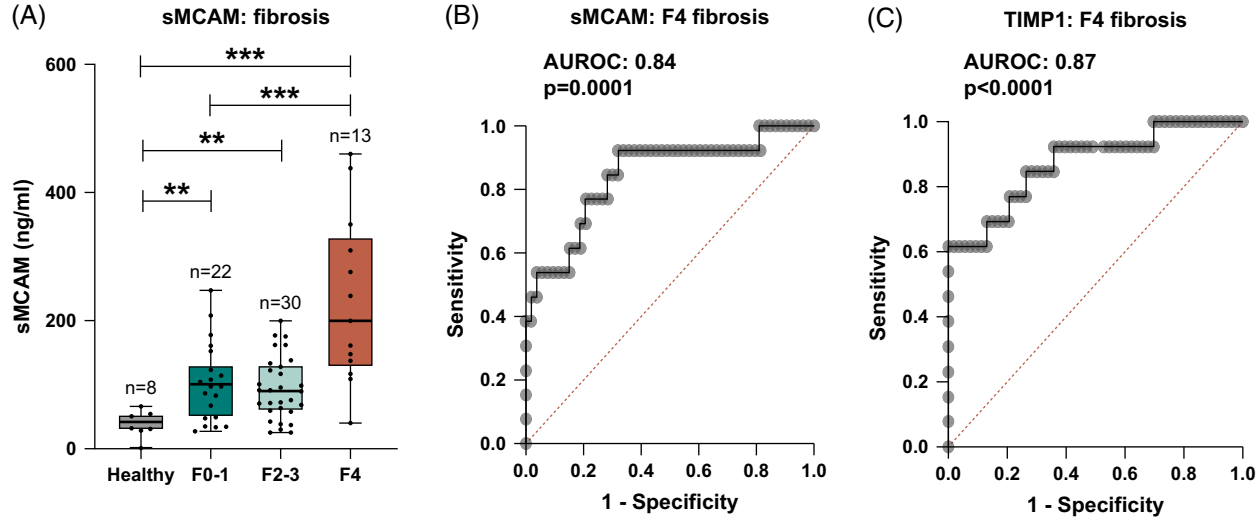

FIGURE 3 sMCAM blood levels show similar diagnostic performance in the diagnosis of liver cirrhosis as TIMP1. (A) sMCAM blood levels in healthy controls (n = 8) versus patients with different stages of chronic liver disease (F0–F4: n = 65) ($p = 0.003$, $p = 0.001$, $p = 0.0001$, $p = 0.0007$, respectively, U test). (B) ROC curve for the performance of sMCAM blood levels in the prediction of F4 fibrosis/liver cirrhosis diagnosis (AUROC = 0.84, $p = 0.0001$). (C) ROC curve for the performance of TIMP1 blood levels in the prediction of F4 fibrosis/liver cirrhosis diagnosis (AUROC = 0.87, $p < 0.0001$). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. Abbreviations: F0–4, stages of chronic liver disease; MCAM, melanoma cell adhesion molecule; ROC curve, receiver operating characteristic curve; sMCAM, soluble melanoma cell adhesion molecule.

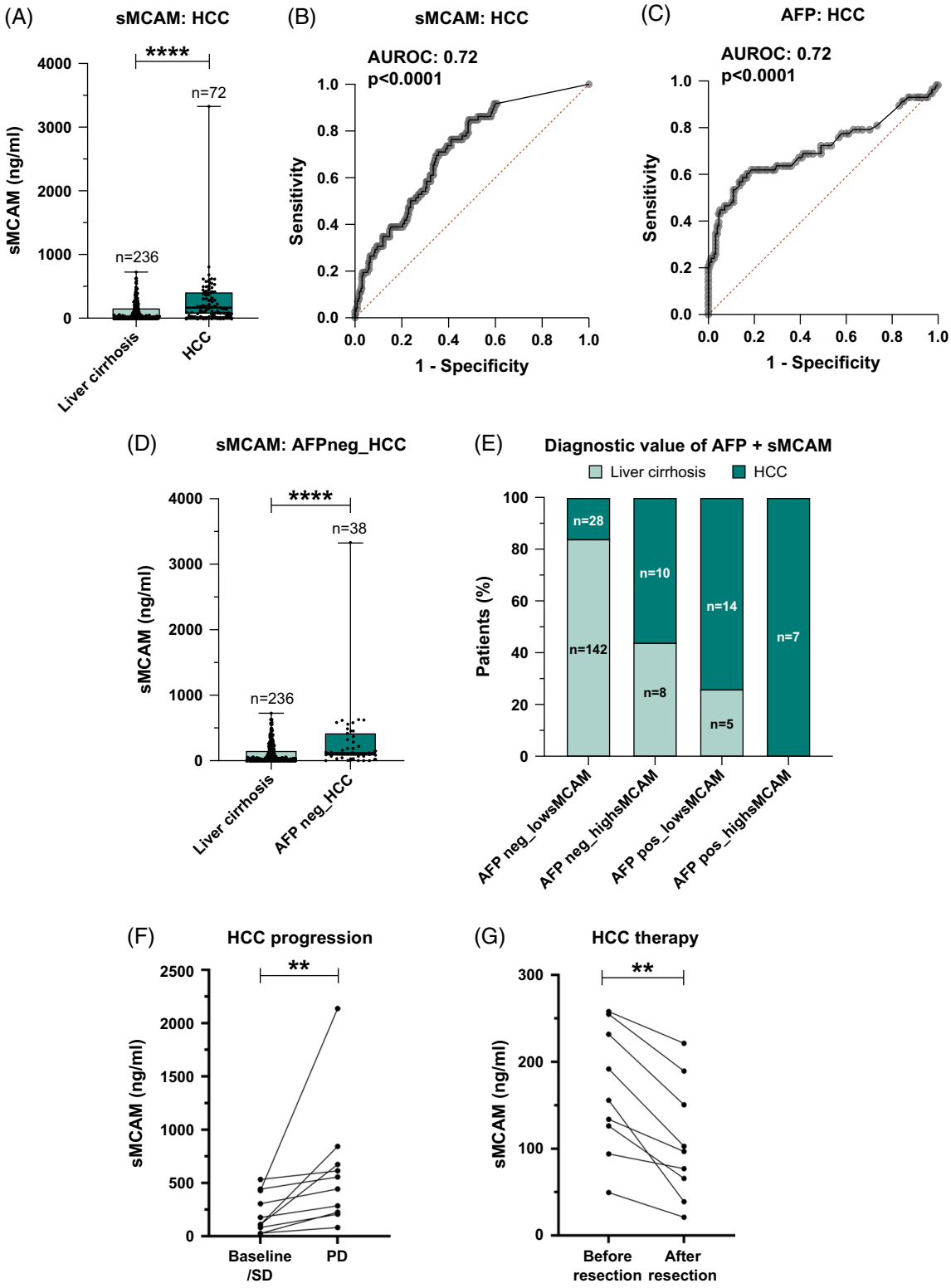

FIGURE 4 sMCAM blood levels are increased in HCC and model clinical tumor course. (A) sMCAM blood levels in patients with liver cirrhosis (n = 236) versus patients with HCC (n = 72) ($p < 0.0001$, *U* test). sMCAM blood levels were detectable in 92% of patients with HCC compared to 61% of patients with liver cirrhosis. (B) ROC curve for performance of sMCAM blood levels in HCC diagnosis (AUROC = 0.72, $p < 0.0001$). (C) ROC curve for the performance of AFP blood levels in the prediction of HCC diagnosis (AUROC = 0.72, $p < 0.0001$). (D) sMCAM blood levels in patients with liver cirrhosis (n = 236) versus patients with AFP-negative HCC (n = 38) ($p < 0.0001$, *U* test). (E) Proportion of patients with liver cirrhosis and HCC in the $AFP_{neg}sMCAM_{low}$-, $AFP_{neg}sMCAM_{high}$-, $AFP_{pos}sMCAM_{low}$-, and $AFP_{pos}sMCAM_{high}$ subgroups. (F) sMCAM blood levels in patients with HCC before versus after radiologically confirmed tumor progression (n = 10, $p = 0.002$, Wilcoxon matched pairs test). (G) sMCAM blood levels in patients with HCC before versus after resection (n = 9, $p = 0.004$, Wilcoxon matched pairs test). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. Abbreviations: AFP, alpha-fetoprotein; MCAM, melanoma cell adhesion molecule; PD, progressive disease; ROC curve, receiver operating characteristic curve; SD, stable disease; sMCAM, soluble melanoma cell adhesion molecule.

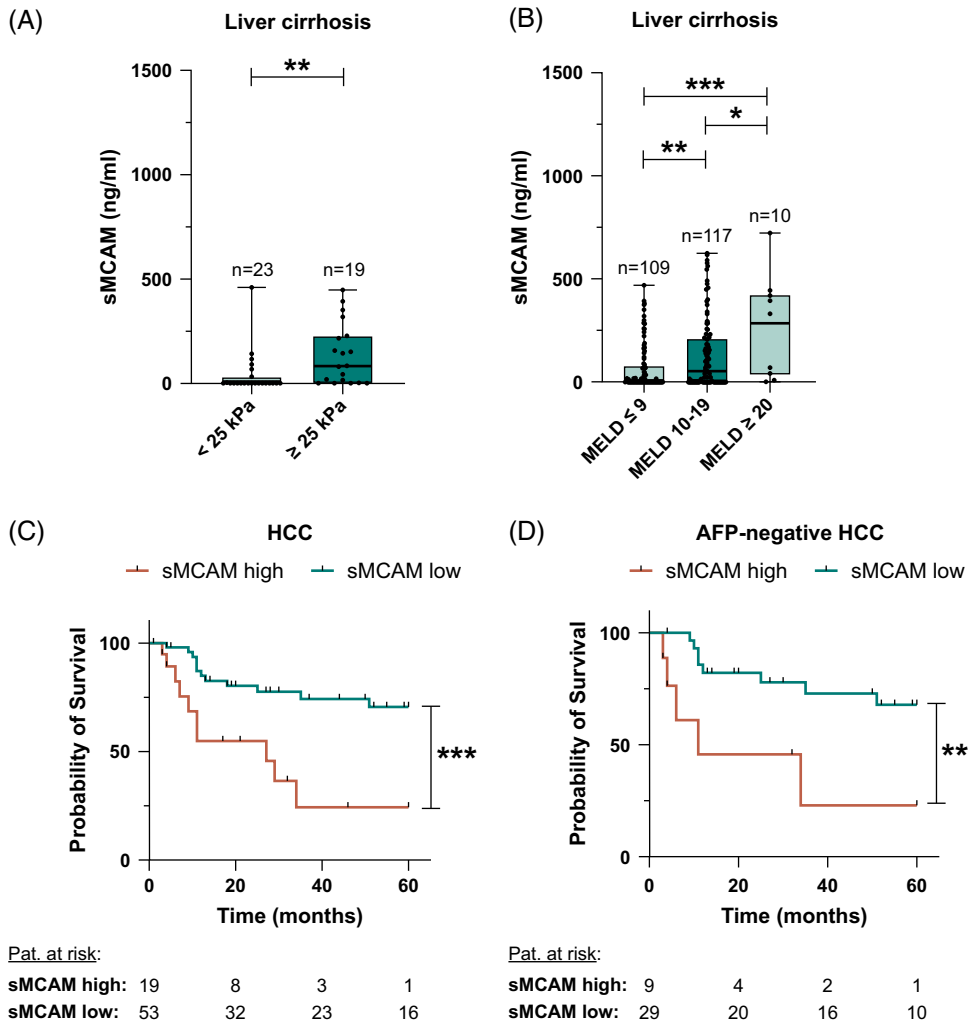

FIGURE 5 sMCAM blood levels correlate with noninvasive markers of liver cirrhosis and are highly prognostic in patients with HCC. (A) sMCAM blood levels in patients with liver cirrhosis and liver stiffness by transient elastography <25 (n = 23) or ≥ 25 kPa (n = 19) ($p = 0.001$, *U* test). (B) sMCAM blood levels in patients with liver cirrhosis and MELD scores ≤ 9 or 10–19 or ≥ 20 points (n = 109, n = 117, and n = 10, respectively) ($p = 0.001$, $p = 0.0005$, $p = 0.04$, respectively, *U* test). (C) Kaplan-Meier survival curves for patients with HCC and high or low sMCAM blood levels ($p = 0.0007$, log-rank test). Cutoff blood level of 407.9 ng/mL was determined using the ROC statistic and the Youden Index for differentiation of patients with liver cirrhosis and HCC with detectable sMCAM levels. (D) Kaplan-Meier survival curves for patients with AFP-negative HCC and high or low sMCAM blood levels (cutoff: 407.9 ng/mL, $p = 0.006$, log-rank test). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. Abbreviations: AFP, alpha-fetoprotein; MCAM, melanoma cell adhesion molecule; sMCAM, soluble melanoma cell adhesion molecule.

Supplemental Table S2, http://links.lww.com/HC9/B47). As shown in Figure 3A, sMCAM levels were significantly elevated in patients with chronic liver disease compared to healthy controls ($p < 0.01$, *U* test). In addition, sMCAM strongly increased in patients with F4 fibrosis stage (median F4 vs. F0/1 = 199.9 vs. 99.9 ng/mL, $p = 0.0007$, Figure 4A). The etiology of liver disease, on the other hand, did not affect sMCAM levels (Supplemental Figure S6, http://links.lww.com/HC9/B47). Notably, AUROC indicated a similar performance of sMCAM in the diagnosis of liver cirrhosis as compared to previously validated fibrosis markers such as TIMP1 and HA[3,4] ($AUROC_{MCAM} = 0.84$ [95% CI = 0.72–0.97]; $AUROC_{TIMP1} = 0.87$ [95% CI = 0.77–1.00]; $AUROC_{HA} = 0.89$ [95% CI = 0.76–0.99], Figures 3B, C, Supplemental Figure S7A, http://links.lww.com/HC9/B47). Yet, in contrast to HA and TIMP1, sMCAM levels did not differ in patients with fibrosis stages F0/1–F3, thus suggesting its sole applicability as a cirrhosis biomarker.

Levels of sMCAM are increased in patients with HCC

Given the strongest overexpression of MCAM in cirrhotic and tumorous liver tissue (Figures 1 and 2), we next investigated sMCAM blood levels in a second independent cohort of patients with liver cirrhosis (n = 236) and HCC (n = 72). In all patient subgroups, alcoholic steatohepatitis was the most common etiology of chronic liver disease (liver cirrhosis: 44.5%, HCC:

TABLE 1 Baseline clinical characteristics of patients with liver cirrhosis and HCC with high or low sMCAM blood levels

	Liver cirrhosis			HCC		
Group	**sMCAM low**	**sMCAM high**	***p***	**sMCAM low**	**sMCAM high**	***p***
Patients included	n = 221	n = 15	—	n = 53	n = 19	—
Age (years)	58.1 ± 11.6	50.4 ± 12.6	***p* = 0.02**	64.1 ± 10.3	65.7 ± 9.5	*p* = 0.69
Female/male	38.9%/61.1%	13.3%/86.7%	***p* = 0.047**	11.3 ± 88.7%	36.8 ± 63.2	***p* = 0.01**
Etiology of chronic liver disease	ASH (43.0%) cHCV (21.7%) Other (17.6%) MASH (13.1%) cHBV (4.5%)	ASH (66.7%) cHCV (13.3%) MASH (13.3%) Other (6.7%)	*p* = 0.42	ASH (37.7%) cHCV (20.8%) MASH (17.0%) cHBV (13.2%) Other (11.3%)	ASH (36.8%) cHCV (31.6%) Other (15.8%) cHBV (10.5%) MASH (5.3%)	*p* = 0.67
CHILD (points)	6.7 ± 1.8	7.3 ± 2.0	*p* = 0.24	6.2 ± 1.5	6.9 ± 2.5	*p* = 0.37
MELD	11.0 ± 4.1	14.1 ± 5.4	***p* = 0.01**	9.6 ± 3.9	10.5 ± 4.4	*p* = 0.56
AST (U/L)	60.1 ± 48.8	46.9 ± 25.0	*p* = 0.09	79.7 ± 71.6	81.4 ± 66.8	*p* = 0.82
ALT (U/L)	40.6 ± 35.5	76.2 ± 35.4	***p* = 0.014**	51.9 ± 42.1	43.7 ± 32.0	*p* = 0.33
Bilirubin (mg/dL)	1.7 ± 1.5	3.6 ± 3.5	***p* = 0.0003**	1.3 ± 0.6	1.8 ± 1.9	*p* = 0.91
INR	1.2 ± 0.2	1.3 ± 0.2	***p* = 0.002**	1.2 ± 0.2	1.2 ± 0.17	*p* = 0.92
BCLC stage	—	—	—	A (39.5%) B (23.7%) C (28.9%) D (7.9%)	A (37.5%) B (37.5%) C (25.0%) D (0.0%)	*p* = 0.76
M1	—	—	—	14.3%	33.3%	*p* = 0.13
AFP-positivity (%)	—	—	—	33.3%	47.0%	*p* = 0.32
Max. tumor size (cm)	—	—	—	4.0 ± 3.5	5.3 ± 3.8	*p* = 0.15
Singular/multinodular	—	—	—	59.1%/40.9%	57.9%/42.1%	*p* = 0.93

Note: Cutoff for sMCAM: 407.9 ng/mL; cutoff for AFP = 20 ng/mL. Continuous variables are shown as mean ± SD. Bold *p* values indicate statistical significance.

Abbreviations: AFP, alpha-fetoprotein; ASH, alcoholic steatohepatitis; BCLC, Barcelona Clinical Liver Cancer; cHBV, chronic Hepatitis B virus infection; cHCV, chronic Hepatitis C virus infection; CHILD, Child-Pugh score; INR, international normalized ratio; M1, distant metastases (TNM classification); MASH, metabolic dysfunction–associated steatohepatitis; sMCAM, melanoma cell adhesion molecule blood level.

TABLE 2 Cox regression model for overall survival in patients with HCC

	HCC					
	Univariable model			Multivariable model		
Group	HR	95% CI	*p*	HR	95% CI	*p*
Age (years)	1.01	0.97–1.05	$p = 0.74$	—	—	—
Bilirubin (mg/dL)	1.99	1.34–2.88	**$p = 0.0003$**	1.50	0.99–2.19	**$p = 0.039$**
Creatinine (mg/dL)	1.90	0.96–3.15	**$p = 0.027$**	1.80	0.80–3.38	$p = 0.095$
High sMCAM	4.59	1.95–10.71	**$p = 0.0004$**	4.40	1.71–11.32	**$p = 0.002$**
Tumor diameter (cm)	1.07	0.92–1.22	$p = 0.31$	—	—	—
Tumor expansion (multifocal vs. solitary)	1.23	0.53–2.76	$p = 0.63$	—	—	—
AFP ≥400 ng/mL	2.78	0.63–8.83	$p = 0.12$	—	—	—

Note: Cutoff for sMCAM: 407.9 ng/mL. Bold *p* values indicate statistical significance.
Abbreviations: AFP, alpha-fetoprotein; sMCAM, melanoma cell adhesion molecule blood level.

37.5%), followed by chronic HCV infection (21.2% and 23.6%, respectively) and metabolic dysfunction–associated steatohepatitis (13.1% and 13.9%, respectively). Patients of all Child-Pugh score clinical stages of liver disease were included, with most patients showing Child-Pugh class A liver cirrhosis and no signs of decompensation (patients' characteristics shown in Supplemental Table S3, http://links.lww.com/HC9/B47). Blood levels of sMCAM were more frequently detectable and further significantly increased in patients with HCC compared to patients with liver cirrhosis ($p < 0.0001$, *U* test, Figure 4A). The best cutoff for diagnosis of HCC with a specificity of 93.64% was 407.9 ng/mL. Remarkably, AUROC indicated comparable performance of sMCAM in diagnosis of HCC among patients with liver cirrhosis as AFP, the currently applied standard HCC blood biomarker ($AUROC_{sMCAM}$ vs. $AUROC_{AFP}$: 0.72, 95% CI = 0.65–0.78 vs. 0.72, 95% CI = 0.64–0.81, respectively, Figures 4B, C). sMCAM levels did not differ in patients with different etiologies of HCC (Supplemental Figure S8A, http://links.lww.com/HC9/B47). Thus, the diagnostic performance of sMCAM could be validated in the most common etiological subgroups of patients with alcoholic steatohepatitis and chronic Hepatitis C virus infection (Supplemental Figures S8B–E, http://links.lww.com/HC9/B47).

An important limitation of AFP as the current standard HCC blood biomarker in clinical routine is the frequency of AFP-negative tumors. Interestingly, blood levels of sMCAM were significantly increased in the subgroup of AFP-negative (<20 ng/mL) HCC compared to patients with liver cirrhosis ($p < 0.0001$, *U* test, Figure 4D). In fact, diagnostic discrimination of patients with liver cirrhosis and those with concomitant HCC significantly improved by simultaneous assessment of AFP and sMCAM (Figure 4E). Finally, sMCAM blood levels not only increased along the progression of liver cirrhosis and development of HCC but also during tumor progression of HCC. Thus, blood levels of sMCAM were significantly higher at the time of radiologically confirmed tumor progress compared to baseline or at the time of stable disease ($p = 0.002$, Wilcoxon matched pairs test, Figure 4F). In patients with curative liver resection, on the other hand, sMCAM levels significantly decreased after surgery ($p = 0.004$, Wilcoxon matched pairs test, Figure 4G).

Taken together, these data indicate sMCAM as a supplemental diagnostic and longitudinal biomarker in patients with HCC.

sMCAM blood levels correlate with liver cirrhosis stage and with overall survival in patients with HCC

Given the strong association of sMCAM blood levels with liver disease progression, we hypothesized its value as a prognostic biomarker. In patients with liver cirrhosis, sMCAM blood levels correlated with liver stiffness, measured by transient elastography. In patients with liver stiffness ≥25 kPa and thus assumable portal hypertension with high risk for hepatic decompensation,[28] sMCAM blood levels were markedly increased compared to patients with lower liver stiffness ($p = 0.001$, *U* test, Figure 5A). In line, patients with a MELD score ≥10–19 and ≥20 points were characterized by significantly higher sMCAM levels compared to patients with MELD ≤9 points and less advanced disease ($p = 0.001$ and $p = 0.0005$, *U* test, respectively, Figure 5B).

To elucidate further clinical characteristics associated with high or low sMCAM levels, we used the previously defined cutoff of 407.9 ng/mL for allocation of patients into subgroups. As shown in Table 1, patients with liver cirrhosis and high sMCAM levels had higher ALT levels, worse liver synthesis parameters (bilirubin and international normalized ratio) as well as overall increased MELD scores (all $p < 0.05$, Table 1). In patients with HCC, on the other hand, we did not find a correlation of sMCAM levels with specific tumor

characteristics, such as tumor size or BCLC (Barcelona Clinical Liver Cancer) stage. However, patients with HCC and high sMCAM levels showed a significantly worse median survival compared to patients with low sMCAM levels (sMCAM high vs. low: 27 mo vs. not reached, $p = 0.0007$, log-rank test, Figure 5C). In fact, sMCAM was the strongest independent predictor of survival among multiple clinical parameters tested, including tumor size and AFP level (Table 2). Thus, sMCAM blood levels >407.9 ng/mL were associated with a 4.4 times increased risk of death within follow-up (*univariable model:* HR = 4.59, $p = 0.0004$ [95% CI: 1.95–10.71], *multivariable model:* HR = 4.40, $p = 0.002$ [95% CI: 1.71–11.32], Table 2). The prognostic value of sMCAM could be validated in the most common etiologies of chronic liver disease in our HCC cohort (alcoholic steatohepatitis, $p = 0.02$; HCV: $p = 0.03$, log-rank test, respectively, Supplemental Figures S9A, B, http://links.lww.com/HC9/B47). Of note, these prognostic associations were also detectable in the subgroup of AFP-negative HCC (median survival sMCAM high vs. low: 11 mo vs. not reached, $p = 0.006$, log-rank test, Figure 5D).

Supporting its role as a stable prognostic blood biomarker, sMCAM blood levels were independent of acute clinical states of decompensation (Supplemental Figure S10, http://links.lww.com/HC9/B47) in patients with liver cirrhosis.

Collectively, these data suggest sMCAM as an independent prognostic blood biomarker in patients with liver cirrhosis and HCC.

MCAM overexpressing tumors show enrichment of a signature predicting response to systemic atezolizumab/bevacizumab therapy

High expression of MCAM in endothelial cells, as well as enrichment of angiogenesis gene signatures in HCCs with high MCAM expression, led us to question an association of MCAM with response to current first-line systemic therapy atezolizumab/bevacizumab that targets endothelial cells and the immune microenvironment. We, therefore, assessed a previously described gene signature predictive for treatment response to atezolizumab/bevacizumab[26] in HCC tissues with high versus low MCAM gene expression. Indeed, HCC tissues with high MCAM expression showed significant enrichment of the atezolizumab/bevacizumab response signature (normalized enrichment score = 2.00, false discovery rate <0.0001, Kolmogorov-Smirnov test, GSE112790,[18] Supplemental Figure S11A, http://links.lww.com/HC9/B47). In a small pilot study, we assessed sMCAM just before receiving atezolizumab/bevacizumab for HCC therapy. Interestingly, patients with partial response to atezolizumab/bevacizumab tended to have higher sMCAM levels compared to those with stable or progressive disease (Supplemental Figure S11B, http://links.lww.com/HC9/B47); however, the small number of patients did not allow final conclusions. These data suggest future studies on sMCAM as a potential predictive marker of the HCC tumor microenvironment mediating response to VEGF inhibitors.

DISCUSSION

The rising number of deaths worldwide due to liver cirrhosis–related complications indicates the need for prognostic biomarkers that identify patients requiring close clinical monitoring.[1] In this study, we identified sMCAM as a potential prognostic biomarker in patients with liver cirrhosis and HCC. A comprehensive investigation of hepatic MCAM gene expression in diverse large-scale transcriptomic data sets from patients with liver disease revealed a previously unknown association of MCAM with fibrogenesis. In fact, our study reveals significant upregulation of hepatic MCAM gene expression along fibrotic disease progression, independent from the underlying etiology. In line with the strong association of MCAM tissue expression with fibrogenesis, our study indicates its soluble form sMCAM as a noninvasive biomarker of liver cirrhosis. In fact, sMCAM levels strongly increased in patients with F4 fibrosis/liver cirrhosis and further correlated with transient elastography and MELD score in patients with cirrhosis. While sMCAM reached comparable diagnostic performance for liver cirrhosis as TIMP1 and HA, these validated fibrosis markers are superior in differentiating lower fibrosis stages.

Overexpression of MCAM in malignant diseases has previously been shown for diverse solid tumors.[10,29] In line, this study revealed highly significant upregulation of MCAM in human HCC tissue on both gene and protein levels. While we could also confirm previous reports of MCAM association with epithelial-mesenchymal transition in malignant HCC tissue, our study further links these functional tumor characteristics with a specific composition of the tumor microenvironment. Thus, in line with our analyses in fibrotic tissue, our single-cell RNA sequencing and cell type enrichment analyses indicate MCAM overexpressing HCCs to be enriched in stromal cell types, particularly in endothelial cells and fibroblasts, which are further the predominant source of MCAM overexpression. Besides MCAM tissue expression, we also found sMCAM blood levels to be highly upregulated in patients with HCC. Of note, sMCAM was also upregulated in patients with AFP-negative HCC and could, therefore, serve as a supplemental tumor biomarker in this subgroup of patients. The association of sMCAM levels with tumor progression and surgical treatment response, as well as overall survival, corroborates its potential as a noninvasive biomarker for

disease monitoring. Interestingly, in our cohort of patients with HCC, the prognostic impact of sMCAM was superior to that of AFP. This might be due to the functional association of MCAM with both tumor and fibrosis progression; however, future studies need to validate this hypothesis in larger patient cohorts.

Finally, the association of MCAM with tumor endothelial cell enrichment and previous reports of sMCAM as a proangiogenic factor[30] suggest its evaluation as a predictor of response to endothelial cell targeting therapies[31] as bevacizumab that is currently applied in combination with atezolizumab in first-line treatment of advanced HCC. Indeed MCAM overexpressing tumors showed enrichment of a well-defined transcriptomic signature predictive for response to atezolizumab/bevacizumab therapy.[26] The mild tendency of higher sMCAM levels in atezolizumab/bevacizumab responders in our pilot study does not allow conclusive assumptions on the role of sMCAM as a predictor of response to VEGF-targeting therapies. Since MCAM overexpression is only associated with endothelial cell enrichment but not with alterations of the immune microenvironment, future studies addressing this question might require a high number of patients but could address the clinically relevant decision of treating patients with atezolizumab/bevacizumab or with the alternative first-line combination of 2 checkpoint inhibitors (tremelimumab/durvalumab).[32]

An important limitation of sMCAM as a blood biomarker in liver cirrhosis and HCC is the absent specificity of MCAM expression for the liver. Thus, MCAM is also expressed by multiple other organs, and sMCAM blood levels thus may be prone to potential confounding origins of MCAM release.[33] Concomitant malignant diseases of the skin, the lung, or the colon that have been reported to overexpress MCAM might, therefore, limit the sensitivity and specificity of sMCAM as a biomarker.[33,34] Importantly, particularly in patients with liver cirrhosis, sMCAM levels were frequently below our detection limit. Since we could not identify clinical characteristics associated with undetectable sMCAM, future studies are needed to evaluate factors affecting its bioavailability. Finally, although the overexpression of MCAM in fibroblasts and endothelial cells, as well as the mechanism of sMCAM blood release by matrix metalloproteinase-mediated cleavage, most likely determine its prognostic role, the detailed pathophysiology behind these observations needs to be elucidated in further studies. The observation of MCAM expression with clinical disease progression further supports the investigation of MCAM as a therapeutic target in both patients with liver fibrosis and cirrhosis as well as HCC.

Taken together, our study identifies MCAM as a marker of nonparenchymal cells associated with liver disease progression and its soluble variant sMCAM as a prognostic biomarker for disease monitoring in patients with liver cirrhosis and HCC.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available within the article or its supplemental materials, http://links.lww.com/HC9/B47. Raw data associated with this paper are available upon reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

Design of the study: Natascha Roehlen; acquisition of data: Eva Stockinger, Mikhail Gromak, Hendrik Luxenburger, Sabine MacNelly, Peter Bronsert, Lukas Sturm, Christopher Berlin, Philipp Holzner, Simon Johannes Gairing, Natascha Roehlen, and Friedrich Foerster; analysis of the data: Eva Stockinger, Natascha Roehlen, David Obwegs, Sagar, and Dominik Bettinger; interpretation of the data: Eva Stockinger, Natascha Roehlen, David Obwegs, Sagar, Bertram Bengsch, and Dominik Bettinger; drafting the manuscript: Natascha Roehlen; revision for important intellectual content: Eva Stockinger, Hendrik Luxenburger, Dominik Bettinger, Maike Hofmann, Christian Labenz, Friedrich Foerster, Simon Johannes Gairing, Tobias Boettler, and Robert Thimme; conceptualization: Natascha Roehlen; methodology: Natascha Roehlen; formal analysis: Eva Stockinger; investigation: Eva Stockinger, Mikhail Gromak, and Sabine MacNelly; resources: Hendrik Luxenburger, Peter Bronsert, Christopher Berlin, Simon Johannes Gairing, and Natascha Roehlen; data curation: Eva Stockinger, Mikhail Gromak, and Natascha Roehlen; writing—original draft: Natascha Roehlen; writing—review and editing: Eva Stockinger, Hendrik Luxenburger, Dominik Bettinger, Tobias Boettler, Maike Hofmann, and Robert Thimme; visualization: Eva Stockinger and Natascha Roehlen; supervision: Maike Hofmann, Robert Thimme, and Natascha Roehlen; project administration: Natascha Roehlen and Maike Hofmann; funding acquisition: Maike Hofmann, Robert Thimme, and Natascha Roehlen. All authors approved the final version of the article, including the authorship.

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CONFLICTS OF INTEREST

Dominik Bettinger is on the speaker's bureau for Gore and Falk Foundation. He received grants from Abbvie. Simon Johannes Garing received grants from Ipsen, Gilead, and Schwiete Foundation. Friedrich Foerster advises and is on the speaker's bureau for AstraZeneca and Roche. He advises BMS and Eisai. He is on the speakers' bureau for MSD and Pfizer. He received grants from Merck KGaA and Servier. Bertram Bengsch advises for Roche. The remaining authors have no conflicts to report.

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REFERENCES

1. Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. Lancet. 2014;383:1749–61.
2. Ascione A, Fontanella L, Imparato M, Rinaldi L, de Luca M. Mortality from cirrhosis and hepatocellular carcinoma in Western Europe over the last 40 years. Liver Int. 2017;37:1193–201.
3. Sanyal AJ, Shankar SS, Yates KP, Bolognese J, Daly E, Dehn CA, et al. Diagnostic performance of circulating biomarkers for non-alcoholic steatohepatitis. Nat Med. 2023;29:2656–64.
4. European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu, Clinical Practice Guideline Panel, Chair:, EASL Governing Board representative:, Panel members: EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis—2021 update. J Hepatol. 2021;75:659–89.
5. Reig M, Forner A, Rimola J, Ferrer-Fàbrega J, Burrel M, Garcia-Criado Á, et al. BCLC strategy for prognosis prediction and treatment recommendation: The 2022 update. J Hepatol. 2022;76:681–93.
6. Cheng J, Wang W, Zhang Y, Liu X, Li M, Wu Z, et al. Prognostic role of pre-treatment serum AFP-L3% in hepatocellular carcinoma: Systematic review and meta-analysis. PLoS One. 2014;9:e87011.
7. Carr BI, Akkiz H, Üsküdar O, Yalçın K, Guerra V, Kuran S, et al. HCC with low- and normal-serum alpha-fetoprotein levels. Clin Pract (Lond). 2018;15:453–64.
8. Wang Z, Yan X. CD146, a multi-functional molecule beyond adhesion. Cancer Lett. 2013;330:150–62.
9. Wang Z, Xu Q, Zhang N, Du X, Xu G, Yan X. CD146, from a melanoma cell adhesion molecule to a signaling receptor. Signal Transduct Target Ther. 2020;5:148.
10. Wang J, Tang X, Weng W, Qiao Y, Lin J, Liu W, et al. The membrane protein melanoma cell adhesion molecule (MCAM) is a novel tumor marker that stimulates tumorigenesis in hepatocellular carcinoma. Oncogene. 2015;34:5781–95.
11. Boneberg EM, Illges H, Legler DF, Fürstenberger G. Soluble CD146 is generated by ectodomain shedding of membrane CD146 in a calcium-induced, matrix metalloprotease-dependent process. Microvasc Res. 2009;78:325–31.
12. Starmann J, Fälth M, Spindelböck W, Lanz KL, Lackner C, Zatloukal K, et al. Gene expression profiling unravels cancer-related hepatic molecular signatures in steatohepatitis but not in steatosis. PLoS One. 2012;7:e46584.
13. Frades I, Andreasson E, Mato JM, Alexandersson E, Matthiesen R, Martínez-Chantar ML. Integrative genomic signatures of hepatocellular carcinoma derived from nonalcoholic fatty liver disease. PLoS One. 2015;10:e0124544.
14. Trépo E, Goossens N, Fujiwara N, Song WM, Colaprico A, Marot A, et al. Combination of gene expression signature and Model for End-Stage Liver Disease score predicts survival of patients with severe alcoholic hepatitis. Gastroenterology. 2018;154:965–75.
15. Wang M, Gong Q, Zhang J, Chen L, Zhang Z, Lu L, et al. Characterization of gene expression profiles in HBV-related liver fibrosis patients and identification of ITGBL1 as a key regulator of fibrogenesis. Sci Rep. 2017;7:43446.
16. Moylan CA, Pang H, Dellinger A, Suzuki A, Garrett ME, Guy CD, et al. Hepatic gene expression profiles differentiate presymptomatic patients with mild versus severe nonalcoholic fatty liver disease. Hepatology. 2014;59:471–82.
17. Grinchuk OV, Yenamandra SP, Iyer R, Singh M, Lee HK, Lim KH, et al. Tumor-adjacent tissue co-expression profile analysis reveals pro-oncogenic ribosomal gene signature for prognosis of

resectable hepatocellular carcinoma. Mol Oncol. 2018;12: 89–113.
18. Shimada S, Mogushi K, Akiyama Y, Furuyama T, Watanabe S, Ogura T, et al. Comprehensive molecular and immunological characterization of hepatocellular carcinoma. EBioMedicine. 2019;40:457–70.
19. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, et al. NCBI GEO: Archive for functional genomics data sets–update. Nucleic Acids Res. 2013;41(Data base issue):D991–5.
20. Aizarani N, Saviano A, Sagar, Mailly L, Durand S, Herman JS, et al. A human liver cell atlas reveals heterogeneity and epithelial progenitors. Nature. 2019;572:199–204.
21. Ma L, Wang L, Khatib SA, Chang CW, Heinrich S, Dominguez DA, et al. Single-cell atlas of tumor cell evolution in response to therapy in hepatocellular carcinoma and intrahepatic cholangiocarcinoma. J Hepatol. 2021;75:1397–408.
22. Lee YT, Wang JJ, Zhu Y, Agopian VG, Tseng HR, Yang JD. Diagnostic criteria and LI-RADS for hepatocellular carcinoma. Clin Liver Dis (Hoboken). 2021;17:409–13.
23. Stauber R. Nichtinvasive Diagnostik bei nichtalkoholischer Fettlebererkrankung (NAFLD). J Gastroenterol Hepatol Erkrank. 2019;17:75–81.
24. Llovet JM, Lencioni R. mRECIST for HCC: Performance and novel refinements. J Hepatol. 2020;72:288–306.
25. Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular Signatures Database (MSigDB) hallmark gene set collection. Cell Syst. 2015;1:417–25.
26. Zhu AX, Abbas AR, de Galarreta MR, Guan Y, Lu S, Koeppen H, et al. Molecular correlates of clinical response and resistance to atezolizumab in combination with bevacizumab in advanced hepatocellular carcinoma. Nat Med. 2022;28: 1599–611.
27. Aran D, Hu Z, Butte AJ. xCell: Digitally portraying the tissue cellular heterogeneity landscape. Genome Biol. 2017;18:220.
28. de Franchis R, Bosch J, Garcia-Tsao G, Reiberger T, Ripoll C, Baveno VII. Faculty. Baveno VII—Renewing consensus in portal hypertension. J Hepatol. 2022;76:959–74.
29. Jiang G, Zhang L, Zhu Q, Bai D, Zhang C, Wang X. CD146 promotes metastasis and predicts poor prognosis of hepatocellular carcinoma. J Exp Clin Cancer Res. 2016;35:38.
30. Stalin J, Nollet M, Garigue P, Fernandez S, Vivancos L, Essaadi A, et al. Targeting soluble CD146 with a neutralizing antibody inhibits vascularization, growth and survival of CD146-positive tumors. Oncogene. 2016;35:5489–500.
31. Ranieri G, Patruno R, Ruggieri E, Montemurro S, Valerio P, Ribatti D. Vascular endothelial growth factor (VEGF) as a target of bevacizumab in cancer: From the biology to the clinic. Curr Med Chem. 2006;13:1845–57.
32. Yang X, Yang C, Zhang S, Geng H, Zhu AX, Bernards R, et al. Precision treatment in advanced hepatocellular carcinoma. Cancer Cell. 2024;42:180–97.
33. An Y, Wei N, Cheng X, Li Y, Liu H, Wang J, et al. MCAM abnormal expression and clinical outcome associations are highly cancer dependent as revealed through pan-cancer analysis. Brief Bioinform. 2020;21:709–18.
34. Rapanotti MC, Campione E, Spallone G, Orlandi A, Bernardini S, Bianchi L. Minimal residual disease in melanoma: Circulating melanoma cells and predictive role of MCAM/MUC18/MelCAM/CD146. Cell Death Discov. 2017;3:17005.

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