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## Retrospective Study

## Prostate-specific membrane antigen expression in hepatocellular carcinoma, cholangiocarcinoma, and liver cirrhosis

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## Abstract

### BACKGROUND

Primary liver cancer includes three subtypes: Hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (CCA), and combined hepatocellular carcinoma. Patients with primary liver cancer experienced poor prognosis and high mortality, so early detection of liver cancer and improved management of metastases are both key strategies to reduce the death toll from liver cancer. Prostate-specific membrane antigen (PSMA) expression in the tumor-associated neovasculature of nonprostate malignancies including liver cancer has been reported recently, but conclusive evidence of PSMA expression based on the pathological type of liver cancer remains limited.

### AIM

To study the expression of PSMA in HCC, CCA, and liver cirrhosis.

### METHODS

A total of 446 formalin-fixed paraffin-embedded (FFPE) liver tumor and liver cirrhosis tissue samples were obtained retrospectively from the Pathology Department of Tongji Hospital. Immunohistochemistry was used to detect PSMA expression in these 446 FFPE liver biopsy specimens (213 HCC, 203 CCA, and 30 liver cirrhosis). The tumor compartment and the associated neovascular endothelium were separately analyzed. PSMA expression was examined by two certified pathologists, and the final results were presented in a 4-point scoring

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system (0-3 points). Correlation between PSMA expression and clinicopathological information was also assessed.

## RESULTS

PSMA was expressed primarily in the neovascular endothelium associated with tumors. The positive rate of PSMA staining in HCC was significantly higher than that in CCA (86.8% vs 79.3%;  $P = 0.001$ ) but was only 6.6% in liver cirrhosis ( $P = 0.000$ ). HCC cases had more 3-score PSMA staining than CCA had (89/213, 41.8% vs 35/203, 17.2%;  $P = 0.001$ ). PSMA expression correlated positively with the stage and grade of HCC and CCA. In both liver cancer subtypes, there were more PSMA<sup>+</sup> cases in stages III-V diseases than in stages I and II. High staining intensity of PSMA was more frequently observed in liver cancers at high grade and advanced stage. There was no significant association of PSMA expression with sex, age, region,  $\alpha$ -fetoprotein, hepatitis B surface antigen, or tumor size in both tumor subtypes.

## CONCLUSION

Neovascular PSMA may be a promising marker to differentiate HCC from liver cirrhosis and a prognostic marker for anti-tumor angiogenesis therapy for HCC.

**Key Words:** Prostate-specific membrane antigen; Hepatocellular carcinoma; Cholangiocarcinoma; Liver cirrhosis; Neovasculture; Immunohistochemistry

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**Core Tip:** Immunohistochemistry was used to detect prostate-specific membrane antigen (PSMA) expression in hepatocellular carcinoma (HCC), cholangiocellular carcinoma (CCA), and liver cirrhosis. PSMA is specifically expressed in tumor-associated vasculature in HCC and CCA. The positive rate of PSMA staining in HCC was significantly higher than that in CCA (86.8% vs 79.3%), meanwhile, it was only 6.6% in liver cirrhosis, thus the potential of using PSMA-targeted imaging to distinguish HCC from liver cirrhosis may be true. PSMA expression correlated positively with stage and grade both in HCC and CCA; high staining intensity of PSMA was more frequently observed in liver cancers at high grade and advanced stage.

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## INTRODUCTION

Primary liver cancer can be categorized according to its pathological characteristics into hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (CCA), and combined hepatocellular carcinoma (CHC)<sup>[1]</sup>. HCC accounts for 85%–90% cases of primary liver cancer, which is highly prevalent in China due to the epidemic of chronic hepatitis B. Most patients with primary liver cancer are diagnosed at advanced stages when treatment options are limited and subsequently experience poor prognosis and high mortality<sup>[1]</sup>. Therefore, early detection of liver cancer as well as improved management of metastases are both critical approaches to reducing the death toll from liver cancer.

Prostate-specific membrane antigen (PSMA), also known as folate hydrolase I or glutamate carboxypeptidase II, is a new biomarker that was initially defined by 7E11 immunoglobulin G monoclonal antibody<sup>[2]</sup>. PSMA is a 100 kDa transmembrane glycoprotein that can transduce extracellular signals into cytoplasm<sup>[3-6]</sup>. Originally found to be highly expressed in prostate cancer and high-grade intraepithelial neoplasia of prostate, PSMA has been extensively studied in recent decades for

prostate cancer imaging and theranostic applications<sup>[7]</sup>. For example, a large number of clinical trials have underpinned the advantage of PSMA-targeted radionuclide therapy for metastatic prostate cancer<sup>[8]</sup>.

Despite its nomenclature, PSMA expression is also observed in the neovasculature of a wide range of nonprostate cancers, including glioblastoma multiforme; esophageal, gastric, breast, ovarian, colorectal, lung, adrenal, hepatocellular, pancreatic, renal cell, bladder, and testicular germ cell carcinoma; malignant melanoma; mesothelioma tumor and malignant neoplasms of the thyroid<sup>[9-26]</sup>. Several case reports have shown that HCC, CCA, and CHC have high uptake of radiotracer in PSMA-targeted positron emission tomography (PET) imaging<sup>[20-23]</sup>. A recent prospective pilot study in seven HCC patients demonstrated that the HCC lesions are hypervascular with <sup>68</sup>Ga-PSMA-positive microvessels, suggesting that <sup>68</sup>Ga-PSMA PET is more suitable for imaging HCC patients than the conventional <sup>18</sup>F-fluorodeoxyglucose (FDG)-PET<sup>[24]</sup>. We recently compared PSMA-PET with FDG-PET in HCC imaging and found that PSMA-PET exhibited higher standardized uptake value in the tumor region and higher tumor-to-background ratios (Figure 1). In addition to the findings from noninvasive imaging, a pathological evaluation of 103 HCC specimens confirmed that PSMA was expressed on 74% of tumor-associated blood vessels. PSMA expression has oncogenic consequences, including an association with tumor stage, differentiation, lymph node metastasis, and Ki67 index<sup>[25]</sup>. High vascular expression of PSMA is correlated with poor prognosis, indicating that it is an independent prognostic factor for liver cancer and subsequently a target for antiangiogenic therapy<sup>[25]</sup>.

However, HCC is often accompanied with cirrhosis, which may acquire a nodular architecture with altered vascularity that resembles the regenerated nodules of early-stage HCC. As a result, the correlation between PSMA expression and the pathological classification of liver cancers remain elusive. In this retrospective study, we examined PSMA expression in 446 liver specimens (213 HCC, 203 CCA, and 30 cirrhosis) by immunohistochemistry (IHC), investigated the relationship between PSMA expression and clinicopathological findings, and discussed the potential of using PSMA-targeted imaging to distinguish HCC from liver cirrhosis.

## MATERIALS AND METHODS

### *Specimen collection, tumor grading, and patient information*

This study was approved by the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology (No. 2019-S951). Formalin-fixed paraffin-embedded liver tumor and liver cirrhosis tissue samples from hospitalized patients were obtained retrospectively from the Pathology Department of Tongji Hospital from January 2013 to December 2017. All samples were deidentified before analysis. A total of 446 liver specimens, including 213 HCC, 203 CCA, and 30 cirrhosis specimens, were studied. HCC and CCA were classified according to the World Health Organization and Edmondson pathological classification criteria as grade I (low), grade II (intermediate), and grade III (high)<sup>[1,26,27]</sup>. Patient characteristics and pathological features are summarized in Table 1.

### *IHC procedure*

IHC was performed as previously described<sup>[11]</sup>. PSMA was stained with an anti-PSMA rabbit monoclonal antibody (ab133579; Abcam, Cambridge, MA, United States; 1:250 dilution) on a Leica Bond-Max autostainer and visualized with the Bond Polymer Refine Detection System (Leica Biosystems Newcastle, Newcastle upon Tyne, United Kingdom). Vascular structures were confirmed by staining with an anti-CD31 rabbit polyclonal (ab28264; Abcam; 1:100 dilution). Primary antibody-null staining was used as a negative control. Prostatic adenocarcinoma specimens with confirmed PSMA expression and tonsil specimens were used as the positive controls for PSMA and CD31 staining, respectively (Figure 2). All specimens were routinely stained with hematoxylin and eosin to verify tumor morphology prior to IHC.

### *IHC evaluation*

The tumor compartment and the associated neovascular endothelium (ANVE) were separately analyzed on a minimum of three randomly chosen sections and observed at three different magnifications (40 ×, 100 ×, and 400 ×) *per* section. Protein expression was examined by two certified pathologists who were blinded to all the clinical data. Each pathologist assigned a score of 0 (no staining on any tumor cells or neovascular

Table 1 Clinicopathological features of liver tissues

Clinicopathological parameters		No. of cases (%)	
		HCC	CCA
Total		213	203
Gender	Male	185 (86.9)	112 (55.2)
	Female	28 (13.1)	91 (44.8)
Age of diagnosis	< 50	106 (49.8)	49 (24.1)
	≥ 50	107 (50.2)	154 (75.9)
Mean (range)		50 (19-85)	57 (41-78)
Region	Country	82 (38.5)	112 (55.2)
	Urban	131 (61.5)	91 (44.8)
AFP	< 400	134 (62.9)	-
	≥ 400	79 (37.1)	-
HBsAg	+	166 (77.9)	140 (69.0)
	-	47 (22.1)	63 (31.0)
Tumor size	< 5 cm	92 (43.2)	98 (48.3)
	≥ 5 cm	121 (56.8)	105 (51.7)
Stage	pT1	9 (4.2)	14 (6.9)
	pT2	73 (34.3)	105 (51.7)
	pT3	24 (11.3)	21 (10.3)
	pT4	107 (50.2)	63 (31.0)
Nodal status	N0	190 (89.2)	182 (89.7)
	N1	23 (10.8)	21 (10.3)
Metastasis	M0	188 (88.2)	182 (89.7)
	M1	25 (11.8)	21 (10.3)
UICC stage at diagnosis	I	16 (7.5)	14 (6.9)
	II	106 (49.8)	98 (48.3)
	III	28 (13.6)	21 (10.3)
	IV	63 (29.6)	70 (34.5)
Tumor grading	I	79 (37.1)	75 (36.9)
	II	76 (35.7)	72 (35.7)
	III	58 (27.2)	56 (27.6)

Data in parenthesis are percentages except the line of "mean". AFP:  $\alpha$ -fetoprotein; CCA: Cholangiocellular carcinoma; HBsAg: Hepatitis B surface antigen; HCC: Hepatocellular carcinoma; UICC: Union for International Cancer Control.

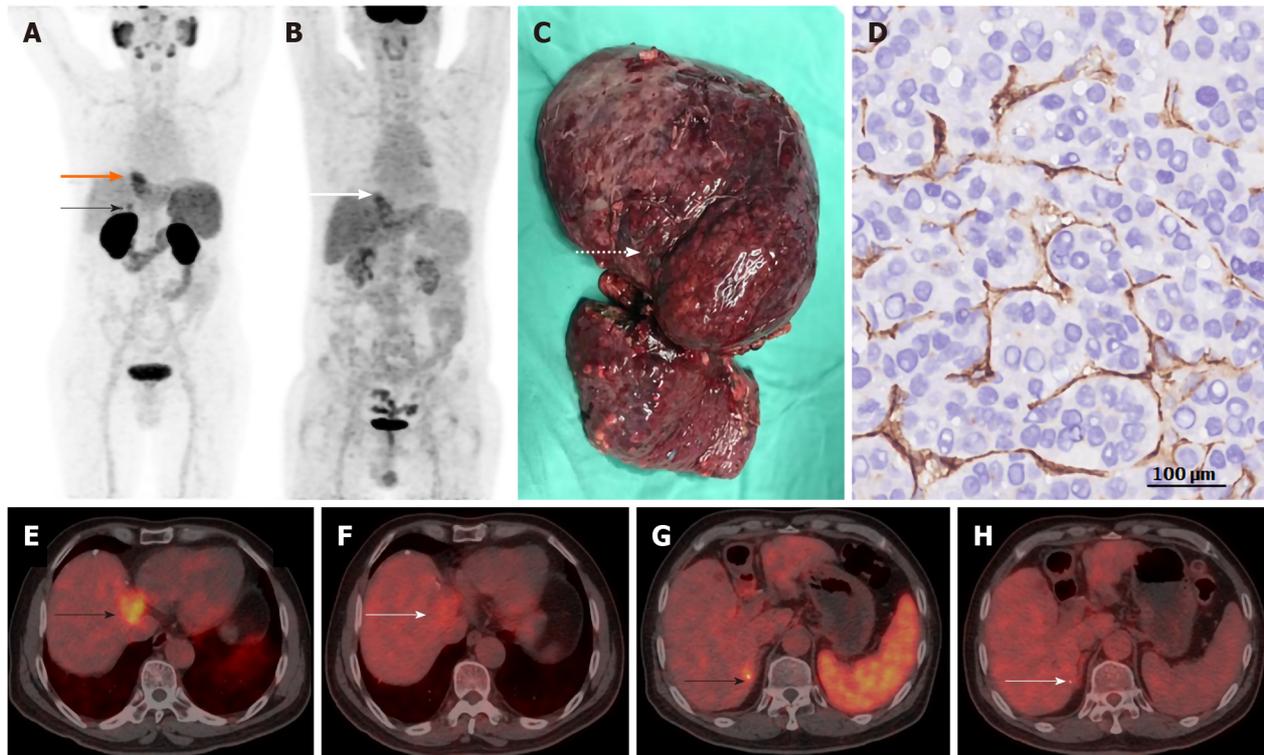
endothelium); 1 (low staining intensity in < 10% of tumor cells or ANVE); 2 (low staining intensity in 10%–50% of tumor cells or ANVE, or high staining intensity in ≤ 25% of tumor cells or ANVE); and 3 (low staining intensity in > 50% of tumor cells or ANVE, or high staining intensity in > 25% of tumor cells or ANVE) (Table 2)<sup>[11]</sup>. The two scores for each section were then averaged to give the final score. A consensus review was performed in case where there was substantial disagreement between the two pathologists.

### Statistical analysis

Data were analyzed using SPSS version 25.0 (SPSS, Armonk, NY, United States).  $P < 0.05$  was considered statistically significant. Quantitative data were expressed as mean  $\pm$  standard deviation. The  $\chi^2$  test was used to compare categorical variables.

Table 2 Standard for evaluation

Score	Stain intensity	Percent of vessels staining
0	None	0
1	Low	≤ 10%
2 (type 1)	Low	10%-50%
2 (type 2)	High	≤ 25%
3 (type 1)	Low	≥ 50%
3 (type 2)	High	> 25%



**Figure 1** Positron emission tomography imaging study on a 75-year-old man with hepatocellular carcinoma.  $^{18}\text{F}$ -Fludeoxyglucose (FDG) and  $^{68}\text{Ga}$ -prostate-specific membrane antigen (PSMA) positron emission tomography/computed tomography imaging was performed. A: Maximal intensity projection,  $^{68}\text{Ga}$ -PSMA revealed focal uptake [bold orange arrow, standardized uptake value (SUV)max: 7.6; black arrow, SUVmax: 5.7]; B: Maximal intensity projection,  $^{18}\text{F}$ -FDG revealed focal uptake (bold white arrow, SUVmax: 4.6), no uptake in right lesion (white arrow); C: Gross section displayed a nodule histologically classified as hepatocellular carcinoma; D: Strong PSMA expression (400 ×, immunohistochemistry, scale bar = 100 μm) was shown in the tumor-associated vascular; E and G: Transaxial fused,  $^{68}\text{Ga}$ -PSMA revealed focal uptake (bold black arrow, SUVmax: 7.6; black arrow, SUVmax: 5.7); F and H: Transaxial fused,  $^{18}\text{F}$ -FDG revealed focal uptake (bold white arrow, SUVmax: 4.6), no uptake in right lesion (white arrow).

Spearman's correlation coefficient (nonparametric) was used to determine the correlation between IHC scores and clinical variables.

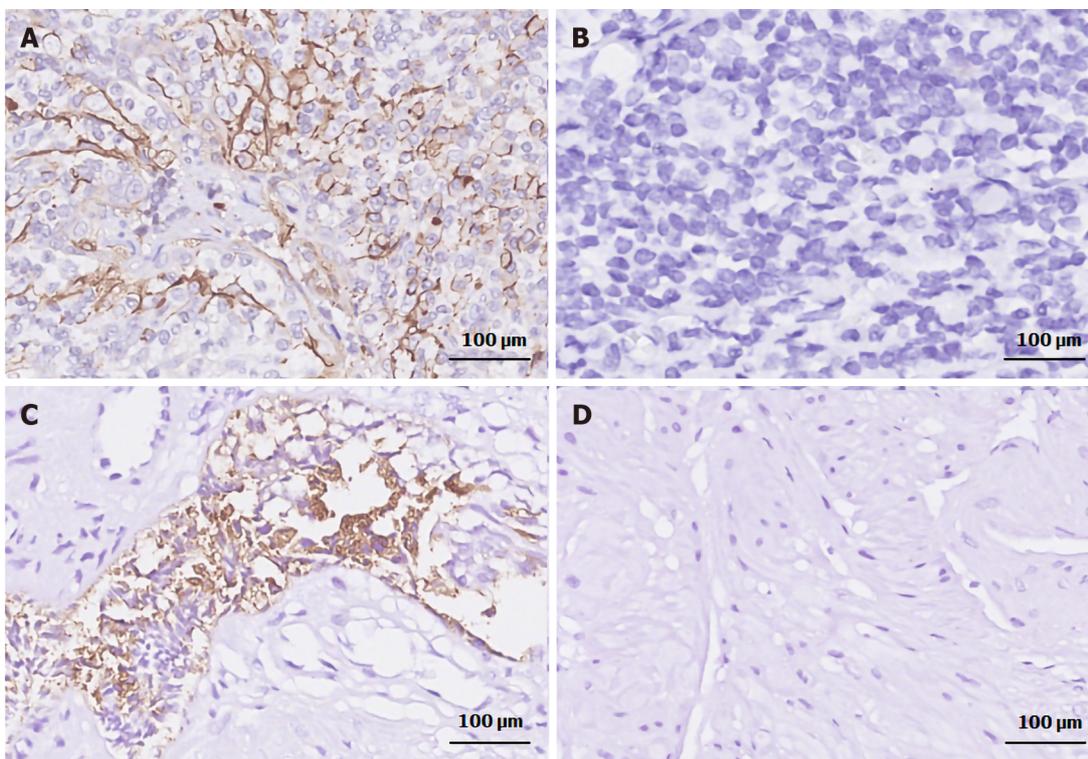
## RESULTS

PSMA was expressed in the tumor-associated neovascular endothelium that was also positively stained with the pan-endothelial marker CD31 (Figures 3 and 4). In contrast, blood vessels in the peritumoral normal tissues were exclusively CD31<sup>+</sup>, indicating that PSMA is a specific marker for the tumor-associated neovasculature. The percentage of PSMA<sup>+</sup> cases in HCC (185/213, 86.8%) and CCA (161/203, 79.3%) was 13- and 12-fold higher, respectively, than that in liver cirrhosis (2/30, 6.6%) ( $P < 0.0001$ , Table 3), while the percentage of PSMA<sup>+</sup> cases in HCC was significantly higher than that in CCA (86.8% vs 79.3%,  $P = 0.001$ ). There were more sections with a score of 3 for PSMA expression in HCC (89/213, 41.8%) than in CCA (35/203, 17.2%,  $P = 0.001$ ).

**Table 3** Cells and tumor-associated neovascular endothelial cells of liver cancers compared with liver cirrhosis

		Number	PSMA expression score, <i>n</i>				Positive staining, <i>n</i> (%)
			0	1	2	3	
HCC	Cells	213	187	8	16	2	26 (12.2)
	NECs	213	29	31	64	89	184 (86.4)
	Total	213	28	32	64	89	185 (86.8)
CCA	Cells	203	196	0	7	0	7 (3.4)
	NECs	203	42	42	84	35	161 (79.3)
	Total	203	42	42	84	35	161 (79.3)
Cirrhosis	Cells	30	28	2	0	0	2 (6.6)

CCA: Cholangiocellular carcinoma; HCC: Hepatocellular carcinoma; NECs: Neovascular endothelial cells.

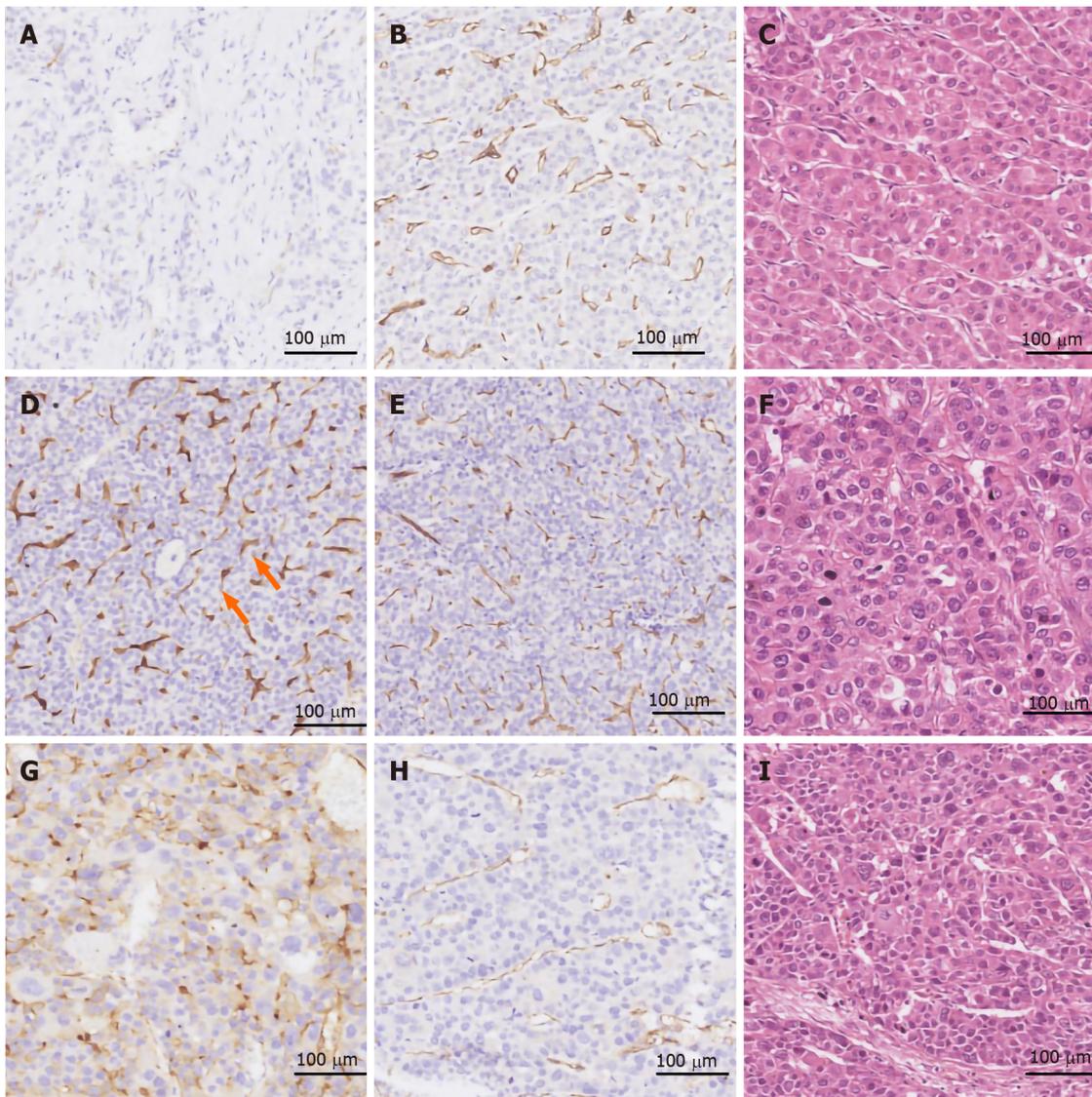


**Figure 2** CD31 staining and prostate-specific membrane antigen staining. A: Positive control, CD31 staining in human tonsils (400 ×, scale bar = 100 μm); B: Negative control, CD31 staining in human tonsils (400 ×, scale bar = 100 μm); C: Anti-prostate-specific membrane antigen (PSMA) positive control, PSMA staining in human prostate cancer tissues (400 ×); D: Anti-PSMA negative control, PSMA staining in human prostate cancer tissues (400 ×).

PSMA expression correlated positively with the stage and grade of HCC and CCA. In both liver cancer subtypes, stages III–V disease had more PSMA<sup>+</sup> cases than stage I and II had, while high staining intensity of PSMA was more frequently observed in liver cancers of high grade and advanced stage. There was no significant association of PSMA expression with sex, age, region, AFP, hepatitis B surface antigen (HBsAg), or tumor size.

#### ***IHC of PSMA expression in HCC***

Neovascular expression of PSMA was observed in 184/213 (86.4%) HCC cases, while no PSMA staining was found in normal vascular endothelial cells or peritumoral normal tissues. Among the 184 cases with PSMA<sup>+</sup> neovasculature, 31 (14.6%) had an expression score of 1, 64 (30.0%) a score of 2, and 89 (41.8%) a score of 3. In comparison, only 26/213 HCC cases had PSMA<sup>+</sup> tumor cells, with most of the staining in the cytoplasm and cell membrane. The PSMA staining score was 1 in eight (3.7%)



**Figure 3** Prostate-specific membrane antigen staining in representative tissues samples of hepatocellular carcinoma with magnification of 400 ×, scale bar = 100 μm. A: Weak prostate-specific membrane antigen (PSMA) staining (score = 1); B, E and H: The corresponding CD31 staining; C, F and I: The corresponding hematoxylin and eosin staining; D: Strong staining (score = 3); D and E: Vessel-like structures within the tumor (bold orange arrow) showed only PSMA staining but no CD31, D and E were from adjacent slides; G: Blood vessel staining and weak staining of cellular elements (score = 3).

cases, 2 in 16 (7.5%) cases, and 3 in two (0.9%) cases (Table 3 and Figure 3). Among these 26 cases, one case showed PSMA staining exclusively in tumor cells, while the remaining 25 cases had PSMA staining in both tumor cells and neovasculature. Furthermore, in 3/213 (1.4%) cases, positive PSMA staining of tumor cells was not accompanied by nearby CD31 expression, which may be attributed to tumor necrosis. In 2/213 cases, the vessel-like structures within the tumor compartment were exclusively stained with PSMA rather than CD31 (score of 3, Figure 3D and E).

PSMA expression correlated positively with stage (Spearman  $r = 0.226$ ,  $P = 0.001$ ) and grade (Spearman  $r = 0.224$ ,  $P = 0.004$ ) of HCC. Eighty-seven of 91 (95.5%) stage III and IV HCC cases were PSMA<sup>+</sup>, which was significantly higher than stage I and II HCC (97/122, 79.5%,  $P = 0.001$ ). There was a higher positive rate for PSMA expression in the neovasculature of grade III (high) HCCs (57/58, 98.2%) than in those of grade II (intermediate, 65/76, 86.5%) or grade I (low, 62/79, 78.4%,  $P = 0.004$ ) HCC cases. There was no significant association of PSMA expression with sex, age, region, alpha fetoprotein (AFP), HBsAg, or tumor size (Table 4).

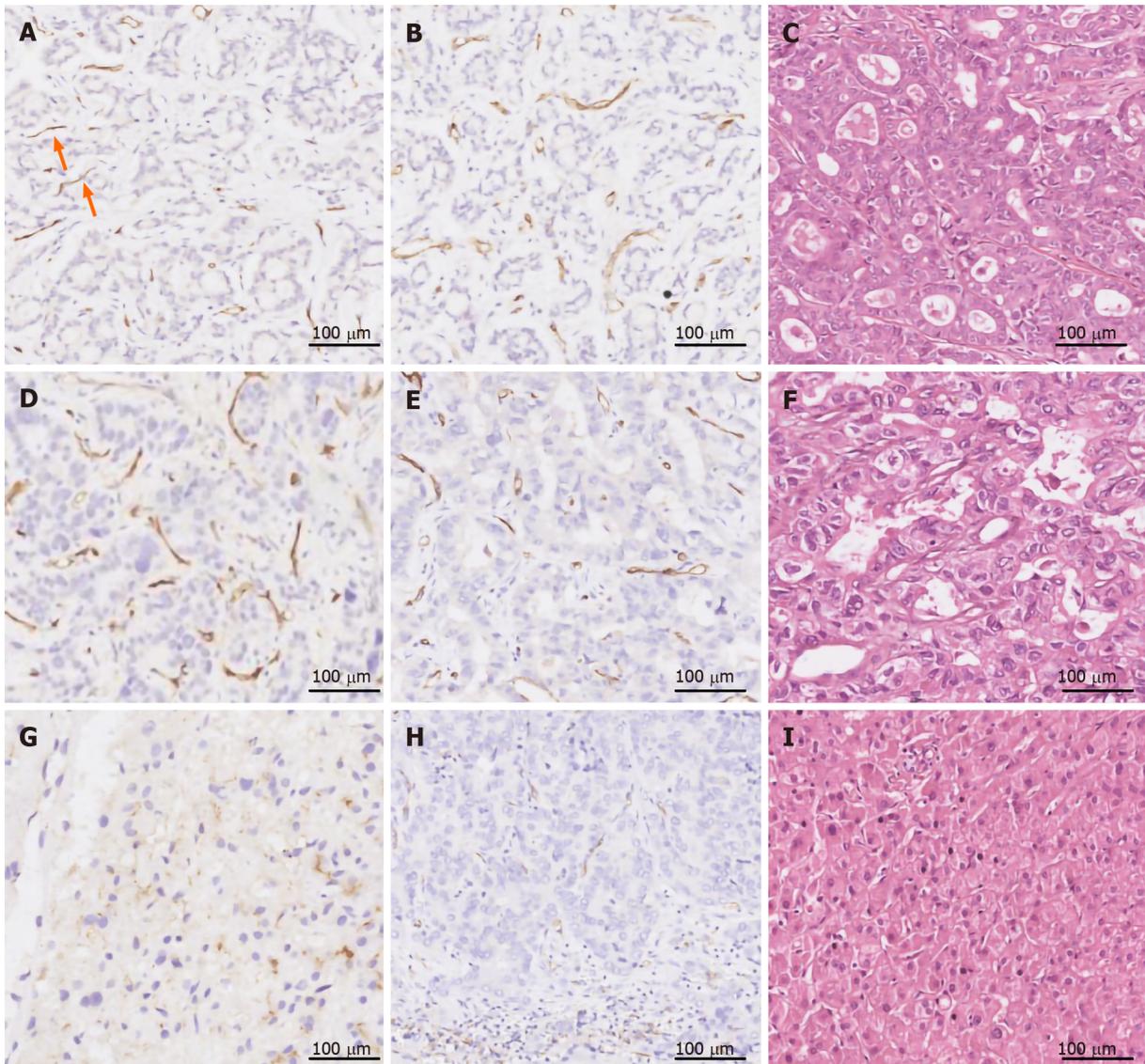
#### PSMA expression by IHC in CCA

Variable levels of PSMA expression were found in tumor neovasculature but in neither normal liver tissue nor peritumoral tissue (Table 3 and Figure 4). One hundred and sixty-one (79.3%) of 203 primary CCA cases were PSMA<sup>+</sup> in the tumor neovasculature,

**Table 4** Expression of prostate-specific membrane antigen in neovascularization of hepatocellular carcinoma and its relationship with clinicopathological parameters

Clinicopathological parameters	No. of cases	Tumor PSMA-positive, <i>n</i>				<i>P</i> value
		0	1	2	3	
Gender						
Male	185	25	27	52	81	0.912
Female	28	4	4	12	8	
Age of diagnosis						
< 50	106	12	14	29	51	0.331
≥ 50	107	17	17	35	38	
Mean (range)	50 (19-85)					
Region						
Urban	131	15	16	44	56	0.080
Country	82	14	15	20	33	
AFP						
< 400	134	21	18	38	57	0.254
≥ 400	79	8	13	26	32	
HBsAg						
+	166	24	23	49	70	0.990
-	47	5	8	15	19	
Tumor size						
< 5 cm	92	14	10	23	45	0.552
≥ 5 cm	121	15	21	41	44	
Stage						
pT1	9	1	2	4	2	0.812
pT2	73	19	15	18	54	
pT3	24	2	4	6	12	
pT4	107	7	10	36	21	
Nodal status						
N0	190	27	24	58	82	0.466
N1	23	2	7	6	7	
Metastasis						
M0	188	23	31	57	77	0.136
M1	25	6	0	7	12	
UICC stage at diagnosis						
I-II	122	25	17	37	43	0.001 <sup>3</sup> , <i>r</i> = 0.226
III-IV	91	4	14	27	46	
Tumor grading						
I	79	17	8	17	37	0.004 <sup>3</sup> , <i>r</i> = 0.224
II	76	11	7	25	33	
III	58	1	16	22	19	
All case	213	29	31	64	89	

<sup>a</sup> $P < 0.01$ . AFP:  $\alpha$ -fetoprotein; HBsAg: Hepatitis B surface antigen; PSMA: Prostate-specific membrane antigen;  $r$ : Spearman  $r$ ; UICC: Union for International Cancer Control.



**Figure 4** Prostate-specific membrane antigen staining in representative tissues samples of cholangiocarcinoma with magnification of 400  $\times$ , scale bar = 100  $\mu\text{m}$ . A: Weak prostate-specific membrane antigen (PSMA) staining (score = 1); A and B: Vessel-like structures within the tumor (bold orange arrow) showed staining exclusively for PSMA with no CD31 staining, A and B were from adjacent slides; B, E and H: The corresponding CD31 staining; C, F, and I: The corresponding hematoxylin and eosin staining; D: Strong staining (score = 3); G: Blood vessel staining and weak staining of cellular elements (score = 1).

among which, 42 cases (20.7%) had an expression score of 1, 84 (41.4%) a score of 2, and 35 (17.2%) a score of 3 (Table 3 and Figure 4). Seven (3.4%) cases had PSMA staining in both tumor cells (cytoplasm and cell membrane) and tumor-associated neovasculature endothelium, with an expression score of 2. Like HCC, one CCA case exhibited vessel-like structures within the tumor compartment that was weakly stained with PSMA (score = 1) but negative with CD31 staining (Figure 4A and B).

PSMA expression correlated positively with the stage (Spearman  $r = 0.211$ ,  $P = 0.002$ ) and grade (Spearman  $r = 0.253$ ,  $P = 0.001$ ) of CCA. Positive staining of PSMA was more frequent in stage III and IV CCAs (81/91, 89.0%) than in stage I and II CCA (80/112, 71.4%,  $P = 0.002$ ). There was a higher rate of positive staining for PSMA in the tumor neovasculature of grade III (high) CCA cases (53/56, 94.6) compared to that of grade II (intermediated, 57/72, 79.0%) or grade I (low, 51/75, 68.0,  $P = 0.001$ ). There was no significant correlation between PSMA expression and other clinicopathological features of CCA patients (Table 5).

**Table 5** Expression of prostate-specific membrane antigen in neovascularization of cholangiocellular carcinoma and its relationship with clinicopathological parameters

Clinicopathological parameters	No. of cases	Tumor PSMA-positive, <i>n</i>				<i>P</i> value
		0	1	2	3	
Gender						
Male	112	24	13	46	29	0.773
Female	91	18	29	38	6	
Age of diagnosis						
< 50	49	8	22	6	13	0.387
≥ 50	154	34	20	78	22	
Mean (range)	57 (41-78)					
Region						
Urban	91	16	23	47	5	0.325
Country	112	26	19	37	30	
HBsAg						
+	140	29	26	69	16	0.990
-	63	13	16	15	19	
Tumor size						
< 5 cm	98	23	27	30	18	0.178
≥ 5 cm	105	19	15	54	17	
Stage						
pT1	14	5	3	4	2	0.293
pT2	105	23	21	49	12	
pT3	21	5	5	7	4	
pT4	63	9	13	24	17	
Nodal status						
N0	182	36	39	74	33	0.346
N1	21	6	3	10	2	
Metastasis						
M0	182	37	40	76	29	0.709
M1	21	5	2	8	6	
UICC stage at diagnosis						
I-II	112	32	26	38	16	0.002 <sup>a</sup> , <i>r</i> = 0.211
III-IV	91	10	16	46	19	
Tumor grading						
I	75	24	18	28	5	0.001 <sup>a</sup> , <i>r</i> = 0.253
II	72	15	18	35	6	
III	56	3	6	21	5	
All case	203	42	42	84	35	

<sup>a</sup>*P* < 0.01. HBsAg: Hepatitis B surface antigen; PSMA: Prostate-specific membrane antigen; *r*: Spearman *r*; UICC: Union for International Cancer Control.

### PSMA expression by IHC in liver cirrhosis

CD31<sup>+</sup> blood vessels were observed in all 30 liver cirrhosis specimens (Figure 5). However, only two of 30 specimens showed weak PSMA staining in the cytoplasm and cell membrane of liver cells (score = 1). The remaining 28 specimens were PSMA<sup>+</sup> in either hepatocytes or vascular endothelium.

## DISCUSSION

HCC is the fourth most common malignancy and the third leading cause of tumor-related death in China, accounting for 85%-90% of all primary liver cancer cases<sup>[1]</sup>. Early radical intervention or effective management at late stage are both important strategies to reduce the death toll from HCC.

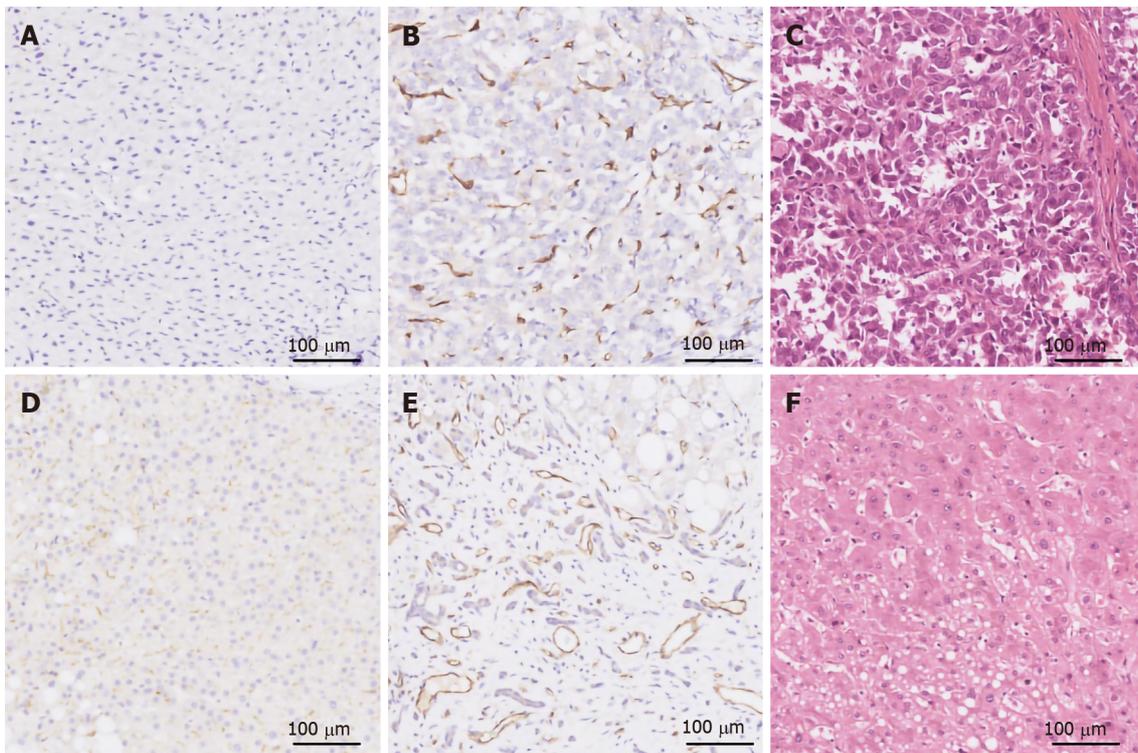
PSMA is a type II transmembrane glycoprotein that has attracted extensive attention due to its specific and high expression in prostate cancer cells. PSMA was first identified by Holmes *et al*<sup>[7]</sup> from a crude membrane extract of an androgen-dependent prostate cancer cell line LNCaP<sup>[7]</sup>. Other than tumor tissue, PSMA is also highly expressed in pancreatic islets and skeletal muscle, moderately expressed in brain and ganglia of gastrointestinal tract, and weakly expressed in prostate, endometrial glands, kidney tubules, and urinary bladder. No PSMA expression was observed in the liver, spleen, or other tissues<sup>[12]</sup>. In addition to prostate cancer cells, PSMA has previously been detected in the tumor-associated neovasculature of solid tumors including HCC<sup>[9-26]</sup>. Notably, PSMA is absent in blood vessels of normal tissue due to the lack of PSMA transcription enhancement regions<sup>[28,29]</sup>.

HCC is a highly vascularized tumor that is characterized by early angiogenesis. The hepatic artery is the main route to supply oxygen and nutrients to HCC, therefore making antiangiogenic therapy promising for HCC. In contrast, PSMA facilitates the invasion of endothelial cells during angiogenic sprouting and thereby supports tumor growth through provision of oxygen and nutrients<sup>[29,30]</sup>. As a result, targeted therapy against PSMA-expressing neovasculature represents a feasible option in treating rapidly growing solid tumors. Recently, several PSMA-targeted PET imaging studies reported high uptake of radiotracers in the tumor region of HCC, CCA, and CHC<sup>[20-22]</sup>. Kuyumcu *et al*<sup>[31]</sup> studied <sup>68</sup>Ga-PSMA PET imaging in 19 patients with liver cancer and found tumor uptake of radiotracers in 16 patients<sup>[31]</sup>. A multi-center phase II trial found that a PSMA-targeted therapy using an antiangiogenic drug mipsagargin led to long-term stable disease in patients with advanced liver cancer<sup>[32]</sup>. Magnetic resonance imaging after the mipsagargin treatment revealed a decrease in blood flow in liver lesions, confirming that PSMA plays an important role in liver cancer progression<sup>[32]</sup>. Jiao *et al*<sup>[25]</sup> found that PSMA was specifically expressed in the vasculature in 76 of 103 (74%) HCC specimens<sup>[25]</sup>. However, PSMA expression in liver cancer subtypes other than HCC remains to be elucidated.

Here, for the first time, we demonstrated that PSMA was expressed in the tumor-associated neovasculature of most HCC (86.8%) and CCA (79.3%) cases in a large sample set. PSMA expression was restricted to the neovasculature of HCC and CCA, while normal liver and peritumoral tissues were largely PSMA<sup>-</sup>. A few vessel-like structures in the tumor compartment was PSMA<sup>+</sup> but CD31<sup>-</sup>, suggesting that PSMA is a useful biomarker for early-stage tumor-associated angiogenesis. This temporal mismatch between PSMA and CD31 underscores the role of PSMA in the invasion of endothelial cells. It is worth mentioning that HCC (86.8%) exhibited a higher positive rate of PSMA staining than CCA (79.3%) did and that the HCC cases had more 3-score PSMA staining than CCA had (89/213, 41.7% *vs* 35/203, 17.2%). Therefore, PSMA could provide better diagnostic power in HCC than in CCA and functions as a valuable therapeutic target in HCC.

In some HCC and CCA cases, PSMA staining was observed in the cytoplasm and cell membrane of tumor cells, albeit with lower staining intensity than in tumor-associated neovasculature. Similarly, Nomura *et al*<sup>[10]</sup> found that < 2% of tumor cells were stained with PSMA in grade II and III glioma<sup>[10]</sup>. In contrast, Kesler *et al*<sup>[24]</sup> recently reported that three out of five HCC specimens had intense PSMA staining in intratumoral microvessels<sup>[24]</sup>. However, they did not observe any PSMA staining in the epithelial tumor cells. Such discrepancies in terms of PSMA expression can be attributed to the difference in sample size and biopsy locations.

Cirrhosis caused by viral hepatitis, especially type B and C, is the leading risk factor for HCC. The regenerated nodules of early-stage HCC are often indistinguishable from the accompanying cirrhosis, which makes ablative therapy more challenging. In our study, only two (6.7%) cases of liver cirrhosis showed weak PSMA staining in tumor



**Figure 5 Prostate-specific membrane antigen staining in liver cirrhosis with magnification of 400 ×, scale bar = 100 µm.** A: Liver cirrhosis showing no prostate-specific membrane antigen (PSMA) staining in blood vessels and hepatocytes (0 point); B and E: The corresponding CD31 staining; C and F: The corresponding hematoxylin and eosin staining; D: Liver cirrhosis showing no PSMA blood vessel staining with a score of 0 and light staining of cellular elements with a score of 1.

cell cytoplasm and cell membrane, with an expression score of 1. In contrast, the positive staining of PSMA was more frequent and with higher intensity in HCC and CCA. Therefore, our study proves that PSMA could be a useful biomarker to distinguish HCC from liver cirrhosis. Accordingly, PSMA-targeted PET imaging can potentially pinpoint the regenerated nodules of HCC.

In this study, PSMA expression correlated positively with the stage and grade of HCC and CCA, and stage III and IV disease tended to have higher positive rate of PSMA than stage I and II diseases. High PSMA expression was more likely to be found in the neovasculature of HCC and CCA with high grade or stage III or IV. There was no significant association of PSMA expression with sex, age, region, AFP, HBsAg, or tumor size in HCC and CCA. Jiao *et al*<sup>[25]</sup> reported that vascular PSMA expression correlated with tumor stage, tumor differentiation, lymph node metastasis, and Ki67 index<sup>[25]</sup>. They did not find any significant association between the vascular PSMA expression and age or sex, which was in accordance with our results.

## CONCLUSION

PSMA was expressed primarily in the tumor-associated neovascular endothelium of liver cancer. We discovered a potential role of PSMA-targeted imaging in the detection and staging of liver cancer patients, especially those with HCC. The PSMA-targeted imaging may also be useful to distinguish liver cancer from cirrhosis. As a result, PSMA-targeted approaches represent a feasible alternative to current antiangiogenic cancer therapy.

## ARTICLE HIGHLIGHTS

### Research background

Prostate-specific membrane antigen (PSMA) is a transmembrane glycoprotein expressed in the neovasculature of various nonprostate malignancies.

**Research motivation**

PSMA expression in the tumor-associated neovasculature of nonprostate malignancies including liver cancer has been reported, but conclusive evidence of PSMA expression based on the pathological type of liver cancers remains limited.

**Research objectives**

This retrospective study was performed to study the expression of PSMA in hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), and liver cirrhosis.

**Research methods**

Immunohistochemistry was used to detect PSMA expression in 446 formalin-fixed paraffin-embedded liver biopsy specimens (213 HCC, 203 CCA, and 30 liver cirrhosis).

**Research results**

PSMA was expressed primarily in the neovascular endothelium associated with tumors. The positive rate of PSMA staining in HCC was significantly higher than that in CCA.

**Research conclusions**

Neovascular PSMA may be used as a promising marker to differentiate HCC from liver cirrhosis and a prognostic marker for antitumor angiogenesis for HCC.

**Research perspectives**

Vascular PSMA may be used as a prognostic marker for anti-tumor angiogenesis therapy for HCC.

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