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Clinicopathological features and differential diagnosis of hepatocellular carcinoma in extrahepatic metastases

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Abstract

Extrahepatic metastasis of hepatocellular carcinoma (HCC) may cause a diagnostic problem. All 195 cases of histologic and immunostained sections were reviewed retrospectively in one center. The expression of arginase-1 (Arg-1), hepatocyte paraffin-1 (HepPar-1), glypican-3 (GPC-3), and α-Fetoprotein (AFP) was evaluated. Eighty cases of metastatic tumors of the liver were also collected to verify their effectiveness. Totally 151 cases had previous history of HCC, in whom 49 had history of liver transplantation. Forty-four cases were diagnosed as metastatic HCC at initial presentation. The most common extrahepatic metastatic sites were bone (57%), followed by lung, lymph node, etc. Around 19 cases were positive for 1 marker, 22 were positive for 2 markers, 95 were positive for 3 markers, and 59 were positive for 4 markers. With the number of antibody increased in the panel, the negative cases decreased. The sensitivity of ARG, GPC-3, HepPar-1, and AFP was 82.6%, 89.2%, 83.6% and 53.8%, and the specificity was 98.3%, 94.8%, 96.2% and 100%, respectively. These data suggest that the panel of ARG-1, GPC-3, HepPar-1 and AFP has a high sensitivity and specificity to differentiate HCC from non-HCC. This study indicated that HCC should be considered when diagnosing metastasis of unclear origin. It is recommended to use the panel of ARG-1, GPC-3, HepPar-1 and AFP to differentiate HCC from non-HCC in extrahepatic metastasis, because of their sensitivity and specificity, especially in poorly differentiated lesions.

Abbreviations: AFP = α-Fetoprotein, Arg-1 = arginase-1, GIST = gastrointestinal stromal tumor, GPC-3 = glypican-3, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, HepPar-1 = hepatocyte paraffin-1, NEC = neuroendocrine carcinoma, PEComa = perivascular epitheliod cell tumor.

Keywords: differential diagnosis, hepatocellular carcinoma, immunohistochemistry, metastases

1. Introduction

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related deaths, which is the third most common cause of cancer-related death and the fifth most common cancer all over the world, followed by lung and stomach cancers.[1] In China, HCC is the fourth most common cancer in male after pulmonary, stomach, esophagus, and sixth in female, which is the fourth cause of cancer death in male and female.[2] The burden of HCC has been increasing in the mainland of China, the high incidence of HCC in China is attributed to the high prevalance of hepatitis B virus (HBV) infection. Therefore, control of HBV and hepatitis C virus (HCV) infections may cause a significantly decreased incidence and mortality trend for HCC in China. Despite the declining trends for this group of cancers, population growth and aging still led to a large and rising number of new cases in 2015.[2,3]

Metastasis is a major cause for the death of HCC patient, with some cases present with metastatic carcinoma before primary liver tumor is found. In metastasis, differentiation of HCC from non-HCC may cause a diagnostic problem, because HCC may show a variety of histologic patterns, mimicking a wide variety of malignant tumors, in addition a number of metastatic tumors may mimic the trabecular, pseudoglandular and solid patterns of HCC. The single routine histopathology can not achieve the diagnosis, so immunostaining was used. Some markers were useful, such as AFP, HepPar-1, and GPC-3, ARG-1, etc.

AFP (α-fetoprotein) is a marker of hepatocellular differentiation, and can express in germ cell tumors (such as yolk sac tumor). AFP is an oncofetal protein produced by liver and yolk sac visceral endoderm.[4]

GPC-3 (glypican-3) is one of the glypican family of glycosylphosphatidylinositol- anchored cell surface heparan sulfate proteoglycans. It stains cytoplasm and/or membrane. Some studies have shown that GPC-3 may be a specific tumor marker to diagnose HCC.[5]

HepPar-1 (hepatocyte paraffin-1) is a mitochondrial urea cycle antigen linking mitochondrial antigens from both malignant and nonmalignant hepatocytes. It is a positive marker for hepatocyte differentiation on paraffin-embedded tissue, which has been used to verify hepatic differentiation. It can not differentiate benign from malignant hepatocyte and expresses poorly in HCC with low differentiation. Sometimes it can express in other tumors.[6]

ARG-1 (Arginase-1) is a binuclear manganese metalloenzyme that hydrolyzes arginine to ornithine and urea as a part of the

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urea cycle, which is specific for hepatocyte. ARG-1 can express in HCC with low differentiation and scirrhous HCC.[7]

Since these markers have also been reported in non-HCC,[8–10] Timek et al[11] recommended to use a panel to differentiate a non-HCC from HCC.

The aim of this study was to assess retrospectively the diagnostic accuracy of a panel of markers (ARG-1, HepPar-1, GPC-3, and AFP) for the diagnosis of extrahepatic metastatic HCC and to summarize the clinicopathological features of metastatic HCC.

2. Materials and methods

The current study was approved by the institutional review board at Peking University People's Hospital. A total of 195 cases, pathologically diagnosed as distant extrahepatic metastatic HCC were reviewed retrospectively from archived specimens during the period 2001 to 2017, accounting for about 12.2% (195/1592) of HCC diagnosed during the same period in our database. All medical records of relevant clinical, radiological, and laboratory data were collected to analyze. All available histologic and immunostained sections were reviewed according to the 2010 WHO classification.[12]

The immunohistochemical expression of ARG-1, HepPar-1, GPC-3, and AFP in 195 cases was evaluated. The information of antibodies and staining conditions are listed in Table 1. Paraffin section immunoperoxidase studies were performed manually on 4 μm deparaffinized, formalin-fixed sections. Immunohistochemical studies were performed by the 2-step EnVision procedure. Appropriate positive and negative controls were prepared. The staining result was assessed as negative (<5% of tumor cells stained) or positive. The immunostained slides were independently evaluated by 2 experienced pathologists (DC and LQ)

In order to verify the effectiveness of ARG-1, HepPar-1, GPC-3, and AFP in differentiating metastatic HCC from non-HCC, 80 cases of metastatic tumors of the liver were also collected, including adenocarcinomas of intestine, biliary duct, breast, kidney, ovary, pancreas, lung, gallblader (58 cases), and other tumors of neuroendocrine carcinoma (NEC), perivascular epithelioid cell tumor (PEComa), hemangiopericytoma (solitary fibrous tumor), gastrointestinal stromal tumor (GIST), osteosarcoma, panceatic solid pseudopapillary tumor, urothelial carcinoma, and squamous cell carcinoma (22 cases).

The SPSS software package version 17.0 for windows was used for all statistical analysis. Data were expressed as numbers and percentages. The data were compared for statistical significance by chi-square (χ^2) and Fischer exact probability tests, and different significance was considered as $P < .05$. Effectiveness of the antibodies was evaluated by sensitivity and specificity. The histopathologic diagnosis was considered as the gold standard.

Table 1

Antibodies used for immunohistochemistry in this study.

Antibody	Code no.	Dilution	Type	Company	Country
HepPar-1	OCH1E5	Ready to use	Monoclonal mouse	Dako	Denmark
AFP	EP209	1:100	Monoclonal rabbit	Dako	Denmark
GPC-3	1G12	1:200	Monoclonal mouse	Dako	Denmark
ARG1	EP261	1:100	Monoclonal rabbit	Dako	Denmark
TTF1	SPT24	1:200	Monoclonal mouse	Dako	Denmark
CDX2	EP25	1:100	Monoclonal rabbit	Dako	Denmark
PSA	EP109	1:100	Monoclonal rabbit	Dako	Denmark

AFP = α-fetoprotein, ARG-1 = arginase-1, CDX-2 = caudalhomeoboxfactor-2, GPC-3 = glypican-3, HepPar-1 = hepatocyte paraffin antigen-1, PSA = prostate specific antigen, TTF-1 = thyroid transcription factor-1.

3. Results

In 195 cases, 172 were males, and 23 were females (male-to-female ratio was 7.5:1). The age ranged from 10 to 78 years (mean: 53.0, median: 53.0). The features of the 195 patients were shown in Table 2. Totally 151 cases had previous history of HCC, in which 49 with history of liver transplantation. Forty-four cases were diagnosed as metastatic HCC at initial presentation, in which 3 cases had metastatic HCC with

Table 2

Clinicopathological characteristics of 195 extrahepatic metastases.

	Number	Percent
Sex		
Male	172	88.2
Female	23	11.8
Age range	10–78	
Mean	53.0 (52.98 ± 11.966)	
Median	53.0	
HBV		
+	152	82.2
−	33	17.8
HCV	11	5.6
HAV	1	0.5
Serum APF		
Elevated	102	64.6
Normal	56	35.4
Cirrhosis	133	68.2
Hepatic focal lesion		
Solitary	41	61.2
Multiple	26	38.8
Sites of metastases		
Bones	111	57.0
Lung	41	21.0
Abdomen	11	5.6
Omentum	7	3.6
Adrenal gland	7	3.6
Lymph node	5	2.6
Soft tissue	2	1.0
Pelvic cavity	2	1.0
brain	2	1.0
Intestine	2	0.5
Stomach	1	0.5
Retroperitoneum	1	0.5
Diaphragm	1	0.5
Ventriculus dexter	1	0.5
Kidney	1	0.5
Umbilical region	1	0.5
Growth pattern		
Trabecular (plate like)	19	9.7
Pseudoglandular (acinar)	1	0.5
Solid	88	45.1
Mixed	87	44.6
Grade of differentiation		
Well	3	1.5
Moderately	90	46.2
poorly	102	52.3
intrahepatic HCC		
Previous history of HCC	151	77.4
Liver transplantation	49	25.1
Coexisting	3	1.5
Initially present with extrahepatic metastases		
Subsequent HCC	41	21.0
Previous history of HCC	1	0.5

AFP = α-Fetoprotein, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HCV = hepatitis C virus.

coexisting intrahepatic masses, 41 cases without known liver primary tumors had no clinical features suggestive of HCC, which were confirmed by the detection of primary HCC subsequently. Their initial presentation was in the form of extrahepatic mass lesion. The symptoms caused by extrahepatic metastasis included pain or fracture caused by bone metastasis, dyspnea caused by multiple lung metastasis, nerve paralysis, and abdominal pain, etc. Totally 133 cases (68.2%) had cirrhosis. The hepatic lesions of 67 cases were focal, of which 41 were solitary (61.2%, 1.5–6 cm in diameter). The focal lesions of 26 cases (38.8%) were multiple (0.5–17 cm). HBV was positive in 152 cases, and 11 were positive for HCV and 1 for HAV. Serum AFP level was elevated in 102 patients (64.6%).

Bone was the most common site for extrahepatic HCC metastases (111 cases, 57.0%). The vertebrae were the most common site of bone (59 cases, 53.2%), followed by the sacrum (16 cases) (Fig. 1), femur (7 cases), pelvis (7 cases), scapula (5 cases), ribs (4 cases), sternum (3 cases), and ilium (3 cases). The sites of the rest cases were the clavicle, humerus, acetabulum (2 cases for each site), and pubis (1 case). Primary or secondary malignant tumors were considered in these cases by clinical and radiological features. (Fig. 2)

Lung metastasis (41 cases) was the second, followed by lymph node, abdomen, omentum, adrenal gland, soft tissue, pelvic cavity, brain, stomach, intestine, retroperitoneum, diaphragm, ventriculus dexter, kidney, and umbilical region (Table 2). A space-occupying lesion or a mass can be seen in radiography, CT scan or ultrasonic examination in these cases.

All metastases demonstrated malignant tumor cells composed of hepatocyte-like cells with mild to severe nuclear atypia and variable mitoses. The cells presented as trabecular, pseudoglandular sinusoidal or solid patterns, some cases showed mixed patterns, just like those of primary HCC (Figs. 3 and 4). In some cases, the tumor cells showed granular cytoplasmic positivity for HepPar-1 (Fig. 5), GPC-3 (Fig. 6), ARG-1 (Fig. 7), and/or AFP (Fig. 8), which accounted for 83.6%, 89.2%, 82.6%, and 53.8%, respectively.

Of 195 cases, 19 were positive for 1 marker, 22 were positive for 2 markers, 95 were positive for 3 markers, and 59 were positive for 4 markers. When 4 antibodies in the panel were used to differentiate metastatic HCC from other adenocarcinomas, there was no negative case, and at least 1 antibody expressed.

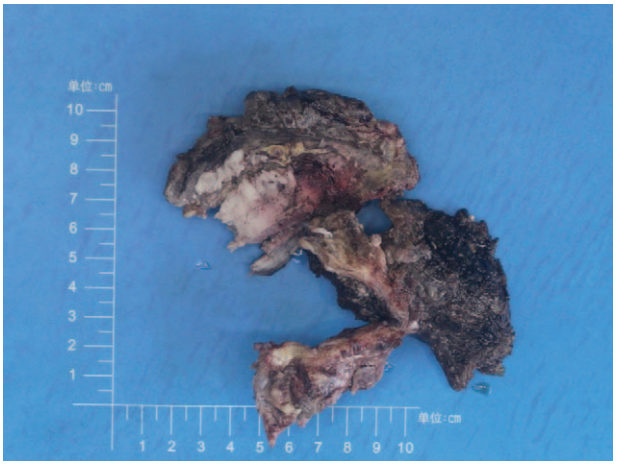

Figure 1. Metastatic HCC of the vertebra. Macroscopic view of a poorly circumscribed tan mass invading the bone. HCC = hepatocellular carcinoma.

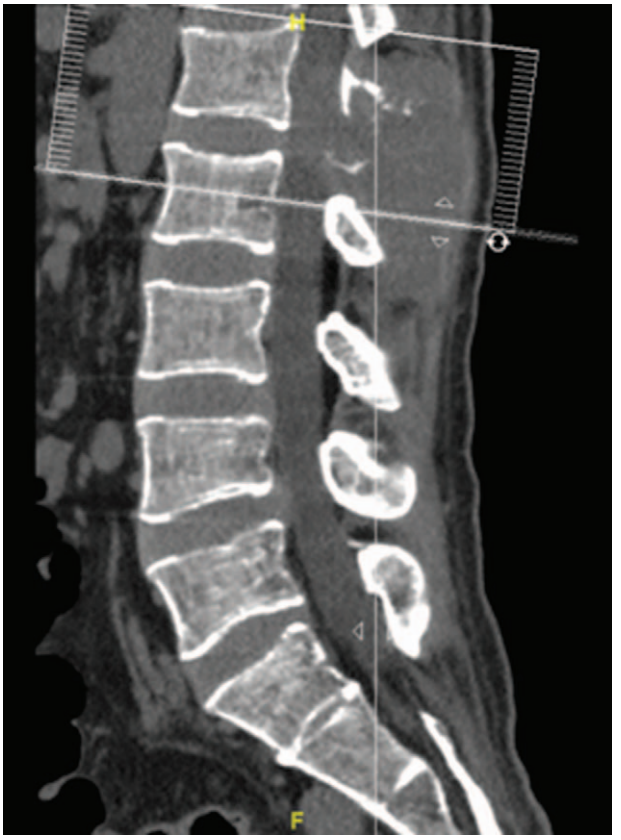

Figure 2. A metastatic mass from HCC infiltrating spinous process of first vertebra with lytic lesion (square) in computed tomography (sagittal reconstruction). HCC = hepatocellular carcinoma.

There was significant difference between expression in HCC and non-HCC for each antibody ($P < .01$). (Tables 3 and 4)

The correlation between expression of makers and different differentiation of HCC was also observed. The ratio of ARG-1, HepPar-1, GPC-3 expression in moderately to well differentiated HCC was higher than that of poorly differentiated HCC (90.5% vs 76.6%, 80.0% vs 74.8%, 92.9% vs 86.5%), whereas it was

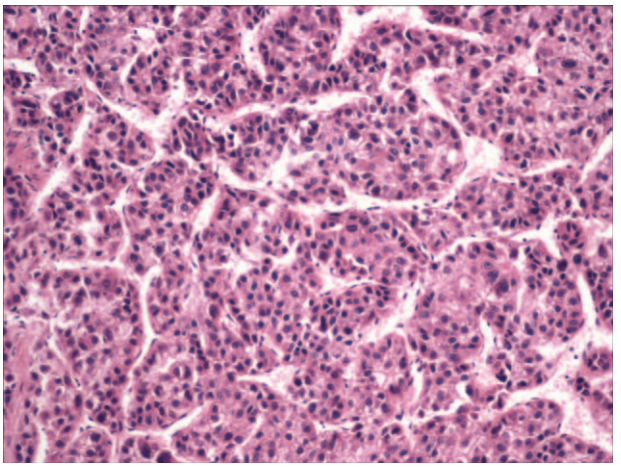

Figure 3. Moderately differentiated HCC with trabecular growth pattern of 3 or more cells in thickness. The tumor cells have abundant eosinophilic cytoplasm, with moderate atypia (HE staining ×100). HCC = hepatocellular carcinoma.

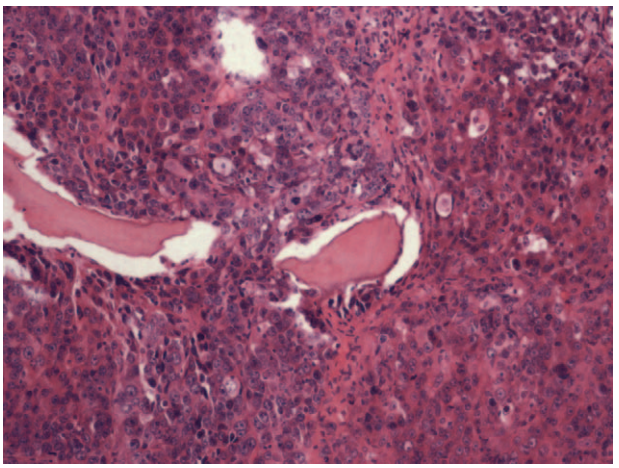

Figure 4. Specimen of the bone lesion shows histologic features of poorly differentiated HCC with solid and adenoid pattern. The tumor cells show marked atypia, with eosinophilic cytoplasm and an increased nucleus: cytoplasm ratio (HE staining ×200). HCC = hepatocellular carcinoma.

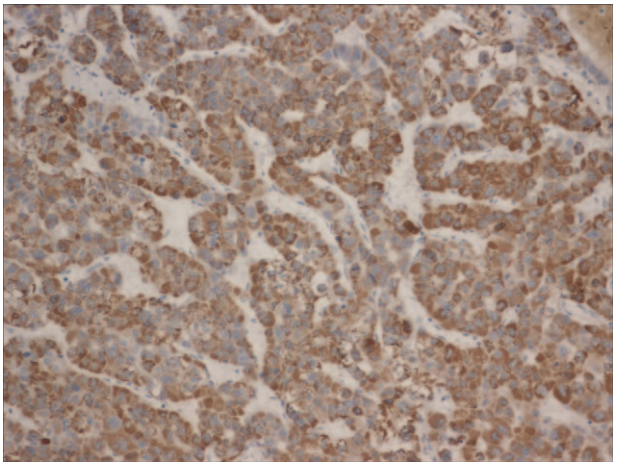

Figure 5. Diffuse HepPar-1 staining (cytoplasmic pattern) on metastatic hepatocellular carcinoma (immunohistochemical staining ×100). HepPar-1 = hepatocyte paraffin-1.

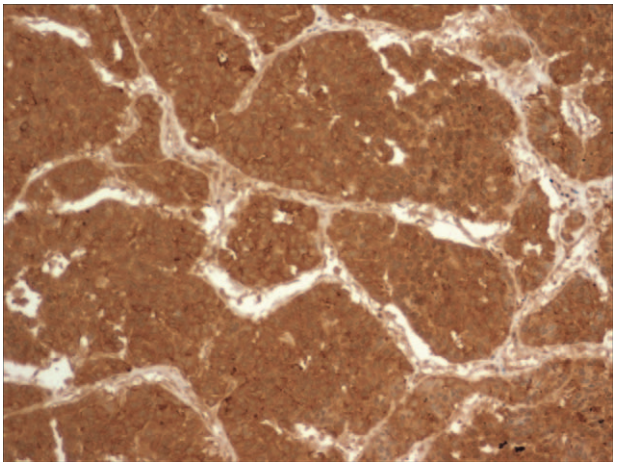

Figure 6. Diffuse GPC-3 staining (cytoplasmic pattern) on metastatic hepatocellular carcinoma (immunohistochemical staining ×200). GPC-3 = glypican-3.

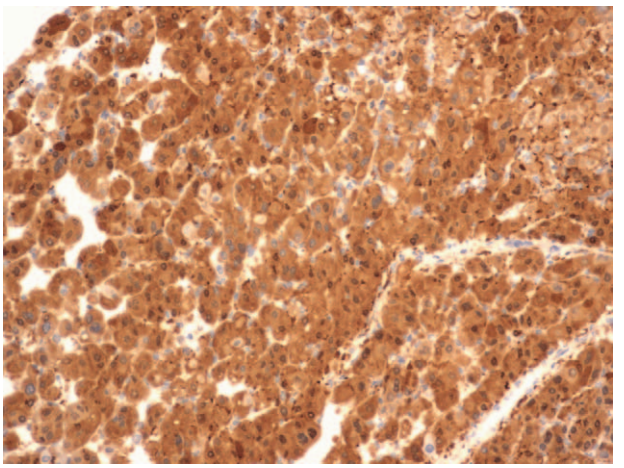

Figure 7. Diffuse ARG-1 staining (both cytoplasmic and nuclear pattern) on metastatic hepatocellular carcinoma (immunohistochemical staining ×100). Arg-1 = arginase-1.

contrary to AFP (41.7% vs 63.1%). When 4 markers were used, no case was negative in different differentiation, and at least 1 marker can be positive. (Table 4)

In order to distinguish HCC from adenocarcinoma from other site, CDX2, TTF1, and PSA were also detected in some cases, which were negative except that CDX2 were focal and weak positive in 3 cases.

The diagnostic sensitivity and specificity of ARG-1, GPC-3, HepPar-1, and AFP were calculated based on the combined numbers of metastatic HCC (195 cases) and collected non-hepatocellular adenocarcinomas (58 cases). The sensitivity of ARG-1, GPC-3, HepPar-1, and AFP was 82.6%, 89.2%, 83.6% and 53.8%, and the specificity was 98.3%, 94.8%, 96.2% and 100%, respectively, which were 100% and 96.3% in the panel (Table 5). Sensitivity and specificity for ARG-1, GPC-3, HepPar-1 were better, whereas sensitivity for AFP was not ideal, but its specificity was quite good. In 4 markers detected cases, 9.7% were positive for 1 marker, 11.3% for 2 markers, 48.7% for 3 markers, 30.3% for 4 markers, and no case was negative for

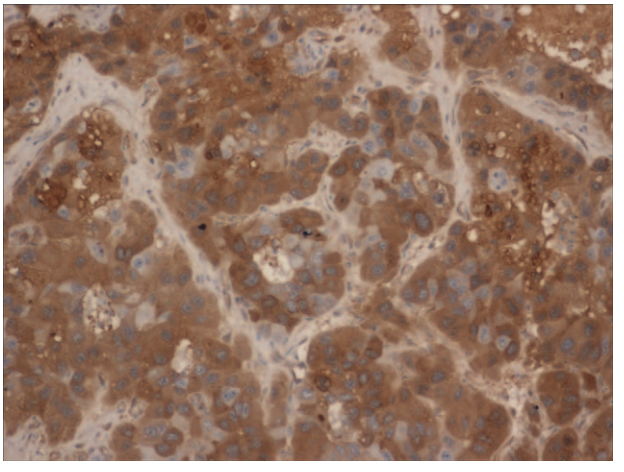

Figure 8. Diffuse AFP staining (cytoplasmic pattern) on metastatic hepatocellular carcinoma (immunohistochemical staining ×200). AFP = α-Fetoprotein.

Table 3

Antibodies used in differentiation of hepatocellular carcinoma in extrahepatic metastases.

Kinds of antibodies used in the panel	Number	Percent
4 Markers		
1/4+	19	9.7
2/4+	22	11.3
3/4+	95	48.7
4/4+	59	30.3
4/4−	0	0
HepPar-1		
+	163	83.6
−	32	16.4
AFP		
+	105	53.8
−	90	46.2
GPC-3		
+	174	89.2
−	21	10.8
ARG		
+	161	82.6
−	34	17.4
CDX2		
+	3	8.3
−	33	91.7
TTF1		
+	0	0
−	83	100
PSA		
+	0	0
−	17	100

AFP = α-Fetoprotein, Arg-1 = arginase-1, HepPar-1 = hepatocyte paraffin-1.

4 markers. It indicated that with the markers increased in the panel, the detection ratio was raised.

In 80 cases of intrahepatic metastatic non-HCC, ARG-1, and HepPar-1 showed focal positive in 1 cholangiocarcinoma,

Table 4

Immunostaining results of hepatocellular carcinoma with different differentiation.

	Type	
Marker	HCC with well to moderate differentiation (n = 84) (%)	HCC with moderate to poor differentiation (n = 111) (%)
ARG-1		
+	76 (90.5)	85 (76.6)
−	8	26
HepPar-1		
+	80 (95.2)	83 (74.8)
−	4	28
GPC-3		
+	78 (92.9)	96 (86.5)
−	6	15
AFP		
+	35 (41.7)	70 (63.1)
−	49	41
4 Markers		
1/4+	4 (4.8)	15 (13.5)
2/4+	4 (4.8)	18 (16.2)
3/4+	46 (54.8)	49 (44.1)
4/4+	30 (35.7)	29 (26.1)
4/4−	0	0

AFP = α-Fetoprotein, Arg-1 = arginase-1, GPC-3 = glypican-3, HepPar-1 = hepatocyte paraffin-1.

Table 5

Comparison of ARG-1, HepPar-1, GPC-3, AFP and combination of 4 markers expression between extrahepatic hepatocellular carcinoma and adenocarcinoma from other site.

Expression	Extrahepatic HCC	Non-HCC	X^2	*P* value	Sensitivity, %	Specificity, %
ARG-1						
+	161	1	126.85	<.001	82.6	98.3
−	34	57				
GPC-3						
+	174	3	150.3	<.001	89.2	94.8
−	21	55				
HepPar-1						
+	163	2	119.23	<.001	83.6	96.2
−	32	51				
AFP						
+	105	0	49.49	<.001	53.8	100
−	90	53				
4 Markers						
≥ 1 +	195	3	260.68	<.001	100	96.3
All −	0	77				

AFP = α-Fetoprotein, Arg-1 = arginase-1, GPC-3 = glypican-3, HepPar-1 = hepatocyte paraffin-1.

HepPar-1 was also focal positive in 1 gall bladder adenocarcinoma, GPC-3 was focal positive in 2 cholangiocarcinoma and 1 ovary high grade serous carcinoma, whereas AFP was negative in all cases. The expression of ARG-1, GPC-3, HepPar-1, and AFP in collected cases other than adenocarcinoma was negative (Table 6).

4. Discussion

The purpose of this study was: to characterize the clinicopathological features of extrahepatic HCC; to verify the specificity and sensitivity of Arg-1 and GPC-3 compared with HepPar-1 and AFP; to recognize the most effective panel of markers to differentiate extrahepatic HCC.

Table 6

Immunostaining results of ARG-1, HepPar-1, GPC-3, and AFP of non-hepatocellular carcinoma tumors*.

Tumor (number of cases)	ARG-1	HepPar-1	AFP	GPC-3
Cholangiocarcinoma (32)	1/32	1/30	0/26	2/28
ovary serous carcinoma (3)	0/3	0/3	0/2	1/2
Intestinal CA (8)	0/8	0/5	0/2	0/8
Breast CA (7)	0/7	0/7	0/4	0/5
Clear cell RCC (2)	0/2	0/2	0/1	0/1
Neuroendocrine neoplasm (7)	0/7	0/7	0/5	0/7
Hemangiopericytoma/solitary fibrous tumor (1)	0/1	0/1	0/1	0/1
Pancreatic adenocarcinoma (3)	0/3	0/3	0/2	0/2
Lung squamous CA (1)	0/1	0/1	0/1	0/1
GIST (3)	0/3	0/3	0/3	0/1
Osteosarcoma (1)	0/1			
urinary epithelial carcinoma (1)	0/1	0/1	0/1	0/1
Liver PEComa (4)	0/4	0/3	0/3	0/3
Panceatic solid pseudopapillary tumor (1)	0/1	0/1		0/1
Neuroendocrine CA (4)	0/4	0/4	0/4	0/3
Pancreatic adenosquamous CA (1)	0/1	0/1	0/1	0/1
Gall bladder CA (1)	0/1	1/1	0/1	0/1

CA = carcinoma, GIST = gastrointestinal stromal tumor, RCC = renal cell carcinoma, PEComa = perivascular epithelioid cell tumor.

* Data are given as positive/number of cases unless otherwise indicated.

The initial aim of this study was to characterize a relatively large number of patients who presented as extrahepatic metastases of HCC, some of which initially manifested as metastases before the primary HCC were confirmed. Most of such reported cases were described in case reports or case series except for 5 studies[13–16] (Table 7). The current study included 195 patients from China mainland, most of whom were HBV positive. The number of extrahepatic metastases constitutes about 12.2% of the total number of HCC cases in our hospital, more than those of the literature (5%-6.5%),[13–16] similar to Natsuizaka's report (13.5%).[17] The male-to-female ratio was 7.5:1 and the median age was 53.0 years in our study. Twenty Chinese cases presented with bone metastases were reported in a larger series.[16] Totally 251 patients with extrahepatic metastases initially diagnosed as HCC were studied in Korea.[14] The Egypt study used 5 antibodies to confirm the diagnosis and 47 HCC patients were HCV-related, but the cause of patients in Korean study was multiple, including HBV, HCV, alcohol, etc., similar to that of our study. Variable etiology of HCC may play a role in its metastatic behavior.

The clinical presentations of patients with extrahepatic metastatic HCC were mostly correlated with the manifestations of the primary tumor and the metastatic presentation were later event. Wu et al[18] and Natsuizaka et al[17] found that HCC patients present with extrahepatic metastatic pattern (lung, followed by bone, distant lymph and brain metastasis). HCC can spread to unusual sites. In the present study, the most common extrahepatic metastatic sites were bone (the most common site was the vertebrae, and the unusual site was pubis), accounting for 57% of the cases, followed by lung, contrasting to Wu's study[18] and Uchino's report,[19] in which lung (followed by bone) was the most common sites in Wu's study and lymph nodes, bone, and adrenal glands in Uchino's report, because there is a large Bone and Soft Tissue Center in our hospital. There were still unusual sites in our study, such as omentum, adrenal gland, soft tissue, brain, retroperitoneum, diaphragm, ventriculus dexter, and kidney. The survival outcome of cases with distant lymph metastases was best while that of cases with brain metastases was the worst in both OS and CSS analysis.[18]

The identification of the metastatic lesion before the primary HCC was diagnosed is an important finding, there were 41 cases presented initially with extrahepatic metastases, which were considered as primary or secondary malignant tumor clinically and radiologically, and were confirmed by the presence of primary HCC subsequently. Totally, 151 cases had primary HCC history, 49 of whom had received liver transplantation, and 3 cases had coexisting intrahepatic HCC.

When the patient has a history of primary HCC, it is easy to consider the metastasis as HCC. However, if there is no HCC history and no manifestation indicating HCC in extrahepatic metastasis, the diagnosis is difficult, especially in poorly differentiated tumor. On the basis of histopathology, hepatocyte like cells arranged in trabecular (plate like), pseudoglandular (acinar), solid or mixed patterns. In some instances, lack of typical features of classical HCC causes difficulty in the accurate diagnosis, thereby IHC is employed to identify metastatic HCC. Timek et al[11] recommended to use 3 markers as a panel in distinguishing HCC from metastatic carcinoma. In general, an immunohistochemical panel including ARG-1, HepPar-1, AFP and GPC-3, TTF-1, napsin-A, GATA3, CDX2, PAX5, PSA serves as a useful ancillary tool in the differential diagnosis between HCC and non-HCC in most metastatic cases. We used HepPar-1, Arg-1, GPC-3 and AFP in the panel of immunohistochemical markers applied for identification of the primary site of metastatic carcinoma.

In our study, AFP showed negative in non-HCC, whose specificity is quite good, and is a highly specific marker for HCC (100%), but its sensitivity is 54.2%, lower than those of the other markers. The sensitivity of ARG-1, GPC-3, HepPar-1 was 82.6%, 89.2%, 83.6%, and the specificity was 98.3%, 94.8%, 96.2%, respectively. The specificity of ARG-1 is better than those of GPC-3 and HepPar-1, but its sensitivity is worse than those of GPC-3 and HepPar-1, different from the report of Yan et al[10] The sensitivity and specificity of GPC-3 are lower than those of Ibrahim's report (96.7% and 100%).[9] The sensitivity of HepPar-1 was lower but its specificity is higher than that of Ibrahim's report (93.3% and 88.9%).[9] In HCC of various differentiation of our study, we found that when we use 3 markers, there were still 7 poorly differentiated HCC were negative for all 3 markers. The ratio of AFP expression in moderately to poorly differentiated HCC was higher than that of moderately to well differentiated HCC, which emphasized the importance of AFP in the diagnosis of poorly differentiated HCC. It indicated that we can increase the number of markers in the panel to improve the accuracy in identification of metastatic HCC. In our panel staining, 19 cases were positive for only 1 marker, and the diagnoses were based on their history of primary HCC, which emphasized the importance of more than 1 marker to identify HCC in metastasis. In poorly differentiated HCC, one or more markers maybe negative or focal, weakly positive. In rare instances, we should keep in mind that ARG-1, GPC-3, HepPar-1 may show focal or weakly positive in non-HCC, such as cholangiocarcinoma, high grade serous carcinoma, and gall bladder adenocarcinoma, etc. These findings reinforce the concept of using a diagnostic panel of these 4 markers to best

Table 7

Comparison of hepatocellular carcinoma initially presented as extrahepatic metastastatic lesions in the literature.

Authors	Publishing time	Number of cases	Age range (median)	Male/ Female	Growth pattern	Sites of metastases
Helal et al[26]	2015	47	40–80 (60)	38/9	Mostly mixed	Lymph nodes, bones, omentum, soft tissue, adrenal land, maxillary sinus, skin, brain
Yoo et al[27]	2011	251	18–85 (51)	212/39	Unclear	Lymph node, lung, adrenal gland, bones, others.
Uka et al[28]	2007	151	21–82 (64)	117/34	Unclear	Lymph nodes, lung, bones, peritoneum, adrenal gland, nasal passages, pancreas.
Liaw et al[29]	1989	20	26–64 (50)	16/4	Mostly trabecular	Ribs, spines, skull, pelvis, scapula, long bone, clavicle, sternum.
The present study	2017	41	10–75 (57)	34/7	Mostly Mixed	Bones, lung, abdomen, omentum, adrenal gland, pelvic cavity, brain, intestine, stomach, retroperitoneum, diaphragm, ventriculus dexter, kidney, unbilical region.

differentiate HCC from non-HCC in metastases. When adenocarcinoma of lung is considered, CK7, TTF1, and NapsinA should be added in the immunohistochemical panel, and CK20, CDX2 for differentiating colorectal carcinoma, villin for stomach, PSA for prostate, CK7, ER, PR, GATA-3 for breast, CK7, ER, WT1, PAX8 for ovary, CK, CD10, Vimentin, PAX8 for renal cell carcinoma, and synaptophysin and/or chromogranin for NET. Timek et al[11] recommended to use ARG-1, GPC-3, HepPar-1 as a panel in distinguishing HCC from non-HCC in liver metastases. The high sensitivity and specificity of Arg-1, GPC-3, and HepPar-1 make them the first choice for demonstrating hepatocellular differentiation. We propose increasing AFP in the panel to improve the specificity of differentiation. Choi et al[20] do not recommend an AFP stain in diagnosing HCC, because AFP has a low sensitivity of 30% to 50% for HCC, and its staining tends to be patchy with high background staining. But we suggest to use AFP in the panel because its high specificity, especially in poorly differentiated HCC.

These data suggest that a panel of ARG-1, GPC-3, HepPar-1, and AFP has a high sensitivity and specificity to differentiate HCC from non-HCC. We recommend the most effective 4 markers as a panel to differentiate HCC from non-HCC in extrahepatic metastases. We can also analyze gene expression and genome to classify tumor origin by molecular methods, especially in poorly differentiated tumor. However, it is infeasible for most cases in routine practice because of the expenses and time.

The unique of this work is trifold: firstly, characterize a relatively large number of patients who presented as ertrahepatic metastases of HCC, some of which initially manifested as metastases before the primary HCC were diagnosed. Also there were more cases who had HCC history, some of whom had received treatment with liver transplantation. Secondly, the present research was about Chinese cases; most of whom were HBV-positive. Extrahepatic metastases of HCC are not rare, and major metastatic organs are the bone, lung, abdomen. Thirdly, an effective imunostaining panel to differentiate HCC from non-HCC in extrahepatic metastases is proposed. It seems that there are no such documented reports in the databases of literature that we have searched.

In summary, the present research emphasizes that metastatic HCC should be put into consideration when evaluating metastatic carcinoma with unclear origin. The most common extrahepatic metastatic sites are bone, lung and abdomen. It is recommended to use a panel of ARG-1, GPC-3, HepPar-1, and AFP to differentiate HCC from non-HCC in extrahepatic metastasis, because of their sensitivity and specificity, especially in poorly differentiated lesions.

Author contributions

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References

[1] Dhanasekaran R, Limaye A, Cabrera R. Hepatocellular carcinoma: current treads in worldwide epidemiology, risk factors, diagnosis, and therapeutics. Hepat Med 2012;4:19–37.
[2] Chen WQ, Zheng RS, Baade PD, et al. Cancer statistics in China, 2015. Ca Cancer J Clin 2016;66:115–32.
[3] Chen JG, Zhang SW. Liver cancer epidemic in China: past, present and future. Semin Cancer Biol 2011;21:50–69.
[4] Kakar S, Gown AM, Goodman ZD, et al. Best practices in diagnostic immunohistochemistry: hepatocellular carcinoma versus metastatic neoplasms. Arch Pathol Lab Med 2007;131:1648–54.
[5] Yasuda E, Kumada T, Toyoda H, et al. Evaluation for clinical utility of GPC3, measured by a commercially available ELISA kit with glypican-3 (GPC3) antibody, as a serological and histological marker for hepatocellular carcinoma. Hepatol Res 2010;40:477e85.
[6] Shafizadeh N, Ferrell LD, Kakar S. Utility and limitations of glypican-3 expression for the diagnosis of hepatocellular carcinoma at both ends of the differentiation spectrum. Mod Pathol 2008;21:1011–8.
[7] Nguyen T, Phillips D, Jain D, et al. Comparision of 5 immunohistochemical markers of hepatocellular differentiation for the diagnosis of hepatocellular carcinoma. Arch Pathol Lab Med 2015;139:1028–34.
[8] Shirakawa H, Kuronuma T, Nishimura Y, et al. Glypican-3 is a useful diagnostic marker for a component of hepatocellular carcinoma in human liver cancer. Int J Oncol 2009;34:649–56.
[9] Ibrahim TR, Abdel-Raouf SM. Immunohistochemical study of Glypican-3 and HepPar-1 in differentiating hepatocellular carcinoma from metastatic carcinomas in FNA of the Liver. Pathol Oncol Res 2015; 21:379–87.
[10] Yan BC, Gong C, Song J, et al. Arginase-1: a new immunohistochemical marker of hepatocytes and hepatocellular neoplasms. Am J Surg Pathol 2010;34:1147–54.
[11] Timek DT, Shi JH, Liu HY, et al. Arginase-1, HepPar-1, and Glypican-3 are the most effective panel of markers in distinguishing hepatocellular carcinoma from metastatic tumor on fine-needle aspiration specimens. Am J Clin Pathol 2012;138:203–10.
[12] Bosman FT, Carneiro F, Hruban RH, et al. WHO Classification of Tumours of the Digestive System. 2010;IARC Press, Lyon:205–216.
[13] Helal TE, Radwan NA, Shaker M. Extrahepatic metastases as initial manifestations of hepatocellular carcinoma: an Egyptian experience. Diagno Pathol 2015;10:82.
[14] Yoo DJ, Kim KM, Jin YJ, et al. Clinical outcome of 251 patients with extrahepatic metastasis at initial diagnosis of hepatocellular carcinoma: does transarterial chemoembolization improve survival in these patients? J Gastroenterol Hepatol 2011;26:145–54.
[15] Uka K, Aikata H, Takaki S, et al. Clinical features and prognosis of patients with extrahepatic metastases from hepatocellular carcinoma. World J Gastroenterol 2007;13:414–20.
[16] Liaw CC, Ng KT, Chen TJ, et al. Hepatocellular carcinoma presenting as bone metastasis. Cancer 1989;64:1753–7.
[17] Natsuizaka M, Omura T, Akaike T, et al. Clinical features of hepatocellular carcinoma with extrahepatic metastases. J Gastroenterol Hepatol 2005;20:1781–887.
[18] Wu WR, He XK, Andayani D, et al. Pattern of distant extrahepatic metastases in primary liver cancer: a SEER based study. J Cancer 2017;8:2312–8.
[19] Uchino K, tateishi R, Shiina S, et al. Hepatocellular carcinoma with extrahepatic metastasis. Cancer 2011;117:4475–83.
[20] Choi WT, Ramachandran R, Kakar S. Immunohistochemical approach for the diagnosis of a liver mass on small biopsy specimens. Hum Pathol 2017;63:1–3.