Int J Clin Exp Pathol 2016;9(7):7748-7754
www.ijcep.com /ISSN:1936-2625/IJCEP0028344

Original Article

Value of the combined detection arginase-1 and glypican-3 expression in distinguishing hepatocellular carcinoma from metastatic tumors

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Received March 16, 2016; Accepted May 26, 2016; Epub July 1, 2016; Published July 15, 2016

Abstract: Background: Hepatocellular carcinoma (HCC) is one of the most heterogeneous malignancies with poor prognosis of human beings. Arginase-1 (Arg-1) and glypican-3 (GPC-3) was separately specific markers for HCC, however, the value of Arginase-1-Glypican-3 (Arg1-GPC3) combined staining in distinguishing hepatocellular carcinoma from metastatic tumor have never been studied in surgical specimens. Methods: Expression of Arg-1 and GPC-3 are measured by immunohistochemistry, including 78 HCCs, 34 metastatic tumors and 228 nonhepatocellular tumors of surgical specimens. Results: The overall sensitivity of Arg-1 and GPC-3 in HCC was 96.1% (75/78) and 64.1% (50/78), respectively; Arg-1 was more sensitivity in well 100% (15/15) and moderately 100% (15/15) differentiated HCC than poorly 86.0% (19/22) differentiated HCC. In contrast, GPC-3 was more sensitivity in poorly 81.8% (18/22) differentiated HCC compared with well 46.70% (7/15) and moderately 61.0% (25/41) differentiated HCC. Single of Arg-1 or GPC-3 positive demonstrated superior sensitivity (98.7%) compared with Arg-1 (96.1%) or GPC-3 (64.1%) alone, and the specificity for both of Arg-1 and GPC-3 in distinguishing HCC from metastatic tumors and nonhepatocellular tumors was better (100%) than that of Arg-1 (99.6%) or GPC-3 (95.4%) alone, none of metastasis and nonhepatocellular tumors had expressed both of Arg-1 and GPC-3. Conclusion: Arg-1 has higher sensitivity and specificity for HCC compared with GPC-3, but the combination of Arg1-GPC3 has better specificity than dose single staining in differentiated diagnosis of HCC from metastasis tumors.

Keywords: Arg-1, GPC-3, Arg1-GPC3 combination, hepatocellular carcinoma, metastatic tumors, nonhepatocellular tumors, immunohistochemistry

Introduction

Hepatocellular carcinoma (HCC) is one of the most heterogeneous malignancies with poor prognosis of human beings. Previous evidence has suggested an estimated 782,500 new liver cancer cases and 745,500 deaths occurred worldwide during 2012, with a high prevalence of HCC in developing countries including China [1, 2]. Although significant advances have been made in HCC research in the past several decades, including dynamic contrast-enhanced magnetic resonance imaging, computed tomography, and serum α-fetoprotein levels, the efficacy for the accurate diagnosis of HCC was still undesirability, particularly in the early stage of HCC and discrimination between primary HCC and metastatic tumor of liver. Several treatment modalities available for HCC were not so effective for none-hepatocellular carcinoma of the liver [3, 4]. Therefore, the discovery of novel biomarkers is urgently required for the HCC diagnosis.

In general, some specific markers of the liver have been widely used in the diagnosis of HCC, such as hepatocyte paraffin antigen (HepPar-1), polyclonal carcinoembryonic antigen (CEA), α-fetoprotein (AFP) and CD10 [5-8], losing of the antigen of these markers in portion of poorly differentiated HCC and expressing in nonhepatocellular tumors, false positive or false negative will give the wrong guide in the diagnosis [9-17]. Until recently, some novel biomarkers were employed for the HCC. Among these, arginase-1 (Arg-1), a specific marker for hepatocyte, hardly expressing in nonhepatocyte, is recently recognized as a useful diagnostic marker in the

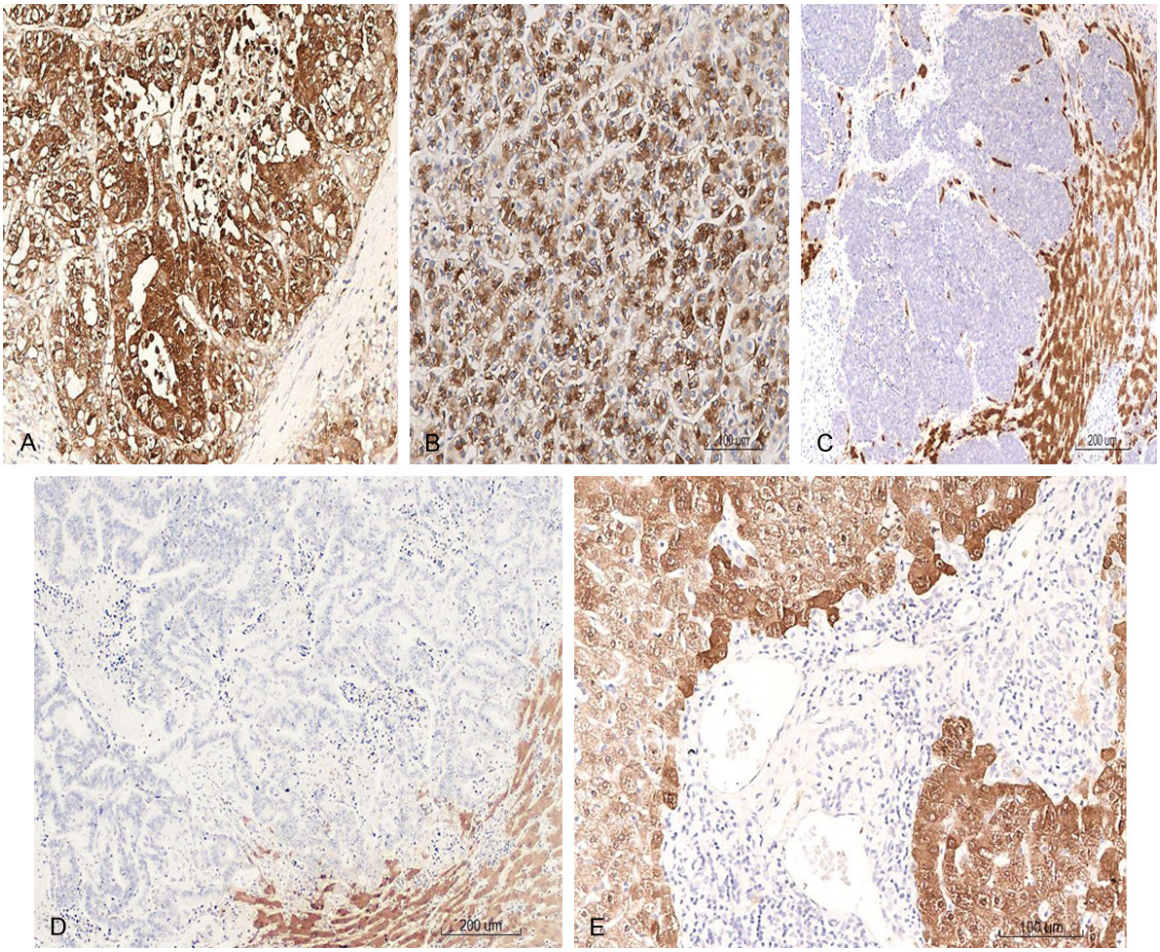

Figure 1. A. Arg-1 staining shows intense cytoplasmic and nuclear staining (original magnification, ×40), B. GPC-3 staining shows intense cytoplasmic staining (original magnification, ×40), C. Arg-1 negative expression in neuroendocrine carcinoma of stomach transferring to the liver, Arg-1 positive expression in normal liver cell (immunohistochemistry; original magnification, ×20), D. Arg-1 negative expression in colonic adenocarcinoma transferring to the liver, Arg-1 positive expression in normal liver cell (immunohistochemistry; original magnification, ×20), E. Negative control for Arg-1 with interlobular duct (original magnification, ×20).

differential diagnosis of HCC from metastatic tumors [16, 18]. And Glypican-3 (GPC-3), a member of the glypian family, a group of heparan sulfate proteoglycans linked to the cell surface through a glycosyl-phosphatidylinositol anchor is another promising biomarker for HCC [19]. However, increased studies indicated that different expression panel may exist between Arg-1 and GPC-3 in the diagnosis of HCC. For example, GPC-3 express can be found in HCC, but not in normal liver cells, cirrhosis and hepatic adenoma, and less expressing in non-hepatocyte [20]; Whereas Arg-1 expressed in normal hepatocyte, well/moderately differentiated HCC, but limited in poorly differentiated HCC; in contrast, GPC-3 expressed in poorly differentiated HCC more than well and moderately differentiated HCC. Although Arg-1 or GPC-3 has been reported in HCC, inadequate samples especially in tumors full of heterogeneity cannot reach a precise diagnosis, Several literatures showed Arginase-1 (Arg-1) was a specific marker for hepatocyte, and glypican-3 (GPC-3) was a specific marker for HCC, these studies did in the small samples size by tissue microarray (TMA) or fine-needle aspiration (FNA) biopsy specimens, however a more precise diagnosis cannot be reached via inadequate samples especially in tumors full of heterogeneity. Taken together, the value of Arg1-GPC3 combined staining need be necessary in surgical specimens.

In this study, we compared the expression of these 2 markers (Arg-1, GPC-3) in HCC, metastatic tumors and nonhepatocellular tumors in

surgical specimens, and we also estimated the value of Arg1-GPC3 combined staining, and determined the diagnostic utility of Arg1-GPC3 immunohistochemistry in distinguishing these tumors.

Materials and methods

Tissue specimens

All of the cases in this study were described previously [21]. Briefly, all 340 formalin-fixed, paraffin-embedded cases including 78 HCC, 34 metastatic tumors and 228 none hepatocellular tumors, were employed from the Department of Pathology in the First Affiliated Hospital of Xinjiang Medical University during 2006-2012. Among the 78 HCC cases, there were 54 male and 24 female, respectively, with an average age of 49 years (range from 35-78 years), and the average size of the main lesion was 3.96 CM (range from 1.2-17.4 CM) in maximum diameter. According to the latest diagnostic criteria of World Health Organization (WHO), There were 15, 41 and 22 for well, moderately and poorly differentiated of HCC, respectively. Furthermore, there were 34 cases of metastatic tumors and 228 cases of nonhepatocellular included in this study.

Information of the whole HCC cases was extracted based on the criteria from the CAP website data (CAP Home > CAP Reference Resources and Publications > Cancer > Cancer Protocols and Checklists). And written informed consent was obtained from all of the participants. All specimens were handled and approved by the hospital's ethics committee.

Reagents and immunohistochemistry

The main antibodies Arg-1 and GPC-3 immunoreactivity were detected by immunohistochemistry. The brief characteristics of these two antibodies were as follows: Arg-1 (polyclonal antibody C60725; 1:3000; cytoplasm/nucleus; Sigma); GPC-3 (polyclonal antibody PA532432; 1:60; cytoplasm; Phermo). Experimental procedures were performed as previously described [22, 23]#. Briefly, serial 5-μm sections from FFPE tissues were collected onto poly-L-lysine coated slides and processed with a standard manual streptavidin peroxidase technique using a biotin free detection system (Dako, Colorado, USA) after a heat-induced antigen retrieval procedure. Appropriate positive and negative controls were routinely performed. Additionally, some biomarkers were routinely used for diagnosing purposes, for example, AFP (polyclonal antibody ZSA06; 1:150; cytoplasm; Zhongshan); HepPar-1 (polyclonal antibody OCH1E5; 1:50; cytoplasm; Dako); CK8 (polyclonal antibody 7S1; ready to use; cytoplasm; MAIXIN); CK18 (polyclonal antibody DC10; ready to use; cytoplasm; MAIXIN); CK7 (polyclonal antibody OV-TL; 1:100; cytoplasm; GENE); CK19 (polyclonal antibody A53-B/A2.26; ready to use; cytoplasm; MAIXIN).

Scoring of immunostaining

Intensity and cellular percentages (HSCORE system) of immunohistochemical scores were assessed blindly by two independent senior pathologist (Qiaoxin Li and Xinxia Li), and any disagreements were resolved with a third reviewer (Wei Zhang). More than 5% of the target cells with immunostaining would be considered positive finding. The intensity of immunostaining was scored on a 4-tiered scale (0 to 3+), using the following semi-quantitative scale: 0, no reactivity (no staining); 1+, weak staining (seen clearly with ×20 or ×40); 2+, moderate staining (seen clearly with ×10); and 3+, strong staining (seen clearly with ×4).

Statistical analysis

Statistical analysis was performed using SPSS version 13.0. Group comparisons of categorical variables were evaluated using the Fisher's exact or Pearson's chi-square test. Finally, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of Arg-1 and GPC-3 were calculated for the significance. All *P*-values were two-sided with less than 0.05 were considered to be statistically significant, and less than 0.01 meant highly significant.

Results

Arg-1 expression

Only cytoplasm and nucleus immunostaining was considered to be positive finding for Arg-1 (**Figure 1**). The overall sensitivity of Arg-1 was 96.1% (75/78) in HCC (**Table 1**) with more sensitivity in well 100% (15/15) and moderately 100% (15/15) differentiated HCC than poorly 86.0% (19/22) differentiated HCC (**Table 2**). As expected, Arg-1 immunoactivity could be found

Table 1. Arg-1 and GPC-3 expression in HCC, metastatic tumors and nonhepatocellular tumors

	HCC (N=78)	Metastatic tumors (N=34)	Nonhepatocellular tumors (N=228)	*P* value[a]
Arg-1	75 (96.1)	0 (0)	1 (0.4)	<0.0001
GPC-3	50 (64.1)	3 (8.8)	9 (3.9)	<0.0001

a: Two-sided Pearson's chi square test for distributions between different tumors in this study. The results were in bold, if *P*<0.05.

Table 2. Arg-1 and GPC-3 expression in well, moderately and poorly differentiated HCC

	Well differentiated HCC (N=15)	Moderately differentiated HCC (N=41)	Poorly differentiated HCC (N=22)	*P* value[a]
Arg-1	15 (100)	41 (100)	19 (86.0)	0.0189
GPC-3	7 (46.7)	25 (61.0)	18 (81.8)	0.0759

a: Two-sided Pearson's chi square test for distributions between different tumors in this study. The results were in bold, if *P*<0.05.

Table 3. Statistical analysis of sensitivity, specificity, and positive and negative predictive value of marker and marker combinations in distinguishing hepatocellular carcinoma from metastatic tumors and nonhepatocellular tumors

Marker and marker combination	HCC (N=78)	Metastatic tumors (N=34)	Nnonhepatocellular tumors (N=228)	Sensitivity	Specificity	PPV	NPV
Arg-1	75	0	1	96.1%	99.6%	98.7%	98.8%
GPC-3	50	3	9	64.1%	95.4%	80.6%	89.9%
Arg-1 or GPC-3	77	3	10	98.7%	95.0%	85.6%	99.6%
Arg-1 and GPC-3	48	0	0	61.5%	100%	100%	89.7%

in HCC, but not in nonhepatocellular derived tumors except one case (*P*<0.0001). Arg-1 staining intensity of immunoreactivity of HCC: 3 cases (3.8%) showed 0, 4 cases (5.1%) showed 1+, 10 cases (12.8%) showed 2+, and 61 cases (78.2%) showed 3+, respectively (Supplementary Table 1). No significant association between Arg-1 staining intensity and histological differentiation was found in this study (data was not showed).

GPC-3 expression

Cytoplasm immunostaining was considered to be positive finding for GPC-3. The overall sensitivity of GPC-3 was much high in HCC (64.1%, 50/78) than that of metastatic tumors (8.8%, 3/34) and nonhepatocellular tumors (3.9%, 9/228), with noteworthy significant association of *P*<0.0001 (**Table 1**). Although GPC-3 expression was more common in poorly differentiated HCC, compared with well and moderately HCC, no significant association was found between (*P*=0.0759) (**Table 2**). GPC-3 Staining intensity of immunoreactivity of HCC: 28 cases (35.9%) showed 0, 9 cases (11.5%) showed 1+, 17 cases (21.8%) showed 2+, and 24 cases (30.8%) showed 3+, respectively (Supplementary Table 1). No significant association between GPC-3 staining intensity and histological differentiation was found in this study (data was not showed). Furthermore, GPC-3 expression can be found in some tumors other than HCC in this study, including 25% (1/4) of metastatic gallbladder carcinoma, 16.7% (1/6) of metastatic gastric adenocarcinoma, 11.1% (1/9) of metastatic colonic adenocarcinoma, 7.1% (2/28) of colonic adenocarcinoma, 8.33% (1/12) of gallbladder carcinoma, 6.25% (1/16) of renal clear cell carcinoma, 8.33% (1/12) of pulmonary adenocarcinoma, 10% (1/10) of uterine adenocarcinoma and 13.6% (3/22) of gastric adenocarcinoma (Supplementary Tables 2, 3).

Combination analysis Arg1-GPC3

If any of Arg-1 or GPC-3 expression is defined as a positive case: the positivity cases were 98.7% (77/78), 8.8% (3/34) and 4.4% (10/228) in HCC, metastatic tumors and nonhepatocellular tumors, respectively. This combination showed the sensitivity, specificity, and positive and negative predictive value of markers in distinguish-

ing hepatocellular carcinoma from metastatic tumors and nonhepatocellular tumors were 98.7%, 95.0%, 85.6% and 99.6%, respectively (**Table 3**). This combination can have higher sensitivity compared with separate marker.

If both of Arg-1 and GPC-3 expression is defined as a positive case: the positivity cases were 61.5% (48/78), 0% (0/34) and 0% (0/228) in HCC, metastatic tumors and nonhepatocellular tumors, respectively. This combination showed the sensitivity, specificity, and positive and negative predictive value of markers in distinguishing hepatocellular carcinoma from metastatic tumors and nonhepatocellular tumors were 61.5%, 100%, 100% and 89.7%, respectively (**Table 3**). This combination can have higher specificity compared with separate marker.

Other statistical analysis

Among all of the 78 cases of HCC, only one case did not express both Arg-1 and GPC-3, this case expressing CK8, CK18 and AFP, the morphology of this case could find certain typical feature of HCC. All of 22 poorly differentiated HCC expressed one or more of CK8, CK18, AFP and HepPar-1, with varying histological features of HCC.

Discussion

HCC is the most common malignancies of the liver. Many markers have been used on diagnosis for HCC and the identification of HCC from other neoplasms. As a specific marker for hepatocyte, Arg-1 has been used on diagnosis for hepatocellular tumors; GPC-3 is a specific marker for HCC, so the combination of Arg1-GPC3 could be better diagnostic value in distinguishing hepatocellular carcinoma from metastatic tumor. Although, some literatures focused on these of two markers in TMA or FNA biopsy specimens, but no consistent result was obtained based on Arg-1 and GPC-3 expression by far; secondly, limitations of sample and heterogeneity of tumor could not aid accurate diagnosis, so the value of Arg1-GPC3 combined immunostaining in distinguishing HCC from metastatic tumor should be necessary in surgical specimens. The aims of our study were to evaluate these two biomarkers (Arg-1, GPC-3) in distinguishing a metastasis from an HCC on surgical specimens in a Chinese HCC population.

Arg-1, an enzyme involving in the urea cycle [24], is recently recognized a useful diagnostic marker in the differential diagnosis of HCC from metastatic tumors [18, 22]. Our study showed that staining intensity of Arg-1 is strong in majority of HCC, the total sensitivities of Arg-1 was 96.1% (75/78) consistent with previous research (95.9%) [25], it also showed that Arg-1 has higher sensitivity of well or moderately differentiated HCC compared with poorly differentiated HCC. No Arg-1 expressing in any metastatic tumors and only one case was found expressed in nonhepatocellular tumors in this study, which meant high specificity (99.6%) in differentiated diagnosis of HCC from metastasis tumors. However, a FNA study showed that Arg-1 expressed in 9.8% (6/61) metastatic tumors [17], we supposed that limited background could made unspecific staining. In our study, metastatic tumors chose the border tissue of liver tissue and metastatic tumor, which can exclude unspecific staining. HCC and intrahepatic cholangiocarcinoma (ICC) are most common malignant tumor in liver, it is difficult on differential diagnosis of HCC from ICC, as we wish, no expression of Arg-1 was found in 17 cases of ICC, which means that Arg-1 is a better marker in differential diagnosis of HCC from ICC. Our study also emphasized the importance in distinguishing HCC form ICC, because ICC cannot benefit from radiotherapy and chemotherapy, whereas HCC can do.

GPC-3, a member of the heparin sulfate proteoglycan family, is expressed in embryonic stages; its expression is greatly reduced in adult tissues [23, 26]. It is proved that GPC-3 plays an important role in HCC growth and it is a specific marker for HCC [17, 27-30]. GPC-3 is upregulated in 50 to 84% of HCC samples, and specificity of 92% in some studies. Our study showed that the sensitivity of GPC-3 was 64.1% for HCC, which showed poorer sensitivity compared of Arg-1. GPC-3 has higher sensitivity of poorly differentiated HCC compared with well or moderately differentiated HCC, and GPC-3 also had high specificity (95.4%) as well as Arg-1. Overall, GPC-3 had general sensitivity and good specificity; it means GPC-3 maybe not a good marker for HCC alone, GPC-3 should need another marker combination and achieve better diagnostic value.

Although three cases of HCC did not express Arg-1, two of these cases expressed GPC-3; 28 cases of HCC did not express GPC-3, while 27 of these cases expressed Arg-1, which means that Arg1-GPC3 combination can increase the

positive rate for HCC. So Arg1-GPC3 combination is necessary in our study.

We estimated the value of Arg1-GPC3 combination in distinguishing HCC from metastatic tumors and nonhepatocellular tumors, the result showed that combination of Arg1-GPC3 can increase sensitivity or specificity compared with single marker. If any of Arg-1 or GPC-3 expression is defined as a positive case, the result showed higher sensitivity (98.7%), and if both of Arg-1 and GPC-3 expression is defined as a positive case, the result showed higher specificity (100%). Although Arg-1 has higher sensitivity for HCC compared with GPC-3, the combination of Arg-1 or GPC-3 expression may has higher sensitivity (98.7%) for HCC diagnosis compared with single Arg-1 expression (96.1%), especially for poorly differentiated HCC. GPC-3 expressed in a few cases of nonhepatocellular tumors consistent with some reports [31, 32], but our study showed that combination of Arg-1 and GPC-3 expression can increase specificity (100%) compared with single GPC-3 (95.4%) expression, in our study, none of metastasis and nonhepatocellular tumors had expressed both of Arg-1 and GPC-3, just only expressed single marker.

Our findings support the conclusion that Arg-1 has higher sensitivity and specificity compared with GPC-3 for HCC, and the separate of Arg-1 or GPC-3 expression has higher sensitivity for HCC diagnosis, and the both of Arg-1 and GPC-3 expression has higher specificity in differentiated diagnosis of HCC from metastasis tumors. So the combination of Arg1-GPC3 has better diagnostic value than dose single staining. However, several limitations of the present study need to be addressed. Firstly, poorly differentiated HCC usually lose antigen, how can we identify the case losing both of these two markers in HCC from metastatic tumor, maybe some other markers should be necessary taken. Nevertheless, Arg-1 may be a good ideal marker for HCC so far, so morphology may be the best reliable method to use. Secondly, limitation of amount of HCC, so subgroup analyses of tumor types cannot be made.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (NO 81460513), and the First Affiliated Hospital of Xinjiang Medical University (NO 2015ZRQN01).

Disclosure of conflict of interest

None.

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References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] Zuo T, Zheng R, Zeng H, Zhang S and Chen W. [Analysis of liver cancer incidence and trend in China]. Zhonghua Zhong Liu Za Zhi 2015; 37: 691-696.

[3] Altekruse SF, McGlynn KA and Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. J Clin Oncol 2009; 27: 1485-1491.

[4] El-Serag HB. Hepatocellular carcinoma. N Engl J Med 2011; 365: 1118-1127.

[5] Fasano M, Theise ND, Nalesnik M, Goswami S, Garcia de Davila MT, Finegold MJ, Greco MA. Immunohistochemical evaluation of hepatoblastomas with use of the hepatocyte-specific marker, hepatocyte paraffin 1, and the polyclonal anti-carcinoembryonic antigen. Mod Pathol 1998; 11: 934-938.

[6] Leong AS, Sormunen RT, Tsui WM and Liew CT. Hep Par 1 and selected antibodies in the immunohistological distinction of hepatocellular carcinoma from cholangiocarcinoma, combined tumours and metastatic carcinoma. Histopathology 1998; 33: 318-324.

[7] Maitra A, Murakata LA and Albores-Saavedra J. Immunoreactivity for hepatocyte paraffin 1 antibody in hepatoid adenocarcinomas of the gastrointestinal tract. Am J Clin Pathol 2001; 115: 689-694.

[8] Murakata LA, Ishak KG and Nzeako UC. Clear cell carcinoma of the liver: a comparative immunohistochemical study with renal clear cell carcinoma. Mod Pathol 2000; 13: 874-881.

[9] Iczkowski KA and Butler SL. New immunohistochemical markers in testicular tumors. Anal Quant Cytol Histol 2006; 28: 181-187.

[10] Ota S, Hishinuma M, Yamauchi N, Goto A, Morikawa T, Fujimura T, Kitamura T, Kodama T, Aburatani H and Fukayama M. Oncofetal protein glypican-3 in testicular germ-cell tumor. Virchows Arch 2006; 449: 308-314.

[11] Zynger DL, Dimov ND, Luan C, Teh BT and Yang XJ. Glypican 3: a novel marker in testicular germ cell tumors. Am J Surg Pathol 2006; 30: 1570-1575.

[12] Zynger DL, Everton MJ, Dimov ND, Chou PM and Yang XJ. Expression of glypican 3 in ovarian and extragonadal germ cell tumors. Am J Clin Pathol 2008; 130: 224-230.
[13] Kakar S, Gown AM, Goodman ZD and Ferrell LD. Best practices in diagnostic immunohistochemistry: hepatocellular carcinoma versus metastatic neoplasms. Arch Pathol Lab Med 2007; 131: 1648-1654.
[14] Kandil DH and Cooper K. Glypican-3: a novel diagnostic marker for hepatocellular carcinoma and more. Adv Anat Pathol 2009; 16: 125-129.
[15] Shirakawa H, Kuronuma T, Nishimura Y, Hasebe T, Nakano M, Gotohda N, Takahashi S, Nakagohri T, Konishi M, Kobayashi N, Kinoshita T and Nakatsura T. Glypican-3 is a useful diagnostic marker for a component of hepatocellular carcinoma in human liver cancer. Int J Oncol 2009; 34: 649-656.
[16] Wang HL, Anatelli F, Zhai QJ, Adley B, Chuang ST and Yang XJ. Glypican-3 as a useful diagnostic marker that distinguishes hepatocellular carcinoma from benign hepatocellular mass lesions. Arch Pathol Lab Med 2008; 132: 1723-1728.
[17] Fujiwara M, Kwok S, Yano H and Pai RK. Arginase-1 is a more sensitive marker of hepatic differentiation than HepPar-1 and glypican-3 in fine-needle aspiration biopsies. Cancer Cytopathol 2012; 120: 230-237.
[18] McKnight R, Nassar A, Cohen C and Siddiqui MT. Arginase-1: a novel immunohistochemical marker of hepatocellular differentiation in fine needle aspiration cytology. Cancer Cytopathol 2012; 120: 223-229.
[19] Bernfield M, Gotte M, Park PW, Reizes O, Fitzgerald ML, Lincecum J and Zako M. Functions of cell surface heparan sulfate proteoglycans. Annu Rev Biochem 1999; 68: 729-777.
[20] Yu J, Ma Q, Zhang B, Ma R, Xu X, Li M, Xu W and Li M. Clinical application of specific antibody against glypican-3 for hepatocellular carcinoma diagnosis. Sci China Life Sci 2013; 56: 234-239.
[21] Sang W, Zhang W, Cui W, Li X, Abulajiang G and Li Q. Arginase-1 is a more sensitive marker than HepPar-1 and AFP in differential diagnosis of hepatocellular carcinoma from nonhepatocellular carcinoma. Tumour Biol 2015; 36: 3881-3886.
[22] Timek DT, Shi J, Liu H and Lin F. 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: 203-210.
[23] Davoodi J, Kelly J, Gendron NH and MacKenzie AE. The Simpson-Golabi-Behmel syndrome causative glypican-3, binds to and inhibits the dipeptidyl peptidase activity of CD26. Proteomics 2007; 7: 2300-2310.
[24] Multhaupt H, Fritz P and Schumacher K. Immunohistochemical localisation of arginase in human liver using monoclonal antibodies against human liver arginase. Histochemistry 1987; 87: 465-470.
[25] Yan BC, Gong C, Song J, Krausz T, Tretiakova M, Hyjek E, Al-Ahmadie H, Alves V, Xiao SY, Anders RA and Hart JA. Arginase-1: a new immunohistochemical marker of hepatocytes and hepatocellular neoplasms. Am J Surg Pathol 2010; 34: 1147-1154.
[26] Okamoto N, Yagi M, Imura K and Wada Y. A clinical and molecular study of a patient with Simpson-Golabi-Behmel syndrome. J Hum Genet 1999; 44: 327-329.
[27] Sawada Y and Nakatsura T. [The cancer specific antigen, glypican-3 (GPC3)-targeted immunotherapy]. Nihon Rinsho 2012; 70: 2136-2141.
[28] Wang YL, Zhu ZJ, Teng DH, Yao Z, Gao W and Shen ZY. Glypican-3 expression and its relationship with recurrence of HCC after liver transplantation. World J Gastroenterol 2012; 18: 2408-2414.
[29] Wang F, Jing X, Wang T, Li G, Li T, Zhang Q, Huang Y, Li J, Wang Y, Gao Y, Han T and Du Z. Differential diagnostic value of GPC3-CD34 combined staining in small liver nodules with diameter less than 3 cm. Am J Clin Pathol 2012; 137: 937-945.
[30] Gao W and Ho M. The role of glypican-3 in regulating Wnt in hepatocellular carcinomas. Cancer Rep 2011; 1: 14-19.
[31] Mounajjed T, Zhang L and Wu TT. Glypican-3 expression in gastrointestinal and pancreatic epithelial neoplasms. Hum Pathol 2013; 44: 542-550.
[32] Lagana SM, Moreira RK, Remotti HE and Bao F. Glutamine synthetase, heat shock protein-70, and glypican-3 in intrahepatic cholangiocarcinoma and tumors metastatic to liver. Appl Immunohistochem Mol Morphol 2013; 21: 254-257.

Supplementary Table 1. staining intensity of Arg-1 and GPC-3 antibodies in HCC

	Arg-1					GPC-3				
Tumor	N (%)	0	1+	2+	3+	N (%)	0	1+	2+	3+
HCC	75 (96.15)	3 (3.8)	4 (5.1)	10 (12.8)	61 (78.2)	50 (64.1)	28 (35.9)	9 (11.5)	17 (21.8)	24 (30.8)

Supplementary Table 2. Arg-1 and GPC-3 expression in metastatic tumors

Metastatic tumors	Cases	Arg-1 (%)	GPC-3 (%)
Mammary carcinoma	2	0	0
Gallbladder carcinoma	4	0	1 (25)
Endocrine carcinoma	5	0	0
Endometrioid carcinoma of ovary	2	0	0
Nasopharyngeal carcinoma	2	0	0
Ductal adenocarcinoma of the pancreas	1	0	0
Renal clear cell carcinoma	3	0	0
Gastric adenocarcinoma	6	0	1 (16.7)
Colonic adenocarcinoma	9	0	1 (11.1)
Total	34	0 (0)	3 (8.8)

Supplementary Table 3. Arg-1 and GPC-3 expression in nonhepatocellular tumors

Types of tumors	Cases	Arg-1 (%)	GPC-3 (%)
Colonic adenocarcinoma	28	0	2 (7.1)
Breast carcinoma	22	0	0
Endometrial adenocarcinoma	31	0	0
Endocrine carcinoma	10	0	0
Intrahepatic cholangiocarcinomas	2	0	0
Ductal adenocarcinoma of the pancreas	6	0	0
Gallbladder carcinoma	12	1 (8.33)	1 (8.33)
Renal clear cell carcinoma	16	0	1 (6.25)
Nasopharyngeal carcinoma	15	0	0
Urothelial carcinoma	16	0	0
Pulmonary adenocarcinoma	12	0	1 (8.33)
Uterine adenocarcinoma	10	0	1 (10)
Esophageal adenocarcinoma	10	0	0
Gastric adenocarcinoma	22	0	3 (13.6)
Total	228	1 (0.4)	9 (3.9)