Int J Clin Exp Pathol 2016;9(8):7878-7888
www.ijcep.com /ISSN:1936-2625/IJCEP0024793

Original Article

Utility of an immunostaining panel for diagnosis of hepatocellular carcinoma in fine needle-aspiration biopsies of the liver

Jiawen Xu[1], Yuhong Sun[2], Chunqin Chu[3], Shujian Ge[4], Xinxing Wang[5], Qiangxiu Wang[1], Zhenhai Zhang[5]

[1]Department of Pathology, [4]Science and Education, [5]Hepatobiliary Surgery, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, Shandong, P. R. China; [2]Department of Pathology, Shandong Cancer Hospital and Institute, Jinan, Shandong, P. R. China; [3]Department of Anesthesiology, The Affiliated Hospital of Qingdao University, Qingdao, Shandong, P. R. China

Received January 24, 2016; Accepted June 20, 2016; Epub August 1, 2016; Published August 15, 2016

Abstract: Background: Histological identification of liver nodules between hepatocellular carcinoma (HCC) and other lesions can be full of challenges sometimes, especially in fine-needle aspiration (FNA) biopsies. Our aim was to investigate the efficacy of combined use of Arginase-1, Glypican-3 (GPC-3), heat shock protein 70 (HSP70) and cytokeratin 7 (CK7) in differentiating these lesions in FNA biopsies. Methods: Immunohistochemistry for these four markers was done in 45 HCCs, 22 metastatic carcinoma, 15 intrahepatic cholangiocarcinoma, 12 hepatocellular adenoma, 8 focal nodular hyperplasia, 12 large regenerative nodules arising in cirrhotic livers, and 10 specimens of normal liver tissues. Results: Arginase-1 reactivity was present in 43 of 45 HCCs (96.0%) and all of the benign liver lesions recruited in this study but not in any of MCs and ICCs ($P < 0.001$). GPC-3 stained 37 of 45 HCCs (82%) and only 1 of 22 MCs (5%), however, all cases of ICCs and the other benign lesions were negative for GPC-3 expression ($P < 0.05$). HSP70 showed markedly immunoreactivity in progressive tumors, 38 of 45 HCCs (84%), 18 of 22 MCs (82%), and 11 of 15 ICCs (73%), but was negative for all of the benign cases ($P < 0.001$). All ICCs showed diffused and strong immunostaining for CK7, but only 3 of 45 HCCs (7%) were detected to react with CK7. The combination of the four immunostaining markers for the diagnosis of HCC could raise the sensitivity and specificity to 98% and 100%, respectively. Conclusion: This study demonstrates that Arginase-1 is an extremely effective and specific immunohistochemistry marker for confirming hepaticorigin, while GPC-3 and HSP70 is typically positive in malignant lesions. Our results indicated the accuracy of diagnosis can be enhanced by their combination of these four markers.

Keywords: Hepatocellular carcinoma, Arginase-1, Glypican-3, heat shock protein 70, cytokeratin 7, fine-needle aspiration

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignant carcinoma, with a high mortality rate and increasing incidence worldwide [1]. HCC, which has a high prevalence in China, is the second leading cause of cancer-related deaths among males and is the third leading cause among females as a result of the high incidence of hepatitis B virus (HBV) infections [2]. Although various therapeutic options are available, the validity of these methods and the prognosis of patients with HCC remain extremely poor. However, the five-year survival rate can be increased to approximately > 70% when patients are diagnosed at an early stage [3]. Recently, much advancement has been achieved in radiological and imaging methods, such as ultrasound, computed tomography, and the magnetic resonance imaging detection of small lesions in the liver [4]. Nevertheless, the differentiation of nodular masses among HCCs and benign or metastatic lesions remains occasionally very difficult. Histopathological diagnosis remains as the golden standard for assessment [5]. For pathologists, distinguishing between low-grade HCC and benign nodular lesions can be very chal-

Table 1. List of antibodies

Antibody	Source	Clone	Dilution	Antigen Retrieval
Arginase-1	Santa Cruz Biotechnology (Santa Cruz, CA, USA)	H52	1:100	PBS, pH 6.0
GPC-3	Santa Cruz Biotechnology	1G12	1:100	PBS, pH 6.0
HSP70	Santa Cruz Biotechnology	W27	1:200	Tris, pH 8.4
CK7	DAKO (Tokyo, Japan)	OV-TL 12/30	1:150	PBS, pH 6.0

Table 2. Summary of different antibody immunostaining patterns

Cases (*n* = 124)	Arginase-1, *n* (%)	GPC-3, *n* (%)	CK7, *n* (%)	HSP70, *n* (%)
HCC (*n* = 45)	43/45 (96)	37/45 (82)	3/45 (7)	38/45 (84)
MC (*n* = 22)	0/22 (0)	1/22 (5)	3/22 (14)	18/22 (82)
HCA (*n* = 12)	12/12 (100)	0/12 (0)	0/12 (0)	0/12 (0)
ICC (*n* = 15)	0/15 (0)	0/15 (0)	15/15 (100)	11/15 (73)
FNH (*n* = 8)	8/8 (100)	0/8 (0)	0/8 (0)	0/8 (0)
Cirrhosis (*n* = 12)	12/12 (100)	0/12 (0)	0/12 (0)	0/12 (0)
Normal (*n* = 10)	10/10 (100)	0/10 (0)	0/10 (0)	0/10 (0)
Combination group (*n* = 30)	30/30 (100)	0/30 (0)	0/30 (0)	0/30 (0)

lenging, particularly in the case of fine-needle aspiration (FNA) biopsies of the liver. The distinction between a primary HCC and other liver lesions, such as intrahepatic cholangiocarcinoma (ICC), metastatic carcinoma (MC), hepatocellular adenoma (HCA) and focal nodular hyperplasia (FNH), is crucial because the treatment methods for these tumors differ. Thus, efficient biomarkers for the valuable diagnosis of HCC are urgently required.

A range of promising biomarkers for the diagnosis of HCCs have recently been distinguished from other mimicked liver lesions. These biomarkers include α-fetoprotein (AFP), hepatocyte paraffin antigen-1 (HepPar-1), glypican-3 (GPC-3), heat shock protein 70 (HSP70), glutamine synthetase (GS), Arginase-1, and the enhancer of zeste homologue 2, among others [4, 6-8]. These markers, when used alone, are sometimes helpful, but the staining results of any single marker can be very confusing because of its limitations in sensitivity and specificity. Thus, a panel of combined markers is much more reliable.

In this study, we investigate the diagnostic value of GPC-3, HSP70, Arginase-1, and cytokeratin (CK7) immunostaining in liver nodular masses of biopsy materials and evaluate the value of this panel of markers in differentiating HCC from other hepatic masses.

Materials and methods

Clinical samples

A total of 124 paraffin-embedded FNAs of liver with their related clinical data were collected from the Department of Pathology, Shandong Provincial Hospital Affiliated to Shandong University from January 2012 to December 2013. The data consisted of 45 cases of HCC, 22 cases of MC, 15 cases of ICC, 12HCA, 8FNH, 12 large regenerative nodules arising in cirrhotic livers, and 10 specimens of normal liver tissues (NL). The diagnoses were reviewed by two gastrointestinal pathologists based on the histology, clinical data, and other assistant methods. The cases were staged and graded according to the Cancer Staging Manual, seventh edition, of the American Joint Committee on Cancer [9]. All the included cases were not treated with chemoembolization or systemic chemotherapy prior to needle biopsy. The primary HCC cases were classified into three groups according to the histological differentiation: 11 cases (24.4%) were well differentiated, 20 cases (44.4%) were moderately differentiated, and 14 cases (31.2%) were poorly differentiated. The MC cases consisted of 22 metastatic neoplasms, including 12 colon adenocarcinomas, 4 duodenum adenocarcinomas, 1 breast adenocarcinoma, 4 metastatic neuroendocrine carcinoma, and 1 pancreas ductal ade-

Table 3. Comparison of the immunoreactivity of different antibodies between HCC and MC

	Arginase-1	GPC-3	CK7	HSP70	GPC-3 or HSP70	GPC-3 and HSP70
HCC (positive/cases)	43/45	37/45	3/45	38/45	44/45	31/45
MC (positive/cases)	0/22	1/22	3/22	18/22	19/22	0/22
P (HCC vs. MC)	< 0.001*	< 0.001*	0.348	0.785	0.064	< 0.001*
Sensitivity for HCC (%)	96	82	7	84	98	69
Specificity for HCC (%)	100	95	86	18	14	100
Positive predictive value (%)	100	97	50	68	70	100
Negative predictive value (%)	92	72	31	36	75	61

*$P < 0.05$.

nocarcinoma. Informed consent was obtained from all the participants included in this work, and the use of the tissue specimens was approved by the Research Ethics Committee of Shandong Provincial Hospital Affiliated to Shandong University (Jinan, China).

Immunohistochemistry

The antibodies used in this study are listed in **Table 1**. Formalin-fixed and paraffin-embedded tissue sections were selected, and 4 μm sections were then dewaxed and rehydrated. The slides were treated with 3% H_2O_2 for 15 min to quench the endogenous peroxidase. Antigen retrieval was conducted by incubating the slides in 0.01 M citrate buffer (pH 6.0) at 100°C for 10 min. A standard immunohistochemical technique was then implemented using a Ventana Benchmark® XT autostainer (Ventana Medical SystemsInc., Tucson, AZ, USA).

The immunoreactivity of Arginase-1, GPC-3, HSP-70, and CK7 was scored by two pathologists. The percentage of reactive cells was quantified as 0 (no reactivity), 1 + (1%-10% staining), 2 + (10%-50% staining), or 3 + (> 50% staining). The intensity of immunostaining was scored as 0 (no staining), 1 + (weak staining), and 2 + (strong staining). The staining value (0-6+) was calculated as the intensity multiplied by the percentage of immunostained cells. A score of 0 indicated a negative value; a score of 1-2 + indicated low expression, and a score of 3-6 + indicated high expression. Only cytoplasmic reactivity or both cytoplasmic and nuclear reactivities were considered positive staining for Arginase-1 and HSP70. For GPC-3, positive staining was defined as coarsely granular cytoplasmic staining. Membrane stains were considered positive for CK7. Known positive and negative controls (without primary antibody) were used for each batch of slides.

Statistical analysis

Data were analyzed by SPSS software version 16.0 (SPSS, Inc., Chicago, IL, USA). The χ^2 test was used to calculate the statistical significance of the variables. A *P* value of less than 0.05 was considered statistically significant.

Results

Summary of different antibody immunostaining patterns

The immunostaining results of the four antibodies in this work are summarized in **Table 2**. For Arginase-1, all benign liver lesions, including HCA, FNH, cirrhosis, and normal liver tissues, showed strong and diffuse immunostaining patterns. Moreover, the Arginase-1 antibody was positive in 43 of 45 (96%) HCCs, but none of the MC and ICC cases showed immunoreactivity with Arginase-1.

GPC-3 stained 37 of 45 (82%) HCCs and only 1 of 22 (5%) MCs (i.e., neuroendocrine carcinomas arising from the stomach). All cases of ICC and the other benign lesions were negative for GPC-3 expression.

CK7 expression was observed in 3 of 45 (7%) HCCs and 3 of 22 (14%) MCs (i.e., 1 case of invasive breast carcinoma, 1 case of neuroendocrine carcinoma transferred from the pancreas, and 1 case of pancreas ductal carcinoma). No benign liver lesion was positive for CK7. However, CK7 showed strong expression in all 15 cases of ICC.

HSP70, which was absolutely negative in all cases of benign liver masses and tissues,

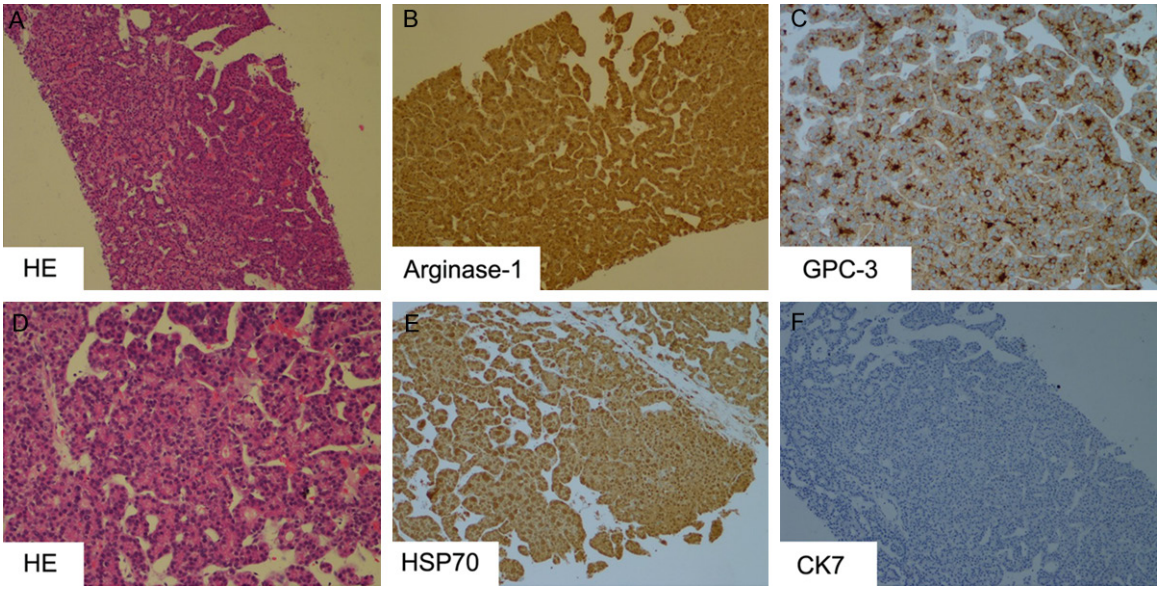

Figure 1. HCC. A. Well differentiated HCC with only focally thickened cell plates and mild cytologic atypia. This morphology could overlap with many benign liver mass lesions (hematoxylin and eosin, × 100), B. Arginase-1 shows strong, diffuse positivity in cell nuclear and cytoplasm (immunohistochemical stain, × 100), C. GPC-3 also shows moderate-strong, diffused positivity in cell cytoplasm (immunohistochemical stain, × 200), D. The same case with A with magnifiation × 200 (hematoxylin and eosin), E. HSP70 also shows strong, diffused positivity in cell nuclear and cytoplasm (immunohistochemical stain, × 100), F. CK7 showed no immunoreactivity in HCC (immunohistochemical stain, × 100).

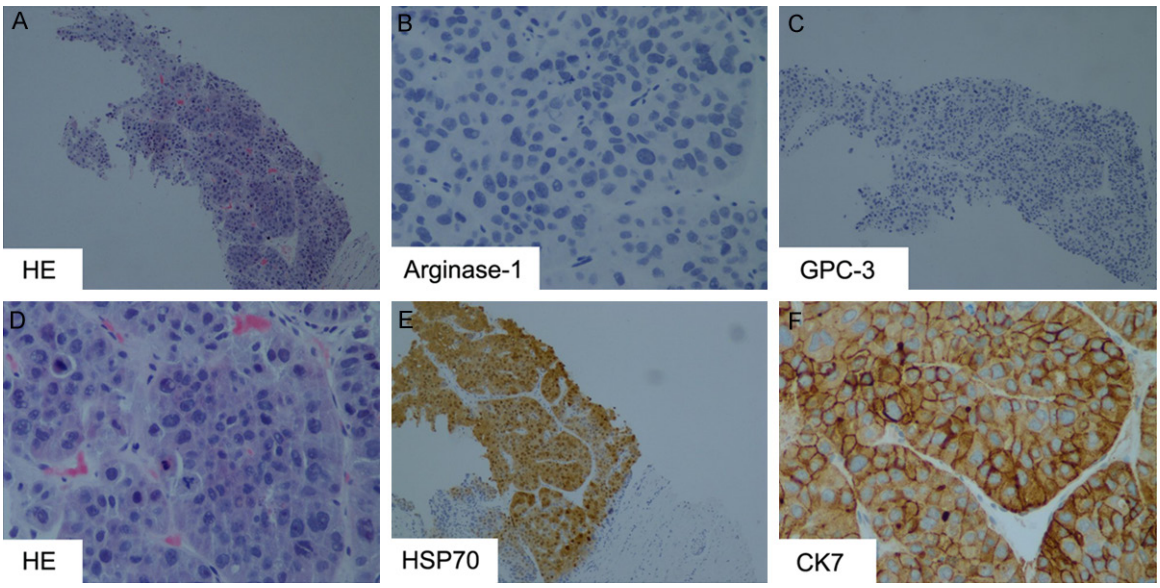

Figure 2. MC. A. Fine-needle biopsy of hepatic metastasis of breast carcinoma made by H&E sections (× 100), B. Arginase-1 showed no immunoreactivity in MC (× 400), C. GPC-3 showed no immunoreactivity in MC (× 400), D. Fine-needle biopsies of breast carcinoma made by H&E sections (× 400), E. Diffused and strong nuclear and cytoplasmic immunostaining for HSP70 (× 100), F. CK7 shows strong, diffuse positivity in membrane (immunohistochemical stain, × 400).

showed apparent immunoreactivity in 38 of 45 (84%) HCCs, 18 of 22 (82%) MCs, and 11 of 15 (73%) ICCs. Among the 18 MC cases, 10 originated from colon carcinoma, 3 from duodenal

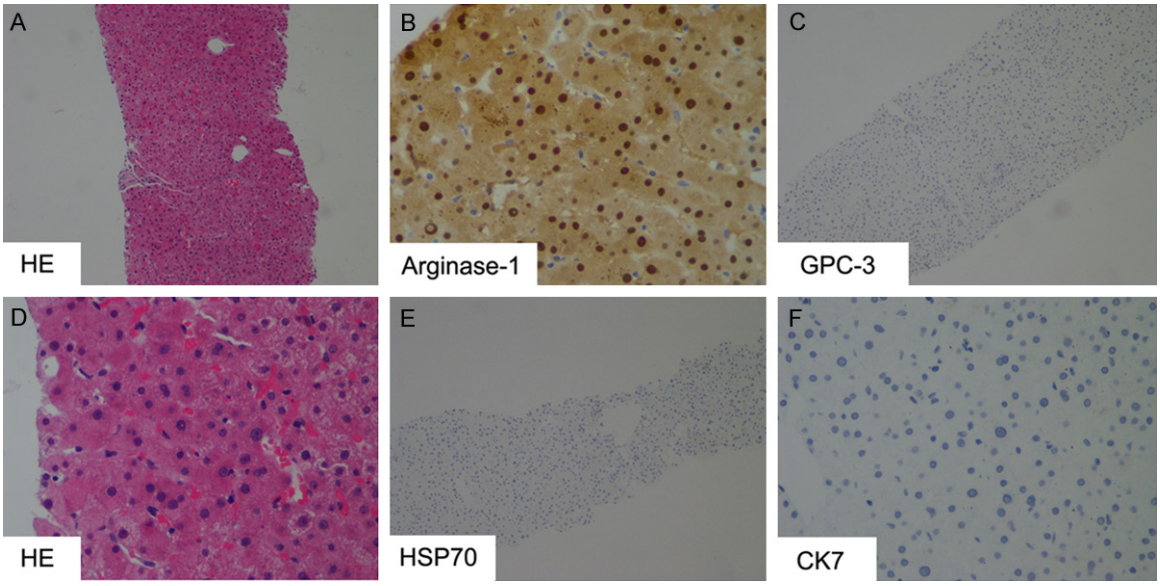

Figure 3. HCA. A. A cell block section of HCA (hematoxylin and eosin, × 100), B. Diffuse nuclear and cytoplasmic immunostaining for Arginase-1 (× 400), C. Negative immunostaining for GPC-3 (× 100), D. The same cell block section of HCA (hematoxylin and eosin, × 400), E. Negative immunostaining for HSP70 (× 100). F. Negative immunostaining for CK7 (× 400).

Table 4. Comparison of the immunoreactivity of different antibodies between HCC and HCA

	Arginase-1	GPC-3	CK7	HSP70	GPC-3 or HSP70	GPC-3 and HSP70
HCC (positive/cases)	43/45	37/45	3/45	38/45	44/45	31/45
HCA (positive/cases)	12/12	0/12	0/12	0/12	0/12	0/12
P (HCC vs. HCA)	0.457	< 0.001*	0.358	< 0.001*	< 0.001*	< 0.001*
Sensitivity for HCC (%)	96	82	7	84	98	69
Specificity for HCC (%)	0	100	100	100	100	100
Positive predictive value (%)	78	100	100	100	100	100
Negative predictive value (%)	0	60	22	63	92	46

*$P < 0.05$.

carcinoma, 1 from invasive breast carcinoma, 3 from neuroendocrine carcinoma, and 1 from pancreas ductal carcinoma.

Comparison of the immunoreactivity of different antibodies in malignant and benign liver nodular lesions

To further investigate the immunoreactivities of the four markers in differentiating HCCs from other malignant or benign liver lesions in the FNA samples, we analyzed the sensitivity, specificity, and predictive value between HCCs and other cases.

In **Table 3**, Arginase-1 and GPC-3 immunoreactivity were diffused and strong in the majority of the HCCs (**Figure 1**) and showed high specificity and positive predictive value for distinguishing HCCs from MCs ($P < 0.001$). None of the MC (**Figure 2**) cases showed immunoreactivity for Arginase-1; only 1 of 22 in the MC cases was positive for GPC-3. The combination of GPC-3 and HSP70 was also useful because the specificity and positive predictive value reached 100% when both GPC-3 and HSP70 were positive ($P < 0.001$).

Arginase-1 stained most of HCCs (43 of 45) and all 12 cases of HCA. By contrast, CK7 had no immunoreactivity in HCAs (**Figure 3**) and stained only a minority of HCCs (3 of 45). Compared with HCAs, 37 of 45 HCCs and 38 of 45 HCCs showed an extreme increase in GPC-3 and HSP70 expression, respectively ($P <$

Table 5. Comparison of the immunoreactivity of different antibodies between HCC and ICC

	Arginase-1	GPC-3	CK7	HSP70	GPC-3 or HSP70	GPC-3 and HSP70
HCC (positive/cases)	43/45	37/45	3/45	38/45	44/45	31/45
ICC (positive/cases)	0/15	0/15	15/15	11/15	11/15	0/15
P (HCC vs. ICC)	< 0.001*	< 0.001*	< 0.001*	0.335	0.003*	< 0.001*
Sensitivity for HCC (%)	96	82	7	84	98	69
Specificity for HCC (%)	100	100	0	27	27	100
Positive predictive value (%)	100	100	17	78	80	100
Negative predictive value (%)	88	65	0	36	80	52

*$P < 0.05$.

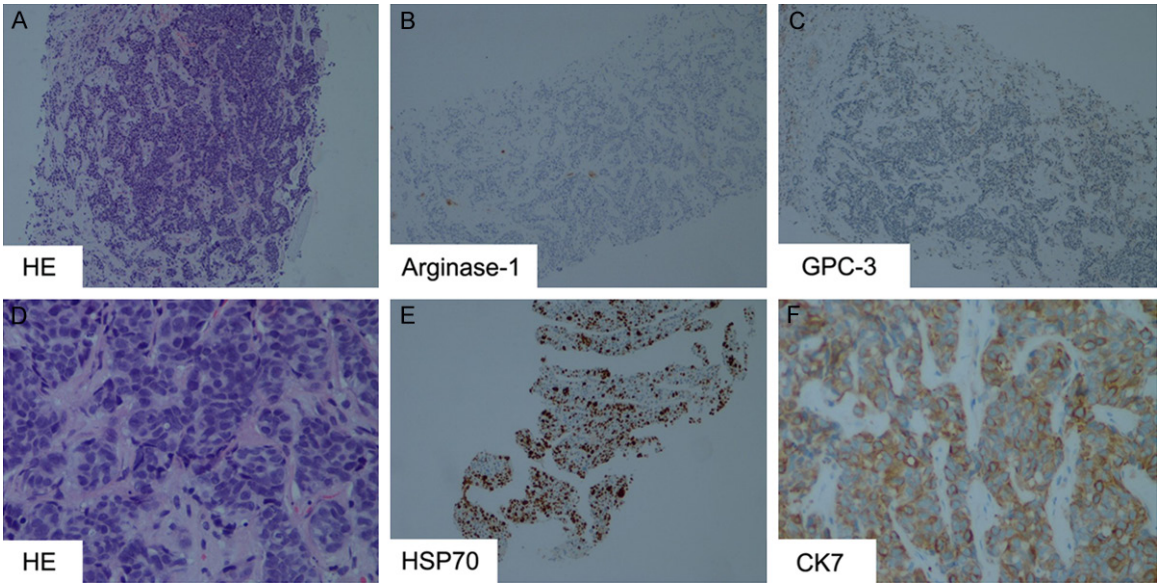

Figure 4. ICC. A. A cell block section of moderate to low differentiated ICC (hematoxylin and eosin, × 100), B. Negative immunostaining for Arginase-1 (× 100), C. Negative immunostaining for GPC-3 (× 100), D. The same cell block section of ICC (hematoxylin and eosin, × 400), E. Diffused and strong nuclear and cytoplasmic immunostaining for HSP70 (× 100), F. Moderate to strong, diffuse positivity for CK7 (immunohistochemical stain, × 400).

0.001). Either GPC-3 and HSP70 and their combination showed 100% specificity and positive predictive values. The sensitivity was 98% when only one marker was positive (**Table 4**).

As shown in **Table 5**, CK7 demonstrated positive immunoreactivity in all 15 (100%) cases of ICC (**Figure 4**), and only 3 of 45 cases of HCC showed positive immunoreactivity for CK7, with focal and weak staining. By contrast, Arginase-1 and GPC-3 showed significantly increased sensitivity and specificity for the diagnosis of HCCs (96%, 100% vs. 82%, 100%). Given that the staining rates for HCC and ICC were high, HSP70 can not differentiate HCCs from ICCs ($P > 0.05$).

As described above, Ariginase-1 showed diffused and strong immunoreactivity in 43 of 45 HCCs and all combination cases (**Figures 5**, **6**), including benign liver lesion masses and normal liver tissues ($P > 0.05$). However, in contrast to the high expression rate in HCC, no cases were positive for GPC-3 and HSP70 immunostaining in the combination groups ($P < 0.001$). GPC-3 and/or HSP70 showed 100% specificity and positive predictive value in differentiating HCCs from benign and normal liver tissues (**Table 6**).

In summary, Ariginase-1 is a sensitive marker for tissues originating from the liver, including HCC, HCA, FNH, cirrhosis, and NL tissues, whereas none of the MC or ICC cases were pos-

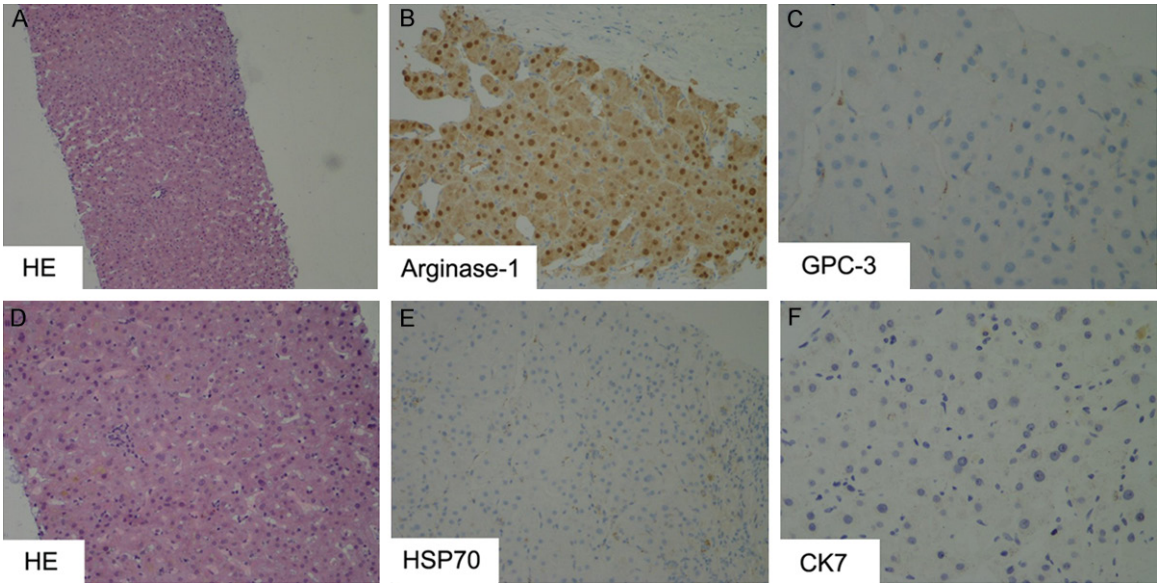

Figure 5. FNH. A. Typical hematoxylin and eosin pattern of biopsies of FNH subtypes (× 100), B. Diffused nuclear and cytoplasmic immunostaining for Arginase-1 (× 200), C. Negative immunostaining for GPC-3 (× 400), D. The same cell block section of FNH (hematoxylin and eosin, × 200), E. Negative immunostaining for HSP70 (× 200), F. Negative immunostaining for CK7 (× 400).

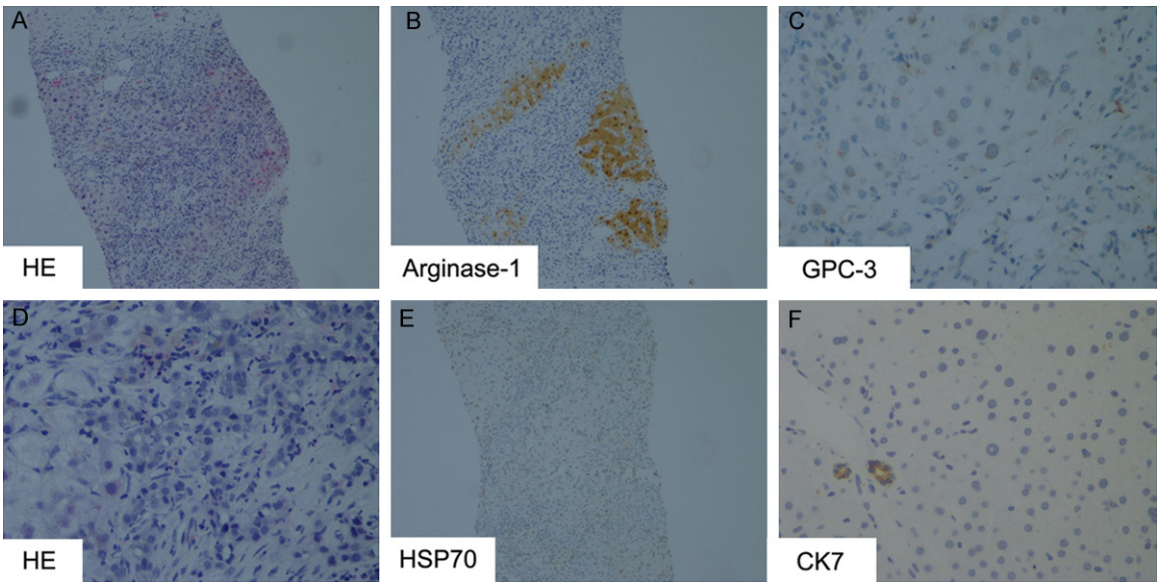

Figure 6. Regenerative Nodules in Liver Cirrhosis. A. Fine-needle biopsy of a case of regenerative nodules in liver cirrhosis made by H&E sections (× 100), B. Diffused nuclear and cytoplasmic immunostaining for Arginase-1 in regenerative liver cells, C. Negative immunostaining for GPC-3 (× 400), D. The same cell block section of liver cirrhosis (hematoxylin and eosin, × 400), E. Negative immunostaining for HSP70 (× 200), F. Negative immunostaining for CK7 in liver cells, but positive in small bile duct (× 400).

itive for Arginase-1. Moreover, 37 among the 45 HCC patients presented an elevated level of immunostaining for GPC-3, and this finding indicated that the expression of GPC-3 may be useful for the diagnosis of HCC. HSP70 showed a high expression rate (38 of 45) in HCCs and was upregulated in MCs (18 of 22) and ICCs (11 of 15). However, all cases of HCA and combination groups were negative for HSP70. Thus, the positive staining of HSP70 was valuable to dif-

Table 6. Comparison of the immunoreactivity of different antibodies between HCC and combination

	Arginase-1	GPC-3	CK7	HSP70	GPC-3 or HSP70	GPC-3 and HSP70
HCC (positive/cases)	43/45	37/45	3/45	38/45	44/45	31/45
Combination (positive/cases)	30/30	0/30	0/30	0/30	0/30	0/30
P (HCC vs. Combination)	0.242	< 0.001*	0.149	< 0.001*	< 0.001*	< 0.001*
Sensitivity for HCC (%)	96	82	7	84	98	69
Specificity for HCC (%)	0	100	100	100	100	100
Positive predictive value (%)	59	100	100	100	100	100
Negative predictive value (%)	0	79	42	81	97	68

*$P < 0.05$.

ferentiate malignant from benign cases and could be complementary to Arginase-1 and GPC-3 in liver FNA diagnosis. In addition, CK7-positive immunostaining demonstrated significant advantages in ICC detection. Although three cases of poorly differentiated HCCs also showed immunoreactivity to CK7, the diagnosis of HCC or ICC can be made after comprehensive consideration of Arginase-1, GPC-3, and CK7 together.

Discussion

HCC is the fifth most common cancer and the second leading cause of cancer-related deaths worldwide [1]. Most patients with HCC are diagnosed at an advanced stage in their illness, and the prognosis is generally poor [10]. Currently, AFP measurement and ultrasound detection are widely and routinely used to screen HCC in China. Nevertheless, the level of serum AFP is not always specific for HCC because upregulated AFP is also detected in patients with viral hepatitis [11, 12]. Under these circumstances, FNA biopsy is necessary for HCC identification, especially when the imaging findings are atypical.

Notably, the clinical application of percutaneous FNA biopsy has been proposed as a safe, effective, and minimally invasive procedure for the diagnosis of liver masses [13]. The sensitivity and specificity of FNA for detecting liver malignancy are around 90% and 100%, respectively [14]. Although morphological observation is often available for pathologists, arrive at a precise final diagnosis remains challenging because of the limited size of samples with core biopsies and the mimicking of histopathological features between liver lesions. Thus, immunohistochemical markers may play a very important role in clinically atypical and indeterminate cases. Recently, a series of diagnostically available immunohistochemical markers for the identification of HCC was applied in routine surgical pathology practice, however, the value of each marker is attributed to tumor heterogeneity and the insufficient sensitivity of each marker. In this work, we proposed Arginase-1, GPC-3, CK7, and HSP70 as an immunohistochemistry biomarker panel for routine diagnostic FNA biopsy and evaluated the specificity and positive predictive value of these markers.

Arginase-1 is a key enzyme of the urea cycle and is found in the liver, this enzyme catalyzes the conversion of L-arginine into L-ornithine and urea. In the past few years, Arginase-1 has been described as a potential and valuable immunohistochemical marker for differentiating HCCs from other tumors [15, 16]. In our study, Arginase-1 demonstrated diffused and strong reactivity in most HCC cases and all benign liver masses, with no immunoreactivity against MC and ICC. However, several studies demonstrated Arginase-1 immunoreactivity in a small portion of pancreatic adenocarcinomas, ICCs, and prostatic adenocarcinomas. Fortunately, all the cases were negative for GPC-3 [6, 16]. The sensitivity for Arginase-1 in HCCs was 96%, and the positive predictive value between HCCs from MCs and ICCs was 100%.

GPC-3 is an oncofetal antigen and a member of the glypican family of glycosyl phosphatidylinositol-anchored cell-surface heparin sulfate proteoglycans. Wang summarized the studies on GPC-3 from 2001 to 2014 and concluded that GPC-3 plays a crucial role in HCC cell proliferation and metastasis. Moreover, GPC-3 is also involved in signaling pathways during hepatocyte malignant transformation [17]. The per-

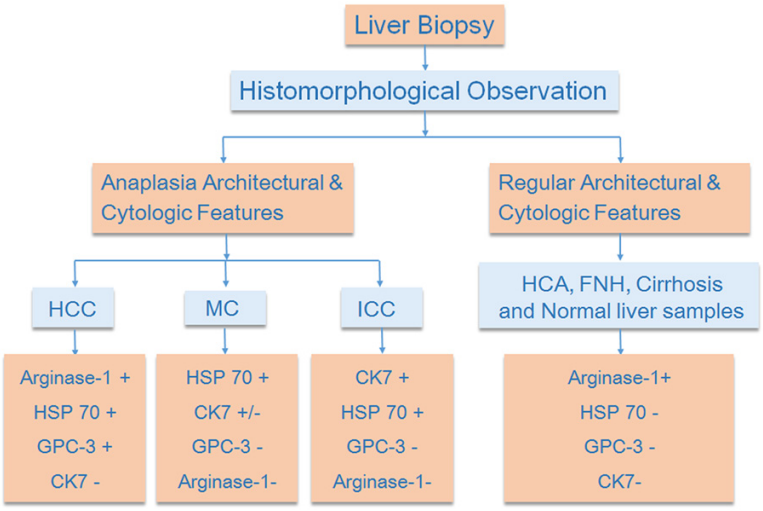

Figure 7. A flow chart for the clinical practical procedure in liver biopsy diagnosis.

centage of GPC-3 expression reported in HCCs ranges from 49% to 97.7%. In our work, GPC-3 was expressed in 37 (82%) HCCs, which is consistent with the results of previous studies [18]. Although GPC-3 positivity was observed in a subset of yolk sac tumor, choriocarcinoma, and melanoma, only one case of neuroendocrine carcinoma arising from the stomach showed immunoreactivity against GPC-3 in our MC group [18, 19]. Moreover, the sensitivity of GPC-3 for identifying HCCs was 82%, whereas the positive predictive value between HCCs and the other groups was 100%. Therefore, the immunoreactivity of GPC-3 cansignificantly distinguish HCCs from other liver masses in FNA biopsy detection.

Over the past few years, the elevation of HSP70 has been assessed in various solid carcinomas [20]. HSP70 may play an important role in protecting cells from DNA damage and perform anti-apoptotic functions. In our series of experiments [21, 22], HSP70 stained 84% of HCC, 82% of MC, and 73% of ICC, without immunoreactivity to benign lesions. Thus, HSP70 can serve as a potential marker of malignancy, although it cannot differentiate HCCs from MCs or ICCs.

CK7 is an effective immunohistochemical diagnostic tool for the study of the origins of tumors from normal epithelial tissues and cancers, especially in adenocarcinoma from the ovary, breast, lung, and bile duct [23, 24]. The utility of CK7 in this work is as a supplementary tool helped to identify ICCs and distinguish some MCs from HCCs [25]. As expected, CK7 was present in all 15 cases of ICC and 3 cases of MC (i.e., 1 case of invasive breast carcinoma, 1 case of neuroendocrine carcinoma transferred from the pancreas, and 1 case of pancreas ductal carcinoma). Although 3 of the HCC cases also showed positive for CK7, they were poorly differentiated and were positive for Arginase-1, GPC-3, and HSP70.

Overall, the findings suggest that a single immunohistochemical marker is insufficient for differentiating HCCs from other liver masses. Thus, we propose Arginase-1, GPC-3, HSP70, and CK7 to comprise a marker panel for routine diagnostic work. (1) Arginase-1 is a reliable marker for confirming the histogenesis of liver cells because of its high specificity and sensitivity. (2) The positive staining of GPC-3 and HSP70 can distinguish malignant components from benign lesions, whereas the negative results do not always indicate benign outcomes. The combination of GPC-3 and HSP70 was also evaluated in our work, and the interpretation of the immunostaining results is an integrated procedure under actual conditions. (3) We considered CK7 to be a vital complementary marker in diagnosis because of the high specificity and sensitivity in ICC differentiation. The positive staining results also prompt the presumption for MC.

The diagnostic differentiation of liver masses is occasionally challenging, especially in FNA samples. On the basis of our results, a flow chart (**Figure 7**) is presented for the clinical practical procedure in liver biopsy diagnosis, which also shows the possible outcomes of potential staining. Further analysis is needed to search for novel biomarkers for HCC and more effective treatment strategies.

Acknowledgements

This work was supported by grants from Key and Development Program of Shandong Province (No.2015GSF118015), the National Na-

tural Science Foundation of China (No.81202092), China Postdoctoral Science Foundation (No.2015M572053), and the Natural Science Foundation of Shandong Province, China (No. ZR2012HM079).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Zhenhai Zhang, Department of Hepatobiliary Surgery, Shandong Provincial Hospital Affiliated to Shandong University, Jinan 250021, Shandong, P. R. China. Tel: +86-531-68776430; Fax: +86-531-87037504; E-mail: mickypiero@aliyun.com

References

[1] Jain D. Tissue diagnosis of hepatocellular carcinoma. J Clin Exp Hepatol 2014; 4: S67-73.

[2] Tanaka M, Katayama F, Kato H, Tanaka H, Wang J, Qiao YL and Inoue M. Hepatitis B and C virus infection and hepatocellular carcinoma in China: a review of epidemiology and control measures. J Epidemiol 2011; 21: 401-416.

[3] Siegel R, Naishadham D and Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013; 63: 11-30.

[4] Tsuchiya N, Sawada Y, Endo I, Saito K, Uemura Y and Nakatsura T. Biomarkers for the early diagnosis of hepatocellular carcinoma. World J Gastroenterol 2015; 21: 10573-10583.

[5] Kobayashi S, Kim SR, Imoto S, Ando K, Hirakawa M, Saito J, Fukuda K, Otono Y, Sakaki M, Tsuchida S, Kim SK, Hayashi Y, Nakano M and Kudo M. Histopathological diagnosis of early HCC through biopsy: efficacy of Victoria blue and cytokeratin 7 staining. Dig Dis 2012; 30: 574-579.

[6] 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.

[7] Cai MY, Tong ZT, Zheng F, Liao YJ, Wang Y, Rao HL, Chen YC, Wu QL, Liu YH, Guan XY, Lin MC, Zeng YX, Kung HF and Xie D. EZH2 protein: a promising immunomarker for the detection of hepatocellular carcinomas in liver needle biopsies. Gut 2011; 60: 967-976.

[8] Lagana SM, Salomao M, Bao F, Moreira RK, Lefkowitch JH and Remotti HE. Utility of an immunohistochemical panel consisting of glypican-3, heat-shock protein-70, and glutamine synthetase in the distinction of low-grade hepatocellular carcinoma from hepatocellular adenoma. Appl Immunohistochem Mol Morphol 2013; 21: 170-176.

[9] Edge SB and Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 2010; 17: 1471-1474.

[10] Shibata T and Aburatani H. Exploration of liver cancer genomes. Nat Rev Gastroenterol Hepatol 2014; 11: 340-349.

[11] Zhou Y, Yin X, Ying J and Zhang B. Golgi protein 73 versus alpha-fetoprotein as a biomarker for hepatocellular carcinoma: a diagnostic meta-analysis. BMC Cancer 2012; 12: 17.

[12] Colli A, Fraquelli M, Casazza G, Massironi S, Colucci A, Conte D and Duca P. Accuracy of ultrasonography, spiral CT, magnetic resonance, and alpha-fetoprotein in diagnosing hepatocellular carcinoma: a systematic review. Am J Gastroenterol 2006; 101: 513-523.

[13] Mahawar KK, Parmar C, Graham Y, Abouleid A, Carr WR, Jennings N, Schroeder N and Small PK. Routine Liver Biopsy During Bariatric Surgery: an Analysis of Evidence Base. Obes Surg 2016; 26: 177-181.

[14] Kuo FY, Chen WJ, Lu SN, Wang JH and Eng HL. Fine needle aspiration cytodiagnosis of liver tumors. Acta Cytol 2004; 48: 142-148.

[15] 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.

[16] 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.

[17] Wang L, Yao M, Pan LH, Qian Q and Yao DF. Glypican-3 is a biomarker and a therapeutic target of hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int 2015; 14: 361-366.

[18] Ibrahim TR and 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: 379-387.

[19] Geramizadeh B and Seirfar N. Diagnostic Value of Arginase-1 and Glypican-3 in Differential Diagnosis of Hepatocellular Carcinoma, Cholangiocarcinoma and Metastatic Carcinoma of Liver. Hepat Mon 2015; 15: e30336.

[20] Ciocca DR and Calderwood SK. Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. Cell Stress Chaperones 2005; 10: 86-103.

[21] Calini V, Urani C and Camatini M. Overexpression of HSP70 is induced by ionizing radiation

in C3H 10T1/2 cells and protects from DNA damage. Toxicol In Vitro 2003; 17: 561-566.
[22] Rerole AL, Jego G and Garrido C. Hsp70: anti-apoptotic and tumorigenic protein. Methods Mol Biol 2011; 787: 205-230.
[23] Reisenbichler ES, Ross JR and Hameed O. The clinical use of a P63/cytokeratin7/18/cytokeratin5/14 antibody cocktail in diagnostic breast pathology. Ann Diagn Pathol 2014; 18: 313-318.
[24] Kriplani D and Patel MM. Immunohistochemistry: A diagnostic aid in differentiating primary epithelial ovarian tumors and tumors metastatic to the ovary. South Asian J Cancer 2013; 2: 254-258.
[25] Ryu HS, Lee K, Shin E, Kim SH, Jing J, Jung HY, Lee H and Jang JJ. Comparative analysis of immunohistochemical markers for differential diagnosis of hepatocelluar carcinoma and cholangiocarcinoma. Tumori 2012; 98: 478-484.