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Original Article

The best immunohistochemical panel for differentiating hepatocellular carcinoma from metastatic adenocarcinoma

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ABSTRACT

It can be difficult to differentiate hepatocellular carcinoma (HCC) from metastatic adenocarcinoma (MA). An appropriate immunohistochemical panel is required for the differential diagnosis. This study aimed at finding the best panel, including hepatocyte-specific antigen (Hepatocyte), pCEA, CD10, Villin, CD34, TTF-1, MOC-31, CK7, and CK20 antibodies. Sixty-eight cases of HCC and 107 cases of MA were investigated. Hepatocyte positivity was seen in 95.6% of HCCs and in 1.9% of MAs. pCEA was expressed in 47.8% of HCCs and in 86.8% of MAs. CD10 stained 73.13% of HCCs and 36.9% of MAs. Villin was positive in 23.5% of HCCs and in 81.0% of MAs. Canalicular staining with pCEA, CD10, and Villin was seen only in HCCs. Sinusoidal CD34 staining was seen only in 42.6% of HCCs. A small subset of HCCs demonstrated cytoplasmic TTF-1 and MOC-31. CK7 was expressed in 29.4% of HCCs and in 29.9% of MAs, whereas CK20 stained 14.7% of HCCs and 62.6% of MAs. In conclusion, Hepatocyte should be combined with pCEA, MOC-31, CD10, and CD34. Canalicular staining with pCEA, CD10, and Villin is specific for HCC. CK7 and CK20 expression may be seen in some HCCs. We suggest that the best panel for discriminating HCC from MA should contain Hepatocyte, MOC-31, pCEA, CD10, and CD34.

Introduction

Hepatocellular carcinoma (HCC) is the most common malignant primary tumor of the liver [1]. HCC affects about a million people every year worldwide [2]. On the other hand, metastatic tumors are widespread in the liver, with metastatic adenocarcinoma (MA) constituting the greatest part [3]. Therefore, the differentiation of HCC from MA in the liver is a frequent problem that the pathologist should solve as a matter of routine. The differential diagnosis may be difficult, especially if the biopsy material is limited and if the tumor shows pseudoglandular or poorly differentiated morphology in a patient with an unknown primary tumor. In such cases, as there are no highly specific markers, an appropriate immunohistochemical panel, including multiple antibodies with different sensitivities and specificities, should be used to establish the correct diagnosis. This study aimed at finding the most effective immunohistochemical panel for the differential diagnosis of HCC and MA in the liver.

Materials and methods

Patients and tissue samples

This study included 68 HCCs and 107 MA cases, diagnosed at Ankara University, Department of Pathology, between 1999 and 2006. Partial or total hepatotectomy, metastasectomy, or wedge biopsy materials were evaluated, while tru-cut biopsies were not included. In all, except for 11 MAs with an unknown primary site, the primary site was confirmed by clinical history and microscopic evaluation of primary resected tumors. The primary sites of the tumors were the colon ($n = 73$ cases), pancreas ($n = 10$ cases), stomach ($n = 5$ cases), breast ($n = 4$ cases), ovary ($n = 3$ cases), and small intestine ($n = 1$ case).

Six tissue microarray (TMA) blocks were constructed using a manual microarrayer (Beecher Instruments, Silver Spring, USA). We reviewed tissue sections (4–5 μm thick) stained by hematoxylin and eosin (H&E) (Fig. 1a and b). HCCs were histologically graded. Each case was represented by three cores of 1.5 mm in diameter.

Immunohistochemistry (IHC)

IHC was performed using antibodies against Hepatocyte, polyclonal CEA (pCEA), CD10, Villin, CD34, TTF-1, MOC-31, CK7, and CK20 with either Zymed ABC Px Kit or Ventana Benchmark

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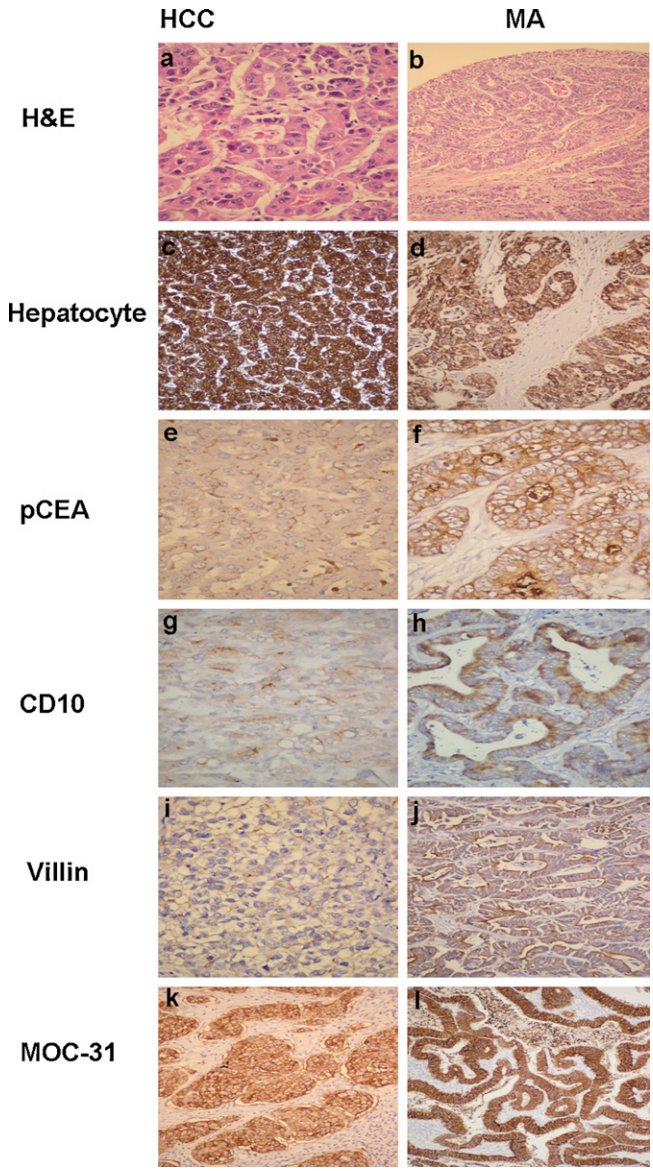

Fig. 1. Example of HCC (a) and MA cases (b) (hematoxylin–eosin, 400×, 200×). The expression of Hepatocyte (c and d), pCEA (e and f), CD10 (g and h), Villin (i and j), and MOC-31 (k and l) in cases of HCC and MA (400×).

automated immunostainer for secondary visualization (Table 1). Appropriate positive and negative tissue controls were put into the arrays.

Staining patterns were recorded for pCEA, CD10, and Villin as canalicular, cytoplasmic, or membranous. CD34 staining of the endothelium surrounding the tumor cells in HCC was considered as positive and indicative of hepatocytic differentiation. TTF-1 positivity was recorded as cytoplasmic or nuclear. The cytoplasmic expressions of Hepatocyte, CK7, and CK20 were scored. MOC-31 was expressed in a membranous pattern.

For all markers, except for CD34, staining results were scored as 0 (negative), 1+ (<5% positive cells), 2+ (5% to 50% positive cells), and 3+ (>50% positive cells). Specific CD34 staining was seen either in a diffuse or focal pattern, and was recorded as diffuse or focal positivity.

For statistical analysis, the highest staining score of three tissue cores was recorded. Because of necrosis and shedding, IHC data for a few IHC markers were not available in all cases. These cases were not included in statistical analysis.

Table 1
Antibodies used for immunohistochemical staining.

Antibody	Clone	Pretreatment	Dilution	Source
Hepatocyte	OCH1E5	Cell condition mild	1:30	Dako
CK7	OV-TL12/30	Protease	1:150	Neomarkers
CK20	Ks20.8	Protease	1:100	Neomarkers
pCEA	Polyclonal	Protease	1:50	Neomarkers
MOC-31	MOC-31	Cell condition mild	1:50	Neomarkers
CD34	QBEnd10	Cell condition mild	1:400	Neomarkers
CD10	56C6	Cell condition extended	1:60	Neomarkers
Villin	CWWB1	Cell condition mild	1:500	Novocastra
TTF-1	SG7G3/1	Cell condition mild	1:200	Zymed

Statistical analysis

The results were evaluated using χ^2 test (p value less than 0.05 was considered significant) and Fisher's exact test. We analyzed the sensitivity and specificity of each immunohistochemical marker for differentiating HCC from MA.

Results

Clinical results

The average age of HCC patients was 56.67 (range 22–83 years) and 59.34 (range 25–101 years) in MA patients. There were 43 males and 25 females in the HCC group, and 59 males and 48 females in the MA group.

Histopathologic results

Histologically, HCCs were differentiated well in 6 cases, moderately in 33 cases, and poorly in 29 cases. Tumor size ranged between 1 cm and 24 cm in diameter (mean 6.3 cm) in HCCs. There was a statistically significant relationship between tumor size and tumor grade in HCCs ($p = 0.007$).

Tumor was solitary in 43 HCCs, and multifocality was noted in 25 HCCs. There was no relationship between multifocality and tumor grade.

Immunohistochemical results

Table 2 summarizes the immunohistochemical results and the specificity–sensitivity of each marker in HCC diagnosis.

Hepatocyte

Sixty-five of 68 HCCs (95.6%) and 2 of 107 MAs (1.9%) demonstrated Hepatocyte positivity (Fig. 1c and d). All but only three cases of grade three HCCs were negative for Hepatocyte. Hepatocyte staining score was (3+) (86.8%) in most of the HCCs. The only two Hepatocyte-positive MAs were stained diffusely (3+). Sensitiv-

Table 2
The immunohistochemical results of HCC and MA cases.

Antibody	HCC ($n = 68$)	MA ($n = 107$)	Sensitivity (%)	Specificity
Hepatocyte	65 (95.6%)	2 (1.9%)	95.6	98.1
pCEA	32 (47.8%)	92 (86.8%)[a]	45.6	100
CD10	49 (73.13%)	38 (36.9%)[a]	50	100
Villin	16 (23.5%)	85 (81.0%)[a]	20.6	100
CD34	29 (42.6%)	0 (0%)	42.6	100
TTF-1	18 (26.5%)	8 (7.5%)	26.5	92.5
MOC-31	13 (19.1%)	106 (99.1%)	99.1[b]	80.9[b]
CK7	20 (29.4%)	32 (29.9%)	29.4	70.1
CK20	10 (14.7%)	67 (62.6%)	14.7	37.4

[a] All cases showed non-canalicular staining.
[b] The results are for MA diagnosis.