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Liver Metastases of Neuroendocrine Tumors Rarely Show Overlapping Immunoprofile with Hepatocellular Carcinomas

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Abstract The distinction of hepatocellular carcinoma (HCC), neuroendocrine tumor (NET) metastatic to the liver, and cholangiocarcinoma (CC) can sometimes be challenging on small biopsies. Tissue microarrays were constructed from HCCs, NETs, and CCs. The immunoprofile was evaluated using HepPar1, glypican-3 (GPC3), synaptophysin (SYN), chromogranin A (CHR), CD56, MOC-31, and pCEA. One hundred thirteen HCCs, 48 NETs, and 44 CCs were included. Of HCCs, 107 (95 %) expressed HepPar1 and/or GPC3, 52 (46 %) both, and 97 (88 %) marked with pCEA (canalicular pattern). Seven (6 %) expressed CD56, of which 3 (3 %) expressed SYN. All 7 HCCs that expressed CD56 and/or SYN also expressed HepPar1 and/or GPC3, and none of the HCCs expressed CHR. Fourteen (13 %) expressed MOC-31. All 48 NETs expressed at least one neuroendocrine marker: 47 (98 %) positive for SYN, 40 (83 %) for CHR, 39 (81 %) for CD56, and 34 (71 %) for all three markers. None expressed HepPar1 or GPC3. All 44 CCs showed at least focal reactivity with MOC-31 and pCEA (membranous/cytoplasmic). One (2 %) was positive for HepPar1, 4 (9 %) for GPC3, 1 (2 %) for SYN and CHR, and 7 (16 %) for CD56. HCCs rarely express CD56 and SYN, while all express either HepPar1 or GPC3. NETs do not express HepPar1 or GPC3 and almost always express SYN, while CHR and CD56 are seen in most cases. Rare CCs focally express HepPar1 and GPC3. Utilizing a limited staining panel can efficiently distinguish HCCs, NETs, and CCs and help avoid diagnostic pitfalls on small biopsies.

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Introduction

Liver is a common site for metastasis of gastrointestinal neoplasms, and in many cases, there is a known primary neoplasm outside of the liver. Not uncommonly, however, liver masses are found in patients with no known history of malignancy, and the primary neoplasm may not be found until later. The distinction of primary carcinoma of the liver, such as hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC), from metastatic carcinoma to the liver can be difficult and is particularly challenging on core or fine needle aspiration biopsies with limited tissue available [1]. Colorectal carcinoma represents the most common primary among liver metastasis; however, distinction from HCC or CC is usually not problematic since most colorectal carcinomas display a characteristic histomorphology and/or have a known primary. Neuroendocrine tumors (NETs), the second most common source of liver metastases [2, 3], often pose challenges in the differential diagnosis because they can show trabecular, nested, and acinar histologic patterns that may be mistaken for HCC or CC.

The number of antibodies that have been shown to be useful in the distinction of HCC from NET and adenocarcinoma is constantly changing as more new antibodies become commercially available [4–7]; however, each has advantages and limitations, making their judicious use imperative particularly in small biopsies.

In the present study, we compared the immunohistochemistry (IHC) staining profile of HepPar1, glypican-3 (GPC3), MOC-31, pCEA, synaptophysin (SYN), chromogranin A

(CHR), and CD56 in a series of HCC, NET liver metastasis, and CC, and to determine how often these tumors show an overlapping pattern of expression using tissue microarray (TMA), a setting mimicking small biopsies.

Materials and Methods

Case Selection and TMA

A total of 205 specimens, including 113 HCCs, 48 NETs metastatic to the liver, and 44 intrahepatic CCs, were retrieved from tissue archive in the Department of Pathology at The Ohio State University Wexner Medical Center from 1992 to 2010. Of 113 HCCs, 110 were conventional HCCs including 85 (77 %) well differentiated (grades I–II of IV, Edmondson and Steiner) and 25 (23 %) moderately to poorly differentiated (grades III–IV of IV, Edmondson and Steiner), and 3 were fibrolamellar variant HCCs. Of 48 NETs, 44 (92 %) were well-differentiated NETs (WHO grades 1–2 of 3) and others were poorly differentiated neuroendocrine carcinomas (NECs) (WHO grade 3 of 3). Thirty NETs were from the gastrointestinal (GI) tract (mostly small intestine), 6 were from pancreas, 2 were from outside of the GI tract/pancreas, and 10 were primary site unclear (including loss of follow-up or medical record unavailable). Of 44 CCs, 38 (86 %) were well to moderately differentiated and others were poorly differentiated. TMA was created from paraffin-embedded tissue using a 1.5-mm-diameter punch (Beecher Instruments, Silver Spring, MD). Duplicate cores were taken from each specimen.

IHC Staining

IHC staining was carried out using avidin-biotin complex technique. Briefly, deparaffinized sections were pretreated with heat-induced antigen epitope retrieval in target retrieval solution (Dako) at pH 6.1. All reactions were carried out using the VECTASTAIN Elite (Vector Laboratories, Burlingame, CA) dictation kit in a Dako Cytomation Autostainer system (DaKo Cytomation, Carpinteria, CA) following manufacturer's instruction. The primary antibodies used in this study include HepPar1, clone M7158 (1:80, Dako, Carpinteria, CA, USA); GPC3, clone B0025 (1:750, Biomosaics, Burlington, VT, USA); SYN, clone NCL-SYNAP-299 (1:100, Leica, Copenhagen, DK); CHR, clone M0869 (1:200, Dako, Carpinteria, CA, USA); CD56, clone NCL-CD56-1B6 (1:50, Novacastra laboratories Ltd., Newcastle, UK); MOC-31 (antihuman epithelial-related antigen, 1:40, Dako, Carpinteria, CA, USA); and pCEA (polyclonal carcinoembryonic antigen, 1:800, Dako, Carpinteria, CA, USA). Positive and negative controls were stained in parallel with each batch and showed appropriate reactivity.

Staining Grading

Two pathologists (XZ, WLF) independently reviewed the IHC stained slides. The tumor cell staining intensity was graded as weak to moderate (+) and strong (++). In addition, the tumor cell staining percentage was graded as no staining for <5 %, focal for 5 to 30 %, and diffuse for >30 %. For pCEA, a canalicular pattern of staining was considered positive for HCC, while membranous and/or cytoplasmic staining was considered positive for CC and NET.

Results

The IHC staining profile of all tumors is shown in Table 1, and examples of stains are demonstrated in Fig. 1.

Of 113 HCCs, 107 (94 %) were immunoreactive for HepPar1 and/or GPC3, 92 (81 %) for HepPar1, 67 (59 %) for GPC3, 52 (46 %) for both, and 97 (86 %) for pCEA with canalicular staining pattern. Of 21 negative for HepPar1, 13 (62 %) were well differentiated, and 8 (38 %) were moderately to poorly differentiated. All 3 fibrolamellar variant HCCs were positive for HepPar1. Of 46 negative for GPC3, 34 (74 %) were well differentiated, 10 (22 %) were moderately to poorly differentiated, and 2 were fibrolamellar variant HCC. Seven HCCs (6 %) showed immunoreactivity for CD56 (4 focal, 3 diffuse, 2 weak to moderate, and 5 strong); of which 3 (3 %) were also positive for SYN (all focal; 1 weak to moderate and 2 strong). None of the HCC was immunoreactive for CHR. None of the three fibrolamellar HCCs showed immunoreactivity to any of the neuroendocrine markers. All 7 HCCs positive for CD56 or SYN showed diffuse expression of HepPar1 or GPC3. Fourteen (12 %; 3 strong, 5 weak to moderate, 2 focal strong, and 4 focal weak to moderate) were positive for MOC-31.

All 48 NET liver metastases expressed at least one neuroendocrine marker. All but one (98 %) NETs were positive for SYN (all strong), 40 (83 %) for CHR (39 strong and 1 weak to moderate), and 39 (81 %, 36 strong, 3 weak to moderate) for CD56. Thirty-four (71 %) expressed all three neuroendocrine markers. Of 4 poorly differentiated NECs, 1 was negative for CHR and SYN but expressed CD56, 1 was negative for CHR and CD56 but expressed SYN, 1 was negative for CHR but expressed SYN and CD56, and 1 was positive for all 3 neuroendocrine markers. No HepPar1 or GPC3 expression was detected in any of the NET/Cs. Forty-six (96 %) were positive for MOC-31 (38 strong, 8 weak to moderate) and 29 (60 %) for pCEA (1 strong, 14 focal weak to moderate, 14 diffuse weak to moderate).

All but one (43 of 44, 98 %) CCs were diffusely positive for MOC-31 (32 strong, 11 weak to moderate) and pCEA (29 strong, 14 weak to moderate; membranous or cytoplasmic). The one CC with 5 % and weak staining for both MOC-31 and

Table 1 Immunohistochemical staining in tumors in the liver

Antibody	HCC ($n = 113$)	NET ($n = 48$)	CC ($n = 44$)
HepPar1	92 (81 %)	0	1 (2 %)
	(83 ++, 9 +)		(f+)
GPC3	67 (59 %)	0	4 (9 %)
	(58 ++, 9 +)		(1 f++, 2 +, 1 f+)
SYN	3 (3 %)	47 (98 %)	1 (2 %)
	(2f++, 1f+)	(All ++)	(f++)
CHR	0	40 (83 %)	1 (2 %)
		(39 ++, 1 +)	(f++)
CD56	7 (6 %)	39 (81 %)	7 (16 %)
	(3++, 2f++, 2f+)	(36 ++, 3 +)	(1f++, 6f+)
MOC-31	14 (12 %)	46 (96 %)	44 (100 %)
	(3++, 5+, 2 f++, 4 f+)	(38 ++, 8 +)	(32 ++, 11 +, 1 f+)
pCEA	97 (86 %)[a]	29 (60 %)[b]	44 (100 %)[b]
	(15++, 62 +, 20 f+)	(1++, 14+, 14 f+)	(29 ++, 14 +, 1f+)

IHC immunohistochemical stain, *HCC* hepatocellular carcinoma, *CC* cholangiocarcinoma, *NET* neuroendocrine tumor, *GPC3* glypican-3, *SYN* synaptophysin, *CHR* chromogranin A, *f* focal, + weak to moderate, ++ strong

[a] Canalicular staining

[b] Membranous and/or cytoplasmic staining

pCEA was an adenocarcinoma with extensive squamous differentiation. None of the 44 CCs expressed HepPar1, while 4 (9 %) were positive for GPC3 (2 focal, 2 diffuse; 3 weak, 1 strong). Of 4 positive for GPC3, 1 was poorly differentiated, and 3 were well to moderately differentiated. One (2 %) was positive for SYN and CHR (both focal but strong), and 7 (16 %) were positive for CD56 (all focal; 6 weak to moderate, and 1 strong).

Discussion

Pathologists are frequently faced with small biopsies of liver tumors. HCC, NET metastatic to the liver, and CC can sometimes appear similar on H&E stains with trabecular, nested, and acinar histologic patterns; thus, the distinction among these can be challenging based on morphology alone. While IHC options are available/known for each tumor, studies directly comparing the staining patterns of these tumors are limited. The current study compared a panel of IHC stains in HCC, NET, and CC using TMAs and evaluated how often they have overlapping immunoprofiles.

Immunoprofile of HCCs

HepPar1, a monoclonal antibody that was developed using formalin-fixed tissue from failed allograft liver, has been used as a sensitive and specific IHC marker for hepatocellular lineage with a high sensitivity and specificity (both greater than 80 %) [5, 7]. We found that 81 % of HCC were positive for HepPar1, consistent with the previously reported findings. All 3 fibrolamellar variant HCCs expressed HepPar1. GPC3, a membrane-anchored heparin sulfate proteoglycan, is designated as an oncofetal protein. Previous studies have shown that GPC3 is expressed in 64 to 85 % of HCC but not in normal liver and benign lesions such as hepatic adenoma [6, 8, 9], although cirrhotic nodules can show positivity [10, 11]. GPC3 has been shown to be more sensitive than HepPar1 for poorly differentiated HCC [11, 12]. In our series, GPC3 was positive in 59 % of HCC cases, close to the 64–85 % range of positivity previously reported. This slightly lower percentage of positive GPC3 immunoreactivity could be explained by a possible under representation of poorly differentiated HCCs in our series (23 % grades III–IV). The pCEA antibody stains normal liver and HCC with a characteristic canalicular pattern because of its cross-reaction with biliary glycoprotein I [13]. We found that 97 (86 %) HCCs were positive for pCEA with canalicular staining. Although pCEA can be very useful, difficulty in interpretation of the canalicular pattern of staining is present in some HCCs [14].

We found that 7 (6 %; 4 focal and 3 diffuse) HCCs were positive for CD56, of which 3 (3 %) were also focally positive for SYN and none were positive for CHR. While strong cytoplasmic staining with SYN or CHR supports a neuroendocrine tumor, focal neuroendocrine differentiation has been described in HCC using various markers including SYN, CHR, and CD56 [15, 16]. In particular, the fibrolamellar variant of HCC has been shown to co-express neuroendocrine markers such as SYN and CHR [17, 18]. However, a study

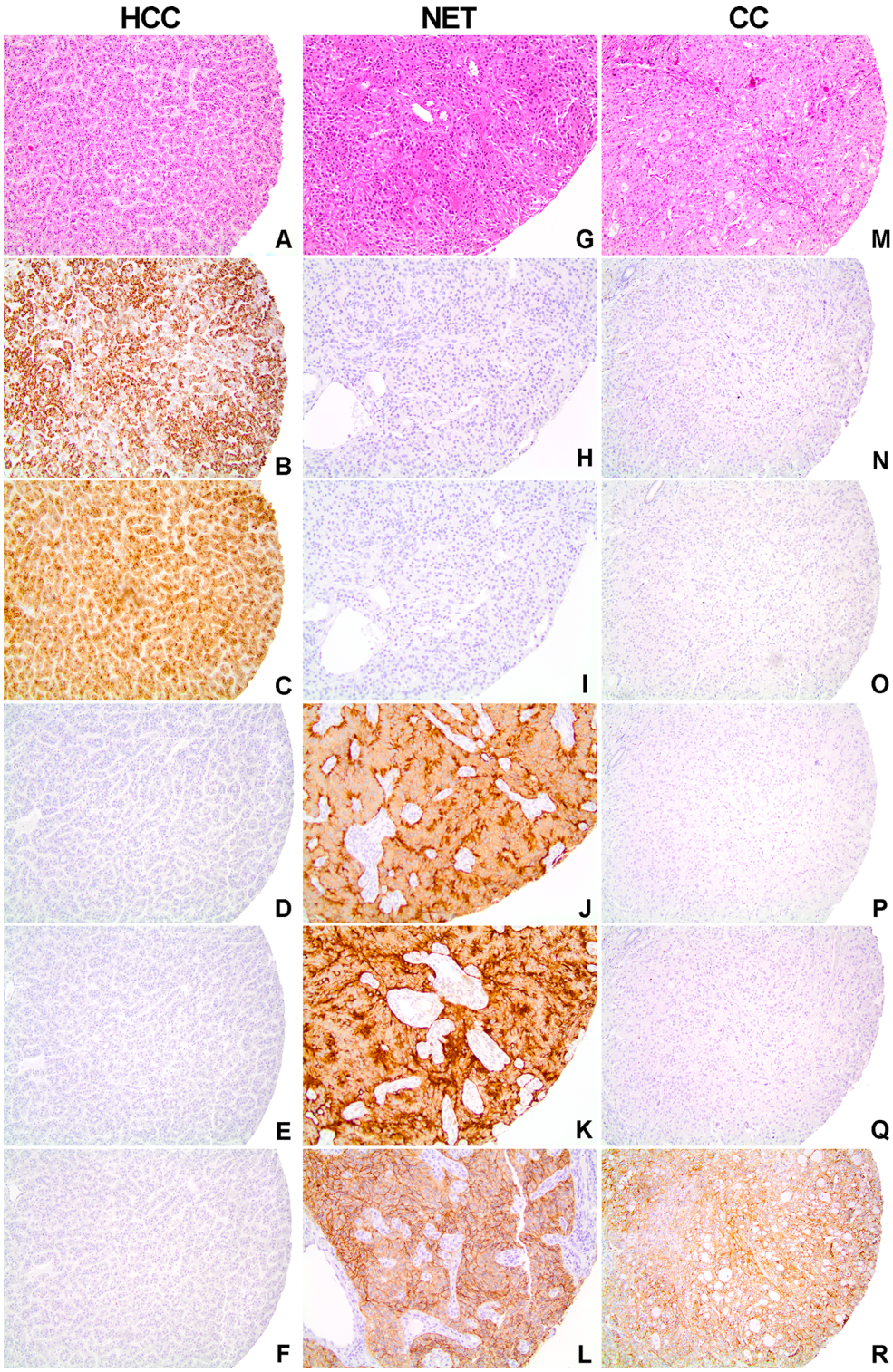

Fig. 1 Representative examples of hepatocellular carcinoma (HCC, *left panel*), neuroendocrine tumor (NET, *middle panel*), and cholangiocarcinoma (CC, *right panel*) (×100). *A*, *G*, *M* H&E stains. *B*, *H*, *N* HepPar1 stain. *C*, *I*, *O* GPC3 stain. *D*, *J*, *P* SYN stain. *E*, *K*, *Q* CHR stain. *F*, *L*, *R* MOC-31 stain. HCC shows positive HepPar1 and GPC3, and negative SYN, CHR, and MOC-31. NET demonstrates positive SYN, CHR, and MOC-31, and negative HepPar1 and GPC3. CC shows positive MOC-31 and negative HepPar1, GPC3, SYN, and CHR

with 26 fibrolamellar HCCs revealed minimal evidence of neuroendocrine differentiation using immunostaining for CD56, SYN, and CHR [19]. The three fibrolamellar HCCs included in our series did not show immunoreactivity to any of the neuroendocrine markers, consistent with the study by Ward et al. Nonetheless, all 7 HCCs positive for CD56 or SYN in our series expressed stronger and more diffuse HepPar1 or GPC3. Although rare HCCs can express CD56 and/or SYN (usually focal), they do not express CHR and do express hepatocellular markers.

Immunoprofile of NETs

SYN, CHR, and CD56 are the most commonly used neuroendocrine markers. All NETs metastatic to the liver that we studied, as expected, expressed at least one of the neuroendocrine markers. Overall, SYN was the most sensitive (98 %) neuroendocrine marker. The single NET without immunoreactivity for SYN and CHR, but with strong positivity for CD56 was a poorly differentiated NEC metastatic to the liver. Concordant with previous finding [15], none of the NETs stained with HepPar1. Mounajjed et al. reported that none of pancreatic NETs expressed GPC3, and very few (2.5 %) GI tract NETs expressed GPC3 [20]. We found that GPC3 was not expressed in any NETs metastatic to the liver. Therefore, NETs metastatic to the liver usually do not show overlapping immunoprofile with HCC.

MOC-31 is an antibody to cell membrane glycoproteins expressed on epithelia and is a general epithelial marker. It is not specific for adenocarcinoma and can be positive in NETs. In our series, 96 % of NETs expressed MOC-31. Neither MOC-31 nor pCEA is useful to distinguish NET from CC.

Immunoprofile of CC

We have previously shown that MOC-31 can be very useful in distinguishing CC and HCC; it consistently stains CC but not usually HCC [20]. The results of the current study are similar to that of previous studies showing that both MOC-31 and pCEA (cytoplasmic and/or membranous staining) are highly sensitive for the distinction of adenocarcinoma from hepatocellular carcinoma [14, 21]. These stains are not useful to distinguish CC from NET.

While some tumors other than HCC, such as adenocarcinomas of the stomach, ovary, and lung, have been reported to show immunoreactivity with HepPar1, CC has been shown to be consistently negative for HepPar1 [4]. Likewise, CC usually does not express GPC3 [10, 11, 20]. Our findings are in agreement with these reports. In our series of 44 CCs, 1 tumor (2 %) showed focal and weak immunoreactivity for HepPar1, and 4 (9 %) were positive for GPC3 (2 focal, 2 diffuse; 3 weak, 1 strong). Of those positive for GPC3, 1 was poorly differentiated, and 3 were well to moderately differentiated.

Seven (16 %) CCs were positive for CD56 (all focal), of which one also showed focal immunoreactivity with SYN and CHR. This finding in CC was similar to that in the HCC cases where predominantly focal staining of CD56 was seen with lack of immunoreactivity for other neuroendocrine markers, suggestive of focal ductular differentiation as described previously [22]. CD56 has been considered as a putative hepatic stem cell/progenitor cell marker [23], found to be expressed in 20 % of HCC components and 60 % of CC components of combined HCC-CC [24]. It is possible that the focal expression of CD56 found in a small subset of HCC and CC in our series could represent the focal stem/progenitor cells.

Summary

We found that HCCs and CCs very rarely express SYN or CHR, and most HCCs express either HepPar1 or GPC3. NETs metastatic to the liver do not express HepPar1 or GPC3 and almost always express SYN, while staining of CHR and CD56 is seen in most cases. While H&E histomorphology as well as clinical history is important for a pathologic diagnosis, utilizing a limited IHC stain panel including HepPar1, GPC3, CHR (and/or SYN), and MOC-31 (or pCEA) can efficiently distinguish HCCs and CCs from NET liver metastases and help to avoid diagnostic pitfalls on small biopsies.

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