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HNF-1β is a More Sensitive and Specific Marker Than C-Reactive Protein for Identifying Biliary Differentiation in Primary Hepatic Carcinomas

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- **Context.**—Intrahepatic cholangiocarcinoma (iCCA) needs to be distinguished from hepatocellular carcinoma (HCC) and metastasis, and in the absence of any specific biliary markers, often is a diagnosis of exclusion. Hepatocyte nuclear factor (HNF)-1β is a transcription factor that plays a critical role in bile duct system morphogenesis.

- **Objective.**—To investigate the diagnostic value of HNF-1β to differentiate iCCA from HCC by immunohistochemistry and compare HNF-1β with C-reactive protein (CRP), a previously identified marker for iCCA.

- **Design.**—Cases of iCCA (n = 75), combined hepatocellular-cholangiocarcinoma (CHCC-CCA) (n = 13) and HCC (n = 65) were included in the study.

- **Results.**—All cases of iCCA (74 of 74, 100%) expressed HNF-1β compared with CRP expressed in 72.60% (53 of 73). The sensitivity and specificity of HNF-1β to differentiate iCCA from HCC was 100% and 92.31%, whereas the sensitivity and specificity for CRP was 75.58% and 7.79%. The expression of HNF-1β was greater in iCCA and the CCA component of CHCC-CCA compared with CRP (87 of 87, 100% versus 65 of 86, 75.58%, P < .001). On the contrary, CRP was more frequently expressed compared with HNF-1β in HCC and HCC component of CHCC-CCA (71 of 77, 92.21% versus 6 of 78, 7.69%; P < .001).

- **Conclusions.**—Our data indicate that HNF-1β is a more sensitive and specific marker than CRP for the diagnosis of iCCA and to identify the CCA component in CHCC-CCA. Lack of HNF-1β expression may be used to exclude iCCA from consideration in cases of adenocarcinomas of unknown primary.

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Intrahepatic cholangiocarcinoma (iCCA) is an intrahepatic malignant epithelial neoplasm with biliary differentiation and is the second most common primary hepatic malignancy (10%−15%) after hepatocellular carcinoma (HCC). Studies show that iCCA shares several risk factors with HCC, including hepatitis B virus and hepatitis C virus infection, alcohol, obesity, diabetes mellitus, and metabolic syndrome. Their histology is often distinct; however, differentiation of HCCs and ICCAs based on morphology sometimes can be challenging. The distinction between HCC and iCCA, and identification of the cholangiocarcinoma (CCA) component to diagnose combined hepatocellular-cholangiocarcinomas (CHCC-CCA) are important due to differences in management. In cases of iCCA and CHCC-CCA surgical resection is the main treatment of choice where feasible, often accompanied by lymph node dissection owing to high frequency of lymph node metastasis. Liver transplant is often contraindicated in iCCA or CHCC-CCA. The treatment options for HCC include resection, ablation, transplantation, transarterial chemotherapy and radiotherapy, and systemic therapy. Resection for HCC does not involve lymph node dissection.

In challenging cases, a wide variety of immunohistochemical (IHC) markers, such as alpha-fetoprotein, polyclonal CEA, CD10, Hep par-1 or Arginase-1, and biliary markers, such as C-reactive protein (CRP), CK7, and CK19, have been suggested to be useful to differentiate iCCA from HCC. However, while some of these markers are highly sensitive and/or specific for the identification of hepatocytic differentiation, they are not very sensitive and/or specific for biliary differentiation. Of these, CRP was recently reported to be a promising IHC marker for the diagnosis of iCCA with a sensitivity of 75.7% and specificity of 91.1%. Hepatocyte nuclear factor-1β (HNF-1β) is a transcription factor that plays a critical role in normal development of the kidney, biliary tract, and pancreas. Mutations in HNF-1β can cause pancreatic agenesis or hypoplasia, cholestasis, abnormal intrahepatic bile duct differentiation and morphogenesis, hypoplastic glomerulocystic kidney, and genital tract malformations. In human malignancies, HNF-1β has been shown to be expressed in ovarian clear cell carcinomas, endometrial carcinomas, colorectal carcinomas, clear cell renal cell carcinomas, and urothelial carcinomas.
Recently, HNF-1β was recognized as a sensitive and specific marker for testicular yolk sac tumors with sensitivity and specificity of 85.4% and 96.5%. Studies show that pancreatic ductal adenocarcinoma expresses HNF-1β in 75.6% to 84.3% of cases. In the liver, HNF-1β expression in iCCAs and its correlation with expression of other biliary markers has been reported. However, the significance of HNF-1β expression in hepatic malignancies and its clinical utility remains controversial. The goal of this study was to study the utility of HNF-1β in differentiating biliary from hepatocytic differentiation and to compare it with CRP, a previously identified marker of iCCAs.

MATERIALS AND METHODS
Case Selection and Pathologic Evaluation
The pathology database was searched from January 2010 to May 2020 for cases of iCCA, and 75 iCCAs, including 52 biopsies and 23 resections, were included in the study where tissue was available to perform IHC. The diagnosis of iCCA was based on careful review of medical records, including clinical history, physical examination, laboratory tests, radiologic evaluation, pathologic examination, and cases with other primary sources of adenocarcinoma with liver metastasis were excluded. To investigate the sensitivity and specificity of the candidate marker, HNF-1β IHC was also performed on 13 resection cases of cHCC-CCA and 27 conventional HCCs (17 resections and 10 biopsies). In addition, tissue microarrays containing 38 HCCs (US Biomax, Rockville, Maryland) were included in this study. The slides from all the cases were reviewed and confirmed by 2 pathologists. The tumor grade, and in case of resection specimens, the tumor (pT) stage and lymphovascular invasion (LVI) were recorded. The study was approved by the institutional review board.

Immunohistochemical Staining and Scoring
One representative block from each case was selected to perform IHC with HNF-1β (HPA002083, 1:500; Sigma) and CRP (ab32412, 1:4000; Abcam) antibodies with the Ventana Benchmark Ultra Stainer. The cytoplasmic expression for CRP and nuclear expression for HNF-1β was semiquantiatively scored. The result was considered negative (score 0) when not expressed or in 5% or less of cells. The result was considered positive when expressed in more than 5% tumor cells and the extent of positivity was scored as follows: 5% to 25% = score 1, more than 25% to 50% = score 2, and more than 50% = score 3. The strength of expression was subjectively scored as weak, moderate, or strong. Tissue was available to perform HNF-1β in 74 of 75 cases of iCCA, and in all cases of HCC and cHCC-CCA. CRP was performed on 73 of 75 cases of iCCA, 64 of 65 cases of HCC, and all cases of cHCC-CCA.

Statistical Analysis
Statistical analysis was performed with GraphPad Prism 8 statistical software using contingency tables. The P values were determined using Fisher exact test. Sensitivity and specificity were determined using Wilson-Brown Method. Differences were considered statistically significant when the P value was less than .05.

RESULTS
Of 75 cases of iCCA, patient age ranged from 31 to 94 years (68.19 ± 12.60) with male to female ratio of 42:33. In the 23 iCCA resection specimens, 12 of those had LVI, and the pT stages were pT1 10 cases, pT2 8 cases, and pT3 5 cases. Of 27 cases of HCC, patient age ranged from 55 to 79 years (66.46 ± 6.38) with male to female ratio of 21:6. In the 17 HCC resection specimens, 8 of those had LVI, and the pT stages were pT1 5 cases, pT2 9 cases, and pT3 2 cases. Of 13 cases of cHCC-CCA, patient age ranged from 51 to 85 years (67.84 ± 9.98) with male to female ratio of 9:4.

Expression of HNF-1β and CRP in Background Liver
In all cases the background liver was available for review. Strong nuclear expression of HNF-1β was seen in the bile duct and bile ductular epithelial cells, and was negative in hepatocytes. Focal cytoplasmic expression of CRP was seen along the perportal/perisepal hepatocytes, and was negative or barely perceivable in epithelium of bile ducts and bile ductules.

Expression of HNF-1β and CRP in iCCA
Among the 75 iCCA cases (Figure 1, A), 74 cases were available for IHC and were all immunoreactive for HNF-1β. Of these, 67 cases (90.54%) showed diffuse (score 3) and strong nuclear HNF-1β expression (Figure 1, B), of which 1 showed concurrent weak cytoplasmic staining. Among the 73 iCCA cases available for CRP IHC, 53 cases (72.60%) were positive for cytoplasmic CRP expression, of which 42 cases (79.25%) showed diffuse positivity (score 3) (Figure 1, C). There was no significant correlation of CRP or HNF-1β expression with tumor grade (P = .68 or P = .12), pT stage (P = .39 or P > .99), and LVI (P = .16 or P > .99). These data indicate that there is a significant difference in the expression of HNF-1β compared with CRP in iCCAs, and HNF-1β is more sensitive than CRP (P < .001) as a diagnostic marker of iCCA (Table 1).
Expression of HNF-1\(\beta\) and CRP in HCC

Of 65 HCCs (Figure 2, A), 59 cases (90.77\%) were negative for HNF-1\(\beta\) (Figure 2, B). Six cases (9.23\%) showed weak nuclear expression in the tumor cells (score 1). CRP was positive in 58 of the 64 HCCs (90.63\%) with available CRP stain, of which 31 (48.44\%) showed strong and diffuse cytoplasmic expression (score 3) (Figure 3, C). There was no significant correlation of CRP or HNF-1\(\beta\) expression with tumor grade \((P = .07\) or \(P = .13\)), pT stage \((P = .20\) or \(P > .99\)), or LVI \((P > .99\) or \(P > .99\)). These data indicate that there is a significant difference in the expression of CRP and HNF-1\(\beta\) in HCC \((P < .001)\), and HNF-1\(\beta\) expression is largely negative in HCCs (Table 1).

Expression of HNF-1\(\beta\) and CRP in cHCC-CCA

To further investigate the performance of these markers in biliary differentiation in cHCC-CCAs, we stained 13 cHCC-CCAs (Figure 3, A). HNF-1\(\beta\) was expressed in the CCA (Figure 2).
component but was negative in the HCC component in all 13 (100%) cases (Figure 3, B). CRP was expressed in the CCA component in 12 (92.31%) and the HCC component in all 13 (100%) cases (Figure 3, C). There was a significant difference in the expression of HNF-1β between the CCA and HCC components of cHCC-CCA (P < .001). However, there was no difference in expression of CRP between the CCA and HCC components of cHCC-CCA (P > .99) (Table 1).

Sensitivity and Specificity of HNF-1β and CRP Expression to Differentiate iCCA From HCC

To further analyze the difference of HNF-1β and CRP expression in iCCA and HCC, we combined the iCCA cases with CCA component of chCC-CCA cases, and the HCC cases with HCC component of chCC-CCA cases to analyze CCA and HCC, respectively. As seen in Table 1, all the iCCAs and the CCA components of chCC-CCAs were positive for HNF-1β expression (87 of 87; 100%). CRP expression was found in 75.58% (65 of 86) of iCCAs and CCA components of chCC-CCAs. Of the tested HCCs and HCC components of chCC-CCAs, 92.31% (72 of 78) were negative for HNF-1β expression. Only 6 cases (7.69%) of HCC showed weak HNF-1β expression (score 1). Majority of the HCCs and all the HCC components of chCC-CCAs were positive for CRP expression (71 of 77; 92.21%). These data further confirm that there are significant differences between HNF-1β and CRP expression in CCAs (P < .001). Furthermore, HNF-1β was found to have 100% sensitivity and 92.31% specificity to differentiate CCA from HCC (P < .001). CRP on the other hand was 75.58% sensitive and 7.79% specific to distinguish CCA from HCC (P = .006). The positive and negative predictive values for HNF-1β were 93.55% and 100%, respectively; for CRP they were 47.79% and 22.22%, respectively (Table 2).

Comparison of HNF-1β With Commonly Used Biliary and Hepatocytic IHC Markers to Diagnose iCCA

CK7 and CK19 are commonly used biliary IHC markers, whereas Hep par-1 and Arginase-1 are commonly used hepatocytic IHC markers. To further investigate the value of HNF-1β in diagnosing iCCA, we reviewed all available stains from the iCCA cohort and correlated them with the histology and final diagnosis. CK7 and CK19 immunostains were available in 74 and 39 of 75 CCAs, respectively, and were positive in all cases. Hep par-1 and Arginase-1 immunostains were available in 18 and 11 of 75 CCAs, respectively, and were all negative, except for 2 cases that showed focal positivity for 1 of these markers. Both cases were positive for CK7 and CK19, in addition to strong positivity for HNF-1β. Of note, molecular tumor profiling showed BAP1 mutation in 1 case, and another case was found to have IDH1 and ARID1A mutations, further supporting the diagnosis of iCCA. The clinical findings in both cases were also consistent with iCCA. In the HCC cohort, 1 poorly differentiated HCC was positive for CK19 and Arginase-1, but negative for Hep Par-1. Finding of TERT promotor mutation by molecular tumor profiling supporting the diagnosis of HCC. Another poorly differentiated HCC was positive for CK19 and focally positive for Hep par-1, but negative for Arginase-1. Based on the high serum alpha-fetoprotein level and positive albumin RNA in situ hybridization, this case was diagnosed as HCC. Of note, both cases were positive for CRP and negative for HNF-1β. These findings further demonstrate the diagnostic utility of HNF-1β to differentiate HCC from CCA is better than CRP.

DISCUSSION

Recognition of hepatocytic and biliary differentiation is very important in the diagnosis of iCCA, HCC, and chCC-CCA.

Table 2. Sensitivity and Specificity of Hepatocyte Nuclear Factor-1β (HNF-1β) and C-Reactive Protein (CRP) to Distinguish Intrahepatic Cholangiocarcinoma From Hepatocellular Carcinoma

<table>
<thead>
<tr>
<th></th>
<th>HNF-1β</th>
<th>CRP</th>
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<tbody>
<tr>
<td>Sensitivity (95% CI)</td>
<td>100% (95.77–100)</td>
<td>75.58% (65.54–83.44)</td>
</tr>
<tr>
<td>Specificity (95% CI)</td>
<td>92.31% (84.22–96.43)</td>
<td>7.79% (3.62–15.98)</td>
</tr>
<tr>
<td>Positive predictive value (95% CI)</td>
<td>93.55% (86.63–97.01)</td>
<td>47.79% (39.58–56.13)</td>
</tr>
<tr>
<td>Negative predictive value (95% CI)</td>
<td>100% (94.93–100)</td>
<td>22.22% (10.61–40.76)</td>
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Abbreviation: CI, confidence interval.
CCA. While the diagnosis is obvious on morphology in some cases, often IHC markers are used in clinical practice to aid in the diagnostic workup. A variety of IHC markers are currently available that have high sensitivity and specificity to confirm hepatocytic differentiation. In comparison, there are only a few markers that help in recognizing biliary differentiation, which include CK7, CK19, and CRP. None of these markers reach the sensitivity or the specificity of hepatocytic markers. In practice, in the context of appropriate morphology the diagnosis of iCCA is supported by negativity for specific hepatocytic markers and positivity for biliary differentiation markers, such as CK7 and CK19. In the current study, we investigated HNF-1β expression and compared it with CRP to recognize biliary differentiation and support the diagnosis of iCCA, HCC, and chCC-CCA. Our results show that compared with CRP, HNF-1β is a highly sensitive and specific IHC marker in diagnosing iCCA and differentiating iCCA from HCC.

Markers of hepatocytic differentiation commonly used in practice include Hep par-1, Arginase-1, Polyclonal CEA, and α-fetoprotein. Arginase-1, an antibody that binds the urea cycle enzyme ARG-1, considered to be the most sensitive and specific hepatocytic marker has been reported to have sensitivities ranging from 83% to 100%. However, a subset (10%) of well-differentiated HCCs is Arginase-1 negative, and pancreatic neuroendocrine tumors and iCCAs may express Arginase-1. Hep par-1, a urea cycle enzyme carbamoyl phosphate synthetase 1, is a sensitive marker for well-differentiated HCC. The sensitivity of Hep par-1 may be as low as 22% in poorly differentiated HCCs. The sensitivity of Hep par-1 was nuclear. However, expression of Hep par-1 is seen in a subset of HCCs, and higher serum levels or overexpression by IHC has been associated with recurrence or poor prognosis. In keeping with prior study, the difference in CRP expression in iCCAs did not differ with tumor grades, pT stage, or LVI. Furthermore, our study demonstrated CRP expression in 75.58% of iCCAs and 92.21% of HCCs. Therefore, CRP immunohistochemistry can be used to diagnose iCCA, but does not aid in the differentiation of iCCA from HCC.

HNF-1β, a transcription factor, plays an important role in the early development of biliary tract, pancreas, and kidney. In the pancreaticobiliary system 84.3% pancreatic ductal adenocarcinomas were reported positive for HNF-1β with either cytoplasmic, membranous, or nuclear expression patterns, of these, nuclear expression of HNF-1β was demonstrated only in 35.5% cases. The same study also reported expression of HNF-1β in various carcinomas with cytoplasmic and/or nuclear expression, including cholangiocarcinomas (86.7%). However, previous studies on renal clear cell carcinoma and urothelial carcinoma, testicular yolk sac tumor, ovarian clear cell carcinoma, and colorectal carcinoma showed only nuclear expression. In background liver, only the biliary epithelial cells show nuclear HNF-1β expression, whereas hepatocytes are negative. In our study, expression of HNF-1β was nuclear and found in cholangiocarcinoma (except 1 case with concurrent weak cytoplasmic staining), background liver bile ducts and bile ductules, and focal weak expression of HNF-1β was seen in 6 cases of HCC. A nuclear transcription factor like HNF-1β is ideally considered positive only when expressed in the nucleus by IHC, and is therefore easier to interpret compared with other markers that are cytoplasmic and/or membranous. Preanalytical factors, such as different antibody resources, different antibody batches from same manufacture, different manufactures, different antigen retrieval method, and/or tissue fixation time, may be responsible. However, the cytoplasmic or membranous expression of HNF-1β needs further investigation.

In our study, all HCC cases were negative for HNF-1β, except 6 cases with focal weak expression in less than 10% cells (score 1). The significance of focal expression of HNF-1β in HCC is unclear. Studies have shown that a subset of HCC without any morphologic iCCA component can show focal or patchy biliary differentiation on IHC. For example,
variable positivity of CK7 and CK19 has been demonstrated in a subset of HCC, and seems to correlate with early recurrence or poor prognosis. Other biliary markers (eg, CK7, CK19, and CRP) and various other biliary markers (eg, CK7, CK19, and CRP) and various histopathologic features, the recommended term is cHCC-CCA. Per the International Consensus Group on the nomenclature of chHCC-CCA and the 2019 World Health Organization classification, 57–59 terms such as mixed hepatobiliary carcinoma, biphenotypic (hepatobiliary) primary liver carcinoma, combined liver cell and bile duct carcinoma, HCC with dual phenotype, HCC with biliary phenotype, HCC with stem/progenitor cell should be abandoned, although it is understood that these tumors do have a component of stem/progenitor cells. Of note, a subset of HCCs with HNF-1β expression (27%) has been reported to recur as iCCA. In this regard, tumors reported as HCC with biliary phenotype and stemness with expression of HNF-1β in a prior study likely represent CHCC-CCA. A recent study found HNF-1β to be negative in all HCC cases. It needs to be recognized that a weak and focal expression of HNF-1β can be seen in HCC, which is in contrast to strong and diffuse nuclear staining in most iCCAs and bile ducts/ductules in the background liver that serve as an internal control. This is a potential pitfall in the interpretation of HNF-1β expression that may be responsible for reported positivity in HCCs in some studies.

Our study has some limitations. One is the small sample size in each cohort, especially the CHCC-CCA cohort. The second is that we only evaluated the role of HNF-1β expression in identifying biliary differentiation in primary liver cancers, including iCCA, HCC, and CHCC-CCA, but we did not study any other metastatic carcinomas to the liver. Prior studies have shown a varying degree of HNF-1β nuclear expression in other carcinomas, such as carcinomas of gallbladder, ampulla, esophagus, and stomach, among others. Cytoplasmic and membranous staining patterns of HNF-1β expression have also been reported in few carcinomas, including pancreatic ductal adenocarcinoma. In practice, differentiation of iCCA from metastasis remains an important issue, and based on current evidence, HNF-1β has limited role in that regard on its own. However, we feel HNF-1β can still be useful when investigating the possibility of metastasis to the liver when used in combination with other biliary markers (eg, CK7, CK19, and CRP) and various site-specific markers that exclude iCCA (eg, PAX8, SALL4, GATA3, etc). However, further studies are needed to explore this possibility.

In summary, HNF-1β is a sensitive and specific marker for biliary differentiation in primary hepatic tumors and very helpful in the diagnosis of iCCA, identification of the CCA component of chHCC-CCA, and differentiation of iCCA and cHCC-CCA from HCC. Our findings support the utility of HNF-1β to identify CCA when the distinction between HCC and CCA is difficult. Lack of expression of HNF-1β can be used to rule out iCCA in adenocarcinomas of unknown primary. Utility of HNF-1β either on its own or in combination with other markers to exclude metastasis needs further exploration.

References


