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Diagnostic Immunomarkers for HepPar-1 Negative Areas of Hepatocellular Carcinoma

by

Tae Hui Lee

Major in Medicine

Department of Medical Sciences

The Graduate School, Ajou University
Diagnostic Immunomarkers for HepPar-1 Negative Areas of Hepatocellular Carcinoma

by

Tae Hui Lee

A Dissertation Submitted to The Graduate School of Ajou University in Partial Fulfillment of the Requirements for the Degree of Master of Medicine

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August, 2011
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June 23rd, 2011
- ABSTRACT –

**Diagnostic Immunomarkers for HepPar-1 Negative Areas of Hepatocellular Carcinoma**

HepPar-1 is a sensitive and specific antibody. Especially in needle biopsy specimens, this diagnostic approach is limited by potential multifocal expression and/or poorly differentiated HCCs, where it may not be expressed. The goal of this study was to determine the antibodies most sensitive to hepatocyte differentiation in HepPar-1 negative areas including: CD13, CD10, polyclonal carcinoembryonic antigen (pCEA), alpha-fetoprotein (AFP) and glypican-3, for needle biopsy. Immunohistochemical staining for hepatocyte and biliary markers – HepPar-1, CD13, glypican-3, pCEA, CD10, AFP, cytokeratin (CK)7, CK19, and epithelial cell adhesion molecule (EpCAM)- was performed from paraffin block samples. HepPar-1 was diffusely negative in 12 cases. Twenty two cases showed partial expression of the HepPar-1 antibody. Among 34 HepPar-1 negative areas, CD13 was the most consistent antibody (85.3%, 29/34) showing the characteristic bile canaliculi pattern. Biliary immune-phenotype with CK7, CK19, and EpCAM showed no significant differences in HepPar-1 negative areas. CD13 could be used for the detection of hepatocyte differentiation in HCCs with HepPar-1 negative expression, especially in needle biopsy specimens. In addition, negative immunoexpression for HepPar-1 was not overtly related to Biliary differentiation from an immunohistochemical viewpoint.

Key words: Hepatocellular carcinoma, needle biopsy, CD13 antigen
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I. INTRODUCTION

The diagnosis of hepatocellular carcinoma (HCC) depends on architectural and cytological evidences of hepatocytic differentiation. Routine hematoxylin and eosin (H&E) staining is usually enough for the evaluation of hepatocytic differentiation. Immunohistochemical staining is often useful for diagnosis of HCCs from metastatic carcinoma or primary cholangiocarcinoma particularly in cases of a poorly differentiated type. (Lau et al, 2002; Saleh et al, 2009) HepPar-1, an antigen of human hepatocyte mitochondria, is a very sensitive and specific positive immunohistochemical marker for HCCs. (Wennerberg et al, 2009; Lamps and Folpe, 2003) It can be used on paraffin block samples and shows broad expression in normal hepatocytes, hepatic adenomas, and HCCs. However, the HepPar-1 antibody has a tendency to be less frequently present in poorly differentiated HCCs, and is not entirely specific for HCC. (Wennerberg et al, 2009) Furthermore, because HepPar-1 often stains in a multifocal inhomogeneous manner, false negative results may be obtained on preoperative needle biopsy samples. Therefore, other immunohistochemical markers for hepatocytic differentiation are necessary as complementary use, in these circumstances. CD13 and glypican-3, introduced recently as positive immunohistochemical markers for HCCs, may exceed the sensitivity of HepPar-1; they have been shown to maintain a positive immunohistochemical reaction in poorly differentiated tumors. (Rocken et al, 2005; Kandil and Cooper, 2009) The goal of this study was to determine the efficacy of a variety of immunohistochemical markers: HepPar-1, CD13, glypican-3, polyclonal carcinoembryonic antigen (pCEA), and CD10 for diagnostic purposes when HepPar-1 is not expressed in HCC.
In addition, immunohistochemical markers for biliary differentiation were assessed to
determine the different histogenesis of HCCs associated with HepPar-1 expression.
II. MATERIAL AND METHODS

A. Cases and slide selection

Fifty cases of HCC were retrieved from the archive of the department of pathology, Ajou University, Korea. All patients had undergone surgical resection including wedge resection, segmentectomy, lobectomy and liver transplantation over the last three years (2007–2009). In most cases hepatitis B viral antigens were identified (40 cases, 80%) and two cases had a background of hepatitis C virus infection (4%). Multiple H&E-stained slides were reviewed for each tumor and we chose a single slide that showed the poorest differentiation for the analysis. Criteria for tumor grades were based on the WHO classification. (Hirohashi et al, 2000)

B. Immunohistochemical staining

Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded, 4μm-thick tissue sections using the polymer chain two-step indirect technique. Deparaffinization of all sections was performed through a series of xylene baths, and rehydration was performed with a series of graded alcohol solutions. To enhance immunoreactivity, microwave antigen retrieval was performed for 30 min in 10 mM of citrate buffer. After hydrogen peroxide blocking for quenching nonspecific endogenous peroxidase activity, immunohistochemical staining was carried out according to the routine process using the Ultra-Vision detection system (Thermo Co., Fremont, CA, U.S.A). The antibodies for hepatocytic differentiation included: HepPar-1 (1:30; Cell Marque Co., Rocklin, CA, U.S.A), CD13
(1:40; Thermo Co., Waltham, MA, U.S.A), CD10 (1:30; Thermo Co., Waltham, MA, U.S.A), pCEA (1:100; Zymed, San Francisco, CA, U.S.A), alpha-fetoprotein (AFP) (1:200; Thermo Co., Waltham, MA, U.S.A), and glypican-3 (1:100; Cell Marque Co., Rocklin, CA, U.S.A). Antibodies for biliary differentiation included cytokeratin (CK) 7 (1:100; Dako Co., Seoul, Korea), CK19 (1:100; Novoceastra Co., Norwell, MA, U.S.A), and epithelial cell adhesion molecule (EpCAM, 1:20; Cell Marque Co., Rocklin, CA, U.S.A). Immunohistochemical antibody results were assessed according to intensity and relevant localization compared to both internal positive and negative controls. AFP and glypican-3 had only negative inner control tissues.

C. Interpretation and grouping

A latticework system was used to evaluate the status of inhomogeneous immunoexpression within a case, a system similar to a tissue microarray. A 5-mm latticework system was added to every cover glass slide preparation in order to divide a certain area of tumor into multiple regular squares of 5 mm. Predetermined coordinates were used to maintain the orientation of the latticework system, so that each area could be referred to correctly and compared to other areas of interest on sequential sections for both H&E stains and immunohistochemical stains. The immunohistochemical results of a certain antibody in a given area were considered positive if clear-cut immunoexpression was present, regardless of the intensity. If HepPar-1 immunoexpression was totally negative in a square of the lattice, we classified the case as group A containing HepPar-1(−) areas. The extent of
smaller areas were variable, from several squares to one half of a square of the latticework system.

However, no one was smaller than the size of conventional liver biopsies. In addition, group A also included cases with total absence of HepPar-1 immunoexpression. Cases with totally positive HepPar-1 immunoexpression were included in group B and considered the control group. Each case was sorted into either group A or group B, the HepPar-1(−) group or HepPar-1(+) group.
### III. RESULTS

There were 34 cases classified into group A and 16 cases were group B. Group A was composed of nine areas with grade 2 HCCs, 16 areas of grade 3 HCCs, and nine areas of grade 4 HCCs. Group B was composed of seven areas of grade 2 HCCs, five areas of grade 3 HCCs, and four areas of grade 4 HCCs. Twenty five out of 34 areas were high grade HCCs (grades 3 and 4) in group A and nine out of 16 areas in group B (Table 1). Group A had 12 cases with total absence of HepPar-1 immunoexpression; in detail, 1 out of 9 grade 2 cases, 7 out of 16 grade 3 cases and 4 out of 9 grade 4 cases. Twenty two cases showed a heterogeneous response to HepPar-1 antibodies, and they had HepPar-1(−) areas of group A. Non-neoplastic hepatocytes showed positive immunoexpression for the HepPar-1 antibody in all cases. Immunoexpression of the CD13 antibody showed a typical bile canalicular pattern in 29 areas of group A (85.3%, 29/34) and 15 areas in group B (94.1%, 15/16) (Fig. 1.)

<table>
<thead>
<tr>
<th>Grade</th>
<th>HepPar-1(-) area</th>
<th>HepPar-1(+) area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 2</td>
<td>9(1)</td>
<td>7</td>
</tr>
<tr>
<td>Grade 3</td>
<td>16(7)</td>
<td>5</td>
</tr>
<tr>
<td>Grade 4</td>
<td>9(4)</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>34(12)</td>
<td>16</td>
</tr>
</tbody>
</table>

†Cases that showed total absence of HepPar-1 immunoexpression.
In group A, the CD13 expression ratio decreased in inverse proportion to the tumor grade. However, the sensitivity was highest in all grades of both groups except for the grade 4 areas of group B (glypican 100% [4/4], CD13 75.0% [3/4]). (Table 2) Sometimes CD13 was identified in the cytoplasm membrane of tumor cells without bile canalicular accentuation, or diffuse cytoplasm staining. Normal hepatocytes also showed sharp bile canalicular immunexpression, and the apical membranes of cholangiocytes were normally reactive to CD13 antibody; these could be used as a positive control. Numerous inflammatory cells, mainly neutrophils and histiocytes, also were reactive to the CD13 antibody in the cytoplasm.

Fig. 1. CD13 immunexpression in hepatocellular carcinoma. CD13 antibody shows clear immunexpression along the cell membrane with a bile canaliculi pattern.
Glypican-3 antibody was detected in 23 areas of group A (67.6%, 23/34) and 13 areas of group B (81.3%, 13/16) with a diffuse cytoplasm granular pattern and occasional accentuation of bile canaliculi (Fig. 2). Glypican-3 showed higher expression in poorly differentiated areas of group B, that is, grade 2 areas (71.4%, 5/7), grade 3 areas (80.0%, 4/5), and grade 4 areas (100%, 4/4). In group A, the glypican-3 immunoexpression ratio was not related to the tumor grades (Tables 2, 3).

Table 2. Expression of antibodies in HepPar-1 negative areas (group A)

<table>
<thead>
<tr>
<th></th>
<th>CD13</th>
<th>Glypican</th>
<th>pCEA</th>
<th>CD10</th>
<th>AFP</th>
<th>CK7</th>
<th>CK19</th>
<th>EpCA</th>
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<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>(100%)</td>
<td>(66.7%)</td>
<td>(88.9%)</td>
<td>(11.1%)</td>
<td>(44.4%)</td>
<td>(33.3%)</td>
<td>(22.2%)</td>
<td>(44.4%)</td>
</tr>
<tr>
<td>Grade3</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14/16</td>
<td>11/16</td>
<td>12/16</td>
<td>3/16</td>
<td>7/16</td>
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</tr>
<tr>
<td></td>
<td>(87.5%)</td>
<td>(68.8%)</td>
<td>(75.0%)</td>
<td>(18.8%)</td>
<td>(43.8%)</td>
<td>(31.3%)</td>
<td>(37.5%)</td>
<td>(43.8%)</td>
</tr>
<tr>
<td>Grade4</td>
<td></td>
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<td></td>
<td>6/9</td>
<td>6/9</td>
<td>3/9</td>
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<td></td>
<td>(66.7%)</td>
<td>(66.7%)</td>
<td>(33.3%)</td>
<td>(0%)</td>
<td>(22.2%)</td>
<td>(22.2%)</td>
<td>(22.2%)</td>
<td>(11.1%)</td>
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<tr>
<td></td>
<td>29/34</td>
<td>23/34</td>
<td>23/34</td>
<td>7/34</td>
<td>11/34</td>
<td>10/34</td>
<td>9/34</td>
<td>12/34</td>
</tr>
<tr>
<td></td>
<td>(85.3%)</td>
<td>(76.7%)</td>
<td>(76.7%)</td>
<td>(20.1%)</td>
<td>(32.4%)</td>
<td>(29.4%)</td>
<td>(26.5%)</td>
<td>(35.3%)</td>
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Table 3. Expression of antibodies in HepPar-1 negative areas (group B)

<table>
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<tr>
<th></th>
<th>CD13</th>
<th>Glypican</th>
<th>pCEA</th>
<th>CD10</th>
<th>AFP</th>
<th>CK7</th>
<th>CK19</th>
<th>EpCAM</th>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7/7</td>
<td>5/7</td>
<td>4/7</td>
<td>3/7</td>
<td>2/7</td>
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<tr>
<td></td>
<td>(100%)</td>
<td>(71.4%)</td>
<td>(57.1%)</td>
<td>(42.9%)</td>
<td>(44.4%)</td>
<td>(28.6%)</td>
<td>(14.3%)</td>
<td>(14.3%)</td>
</tr>
<tr>
<td>Grade3</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>5/5</td>
<td>4/5</td>
<td>4/5</td>
<td>2/5</td>
<td>3/5</td>
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<tr>
<td></td>
<td>(100%)</td>
<td>(80.0%)</td>
<td>(80.0%)</td>
<td>(40.0%)</td>
<td>(60.0%)</td>
<td>(20.0%)</td>
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<td>(60.0%)</td>
</tr>
<tr>
<td>Grade4</td>
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<td></td>
</tr>
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<td></td>
<td>3/4</td>
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</tr>
<tr>
<td></td>
<td>(75.0%)</td>
<td>(100%)</td>
<td>(50.0%)</td>
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<td>(50.0%)</td>
<td>(0%)</td>
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<tr>
<td></td>
<td>15/16</td>
<td>13/16</td>
<td>10/16</td>
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<td>5/16</td>
<td>1/16</td>
<td>4/16</td>
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<tr>
<td></td>
<td>(93.8%)</td>
<td>(81.3%)</td>
<td>(62.5%)</td>
<td>(50.0%)</td>
<td>(31.3%)</td>
<td>(31.3%)</td>
<td>(6.3%)</td>
<td>(25.0%)</td>
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Fig. 2. Glypican-3 immunoexpression in hepatocellular carcinoma. Glypican-3 shows a strong cytoplasm immunoexpression with accentuation of the bile canaliculi. Glypican-3 antibody only was present in tumor components, no reactivity was identified in any other nonneoplastic tissues in the liver, including hepatocytes, bile ducts, and inflammatory cells. pCEA was identified in 23 areas of group A (67.6%, 23/34) and in 10 areas of group B (62.5%, 10/16)(Tables 2, 3). It showed a characteristic bile canalicular pattern in both tumor cells and non neoplastic hepatocytes (Fig. 3). In group A, the grades were inversely correlated with the pCEA expression ratio (Table 2). Another bile canaliculi marker, the CD10 antibody, was less sensitive than other bile canalicular markers (CD13, pCEA) in both groups (Tables 2, 3). CD10 often showed a diffuse cytoplasm reaction with
or without bile canalicular accentuation, and was expressed in non neoplastic hepatocytes, bile ducts and inflammatory cells. AFP, an oncofetal protein, was expressed in 11 areas of group A (32.4%, 11/34) and 5 areas of group B (31.3%, 5/16) with a diffuse cytoplasm pattern. It was totally negative in the grade 4 areas in both groups (Tables 2,3). CK7, a biliary marker, was positively expressed in the cytoplasm of some morphologically typical HCCs and in a case of combined HCC-cholangiocarcinoma. The detection rate of CK7 was 29.4%(10/34) in group A and 31.3% (5/16) in group B (Fig. 4).

![Fig. 3. Polyclonal carcinoembryonic antigen immunoexpression in hepatocellular carcinoma. Polyclonal carcinoembryonic antigen is noted focally along the bile canaliculi.]
CK19, another biliary marker, was identified in 9 areas of group A (26.5%, 9/34) and 1 area of group B (6.3%, 1/16) (Fig. 5). The CK7 and CK19 immunoexpression were not different in their association with HepPar-1 immunoexpression and tumor grades (Tables 2,3). The EpCAM antibody delineated the cytoplasm membrane of the tumor cells in 12 areas of group A (35.3%, 12/34) and 4 areas of group B (25.0%, 4/16) (Tables 2,3, Fig. 6). The EpCAM antibody also stained the cytoplasm membrane of normal bile ducts and nonneoplastic hepatocytes had negative immunoexpression with the EpCAM antibody.

Fig. 4. Cytokeratin 7 immunoexpression in hepatocellular carcinoma. Cytokeratin 7 antibody is reactive in the cytoplasm of scattered tumor cells.
Fig. 5. Cytokeratin (CK) immunoexpression in hepatocellular carcinoma. Cytokeratin (CK) 19 antibody is present in more tumor cells than CK7.
Fig. 6. Epithelial cell adhesion molecule (EpCAM) immunoreactivity in 
hepatocellular carcinoma. Epithelial cell adhesion molecule antibody is identified along 
the cell membrane of tumor cells.
IV. DISCUSSION

HCC, the most common primary hepatic malignancy, consists of heterogeneous components including: tumor grade, degree of desmoplasia, and the architectural pattern in a given case. (Leong et al, 1998) Although many cases of HCC can be characterized solely by the histopathological findings, there are limitations with this approach, especially using needle biopsy specimens. These problematic cases of HCC have clear cell morphology, prominent desmoplasia, or severe tumor cell anaplasia, and must be differentiated from other primary hepatic malignancies, such as cholangiocarcinoma and metastatic carcinomas, the most common malignancies found in the liver. (Lau et al, 2002; Wu et al, 1996) Immunohistochemistry assessments might help with hepatic differentiation in these situations.

Among a variety of antibodies, the HepPar-1 antibody is very sensitive and fairly specific for detection of hepatocytic features. (Wennerberg et al, 1993; Leong et al, 1998) The HepPar-1 antibody is thought to recognize the epitope within the mitochondrial membrane of hepatocytes, and does not appear to be expressed in other normal tissues. (Wennerberg et al, 1993) Other studies have demonstrated moderate to high sensitivity of HepPar-1 in HCCs, ranging from 75 to 90%. (Lamp and Folpe, 2003; Kumagai et al, 2001) In this study, tumor portions in 12 areas of group A cases totally lacked HepPar-1 immunoexpression. Therefore, 38 cases had diffuse or at least focal areas of distinct positive immunoexpression of HepPar-1 antibody. The overall sensitivity of the HepPar-1 antibody in this study was 76%. This result is consistent with the average sensitivity of
HepPar-1 antibody previously reported. (Saleh et al, 2009; Lamp and Folpe, 2003; Kumagai et al, 2001) In addition, we found there was a decreased tendency for immunoexpression of HepPar-1 in poorly differentiated HCC, consistent with a prior report.(Kumagai et al, 2001) Although HepPar-1 is specific for hepatocytic neoplasms, it is not perfectly specific. Gastric adenocarcinomas frequently react with HepPar-1 regardless of the histopathological hepatic differentiation, and ovarian germ cell tumors can stain with the HepPar-1 antibody.(Wennerberg et al, 1993; Leong et al, 1998; Fan et al, 2003) Even cholangiocarcinoma can in unusual cases express HepPar-1.(Leong et al, 1998) Some studies have pointed out that the heterogeneous reaction of HepPar-1 may result in false negative results with small biopsy specimens.(Leong et al, 1998) In this study, 22 cases had localized focal or multifocal areas of HepPar-1 negative immunoexpression surrounded by HepPar-1 positive areas. The size of the HepPar-1(−) areas were variable, some filled a few squares of latticework and some were confined to a single square. However, all HepPar-1(−) areas were large enough to be a potential target for needle biopsies; however, small amounts of biopsy tissue might have been reported as HepPar-1 negative in these cases. Therefore, although the HepPar-1 antibody is an excellent marker for the diagnosis of HCC, the results are limited by small biopsy specimens and high grade tumors where the expression is decreased and the specificity is not perfect. Therefore, a panel of immunological markers could provide additional information and reduce the false negative rate while improving the specificity for the tissue components of hepatocytic differentiation.

CD13, aminopeptidase N, is a zinc-dependent metalloproteinase of the cell membrane that can be used as positive immunohistochemical marker for hepatocellular differentiation.
It is clearly observed along with a bile canaliculi pattern, nearly identical to pCEA and CD10. (Rocken et al, 2005) CD13 has been under investigation with regard to tumorigenesis as well as expression in a variety of organs in addition to granulocytes, histiocytes, and hematological malignancies. (Mina-Osorio, 2008; Luan, 2007) CD13 antibody could be reactive in a variety of malignant solid tumors in the cytoplasm or cell membranes. HCC itself could have similar areas of CD13 immunoexpression. Thus, only the bile canaliculi pattern is meaningful for the detection of the hepatocytic structure. (Rocken et al, 2005) In this study, CD13 was the best immunohistochemical marker for positive hepatocytic differentiation in the absence of HepPar-1 immunoexpression. Thus, the CD13 antibody could be used for the diagnosis of HCC when other immunomarkers including HepPar-1 do not provide meaningful information. The CD13 detection rate in group B was 93.8%, which was the highest among the tested antibodies for hepatocytic differentiation in the presence of HepPar-1 immunoexpression. One prior paper has reported on the diagnostic application of CD13 in HCC. (Rocken et al, 2005) Rocken et al. reported that CD13 was detected in 51 out of 53 HCC specimens by delineating the bile canalicular pattern (about 96% sensitivity). In our study, the detection rate of the CD13 antibody was 88% (44/50) including both groups A and B. The variations in the study design might be responsible for the different results; 35 out of 53 cases (64.2%, 35/53) were moderately differentiated HCCs (grades 2 and 3) and only 4 cases (7.5%, 4/53) of poorly differentiated (grade 4) HCCs were included in the prior paper.5 In our study, grade 4 HCCs constituted a much higher proportion of cases (26%, 13/50). In addition, our study was designed primarily to evaluate the expression of a variety of immunohistochemical markers especially at foci of HepPar-1 negative areas;
therefore, a sample collection bias might have been present. However, the overall sensitivity of CD13 in this study was 94% (47/50) almost approaching the results of the previous study, and higher than the overall HepPar-1 sensitivity (76%, 34/50). Needle biopsy specimens were the main samples studied in the report of Rocken et al. (Rocken et al, 2005) However, our study was designed to use entirely resected specimens. Therefore, we confirmed that the CD13 reactivity could be diagnostically meaningful in resected specimens, in addition to the prior confirmation of its efficacy on needle biopsy specimens. In this study, the standard slide preparations always included a non neoplastic portion of liver tissue for comparison.

Glypican-3 antibody, thought to be a type of oncofetal protein similar to AFP, is a newly developed immunohistochemical and immunocytochemical marker that is known to be a highly sensitive and specific immunohistochemical marker for HCC and some premalignant lesions.6,14 Similar to previous studies, we confirmed that glypican-3 had a relatively constant level of sensitivity in both well and poorly differentiated HCC, unlike HepPar-1. (Kandil and Cooper, 2009; Wang et al, 2006; Yamauchi et al, 2005) This might aid in the diagnoses of HCCs, when other findings are equivocal or negative for the hepatocytic phenotype in poorly differentiated cases of HCCs. pCEA or CD10 could be reactive in the bile canalicular membrane of neoplastic and non neoplastic hepatocytic cells, and this could be a very specific sign of hepatocytic differentiation. This is because the bile canalicular structure is very unique in neoplastic and non neoplastic hepatocytes. However, the sensitivity is not very good, and surrounding biliary structures or inflammatory infiltrates could confuse the interpretation of the immunoreactivity of the bile canaliculi. (Borcheri et al, 2001; Ahuja et al, 2008; Wang et al, 2006)
In this study, HCCs sometimes showed HepPar-1(−) islands in the background of diffuse HepPar-1(+) portions, which might represent clonal dedifferentiation. With regard to tumorigenesis, we thought that the HepPar-1(−) areas would be rather poorly differentiated or undifferentiated portions of the tumor and would more frequently show biliary differentiation or liver cancer stem cell properties. (Wu et al, 1996; Yoon et al, 1999; Tickoo and Zee, 2002; Yamashita et al, 2008) We tried other immunohistochemical markers for liver cancer stem cells; however, there were no significant results from these experiments. (Becker et al, 2007; Alison and Lovell, 2005; Fujii et al, 2008) In addition, we found that the biliary antibody expression ratio was not significantly different in both study groups. (Aishima et al, 2007) Sampling errors must also be considered because only one sample from each case was evaluated at a single representative area. That is, two or three representative areas from each case might have changed the results. In addition, the size of the experimental group was quite small; therefore, more cases are needed for statistically significant results.

In conclusion, HepPar-1 remains as a very sensitive and specific positive immune-marker for HCC. However, it is less sensitive in tumors of higher grades and might be falsely negative in needle biopsy specimens. In these circumstances, CD13 is a reliable alternative marker, especially for high grade tumors. The results of this study suggest that HepPar-1 antibody reactivity was not involved in hepatocellular carcinogenesis.
V. CONCLUSION

We found that CD13 could be a reliable alternative marker for HepPar-1 because CD13 persistently is expressed in a unique fashion, that is, bile canaliculi pattern for even high grade hepatocellular carcinomas which often has areas of negative immunoreactivity to HepPar-1, a more popular diagnostic immunomarker for hepatocellular carcinoma.
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- 국문요약 -

**HepPar-1 음성인 간세포암종 영역에 대한 진단적 면역표지자**

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HepPar-1은 민감도와 특이도가 높은 면역표지자이다. 하지만, 간세포암종의 진단에 있어서, 특히 작은 조직을 이용하는 점검결과의 경우, HepPar-1의 특징적인 부분적 염색성으로 인해 진단에 도움을 받을 수 없는 경우가 있다. 본 연구의 목적은 이런 HepPar-1 음성 영역에 대해 보다 민감도가 높은 면역표지자를 찾는데 있다. 50개의 수술검체로 확인된 간세포암종에서 대표적 절편을 고려 HepPar-1, CD13, glypican-3, pCEA, CD10, AFP, cytokeratin(CK)7, CK19, 그리고 EpCAM을 시행하여 비교하였다. HepPar-1이 미만성으로 음성인 12사례와 부분적인 음성을 나타낸 22사례를 합하여 실험군으로 하고, 나머지 16사례를 대조군으로 설정하였다. 실험군에서 CD13이 가장 일관적으로 양성소견을 나타내었고(85.3%, 29/34) 특정적인 담소관 양상으로 나타난다는 점에서 진단적 의미가 있었다. 따라서 CD13 은 HepPar-1 음성인 간세포암종에서, 특히 점검결과 작은 조직에서 간세포분화를 확인할 수 있는 유용한
면역표지자이다. 또한 HepPar-1의 발현과 기타 담관분화를 나타내는 면역표지자의 발현과는 특별한 상관관계가 없었다.

핵심어: 간세포암종, 침생검, CD13