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**Original contribution**

Immunohistochemical distinction between intrahepatic cholangiocarcinoma and pancreatic ductal adenocarcinoma

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Summary Distinction between primary intrahepatic cholangiocarcinoma (ICC) and metastatic pancreatic ductal adenocarcinoma (PDA) on a liver biopsy is essentially impossible histologically but has important clinical implications. In this study, 41 ICCs and 60 PDAs were immunohistochemically evaluated for the expression of S100P, pVHL, IMP3, maspin, MUC5AC, and CK17 proteins. The results showed pVHL expression in 29 (71%) ICCs but in only 3 (5%) PDAs. S100P, MUC5AC, and CK17 were frequently expressed in PDAs, seen in 57 (95%), 40 (67%), and 36 (60%) cases, respectively. In contrast, only 11 (27%), 5 (12%), and 5 (12%) ICC cases expressed these proteins. IMP3 was expressed in 37 (90%) ICC and 54 (90%) PDA cases with equal frequency. All 60 (100%) PDA and 30 (73%) ICC cases showed positive maspin immunoreactivity. A S100P–/pVHL+/MUC5A C–/CK17– staining pattern was essentially indicative of ICC, whereas the S100P+/pVHL–/MUC5AC+/CK17+ and S100P+/pVHL–/MUC5AC–/CK17+ staining patterns were suggestive of PDA. These observations demonstrate that S100P, pVHL, MUC5AC, and CK17 are a useful immunohistochemical panel that may help distinguish primary ICC from metastatic PDA.

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1. Introduction

Distinction between primary intrahepatic cholangiocarcinoma (ICC) and metastatic pancreatic ductal adenocarcinoma (PDA) on a liver biopsy is essentially impossible histologically. It is a frequently asked clinical question, however, because the distinction may have important implications in patient management in terms of surgical options, chemotherapy regimens, and prognosis assessment [1]. Several biomarkers have been studied in this regard, including human pancreatic cancer fusion 2 (HPC2), N-cadherin, and podoca-

lyxin-like protein 1 (PCLP1) [2,3]. However, there has been no single reliable marker proven to be highly sensitive and specific for the distinction between ICC and PDA.

We have recently established the diagnostic value of S100P, the von Hippel-Lindau gene product (pVHL), insulin-like growth factor-II messenger RNA-binding protein-3 (IMP3), and mammary serine protease inhibitor (maspin) for PDA, gallbladder adenocarcinoma, and adenocarcinoma of the extrahepatic bile ducts [4–10]. These studies demonstrated that these biomarkers constitute a useful diagnostic immunohistochemical panel for confirming the diagnosis of adenocarcinoma in difficult cases, which can be helpful in the distinction from normal or reactive epithelium of the pancreas, gallbladder, and extrahepatic bile ducts in surgical, biopsy, and fine-needle aspiration specimens. However, the expression characteristics of

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these biomarkers have not been well studied in ICC. Similarly, immunohistochemical expression of MUC5AC and cytokeratin 17 (CK17) proteins in adenocarcinomas of the pancreas, gallbladder, and bile ducts has been examined in several studies [11-16], but their use in the distinction between ICC and PDA has not been specifically investigated.

In the present study, we specifically compared the expression patterns of S100P, pVHL, IMP3, maspin, MUC5AC, and CK17 proteins between ICC and PDA. Our data demonstrate that an immunohistochemical panel consisting of S100P, pVHL, MUC5AC, and CK17 is useful in distinguishing ICC from PDA.

2. Materials and methods

2.1. Case selection

A total of 41 surgically resected ICCs and 60 surgically resected PDAs retrieved from authors' institutions between 2006 and 2010 were included in this study. Hematoxylin and eosin-stained slides, pathology reports, and pertinent medical records for each case were reviewed to confirm the diagnoses. Cases of hilar or perihilar cholangiocarcinoma were excluded from this study. The ages of the patients with ICC ranged from 36 to 69 years (mean, 58.3 years; median, 58.5 years). The ages of the patients with PDA ranged from 49 to 91 years (mean, 67.7 years; median, 69.0 years). Five ICCs were well differentiated, 22 were moderately differentiated, and 14 were poorly differentiated. Among PDAs, 10 were well differentiated, 35 were moderately differentiated, and 15 were poorly differentiated. All PDAs included in this study were conventional tubular-type ductal carcinomas. Mucinous noncystic carcinomas (colloid carcinomas), signet ring cell carcinomas, and undifferentiated carcinomas were not included. The study was approved by institutional review boards at authors' institutions.

2.2. Immunohistochemistry

Immunohistochemical stains for S100P, pVHL, IMP3, maspin, MUC5AC, and CK17 were performed on 41 ICC cases on routine tissue sections and 60 PDA cases (20 on

routine tissue sections and 40 on tissue microarray sections) using previously published protocols on a Dako autostaining system (Dako North America, Inc, Carpinteria, CA) [6,7]. Each PDA case on the tissue microarray section contained two 1.0-mm punches from tumor areas. Detailed information about antibodies and staining conditions is summarized in Table 1. Positive controls included normal kidney tissue for CK17 and pVHL, normal gastric mucosa for MUC5AC, breast tissue (myoepithelial cells) for maspin, placental tissue for S100P, and PDA for IMP3. Negative controls were also included for the stains in which the primary antibodies were replaced with nonhuman-reactive rabbit or mouse serum.

2.3. Evaluation of immunohistochemical stains

Immunohistochemically stained slides were evaluated by 3 investigators (J.L., F.L., H.L.W.). A stain was considered positive if at least 5% of the tumor cells exhibited immunoreactivity. Positive staining was further graded as 1+ (5%-25% of the tumor cells stained), 2+ (26%-50%), 3+ (51%-75%), or 4+ (>75%), as well as weak, intermediate, or strong for staining intensity. Nuclear or nuclear/cytoplasmic staining was considered positive for S100P and maspin; the stain was considered negative if only cytoplasmic staining was detected. Cytoplasmic staining was considered positive for IMP3, MUC5AC, and CK17. Membranous and cytoplasmic staining was considered positive for pVHL.

2.4. Statistical analysis

SPSS 11.5 for Windows software (SPSS Inc, Chicago, IL) was used for statistical analysis. Differences between ICC and PDA were determined by 2-tailed χ^2 test with Yates continuity correction or 2-tailed Fisher exact test. A *P* value less than .05 was considered statistically significant.

3. Results

3.1. Immunohistochemical findings in ICCs

Cytoplasmic IMP3 immunoreactivity was demonstrated in 37 (90%) of 41 ICC cases, with 25 (68%) cases showing

Table 1 Summary of antibody information and staining conditions

Antibody	Vendor	Clone	Dilution	Incubation time (min)	Antigen retrieval
S100P	BD	16	1:100	30	Proteinase K, pH 7.5, RT, 12 min
pVHL	Santa Cruz	Polyclonal	1:50	30	Proteinase K, pH 7.5, RT, 9 min
IMP3	DAKO	69.1	1:50	40	EDTA, pH 8.0, 100 °C, 15 min
Maspin	BD	G167-70	1:200	40	EDTA, pH 8.0, 100 °C, 15 min
MUC5AC	Vector	CLH2	1:50	60	High pH (9.9), 99 °C, 20 min
CK17	DAKO	E3 (1)	1:80	30	EDTA, pH 8.0, 100 °C, 15 min

NOTE. BD, Becton Dickinson Immunocytometry Systems (BD Biosciences), San Jose, CA; Santa Cruz, Santa Cruz Biotechnology, Inc, Santa Cruz, CA; DAKO, Dako North America, Inc, Carpinteria, CA; Vector, Vector Laboratories, Inc, Burlingame, CA; RT, room temperature; EDTA, ethylenediaminetetraacetic acid.

Table 2 Summary of immunohistochemical findings in ICCs (n = 41)

Antibody	Staining extent					Total positivity, n (%)	Staining intensity		
	Negative	1+	2+	3+	4+		Strong	Intermediate	Weak
S100P	30	2	1	2	6	11 (27)	10	1	0
IMP3	4	4	3	4	26	37 (90)	22	7	8
pVHL	12	3	2	6	18	29 (71)	27	2	0
maspin	11	5	2	2	21	30 (73)	16	8	6
MUC5AC	36	1	1	1	2	5 (12)	5	0	0
CK17	36	1	2	0	2	5 (12)	4	1	0

a diffuse (3+ or 4+) and strong or intermediate staining pattern. Positive membranous and cytoplasmic staining for pVHL was observed in 29 (71%) cases, with 22 (76%) cases showing a diffuse and strong staining pattern. Positive nuclear and cytoplasmic staining for maspin was observed in 30 (73%) cases, with 13 (43%) cases showing a diffuse and strong staining pattern. Eleven cases (27%) showed positive nuclear and cytoplasmic staining for S100P, with diffuse and strong staining seen in 6 (55%) cases. Only 5 (12%) cases showed positive cytoplasmic staining for MUC5AC and CK17, respectively, among which only 1 case overlapped and showed positive staining for both MUC5AC and CK17. These findings are detailed in Table 2.

3.2. Comparison of immunostaining results between ICCs and PDAs

Detailed immunostaining results for PDA cases have been reported in the previous study [5]. As shown in Table 3, both ICCs and PDAs frequently showed positive IMP3 immunostaining. In contrast to PDAs, ICCs much less frequently expressed S100P, MUC5AC, and CK17, but much more frequently expressed pVHL. Although statistically significant in comparison with PDAs (100%), most ICC cases (73%) showed maspin expression.

3.3. Comparison of immunostaining patterns between ICCs and PDAs

Because S100P, pVHL, MUC5AC, and CK17 expression showed marked differences between ICCs and PDAs, their

staining patterns were further compared. As shown in Table 4, ICCs frequently exhibited a S100P-/pVHL+/MUC5AC-/CK17- staining pattern (Fig. 1), seen in 24 (59%) cases. None of the 60 PDA cases we examined showed this combinatorial pattern. In contrast, PDAs more frequently showed S100P+/pVHL-/MUC5AC+/CK17+ (Fig. 2) and S100P+/pVHL-/MUC5AC-/CK17+ (Fig. 3) staining patterns, seen in 22 (37%) and 11 (18%) cases, respectively. These 2 patterns were seen in only 1 (2%) ICC case. PDAs also more frequently showed a S100P+/pVHL-/MUC5AC+/CK17- staining pattern, seen in 16 (27%) cases. This pattern was seen in only 4 (10%) ICCs, although the difference did not reach statistical significance.

4. Discussion

In this study, we analyzed the diagnostic value of S100P, pVHL, IMP3, maspin, MUC5AC, and CK17 in the distinction between ICC and PDA. Our results indicate that ICCs more frequently express pVHL but less frequently express S100P, MUC5AC, and CK17 in comparison with PDAs. Combined use of S100P, pVHL, MUC5AC, and CK17 as an immunohistochemical panel is helpful in distinguishing ICC from PDA. IMP3 and maspin are expressed in both ICC and PDA with high frequencies and thus lack diagnostic value in the distinction.

S100P is a member of the S100 calcium-binding protein family, initially isolated from human placenta [17]. Overexpression has been reported in several epithelial cancers including PDA, adenocarcinoma of extrahepatic bile ducts, and gallbladder adenocarcinoma [4,6-8,10,18,19]. Our current findings showed positive S100P staining in 95% of PDA, which was in line with our previous observations [7,9,10]. However, only 27% of ICCs examined in this study showed S100P immunoreactivity. Similar observations have also been reported by Aishima et al [20], who showed positive nuclear S100P staining in 8 (12%) of 69 peripheral-type ICCs, in contrast to 28 (68%) of 41 perihilar cholangiocarcinomas. Tsai et al [21] reported a higher frequency of S100P expression in peripheral ICCs (53%), but in most of their positive cases, the staining was heterogeneous with mixed nuclear, cytoplasmic, and

Table 3 Comparison of immunostaining results between ICCs and PDAs

Antibody	ICC (n = 41), n (%)	PDA (n = 60), n (%)	P
S100P	11 (27)	57 (95)	<.0001
IMP3	37 (90)	54 (90)	1.0000
pVHL	29 (71)	3 (5)	<.0001
Maspin	30 (73)	60 (100)	<.0001
MUC5AC	5 (12)	40 (67)	<.0001
CK17	5 (12)	36 (60)	<.0001

Table 4 Comparison of immunostaining patterns between ICCs and PDAs

Pattern	ICC (n = 41), n (%)	PDA (n = 60), n (%)	P
S100P-/pVHL+/MUC5AC-/CK17-	24 (59)	0	<.0001
S100P+/pVHL-/MUC5AC+/CK17+	1 (2)	22 (37)	.0002
S100P+/pVHL-/MUC5AC+/CK17-	4 (10)	16 (27)	.0656
S100P+/pVHL-/MUC5AC-/CK17+	1 (2)	11 (18)	.0248
S100P+/pVHL-/MUC5AC-/CK17-	2 (5)	6 (10)	.4676
S100P-/pVHL-/MUC5AC-/CK17-	4 (10)	1 (2)	.1551

NOTE. Only the staining patterns that were statistically significant or appeared meaningful were included in this table. These patterns were observed in 36 of 41 ICCs and 56 of 60 PDAs. The remaining 5 ICCs and 4 PDAs showed several other patterns, with each pattern seen in 1 to 2 cases. These patterns were considered insignificant and were not listed in this table.

extracellular patterns. It is unclear how many of these cases showed nuclear S100P expression. In addition, half of their positive cases exhibited only focal staining. Taken together, these findings suggest that ICC is molecularly different from adenocarcinoma of the extrahepatic bile ducts (including perihilar cholangiocarcinoma), gallbladder adenocarcinoma, and PDA.

Loss of expression of the tumor suppressor protein pVHL has been demonstrated in more than 90% of PDA, gallbladder adenocarcinoma, and adenocarcinoma of extrahepatic bile ducts in our previous studies [6-9,22]. Interestingly, most ICC cases examined in this study showed preserved pVHL expression, suggesting that inactivation of pVHL expression does not serve a major role in ICC tumorigenesis. In this regard, it has been shown that loss of pVHL expression is an early event in pancreatic tumorigenesis, observed in 96% of pancreatic intraepithelial neoplasia

(PanIN)-1A lesions, and in all examined PanIN-1B, PanIN-2, and PanIN-3 lesions [7].

MUC5AC is a high-molecular-weight glycoprotein of the mucin family, which has been shown to serve an important role in pancreatic tumorigenesis [23,24]. Overexpression of MUC5AC in PDA has been reported in 70% to 100% of the cases in several studies [13,14,25]. Similarly, a high frequency of MUC5AC expression has been reported in cholangiocarcinoma [11,13,14,26-29] and gallbladder adenocarcinoma [11,15]. In the studies where ICC and extrahepatic cholangiocarcinoma (ECC) were separately evaluated, it was apparent that ECCs more frequently expressed MUC5AC than ICCs. For example, in the study by Park et al [11], positive MUC5AC staining was observed in 71% of ECCs in contrast to 47% of ICCs. Similar findings were also reported by Lee et al [14], who found MUC5AC positivity in 60% of ECCs but 44% of ICCs. Another

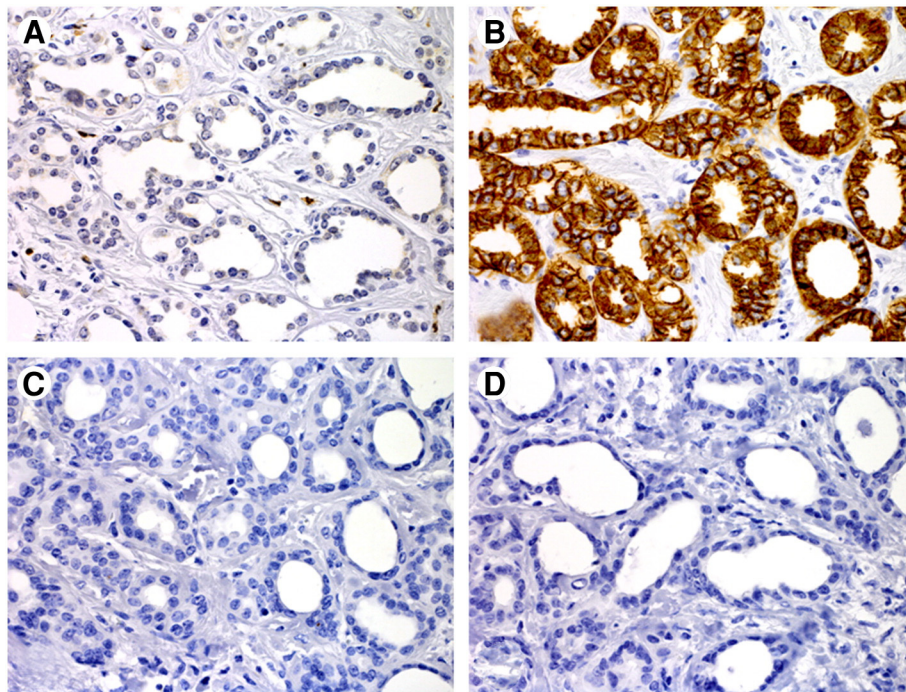


Fig. 1 A representative ICC case showing negative staining for S100P (A), MUC5AC (C), and CK17 (D), but positive staining for pVHL (B) (original magnification $\times 400$). None of the PDA cases showed this S100P-/pVHL+/MUC5AC-/CK17- staining pattern.

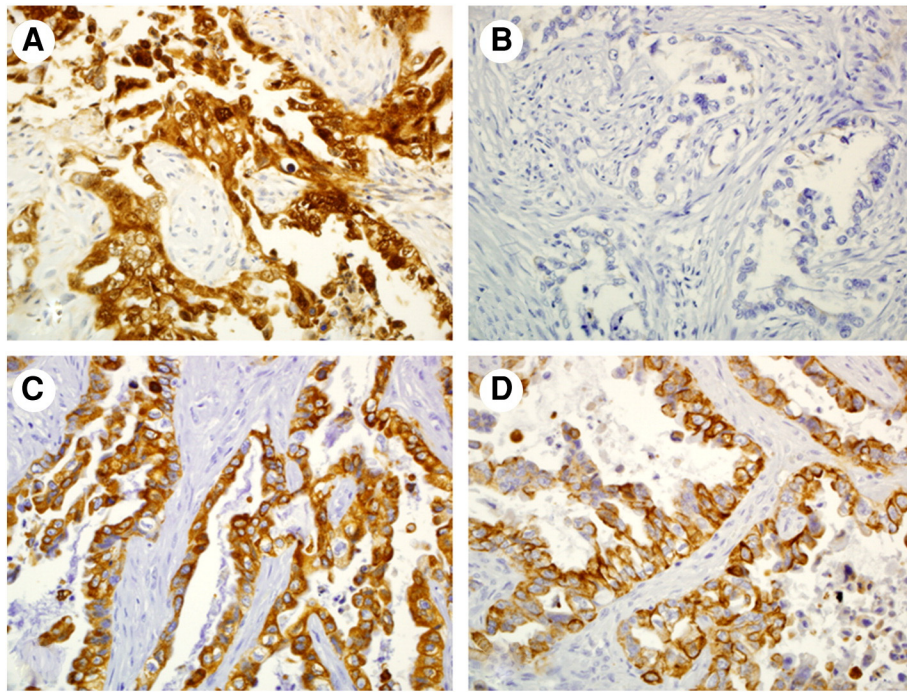


Fig. 2 A representative PDA case showing positive staining for S100P (A), MUC5AC (C), and CK17 (D), but negative staining for pVHL (B) (original magnification $\times 400$). This S100P+/pVHL-/MUC5AC+/CK17+ staining pattern was only rarely seen in ICCs.

interesting observation is that peripheral-type ICCs show MUC5AC expression less commonly than hilar cholangiocarcinomas. In the study by Guedj et al [29], 32 (62%) of 52 hilar cholangiocarcinomas showed positive MUC5AC immu-

nostaining in contrast to 13 (22%) of 59 peripheral ICCs. Similarly, Aishima et al [28] reported positive MUC5AC staining in 69% to 72% of hilar tumors but in only 25% to 27% of peripheral ICCs (2 different anti-MUC5AC

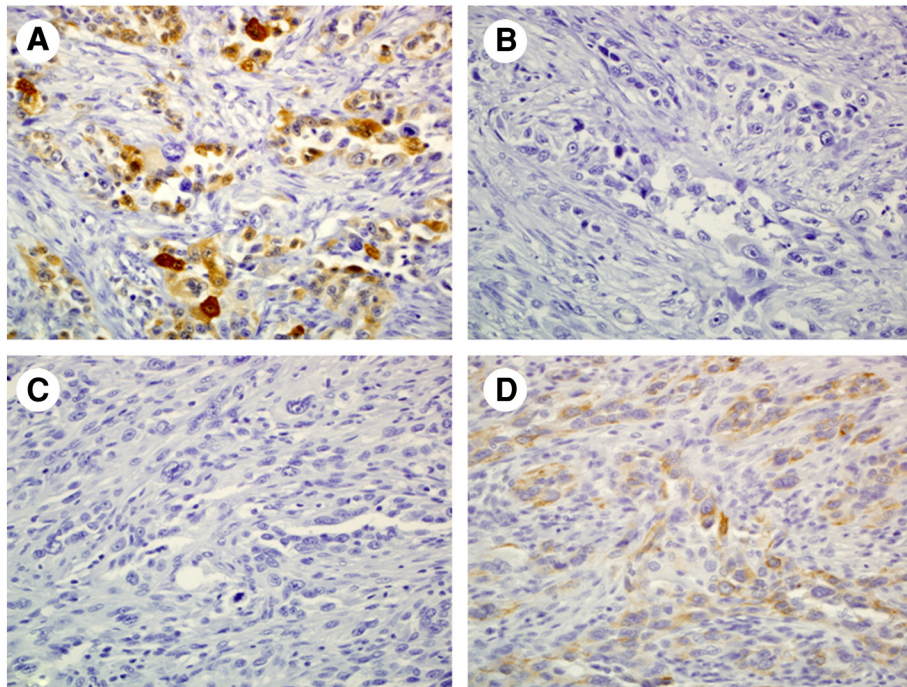


Fig. 3 A representative PDA case showing positive staining for S100P (A) and CK17 (D), but negative staining for pVHL (B) and MUC5AC (C) (original magnification $\times 400$). This S100P+/pVHL-/MUC5AC-/CK17+ staining pattern was only rarely seen in ICCs.

antibodies were used in this study). Our results showed a higher frequency of MUC5AC expression in PDAs (67%) than in ICCs (12%), consistent with previous observations.

CK17 is a low-molecular-weight keratin that is normally expressed in myoepithelial and basal cells and subsets of hair shaft epithelia [13]. A few studies have demonstrated it to be of value in the distinction between pancreaticobiliary and nonpancreaticobiliary adenocarcinomas [13,16,30,31]. In accordance with published results, our data showed frequent CK17 expression in PDAs, seen in 60% of the cases. However, CK17 expression was detected in only 12% of ICC cases we examined. Our findings in ICCs seem to differ from those reported by Chu et al [13], who detected CK17 expression in 17 (71%) of 24 ICCs. However, it is unclear whether hilar or perihilar cholangiocarcinomas were included in that study. To substantiate this argument, Sarbia et al [30] showed positive CK17 staining in 10 (59%) of 17 adenocarcinomas of the extrahepatic bile ducts, a frequency similar to that reported for PDAs.

In summary, the data presented in this study demonstrate that S100P, pVHL, MUC5AC, and CK17 are of diagnostic value in the distinction between ICC and PDA. None of these markers appears sufficient to differentiate the tumor origin when used alone, but combined use as an immunohistochemical panel is helpful. A S100P-/pVHL+/MUC5AC-/CK17- staining pattern is essentially indicative of ICC, whereas the S100P+/pVHL-/MUC5AC+/CK17+ and S100P+/pVHL-/MUC5AC-/CK17+ staining patterns are suggestive of PDA. Our data also support the notion that ICC is molecularly distinct from PDA, adenocarcinoma of the extrahepatic bile ducts, and gallbladder adenocarcinoma.

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