Published in final edited form as:

Hum Pathol. 2012 October ; 43(10): . doi:10.1016/j.humpath.2011.11.012.

The Novel Monoclonal Antibody HPC2 and N-cadherin Distinguish Metastatic Pancreatic Ductal Adenocarcinoma from Cholangiocarcinoma

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Abstract

Metastatic pancreatic ductal adenocarcinoma and primary cholangiocarcinoma are morphologically very similar and therefore challenging to distinguish in liver biopsies. The distinction is important because surgical management and prognosis differ significantly. A number of immunohistochemical markers have been evaluated to aid this diagnosis, but aside from N-cadherin, which labels cholangiocarcinoma, few provide the combination of good sensitivity and specificity. Our laboratory recently developed a novel monoclonal antibody HPC2 that recognizes pancreatic cancer. We hypothesized the combination of our new marker and N-cadherin would reliably distinguish metastatic pancreatic cancer from cholangiocarcinoma. We immunostained 60 pancreatic ductal adenocarcinomas and 31 cholangiocarcinomas for the HPC2 and N-cadherin antigens. We also stained 24 gallbladder adenocarcinomas, 12 ampullary adenocarcinomas, and 10 metastatic colonic adenocarcinomas to the liver. Sections were independently scored by two pathologists with good agreement using both markers (kappa statistics 0.62–0.64, $p < 0.0001$). HPC2 was observed in 80% of pancreatic cancers (48/60), 75% of ampullary (9/12), and 32% (10/31) of cholangiocarcinomas. N-cadherin stained 27% (16/60) of the pancreas cases and 58% (18/31) of the cholangiocarcinomas. Gallbladder and colon cancers were usually double negative (18/24 and 8/10 respectively). Each marker provided significant likelihood ratios to separate pancreatic cancer (HPC2: 2.48 [1.46–4.19], $p < 0.0001$) from cholangiocarcinoma (N-cadherin: 2.17 [1.3–3.64], $p < 0.01$). The combination of both markers provided even better specificity and positive likelihood ratios. We conclude that HPC2 and N-cadherin reliably distinguish pancreatic cancer from cholangiocarcinoma.

Keywords

Pancreatic ductal adenocarcinoma; cholangiocarcinoma; HPC2; N-cadherin

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INTRODUCTION

Differentiating pancreatic ductal adenocarcinoma from cholangiocarcinoma is a complex diagnostic dilemma. Clinical and imaging data are often not sufficient to distinguish the most likely origin of the cancer. Histologically, both carcinomas are similar with infiltrating ductal architecture and mild to moderate nuclear atypia. This is an especially important clinical problem, because distinguishing pancreatic ductal adenocarcinomas from cholangiocarcinoma has significant implications for surgical management, chemotherapy, and patient prognosis².

A long list of immunohistochemical markers have been tested to aid pathologists with this challenging differential diagnosis^{1,3–18} and various anti-cytokeratin and mucin stains have been used to separate “pancreaticobiliary” liver tumors from other metastases. However, most of these previously reported markers lack the sensitivity, specificity, or positive likelihood ratio to warrant use in clinical practice. For example, anti-cytokeratin 7 reportedly stained 92% of pancreatic adenocarcinomas, but it also stained 93% of cholangiocarcinomas in a series of 435 cases⁵. Although cytokeratin 17 has been cited as more likely to react with pancreatic than biliary tract tumors, 70–80% of cholangiocarcinomas are positive for this marker^{5,6}. The utility of K homology domain containing protein over-expressed in cancer (KOC) and S100p are also limited, because they immunostain a high percentage of cholangiocarcinomas^{10,11}.

Thus far the most promising marker has been N-cadherin. It appears to consistently distinguish intrahepatic biliary tumors from other gastrointestinal tumors with a specificity reported as high as 98% if used in conjunction with cytokeratin 7 immunostaining^{9,12}. However, it also stains hepatocellular carcinoma and gallbladder adenocarcinoma¹².

It would be useful to identify a reliable marker that specifically immunostains pancreatic cancer, but not cholangiocarcinoma, gallbladder adenocarcinoma or other common metastases to the liver. We have recently developed a novel mouse monoclonal antibody, HPC2, against a 55–65 kD cell-surface glycoprotein that is expressed by pancreatic ductal adenocarcinoma cells [Morgan TK, Hardiman K, Corless C, et al. (2011) HPC2: A Novel Monoclonal Antibody to Screen for Pancreatic Ductal Dysplasia, manuscript submitted]. The utility of HPC2 may be its improved sensitivity and specificity for pancreatic cancer compared with existing markers such as KOC.^{10, 17, 18} Our objective in the current study was therefore to test whether the combination of HPC2 and N-cadherin could reliably distinguish pancreatic cancer from cholangiocarcinoma.

MATERIALS AND METHODS

Tissue samples

Using an IRB approved protocol, we identified 137 cases including pancreatic adenocarcinoma (n=37 primary, n=23 metastatic), cholangiocarcinoma (n=31), gallbladder adenocarcinoma (n=24), ampullary carcinoma (n=12), and metastatic colon cancer (n=10) in the Oregon Health & Science University, Department of Pathology, Tissue Bank Archives (2000–2009). Routine H&E stained histologic sections were used to confirm the pathologic diagnosis, including verification of the primary in cases of metastatic disease. All diagnoses required consensus between two independent pathologists for inclusion in the study.

Immunohistochemistry

Histologic sections were stained for N-cadherin on an automated Ventana XT instrument (Ventana, Tucson, AZ). Slides were pretreated with cell conditioning 1 buffer with standard time; primary N-cadherin antibody (clone 3B9, Invitrogen) was applied at a dilution of 1:50.

Secondary antibody incubation and detection were performed with the Ventana Ultraview detection kit. HPC2 staining was performed by hand using citrate buffer (pH 6.0) antigen retrieval (Target Retrieval Solution, Citrate pH 6, Dako, S2369). After blocking with 2.5% Normal Horse Serum (Vector, ImmPress Kit blocking solution), slides were incubated for one hour with HPC2 monoclonal antibody (diluted 1:10 from hybridoma culture supernatant) at room temperature. Slides were then incubated in ImmPress Anti-Mouse reagent (Vector Labs, Burlingame, CA) and visualized using 3,3-diaminobenzidine tetrahydrochloride (DAB) chromogen. Slides were counterstained with hematoxylin. Mouse IgG was used as the negative primary antibody control.

Scoring

HPC2 and N-cadherin labeling was scored as positive if greater than 10% of the tumor cells showed staining. HPC2 appeared predominantly on the luminal surface and in the luminal secretions of glandular groups. N-cadherin staining was membranous. Slides were evaluated by two pathologists and discordant results were adjudicated by review and consensus diagnosis.

Statistics

Data were compared by Chi-Square analysis using the Fisher Exact test and SAS software (version 9.1.3; SAS Institute Inc. NC). Test performance of HPC2 and N-cadherin were calculated using standard 2×2 contingency tables with binomial 95% confidence intervals.

RESULTS

HPC2 Immunostains Pancreatic Ductal and Ampullary Adenocarcinoma

The HPC2 antibody predominantly immunostained the luminal surface of tumor cells in pancreatic ductal and ampullary adenocarcinomas and was also present in luminal secretions (Fig 1). Staining was specific with no signal in normal pancreas or liver parenchymal cells. There was occasional weak staining of the basement membrane of normal pancreatic ducts and a weak blush in some islet cells.

We observed good reproducibility between pathologists scoring sections for HPC2 (kappa statistic 0.62, $p < 0.0001$). Using adjudicated staining results for subsequent analysis, the frequency of positive HPC2 staining was similar in both primary pancreatic ductal adenocarcinoma (32/37, 86%) and metastatic pancreatic cancer (16/23, 70%). In fact, Chi-square analysis showed no significant difference in HPC2 and N-cadherin immunophenotypes between primary tumors and metastatic disease for any of the neoplasms tested (Table 1). Subsequent analysis therefore combined results for primaries and metastases for each neoplasm.

Ampullary carcinomas showed similar staining results as the pancreatic cancers with 9/12 (75%) staining for HPC2. Gallbladder carcinoma stained for HPC2 in only 4/24 (17%) cases and 2/10 (20%) metastatic colon cancers were positive.

In order to evaluate the test characteristics of HPC2 and N-cadherin when comparing metastatic pancreatic cancer to primary cholangiocarcinoma, we used 2×2 contingency tables to calculate sensitivity, specificity, and the positive likelihood ratio of a positive stain to predict metastatic pancreatic adenocarcinoma over primary bile duct cancer. Comparing the 16/23 (70%) HPC2 positive cases of metastatic pancreatic cancer with the 10/31 (32%) primary cholangiocarcinomas, HPC2 had a sensitivity of 70% and specificity of 68%. The likelihood ratio of a positive test correctly classifying the neoplasm as metastatic pancreatic cancer was 2.15 [1.21–3.84]; Fisher exact p -value=0.01.

N-cadherin and Cholangiocarcinoma

Scoring sections stained for N-cadherin again showed good reproducibility between pathologists with a kappa statistic of 0.64 (p-value <0.0001). N-cadherin showed the expected membranous staining pattern (Fig. 1) and stained most of the cholangiocarcinomas (18/31), but it also stained normal bile ducts, benign liver, and a subset of the metastatic pancreatic adenocarcinoma cases (5/23). A subset of 3/24 gallbladder cancers were positive for N-cadherin. All ampullary cancers and metastatic colon cases were negative.

Comparing the 18/31 (58%) N-cadherin positive cases of primary cholangiocarcinoma to the 5/23 (22%) cases of metastatic pancreatic cancer, this marker had a sensitivity of 58% and specificity of 78%. The likelihood ratio of a positive test correctly classifying the neoplasm as primary cholangiocarcinoma was 2.67 [1.16–6.13] with a significant Fisher exact p-value of 0.01.

Combined Scoring of HPC2 and N-cadherin

The most common HPC2/N-cadherin immunophenotype for pancreatic adenocarcinoma was HPC2 positive and N-cadherin negative (36/60). Ampullary carcinoma shared the same immunophenotype in 9/12 cases. Combined N-cadherin positive and HPC2 negative results favored cholangiocarcinoma (Table 2). Gallbladder and colon cancer were usually double negative (75% and 80%, respectively)

Utilizing HPC2 and N-cadherin together improved the specificity and positive likelihood ratios of the assay (Table 3). The HPC2 positive/Ncadherin negative immunophenotype yielded a low sensitivity (52%), but good specificity (81%) for pancreatic cancer compared with cholangiocarcinoma. In contrast, the HPC2 negative/N-cadherin positive immunophenotype provided an excellent specificity (93%) for cholangiocarcinoma. Although the likelihood ratio confidence intervals slightly overlap, combined HPC2 and N-cadherin staining provided positive ratios which were double those provided by either antibody alone (Table 3).

DISCUSSION

Metastatic pancreatic ductal adenocarcinoma and primary cholangiocarcinoma are very challenging to distinguish in liver biopsies. A number of immunohistochemical markers have been tested to aid this diagnosis, but few antibodies, other than perhaps N-cadherin are employed in clinical practice. We show that our new monoclonal antibody HPC2 used either alone or in combination with N-cadherin can significantly improve accurate classification of these neoplasms.

HPC2 is a novel monoclonal antibody we generated following immunization of mice with enzyme-dispersed tissue samples of human pancreatic ductal adenocarcinoma [Morgan TK, Hardiman K, Corless C, et al. (2011) HPC2: A Novel Monoclonal Antibody to Screen for Pancreatic Ductal Dysplasia, manuscript submitted]. Through rigorous selection criteria, one hybridoma yielded an antibody that recognized pancreatic cancer and precancerous intraductal papillary mucinous neoplasms, but not benign or reactive ductal cells. Our data suggest that HPC2 recognizes a 55–65 kD glycoprotein that is present on the luminal surface of pancreatic cancer cells and is secreted. HPC2 compares favorably with previously reported markers for pancreatic cancer (Table 3). For example, it has better sensitivity than Podocalyxin-like protein 1¹³ and better specificity than S100p¹¹. Conversely, a marker like pVHL may provide excellent negative predictive value (0/56 pancreatic cancers stained for pVHL in the Lin et al 2008 study), but its sensitivity for cholangiocarcinoma was poor (21%)¹⁴. Combining S100p with pVHL¹¹ provides additional sensitivity for pancreatic adenocarcinomas, but many cholangiocarcinomas (17/28, 61%) also stained for S100p.

Diagnostic conclusions would thereby rely on a negative test for a positive classification, which is not ideal ¹¹.

The KOC marker also appeared promising, because it immunostains pancreatic ductal adenocarcinoma^{10,17,18}. Unfortunately, it is not specific. KOC stained most of the bile duct tumors (3/4) in a cytology series reported by Ligato et al (2008)¹⁰. S100 staining had the same limitation in that series of bile duct brushings ¹⁰. Although studies in surgical specimens are needed, it appears that KOC stains intrahepatic cholangiocarcinomas and thus may not distinguish bile duct tumors from metastatic pancreatic adenocarcinoma.

Our N-cadherin staining results compared well with the prior study performed by Mosnier et al (2009)¹², who showed that 30/45 (67%) cholangiocarcinomas were positive for this marker. Staining frequency in cholangiocarcinomas was similar in our study (18/31, 62%), but we also identified a subset of pancreatic adenocarcinoma cases that stained for N-cadherin (16/60, 27%); whereas all 18 of the prior study's pancreatic cancer cases were negative. Restricting our analysis only to metastatic pancreatic cancer we still observed 5/23 (22%) of cases positive for N-cadherin. The reason for this discrepancy with Mosnier's study is uncertain. We note that both studies used the same monoclonal antibody clone (3B9), but differences include the greater number of cases, and the evaluation of whole tissue sections in our study, while they scored 1-mm cores on a tissue microarray ¹². Regardless, N-cadherin appears to be the most practical current marker available to label cholangiocarcinoma. It is more sensitive than pVHL, and more specific than other markers, because it only stains about 20–25% of pancreatic cancer cases.

We consider our sample size and statistical analysis to be relative strengths in our study, but we note the possibility of prevalence bias because our study is based on archived tissue samples. The accuracy of our test characteristics for each antibody may be influenced by the ratio of pancreas cases to cholangiocarcinoma. However, we note that the number of pancreatic cancer cases used for analysis (n=60) compared with the number of cholangiocarcinomas (n=31) roughly approximates the expected 2:1 prevalence ratio in the general population [4/100,000 (metastatic pancreas) and 2/100,000 (cholangiocarcinoma), respectively]. We conclude our reported test characteristics are therefore reasonably accurate.

In summary, our results support the utility of N-cadherin to highlight cholangiocarcinoma and we introduce HPC2 as an additional marker useful in staining pancreatic and ampullary carcinomas. The combination of HPC2 and N-cadherin may aid the surgical pathologist when diagnosing these challenging cases.

Acknowledgments

Financial support: Dr. Morgan's contribution was funded in part by the Office of Research on Women's Health and the National Institute of Child Health and Human Development, Oregon BIRCWH HD043488-08. Dr. Streeter was supported by the Oregon Stem Cell Center, the OHSU Knight Cancer Institute, and a generous gift from Randy and Mary Huebner. Several of the authors (TKM, MG, CC, and PS) are listed on a patent application for the antibody HPC2.

The authors gratefully acknowledge the support of Dr. Hardiman, Kelsea Lanxon-Cookson, and Stephanie Abraham in the generation and characterization of HPC2; and we thank Dr. Dawn Peters for her assistance with statistical analysis. We also thank Carolyn Gendron, Cara Poage and Linh Matsumura for their expert technical assistance preparing and staining the histologic sections.

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Figure 1. HPC2 and N-cadherin immunostaining of pancreatic ductal adenocarcinoma and cholangiocarcinoma

(A) Pancreatic ductal adenocarcinoma shows predominantly luminal staining for HPC2 (B) and is usually negative for N-cadherin (C). In contrast, cholangiocarcinoma (D) is usually negative for HPC2 (E) and positive for N-cadherin (F). Sections A, D were stained with hematoxylin & eosin (H&E). Sections B–C, E–F have brown DAB chromagen staining if positive for marker and sections were counterstained with hematoxylin. Photomicrographs A–F were photographed using the 20x objective. (Leica, Wetzlar, Germany).

Table 1

Immunophenotypes in primary and metastatic adenocarcinoma.

Neoplasm	Location	Immunophenotype, n (% neoplasm at location)			
		HPC2 +/NCAD –	HPC2 –/NCAD +	HPC2 +/NCAD +	HPC2 –/NCAD –
Pancreas n=60	Primary (n=37)	24 (65%)	3 (8%)	8 (22%)	2 (5%)
	Metastasis (n=23)	12 (52%)	1 (4%)	4 (17%)	6 (26%)
Cholangiocarcinoma n=31	Primary (n=31)	6 (19%)	14 (45%)	4 (13%)	7 (23%)
	Metastasis (n=0)	0	0	0	0
Gallbladder n=24	Primary (n=22)	3 (14%)	2 (9%)	1 (5%)	16 (73%)
	Metastasis (n=2)	0	0	0	2
Ampulla n=12	Primary (n=11)	9 (82%)	0	0	2 (18%)
	Metastasis (n=1)	0	0	0	1
Colon n=10	Primary (n=0)	0	0	0	0
	Metastasis (n=10)	2 (20%)	0	0	8 (80%)

There are no significant differences in immunophenotype when comparing primary location with metastasis for each type of neoplasm using 2×4 table and Chi-square analysis. HPC2 +/-, or N-cadherin (NCAD) +/-, is positive or negative result, respectively.

Table 2

HPC2 and N-cadherin immunophenotypes in 137 surgical cases.

Neoplasm/Immunophenotype	HPC2+/NCAD–	HPC2–/NCAD+	HPC2+/NCAD +	HPC2–/NCAD–	X ² Test
Cholangiocarcinoma (n=31)	6 (19%)	14 (45%)	4 (13%)	7 (23%)	reference
Pancreas (n=60)	36 (60%)	4 (7%)	12 (20%)	8 (13%)	p<0.0001
Gallbladder (n=24)	3 (14%)	2 (8%)	1 (4%)	18 (75%)	p=0.001
Ampulla (n=12)	9 (75%)	0	0	3 (25%)	p=0.002
Colon (n=10)	2 (20%)	0	0	8 (80%)	p=0.006

Pancreatic ductal adenocarcinoma and ampullary adenocarcinoma are more likely to be positive for HPC2 than cholangiocarcinoma when analyzed by 2 × 4 table Chi-square analysis. Gallbladder and colon are more likely to be negative for both markers. Cholangiocarcinoma is more likely to be positive for N-cadherin and negative for HPC2 by post hoc cell contributions.

Table 3

Test characteristics of immunostains commonly employed to distinguish pancreatic cancer and cholangiocarcinoma.

Study ^{reference}	Antibody	n	Pancreatic	Cholangiocarcinoma	SN	SP	LR [95% CI]	p-value
Chu et al, 2003 ⁴	CD5*	27	6/13 (46%)	12/14 (85%)	86	54	1.9 [0.99–3.46]	p=0.05
Abe et al, 2003 ¹	HMGAI	11	6/6 (100%)	2/5 (40%)	100	60	2.5 [0.85–7.31]	p=0.06
Chu et al, 2005 ³	MUC1	70	41/46 (89%)	20/24 (83%)	89	17	1.1 [0.87–1.31]	N.S.
Chu et al, 2005 ³	CK17	70	38/46 (83%)	17/24 (71%)	83	29	1.2 [0.87–1.55]	N.S.
Ney et al, 2006 ¹³	PODXL-1	178	71/160 (44%)	0/18 (0%)	44	100	8.0 [1.17–54.1]	p<0.001
Lin et al, 2008 ¹¹	S100P	84	56/56 (100%)	17/28 (61%)	100	39	1.6 [1.22–2.21]	p<0.0001
Lin et al, 2008 ¹¹	pVHL*	84	0/56 (0%)	6/28 (21%)	21	100	1.2 [1.03–1.41]	p<0.001
Mosnier et al, 2009 ¹²	N-cadherin*	63	0/18 (0%)	30/45 (67%)	67	100	3.0 [2.0–4.53]	p<0.0001
Current Study	HPC2 1-B3	91	48/60 (80%)	10/31 (32%)	80	68	2.48 [1.46–4.19]	p<0.0001
Current Study	N-cadherin*	91	16/60 (27%)	18/31 (62%)	58	73	2.17 [1.30–3.64]	p<0.01
Current Study	HPC2+/Ncad–	91	36/60 (60%)	6/31 (19%)	60	80	3.10 [1.46–6.54]	p<0.001
Current Study	HPC2–/Ncad+*	91	4/60 (6%)	14/31 (45%)	45	93	6.77 [2.43–18.8]	p<0.0001

Sensitivity (SN), specificity (SP), and likelihood ratio (LR) with 95% confidence intervals. When the antibody is designated with an asterisk (*) the test characteristics were calculated to detect cholangiocarcinoma rather than pancreatic cancer. P-values were determined by Fisher exact test. N.S. is not significant.