Modern Pathology (2005) 18, 752–761

www.modernpathology.org

Expression of novel markers of pancreatic ductal adenocarcinoma in pancreatic nonductal neoplasms: additional evidence of different genetic pathways

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Solid pseudopapillary tumor, pancreatoblastoma, undifferentiated carcinoma with osteoclastic-like giant cells, and acinar cell carcinomas are rare pancreatic nonductal neoplasms. Compared to the significant advances in our understanding of the pathogenesis of pancreatic ductal adenocarcinomas in the last decades, the molecular mechanisms underlying pancreatic nonductal neoplasms are poorly understood. In order to elucidate their molecular pathogenesis, we constructed tissue microarrays to study the expression of some novel pancreatic ductal adenocarcinoma-associated tumor markers in these nonductal pancreatic neoplasms. We analyzed nine markers including tumor suppressor gene (*14-3-3 sigma*), proliferation marker (topoisomerase II alpha), epithelial markers (prostate stem cell antigen, mesothelin and cytokeratin 19), stromal markers (fascin, hsp47 and fibronectin), and gamma-synuclein whose function is not delineated. In addition, we included tumor suppressor gene *DPC4* and oncogene *Beta-catenin* to further confirm their expression in pancreatic nonductal tumors. Our results showed that in contrast to pancreatic ductal adenocarcinomas that show loss of Dpc4 protein in 55% of cases, loss of Dpc4 expression is absent in pancreatic nonductal neoplasms. Expression of 14-3-3 sigma is frequently seen in both pancreatic nonductal neoplasms (25–100%) and ductal adenocarcinomas (89%). Aberrant nuclear expression of beta-catenin is common in pancreatic nonductal neoplasms, specifically in solid pseudopapillary tumors (88%) and pancreatoblastomas (100%) but is rarely seen in pancreatic ductal adenocarcinomas (<5%). Expression of topoisomerase II alpha is not seen in solid pseudopapillary tumors and undifferentiated carcinomas with osteoclastic-like giant cells but is focally seen in pancreatoblastomas (50%) and acinar cell carcinomas (85%). Expression of PSCA and mesothelin was observed in pancreatic nonductal neoplasms but their expression was seen less frequently (0–50%) and weaker than that in pancreatic ductal adenocarcinomas (60–100%). CK19, a marker of pancreatic ductal adenocarcinomas, is not expressed in pancreatic nonductal neoplasms. Expression of gamma-synuclein as well as stromal markers (fascin, hsp47 and fibronectin) is frequently seen in both. Our findings indicate pancreatic nonductal neoplasms have distinctive patterns of protein expression relative to pancreatic ductal adenocarcinomas and suggest that pancreatic nonductal neoplasms have different genetic pathways from the more common pancreatic ductal adenocarcinomas.
Modern Pathology (2005) **18,** 752–761, advance online publication, 14 January 2005; doi:10.1038/modpathol.3800363

Keywords: solid pseudopapillary tumor; pancreatoblastoma; undifferentiated carcinoma with osteoclastic-like giant cells; acinar cell carcinoma; pancreatic ductal adenocarcinoma; tissue microarray; novel markers

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Received 23 September 2004; revised 11 November 2004; accepted 12 November 2004; published online 14 January 2005

Solid pseudopapillary tumor, pancreatoblastoma, undifferentiated carcinoma with osteoclastic-like giant cells, and acinar cell carcinomas are rare pancreatic neoplasms, accounting for less than 10% of pancreatic neoplasms.[1,2] The vast majority of solid pseudopapillary tumors (90%) occur in

women[3] and are considered to have a low malignant potential; however, as many as 10% of solid pseudopapillary tumors have metastasized at the time of diagnosis.[3] Pancreatoblastomas are extremely rare neoplasms that can show multiple directions of differentiation including acinar, endocrine, and, less commonly, ductal differentiation. They also have distinctive squamoid corpuscles. Most pancreatoblastomas arise in infants and young children, although up to one-third occur in adults.[4] As many as one-third of pancreatoblastomas have metastases at diagnosis.[5] Acinar cell carcinomas are rare carcinomas of the exocrine pancreas, comprising less than 1% of primary pancreatic neoplasms.[6] Acinar cell carcinomas are highly malignant with a poor prognosis and a high rate of recurrence.[7] Undifferentiated carcinomas with osteoclastic-like giant cells are also extremely rare tumors with distinctive non-neoplastic osteoclast-like giant cells, and carry a similar poor prognosis to ductal adenocarcinoma.[8] Since many undifferentiated carcinomas with osteoclastic-like giant cells have associated ductal neoplasms (either components of conventional ductal adenocarcinoma or noninvasive precursors such as mucinous cystic neoplasms), they are regarded as undifferentiated carcinomas related to ductal adenocarcinoma. They also share the same K-ras mutations found in the coexisting ductal components.[9] However, the neoplastic cells in the osteoclast-containing areas generally lose all phenotypic evidence of ductal origin, including the loss of keratin expression.

Compared to the significant advances in our understanding of the pathogenesis of pancreatic ductal adenocarcinomas in the last decades, the molecular mechanisms underlying pancreatic nonductal neoplasms are poorly understood. In an effort to elucidate the underlying molecular mechanisms of pancreatic nonductal neoplasms, we constructed tissue microarrays of a series of pancreatic nonductal neoplasms using archival tissues. Tissue microarray technology has been proven as a valid and cost-effective method to study pancreatic neoplasms.[10,11] In this study, we used tissue microarrays and immunohistochemical labeling to investigate expression of a panel of novel tumor markers of ductal adenocarcinoma in the above-mentioned four nonductal pancreatic neoplasms. These markers had been previously identified through global gene expression analyses and include: tumor suppressor gene (*14-3-3 sigma*),[12] proliferation marker (topoisomerase II alpha),[13] epithelial proteins (PSCA,[14] mesothelin,[15] and CK19[16]), stromal markers (fascin,[17] hsp47,[17] fibronectin[12]), and gamma-synuclein with unknown function.[12] We also included immunolabeling for *DPC4*, a tumor suppressor gene frequently inactivated in pancreatic ductal adenocarcinoma,[18–20] and *Beta-catenin*, of the Wnt pathway mediator as an oncogene.

Materials and methods

The Internal Review Board of the Johns Hopkins Hospital approved this study.

Tissue Microarray Construction

The surgical pathology files of the Johns Hopkins Hospital, Memorial Sloan-Kettering Cancer Center and Karmanos Cancer Center were searched for solid pseudopapillary tumor, pancreatoblastoma, undifferentiated carcinoma with osteoclastic-like giant cells and acinar cell carcinoma with available tissue blocks. Eight solid pseudopapillary tumors, four pancreatoblastomas, four undifferentiated carcinomas with osteoclastic-like giant cells, and 13 acinar cell carcinomas were selected for this study. Tissue microarrays were generated from formalin-fixed paraffin-embedded archival tissues. Each case was represented by four 1.8 mm tissue cores: two cores were arrayed from the neoplastic compartment in order to account for potential tumor heterogeneity, and two cores were arrayed from adjacent normal pancreatic parenchyma as an internal control.

Immunohistochemistry

Unstained 4-μm sections were cut from each tissue microarray and deparaffinized by routine techniques before placing in 200 ml Target Retrieval Solution, pH 6.0 (Dako, Envision Plus Detection Kit, Carpinteria, CA, USA) for 20 min at 100°C. After cooling for 20 min, slides were quenched with 3% H_2O_2 for 5 min, before incubating with the appropriate dilution of each primary antibody (see Table 1 for antibody information) for 60 and 30 min, respectively, using the Dako Autostainer. Labeling was detected with the Dako Envision system as per the manufacturer's protocol. All sections were counterstained with Giles' hematoxylin. Three of the authors (DC, AM, and RHH) evaluated the staining first individually and then collectively under a multiheaded microscope with agreement reached on all cases. The staining was reported as negative ($<5\%$ expression), focally positive (5–25%) and diffuse positive ($>25\%$). Only labeling of the appropriate cellular compartment (cell membrane, cytoplasm, or nucleus) was considered for evaluation (see Table 1 for details of the appropriate cellular compartment for each marker). For example, only absence of cytoplasmic and nuclear staining of Dpc4 in more than 95% of neoplastic cells was considered negative. For beta-catenin and topoisomerase II alpha, only nuclear labeling was considered abnormal.

Results and discussions

A summary of immunolabeling of these markers in solid pseudopapillary tumors, pancreatoblastomas,

Table 1 Antibodies used in immunohistochemical study

Antigen	*Clone*	*Dilution*	*Source*	*Staining pattern*
Dpc4	B8	1:100	Santa Cruz Biotechnology, Santa Cruz, CA, USA	Cytoplasm and nucleus
14-3-3 sigma		1:100	NeoVision Laboratories, Freemont, CA, USA	Cytoplasm/membrane
Beta-catenin	Clone 14	1:500	Beckton Dickinson Transduction Laboratories, Lexington, KY, USA	Nuclear
Topoisomerase II alpha	3F6	1:3200	Neomarkers, Freemont, CA, USA	Nucleus
PSCA	1G8	1:200	Dr. Robert E. Reiter, UCLA, USA	Cytoplasm/membrane
Mesothelin	5B2	1:20	Novocastra, Newcastle upon Tyne, UK	Cytoplasm/membrane
CK19	BA17	1:100	Serotec Inc., Raleigh, NC, USA	Cytoplasmic
Fascin	55K-2	1:500	Dako, Carpinteria, CA, USA	Cytoplasm/membrane
hsp47	M16.10A1	1:800	Stressgen Biotechnologies, Victoria, British Columbia	Cytoplasmic
Fibronectin	A0245	1:1200	Dako, Carpinteria, CA, USA	Cytoplasmic
Gamma-synuclein	E-20	1:100	Santa Cruz Biotechnology, Santa Cruz, CA, USA	Cytoplasmic

Table 2 Reactivity of Dpc4, 14-3-3 sigma, beta-catenin, topoisomerase II alpha, PSCA, mesothelin, CK19, fascin, hsp47, fibronectin and gamma-synuclein in nonductal pancreatic neoplasms

Markers	*No. of positive cases*				*Pancreatic duct adenocarcinoma Percentage (reference)*
	SPTs[a] (N = *8*)	*PBs*[b] (N = *4*)	*UCOGCs*[c] (N = *4*)	*ACCs*[d] (N = *13*)	
Dpc4	8 (6+2)[e] (100%)	4 (3+1) (100%)	4 (3+1) (100%)	12[f] (9+3) (100%)	45% (20)
14-3-3 sigma	7 (7+0) (88%)	4 (4+0) (100%)	1 (0+1) (25%)	13 (13+0) (100%)	89% (12)
Beta-catenin[g]	7 (7+0) (88%)	4 (2+2) (100%)	0	1 (0+1) (8%)	0% (56)
Topoisomerase II alpha	0	2 (0+2) (50%)	0	11 (0+11) (85%)	Expressed but no percentage given (13)
PSCA	4 (2+2) (50%)	1 (1+0) (25%)	1 (1+0) (25%)	3 (3+0) (23%)	60% (14)
Mesothelin	4 (1+3) (50%)	1 (1+0) (25%)	0	0	100% (15)
CK19	0	0	0	0	100% (16)
Fascin	7 (7+0) (88%)	4 (4+0) (100%)	4 (3+1) (100%)	4 (3+1) (31%)	95% (17)
hsp47	2 (2+0) (25%)	3 (3+0) (75%)	4 (3+1) (100%)	3 (2+1) (23%)	65% (17)
Fibronectin	6 (4+2) (75%)	3 (1+2) (75%)	4 (2+2) (100%)	11 (7+4) (85%)	25% (12)
Gamma-synuclein	6 (4+2) (75%)	1 (1+0) (25%)	0	4 (4+0) (31%)	25% (12)

[a]SPT = solid pseudopapillary tumor.
[b]PB = pancreatoblastoma.
[c]UCOGC = undifferentiated carcinoma with osteoclastic-like giant cells.
[d]ACC = acinar cell carcinoma.
[e]*N* (*N*1+*N*2) = combined diffusely and focally positive cases (diffuse ones+focal ones).
[f]One case was excluded because the internal positive control cells were not labeled.
[g]Nuclear staining.

undifferentiated carcinomas with osteoclastic-like giant cells and acinar cell carcinomas is tabulated in Table 2. The result of each protein expression in these tumors is described in the following discussion. For a better comparison, we also include the expression profiles of these markers in ductal adenocarcinomas in the right-most column, previously reported by our group and others.

Tumor Suppressor Genes (*DPC4* and *14-3-3* Sigma)

DPC4

DPC4 is a tumor suppressor gene on chromosome 18q21.1[18] and its gene product, Dpc4, functions in the TGF-beta signaling pathway.[19] The *DPC4* gene is inactivated in 55% of pancreatic ductal adenocarcinomas.[20] In this study, Dpc4 protein expression was intact in all eight solid pseudopapillary tumors (Figure 1) as previously reported.[21] Weak, but intact expression of Dpc4, was seen in all four pancreatoblastomas (Figure 1) but one case showed only focal positivity. This was consistent with a previous study.[22] All four undifferentiated carcinomas with osteoclastic-like giant cells retained expression of Dpc4 but again the labeling was weak (Figure 1). This finding confirmed a previous report that Dpc4 expression was not lost in undifferentiated carcinomas with osteoclastic-like giant cells.[23]

In our study, one acinar cell carcinoma did not have any expression of Dpc4 in the neoplastic cells. However, the internal positive control (ie, the nonneoplastic stroma cells) also did not shown expression of Dpc4, and this case was excluded from our analysis. The remaining 12 cases all showed intact expression of Dpc4 (Figure 1) as has been reported by Abraham *et al*.[24] These findings indicate that acinar cell carcinomas and pancreatic ductal adenocarcinoma arise through fundamentally genetically distinct pathways.

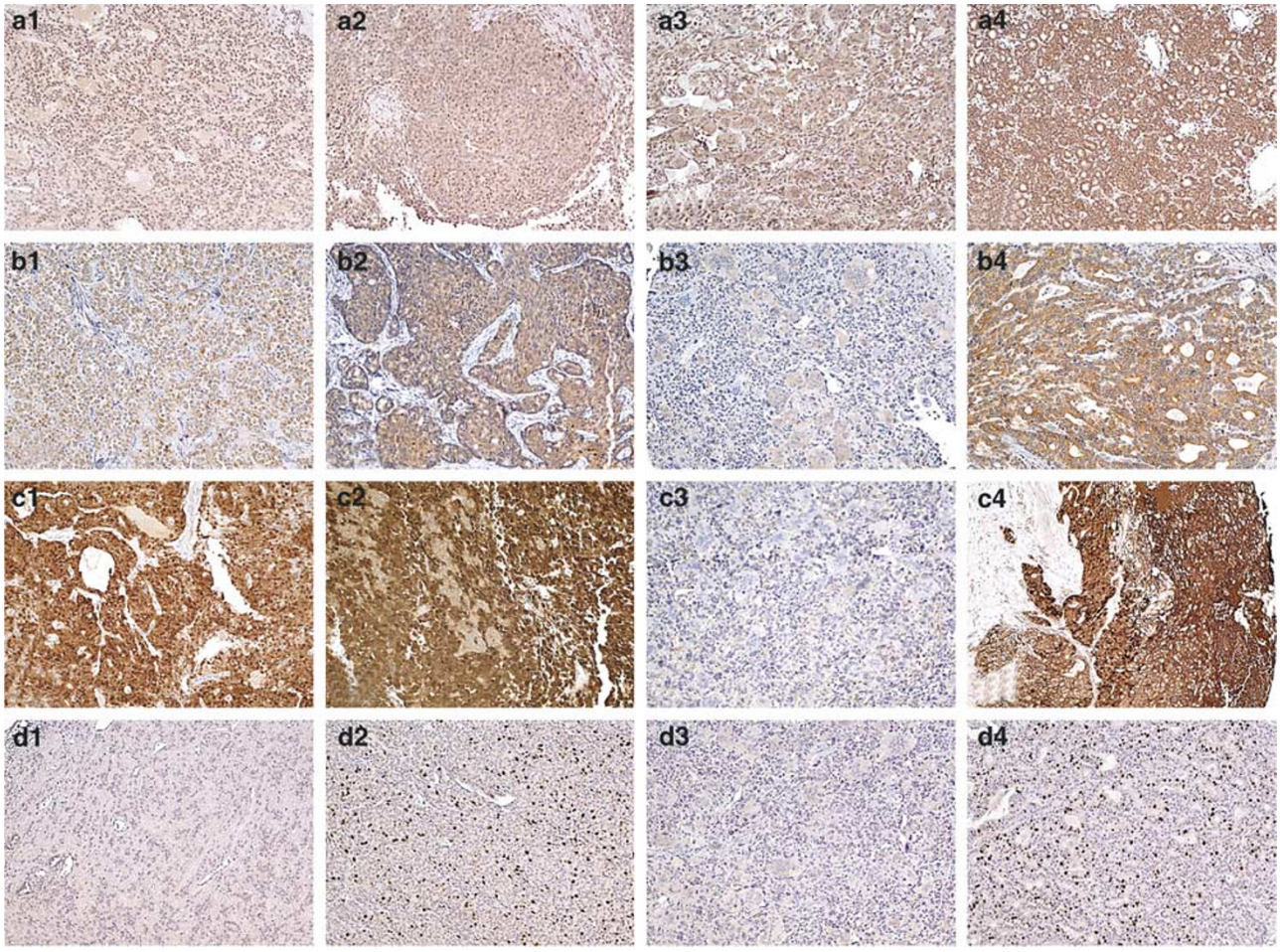

Figure 1 Expression of pancreatic duct adenocarcinoma-associated markers Dpc4, 14-3-3 sigma, beta-catenin, topoisomerase II alpha in solid pseudopapillary tumors, pancreatoblastomas, undifferentiated carcinomas with osteoclastic-like giant cells, and acinar cell carcinomas. Dpc4 expression was seen in all solid pseudopapillary tumors (**a1**), pancreatoblastomas (**a2**), undifferentiated carcinomas with osteoclastic-like giant cells (**a3**), and acinar cell carcinomas (**a4**). All but one solid pseudopapillary tumors (**b1**), pancreatoblastomas (**b2**), and acinar cell carcinomas (**b4**) expressed 14-3-3 sigma but only one of four undifferentiated carcinomas with osteoclastic-like giant cells (**b3**) did. Nuclear beta-catenin staining was seen in seven of eight solid pseudopapillary tumors (**c1**), all four pancreatoblastomas (**c2**) but only focally in one of 13 acinar cell carcinomas (**c4**). No undifferentiated carcinoma with osteoclastic-like giant cells showed nuclear staining of beta-catenin (**c3**). None of the solid pseudopapillary tumors (**d1**) and undifferentiated carcinomas with osteoclastic-like giant cells (**d3**) expressed topoisomerase II alpha (nuclear staining), whereas half of the pancreatoblastomas (**d2**) and most of the acinar cell carcinomas (**d4**) showed focal staining.

14-3-3 sigma

14-3-3 sigma, or *stratifin*, is thought to act as a tumor suppressor gene that inhibits G2/M progression.[25,26] Loss of expression of 14-3-3 sigma has been reported in several types of cancer including breast carcinoma,[27] bladder transitional cell carcinoma,[28] oral[29] and vulvar squamous cell carcinomas,[30] hepatocellular carcinoma,[31] pulmonary small-cell carcinoma[32] and basal cell carcinoma of the skin.[33] In contrast to the downregulation of 14-3-3 sigma in these tumors, the expression of 14-3-3 sigma was paradoxically elevated (in contrast to normal ducts) in pancreatic ductal adenocarcinoma.[12] In this study, we found expression of 14-3-3 sigma in solid pseudopapillary tumors (seven of eight cases), pancreatoblastomas (four of four cases), undifferentiated carcinomas with osteoclastic-like giant cells (one of four cases) and acinar cell carcinomas (13 of 13 cases) (Figure 1). It should be noted that 14-3-3 sigma is normally expressed in acinar cells and islet cells (more intense in acinar cells than in islet cells) but not in ductal epithelial cells. Compared to normal acini, 14-3-3 sigma expression actually was more intense or upregulated in only two acinar cell carcinomas, and weaker or downregulated in three acinar cell carcinomas. All four pancreatoblastomas were positive for 14-3-3 sigma: one had weaker staining than that seen in normal acini and three had similar intense staining to normal acini. Solid pseudopapillary tumor is a neoplasm with uncertain histogenesis. Compared to normal acini, the expression in all solid pseudopapillary tumors was downregulated including one showing loss of expression but compared to ductal cells, seven of eight solid pseudopapillary tumors showed overexpression of 14-3-3 sigma since normal ductal cells do not express this protein.

The expression level of 14-3-3 sigma in tumors has been related to the methylation status of its gene, with the overexpression of 14-3-3 sigma associated with hypomethylation[12] and downregulation associated with hypermethylation.[27–33]

Oncogene (*Beta-catenin*)

Beta-catenin protein is a member in the cadherin-mediated cell adhesion system. It also acts as a downstream transcriptional activator in the Wnt (wingless-type) signaling pathway.[34–37] The *APC* tumor suppressor gene promotes the phosphorylation of beta-catenin.[35] Events disrupting the degradation of beta-catenin, including activating point mutations in *beta-catenin* and inactivating mutations of the *APC* or *AXIN* genes[35,37,38] will lead to abnormal subcellular accumulation.

In this study, abnormal nuclear accumulation of beta-catenin was seen in four of four pancreatoblastomas (Figure 1). Two previously studies also reported nuclear labeling of beta-catenin in seven of nine cases[22] and seven of seven cases.[39] In these two studies mutation in the *beta-catenin* gene was found in five of nine cases[22] and two of five cases.[39] Biallelic *APC* inactivation was also reported in a FAP-associated pancreatoblastoma.[22] Our findings further confirm that nuclear labeling is a common occurrence in pancreatoblastoma.[22,39]

Of eight solid pseudopapillary tumors, seven showed nuclear labeling of beta-catenin (Figure 1), reflecting previous reports that almost all solid pseudopapillary tumors harbor mutations in the *beta-catenin* gene.[21] Our data therefore further confirm the concept that solid pseudopapillary tumors are genetically distinct from pancreatic ductal adenocarcinoma.[21] Of note, the case without nuclear staining of beta-catenin showed complete loss of beta catenin, that is, even membranous pattern was not observed. The reason was not clear. The *beta-catenin* gene might not function (mutated) or the protein was unstable. Another possibility might be poor tissue fixation. However, other markers such as Dpc4, fibronectin, gamma-synuclein were positive in the neoplastic cells. The stroma cells in this case were positive for fascin and hsp47, although the neoplastic cells were negative for them.

We observed focal nuclear accumulation of beta-catenin in one of 13 acinar cell carcinomas (Figure 1). Alternations in the *APC/Beta-catenin* pathway and nuclear staining of beta-catenin have been reported in four of 17 cases (24%) and three of 20 cases (15%), respectively in a previous study.[24] None of the four undifferentiated carcinomas with osteoclastic-like giant cells showed any labeling of the beta-catenin protein (Figure 1).

Proliferation Marker (Topoisomerase II Alpha)

Topoisomerase II alpha is an enzyme involving DNA metabolism.[40] Topoisomerase II alpha is expressed in proliferating cells only.[41] In the normal pancreas, nuclear expression of topoisomerase II alpha is only seen in rare acinar cells. This protein is also expressed in some pancreatic ductal adenocarcinomas.[13] In this study, we found that topoisomerase II alpha was not expressed in solid pseudopapillary tumors and undifferentiated carcinomas with osteoclastic-like giant cells (Figure 1). Two of 4 pancreatoblastomas and 11 of 13 acinar cell carcinomas showed focal expression (5–25% cells) (Figure 1). In previous studies, topoisomerase II alpha expression correlated with cell proliferation,[11] and solid pseudopapillary tumors have the lowest proliferation rates of the neoplasms examined here.

Epithelial Markers (PSCA, Mesothelin and CK19)

PSCA

Prostate stem cell antigen (PSCA) is a glycoprotein expressed in normal prostate and is a marker of late intermediate prostate epithelial cells.[42] Normally, it is not expressed in pancreatic ductal epithelium, but it is expressed in 43-57% PanINs[11] and in 60% pancreatic ductal adenocarcinoma.[14] The expression of PSCA has also been reported in various tumors such as prostate cancer,[43] transitional cell carcinoma,[44] and ovarian mucinous carcinoma.[45] In this study, we demonstrated that four of eight solid pseudopapillary tumors showed weak expression of PSCA. Weak expression of PSCA was also observed in one of four undifferentiated carcinomas with osteoclastic-like giant cells, one of four pancreatoblastomas and three of 13 acinar cell carcinomas. The expression of PSCA is of particular interest because immunotherapies targeting this protein have recently been developed.[46]

Mesothelin

Mesothelin is a cell surface glycoprotein whose function is not well delineated. Mesothelin expression has been seen in many neoplasms including such as epithelial mesotheliomas,[47–49] gastrointestinal cancers (esophageal, gastric, and colonic adenocarcinomas),[50] cholangiocarcinoma,[50] breast adenocarcinoma,[50] lung adenocarcinoma, and squamous cell carcinoma,[48,50] uterine endometrioid adenocarcinoma, and ovarian mucinous carcinoma.[45,49,50] Mesothelin is also expressed in almost all pancreatic ductal adenocarcinomas.[15] In this study, we found mesothelin expression in one of four pancreatoblastomas and four of eight solid pseudopapillary tumors had weak expression of this antigen. None of the undifferentiated carcinomas with osteoclastic-like giant cells or acinar cell carcinomas showed any expression. Our findings indicate that mesothelin expression can also be seen in some pancreatic nonductal tumors, but the expression of this antigen is weak in contrast to the strong and uniform expression in pancreatic ductal carcinomas.[15]

CK19

Cytokeratin 19 is an intermediate type I keratin. In the pancreas, its expression is normally found only in ductal epithelial cells.[51] Pancreatic ductal adenocarcinoma usually retains expression of this protein.[16,52] Expression of CK19 was previously reported in solid pseudopapillary tumors (two of 15),[9] acinar cell carcinomas[9,53] and pancreatoblastomas.[53] In this study, we did not see any expression of CK19 in any of solid pseudopapillary tumors, pancreatoblastomas, undifferentiated carcinomas with osteoclastic-like giant cells, and acinar cell carcinomas.

Stromal Markers (Fascin, hsp47, and Fibronectin)

Fascin

Fascin is an actin-bundling protein regulating cell mobility with normal expression in stromal cells.[54] Previous studies have shown that the expression of fascin increases with the grade of PanIN (25–28% in PanINs 1, 57% in PanINs 2 and PanINs 3[11]) and that fascin is expressed in 95% of infiltrating pancreatic ductal adenocarcinomas.[17] In the current study, we observed expression of fascin in seven of eight of solid pseudopapillary tumors, four of four pancreatoblastomas, four of four undifferentiated carcinomas with osteoclastic-like giant cells, and four of 13 acinar cell carcinomas (Figure 2). These findings indicate that expression of fascin by neoplastic cells is a very common occurrence in pancreatic neoplasms (ductal and nonductal).

The mechanism by which fascin is upregulated in these neoplasms is of interest. It has been suggested that Wnt signaling pathway abnormalities such as stabilizing *beta-catenin* mutations or inactivation of *APC* gene[55] might upregulate fascin. Fascin competes with E-cadherin to form complexes with beta-catenin.[55] In our study seven of eight solid pseudopapillary tumors, all of which showed abnormal nuclear accumulation of beta-catenin, expressed fascin and the only solid pseudopapillary tumor without expression of fascin in the neoplastic cells. The stromal cells in all eight cases were strongly positive for fascin did not have nuclear beta-catenin accumulation. We have previously shown that 90% of solid pseudopapillary tumors harbor *beta-catenin* mutation in codons 33–37 in exon 3 and that solid pseudopapillary tumors with mutated *beta-catenin* gene show nuclear accumulation of beta-catenin.[21] All four pancreatoblastomas expressed both nuclear

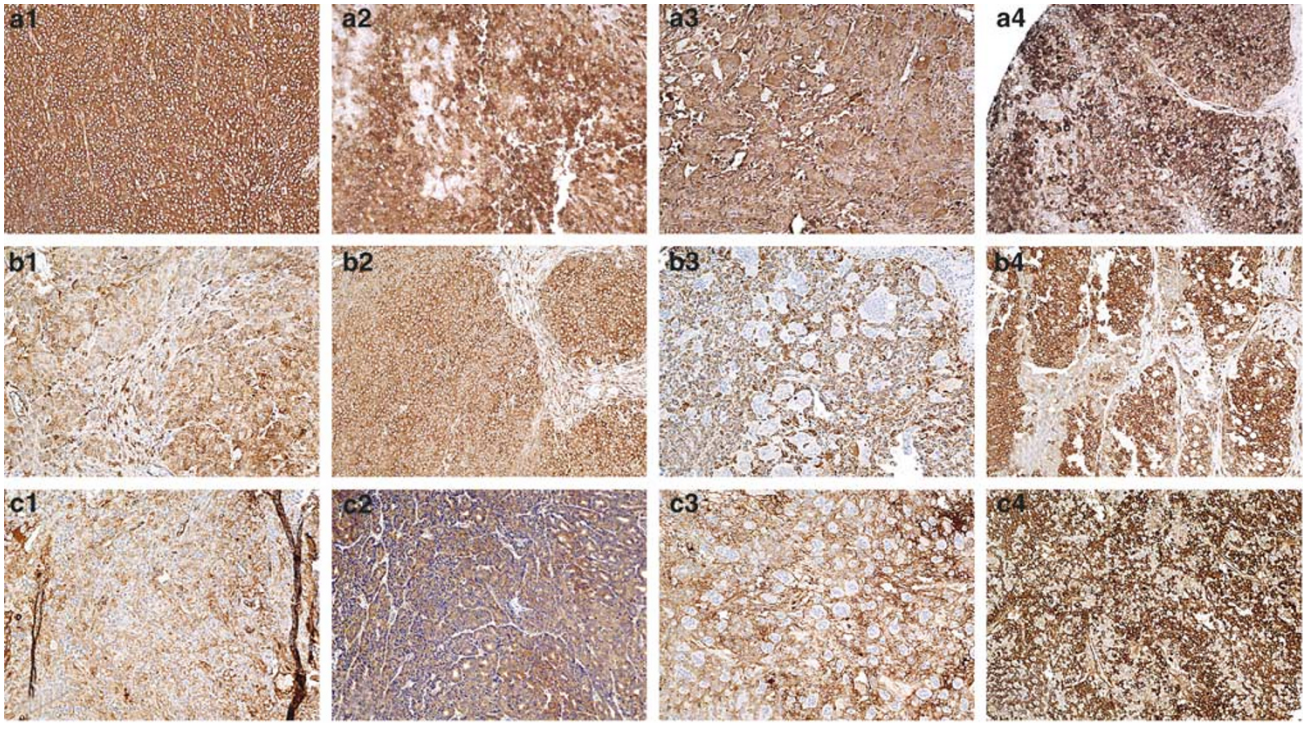

Figure 2 Expression of pancreatic duct adenocarcinoma-associated stromal markers fascin, hsp47, and fibronectin in solid pseudopapillary tumors, pancreatoblastomas, undifferentiated carcinomas with osteoclastic-like giant cells, and acinar cell carcinomas. All but one solid pseudopapillary tumors (**a1**), all pancreatoblastomas (**a2**) and undifferentiated carcinomas with osteoclastic-like giant cells (**a3**) showed expression of fascin but only about one-third of acinar cell carcinomas (**a4**) did. All four types of tumors showed expression of hsp47 expression, but the frequency was much lower in solid pseudopapillary tumors (two of eight) (**b1**) and acinar cell carcinomas (three of 13) (**b4**) than that in pancreatoblastomas (three of four) (**b2**) and undifferentiated carcinomas with osteoclastic-like giant cells (four of four) (**b3**). Expression of fibronectin was seen in all undifferentiated carcinomas with osteoclastic-like giant cells (**c3**) and in the majority of solid pseudopapillary tumors (**c1**), pancreatoblastomas (**c2**), and acinar cell carcinomas (**c4**). The osteoclastic-like giant cells in undifferentiated carcinomas with osteoclastic-like giant cells did not express hsp47 (**b3**) and fibronectin (**c3**).

beta-catenin and fascin, and in these cases the expression of nuclear beta-catenin correlated with fascin expression. Moreover, the only acinar cell carcinoma with focal nuclear beta-catenin labeling also expressed strong fascin in that area. These findings support a correlation between beta-catenin abnormalities and fascin expression; however, it should be noted that pancreatic adenocarcinomas overexpress fascin but do not harbor *beta-catenin* mutations.[17,56] Therefore other mechanisms must be also involved in up-regulating fascin overexpression.[57]

hsp47

hsp47, heat shock protein 47 or colligin, is a collagen-specific chaperone that is essential for development and collagen molecular maturation in the ER.[58] In pancreatic ductal adenocarcinoma, hsp47 is expressed in neoplastic epithelial cells and in stroma fibroblasts.[17] In this study, three of four pancreatoblastomas and all four undifferentiated carcinomas with osteoclastic-like giant cells expressed hsp47 (Figure 2). Less frequent expression was also seen in acinar cell carcinomas (three of 13 cases; 23%) and solid pseudopapillary tumors (two of eight cases; 25%) (Figure 2). The stromal cells in all tested cases were positive for hsp47. Therefore, although the prevalence of expression is not as high as it is for fascin, hsp47 is also a common antigen expressed in pancreatic nonductal neoplasms. hsp47 expression on the surface of neoplastic cells has been suggested associated with tumor invasiveness.[59–62]

Fibronectin

Fibronectin is an also extracellular protein with normal expression in stromal cells. In 25% of pancreatic ductal adenocarcinoma, the neoplastic epithelial cells also express this protein.[12] In this study, fibronectin was expressed in neoplastic cells of the majority of nonductal pancreatic neoplasms (six of eight solid pseudopapillary tumors, three of four pancreatoblastomas, four of four undifferentiated carcinomas with osteoclastic-like giant cells and 11 of 13 acinar cell carcinomas) (Figure 2). As expected, the stromal cells in all tested cases are positive. Fibronectin expression has also been observed in ovarian carcinoma,[63] head and neck tumors,[64] non-small lung cancer,[65] and acute myeloid leukemia with dendritic cell differentiation.[66] The expression of fibronectin may give the neoplastic cells some growth advantages through different mechanisms, for example, the acquisition of anticancer drug resistance[67] and decreased apoptosis in pancreatic cancer,[68] enhanced cancer growth in small-cell lung cancer through PI3-K pathway[69] and in prostate cancer through the AKT/survivin pathway.[70]

It was noted that the mononuclear cells and osteoclastic-like giant cells showed different patterns of expression in two stromal markers (Figure 2). Only the mononuclear cells expressed hsp47 and fibronectin, while the osteoclastic-like giant cells did not. On the other hand, expression of fascin was seen in both the osteoclast-like giant cells and the infiltrating mononuclear cells.

Novel Marker with Unknown Function (Gamma-synuclein)

Gamma-synuclein, also referred as the breast carcinoma-specific gene 1 *(BCSG1*) protein, is small cytoplasmic protein that is expressed primarily in the peripheral nervous system and retina. Its normal function has not yet been delineated. In normal pancreas, islet cells and some acinar cells label with antibodies to this protein but the ductal epithelium is negative.[71,72] In our study, six of eight solid pseudopapillary tumors showed expression of gamma-synuclein, more frequently than acinar cell carcinomas (four of 13) and pancreatoblastomas (one of four) did. No expression of this protein was observed in undifferentiated carcinomas with osteoclastic-like giant cells. A previous study reported that 25% (two of eight) pancreatic ductal adenocarcinoma showed focal expression of gamma-synuclein.[12] Aberrant expression of gamma-synuclein has also been observed in breast[73] and ovarian carcinoma,[74] and expression of this antigen was reported to be associated with a high stage in breast cancer.[75] In breast carcinoma and ovarian carcinoma, the aberrant expression of gamma-synuclein is thought to be promoted by hypomethylation of the CpG islands of the *gamma-synuclein* gene.[76] Once overexpressed, gamma-synuclein may promote cancer cell survival and inhibit stress- and chemotherapy drug-induced apoptosis by modulating MAPK pathways.[77]

Comparison between Acinar Cell Carcinomas and Pancreatoblastomas

Acinar cell carcinomas are neoplasm of acini and pancreatoblastomas have an acinar component. They share some common patterns in the expression of some markers. Both types of tumor showed intact Dpc4 and expression of 14-3-3 sigma in all cases (Figure 1). Expression of topoisomerase II alpha (Figure 1), fibronectin (Figure 2) and gamma-synuclein was seen in comparable percentages of cases in acinar cell carcinomas and pancreatoblastomas. However, a much higher percentage of pancreatoblastomas showed expression of fascin, hsp47 than acinar cell carcinomas did. The most striking difference probably lied in the nuclear expression of beta-catenin. All pancreatoblastomas showed nuclear staining of beta-catenin but only one of 13 acinar cell carcinomas showed focal expression of nuclear beta-catenin (Figure 1). These findings indicate that acinar cell carcinomas and pancreatoblastomas are genetically different.

Comparison between Pancreatic Nonductal Neoplasms and Ductal Adenocarcinoma

In contrast to ductal carcinomas showing loss of Dpc4 in 55% cases, no loss of Dpc4 expression occurs in nonductal neoplasms. In this study, all solid pseudopapillary tumors, pancreatoblastomas, undifferentiated carcinomas with osteoclastic-like giant cells and acinar cell carcinomas retained expression of Dpc4, although expression in some cases was weak. Aberrant nuclear expression of beta-catenin is common in nonductal neoplasms, specifically in solid pseudopapillary tumor (88%) and pancreatoblastoma (100%) but rarely seen in ductal adenocarcinoma (<5%). Expression of PSCA and mesothelin was observed in nonductal neoplasms, but their expression was seen less frequent (0–50%) and weaker than that in pancreatic ductal adenocarcinoma (60% for PSCA and 100% for mesothelin). CK19, a marker of ductal adenocarcinomas, was not expressed in nonductal neoplasms, suggesting that this marker could be useful in establishing a diagnosis. Therefore, expression profile of most markers in pancreatic nonductal neoplasms is very different from that in pancreatic ductal adenocarcinomas though frequent expression of gamma-synuclein and stromal markers (fascin, hsp47, and fibronectin) is seen in both.

In summary, we used high-throughput tissue microarrays to study expression of a panel of pancreatic ductal adenocarcinoma-associated tumor markers in a series of nonductal neoplasms. Our results confirmed that nonductal pancreatic neoplasms have distinctive genetic pathways from the more common pancreatic ductal adenocarcinomas, and distinctive patterns of protein expression.

Acknowledgements

This work was supported by the NIH SPORE (Specialized Programs of Research Excellence) in Gastrointestinal Cancer Grant CA62924, the Michael Rolfe Foundation for Pancreatic Cancer Research, and the Goldman Family.

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