

# Carboxypeptidase A1 (CPA1) Immunohistochemistry Is Highly Sensitive and Specific for Acinar Cell Carcinoma (ACC) of the Pancreas

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**Abstract:** Carboxypeptidase A1 (CPA1) is a zinc metalloprotease that is produced in pancreatic acinar cells and plays a role in cleaving C-terminal branched-chain and aromatic amino acids from dietary proteins. This study assessed the utility of immunohistochemical CPA1 staining for diagnosing pancreatic acinar cell carcinoma (ACC). A total of 12,274 tumor samples from 132 different tumor types and subtypes as well as 8 samples each of 76 different normal tissue types were interpretable by immunohistochemistry in a tissue microarray format. CPA1 was strongly expressed in acinar cells of all normal pancreas samples but not in any other normal tissues. CPA1 immunostaining was detected in 100% of 11 pancreatic ACCs and 1 mixed acinar endocrine carcinoma, but absent in 449 pancreatic ductal adenocarcinomas, 75 adenocarcinomas of the ampulla Vateri, and 11,739 other evaluable cancers from 128 different tumor entities. A weak to moderate diffuse staining of epithelial and stromal cells of cancer tissues immediately adjacent to non-neoplastic pancreatic acinar cells often occurred and was considered to be caused by the diffusion of the highly abundant CPA1 from normal acinar cells that

may have suffered some autolytic cell damage. In conclusion, our data show that CPA1 is a highly sensitive and largely specific marker for normal and neoplastic pancreatic acinar cells. CPA1 immunohistochemistry greatly facilitates the otherwise often difficult diagnosis of pancreatic ACC.

**Key Words:** carboxypeptidase A1, immunohistochemistry, tissue micro array, acinar cell carcinoma, pancreas

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Acinar cell carcinoma (ACC) of the pancreas represents a rare cancer type derived from pancreatic acinar cells that accounts for <2% of all pancreatic neoplasms.<sup>1</sup> Most ACCs occur as pure ACCs, but mixed carcinomas with >25% of additional cell types also occur and include mixed acinar-neuroendocrine carcinoma (MAEC) and mixed acinar-ductal carcinoma.<sup>2</sup> A recent analysis of 57,804 pancreatic cancer patients who underwent surgical resection showed a median overall survival (mOS) of 67.5 months and 51% 5-year OS for ACC.<sup>3</sup> In case reports, OS reached up to 123 months.<sup>4</sup> Accordingly, the prognosis of pancreatic ACC is markedly better than the 22% 5-year OS of resected ductal adenocarcinoma but significantly worse than the 84% 5-year OS of neuroendocrine tumors (NETs) of the pancreas.<sup>3</sup> Considerable differences in the molecular alterations seen between these tumor entities are likely to be responsible for marked differences in cancer aggressiveness and also in response to chemotherapy.<sup>5</sup> A precise distinction of these pancreatic tumor entities by the pathologist is thus of high importance.

Due to its relatively broad morphologic spectrum caused by some morphologic and immunohistochemical overlap with NETs, ACC can represent a difficult diagnosis for pathologists, and false diagnoses do occur both on histologic and cytologic samples.<sup>6–8</sup> The correct diagnosis in

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cases with nonprototypic morphology requires the use of immunohistochemical markers such as trypsin, chymotrypsin, BCL-10, cytokeratin 19, and cytokeratin 7.<sup>9</sup> However, none of these antibodies are completely sensitive and specific for ACC.<sup>10,11</sup> Carboxypeptidase A1 (CPA1) is a zinc metalloprotease coded by the CPA1 gene located at 7q32.2. It is a 34.6 kDa protein which is produced in the pancreatic acinar cells. It is involved in zymogen inhibition and was shown to preferentially cleave C-terminal branched-chain and aromatic amino acids from dietary proteins.<sup>12</sup> Mutations of CPA1 gene have been linked to chronic pancreatitis.<sup>13,14</sup> Elevated CPA1 serum protein levels have been described in patients with pancreatic cancer.<sup>15</sup> CPA1 immunohistochemistry may be suited for facilitating a safe diagnosis of ACC. In a recent study, analyzing 14 ACC and 5 MAEC as well as 80 nonacinar pancreatic tumors, Said et al<sup>16</sup> found a 100% sensitivity and a 95% specificity of a positive CPA1 immunostaining for ACC.

To further assess the potential of CPA1 immunostaining for securing the diagnosis of pancreatic ACC an extensive survey of CPA1 immunostaining in nonpancreatic tumor types is needed. In this study, CPA1 expression was thus analyzed in 15,680 tumor tissue samples from 132 different tumor types and subtypes as well as 76 different non-neoplastic tissue types by immunohistochemistry in a tissue microarray (TMA) format.

## MATERIALS AND METHODS

### Tissue Microarrays

Preexisting TMAs, which have been used in several previous studies<sup>17–20</sup> were also used for this study. Our normal tissue TMA was composed of 8 samples from 8 different donors for each of 76 different normal tissue types (608 samples on 1 slide). The cancer TMAs contained a total of 15,680 primary tumors from 132 tumor types and subtypes. The composition of both normal and cancer TMAs is described in detail in the Results section. Our “pancreatic heterogeneity TMA” contained up to 9 samples (mean: 6.7) each from 224 pancreatic ductal adenocarcinomas that were taken from 3 to 9 (mean: 5.5) available tumor containing tissue blocks per patient. These tumors partially overlapped with the set analyzed in the primary tumor TMAs. All samples were from the archives of the Institutes of Pathology, University Hospital of Hamburg, Germany, the Institute of Pathology, Clinical Center Osnabrueck, Germany, and Department of Pathology, Academic Hospital Fuerth, Germany. Tissues were fixed in 4% buffered formalin and then embedded in paraffin. Decalcification was not performed on any tissue samples. Time of tissue acquisition to fixation and fixation duration was not standardized. TMA tissue spot diameter was 0.6 mm. The use of archived remnants of diagnostic tissues for manufacturing of TMAs and their analysis for research purposes as well as patient data analysis has been approved by local laws (HmbKHG, §12) and by the local ethics committee (Ethics commission Hamburg, WF-049/09). All work has been carried out in compliance with the Helsinki Declaration.

### Immunohistochemistry

Freshly cut TMA sections were immunostained on 1 day and in 1 experiment. Slides were deparaffinized with xylol, rehydrated through a graded alcohol series, and exposed to heat-induced antigen retrieval for 5 minutes in an autoclave at 121°C in pH 7.8 DakoTarget Retrieval Solution (Agilent, California; #S2367). Endogenous peroxidase activity was blocked with Dako Peroxidase Blocking Solution (Agilent; #52023) for 10 minutes. A primary antibody specific for CPA1 (mouse monoclonal, MSVA-601M, MS Validated Antibodies, Hamburg, Germany, ms-validatedantibodies.com) was applied at 37°C for 60 minutes at a dilution of 1:150. Bound antibody was then visualized using the EnVision Kit (Agilent; #K5007) according to the manufacturer's directions. The sections were counterstained with haemalaun. One pathologist (S.M.) evaluated all TMA slides. For normal tissues, the immunostaining staining intensity of positive cells was semiquantitatively recorded (+, ++, +++). For tumor tissues, the percentage of positive neoplastic cells was estimated, and the immunostaining staining intensity was semiquantitatively recorded (0, 1+, 2+, 3+). For statistical analyses, the staining results were categorized into 4 groups. Tumors without any staining were considered negative. Tumors with 1+ staining intensity in  $\leq 70\%$  of cells and 2+ intensity in  $\leq 30\%$  of cells were considered weakly positive. Tumors with 1+ staining intensity in  $> 70\%$  of cells, 2+ intensity in 31% to 70%, or 3+ intensity in  $\leq 30\%$  were considered moderately positive. Tumors with 2+ intensity in  $> 70\%$  or 3+ intensity in  $> 30\%$  of cells were considered strongly positive.

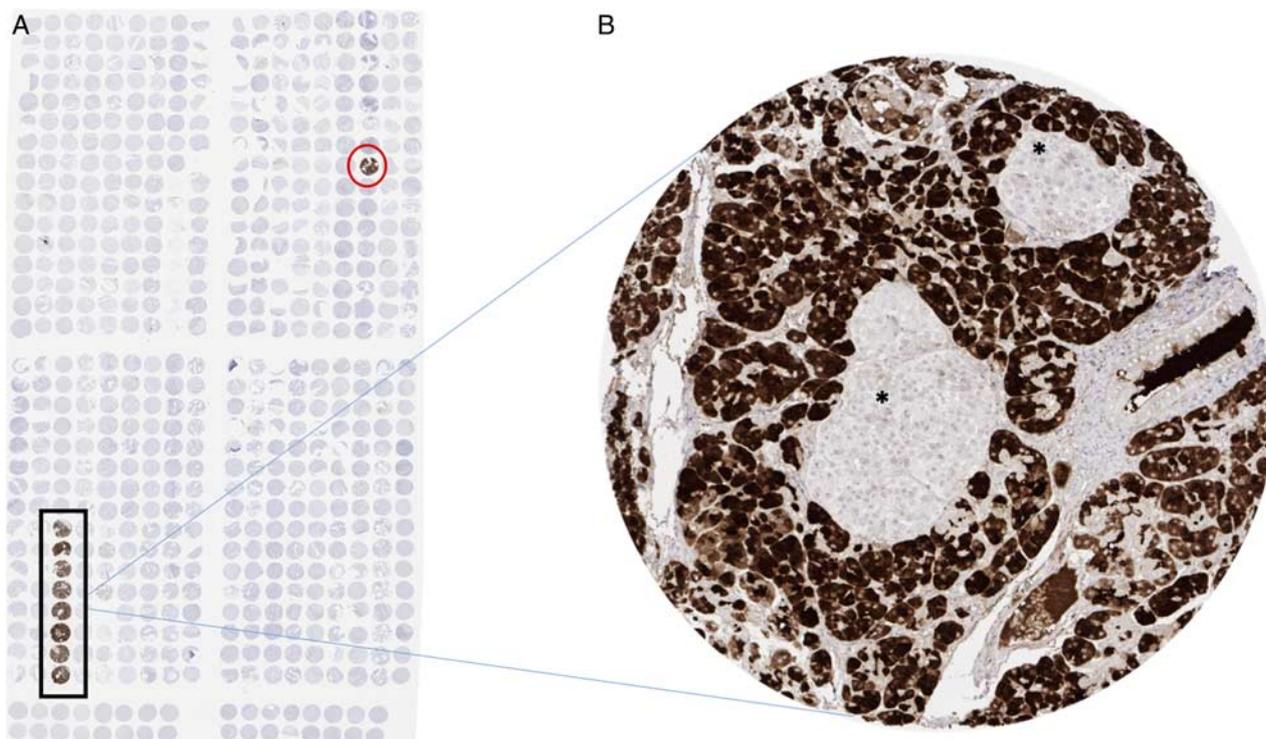
## RESULTS

### Technical Issues

A total of 12,274 (78.3%) of 15,680 tumor samples were interpretable in our TMA analysis. Noninterpretable samples demonstrated a lack of unequivocal tumor cells or loss of the tissue spots during technical procedures. At least 4 samples of each normal tissue type were evaluable.

### CPA1 in Normal Tissues

A strong (3+) cytoplasmatic CPA1 staining was only found in the pancreas where staining was most intense in acinar cells (Fig. 1). However, in cases with particularly strong acinar cell positivity, some fading of the staining into adjacent other cell types was occasionally observed. CPA1 staining was completely absent in all analyzed extrapancreatic tissues including skeletal muscle, heart muscle, smooth muscle, myometrium of the uterus, corpus spongiosum of the penis, ovarian stroma, fat, skin (including hair follicle and sebaceous glands), oral mucosa of the lip, oral cavity, surface epithelium of the tonsil, and transitional mucosa of the anal canal, ectocervix, squamous epithelium of the esophagus, urothelium of the renal pelvis and urinary bladder, decidua, placental trophoblastic cells, lymph node, spleen, thymus, tonsil, mucosa of the stomach, duodenum, ileum, appendix, colon, rectum and gall bladder, liver, parotid gland, submandibular gland, sublingual gland, Brunner gland of the duodenum, cortex and medulla of the kidney, prostate, seminal vesicle, epididymis, testis, respiratory epithelium and glands of



**FIGURE 1.** CPA1 immunostaining in normal tissues. A, Overview of the normal tissue microarray. Eight spots of acinar parenchyma in 1 row show a strong CPA1-staining (black rectangular). In the row of thymic tissue, acinar parenchyma has been mistakenly used (red circle). None of the other normal tissues show any positive CPA1-staining. B, Enlargement of one tissue microarray spot showing strong CPA1-staining in acinar parenchyma of the pancreas. Absent staining in Islets of Langerhans\*.

bronchi and sinus paranasales, lung, breast, endocervix, endometrium, fallopian tube, corpus luteum and follicular cyst of the ovary, adrenal gland, parathyroid gland, cerebellum, cerebrum, and pituitary gland.

### CPA1 in Neoplastic Tissues

A strong cytoplasmic CPA1 immunostaining was observed in 6 of 6 previously diagnosed ACC (Fig. 2). A strong CPA1 immunostaining was also seen in 5 tumors initially classified as NET (G3; n=1), neuroendocrine carcinoma (NEC; n=2) or adenocarcinoma of the pancreas (n=2), and in 1 tumor initially classified as NEC, large cell variant. However, after comprehensive histologic reevaluation by 4 pathologists (S.M., G.S., T.S.C., and F.J.), these tumors were subsequently reclassified as pancreatic ACC and mixed acinar endocrine carcinoma (MAEC) (see the Discussion section). CPA1 immunostaining was completely absent in all other 12,262 evaluable tumors from 130 different tumor types and subtypes (Table 1). This included 128 evaluable acinic cell carcinomas derived from salivary glands.

### CPA1 Heterogeneity Analyses

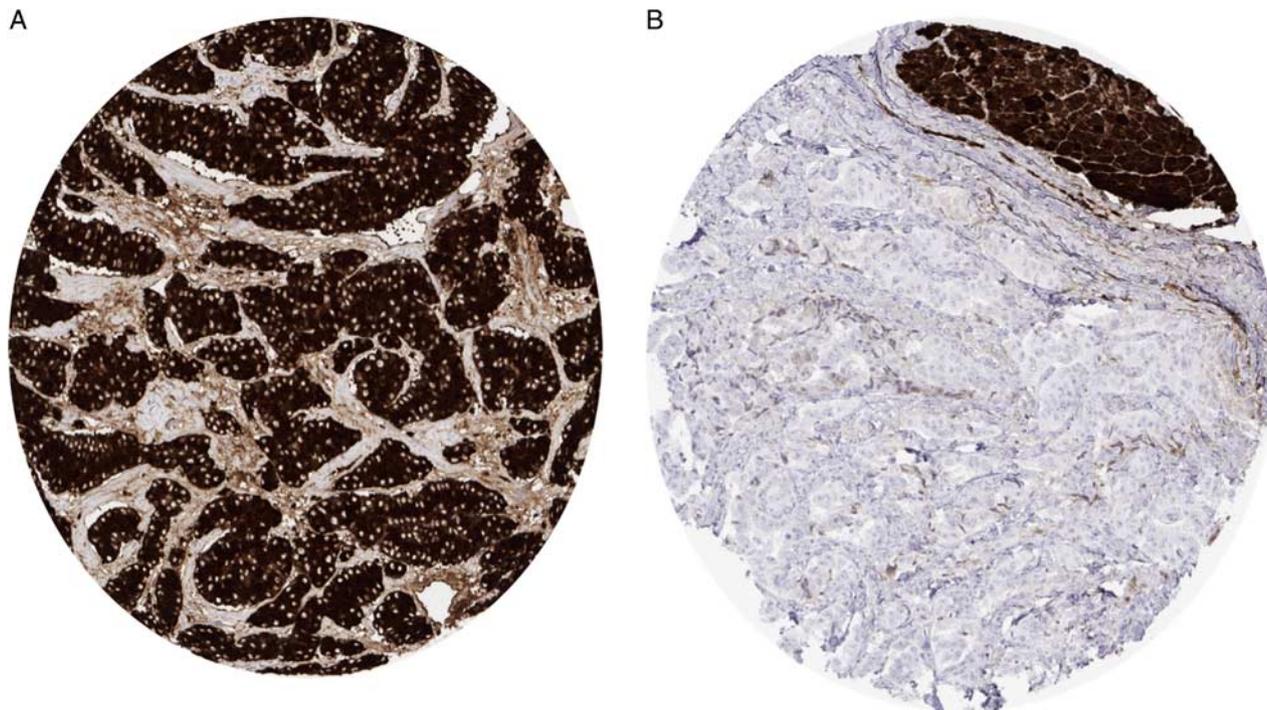
In an attempt to identify focal CPA1 positivity in pancreatic tumors, 17 large sections of ductal adenocarcinomas and 20 large section of NETs were analyzed as well as a heterogeneity TMA containing up to 9 samples from 224 pancreatic ductal adenocarcinomas. In 219 of 224 primary tumors a minimum of 2 of 6 samples were evaluable (a minimum of 3 spots were evaluable in 206,  $\geq 4$  in 182,  $\geq 5$  in 134

and all 6 in 56 primary tumors). The range of evaluable samples was 0 to 6 for primary tumors and 0 to 3 for metastasis. A strong CPA1 immunostaining was found in 1 ACC (case #5, see above). In the remaining 223 tumors CPA1 staining was not observed in any tumor cells of these samples. In several instances, however, a weak to moderate CPA1 staining was seen immediately adjacent to highly CPA1 expressing normal pancreatic tissue that equally involved tumor and stromal cells. This staining was considered to reflect diffusion of highly abundant CPA1 from normal acinar cells that may have suffered some autolytic cell damage.

### DISCUSSION

The results of this study demonstrate a close to perfect sensitivity and specificity of anti-CPA1 clone MSVA-601M for diagnosing pancreatic ACC.

According to The International Working Group for Antibody Validation (IWGAV), suitable validation strategies for immunohistochemistry on formalin-fixed tissues include comparison with expression data obtained by another independent method.<sup>21</sup> This is particularly straightforward for CPA1, because 3 independent RNA screening studies, including the FANTOM5 project,<sup>22,23</sup> the Genotype-Tissue Expression (GTEx) project,<sup>24</sup> and The Cancer Genome Atlas Program (TCGA)<sup>25</sup> (which are all summarized in the protein atlas<sup>26</sup>), demonstrate that CPA1 expression is strictly limited to pancreatic tissue. This is in perfect agreement with our immunohistochemistry data.



**FIGURE 2.** CPA1 immunostaining in tumors. A, Strong positive CPA1 staining in acinar cell carcinoma. B, Absent CPA1 staining in a neuroendocrine tumor of the pancreas. Positive staining in non-neoplastic acinar parenchyma of the pancreas.

A very high expression of CPA1 in pancreatic tissue is confirmed by our normal tissue analysis which in addition localizes the CPA1 protein to acinar cells and suggests that other cell types such as Islets of Langerhans and excretory ducts do not express CPA1 at measurable levels. The limitation of CPA1 expression to acinar cells is consistent with the function of CPA1, which is among the most important secretory enzymes.<sup>27</sup> The strict limitation of CPA1 expression to all cases of pancreatic acinar cells obtained from 8 independent donors in our extensive normal tissue analysis demonstrates that CPA1 expression is indeed limited to this cell type and validates specificity and sensitivity of MSVA-601M. It is of note that negative results from RNA screening databases cannot rule out CPA1 expression in structures constituting only small fractions of the total cells of an organ. RNAs derived from small structures or rare cell types are largely under-represented and thus potentially missed in RNA analyses. The absence of CPA1 immunostaining in any other normal tissue samples also argues against the potential cross-reactivity of our antibody in formalin-fixed tissues. Notably, the analysis of 76 different tissue categories ensures that a very large fraction of proteins that are being expressed in cells of adult humans have been exposed to the antibody and tested for cross-reactivity in this study.

The analysis of 12,274 human tumors, including samples from 132 different tumor types and subtypes, resulted in a similar “black and white” picture as seen in our normal tissue analysis and identified 12 strongly positive carcinomas and 12,262 entirely negative tumors. It is remarkable that 6 of these 12 CPA1 positive cancers had

initially not been classified as ACC. Due to the high level of CPA1 expression in normal pancreatic acinar cells, the absolute specificity of our assay for pancreatic acinar cells among normal tissues, and the fact that these tumors were all located in the pancreas and showed high-level CPA1 expression, we challenged our initial diagnosis in these cases. In retrospect, 3 of these tumors (case #1, #5, and #6) were reclassified as definite ACC, since apart from CPA1 positivity, the tumors fulfilled at least some of the “classic” criteria for diagnosing an ACC. Two tumors (case #2 and #3) were reclassified as “probably ACC—see comment,” since these tumors apart from a strong CPA1 staining, did not fulfill the “classic” histomorphologic criteria, showed a focal weak or absent chymotrypsin immunostaining and a focal synaptophysin immunostaining. One tumor (case #4), initially classified as NEC, large cell variant, was newly diagnosed as probably MAEC, since parts of the tumor showed the typical morphology of a NEC (large cell variant) and part of the tumor showed moderate to strong CPA1 staining. Morphologic and immunohistochemical results of these tumors are shown in Figure 3. Reclassification of these tumors is not surprising, since ACCs can show several architectural patterns apart from the “classic” acinar pattern. The fact, that 5 of 11 ACCs and 1 mixed acinar endocrine carcinoma were initially not recognized reflects the well-known difficulties in correctly diagnosing this rare pancreatic cancer subtype. Even “experts” in gastrointestinal pathology will only rarely come across these tumors. In a retrospective study at John Hopkins only 14 ACCs were identified over a period of 18 years.<sup>28</sup> Several other studies have emphasized on the difficulties in recognizing this rare subtype of

TABLE 1. CPA1 Immunostaining in Tumors

Tumor Entity		CPA1 Immunostaining					
		On TMA (n)	Analyzable (n)	Negative (%)	Weak (%)	Moderate (%)	Strong (%)
Tumors of the skin	Pilomatrixoma	35	32	100	0	0	0
	Basal cell carcinoma	88	56	100	0	0	0
	Benign nevus	29	26	100	0	0	0
	Squamous cell carcinoma of the skin	90	82	100	0	0	0
	Malignant melanoma	48	44	100	0	0	0
	Merkel cell carcinoma	46	44	100	0	0	0
Tumors of the head and neck	Squamous cell carcinoma of the larynx	110	96	100	0	0	0
	Squamous cell carcinoma of the pharynx	60	46	100	0	0	0
	Oral squamous cell carcinoma (floor of the mouth)	130	118	100	0	0	0
	Pleomorphic adenoma of the parotid gland	50	44	100	0	0	0
	Warthin tumor of the parotid gland	104	91	100	0	0	0
	Adenocarcinoma, not otherwise specified (NOS) (papillary cystadenocarcinoma)	14	12	100	0	0	0
	Salivary duct carcinoma	15	12	100	0	0	0
	Acinic cell carcinoma of the salivary gland	181	128	100	0	0	0
	Adenocarcinoma NOS of the salivary gland	109	69	100	0	0	0
	Adenoid cystic carcinoma of the salivary gland	180	99	100	0	0	0
	Basal cell adenocarcinoma of the salivary gland	25	21	100	0	0	0
	Basal cell adenoma of the salivary gland	101	86	100	0	0	0
	Epithelial-myoepithelial carcinoma of the salivary gland	53	51	100	0	0	0
	Mucoepidermoid carcinoma of the salivary gland	343	239	100	0	0	0
	Myoepithelial carcinoma of the salivary gland	21	20	100	0	0	0
	Myoepithelioma of the salivary gland	11	9	100	0	0	0
	Oncocytic carcinoma of the salivary gland	12	12	100	0	0	0
	Polymorphous adenocarcinoma, low grade, of the salivary gland	41	32	100	0	0	0
	Pleomorphic adenoma of the salivary gland	53	32	100	0	0	0
Tumors of the lung, pleura, and thymus	Adenocarcinoma of the lung	246	160	100	0	0	0
	Squamous cell carcinoma of the lung	130	65	100	0	0	0
	Small cell carcinoma of the lung	20	16	100	0	0	0
	Mesothelioma, epitheloid	39	32	100	0	0	0
	Mesothelioma, other types	76	63	100	0	0	0
	Thymoma	29	29	100	0	0	0
Tumors of the female genital tract	Squamous cell carcinoma of the vagina	78	63	100	0	0	0
	Squamous cell carcinoma of the vulva	130	116	100	0	0	0
	Squamous cell carcinoma of the cervix	130	124	100	0	0	0
	Endometrioid endometrial carcinoma	236	223	100	0	0	0
	Endometrial serous carcinoma	82	72	100	0	0	0
	Carcinosarcoma of the uterus	48	38	100	0	0	0
	Endometrial carcinoma, high grade, G3	13	13	100	0	0	0
	Endometrial clear cell carcinoma	8	7	100	0	0	0
	Endometrioid carcinoma of the ovary	110	91	100	0	0	0
	Serous carcinoma of the ovary	559	462	100	0	0	0
	Mucinous carcinoma of the ovary	96	71	100	0	0	0
Clear cell carcinoma of the ovary	50	40	100	0	0	0	
Carcinosarcoma of the ovary	47	38	100	0	0	0	
Brenner tumor	9	9	100	0	0	0	
Tumors of the breast	Invasive breast carcinoma of no special type	1391	1185	100	0	0	0
	Lobular carcinoma of the breast	294	236	100	0	0	0
	Medullary carcinoma of the breast	26	26	100	0	0	0
	Tubular carcinoma of the breast	27	26	100	0	0	0
	Mucinous carcinoma of the breast	58	44	100	0	0	0
	Phyllodes tumor of the breast	50	47	100	0	0	0
Tumors of the digestive system	Adenomatous polyp, low-grade dysplasia	50	49	100	0	0	0
	Adenomatous polyp, high-grade dysplasia	50	49	100	0	0	0
	Adenocarcinoma of the colon	956	721	100	0	0	0
	Gastric adenocarcinoma, diffuse type	226	129	100	0	0	0
	Gastric adenocarcinoma, intestinal type	224	134	100	0	0	0
	Gastric adenocarcinoma, mixed type	62	47	100	0	0	0
	Adenocarcinoma of the esophagus	133	60	100	0	0	0
	Squamous cell carcinoma of the esophagus	124	42	100	0	0	0
	Squamous cell carcinoma of the anal canal	91	78	100	0	0	0
	Cholangiocarcinoma	114	108	100	0	0	0
Hepatocellular carcinoma	50	50	100	0	0	0	

TABLE 1. (continued)

	Tumor Entity	CPA1 Immunostaining					
		On TMA (n)	Analyzable (n)	Negative (%)	Weak (%)	Moderate (%)	Strong (%)
	Ductal adenocarcinoma of the pancreas	662	449	100	0	0	0
	Pancreatic/Ampullary adenocarcinoma	119	75	100	0	0	0
	Acinar cell carcinoma of the pancreas	11	11	0	0	18.2	81.8
	Mixed acinar endocrine carcinoma of the pancreas	1	1	0	0	0	100
Tumors of the urinary system	Gastrointestinal stromal tumor (GIST)	50	49	100	0	0	0
	Urothelial carcinoma, pT2-4 G3	1207	613	100	0	0	0
	Small cell neuroendocrine carcinoma of the bladder	18	18	100	0	0	0
	Sarcomatoid urothelial carcinoma	25	24	100	0	0	0
	Clear cell renal cell carcinoma	858	759	100	0	0	0
	Papillary renal cell carcinoma	255	208	100	0	0	0
	Clear cell (tubulo) papillary renal cell carcinoma	21	19	100	0	0	0
	Chromophobe renal cell carcinoma	131	118	100	0	0	0
	Oncocytoma	177	147	100	0	0	0
	Tumors of the male genital organs	Adenocarcinoma of the prostate, Gleason 3+3	83	79	100	0	0
Adenocarcinoma of the prostate, Gleason 4+4		80	73	100	0	0	0
Adenocarcinoma of the prostate, Gleason 5+5		85	80	100	0	0	0
Adenocarcinoma of the prostate (recurrence)		261	231	100	0	0	0
Small cell neuroendocrine carcinoma of the prostate		17	16	100	0	0	0
Tumors of endocrine organs	Seminoma	621	444	100	0	0	0
	Embryonal carcinoma of the testis	50	39	100	0	0	0
	Yolk sack tumor	50	32	100	0	0	0
	Teratoma	50	44	100	0	0	0
	Squamous cell carcinoma of the penis	80	66	100	0	0	0
	Adenoma of the thyroid gland	114	108	100	0	0	0
	Papillary thyroid carcinoma	392	361	100	0	0	0
	Follicular thyroid carcinoma	158	147	100	0	0	0
	Medullary thyroid carcinoma	107	100	100	0	0	0
	Anaplastic thyroid carcinoma	45	43	100	0	0	0
	Adrenal cortical adenoma	50	44	100	0	0	0
	Adrenal cortical carcinoma	26	26	100	0	0	0
	Phaeochromocytoma	50	50	100	0	0	0
	Appendix, neuroendocrine tumor (NET)	22	12	100	0	0	0
	Colorectal, NET	11	10	100	0	0	0
	Ileum, NET	49	46	100	0	0	0
	Lung, NET	19	17	100	0	0	0
Pancreas, NET	98	87	100	0	0	0	
Colorectal, NEC	12	10	100	0	0	0	
Gallbladder, NEC	4	4	100	0	0	0	
Pancreas, NEC	14	14	100	0	0	0	
Tumors of hematopoietic and lymphoid tissues	Hodgkin lymphoma	103	76	100	0	0	0
	Non-Hodgkin lymphoma	62	54	100	0	0	0
	Small lymphocytic lymphoma, B-cell type (B-SLL/B-CLL)	50	30	100	0	0	0
	Diffuse large B-cell lymphoma (DLBCL)	114	94	100	0	0	0
	Follicular lymphoma	88	63	100	0	0	0
	T-cell non-Hodgkin lymphoma	24	16	100	0	0	0
	Mantle cell lymphoma	18	13	100	0	0	0
	Marginal zone lymphoma	16	10	100	0	0	0
	DLBCL in the testis	16	13	100	0	0	0
	Burkitt lymphoma	5	1	100	0	0	0
Tumors of soft tissue and bone	Tenosynovial giant cell tumor	45	44	100	0	0	0
	Granular cell tumor	53	44	100	0	0	0
	Leiomyoma	50	48	100	0	0	0
	Leiomyosarcoma	87	84	100	0	0	0
	Liposarcoma	132	129	100	0	0	0
	Malignant peripheral nerve sheath tumor (MPNST)	13	11	100	0	0	0
	Myofibrosarcoma	26	26	100	0	0	0
	Angiosarcoma	73	66	100	0	0	0
	Angiomyolipoma	91	91	100	0	0	0
	Dermatofibrosarcoma protuberans	21	18	100	0	0	0
	Ganglioneuroma	14	13	100	0	0	0
	Kaposi sarcoma	8	6	100	0	0	0
	Neurofibroma	117	96	100	0	0	0

TABLE 1. (continued)

Tumor Entity	CPA1 Immunostaining					
	On TMA (n)	Analyzable (n)	Negative (%)	Weak (%)	Moderate (%)	Strong (%)
Sarcoma, NOS	75	59	100	0	0	0
Paraganglioma	41	37	100	0	0	0
Primitive neuroectodermal tumor (PNET)	23	18	100	0	0	0
Rhabdomyosarcoma	7	7	100	0	0	0
Schwannoma	121	106	100	0	0	0
Synovial sarcoma	12	11	100	0	0	0
Osteosarcoma	43	35	100	0	0	0
Chondrosarcoma	38	17	100	0	0	0

All reclassified cases are included in this table. All positive cases are highlighted in gray.

pancreatic neoplasm.<sup>6,7</sup> In a study by Basturk et al<sup>6</sup> 17 of 107 tumors initially diagnosed as poorly differentiated NECs were reevaluated and in retrospect diagnosed as pure ACC or mixed acinar-NEC.

Our data strongly suggest that CPA1 immunostaining should be broadly applied to newly diagnosed pancreatic tumors with “equivocal morphology” (ie, pancreatic tumors lacking a “classic” acinar pattern, without distinct PAS-positive cytoplasmatic zymogen granules and a disputable chymotrypsin staining) and that this examination has the potential to prevent relevant diagnostic errors. The high sensitivity for ACC detection seen in our study fits well with the findings with a recent study on CPA1 expression in pancreatic cancers where Said et al<sup>16</sup> described strong diffuse CPA1 positivity in all 14 cases of ACC and 5 cases of MAEC

analyzed. These authors, however, described a mostly focal or patchy CPA1 positivity to also occur in 2 of 20 ductal adenocarcinomas, 10 of 20 NETs, 2 of 20 mucinous cystic neoplasms, and 1 of 20 solid pseudopapillary tumor but still found a 95% specificity of CPA1 positivity for ACC if only patchy and diffuse staining was considered. While it cannot be excluded that some CPA1 positive non-ACCs also reflect diagnostic borderline cases in this study, it appears more likely that differences in the antibodies and protocols used for these studies are responsible for the higher rate of CPA1 positive non-ACCs. Our reclassification of tumors could be interpreted as a weakness of our study since this obviously could account for the high specificity of the marker. A comparison of the specificity of CPA 1 immunostaining before (99.5%) and after (100%) reclassification shows,

	Example of a “straightforward” acinar cell carcinoma	Case #1	Case #2 *	Case #3 *	Case #4 **	Case #5	Case #6
Hematoxylin eosin staining							
Chymotrypsin immunostaining							
Synaptophysin immunostaining							
Initial diagnosis	Acinar cell carcinoma	Ductal Adenocarcinoma of the pancreas (G3)	Neuroendocrine Tumor (NET, G3)	Neuroendocrine Carcinoma, large cell variant (NEC)	Neuroendocrine Carcinoma, large cell variant (NEC)	Adenocarcinoma of the pancreas	Neuroendocrine Carcinoma (NEC)
CPA1 immunostaining							
Final diagnosis	Acinar cell carcinoma	Acinar cell carcinoma	probably Acinar cell carcinoma – see comment*	probably Acinar cell carcinoma – see comment*	probably Mixed acinar-endocrine carcinoma (MAEC) – see comment*	Acinar cell carcinoma	Acinar cell carcinoma

\*Comment: Case #2 and Case #3 do not fulfill the “classic” criteria for an acinar cell carcinoma, but since they show a strong CPA1 immunostaining we would favour the diagnosis acinar cell carcinoma. \*\*Comment: Case #4 shows the typical morphology of a neuroendocrine carcinoma (large cell variant). Since parts of the tumor show moderate to strong CPA1 staining, we would favour the diagnosis mixed acinar-endocrine carcinoma (MAEC).

FIGURE 3. Morphologic and immunohistochemical findings of 6 cancers reclassified in this study.

however, that the specificity was not markedly impacted. The sensitivity is obviously unaffected and remains 100%.

It is of note that both the staining of large sections of 20 NETs and 17 ductal adenocarcinomas and the analysis of a pancreatic adenocarcinoma heterogeneity TMA containing up to 9 samples retrieved from different tumor blocks of 224 pancreatic cancers provided no evidence for even a minimal focal CPA1 immunostaining in non-ACCs. A weak CPA1 staining that involved both tumor cells and stroma cells that was occasionally seen immediately adjacent to highly CPA1 expressing normal pancreatic tissue was considered to be caused by diffusion of the highly abundant CPA1 from normal acinar cells that may have suffered some autolytic cell damage.

In summary, our data show the outstanding specificity of CPA1 immunostaining for acinar differentiation in the pancreas to such an extent that we suggest to include CPA1 immunostaining when faced with a pancreatic tumor with “equivocal morphology.” The data also demonstrate the strength of a large-scale tissue screening approach for clarifying the specificity and diagnostic utility of antibodies.

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