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Original Article

BCL10 as a useful marker for pancreatic acinar cell carcinoma, especially using endoscopic ultrasound cytology specimens

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Acinar cell carcinomas (ACCs) of the pancreas are characterized by the histological and immunohistochemical features of acinar cell differentiation. Recently, BCL10, originally identified as a recurrent t(1;14)(p22;q32) translocation in MALT B-cell lymphoma, was found to be immunohistochemically positive in some solid tumors, including ACC. To evaluate its diagnostic efficacy, we performed BCL10 immunohistochemistry and evaluated molecular markers correlated to pancreatic tumor lineages (neuroendocrine markers and a mutation analysis of *KRAS* and *GNAS*) using samples from 126 pancreatic tumors (17 ACCs, 24 pancreatic ductal adenocarcinomas, 4 adenosquamous carcinomas, 9 intraductal papillary mucinous neoplasms, 10 mucinous cystic neoplasms, 44 neuroendocrine tumors, 9 serous cystic tumors and 10 solid-pseudopapillary neoplasms). BCL10 was exclusively expressed in normal acini. In pancreatic tumors, 14 of 17 (82%) ACCs and 2 of 4 (50%) adenosquamous carcinomas were positive, while the other subtypes were almost negative. We subsequently examined the diagnostic utility of BCL10 in endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) specimens using 57 pancreatic tumors. BLC10 correctly identified ACCs (9/13) and adenosquamous carcinomas (2/4) but none of the other subtypes (n = 41). Therefore, we suggested that BCL10 expression is a useful marker for acinar cell differentiation, particularly in the diagnosis of EUS-FNA specimens.

Key words: acinar cell carcinoma, BCL10, differential diagnosis, immunohistochemistry, pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDA) is a major subtype of pancreatic cancer that is followed by neuroendocrine tumors. These two major subtypes represent the cellular composition of the pancreas. Although most other neoplasms are associated with ductal epithelial differentiation, i.e. intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs), some tumors show acinar features. Pancreatic acinar cell carcinoma (ACC) is a tumor characterized by acinar cell features and comprises less than 2% of all pancreatic neoplasms.[1,2] This subtype is clinically and pathologically distinct from typical PDAs. The median age of the patients with ACC is slightly older than PDA, and ACC shows a better prognosis despite more frequent distant metastasis. Histologically, ACC has characteristic acinar differentiation that is illustrated by immunohistochemical staining for trypsin, chymotrypsin, and/or lipase.[3–5] Furthermore, ACCs have been reported to lack a *KRAS* mutation, suggesting that they develop via different molecular pathways than PDAs. Abnormalities in tumor suppressor genes, such as *TP53*, *DPC4/Smad4* and *p16*, are less common than in PDAs.[3,6–9]

BCL10 was recently identified through the cloning of a (1;14)(p22;q32) translocation breakpoint in several cases of low-grade mucosa-associated lymphoid tissue (MALT) B-cell lymphoma.[10,11] This gene, localized to chromosome band 1p22, is a cellular homolog of the equine herpesvirus-2 E10 gene; both contain an amino-terminal caspase recruitment domain (CARD) homologous to that found in several apoptotic molecules.[12] Mutation analyses of BCL10 have implicated this gene in MALT lymphomas and other lymphoid tumors of the B- or T-cell lineage without t(1;14)(p22;q32) translocation.[13–15] In addition, BCL10 abnormalities have been reported in solid cancer cell lines and tumors, including malignant mesotheliomas, germ cell tumors, and colon carcinomas, suggesting that BCL10 can contribute to the pathogenesis of several types of neoplasia.[10,16] In contrast to a number of articles about BCL10 expression in lymphoid

malignancies, only a limited number of articles have documented its expression in solid cancers. Chang *et al*. have reported that BCL10 expression is significantly associated with the progression and prognosis of oral squamous cell carcinomas, while BCL10 has been reported by Kuo *et al*. to play an important role in controlling the growth of cervical cancer cells through NF-κB-dependent cyclin D1 regulation.[17,18] In the pancreas, La Rosa *et al*. recently reported that BCL10 was expressed specifically in acinar cells and acinar cell carcinomas.[5,19]

Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) was introduced into clinical practice in the early 1990s and is now considered one of the most useful methods for the histological diagnosis and staging of pancreatic cancers.[20] However, specimens obtained by EUS-FNA are tiny and fragmented, and a definitive diagnosis is frequently challenging for pathologists. We recently reported a diagnostic scheme for EUS-FNA specimens of three major pancreatic tumor types using a minimal number of markers, including CK7, CDX2, synaptophysin, chromogranin A and *KRAS* mutations.[21] In that study, ACC was characterized by occasional expression of CK7 and CDX2, lack of a *KRAS* mutation, and various expression patterns of neuroendocrine markers. When heterogeneous expression and staining errors were considered, it was determined that some positive markers were required in this panel. In the present study, we found that BCL10 is expressed exclusively in ACC, implying that it could serve as a useful marker for labeling this rare, well-differentiated subtype, particularly when diagnosing EUS-FNA specimens.

MATERIALS AND METHODS

Patients and tissues

A total of 126 pancreatic tumors from 124 patients were selected from the database of the Department of Pathology and Molecular Diagnostics, Aichi Cancer Center Hospital, Nagoya, Japan. This cohort was composed of 17 ACCs, 23 PDAs, 4 adenosquamous carcinomas, 9 IPMNs, 10 MCNs, 44 neuroendocrine tumors (including 41 well-differentiated tumors and three poorly differentiated tumors, according to the WHO classification[2]), 9 serous cystic tumors and 10 solid-pseudopapillary neoplasms. Of these, surgical materials were available in 116 tumors. EUS-FNA procedures were performed in 58 tumors, of which 48 underwent subsequent surgical procedures. All specimens were fixed with formalin and embedded in paraffin. Aspirates were also fixed with formalin and then processed to make cell blocks. This study was a part of a comprehensive research program of the tissue bank in Aichi Cancer Center that had been approved by an institutional review board.

Immunohistochemistry

Immunohistochemistry was performed using an Autostainer Link 48 (Dako, Copenhagen, Denmark) according to the manufacturer's instructions. Anti-BCL10 mouse monoclonal antibody (Clone 331.3; Santa Cruz, San Francisco, CA, USA) was used at a 1200-fold dilution, and anti-trypsin mouse monoclonal antibody (Clone 430; Biodesign/Meridian Life Science, Memphis, TN, USA) was used at a 1000-fold dilution. The antigens were retrieved by PT Link (Dako) for 30 min in a High Buffer Solution (pH 9.0, Dako). The staining patterns of positive BCL10 and trypsin reactions were classified into the four-tiered scoring system: negative; 1+, faint and focal staining (in less than 50% of the total area); 2+, faint but diffuse staining (in more than 50% of the total area) or strong but focal staining (in less than 50% of the total area); and 3+, strong and diffuse staining (in more than 50% of the total area). We evaluated the staining as positive when the tumor cells showed moderate or greater intensity in 50% of the area (2+, or 3+). Detection of synaptophysin and chromogranin A expression has been reported elsewhere.[21]

Mutation analysis

The *KRAS* mutation status of the individual tumors was obtained from our database, and the details of the methods used to detect the mutations have been previously reported.[21,22] For the detection of the *GNAS* mutation, we developed a sensitive method using a cycleave PCR technique similar to that for *KRAS* mutation detection. The details are reported elsewhere (personal communication).

Statistical analysis

Fisher's exact test, a χ^2 test for independence and an unpaired *t*-test were used to compare gene expression between histological subtypes. Logistic regression models were constructed to analyze more complex relationships using the SYSTAT software (SYSTAT Software Inc., Richmond, CA, USA). $P < 0.05$ was considered to be statistically significant.

RESULTS

BCL10 expression of the normal pancreas and pancreatic cancers

We first examined the expression pattern of BCL10 in normal pancreatic tissue. As shown in Fig. 1, BCL10 expression was observed exclusively in the cytoplasm of acinar cells. This

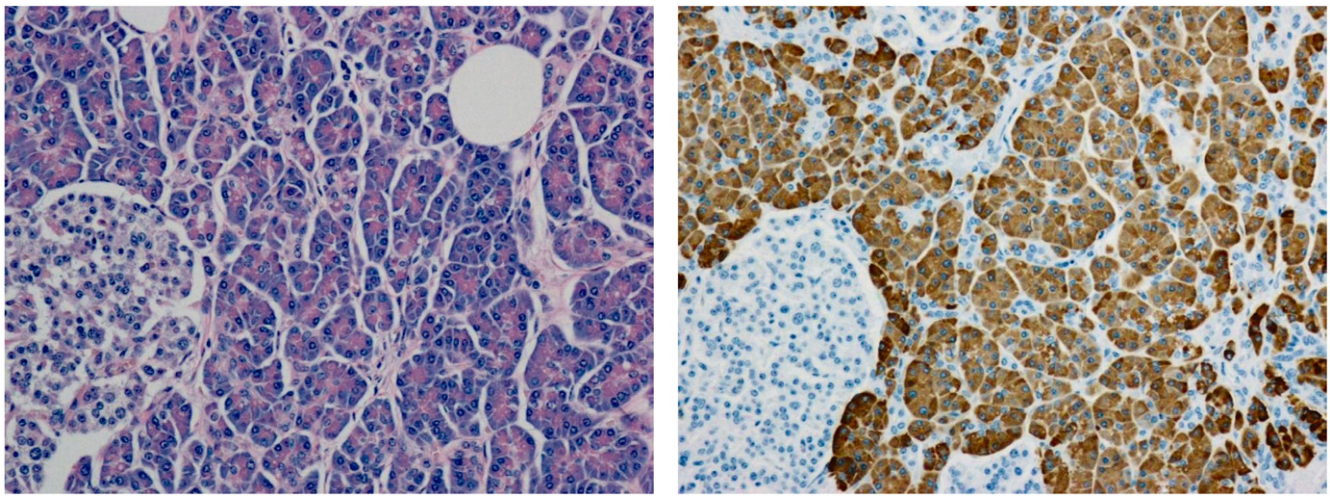

Figure 1 BCL10 expression in the normal pancreas. BCL10 uniformly labels acinar cells but not islet cells.

pattern was highly contrasted to those of CDX2 and CK7 in the ductal epithelial cells from the centroacinar cells to the main pancreatic ducts and the centroacinar cells to the intercalated ducts, respectively, as reported previously.[21] No other cells were positive for BCL10, suggesting that BCL10 could serve as a good marker for acinar cells.

This restricted pattern was also present in the pancreatic cancer tissues. We examined a wide range of pancreatic tumors in which intensely and diffusely positive reactions were detected in the majority of ACCs (14/17, 82%) (Fig. 2). Two of four adenosquamous carcinomas were positive, and the reaction was notably restricted to carcinoma cells that showed distinct keratinocytic differentiation (Fig. 3a,b). Other subtypes of pancreatic tumors were mostly negative (Table 1).

Lack of *KRAS* and *GNAS* mutations in BCL10-expressing cancers (Table 1)

Some gene mutations occur in a cancer type-specific manner. It has been shown that *KRAS* is mutated exclusively in PDAs, IPMNs and MCNs, while the *GNAS* mutation has been reported to be specific to IPMNs.[23–27] Therefore, we examined whether BCL10 expression is related to these cancer type-specific mutations and neuroendocrine markers of neuroendocrine tumors. BCL10 was positive in three of 30 *KRAS*-mutated tumors, and they were histologically classified as adenosquamous carcinoma (two cases) and IPMN (one case). *GNAS* mutations were found in 3 of 114 tumors, all of which were diagnosed as IPMN. No neuroendocrine tumors were positive for BCL10. PDAs, IPMNs and neuroendocrine tumors account for 92% of pancreatic cancers,[1] and BCL10 was not expressed in these major pancreatic cancers. This result genotypically supported the ACC-specific expression of BCL10.

Specific BCL10 staining in ACC in EUS-FNA samples (*n* = 13, Table 2)

Currently, EUS-FNA is the standard in the histological diagnosis of pancreatic cancers. However, specimens obtained by this method are tiny and fragmented, and a definitive diagnosis is frequently challenging for pathologists. Therefore, we examined whether BCL10 expression could be used as a differential immunohistochemical marker in EUS-FNA samples. BCL10 was labeled in nine of 13 ACCs, whereas none of the other 43 tumors, except two adenosquamous carcinomas, were positive for BCL10 (Table 2, Fig. 3c,d).

DISCUSSION

Various cellular components are present in the pancreas, and the current classification of pancreatic cancer is based on the knowledge of such normal counterparts. ACC is thought to be derived from pancreatic acinar cells, and ACC mimics the morphology and phenotype of the normal counterpart, acinar cells, including trypsin expression. However, ACC is frequently positive for neuroendocrine makers, despite early divergence in the development of the pancreas.[28] As shown in Tables 1 and 2, approximately one third of ACCs expressed neuroendocrine markers. Furthermore, both tumors shared morphological characteristics including

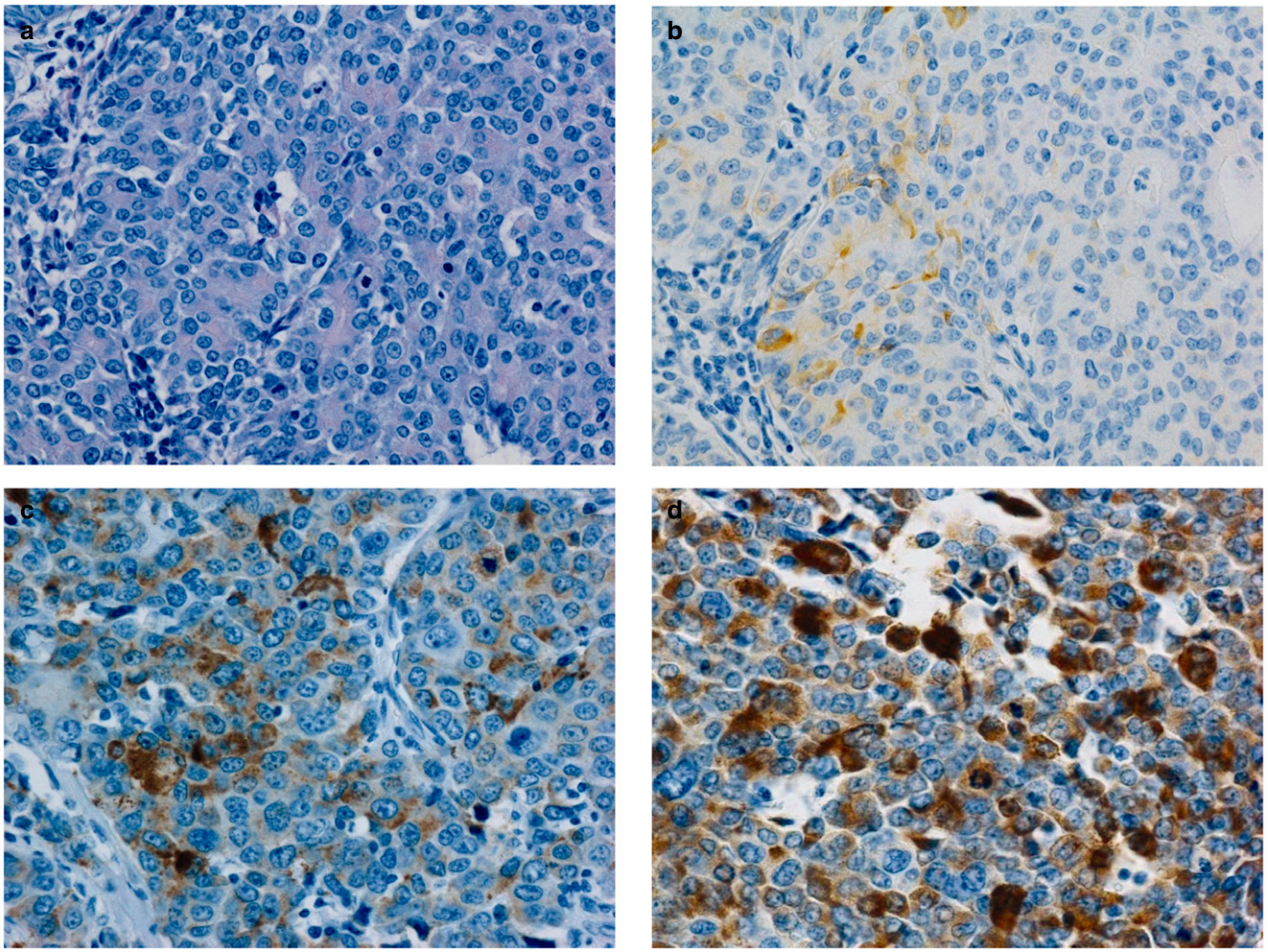

Figure 2 Typical acinar cell carcinoma. (**a**) An acinar cell carcinoma showing small acinar-like nests composed of small round nuclei and ample basophilic cytoplasms. Focal positive reactions for (**b**) synaptophysin and (**c**) trypsin were observed. (**d**) Despite divergence of the intensity, most tumor cells were positive for BCL10.

small round nuclei, central localization of the nuclei in the cytoplasm, relative ample cytoplasm, and sheet-like growth. These morphological characteristics, in addition to the frequent expression of neuroendocrine markers, make the differential diagnosis between ACC and neuroendocrine tumor difficult. However, this study clearly demonstrated that BCL10 could be used in this setting. None of the 44 neuroendocrine tumors, which included three cases of poorly differentiated neuroendocrine carcinomas, were positive for BCL10, but 82% (14/17) of ACCs expressed BCL10. This specific expression is especially useful for EUS-FNA samples because only a tiny piece of the tissues is allowed to be examined, and in many cases, no normal acini are included for control staining. Indeed, the commonly used ACC markers, such as trypsin and chymotrypsin, sometimes stained weakly and focally. Even though overall frequency of positive trypsin was higher than that of BCL10, distinctively positive reactions (2+ and 3+ in Table 2) were limited to only one third of ACCs. Furthermore, faint reaction (1+) in two surgical specimens was not detected in the EUS-FNA samples. This is in sharp contrast to BCL10, in which we found clear labeling (all 2+ or 3+) in EUS-FNA samples of ACCs. Such clear reaction is particularly crucial for diagnosis with EUS-FNA samples. Although two articles from the same group have reported specific expression of BCL10 in ACC using surgical specimens,[5,19] we confirmed the finding with detailed genotypes, and found that the expression was useful particularly in diagnosis using EUS-FNA samples.

In addition to neuroendocrine tumors, ACC with prominent ductal differentiation may be problematic in differential diagnoses. Stelow *et al*. have recently reported 11 such cases, five and six of which showed some morphological features of mucinous carcinoma and typical ductal carcinoma, respectively.[29] For this type of tumor, a differential diagnosis with

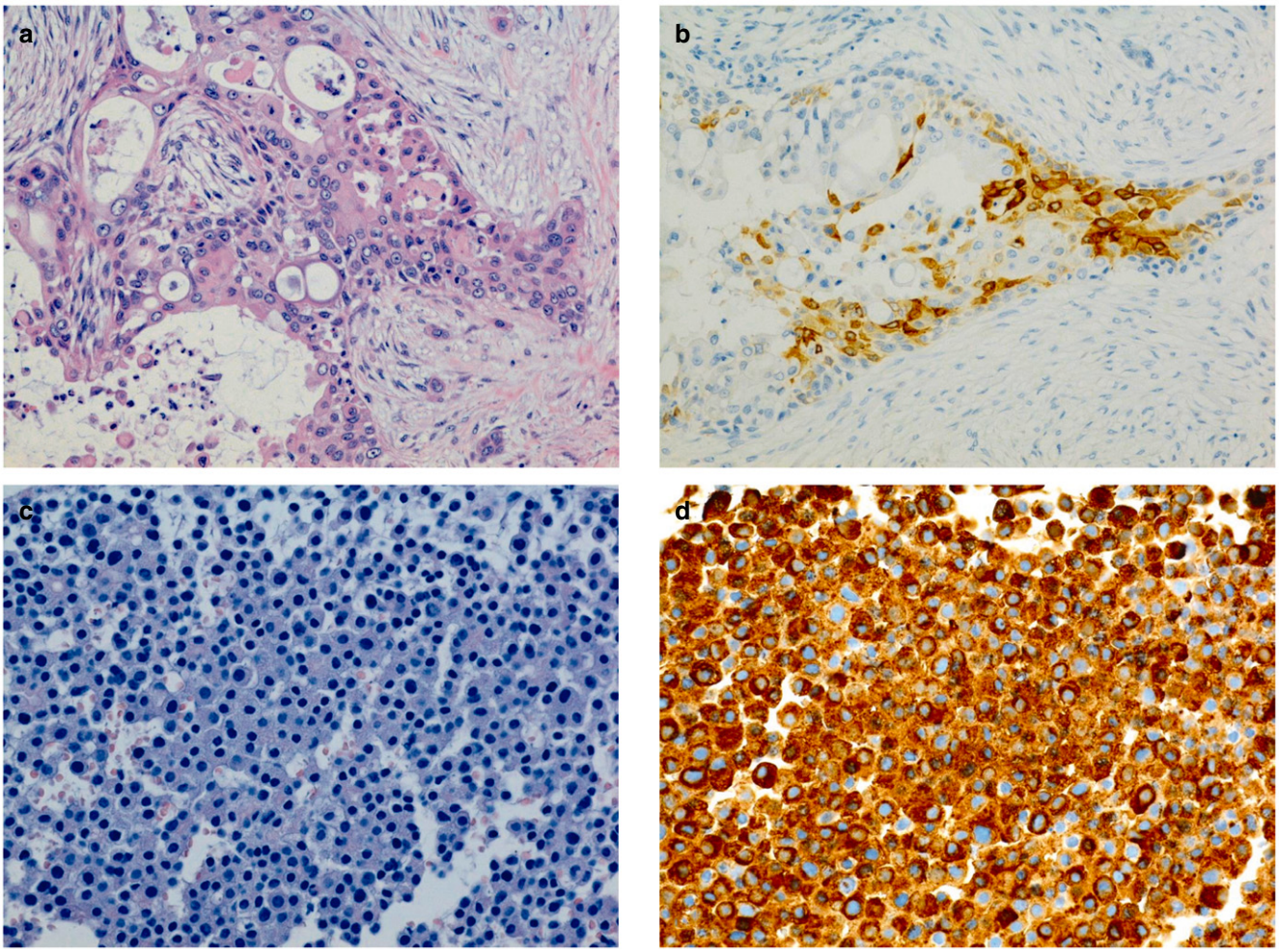

Figure 3 (**a,b**) Adenosquamous carcinoma and (**c,d**) an EUS-FNA specimen of an acinar cell carcinoma. Some pancreatic cancers showed differentiation to both glandular and squamous cells, and these tumors were classified as adenosquamous carcinoma. (**b**) With immunohistochemical staining of BCL10, squamous but not adenocarcinomatous components were positive. The lower panel is a representative case of acinar cell carcinoma in an EUS-FNA sample. (**c**) Only a limited number of tumor cells were obtained, and the acinar cell carcinoma shared morphological characteristics with neuroendocrine tumors. Thus, the differential diagnosis is challenging in cases such as this. However, (**d**) the specific expression of BCL10 in acinar cell carcinoma is quite helpful.

Table 1 Expression of BCL10 and neuroendocrine markers and genetic analyses of the *KRAS* and *GNAS* genes in 126 pancreatic tumors

		BCL-10								
		Positive		Negative			*KRAS*	*GNAS*	CGA	SYN
Subtype	Total (*n*)	3+	2+	1+	0	Positive rate (%)	Mutation rate (%)	Mutation rate (%)	Positive (%)	Positive (%)
ACC	17	10	4		3	14/17 (82%)	0/16 (0%)	0/16 (0%)	6/17 (35%)	8/17 (47%)
ADSQ	4		2	1	0	2/4 (50%)	4/4 (100%)	0/4 (0%)	0/4 (0%)	0/4 (0%)
PDA	23			1	22	0/23 (0%)	20/22 (91%)	0/21 (0%)	0/20 (0%)	0/20 (0%)
IPMN	9		1	3	5	1/9 (11%)	3/8 (38%)	3/8 (38%)	n.a.	n.a.
MCN	10		1	3	6	1/10 (10%)	1/5 (20%)	0/6 (0%)	n.a.	n.a.
NET	44			1	43	0/44 (0%)	2/44 (5%)	0/43 (0%)	43/44 (98%)	44/44 (100%)
SCN	9			1	8	0/9 (0%)	0/7 (0%)	0/7 (0%)	n.a.	n.a.
SPN	10		1	2	7	1/10 (10%)	0/9 (0%)	0/9 (0%)	2/7 (29%)	5/7 (71%)

ACC, acinar cell carcinoma; ADSQ, adenosquamous carcinoma; CGA, chromogranin A; IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm; n.a., not assessed; NET, neuroendocrine tumor; PDA, pancreatic ductal adenocarcinoma; SCN, serous cystic neoplasm; SPN, solid pseudopapillary neoplasm; SYN, synaptophysin.

Table 2 Results of the immunohistochemical and genetic analyses of 58 pancreatic tumors using EUS-FNA specimens

		EUS-FNA						Resected	
Case ID.	Subtype	*KRAS* mutation	*GNAS* mutation	CGA	SYN	BCL10	Trypsin	BCL10	Trypsin
1	ACC	WT	WT	+, focal	–	2+	2+	3+	2+
2	ACC	WT	WT	–	–	3+	–	3+	2+
3	ACC	WT	WT	–	–	–	equivocal	2+	2+
4	ACC	WT	WT	–	–	2+	1+	3+	1+
5	ACC	WT	WT	–	+, focal	3+	1+	3+	1+
6	ACC	WT	WT	–	–	–	–	–	1+
7	ACC	WT	WT	–	–	–	–	–	1+
8	Mixed Ductal-ACC	WT	WT	+	+	3+	equivocal	2+	2+
9	ACC	WT	WT	–	–	3+	1+		
10	ACC	WT	WT	+	+	3+	2+		
11	ACC	WT	WT	–	+, focal	2+	3+		
12	ACC	WT	WT	–	+, focal	2+	2+		
13	ACC	WT	WT	+, focal	+, focal	–	1+		
	PDA (n = 18)	15/17 (88%)	0/14 (0%)	0/15 (0%)	0/15 (0%)	0/18 (0%)			
	ADSQ (n = 4)	4/4 (100%)	0/4 (0%)	n.a.	n.a.	2/4 (50%)			
	NET (n = 17)	2/17 (12%)	0/16 (0%)	17/17 (100%)	17/17 (100%)	0/17 (0%)			
	SPN (n = 6)	0/6 (0%)	0/6 (0%)	2/3 (67%)	2/3 (67%)	0/6 (0%)			

Two cases of acinar cell carcinomas showed equivocal results, in which non-specific staining was too pronounced to make a definite evaluation.
ACC, acinar cell carcinoma; ADSQ, adenosquamous carcinoma; CGA, chromogranin A; n.a., not assessed; NET, neuroendocrine tumor; PDA, pancreatic ductal adenocarcinoma; SPN, solid pseudopapillary neoplasm; SYN, synaptophysin; WT, wild-type.

PDA, IPMN and MCN is needed. Fortunately, good genetic markers are available: *KRAS* and *GNAS*. *KRAS* mutations were previously detected in 95% or more of PDAs, 40–80% of IPMNs, and 30% of MCNs, while *GNAS* mutations were specific to IPMNs.[23–27] This study revealed that BCL10 was expressed in a mutually exclusive fashion to *KRAS* and *GNAS* mutations, suggesting that BCL10-expressing tumors are distinct from PDAs, IPMNs and MCNs.

It is also interesting that adenosquamous carcinomas expressed BCL10, although the number of examined samples was limited. Prior to this study, we examined BCL10 expression in 130 tumors of various organs using tissue microarray (data not shown, but could be provided as supplementary data if requested). Five of 33 squamous carcinomas but no adenocarcinomas were positive for BCL10. Indeed, some reports have noted that BCL10 is expressed in most oral squamous cell carcinomas[17,30] and that the intensity is associated with cancer progression and prognosis.[17] Therefore, squamous cell differentiation could be a pitfall of the interpretation of BCL10 expression in pancreatic tumor subtyping.

In summary, we examined BCL10 expression in pancreatic cancer. BCL10 was specifically expressed in ACCs, and none of the BCL10-expressing tumors harbored the *KRAS* or *GNAS* mutations that are frequently mutated in PDAs, IPMNs and MCNs. ACC is often difficult to distinguish from neuroendocrine tumors, particularly when limited samples are obtained, because approximately one third of ACCs are positive for neuroendocrine markers and the two tumors share morphological characteristics. BCL10 could be a useful marker in this setting.

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