Monoclonal antibody 16D10 to the COOH-terminal domain of the feto-acinar pancreatic protein targets pancreatic neoplastic tissues

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Abstract

We have shown that the 16D10 antigen located on the mucin-like COOH-terminal domain of the feto-acinar pancreatic protein (FAPP) is expressed at the surface of human pancreatic tumor cell lines such as SOJ-6 cell line. Furthermore, an in vivo study indicates that targeting this cell-membrane glycopeptide by the use of the monoclonal antibody (mAb) 16D10 inhibits the growth of SOJ-6 xenografts in nude mice. To validate the potential use of the mAb16D10 in immune therapy, this study examined the expression of 16D10 antigens at the surface of human pancreatic adenocarcinomas versus control tissues. We examined the reactivity of mAb16D10 and mAb8H8 with pancreatic ductal adenocarcinomas (PDAC) compared with controls by using immunohistochemistry and confocal laser scanning microscopy. mAb8H8 does react with control or nontumoral human pancreatic tissues. mAb16D10 has a strong and specific reactivity with PDAC and does not react with other cancers of epithelia or normal tissues tested. Notable, mAb16D10 mostly recognizes membrane of tumoral cells. Furthermore, mAb8H8 and mAb16D10 recognized a protein of 110 to 120 kDa in homogenates of nontumoral and tumoral human pancreatic tissues, respectively. This size correlates with that of FAPP or with that of the normal counterpart of FAPP, the so-called bile salt-dependent lipase. The results suggest that mAb16D10 presents a unique specificity against PDAC; consequently, it could be effective in immune therapy of this cancer. Furthermore, mAb16D10 and mAb8H8 pair might be useful for diagnosis purpose in discriminating tumoral from nontumoral human pancreatic tissues. [Mol Cancer Ther 2009;8(2):282 – 91]

Introduction

The bile salt-dependent lipase (BSDL; E.C. 3.1.1.13) is found in the pancreatic secretions of all species from fish to human (1). This enzyme participates in the intestinal processing of cholesteryl esters (2–4). During its transport from the endoplasmic reticulum up to the trans-Golgi network, BSDL is associated with intracellular membranes by means of a complex that involves glycosphingolipids of rafts (5) and the 94-kDa glucose-related protein (6, 7). This Grp 94 controls the late folding steps and the sorting of the active enzyme toward secretion (8). Once in the trans-Golgi network and after completion of glycosylation (9, 10), the complex is released from membranes on the phosphorylation of the threonine residue at position 340 (11) by a protein kinase CKII (12, 13). Once released from intracellular membranes, BSDL enters into the secretion route until the duodenum where it accomplishes its physiologic role.

The feto-acinar pancreatic protein (FAPP) is a specific component of acinar cells of the human pancreas, which is associated with the ontogenesis and the development of the gland (14). Maximum synthesis of FAPP, determined as the emergence of the J28 epitope recognized by the monoclonal antibody (mAb) J28 (15), occurs when acinar cell proliferation is maximal between 20 and 22 weeks of gestation; thereafter, it declines to parturition (16). In adult, FAPP defined by the expression of the J28 epitope behaves as an oncodevelopment-associated antigen (14). FAPP (a 100-120 kDa protein) presents strong homologies with BSDL (a 100 kDa protein; ref. 17) and its cloning from human pancreatic tumoral cells (18) indicates that the NH2-terminal domain encoded by exons 1 to 10 is identical to that of BSDL. However, the sequence corresponding to

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glycotope requires the core 2 COOH-terminal domain of FAPP. The formation of the mAb16D10, two mAbs directed against the degradation is specifically recognized by mAbJ28 and presented at the surface of tumoral cells such as SOJ-6 cells domain of FAPP that carries out oncofetal epitopes is distributed within the endoplasmic reticulum and the Golgi (19). FAPP is then degraded (22) and the COOH-terminal domain of FAPP that carries out oncofetal epitopes is presented at the surface of tumoral cells such as SOJ-6 cells (23). This 32-kDa glycotope issued from the FAPP degradation is specifically recognized by mAbJ28 and mAb16D10, two mAbs directed against the O-glycosylated COOH-terminal domain of FAPP. The formation of the J28 glycopeptide requires the core 2 β1,6-N-acetylglucosaminyl-transferase and the α1,3/4-fucosyltransferase (24, 25), two glycosyltransferases whose expression is up-regulated in pancreatic cancer (26). Furthermore, we have shown that the growth of xenografted SOJ-6 cells in nude mice was significantly decreased by preventative injections of mAb16D10 (23). To validate the potential use of this mAb16D10 in immune therapy, it was important to determine the expression of 16D10 antigen on human pancreatic adenocarcinomas. In this study, we have shown that mAb16D10 is capable to discriminate specifically the human pancreatic tumoral tissue from nontumoral tissue. This study was carried out on both frozen tissues and formalin-fixed, paraffin-embedded archival tissues.

Materials and Methods

Human Tissues

Seven fresh frozen tissue sections of primary malignant pancreatic ductal adenocarcinomas (PDAC) were collected from the Department of Pathology (Ste Marguerite Hospital). Frozen tissue slides of a PDAC provided by BioChain were also studied. Adjacent nontumoral areas from three cases of pancreas neoplasia, one benign pancreatic tumor, and one biopsy obtained during transplantation process were used as nontumoral controls. Samples were collected from recently diagnosed adenocarcinomas. Median age of patients was 66 years (range, 42-75). Table 1 gives histopathologic data of each patient. A lung adenocarcinoma case was provided by the Department of Thoracic Surgery (Ste Marguerite Hospital). Carcinomas of colon, esophagus, liver, stomach, and thyroid were studied as negative tissue controls (BioChain).

Formalin-fixed, paraffin-embedded tissue sections from 10 patients with histologic nontumoral pancreas were studied as controls. Four cases were provided by the Department of Pathology. Three cases were previously studied on frozen sections and six other cases were provided by BioChain (Table 1). Twenty-one archival PDAC cases were studied. Six cases were previously studied on frozen sections. Median age of patients was 66 years (range, 42-82).

Histopathologic data of each patient are also provided in Table 1. Samples of PDAC were collected from patients without prior chemotherapy or radiotherapeutic treatments. One sample from bladder metastasis of pancreatic cancer and one sample from a patient with a preoperative gemcitabine treatment before the tumor resection were provided by the Department of Pathology.

Table 1.  Clinicopathologic data of nontumoral pancreas and PDAC

<table>
<thead>
<tr>
<th>Cases</th>
<th>Age</th>
<th>Gender</th>
<th>Histopathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1*</td>
<td>78</td>
<td>F</td>
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<td>Normal tissue obtained in a case of kidney tumor resection</td>
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<td>F</td>
<td>Chronic obstructive pulmonary disease</td>
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<tr>
<td>C8†</td>
<td>83</td>
<td>F</td>
<td>Congestive heart failure</td>
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<tr>
<td>C9†</td>
<td>86</td>
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<td>M†</td>
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<td>Bladder metastasis of pancreatic cancer</td>
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<tr>
<td>T†</td>
<td>73</td>
<td>F</td>
<td>Ductal adenocarcinoma obtained after a preoperative gemcitabine treatment</td>
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</table>

*Frozen sections.
†Formalin-fixed, paraffin-embedded sections.
‡Tissue provided by BioChain. All controls and patients were Caucasians, except PDAC7, which was Asiatic.
The 16D10 antigen expression on human normal tissues has been determined by MDS Pharma Services. The human normal tissue bank was prepared in accordance to the French Bioethical Law nb. 2004-800 of August 2004 and under the control of the Institut de Médecine Légale. Placenta was obtained just after parturition and came from the appropriate medical service of the Centre Hospitalier Universitaire de Lyon with the same safety and scientific precautions.

**Tissue Treatment and Histologic Study**

All tissues from the human normal tissue bank and freshly collected pancreatic and lung tissues were frozen in liquid nitrogen, stored at -80°C, and further cut into 5 μm thick cryotome sections at -20°C. The frozen sections were dried, fixed at 4°C in acetone, and stained with hematoxylin-phloxine-saffron. Formalin-fixed, paraffin-embedded tissue specimens were routinely fixed in 10% formalin, embedded in paraffin, and further cut into 5 μm sections stored at 4°C or stained with hematoxylin-phloxine-saffron. Each sample was processed for histopathologic diagnosis, which was done by a senior anatomopathologist (M-J.P-D.) specialized in pancreatic histology. All the examined patients were in stage 0 or I.

**Antibodies**

The patented IgM mAb16D10 directed against O-glycosylated repeated COOH-terminal mucin domain of FAPP (23), the patented IgG mAb8H8 against BSDL, and the sylated repeated COOH-terminal mucin domain of FAPP were homemade. mAbJ28 specific for the fucosylated J28 glycopeptide, and irrelevant mouse IgM, IgG, and rabbit IgG were from Sigma.

**Immunohistochemistry**

Cryotome tissue sections were dried, treated for 10 min with acetone at 4°C, and rehydrated in TBS (pH 7.6). Unspecific binding sites were blocked 30 min at room temperature with a nonimmune goat serum. The sections were then incubated (1 h, room temperature) with mAb8H8 (20 μg/mL), pAbL64 (20 μg/mL), or mAb16D10 (20 μg/mL), washed in PBS, and incubated (1 h, room temperature) with secondary antibodies diluted to 1:50 [biotin-conjugated F(ab')2 fragment goat anti-mouse IgG or biotin-conjugated F(ab')2 fragment goat anti-mouse IgG (Immunotech)] or mAb8H8 (20 μg/mL), or mAb16D10 (100 μg/mL), washed in PBS, and incubated (1 h, room temperature) with secondary antibodies diluted to 1:50 dilution of streptavidin-fluorescein (Immunotech). Sections were washed in PBS, counterstained in Mayer’s hematoxylin solution allowing the correct determination of the studied cellular zones, and then washed with water and mounted in aqueous permanent mounting medium. For all controls, slides were incubated with irrelevant IgM or IgG primary antibodies or adequate secondary antibodies.

**Confocal Laser Scanning Microscopy**

Dry cryotome tissue sections were fixed for 10 min with acetone at 4°C and rehydrated in PBS (pH 7.2). Sections were incubated (1 h, room temperature) with mAb8H8 (20 μg/mL), pAbL64 (20 μg/mL), or mAb16D10 (20 μg/mL), washed in PBS, and incubated (1 h, room temperature) with secondary antibodies diluted to 1:50 [biotin-conjugated F(ab')2 fragment goat anti-mouse IgG or biotin-conjugated F(ab')2 fragment goat anti-mouse IgG (Immunotech)]. The sections were washed in PBS and treated (1 h, room temperature) with 1:50 dilution of streptavidin-fluorescein (Immunotech). Sections were washed in PBS, counterstained in Mayer’s hematoxylin solution allowing the correct determination of the studied cellular zones, and then washed with water and mounted in aqueous permanent mounting medium. For all controls, slides were incubated with irrelevant IgM or IgG primary antibodies or adequate secondary antibodies.

Antigen retrieval was done on formalin-fixed, paraffin-embedded sections as described previously for immunohistochemistry. After rinsing with water and PBS, the slides were incubated (2 h, room temperature) with mAb8H8 (20 μg/mL), pAbL64 (20 μg/mL), or mAb16D10 (100 μg/mL), washed in PBS, and incubated (1 h, room temperature) with secondary antibodies diluted to 1:50 [biotin-conjugated F(ab')2 fragment goat anti-mouse IgG or biotin-conjugated F(ab')2 fragment goat anti-mouse IgG (Immunotech)]. The sections were washed, treated with streptavidin-fluorescein, and processed as for frozen sections. Sections were observed by means of a confocal laser scanning microscope (Leica) with a ×25 objective.

**Image Processing**

Images were processed as described previously (27). For each primary antibody, the staining was calculated as the ratio between the total fluorescence of the area (total specific fluorescence) and the surface of this area [mean specific fluorescence (MSF)]. The mean values of six stained areas for each biopsy were then calculated.

**SDS-PAGE**

Frozen tissues were collected from glass slides using a razor blade, homogenized in Laemmlı’s loading buffer (28), centrifuged (2 min, 1,000 rpm), and analyzed on 10%
polyacrylamide gel in the presence of 1% SDS. Proteins were electrotransferred onto nitrocellulose membranes and the immunodetection was done using mAb and alkaline phosphatase-labeled secondary antibodies as required.

**Statistical Analysis**

All results are expressed as mean ± SE. Differences between controls and biopsies were tested using ANOVA. The significance was calculated with Fisher’s post hoc least significant difference test and/or the Scheffe’s F test. Differences were considered significant when \( P < 0.05 \).

**Results**

**Determination of 8H8 and 16D10 Antigen Expression by Immunohistochemistry**

On frozen sections, the plasma membrane and cytoplasm of acinar cells of all control cases were stained with mAb8H8 (Fig. 1). Endocrine cells of the islet of Langherans (Fig. 1, arrowhead) were always negative to mAb8H8. Tumor cells of PDAC were not reactive to mAb8H8 (Fig. 1) or very slightly stained for few cases (Fig. 1). No staining was found in nontumoral tissue with mAb16D10, whereas PDAC sections were all positive to mAb16D10 (Fig. 1). A strong staining was found at the plasma membrane and around the tumoral cells or in cytoplasm.

Results obtained with formalin-fixed, paraffin-embedded sections were quite similar to those found with frozen sections. With mAb8H8, the staining was mainly found in cytoplasm of acinar cells. Pancreatic ducts were slightly stained (Fig. 1, arrow). Any or slight staining was observed in PDAC cells (Fig. 1). No staining was found in nontumoral tissue with mAb16D10, whereas all PDAC cases were stained with mAb16D10. The staining was mainly found in cytoplasm of tumor cells and at the plasma membrane (Fig. 1).

We also used pAbL64, a pAb directed against BSDL and FAPP, on frozen and formalin-fixed, paraffin-embedded sections. As expected, pictures showed a strong staining localized at the plasma membrane and in the cytoplasm of tumoral cells. The cytoplasm of acinar cells of control cases was also decorated by pAbL64 (Fig. 1).

**Determination of 8H8 and 16D10 Antigen Expression by Confocal Laser Scanning Microscopy**

To determine the precise localization of 16D10 antigen in the tumoral cells, we examined the reactivity of mAb8H8 and mAb16D10 with PDAC and control cases by using confocal laser scanning microscopy (CLSM). Using mAb8H8, a strong fluorescence was found on frozen control sections at the plasma membrane and in the cytoplasm, whereas PDAC cases showed a negative or slight staining (Fig. 2A). With mAb16D10, the staining was negative or extremely faint in control cases (Fig. 2A). The staining of all cases of ductal adenocarcinomas was very strong at the plasma membrane. This labeling of cell plasma membranes by mAb16D10 is clearly visible on enlarged Fig. 2B showing the same area counterstained with Mayer’s hematoxylin. CLSM of six stained areas from each image were quantified by measuring the MSF.
intensity of stained areas. The average of the MSF intensity found with mAb8H8 was significantly decreased ($P < 0.001$) in PDAC (mean = 16 ± 6) compared with controls (mean, 113 ± 7; Fig. 3A). On the other hand, a significant increase in MSF ($P < 0.001$) was found with mAb16D10 (Fig. 3B) in each PDAC (mean, 131 ± 2) compared with controls (mean, 8 ± 2).

Labeling obtained with formalin-fixed, paraffin-embedded sections was stronger than those found with frozen sections. A strong fluorescence was found in cytoplasm in all control cases using mAb8H8 (Fig. 2A). This difference observed between frozen section and formalin-fixed, paraffin-embedded sections could be due to heat-induced antigen retrieval increasing the accessibility of epitopes to the antibodies. Another possibility was that soluble FAPP in the cytoplasm could be partially fixed and extracted during immunohistochemical staining of fresh frozen tissue sections (29).

On the contrary, this antibody did not or slightly react with PDAC. With mAb16D10, the staining was negative or extremely faint in control cases (Fig. 2A). In all cases of PDAC, the staining was very strong on plasma membranes and the cytoplasm was sporadically decorated. This labeling of cell plasma membranes by mAb16D10 is clearly visible on enlarged Fig. 2B associated to the same areas counterstained with Mayer’s hematoxylin.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Representative results of CLSM staining with mAb8H8 and mAb16D10 in frozen and formalin-fixed, paraffin-embedded sections. **A,** all nontumoral pancreatic tissue are stained with mAb8H8. Arrowhead, endocrine tissue of the islet of Langherans is not stained. In PDAC, no detectable or very slight staining is found with mAb8H8. With mAb16D10, no staining or slight staining is found in nontumoral pancreas. In PDAC, a strong staining is found in plasma membrane and cytoplasm. **B,** higher magnification of images stained with mAb16D10 with Mayer’s hematoxylin counterstaining before CLSM observation. Original magnification, ×300.
As for frozen sections, CLSM of six stained areas from each image was quantified by measuring the MSF intensity of stained areas. With mAb8H8, the average of the MSF intensity was significantly decreased ($P < 0.001$) in PDAC (mean, 34 ± 3; Fig. 3C). On the other hand, a significant increase in MSF ($P < 0.001$) was found with mAb16D10 (Fig. 3D) in each PDAC (mean, 226 ± 3) compared with controls (mean, 31 ± 5).

16D10 Antigen Expression on Human Cancer and Normal Tissues

The expression of the 16D10 antigen was recorded on six human cancer tissues. mAb16D10 did not react with other epithelial neoplastic cells such as colon, esophagus, liver, stomach, thyroid, and lung (Fig. 4).

The expression of the 16D10 antigen was also recorded on 18 human normal tissues including epithelium such as colon, duodenum, skin + breast, ileum, jejunum, liver, lung, pancreas, thyroid, placenta, stomach, endothelium such as artery and vein endothelial tissues, and cerebellum, cerebrum, pituitary gland, and striated muscle. No staining was found in all tissues treated with mAb16D10, irrelevant mouse IgM, or labeled secondary antibodies (data not shown).

16D10 Antigen Expression in Metastasis of Pancreatic Cancer and in Tumor from Patient with a Preoperative Chemotherapy

To determine whether the mAb16D10 could be used in advanced cancers and in patients after standard therapies, the expression of the 16D10 and 8H8 antigens was recorded on human bladder metastasis of pancreatic cancer and cancer tissue from a patient with a preoperative gemcitabine treatment. A slight staining was observed with mAb8H8, whereas these two tumors were strongly stained with mAb16D10 (Supplementary Fig. S1A and B). CLSM

Figure 3. Quantitative determination of MSF in frozen sections (A and B) and formalin-fixed, paraffin-embedded sections (C and D). mAb8H8 (A and C) and mAb16D10 (B and D). Six areas in each case were measured. Data obtained with one case were compared to each other. Mean ± SE. Comparison were made by ANOVA followed by Fisher’s and Scheffe’s tests. $P < 0.001$.

9 Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).
of six stained areas from each image was quantified by measuring the MSF intensity of stained areas. With mAb8H8, the average of the MSF intensity in pancreatic bladder metastasis (mean, 36 ± 1) and tumor from patient with a preoperative chemotherapy (mean, 34 ± 2) was similar to that found with PDAC (mean, 34 ± 3) and was significantly decreased (P < 0.001) compared with controls (mean, 144 ± 3; Supplementary Fig. S1C). On the other hand, a significant increase in MSF (P < 0.001) was found with mAb16D10 in pancreatic bladder metastasis (mean, 123 ± 6) and tumor from patient with a preoperative chemotherapy (mean, 189 ± 5) compared with controls (mean, 31 ± 5), although CLSM was less important in these tissues compared with that of PDAC (mean, 226 ± 3; Supplementary Fig. S1C).

**SDS-PAGE Analysis of Pancreatic Tissues**

We next checked that mAbs used in the present study reacted with BSDL or FAPP in pancreatic tissues. For this purpose, nontumoral and tumoral tissues corresponding to sections of nontumoral control tissue C3 and tumoral tissue PDAC3, respectively, were removed from slide and analyzed by SDS-PAGE and immunodetection. As shown on Fig. 5A, mAb8H8 specifically and strongly reacted with a protein, the Mr of which (~110-120 kDa) corresponds to that of BSDL also recognized by pAbL64. mAb16D10 reacted very slightly with a protein present in nontumoral control tissue. In that concerning tumoral tissue, no mAb8H8-immunoreactive protein can be detected; however, proteic material present in lysate reacted with mAb16D10. In particular, this mAb, as well as mAbj28 and pAbL64, recognized a 120-kDa protein, the Mr of this material corresponds to that of FAPP (Fig. 5B). Interestingly, this tumor presents two isoforms of FAPP due to a polymorphism located in the exon 11 encoding the COOH-terminal domain of the protein (30). mAb16D10 also recognized peptides presenting higher migration; in part, it reacted with 40- to 60- and 25- to 35-kDa peptides. This material could be representative of different proteolysis products of the COOH-terminal domain of FAPP, which has been recently detected at the level of the plasma membrane of pancreatic tumoral cells (23). Furthermore, mAb16D10 also binds to material with a very low migration; this material could be aggregated molecules of FAPP or FAPP associated with membrane lipid in cell lysate (5). These data strengthen immunohistochemical results and show that mAb8H8 recognizes the BSDL in nontumoral pancreatic tissue and does not react with the tumoral tissue. Contrarily, mAb16D10 reacts with FAPP in tumoral tissue and has no reactivity with protein in normal tissue.

**Discussion**

During the last decade, no significant progress has been made against the cancer of the exocrine pancreas, although chemotherapy associated with radiotherapy has some positive effect on the survival of patients (31). The curative resectability rate is very low because the cancer is asymptomatic and its diagnosis in most patients occurs at a late stage and had already formed distant metastases (32). At this stage, the prognosis is extremely poor and efforts are urging to identify and evaluate diagnostic and therapeutic markers. Specific markers to target pancreatic tumoral cells are sparse, albeit many mAbs were generated against malignant pancreatic epithelial cells. These include Span-1 (33), Du-Pan-2 (34), CA 19-9 (35), CA50 (36), CAR-3 (37), and CA242 (38). These antibodies displayed distinct patterns of tissue staining, being more highly expressed in certain tissues and tumors than others. Furthermore, the reactivity of these antibodies depends on the genotype of patients (39). Consequently, the sensitivity and the

![Figure 4](http://mct.aacrjournals.org/content/suppl/2009/02/03/MCT-08-0471.DC1.large.png)

**Figure 4.** Immunohistochemical results in nonpancreatic carcinoma cases with mAb16D10. CCA, colon adenocarcinoma; ECA, esophagus adenocarcinoma; LiCA, liver adenocarcinoma; LuCA, lung adenocarcinoma; SCA, stomach adenocarcinoma; TCA, thyroid adenocarcinoma. No staining using mAb16D10 is found in all epithelial tissues examined. Original magnification, ×250.
Two proteins, PAGE and immunodetected using pAbL64, pAbs that reacted with these each protein were loaded on the top of the gel) were also analyzed on SDS-mAb8H8, mAbJ28, mAb16D10, and pAbL64. BSDL and FAPP (50 ng of analysis and immunodetection with anti-Mol Cancer Ther 2009;8(2) . February 2009

immunoreactivity of tissue homogenates with antibodies. Tissue sections were removed from slide C3 (control tissue; A) and slide PDAC3 (tumoral tissue; B). Tissues were homogenized before SDS-PAGE analysis and immunodetection with anti-β-actin antibody and antibodies mAb8H8, mAbJ28, mAb16D10, and pAbL64. BSDL and FAPP (50 ng of each protein were loaded on the top of the gel) were also analyzed on SDS-PAGE and immunodetected using pAbL64, pAbs that reacted with these two proteins.

Figure 5. Immunoreactivity of tissue homogenates with antibodies. Tissue sections were removed from slide C3 (control tissue; A) and slide PDAC3 (tumoral tissue; B). Tissues were homogenized before SDS-PAGE analysis and immunodetection with anti-β-actin antibody and antibodies mAb8H8, mAbJ28, mAb16D10, and pAbL64. BSDL and FAPP (50 ng of each protein were loaded on the top of the gel) were also analyzed on SDS-PAGE and immunodetected using pAbL64, pAbs that reacted with these two proteins.

specificity of these markers are not ideal. The combined use of some of these antigens expression may potentiate the specificity for pancreatic diagnosis (40). Mucin expression pattern may be interesting as overexpression of MUC1 and MUC6 and de novo expression of MUC5AC occurred early in the development of pancreatic adenocarcinoma, whereas sialyl Tn antigen expression occurs later, at the stage of invasive adenocarcinoma (41). Unfortunately, mucins are expressed by normal epithelia (MUC1) or by various normal gastric cells (MUC5AC and MUC6; refs. 42, 43). Although interesting progresses have been done with mesothelin and the prostate stem cell antigen (44), physicians are still awaiting for a specific marker of the pancreatic malignancy with interest both in diagnostic and pancreatic cancer in combination with chemotherapies as described for cetuximab (49) or bevacizumab (50).

In this article, we showed that the mAb16D10 directed against the O-glycosylated COOH-terminal domain of FAPP recognized structures present at the cell surface of human pancreatic tumoral tissues. This mAb recognized 22 tumoral tissues over the 22 tested independently of the tissue pretreatment (frozen sections or formalin-fixed, paraffin-embedded sections) and presented a unique specificity for membranes of neoplastic cells. Furthermore, the mAb16D10 had no reactivity with all nontumoral tissues examined and with all nonpancreatic tumoral and normal tissues tested. Another mAb, the mAb8H8, seemed to be the counterreactive antibody of mAb16D10 as it recognized nontumoral pancreatic tissue but had very poor or even no affinity for tumoral tissue. The same pattern of antibodies reactivity was obtained with pancreatic tissue homogenates in which a protein of the same Mr than that of BSDL or of FAPP was detected in nontumoral tissue using mAb8H8 and in tumoral tissues using mAb16D10, respectively.

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Although the size of the cohort used here is still limited, this study confirmed the unique specificity of mAb16D10 for the tumoral human pancreatic tissues. Furthermore, the mAb16D10 and mAb8H8 pair of mAbs might be useful for diagnosis purpose in discriminating non-tumoral from tumoral human pancreatic tissues. The potential use of mAb16D10 in the identification of precancerous lesions is actually under investigation. Overall, mAb16D10, which does not react with normal human tissues examined here, could be effective in passive immunologic therapy of exocrine pancreatic tumors as suggested by recent results obtained with model animal (23).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

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