

A Case of Nonfunctioning Pancreatic Endocrine Tumor with Atypical Imaging Findings due to Prominent Fibrosis of the Tumor Stroma

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Abstract

The patient, a 56-year-old woman, was found during routine checkup to have a disorder of hepatic function. Abdominal ultrasonography showed an ill-defined hypoechoic mass in the head and body of the pancreas; however, no blood-flow signal was observed within the tumor on Doppler ultrasonography. Abdominal computed tomography showed a low-density area in the arterial and portal venous phases. The lesion was visualized as an area of low signal intensity on both T1- and T2-weighted magnetic resonance images, whereas fluorodeoxyglucose positron emission tomography showed fluorodeoxyglucose accumulation in the tumor. Although a preoperative diagnosis was difficult to make, a rapid cytologic examination revealed evidence of a pancreatic endocrine tumor, and subtotal stomach-preserving pancreaticoduodenectomy with portal vein resection was performed. Histopathological examination showed tumor cell nests scattered in abundant fibrotic tissue; the tumor cells had proliferated in a cord-like fashion and showed immunostaining for chromogranin A. Staining for fibroblast activation protein α was seen in the fibroblastic cells contained within the fibrous stroma surrounding the tumor cell nests, whereas both the fibroblastic cells in the tumor and those in the stroma showed a high rate of staining for thrombospondin. We presume that tumor-associated fibroblasts were involved in the fibrosis of the tumor stroma.

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Key words: pancreatic endocrine tumor, desmoplastic stroma, tumor-associated fibroblasts

Introduction

Pancreatic endocrine tumors, which are normally

hypervascular tumors, often grow in an expansive manner and are visualized as well-defined, deeply-staining tumors on contrast-enhanced computed tomography (CT)¹. Therefore, preoperative diagnosis

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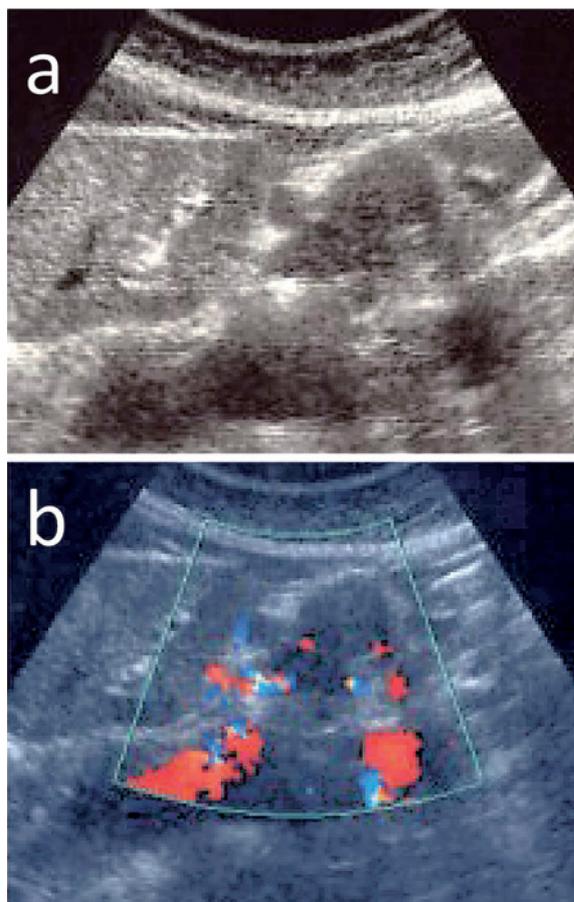


Fig. 1 **a:** Abdominal ultrasonography showing an irregular, ill-defined, hypoechoic tumor in the body of the pancreas. **b:** Doppler ultrasonography revealed no blood-flow signals within the tumor.

is rarely difficult. However, diagnosis may be difficult in patients with cyst formation² or prominent fibrosis of the tumor stroma^{3,4}.

Many recent reports suggest that tumor-associated fibroblasts (TAFs) are involved in the prominent fibrosis of the stroma of malignant tumors, promote the infiltration and metastasis of cancer cells, and reflect the malignant grade of the tumor^{5,6}.

Herein, we report on a patient with a pancreatic endocrine tumor that was difficult to diagnose because of the prominent fibrosis of the tumor stroma; histopathological examination was performed to examine the involvement of TAFs in the stromal fibrosis.

Case Presentation

The patient was a 56-year-old woman who was found during a routine checkup to have a disorder of hepatic function and was referred to our department for further investigation in September 2009. She was otherwise in good health and without complaint but had undergone total hysterectomy for uterine myoma at the age of 36 years. Because CT and magnetic resonance (MR) imaging performed at the outpatient department suggested the possibility of a pancreatic tumor, the patient was admitted to our department for further medical examination and treatment.

Findings on physical examination on admission were as follows: height, 155.0 cm; weight, 52.0 kg; blood pressure, 100/61 mm Hg; pulse, 75/minute, regular; and body temperature, 36.5°C. There was no conjunctival pallor, no evidence of jaundice, and no abnormal findings on examination of the heart or lungs. The abdomen was flat and soft. The liver, spleen, and kidney were not palpable. There was no abdominal tenderness, and no masses was palpable.

Laboratory studies on admission showed mild elevations of the serum levels of aspartate aminotransferase, alanine aminotransferase, and γ -glutamyl transpeptidase; significantly elevated fasting blood glucose (191 mg/dL) and HbA1c (7.8%); normal serum levels of tumor markers (carbohydrate antigen 19-9, carcinoembryonic antigen, and Duke pancreatic monoclonal antigen type 2); normal serum levels of immunoglobulin (Ig) G and IgG4; and a negative serum test for antinuclear antibody.

Abdominal ultrasonography showed an ill-defined hypoechoic mass with an irregular margin, measuring 40 × 25 mm, in the head and body of the pancreas. Doppler ultrasonography showed blood-flow signals in a part of the tumor margin but not within the tumor itself (**Fig. 1**). Abdominal CT showed an ill-defined low-density lesion measuring 3.5 × 1.6 cm in the head and body of the pancreas which was visualized as having isodensity to low density in the portal venous phase (**Fig. 2**). Although mild atrophy and calcification were observed in the

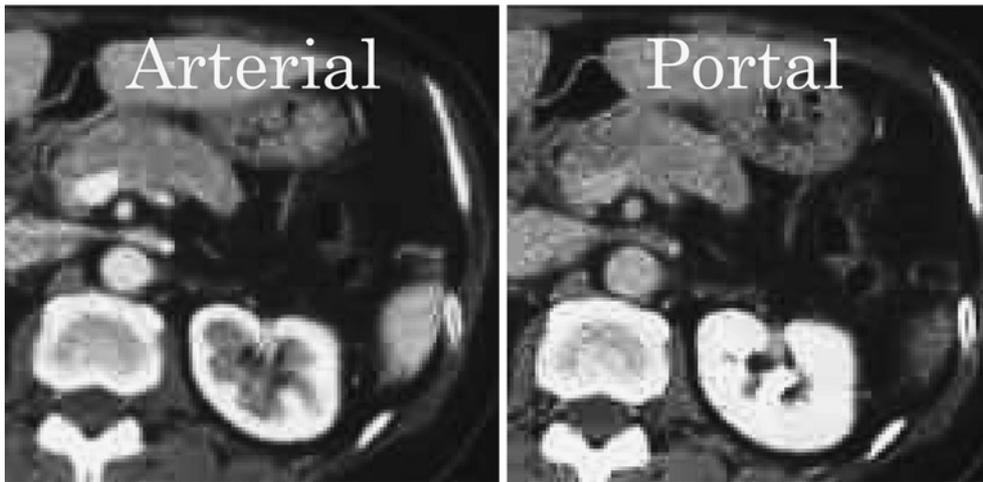


Fig. 2 Abdominal CT scan showing an ill-defined, low-density area in the arterial phase and a low-density or isodense area in the portal phase.

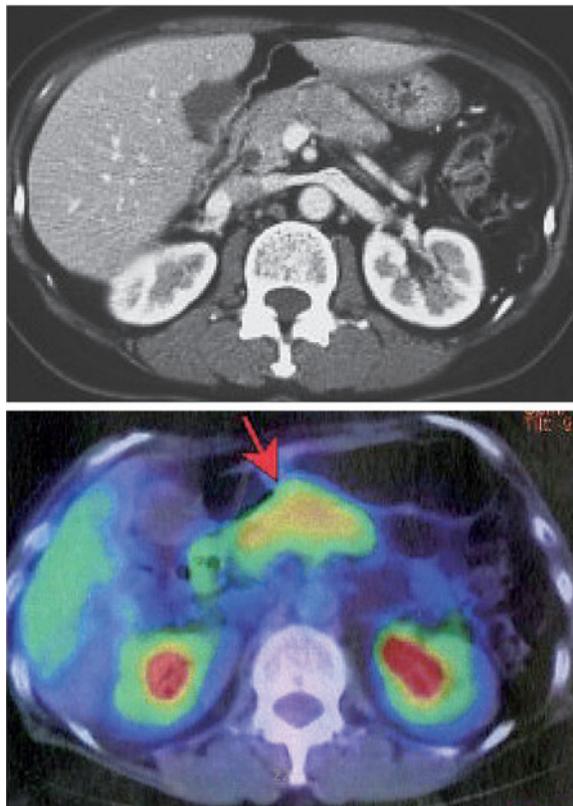


Fig. 3 FDG-PET demonstrated high uptake within the mass in the body of the pancreas (red arrow).

pancreatic tail, no dilatation of the main pancreatic duct was observed. Abdominal MR T1- and T2-weighted images showed low-signal intensity in the head and body of the pancreas. Endoscopic retrograde cholangiopancreatography was

performed, but because of hemispheric enlargement of the papilla of Vater, intubation of the pancreatic duct was difficult, and pancreatic duct imaging was impossible; however, the bile duct images were normal. Fluorodeoxyglucose (FDG) positron emission tomography (PET) showed uptake in the enlarged head and body of the pancreas (standard uptake value: 5.4 at 60 minutes, 7.0 at 120 minutes) (Fig. 3).

On the basis of these imaging findings and despite the difficulty in arriving at a definitive preoperative diagnosis, laparotomy was performed for suspected conventional pancreatic cancer or tumor-forming pancreatitis. The entire pancreas was markedly hardened, and the head and body of the pancreas were enlarged. Intraoperative rapid diagnosis with fine-needle aspiration cytology showed evidence of a pancreatic endocrine tumor. Because portal infiltration was observed, subtotal stomach-preserving pancreaticoduodenectomy, portal vein resection, and D2 lymph node dissection were performed. The postoperative course was uneventful and without complications. The patient recovered fully and was discharged on day 32 after the operation.

On gross examination the cut-surface of the resected specimen was grayish white and entirely fibrotic. Histopathological examination revealed scattered tumor cell nests in an abundant fibrotic stroma (Fig. 4a). The tumor was highly infiltrative, showing partial nerve infiltration. The tumor cells

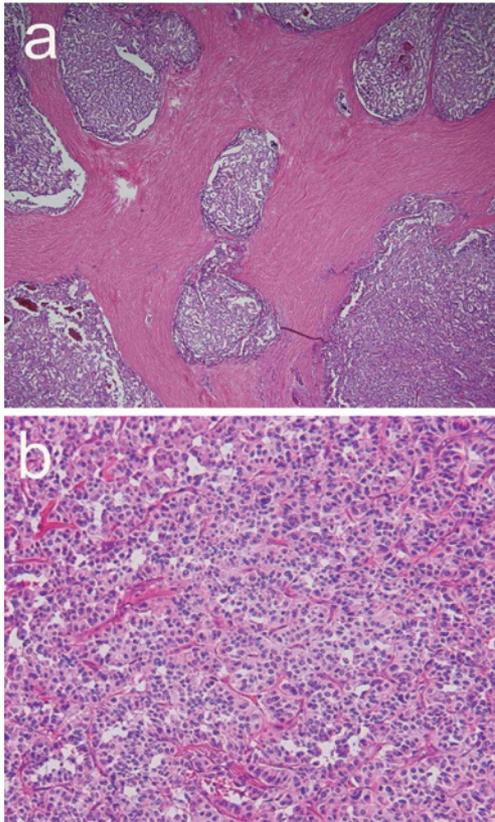


Fig. 4 **a:** Nests of tumor cells were embedded in prominent fibrous stroma (hematoxylin and eosin staining, $\times 40$). **b:** This tumor nest was composed of cuboidal cells with a high nuclear/cytoplasmic ratio, hyperchromatic nuclei, and mitotic figures ($\times 400$).

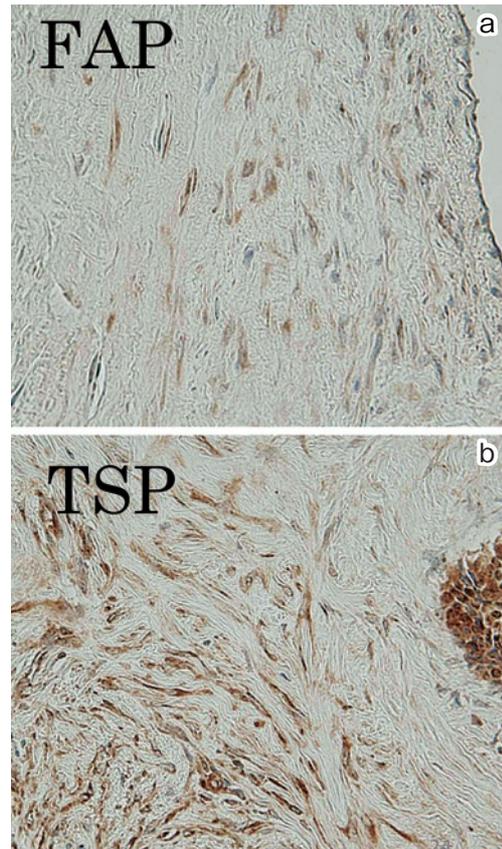


Fig. 5 **a:** Staining for FAP was positive in the fibroblasts in the stroma surrounding the tumor but not in the fibroblasts within the tumor-adjacent stroma. **b:** Both tumor cells and fibroblasts were positive for thrombospondin ($\times 400$).

had proliferated in cord-like and follicular patterns, with a high tumor nuclear/cytoplasmic ratio and abundant chromatin; mitotic figures were also seen (**Fig. 4b**).

Immunohistochemical studies showed staining for chromogranin A, neuron-specific enolase, and CD56 and but showed no staining for insulin, glucagon, gastrin, or somatostatin. With regard to the growth potential of the tumor, 2% or more cells showed staining for Ki-67. Accordingly, a nonfunctioning, well-differentiated pancreatic endocrine tumor was diagnosed.

According to the General Rules for the Study of Pancreatic Cancer 5th Edition, the tumor was classified as Pbh, $3 \times 2 \times 10$ -cm, mixed type, pT5, pCH (-), pDU (-), pS (-), pRP (-), pPV (-), pA (-), pPL (-), pOO (-), pN0, cM0, and stage III.

To examine the involvement of the TAFs in the fibrosis of the tumor stroma, immunostaining was

performed for fibroblast activation protein (FAP) α , a fibroblastic cell marker, and thrombospondin, a marker of tumor infiltration. After antigen retrieval, polyclonal antibodies against FAP- α (Abcam plc, Cambridge, UK) and thrombospondin (Abcam) diluted 1 : 100 were used as the primary antibodies, followed by staining with Autostainer (Dako, Glostrup, Denmark) by means of the EnVision method (Dako). Although some of the myofibroblastic cells in the abundant fibrous stroma surrounding the tumor were positive for FAP (**Fig. 5**), none of the fibroblastic cells adjacent to the tumor were positive for FAP. A high positivity rate for thrombospondin was observed in both the tumor cells and in the fibroblastic cells in the tumor stroma (**Fig. 5**). Neither thrombospondin nor FAP was expressed in the fibroblastic cells in the normal pancreatic tissue.

Discussion

Pancreatic endocrine tumors are rare tumors accounting for 1% to 2% of all pancreatic tumors; about 40% of all pancreatic endocrine tumors are reported to be nonfunctioning pancreatic endocrine tumors^{7,8}. Nonfunctioning pancreatic endocrine tumors produce no hormones or produce hormones at low levels, and no characteristic symptoms associated with the hormone secretion are observed³. Therefore, the diagnosis is often made after the tumors have become large, and symptoms, such as an abdominal mass, abdominal pain, and jaundice, are manifested; sometimes, these tumors are detected at routine checkups³, as in the present patient.

Our patient had imaging findings that differed from those of patients with typical pancreatic endocrine tumors. Normally, pancreatic endocrine tumors are hypervascular, and the imaging findings reflect their vascularity. Abdominal ultrasonography commonly shows a well-defined hypoechoic mass with a regular margin, and Doppler ultrasonography often shows blood-flow signals⁹. Plain CT reveals a well-defined low-density area, and contrast-enhanced CT, the most useful examination for preoperative diagnosis, shows marked enhancement in the arterial phase¹. In general, these tumors show low signal intensities on T1-weighted MR images and high signal intensities on T2-weighted MR images, reflecting the high density of the tumor cells^{10,11}. The mass in our patient was ill-defined and did not show blood-flow signals on Doppler ultrasonography or enhancement on contrast-enhanced CT but showed low signal intensity on both T1- and T2-weighted MR images. Histopathological examination showed prominent fibrosis of the tumor stroma; both tumor cell density and vascular density were low. These imaging findings seem to reflect the fibrosis of the tumor stroma. Sugawara et al.⁴ have reported a case of malignant pancreatic endocrine tumor in which the main imaging finding was fibrosis of the tumor. Ikenaga et al.³ have also reported a case of small nonfunctioning pancreatic endocrine tumor with fibrosis in which the lesion showed low signal

intensity on T2-weighted images, reflective of fibrosis, as in our patient. Irie et al.¹¹ have reported that the percentage of fibrosis in the stroma of nonfunctioning tumors is as high as 82%, as reflected by imaging findings that vary much more than those of functioning tumors.

Although our patient was first suspected to have pancreatic duct cancer, which is a nonhypervascular tumor, she had normal serum levels of tumor markers and no jaundice or dilatation of the main pancreatic duct. Although tumor-forming pancreatitis and autoimmune pancreatitis were also suspected, the patient had no history of alcohol intake or pancreatitis, had normal levels of IgG and IgG4, and was seronegative for antinuclear antibody. Subsequently, FDG-PET revealed FDG accumulation, and surgery was performed for a suspected malignant tumor; a definitive preoperative diagnosis could not be made.

Fibrosis of the tumor stroma involving TAFs reportedly promotes infiltration and proliferation of tumor cells and reflects the malignant grade of the tumor^{5,6}. Wels et al.¹² have proposed "migratory neighbors and distant invaders" as the sources of the TAFs, referring to 1) the involvement of the epithelial-mesenchymal transition (epithelial cells in the tumor tissues), 2) the presence of fibroblastic cells in the tumor stroma, and 3) the presence of bone marrow-derived stem cells. TAFs are present in the tumor stroma and are considered to be fibroblastic cells functioning in a microenvironment promoting tumor progression; unlike normal fibroblastic cells, they contain various tumor growth-promoting factors and fibroblastic cell markers^{5,13}. Normally, TAFs are identified on the basis of the expression of 1) fibroblastic cell markers (e.g., FAP), 2) thrombospondin 1 and stromelysin 1 as infiltration markers of the tumor cells, and 3) myofibroblastic cell markers and various growth factors (e.g., transforming growth factor β , vascular endothelial growth factor)^{6,13}.

In the present study, fibroblastic cells in the stroma surrounding the tumor expressed both FAP and thrombospondin 1, whereas the normal fibroblastic cells adjacent to the tumors did not express FAP, suggesting that the fibroblastic cells

are involved in promoting the prominent fibrosis of the tumor stroma. Many reports have described high rates of FAP expression in the TAFs in the tumor stroma, but not in the fibroblastic cells in the adjacent normal tissue, in colorectal cancer, ovarian cancer, and lung cancer^{14,15}. Cohen et al.¹⁶ have reported a high rate of FAP expression in invasive pancreatic cancer and have suggested that a high rate of FAP expression in TAFs surrounding the tumor correlates with the presence of lymph-node metastasis, tumor recurrence, and the extent of tumor invasion; moreover, they showed that low FAP expression in the fibroblastic cells adjacent to the tumor correlates with fibrosis of the tumor stroma. High FAP expression is considered to induce denaturation of the extracellular matrix and to inhibit fibrosis¹⁶, suggesting that the low FAP expression in the TAFs adjacent to the tumor promotes fibrosis of the tumor stroma. Meanwhile, thrombospondin, a glycoprotein observed in platelet α granules and the extracellular matrix, has been reported to promote tumor neovascularization and is closely involved in infiltration and metastasis in cancer tissues¹⁷. Kasper et al.¹⁸ have reported that the rate of thrombospondin expression is high in pancreatic duct cancer and that tumors expressing thrombospondin show a high capacity for infiltration and metastasis. In the present patient, high thrombospondin expression was noted in fibroblastic cells in the tumor stroma, suggesting the involvement of these cells in tumor infiltration, such as nerve infiltration. In the limited study in this patient, we were not able to confirm that the TAFs are involved in the prominent fibrosis of the tumor stroma. Further study of a larger number of patients is necessary in the future.

Conflict of Interest: Arichika Hoshino and other co-authors have no conflict of interest.

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