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Immunohistochemical analysis of IMP3 and p53 expression in endoscopic ultrasound-guided fine needle aspiration and resected specimens of pancreatic diseases

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ABSTRACT

Background: Insulin-like growth factor II messenger ribonucleic acid-binding protein 3 (IMP3) is a valuable marker that distinguishes malignant from benign lesions and predicts prognosis.
Methods: First, we evaluated IMP3 expression in 77 resected specimens of pancreatic ductal adenocarcinoma (PDAC), intraductal papillary mucinous neoplasm (IPMN), and chronic pancreatitis (CP). Eleven PDAC patients preoperatively underwent endoscopic ultrasound-guided fine needle aspiration (EUS-FNA). Survival analysis of IMP3 and clinicopathological factors was performed. IMP3 and p53 expression was evaluated in another 127 EUS-FNA samples of solid pancreatic masses to compare the diagnostic value of routine and immunohistochemical staining.
Results: IMP3 expression was detected in 72.3%, 50%, 20%, and 0% of PDAC, malignant IPMN, benign IPMN, and CP, respectively. Evaluation of IMP3 expression in EUS-FNA specimens coincided with that in resected specimens in 10 of 11. IMP3 expression correlated with tumor differentiation in PDAC samples ($p = .006$) and with poor prognosis through univariate analysis ($p = .045$). Tumor differentiation and lymph node metastasis were significantly associated with poor prognosis through multivariate analysis. In EUS-FNA specimens, the sensitivity, specificity, and accuracy of cytohistological analysis were 80.8%, 100%, and 85.0%, respectively. IMP3 and p53 expression were detected in 80.8% and 44.9% of malignant and 0% and 5% of benign lesions. Combined with IMP3 immunostaining, the sensitivity, specificity and accuracy of cytohistological analysis significantly increased to 87.9%, 100%, and 90.8% ($p = .016$), respectively. Meanwhile, p53 staining had no impact on the results.
Conclusions: IMP3 immunohistochemical staining can improve the diagnostic accuracy of EUS-FNA for malignant pancreatic tumors.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive malignant tumors with a 5-year survival rate of only 4% [1]. Therefore, pathological diagnosis of malignancy before treatment is important in suspected cases. It is reported that endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is a safe and effective technique for diagnosis of pancreatic tumors with a sensitivity of 78%–95% [2–5]. However, specimens obtained by EUS-FNA are very small, thus differential diagnosis of malignant disease from benign disease is sometimes challenging. To improve the sensitivity and accuracy of EUS-FNA for pancreatic tumors, various type of puncture needles, puncture techniques, and

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molecular biological approaches, including immunohistochemical staining and detection of gene mutations, have been investigated [6–11].

Immunohistochemical markers to differentiate between malignant and benign tissue would be beneficial. Insulin-like growth factor II messenger ribonucleic acid (mRNA) binding protein 3 (IMP3) is an oncofetal protein that is expressed during embryogenesis and is almost silenced in normal mature tissues. IMP3 may play an important role in mRNA trafficking and stabilization, localization, cell growth, and cell migration [12]. IMP3 is expressed in many malignant neoplasms, including renal cell carcinoma [13], ovarian carcinoma [14], urothelial carcinoma [15], lung carcinoma [16], colorectal cancer [17], and PDAC [18–24]. It was recently reported that IMP3 overexpression in PDAC was significantly associated with poor prognosis and IMP3 may be potential therapeutic target for PDAC.

A few studies have shown that IMP3 expression in PDAC is a valuable marker to distinguish this entity from a benign lesion using histological samples obtained with a core biopsy needle or cytological samples obtained with a FNA needle [11,18]. However, it remains unclear whether cytohistological analysis combined with IMP3 expression significantly improved the diagnostic value, and there has been no report on the diagnostic usefulness of IMP3 expression in histological samples obtained with conventional FNA needles.

The p53 tumor suppressor gene is significantly mutated in many tumors, including human pancreatic cancer (38.2–81.1%). Hence, immunohistochemical staining of p53 might improve the diagnostic accuracy of PDAC [25–30].

The aims of the present study were to evaluate IMP3 expression in surgical resected specimens by immunohistochemical staining and analyze the relationship between clinicopathological factors and prognosis of PDAC. In addition, IMP3 expression was examined in paired EUS-FNA specimens and 11 resected PDAC specimens. Finally, the diagnostic values of IMP3 and p53 in EUS-FNA specimens of solid pancreatic masses were evaluated.

Materials and methods

Case selection

Large tissue sections were randomly collected from patients with pancreatic lesions, including 47 with PDAC, 10 with malignant intraductal papillary mucinous carcinoma (IPMN), 10 with benign IPMN, and 10 with chronic pancreatitis (CP) who underwent pancreatic resection from June 2010 to July 2013 at Chiba University Hospital (Chiba, Japan). In this study, IPMN was classified into four groups: IPMN with low-grade dysplasia (LGD), intermediated-grade dysplasia (IGD), high-grade dysplasia (HGD), and an associated invasive carcinoma, according to the World Health Organization classification [31]. Malignant IPMN was defined as HGD and invasive carcinoma. Benign IPMN was defined as LGD and IGD. Among the resected samples, 11 PDAC samples were collected by EUS-FNA before surgery.

First, to evaluate IMP3 expression in large tissue sections, IMP3 staining was performed for all 77 resected specimens. Next, IMP3 expression was evaluated in 11 EUS-FNA specimens and correlated to IMP3 expression of PDAC resected specimens. Moreover, an additional 127 EUS-FNA samples of solid pancreatic masses were collected from 122 patients (83 men, 39 women; mean age, 65.8 ± 10.4 years; age range, 36–84 years) between June 2013 and June 2015 in our institution.

The study protocol was approved by the institutional ethics review board of Chiba University Hospital (approval number: #242, June 29th, 2016).

Resection specimens

Seventy-seven resected specimens were histologically examined according to the 7th TNM classification system [32]. In PDAC, we evaluated the relationship between IMP3 expression and clinicopathological factors, including sex, age, tumor location, tumor size, residual tumor classification, lymph node metastasis, tumor differentiation, and expression levels of carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9). Also, the relationships between IMP3 expression and clinicopathological factors and prognosis were evaluated.

Immunohistochemical staining of resected specimens

Paraffin blocks were sliced into 4 μm-thick sections. After deparaffinization and rehydration, heat-induced antigen retrieval was performed by immersing the sections in high pH buffer solution (pH 9.0) at 97 °C for 20 min. Then, endogenous peroxidase activity was blocked by incubation with blocking agent for 5 min at room temperature. Afterward, the sections were incubated for 20 min at room temperature with a mouse monoclonal antibody against IMP3 (clone 69.1; Dako, Glostrup, Denmark) at a dilution of 1:100. The primary antibodies were detected by incubating the sections with polymer reagent for 20 min at room temperature. The sections were subjected to color development with 3,3′-diaminobenzidine and counterstained with hematoxylin. Normal placental tissue was used as a positive control for IMP3 immunostaining. All slides of the resected specimens stained with hematoxylin and eosin (HE) and immunohistochemical slides were reviewed by the same experienced pathologist.

Specimens obtained by EUS-FNA

Most patients underwent EUS-FNA for pathological diagnosis of pancreatic tumors before surgery or chemotherapy. EUS-FNA was performed with a 19, 22, or 25 gauge needle and an average of 3.1 ± 1.0 passes per session (Table 3). The aspirate biopsy materials were pushed onto filter paper by re-inserting the stylet and into formalin-filled container for histological analysis. The residual material was smeared onto a glass slide by air pressure and fixed with 95% ethanol for cytological analysis. Biopsy specimens fixed in formalin were embedded in paraffin and stained with HE for histological analysis. By histological analysis, the specimens were classified into one of five types: malignancy, suspected malignancy, atypical cells, no evidence of malignancy, or insufficient material. In this study, specimens classified as malignancy and suspected malignancy by HE staining were defined as positive for malignancy, and those classified as atypical cell, no evidence of malignancy, and insufficient materials were defined as negative for malignancy.

The final diagnosis was based on the following: (i) histological analysis of surgically resected specimens; (ii) cytopathological detection of cancer cells from other organs coupled with clinical and/or radiological evidence of progressive disease, and benign lesion was diagnosed by surgically resected specimen or clinical follow-up for at least 6 months, with no evidence of progressive disease.

Immunohistochemical staining of EUS-FNA specimens

Of 127 consecutive cases, 75 sections were stained with HE and immunohistochemically stained with anti-IMP3 and *anti*-p53 antibodies at the same time, while 52 sections immunohistochemically stained against IMP3 and p53 were prepared later. The primary antibodies used for IMP3 immunohistochemistry were mouse monoclonal anti-human IMP3 (clone 69.1; Dako) at a

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