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ORIGINAL ARTICLE

Intranuclear cytoplasmic inclusions are a specific feature of intraductal papillary mucinous neoplasms that distinguish contaminating gastric epithelium

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Introduction Low-grade intraductal papillary mucinous neoplasms (IPMN) are challenging to diagnose because of an absence of reliable morphologic or immunohistochemical features to distinguish them from contaminating gastric foveolar epithelium. After noting intranuclear cytoplasmic inclusions (ICIs) in some cases of IPMN, we investigated whether ICIs could be used as a specific feature to distinguish IPMN from gastric foveolar epithelium.

Materials and methods A consecutive cohort of 61 transduodenal endoscopic fine-needle aspirations of histologically or clinically verified pancreatic IPMNs without high-grade dysplasia from 2005 to 2012 were identified. A control cohort of 24 endoscopic fine-needle aspirations containing gastric epithelium was selected from transgastric specimens of nonpancreatic targets from the same period. Every fragment of mucinous epithelium in the 2 cohorts was examined in alcohol-fixed and cell block sections at high magnification to identify ICIs.

Results ICIs were observed in 31% (19 of 61) of cases in mucinous epithelial fragments obtained by fine-needle aspirations from low-grade IPMNs. When present, they were seen in about 1% of all cells. No ICIs were identified in the control cohort of 24 patients with normal gastric epithelium ($P = 0.001$ Fisher exact test). *BRAF* mutation (V600E) testing was performed on 5 IPMN cases, and was negative in all cases including 2 with and 3 without ICIs. *KRAS* mutation testing was performed on 9 cases of

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IPMN cases. Two cases with ICIs tested positive for *KRAS* mutations. Four cases without ICIs also tested positive, and 3 cases without ICIs tested negative.

Conclusions ICIs are a specific morphologic feature found in about one third of low-grade IPMNs, but absent in gastric foveolar epithelium. There is no obvious molecular correlate with the presence of ICIs.

Introduction

Intraductal papillary mucinous neoplasms (IPMN) are challenging to diagnosis. The standard diagnostic approach relies on endoscopic ultrasound, and existing endoscopes are not well suited for core biopsy sampling for histologic evaluation of the wall of the cyst. Instead, fine-needle aspiration (FNA) sampling is used during endoscopic ultrasound. FNA samples of IPMN are difficult to interpret.[1,2] Low-grade IPMN are typically diploid, and by definition, there is no cellular stratification in the absence of high-grade pancreatic intraepithelial neoplasia. The diploid nature of low-grade IPMN is reflected in a population of cells in which the total degree of hematoxylin staining is identical to normal gastrointestinal mucosa—ie, there is no hyperchromasia. The nonstratified nature is reflected in the FNA samples as flat 2-dimensional groups of epithelium similar to normal (nonstratified) glandular cells. Crowding of nonstratified epithelial cells, resulting in a "drunken honeycomb pattern," may be suggestive of a low-grade neoplastic proliferation, but the reproducibility of this criterion for diagnosis is not well established, and reparative change in glandular epithelium (eg, changes in gastric surface epithelium in the setting of gastritis) can lead to some degree of irregular cellular spacing. Low-grade IPMN may contain goblet cells, reminiscent of Barrett mucosa in the esophagus, but goblet cells are not invariably present, and they may be difficult to recognize in cytology samples.

A major problem with FNA sampling is that FNAs commonly contain contaminating epithelium from the gastrointestinal tract, in spite of the use of a stylet.[1,3] Masses in the head of the pancreas are usually biopsied using a transduodenal approach, whereas the lesions in the body and tail are approached using a transgastric approach.[1] Gastrointestinal contamination in pancreatic FNA cytology specimens causes diagnostic problems because gastric foveolar-type epithelium can closely resemble IPMN cytology, which can result in an erroneous diagnosis.[2,3] Duodenal-/intestinal-type epithelium is easier to distinguish from the epithelium of an IPMN because of the presence of a well-defined brush border.[4]

Immunohistochemical markers can distinguish between ductal adenocarcinoma from IPMN and reactive processes.[5-9] However, no reliable markers have been developed that specifically can distinguish IPMN from benign gastric epithelium. Molecular studies for *KRAS* and *GNAS* mutations demonstrate potential use for the diagnosis of IPMN with a rate 47% and 41%, respectively.[10-12]

In this paper, we show that a purely cytomorphologic feature–intranuclear cytoplasmic inclusions (ICIs)—are present in about one-third of low-grade IPMN, and these are specific for low-grade IPMN, allowing its distinction from contaminating gastric foveolar mucosa.

Material and methods

The cytopathology files were searched for pancreatic FNAs bearing the word "mucin" or "mucinous" in the diagnosis between January 1, 2005 and December 31, 2012. This search field yielded 168 cases. We restricted this set to cases in which the diagnosis or follow-up was consistent with intraductal papillary mucinous neoplasm. Cases with insufficient follow-up or vague diagnostic terminology were excluded. We excluded cases in which high-grade dysplasia or worse were found, and we only included cases in which the FNA was performed in a transduodenal approach. By including only cases that were transduodenal, we expected to be able to be relatively certain that contaminating gastric-type mucosa was not being evaluated. We included 61 consecutive cases in the study group. A control group was selected by searching for transgastric endoscopic ultrasound—FNAs of nonpancreatic lesions. By restricting attention to transgastric FNAs of nonpancreatic targets, we expected that any gastric contaminating epithelium would be unambiguous. We pulled 57 cases from the files to search for any possible gastric epithelium in the samples. Of the 57 cases, 25 cases contained cytologically definitive contaminating gastric epithelium. On-site evaluations for specimen adequacy were prepared as Diff-Quik (American Scientific Products, McGraw Park, Ill.) stained air-dried smears. Additional smears were alcohol fixed on-site for Papanicolaou staining. Every fragment of mucinous epithelium in the 2 groups was examined by 1 observer (P.L.) at 40× magnification to search for the presence of ICIs. For this evaluation, only alcohol-fixed mucinous fragments were studied, including direct smears and ThinPrep slides (Hologic, Inc., Bedford, Mass.), both stained by Papanicolaou technique, and Cellient (Hologic, Inc.) cell block sections stained with hematoxylin and eosin. All cases with ICIs were verified by a second observer (A.F.) and photographed. An ICI was defined as a partial invagination of a spherical to ovoid portion of cytoplasm into the nucleus, the edges of which were sharply demarcated from the chromatin by a rim of heterochromatin on the nuclear side of the inclusion.

Mutation testing either on a sample of cyst aspirate fluid or on resected samples started in 2008 at the University of