Mutant *TP53* in Duodenal Samples of Pancreatic Juice From Patients With Pancreatic Cancer or High-Grade Dysplasia

MITSURO KANDA,[*] YOSHIHIKO SADAKARI,[*] MICHAEL BORGES,[*] MARK TOPAZIAN,[‡] JAMES FARRELL,[§] SAPNA SYNGAL,[¶] JEFFREY LEE,[‖] IHAB KAMEL,[#] ANNE MARIE LENNON,[**] SPENCER KNIGHT,[*] SHO FUJIWARA,[*] RALPH H. HRUBAN,[*,‡‡] MARCIA IRENE CANTO,[**] and MICHAEL GOGGINS[*,**,‡‡]

[*]Department of Pathology, [#]Department of Radiology, [**]Department of Medicine, [‡‡]Department of Oncology, the Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins Medical Institutions, Baltimore, Maryland; [‡]Mayo Clinic, Rochester, Minnesota; [§]Dana Farber Cancer Institute, Boston, Massachusetts; [‖]MD Anderson Cancer Center, Houston, Texas; and [¶]University of California at Los Angeles, Los Angeles, California

BACKGROUND & AIMS: **Imaging tests can identify patients with pancreatic neoplastic cysts but not microscopic dysplasia. We investigated whether mutant *TP53* can be detected in duodenal samples of secretin-stimulated pancreatic juice, and whether this assay can be used to screen for high-grade dysplasia and invasive pancreatic cancer.**

METHODS: **We determined the prevalence of mutant *TP53* in microdissected pancreatic intraepithelial neoplasias (PanINs), intraductal papillary mucinous neoplasms (IPMNs), and invasive adenocarcinomas. *TP53* mutations were quantified by digital high-resolution melt-curve analysis and sequencing of secretin-stimulated pancreatic juice samples, collected from duodena of 180 subjects enrolled in Cancer of the Pancreas Screening trials; patients were enrolled because of familial and/or inherited predisposition to pancreatic cancer, or as controls.**

RESULTS: ***TP53* mutations were identified in 9.1% of intermediate-grade IPMNs (2 of 22), 17.8% of PanIN-2 (8 of 45), 38.1% of high-grade IPMNs (8 of 21), 47.6% of PanIN-3 (10 of 21), and 75% of invasive pancreatic adenocarcinomas (15 of 20); no *TP53* mutations were found in PanIN-1 lesions or low-grade IPMNs. *TP53* mutations were detected in duodenal samples of pancreatic juice from 29 of 43 patients with pancreatic ductal adenocarcinoma (67.4% sensitivity; 95% confidence interval, 0.52–0.80) and 4 of 8 patients with high-grade lesions (PanIN-3 and high-grade IPMN). No *TP53* mutations were identified in samples from 58 controls or 55 screened individuals without evidence of advanced lesions.**

CONCLUSIONS: **We detected mutant *TP53* in secretin-stimulated pancreatic juice samples collected from duodena of patients with high-grade dysplasia or invasive pancreatic cancer. Tests for mutant *TP53* might be developed to improve the diagnosis of and screening for pancreatic cancer and high-grade dysplasia. Clinical Trial numbers: NCT00438906 and NCT00714701.**

Keywords: Tumor; Biomarker; Diagnostic; Early Detection.

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Pancreatic cancer is the fourth leading cause of cancer-related deaths in the United States.[1] Most patients with pancreatic ductal carcinoma are diagnosed with advanced disease. Patients who present with early stage disease have the best outcome, but surgery is only an option for approximately 15% of patients with pancreatic cancer.[1] Early detection is considered potentially one of the most effective approaches to improving the prognosis of this dismal disease.

Effective early detection necessitates detecting potentially curable lesions before they become symptomatic such as subcentimeter invasive cancers and precursors with high-grade dysplasia.[2] The most common precursors to pancreatic adenocarcinoma are pancreatic intraepithelial neoplasias (PanINs). PanINs are microscopic lesions (by definition, <5 mm diameter); intraductal papillary mucinous neoplasm (IPMNs) are larger cystic pancreatic precursor neoplasms[3] with an estimated prevalence of approximately 2% of older adults.[4] PanINs and

Abbreviations used in this paper: CAPS, Cancer of the Pancreas Screening; ERCP, endoscopic retrograde cholangiopancreatography; EUS, endoscopic ultrasonography; HRM, high-resolution melt-curve analysis; IPMN, intraductal papillary mucinous neoplasm; MRCP, magnetic resonance cholangiopancreatography; MRI, magnetic resonance imaging; PanIN, pancreatic intraepithelial neoplasia; PCR, polymerase chain reaction.

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IPMNs are generally asymptomatic and are identified only incidentally or by screening.

Screening is justifiable only when offered to individuals at sufficient risk of developing pancreatic cancer and when the screening test is safe and effective. An ideal screening test would be a highly accurate blood test; however, no blood test has been shown to be as accurate as pancreatic imaging tests (computed tomography, endoscopic ultrasonography [EUS]) for diagnosing symptomatic pancreatic cancers, never mind small asymptomatic cancers and precursor neoplasms.

Screening protocols for individuals with a strong family history of pancreatic cancer use pancreatic imaging tests (usually EUS and/or magnetic resonance imaging/magnetic resonance cholangiopancreatography [MRI/MRCP]).[5–12] Individuals at significantly increased risk can be identified based on their family history of the disease, but we still lack an effective screening test to offer these individuals.[13] EUS and MRI/MRCP are very effective for identifying small pancreatic cysts,[5] but PanINs are generally too small to be identified by these tests[14]; they are only identified after histologic examination of resected pancreata. Low-grade PanIN-1 lesions are prevalent in older adults but PanIN-3 lesions (high-grade dysplasia) usually are found in pancreata of patients with invasive pancreatic cancer and in subjects undergoing pancreatic screening.[15,16] The inability to identify PanINs preoperatively highlights the need for novel diagnostic approaches to identify PanINs. One promising approach is to analyze pancreatic juice for mutations arising from pancreatic neoplasms. Markers of pancreatic cancer in ductal pancreatic juice collected during endoscopic retrograde cholangiopancreatography (ERCP) have been studied,[17,18] but ERCP is too invasive to use for pancreatic screening. We recently reported on the diagnostic potential of secretin-stimulated pancreatic juice samples collected from the duodenum during upper endoscopy.[19] We found that *guanine nucleotide-binding protein* mutations, a highly specific marker of IPMNs, were detected reliably in these samples in subjects with IPMNs and diminutive cysts (<5 mm), suggesting that pancreatic juice is a reliable sample for detecting molecular alterations in the pancreatic ductal system.[19]

Useful markers of pancreatic neoplasia need to accurately distinguish early invasive pancreatic cancers and high-grade dysplasia (PanIN-3 and IPMNs with high-grade dysplasia) from lesions with low-grade dysplasia. Mutant *TP53* may be one such marker. It is mutated in approximately 75% of invasive pancreatic cancers,[20,21] with a similar prevalence in familial and sporadic pancreatic cancers,[21,22] and immunohistochemical studies have suggested that *TP53* mutations occur late in the progression of PanIN lesions.[23] In contrast, other genes commonly mutated in pancreatic ductal adenocarcinomas, *KRAS*, *p16*, and *SMAD4*, do not have these diagnostic characteristics: *KRAS* mutations are present in more than 90% of PanIN-1 lesions,[24] and commonly are detected in the pancreatic juice of controls.[18,24] *P16* mutations are also thought to arise throughout PanIN development, therefore these mutations may not distinguish low-grade from high-grade PanINs. Genetic inactivation of *SMAD4* is thought to be specific for PanIN-3 and invasive cancer,[25] but *SMAD4* commonly is inactivated by homozygous deletion and such alterations have been detected only in secondary fluids when the deletion has been characterized first in the primary cancer.[26]

In this study, we determined the prevalence of *TP53* mutations in PanIN and IPMNs, and used digital high-resolution melt-curve analysis (HRM) and sequencing to measure *TP53* mutation concentrations in duodenal collections of pancreatic juice of individuals undergoing pancreatic evaluation.

Materials and Methods

All elements of this investigation were approved by The Johns Hopkins Medical Institutional Review Board and written informed consent was obtained from all patients. All authors had access to the study data and reviewed and approved the final manuscript.

Patients and Specimens

Pancreatic juice samples and subject data for this study were obtained from 180 participants enrolled in the Cancer of the Pancreas Screening (CAPS)2, CAPS3, and CAPS4 clinical trials[5,9] (http://clinicaltrials.gov, NCT00438906 and NCT00714701). Subjects enrolled for screening were asymptomatic with either (1) a strong family history of pancreatic cancer (at least 2 affected blood relatives with pancreatic cancer related by first-degree); the eligibility age in CAPS4 was 50 to 80 years or 10 years younger than the youngest pancreatic cancer in the kindred; (2) germ line mutation carriers (*BRCA2*, *p16*, *BRCA1*, hereditary nonpolyposis colorectal cancer genes) with a family history of pancreatic cancer; or (3) Peutz–Jeghers syndrome. Disease controls undergoing pancreatic evaluation also were enrolled to evaluate pancreatic juice markers, and we included subjects from CAPS2 through CAPS4 to have sufficient disease controls and adequate follow-up evaluation subsequent to pancreatic juice collections to identify prospective cancers. See the Supplementary Materials and Methods section for further details.

Pancreatic juice secretion was stimulated by infusing intravenous human synthetic secretin (0.2 μg/kg over 1 min), 10 to 20 mL of juice then was collected from the duodenal lumen for approximately 5 minutes by suctioning fluid through the echoendoscope channel. Secretin was provided for CAPS3 and CAPS4 (ChiRhoClin, Inc, Burtonsville, MD), and CAPS2 (Repligen, Corp, Waltham, MA). Juice aliquots (10–20) were stored without additional processing at -80°C before use. Extracted DNA was quantified by real-time polymerase chain reaction (PCR).[19]

Laser Capture Microdissection

PanINs, IPMNs, and normal duct samples identified during intraoperative frozen section analysis of resected pancreata by R.H.H. (from 2007 to 2010) were selected for *TP53* analysis as previously described.[24] For comparison, we analyzed frozen sections of pancreatic ductal adenocarcinomas (Table 1 and Supplementary Materials and Methods).

High-Resolution Melt-Curve Analysis

To evaluate DNA from PanIN, IPMNs, and ductal adenocarcinoma tissues for mutations, HRM was performed in triplicate as previously described.[24] We evaluated the limit of detection and accuracy of digital HRM (Supplementary Figure 1).

For juice analysis, digital HRM analysis was used and assays almost always were performed blinded to the final diagnosis. In each 96-well plate, 900 genome equivalents of pancreatic juice DNA were dispensed into 90 wells (10 genome equivalents per