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CCR Translations

Clinical Cancer Research

Sequencing Pancreatic Juice: Squeezing the Most Out of Surveillance

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Next-generation sequencing of pancreatic juice can detect and quantify tumor-promoting mutations, supporting imaging and cytology findings to predict the degree of dysplasia in patients at high risk for pancreatic cancer. Future studies are needed to optimize this approach and determine how it best fits into clinical practice. *Clin Cancer Res; 24(12); 2713–5.* ©2018 AACR.

See related article by Suenaga et al., p. 2963

In this issue of *Clinical Cancer Research*, Suenaga and colleagues (1) describe that next-generation sequencing of pancreatic juice collected from patients at high risk for pancreatic cancer can identify the presence and concentration of mutations that support imaging and cytologic findings and inform risk stratification. Late onset of symptoms, frequent metastasis, and lack of effective therapies all contribute to the dismal prognosis for patients with pancreatic cancer, highlighting the need for tools that aid in early detection and accurate surveillance of patients known to be at high risk because of family history, known genetic predisposition, and incidentally discovered pancreatic masses that are becoming increasingly recognized due to widespread use of high-resolution imaging modalities in routine care.

Guidelines for surveillance of individuals at high risk for pancreatic cancer due to familial and/or inherited susceptibility have been developed in the past decade because of efforts by groups such as the International Cancer of the Pancreas Screening (CAPS) Consortium (2). Imaging by MRI and endoscopic ultrasound (EUS) is currently recommended, but important factors, such as age of initiation, frequency, and improvements in outcomes provided by surveillance, are still unclear. There is hope that any number of serum or pancreatic biomarkers, including mutation analysis of pancreatic juice, might one day enable detection of premalignant lesions and cancer in these patients before becoming visible by imaging.

Workup for asymptomatic pancreatic cystic neoplasms (PCN) also relies on these imaging modalities and fine needle aspiration (FNA) taken during EUS to assess cyst morphology, cytology, and cyst fluid biomarkers like carcinoembryonic antigen (CEA). Although these techniques can reliably differentiate the major classes of PCNs and direct patients with low-risk serous cysts into conservative surveillance, a large percentage of patients are found to have premalignant mucinous cysts for which imaging and FNA are unable to adequately estimate risk. Current guidelines have tended toward aggressive surgical management (3), but it has become increasingly clear that many patients with only low-grade neoplasms undergo aggressive surgical resections associated with significant morbidity and mortality. Moreover, the small lesions now thought to be the predominant precursor to most invasive cancers, pancreatic intraepithelial neoplasias (PanIN), can be missed at early and more curative stages.

Next-generation sequencing of pancreatic juice, the method described by Suenaga and colleagues (1), has the potential to overcome some of these limitations of current surveillance and diagnostic techniques. Advances in technology now allow detection of mutations from low-abundance DNA, which this study leverages to analyze duodenal aspirates of pancreatic juice for mutations in known drivers of pancreatic tumorigenesis. Pancreatic juice was analyzed from 67 patients from the CAPS Consortium with diverse clinical findings, including pancreatic cancer, resected low- or high-grade dysplastic neoplasms, familial or inherited risk without imaging abnormalities, and normal pancreas controls. Sequences of 12 genes were analyzed to derive associations connecting mutations with known deleterious effects to imaging and pathology results. There was striking concordance between known pancreatic cancer or high-grade dysplasia and the overall concentration of juice mutations found in pancreatic juice, especially mutations in *TP53* and *SMAD4*, two known markers of advanced pancreatic dysplasia.

From a practical standpoint, collection of pancreatic juice could be integrated into the EUS-FNA procedure performed to classify concerning PCNs, allowing for stepwise analyses of FNA cytology, cyst fluid biomarkers, and pancreatic juice mutations (Fig. 1). The relatively high specificity of FNA for detecting invasive cancer suggests that sequencing analysis of pancreatic juice may be best suited following inconclusive cytology and cyst fluid results. Juice mutation in genes like *TP53* or *SMAD4* could then inform decisions to increase frequency of follow-up or trigger surgical resection when combined with higher risk imaging or cytologic findings. Conversely, lack of mutations might lead patients toward more conservative MRI surveillance. Studies in larger cohorts of patients with a variety of pancreatic

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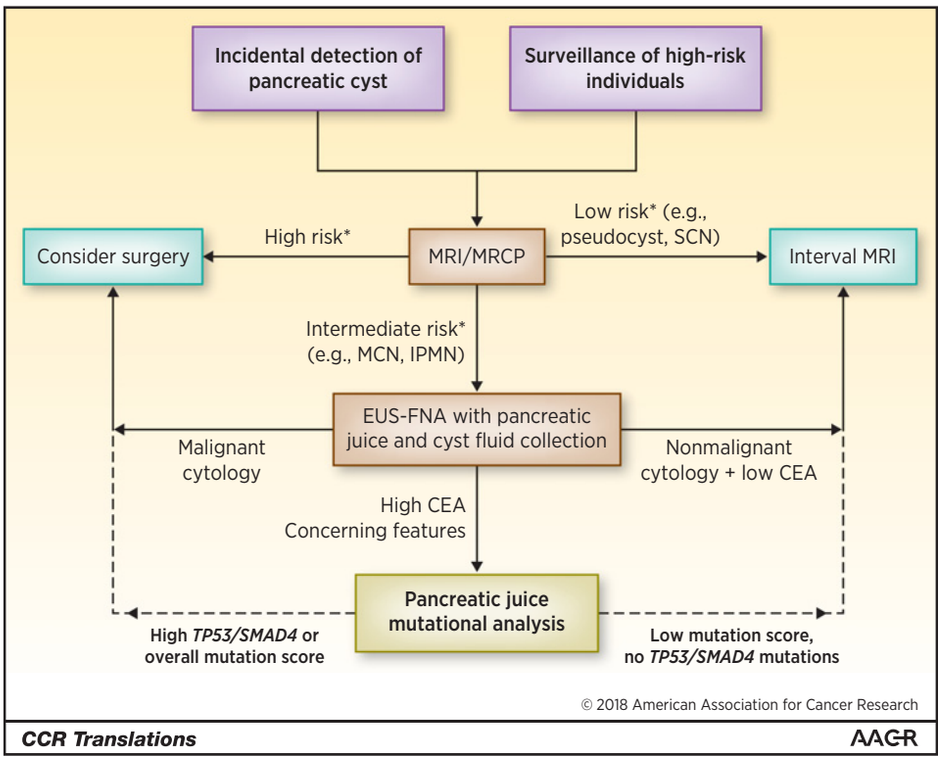

Figure 1.
Potential role for pancreatic juice mutational analysis within clinical management of pancreatic masses. Theoretically, pancreatic juice collected during EUS-FNA is sequenced following indeterminate imaging, cytology, and cyst fluid findings to inform risk stratification and guide treatment decisions. *, Based on imaging features such as size, ductal involvement, and solid component (see ref. 2). IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm; MRCP, magnetic resonance cholangiopancreatography; SCN, serous cystic neoplasm.

pseudocyst and cyst types are needed to clarify the relative effectiveness and practicality of pancreatic juice mutations versus imaging and cytologic studies in dictating effective treatment decisions.

The exciting possibilities for advances in diagnostic sequencing are exemplified by the Goggins group's history of studying pancreatic juice mutations. Ten years ago, pancreatic juice was being analyzed for mutations at only codon 12 of the *KRAS* oncogene using creative but very low-throughput PCR-based methods (4). In 2015, pancreatic juice mutations in specific regions of *GNAS* and *KRAS* were assessed using digital high-resolution melt-curve analysis and pyrosequencing (5). Accurate detection of low-abundance mutations across 12 genes in a minimally cellular sample like pancreatic juice is a sign that even more comprehensive and clinically meaningful analyses are on the horizon.

Despite its promise, unanswered questions remain as to how this method fits into both surveillance of high-risk individuals and the diagnosis of pancreatic neoplasms. Although imaging and juice mutations in this study often agreed with one another, in some cases, they pointed to drastically different results that would have provided distressing mutation information to patients and potentially increased the likelihood of aggressive surgical intervention without clear benefit.

Over half (8/14) of the patients undergoing surveillance for familial pancreatic cancer or germline *BRCA1/2* mutations with no identifiable imaging abnormalities were found to harbor juice mutations in one or more of *KRAS* (7/14), *GNAS* (3/14), and, especially concerningly, *TP53* (3/14), a marker of advanced dysplasia. A third (3/9) of normal control individuals for whom no testing would be indicated were found to have mutations in *KRAS* (3/9). In these patients, clinical and imaging results would indicate continued infrequent monitoring or no monitoring at all, whereas juice mutations suggest the possibility of latent high-grade dysplasia or cancer but do not provide information about anatomic location or the level of urgency to intervene. Larger longitudinal studies to track outcomes in patients harboring diverse juice mutations will allow for proper interpretation of their significance in the context of general screening protocols for high-risk individuals.

Like other imaging and cytologic tests, pancreatic juice sequencing alone lacks the sensitivity needed to reliably diagnose pancreatic neoplasms. For example, five of 14 patients with known cancer had juice mutation scores of <12, and the metric with the highest diagnostic accuracy, overall mutation scores combined with any mutations in *TP53* or *SMAD4*, still did not detect two pancreatic cancer cases. Inability to distinguish invasive cancers within this highly selected patient population relatively uncomplicated by other pancreatic pathology and types of PCNs suggests caution when applying these results to broader patient populations and necessitates larger and more inclusive studies before incorporation as a routine diagnostic workup for PCNs.

There is ample cause for optimism that the results of this study will catalyze advances toward use of pancreatic juice analysis as part of standard clinical management of PCNs. Further insight into additional genes with meaningful mutations, elucidation of functions of variants of unknown significance that were excluded from this study's analyses, and potential inclusion of tumor-promoting epigenetic or proteomic juice changes could all improve the method's diagnostic accuracy and clinical applicability. In the future, pancreatic juice may also be able to act as a proxy for easily overlooked subpopulations of advanced premalignant or invasive cancer

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cells, minimizing unnecessary surgeries while promoting treatment of cancer at its most manageable stages.

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No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: M.B. Lipner
Development of methodology: M.B. Lipner
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.J. Yeh
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.B. Lipner, J.J. Yeh
Writing, review, and/or revision of the manuscript: M.B. Lipner, J.J. Yeh

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