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MicroRNA Dissects Out Dangerous Pancreatic Cysts from All the Rest

Ryan M. Thomas and Jason B. Fleming

Malignant transformation of pancreatic cysts occurs in only a fraction of patients. The diagnostic dilemma is identifying which cysts pose a cancerous threat. Cyst fluid has been analyzed in a variety of ways to answer this question but the microRNA (miRNA) profile of the fluid may finally hold the answer. *Clin Cancer Res; 18(17); 4482–4. ©2012 AACR.*

In this issue of *Clinical Cancer Research*, Matthaei and colleagues provide evidence that examination of miRNA-could provide the needed testing approach to discern malignant potential in cystic neoplasms of the pancreas (1). Three different precursor lesions to invasive pancreatic ductal adenocarcinoma (PDAC) have been described: pancreatic intraepithelial neoplasia (PanIn), intraductal papillary ductal mucinous neoplasm (IPMN), and mucinous cystic neoplasms (MCN). Both IPMN and MCN manifest as cystic lesions within the pancreas but represent only a fraction of the large number of incidental cystic pancreatic tumors identified in clinical medicine. The fluid within the cyst can be sampled and tested, but predictive biomarkers from cystic fluid have failed to accurately predict malignant potential and ultimately guide clinical decision making

Surgical removal of a pancreatic cyst is the only effective therapy, but this requires a pancreatectomy and the therapeutic risks of the procedure must be weighed against the estimated malignancy risk posed by the cyst. For that reason, accurate discrimination of the malignant or benign nature of a cyst is a critical, unanswered clinical question. Current practice broadly defines pancreatic cysts as serous or mucinous. Mucin-producing cystic neoplasms are considered either premalignant, with potential to progress into invasive cancer, or frankly malignant. Consequently, presence of mucin is an important finding. Fortunately, it is typically not difficult to detect mucinous lesions during pathologic examination of direct smears of cyst contents obtained by aspiration. However, distinguishing a cyst as mucinous is insufficient for accurate decision making as the malignant potential varies considerably within subgroups of mucinous neoplasms (2).

IPMN tumors comprise 25% of cystic neoplasms and show the greatest need for biomarkers to guide management. Although the available data are retrospective and biased toward patients who received surgical resection, these observations are the basis for current clinical management. Main duct IPMN, defined as segmental or diffuse dilatation (>5 mm) of the main pancreatic duct, has a reported malignancy rate of roughly 62%, whereas branch-duct IPMN, identified as round cystic tumors of more than 5 mm, has a much lower reported malignancy (24.4%; ref. 3). For this reason, surgery is recommended for patients with main-duct IPMN. For branch-duct IPMN, cyst size is used as a surrogate for risk of malignancy, and therefore surgical resection is recommended for patients with branch-duct IPMN tumors of more than 3 cm. The clinical appearance of MCN tumors is very similar to that of branch-duct IPMNs, but surgical resection is recommended for all MCN regardless of size because malignant progression is considered inevitable. Discriminating a small branch-duct IPMN from an MCN is difficult if not impossible, underscoring the absence of critical biologic information about IPMN and MCN. Specifically lacking is any knowledge about the natural history of malignant progression within IPMN or MCN tumors: Do all tumors within these broad categories pose the same risk of malignant transformation? How long does this take to occur?

Genetically engineered mouse models have advanced our understanding of pancreatic cancer and provide a molecular framework for its development. These studies support the central role of oncogenic *Kras* in all 3 types of precursor lesions. Expression of mutant *Kras* in the mouse pancreas has been shown to induce PanIN lesions and PDAC (4). Cystic lesions resembling IPMN or MCN can be generated in the pancreas when oncogenic *Kras* expression is combined with overexpression of *TGFA* or the loss of the tumor suppressor gene *SMAD4*, respectively (5, 6). Advanced molecular pathologic techniques have confirmed that nearly all human PanIN lesions harbor *Kras* mutations, and recent studies show that *Kras* mutations are detected within cyst fluid of 41% to 47% of premalignant and 52% to 83% of malignant mucinous lesions (7, 8). However, is *Kras* mutation really the

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biomarker needed for clinical discrimination? Autopsy studies have identified that progressive PanIN lesions (PanIN II) are present in the pancreas of 96% of individuals after 40 years of age (9). PanIN III lesions, the last stage before noninvasive cancer, were conspicuously absent in the autopsy study. Thus, clear identification and demarcation of PanIN III lesions versus others is the "sweet spot" for any early PDAC detection strategy. This suggests that the molecular knife used to dissect out the

cysts requiring surgery needs to cut with a finer edge than Kras.

Small RNAs could provide the needed edge. Unique in their method of production and activation, small RNAs, 21 to 30 nucleotides in length, regulate transcription, chromatin structure, genome integrity, and most commonly, mRNA stability. One class of small RNA, miRNA, is predicted to regulate at least one third of all human genes (Fig. 1; ref. 10). Studies of miRNA expression in cancer have
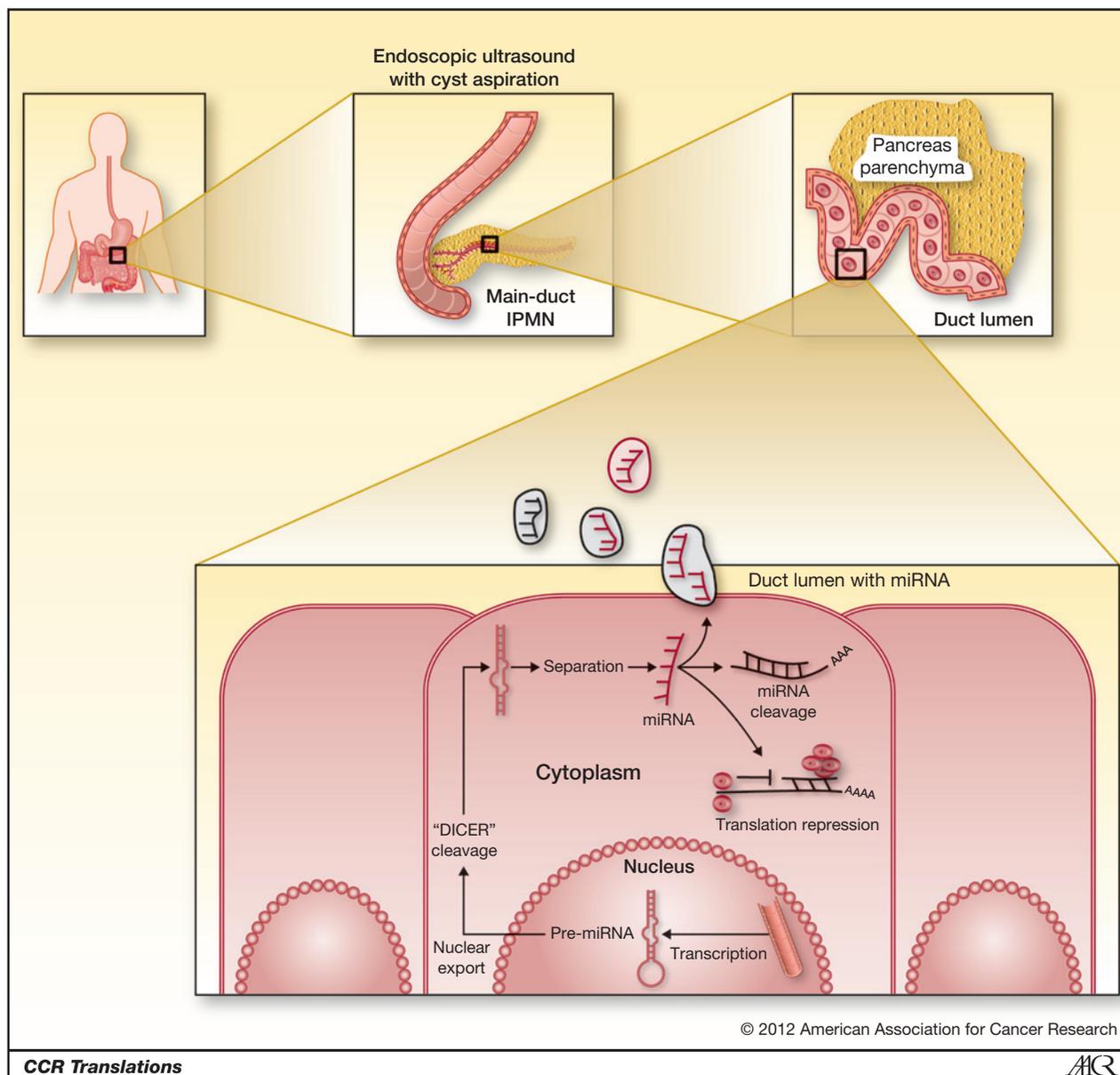

Figure 1. Schematic for the potential utilization of miRNA in risk stratifying patients with cystic neoplasms of the pancreas. On the basis of the malignant potential of the cells lining the cyst cavity, transcription of miRNA genes occurs yielding pre-miRNA that are subsequently exported from the nucleus into the cytoplasm of the cell. An enzymatic cleavage occurs by the "Dicer" enzyme that removes the hairpin loop, leaving a double-stranded structure that subsequently separates, yielding the mature miRNA. Typically, only 1 of the 2 strands is involved in subsequent gene regulation that can involve mRNA cleavage, translational repression, or other regulatory processes. A portion of the miRNA is shed from the cell into the cyst lumen, which ultimately can be aspirated during endoscopic ultrasound and measured, potentially giving insight into the malignant potential of the cells on the basis of their miRNA profile.

identified an extraordinary diversity of miRNA species across cancers prompting investigators to hypothesize that a modest number of miRNAs (~200 in total) might be sufficient to classify all human cancers. In addition, investigators have learned that testing using miRNA is entirely feasible, facilitated by their size and ability to remain intact in routinely collected, formalin-fixed, paraffin-embedded (FFPE) clinical tissues (11). Importantly, Yu and colleagues have recently reported that expression patterns of specific miRNA can achieve the critical discrimination between PanIN II and PanIN III lesions (12).

Matthaei and colleagues have harnessed the miRNA approach to examine cystic lesions of the pancreas and identify cysts likely to need surgical removal versus those that can usually be observed. To show this, they first microdissected low-grade and high-grade dysplasitic cells from FFPE IPMN samples, identified differentially expressed miRNA candidates, and validated them using separate FFPE samples. Similar differential patterns of miRNA were identified within fluid aspirated from pancreatic cystic neoplasms. From these experiments, Matthaei and colleagues identified a subset of 18 miRNAs that segregated cysts typically requiring surgery (e.g., high-grade IPMN) from less-malignant lesions that can be observed (e.g., low-grade IPMN and serous cystadenoma). A logistic regression model was then formulated using 9 miRNAs, and this predicted cyst pathology treated with resection versus observation with a sensitivity of 89% and a specificity of 100%.

The work presented by Matthaei and colleagues suggests a method for risk stratification of patients with pancreatic cystic neoplasms that could solve the clinical dilemma. As with any advance, real-time, prospective testing of this application will be necessary before adoption of this approach in clinical practice can be endorsed. Nevertheless, these data implicate miRNA expression patterns as a powerful tool that can help clinicians know when surgery is the right therapy for cystic lesions in the pancreas.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: R.M. Thomas, J.B. Fleming
Development of methodology: R.M. Thomas
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R.M. Thomas
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R.M. Thomas
Writing, review, and/or revision of the manuscript: R.M. Thomas, J.B. Fleming
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R.M. Thomas, J.B. Fleming
Study supervision: R.M. Thomas, J.B. Fleming

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