# Five plus Three for the Pancreas

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## SUMMARY

Preneoplastic high-risk lesions in the pancreas need to be differentiated from low-risk lesions warranting surveillance and eventually surgical intervention. Imaging is used so far; however, certain imaging features are subject to interpretation and hence have their intrinsic flaws. In a recent article, a liquid biopsy with protein and RNA markers demonstrates differentiation based on a blood test.

*See related article by Zhang et al., p. 1535*


In this issue of *Clinical Cancer Research*, Zhang and colleagues report on a blood-based test consisting of five proteins and three miRNA to differentiate high-risk from low-risk premalignant cystic lesions in the pancreas (1).

Pancreatic cancer, the malignancy developing from such cystic lesions called pancreatic intraductal papillary mucinous neoplasm (IPMN), has the worst prognosis of all solid tumors and represents one of the largest challenges in oncology (2). Pancreatic cancer in the majority of patients cannot be cured due to late diagnosis, then low resection rate, resistance to conventional, targeted and immune therapy (3). Therefore, one focus has been to identify individuals at risk, such as patients with IPMN, and to describe their surveillance in guidelines (4, 5). With the increasing awareness towards these preneoplastic and potentially curable lesions, a tsunami of requests came upon radiology and referrals to specialized gastroenterologists and surgeons who clearly were not ready for the magnitude of the problem: in the largest population-based study investigating healthy subjects with MRI, 49% had cysts in the pancreas – the trigger for referrals, however, only 6% were larger than 1 cm that were eventually considered clinically meaningful (i.e., IPMN) warranting follow-up (6). Consequently, with cross-sectional imaging as a starting point, efforts have been made to better describe "high-risk stigmata" or "worrisome features", however, all these approaches would still make use of regular imaging (7), stratification tools (8) or even engaging AI-supported algorithms (9).

An elevated CA 19–9 is part of the high-risk stigmata, the only tumor marker for pancreatic adenocarcinoma. So far, no other blood test nor liquid biopsy has made it into clinical practice, certainly not for a lack of trying. Liquid biopsy—recently highlighted as key discovery in the 2020 Nature Milestones publication—is defined as the detection and characterization of tumor cells and tumor cell products in the blood or other body fluids. Besides proteins and miRNAs in particular circulating cell-free DNA (ctDNA) has become a prime target for studies on early cancer diagnosis (10). However, the amount of ctDNA molecules in the blood of patients with premalignant and early cancer lesions are extremely small, while circulating proteins and RNAs are more abundant biomarkers.

The study by Zhang and colleagues from one of the largest pancreatic surgical centers worldwide, analyzing the blood of more than 300 patients with IPMN together with their clinical data and final (surgical) morphology demonstrated a combination panel of five proteins and three miRNA's to be able to discriminate high-risk from low-risk IPMN (**Fig. 1**; ref. 1). After going through several rounds of computations, the proteins EEF1A1, RHP3AL, NCOR1, L1CAM, and TMEM161A together with the miRNAs 146a-5p, 155–5p, and 375 reached an AUC of 0.97 in the validation cohort. This is, by far, the best result of a biomarker in IPMN discrimination so far and much better than the values for stratification tools and AI-supported algorithms.

Upon first sight, little is known about an association of these markers with pancreatic cancer or even IPMN. However, it is worth penetrating the known tumor biology of some of these molecules:

The transcription factor EEF1A1 is a drug target for a natural product modified with the Nobel price-winning click chemistry (11). NCOR1 is part of a chromatin histone modifier pathway altered in pancreatic cancer (12). L1CAM induces perineural invasion of pancreas cancer cells via TGFß1 and is a target for celecoxib (13). Of the Ras-associated binding proteins Rab3A (RPH3AL) we only know its expression in the tumorous pancreas (14). The epithelial protein TMEM161A is overexpressed in tumors and cross-reactive epitopes from Epstein–Barr virus and E. coli (15).

Of the miRNAs, 146a-5p expression was significantly decreased in pancreatic ductal adenocarcinoma (PDAC) tissues compared with adjacent normal tissues, and miR-146a-5p expression correlated with prognosis in patients with PDAC; functional studies indicated that miR-146a-5p suppressed PDAC cell proliferation and sensitized PDAC cells to gemcitabine chemotherapy (16). The miRNA 155–5p has repeatedly shown to be elevated in the blood of pancreatic cancer patients and promote immune evasion (17). Finally, the miRNA 375 has been associated before with the malignant conversion of IPMN to invasive pancreatic adenocarcinoma (18), thus confirming this biomarker.

Taken together, these markers seem to make sense in light of the biology behind these molecules. In addition, they may open up avenues not only for diagnosing high-risk IPMN but also suggest interventional studies to prevent progress and malignant conversion into pancreatic cancer. For example, COX-2 inhibition with drugs such as celecoxib has been successful in slowing down pancreatic cancer in preclinical models (19). Celecoxib being one of the most potent substances used in preventing colorectal adenomas (20).

Broad clinical usage of liquid biopsy markers will depend on standardization of both pre-analytical and analytical procedures (10). This task requires large initiatives such as the European Liquid Biopsy Society (ELBS) consortium (www.elbs.eu). The establishment

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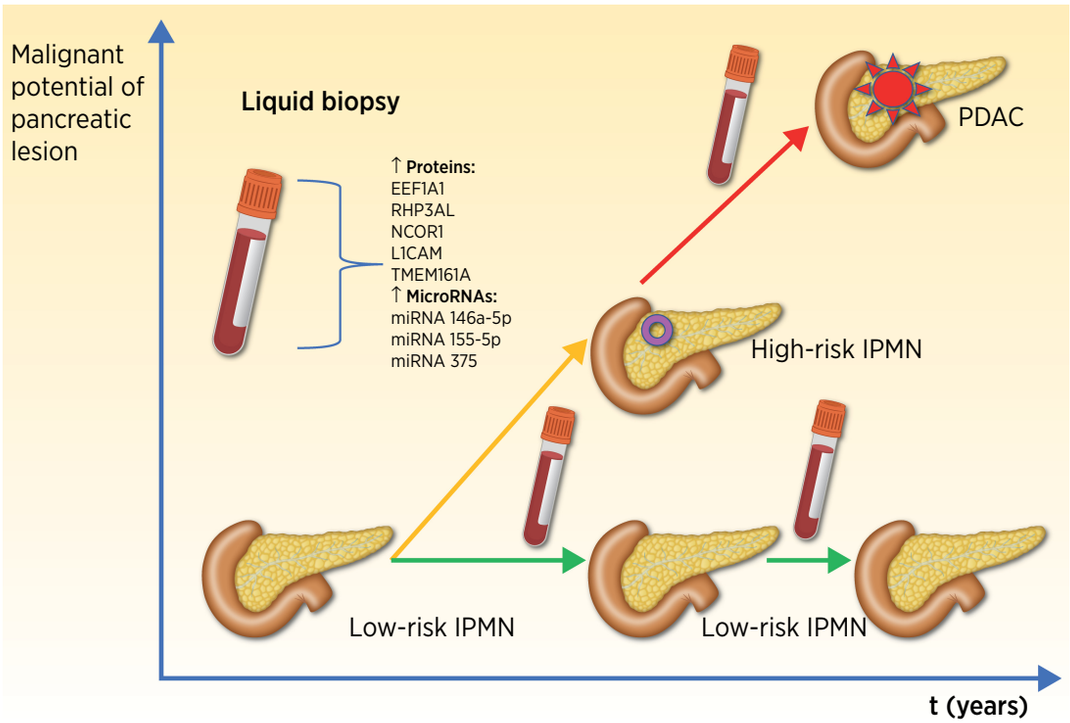


**Figure 1.**
Liquid biopsy with five protein and three miRNA markers to differentiate low-risk from high-risk pancreatic IPMN, the latter with the potential to develop into invasive PDAC.

of standard operating procedures and appropriate reference materials will also contribute to more standardized quality controls, quantification, and reporting among laboratories. In particular, the quantification of circulating miRNAs requires rigorous standardization to obtain reproducible results among different laboratories (21).

This solid though initial study must trigger further prospective studies with these biomarkers in IPMN, especially investigating the use in discriminating low-risk from high-risk IPMN, to lessen the burden on the health care systems. For an application in a wider clinical setting, the performance of the defined biomarker panels has to be reevaluated in a multicenter setting, as suggested already by the authors. Furthermore, exploitation of these molecules as drug targets, e.g., by repurposing known substances such as celecoxib or acetyl salicylc acid (ASS) for the prevention of progression would be feasible. Whilst there is no mouse model for IPMN, well-established genetically engineered mouse models using KRAS and TP53 (KPC model) are available and presumably suitable because IPMN possess these key genetic drivers as well.

## Authors' Disclosures

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