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Conflicts of interest
The authors have made the following disclosures:, HKCJ and SL have no conflicts of interest. AKS is one of the inventors of Minnelide, which has been licensed to Minneamrita Therapeutics by the University of Minnesota; and is its cofounder and CSO.

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Liquid Biopsy for Identification of High-Risk Cystic Lesions of Pancreas

See "Extracellular vesicle analysis allows for identification of invasive IPMN," by Yang KS, Ciprani D, O'Shea A, et al, on page 1345.

Pancreatic cystic lesions (PCLs) are clinically identifiable and histologically diverse groups of precursor lesions that carry an increased risk of malignant transformation into pancreatic ductal adenocarcinoma (PDAC).[1] An estimated 3%–13% of patients undergoing computed tomography or magnetic resonance imaging are incidentally discovered to harbor PCLs, making them a prime target population for screening and surveillance modalities. However, complexities arise because cystic lesions present a variable risk for malignant progression, and while some PCLs, including intraductal papillary mucinous neoplasms (IPMN), carry up to a 3-fold increased risk of developing PDAC,[2,3] others present with a marginal risk or low probability of developing into PDAC.[4] With limited knowledge on the timing and frequency of malignant progression, patients harboring IPMN are usually followed by serial imaging to prevent unnecessary highly morbid surgeries and overtreatment. Therefore, although patients carrying IPMN provide an opportunity for surveillance and screening to prevent malignant PDAC, their clinical management can be challenging owing to the variable risk for malignant progression. Current approaches for the risk stratification of PCLs rely predominantly on radiologically determined anatomical features and histologic examination of invasively obtained endoscopic ultrasound fine-needle aspirates and have limited specificity.

Yang et al[5] demonstrated the potential of blood-based extracellular vesicles (EVs) for differentiating high-risk IPMN from benign PCLs in this issue of *Gastroenterology*.[5] With the abundant release of EVs in patients with cancer compared with normal healthy individuals, there is high confidence that EVs can serve as potential sources of minimally invasive biomarkers. Small size, endocytic release, and resemblance to host–cell signatures make EVs potential candidates for liquid biopsy-based biomarker panels. A wide variety of EVs with varying size and content, including microvesicles, exosomes, exomeres, and oncosomes, are secreted by the cells.[6,7] However, the heterogeneity of size and origin, the ambiguity of surface and luminal markers, and the need for ultrasensitive technologies to detect low-frequency markers within individual EVs are major challenges in harnessing their diagnostic potential.

To address these challenges, Yang et al[5] carried out a 22 well-studied PDAC marker screen in a plasma-based discovery cohort (n = 86) composed of healthy, age-matched benign controls and patients harboring low-grade (LG), high-grade (HG), and invasive/HG (inv/HG) IPMNs. Considering the mucin-producing characteristic of PCLs, Yang et al[5] analyzed the majority of mucin-based markers (MUC1, MUC2, MUC4, MUC5AC, MUC6, and MUC13) that were observed to be differentially expressed in PDAC.[8] Additionally, the investigators evaluated EpCAM, EpHA2, Glypican 1, STMN1, and TSP1, molecules that have been specifically

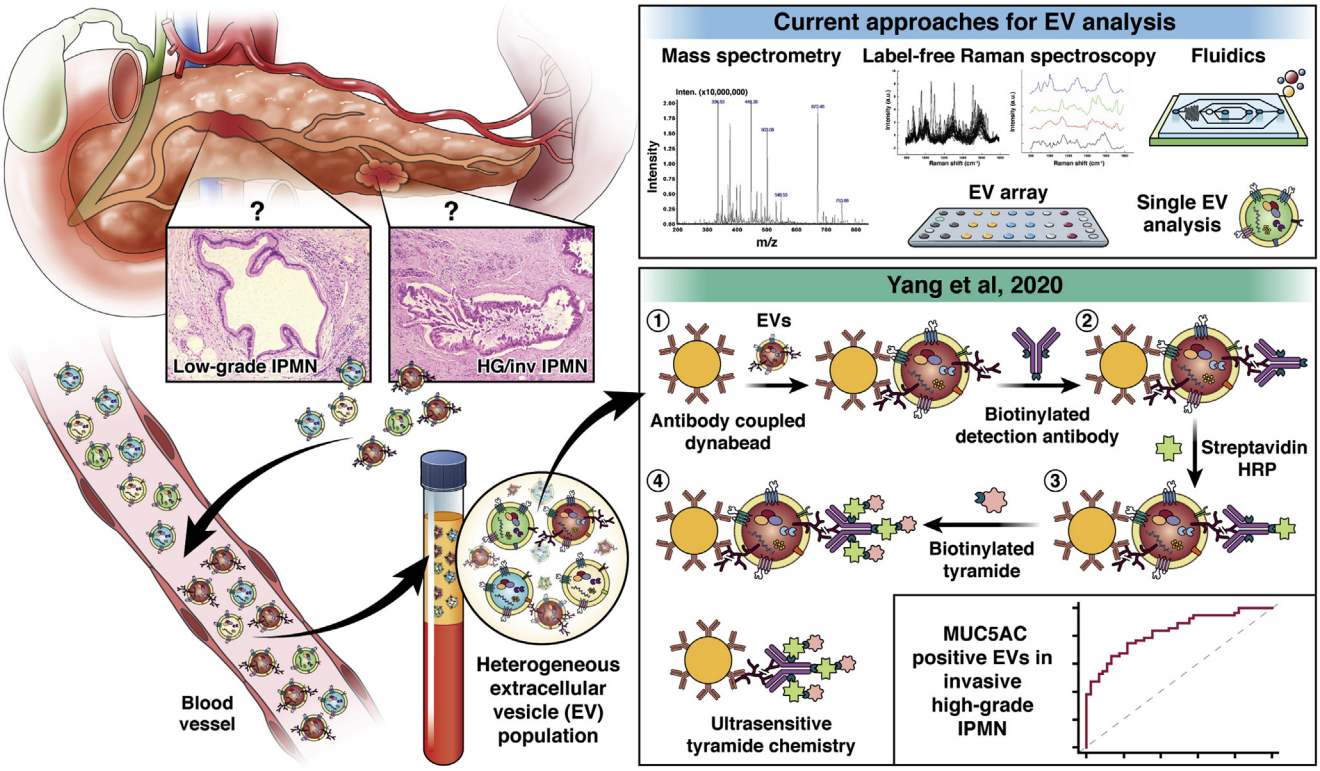

Figure 1. Diagnostic significance of extracellular vesicles (EVs) in the detection of invasive high-grade (HG) intraductal papillary mucinous neoplasm (IPMN). Schematic representation of the morphologic features of high- and low-risk PCLs. Current imaging modalities fail to distinguish high-risk and low-risk PCLs, and there is a lack of circulating biomarkers that can detect the presence of HG cystic lesions with invasive features. EVs secreted by various precursor lesions are released in the circulation, where they are diluted by EVs originating from other cell types and tissues. In this issue of *Gastroenterology*, Yang et al[5] used a magnetic bead-based digital EV screening technique (DEST). This approach relies on capture-detection antibody pair for markers of interest and tyramide-based signal amplification for sensitive detection of markers. Briefly, antibody-coated dyanabeads are used to capture EVs or circulating antigens (1), which are detected using biotinylated detection antibody (2). The signal from the antigen–antibody complex is developed using streptavidin horseradish peroxidase (3) that is further amplified using biotinylated tyramide (4) chemistry. Among various markers, MUC5AC-decorated EVs differentiated patients with invasive HG IPMNs from those with low-grade (LG) and HG IPMN with very high sensitivity and absolute specificity. Further, various other approaches including mass spectrometry, EV array, label-free technologies, and fluidics for single EVs analyses are being employed to characterize and use EVs as biomarkers for differentiating disease cases from controls for prognostication.

associated with PDAC EVs in earlier studies.[6,9–11] The magnetic bead-based digital EV screening technique (DEST) with the capture and detection antibody pair and tyramide-based signal amplification chemistry was employed to detect these markers with high sensitivity (Figure 1). The DEST assay reliably detected 16 markers present on the surface of intact EVs and/or released from the lumen of lysed EVs. With the sensitivity to detect a single EV (approximately 10,000-fold more sensitive than the conventional enzyme-linked immunosorbent assay), the DEST assay was reliable in detecting markers in EVs as confirmed by parallel screens of DEST assay in PC and PDX cell lines. Although several markers were elevated across patients harboring both LG and HG lesions, only secreted mucin MUC5AC was observed to be significantly higher in HG lesions. Among HG cases, MUC5AC expression was restricted to EVs from HG lesion with invasive features (inv/HG, 9/11) in comparison with HG dysplasia (1/11) alone cases. MUC5AC exhibited 100% sensitivity, 82% specificity, and 96% diagnostic accuracy for differentiating inv/HG IPMN from LG-IPMN in the training cohort. Across the validation cohort (n = 44), MUC5AC expression was observed in all 3 cases harboring inv/HG IPMN. In the combined cohort, MUC5AC exhibited 97%–100% specificity with 33%–50% sensitivity, and an area under the curve of 0.65–0.73 for identifying invasive IPMNs. Integrating EV MUC5AC analysis improved the efficiency of imaging for identifying high-risk IPMNs requiring surgical intervention. In cases with worrisome imaging features, MUC5AC expression analysis on EVs detected inv/HG IPMN in 36% of cases that would have been otherwise missed by imaging alone. Overall, this study is significant, with high clinical relevance, and has added a new dimension to MUC5AC as a biomarker for improved clinical decision-making in subjects with high-risk PCLs.

Previous transcriptomic studies identified MUC5AC among the top differentially overexpressed genes in PDAC.[12] In a large-scale tissue- and serum-based study from our

group (>1000 cases), MUC5AC emerged as a potential marker exhibiting better performance than the gold standard biomarker CA19-9 in distinguishing patients with resectable PDAC from patients with benign pancreatic diseases (chronic pancreatitis, benign control groups).[13] Interestingly, MUC5AC serves as a carrier protein for CA19.9, and the sensitivity for PDAC detection is improved when MUC5AC-associated CA19.9 is measured.[14] Further, glycoforms of MUC5AC can specifically distinguish HG cystic lesions from moderate-grade and LG mucinous cysts.[15] However, owing to the direct use of plasma to quantify EVs in the current validation cohort, it remains unclear whether the captured MUC5AC is present in EVs or is circulating freely. Because circulating EVs represent a heterogeneous population of variable sizes, composition, and origin, including microvesicles, EV-L, EV-S, and exomeres, it will be important to characterize the EV population that is specifically enriched for MUC5AC in inv/HG IPMN cases. Although MUC5AC expression has been observed in all histologic subtypes of IPMNs,[16] and is an essential criterion for their classification, it is intriguing that its presence is observed only in circulating EVs in patients with inv/HG IPMN. It remains to be seen if this is a consequence of increased tissue expression of MUC5AC in inv/HG lesion or a result of increased EV biogenesis, secretion, or selective packaging of MUC5AC in EVs.

In earlier studies on PCLs at the tissue level, overexpression of transmembrane MUC4 was observed specifically in patients with high-risk intestinal-type IPMNs and gastric-type IPMNs exhibiting HG dysplasia.[17] However, no significant differences were observed across HG and LG IPMN cases for MUC4-positive EVs. Considering that MUC4 is packaged into EVs,[18] the observed difference could be due to the lack of specificity of monoclonal antibody 1G8 that was used to detect MUC4 in the present study. Generated against rat MUC4, monoclonal antibody 1G8 does not exhibit specific reactivity to human MUC4 in cell lines and tissues as observed in the present[5] or an earlier study.[17] Before its use in clinical decision-making, it will be necessary to evaluate MUC5AC in a larger cohort of PCLs with another set of potential EVs specific markers observed in high-throughput proteomics studies.[9] Beyond their diagnostic significance, both EVs and MUC5AC have been functionally implicated in the pathogenesis of pancreatic malignancies. Although exosomes have been shown to establish a premetastatic niche and drive metastatic organotropism, we have recently demonstrated that MUC5AC is mechanistically involved in neoplastic progression, chemoresistance, and metastasis.[19,20] It will thus be essential to determine if exosomes, MUC5AC, and EV-MUC5AC functionally contribute to the malignant progression of IPMNs. Although the current study has firmly established the usefulness of EV MUC5AC as an important biomarker for IPMN management, its potential as a therapeutic target, if realized, may result in nonsurgical interventions to prevent PDAC in high-risk, clinically identifiable populations and patients.

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