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REVIEW

Pancreatic cancer early detection: Expanding higher-risk group with clinical and metabolomics parameters

Shiro Urayama

Shiro Urayama, Division of Gastroenterology and Hepatology, Department of Internal Medicine, University of California, Davis, Sacramento, CA 95817, United States

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Correspondence to: Shiro Urayama, MD, Professor, Division of Gastroenterology and Hepatology, Department of Internal Medicine, University of California, Davis, 4150 V Street, PSSB Suite 3500, Sacramento, CA 95817, United States. surayama@ucdavis.edu
Telephone: +1-916-7347021
Fax: +1-916-7347908

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is the fourth and fifth leading cause of cancer death for each gender in developed countries. With lack of effective treatment and screening scheme available for the general population, the mortality rate is expected to increase over the next several decades in contrast to the other major malignancies such as lung, breast, prostate and colorectal cancers. Endoscopic ultrasound, with its highest level of detection capacity of smaller pancreatic lesions, is the commonly employed and preferred clinical imaging-based PDAC detection method. Various molecular biomarkers have been investigated for characterization of the disease, but none are shown to be useful or validated for clinical utilization for early detection. As seen from studies of a small subset of familial or genetically high-risk PDAC groups, the higher yield and utility of imaging-based screening methods are demonstrated for these groups. Multiple recent studies on the unique cancer metabolism including PDAC, demonstrate the potential for utility of the metabolites as the discriminant markers for this disease. In order to generate an early PDAC detection screening strategy available for a wider population, we propose to expand the population of higher risk PDAC group with combination clinical and metabolomics parameters.

Key words: Pancreatic cancer; Endoscopic ultrasound; Metabolomics; Early detection; Biomarkers

Core tip: This is a summary of current pancreatic cancer cohort early detection studies and a potential approach being considered for future application. This is an area that requires heightened efforts as lack of effective treatment and screening scheme for wider population is leading this particular disease to be the second lethal cancer by 2030.

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INTRODUCTION

Currently, pancreatic ductal adenocarcinoma (PDAC) is the fourth major cause of cancer mortality in the United States[1]. It is predicted that 46420 new cases and 39590 deaths would result from pancreatic cancer in the United States in 2014[2]. Worldwide, there were 277668 new cases and 266029 deaths from this cancer in 2008[3]. In comparison to other major malignancies such as breast, colon, lung and prostate cancers with their respective 89%, 64%, 16%, 99% 5-year survival rate, PDAC at 6% is conspicuously low[2]. For PDAC, the only curative option is surgical resection, which is applicable in only 10%-15% of patients due to the common discovery of late stage at diagnosis[4]. In fact, PDAC is notorious for late stage discovery as evidenced by the low percentage of localized disease at diagnosis, compared to other malignancies: breast (61%), colon (40%), lung (16%), ovarian (19%), prostate (91%), and pancreatic cancer (7%)[5]. With the existing effective screening methods, the decreasing trends of cancer death rate are seen in major malignancies such as breast, prostate and colorectal cancer. In contrast, it is estimated that PDAC is expected to be surfacing as the second leading cause of cancer death by 2030[6].

With the distinct contribution of late-stage discovery and general lack of effective medical therapy, a critical approach in reversing the poor outcome of pancreatic cancer is to develop an early detection scheme for the tumor. In support of this, we see the trend that despite the poor prognosis of the disease, for those who have undergone curative resection with negative margins, the 5-year survival rate is 22% in contrast to 2% for the advanced-stage with distant metastasis[7,8]. An earlier diagnosis with tumor less than 2 cm (T1) is associated with a better 5-year survival of 58% compared to 17% for stage ⅡB PDAC[9]. Ariyama *et al*[10] reported complete survival of 79 patients with less than 1 cm tumors after surgical resection. Furthermore, as a recent report indicates, the estimated time from the transformation to pre-metastatic growths of pancreatic cancer is approximately 15 years[11]; there is a wide potential window of opportunity to apply developing technologies in early detection of this cancer.

In this article, we will review the recent studies on the PDAC early detection approaches and ongoing research endeavors in developing early detection schemes for this devastating disease, with specific attention to application of combined clinical and metabolomics parameters.

PDAC EARLY DETECTION AND DIAGNOSIS - IMAGING-BASED TESTS

Over the past few decades, endoscopic ultrasound (EUS) has proven itself to be a superior imaging study for detection of a small or early-stage pancreatic neoplasm as compared to other modalities such as transabdominal ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography scans and angiography[12-14]. Yasuda *et al*[15] and Rösch *et al*[16] had initially demonstrated the superiority of EUS in detection of small pancreatic lesions. More recently, DeWitt *et al*[17] had verified the superiority of EUS as compared to multi-detector CT scan. In another study, Khashab *et al*[18] demonstrated that the sensitivity of EUS in detecting a pancreatic mass was significantly greater than that of CT images, and particularly for pancreatic neuroendocrine tumors, which commonly consist of smaller lesions. In addition, EUS detected CT-negative tumors in more than 90% of the cases. As an additional diagnostic modality, EUS-guided fine needle aspiration (FNA) provides success rates of 90%-95%, with an overall sensitivity and specificity of 85%-90% and 98%-99%, respectively[19-22]. Thus, the utility and the advantage of EUS enable visualization and targeting of small pancreatic masses. Lesions of 5 mm or less could be visualized and sampled, which might not have been accessible or identifiable by other imaging modalities[23].

DIAGNOSTIC MOLECULAR MARKERS AND PANCREATIC CANCER

In order to enhance the diagnostic accuracy of PDAC, molecular markers on EUS-FNA samples have been evaluated in recent years. Utilities of DNA mutations and loss of heterozygosity are being reported as potential surrogate markers of the cancer[24,25]. In a recent study, Takahashi *et al*[26] assessed *k-ras* point mutations in PDAC and chronic focal pancreatitis samples obtained by EUS-FNA[27,28]. The study revealed the presence of point mutations of *k-ras* in 74% of patients with PDAC compared to no mutations in chronic focal pancreatitis. In another study, Tada *et al*[29] reported a high *k-ras* gene mutation rate in 20 of 26 cases of EUS-FNA specimens (77%) and in 12 of 19 cases of pancreatic juice (63%) in PDAC. However, the presence of *k-ras* mutations in a benign condition such as chronic pancreatitis and premalignant lesions such as intraductal papillary mucinous neoplasm (IPMN) in addition to lack of such mutations in 20% of PDAC limit the usage of this test solely as a diagnostic or a detection tool. Other studies analyzing p53 by immunohistochemistry[30], telomerase activity with a ribonucleoprotein enzyme[31], and a broad panel of microsatellite allele loss markers demonstrated similar results[32]. In the presence of inconclusive EUS-FNA cytology, molecular markers could potentially complement EUS-FNA cytology results to help establish the diagnosis of malignancy.

Table 1 Pancreatic ductal adenocarcinoma related genetic syndromes

Syndrome	Inheritance	Gene mutation	Risk of PDAC
Peutz-Jeghers syndrome[38]	AD	*STK11/LKB1*	SIR = 132
Hereditary pancreatitis[39-41]	AD	*PRSS1*	OR = 69.9
		SPINK1	
Familial atypical multiple mole melanoma syndrome[42-44]	AD	*CDKN2A*	SIR = 13-38
Hereditary breast-ovarian cancer syndrome[45-51]	AD	*BRCA2*	BRCA2: OR = 3.5-10-fold increased risk
		BRCA1	BRCA1: OR = 2.26 times average population
Lynch syndrome[52]	AD	*MLH1, MSH2, MSH6* or *PMS2*	SIR = up to 8.6
Cystic fibrosis[53]	Autosomal recessive	*CFTR*	OR = 5.3-6.6

AD: Autosomal dominant; PDAC: Pancreatic ductal adenocarcinoma; SIR: Standardized incidence ratio.

SELECT POPULATION-BASED RESEARCH FOR EARLY DETECTION SCHEME DEVELOPMENT

PDAC screening in high-risk individuals

Currently, a general population-screening program for PDAC is not cost-effective because of low relative disease incidence and non-availability of simple, cheap, highly accurate non-invasive tests. The main goal of the screening is to identify clinically significant precursor or early stage PDAC. However, since overwhelming majority of premalignant and small PDAC lesions is asymptomatic, we do not have a definite surrogate marker to identify a subset population for screening. Consequently, as one of the approaches in investigating the risks, research has focused on identification of a subset of individuals with a higher-risk for PDAC development in order to elucidate the genetic predilection. Up to 10% of PDAC patients have a familial/genetic basis and they have increased risk of developing both pancreatic and extra-pancreatic malignancies[33-37]. Classic categorization of high-risk patients are based on the highly associated genetic risks defined as those who have significant family history of the cancer or have an inherited PDAC syndrome with a known genetic abnormality (Table 1).

Familial pancreatic cancer: Familial pancreatic cancer (FPC) cohort (cancer in two or more first-degree relatives (FDRs) or in three or more affected family members - including one first-degree relative) is considered a high-risk and a candidate for screening program[47,54,55]. Currently, the genetic foundation for FPC is not fully understood. Various investigations have demonstrated the presence of a germline mutation in the *BRCA2* gene[47-49], association of BRCA1[46,56], *paladin* gene mutation[57] as well as other genes such as apolipoprotein A4, CEA, keratin 19, stratifin, trefoil factor, and S100A6[58,59] in FPC, and more recently identification of PALB2[60], as a pancreatic cancer susceptibility gene. These facts suggest that multiple and heterogeneous factors are likely at play for the genesis of PDAC in this subset.

Analysis of the PDAC kindred data from Johns Hopkins' National Familial Pancreas Tumor Registry has shown that the risk of PDAC in individuals with two afflicted FDRs is 6.4% and the lifetime risk is 8%-12%; for persons with three afflicted FDRs, the relative and lifetime risks for PDAC increase to 32% and 16%-32%, respectively[36]. Brune *et al*[61] in their recent article reported a higher risk of PDAC among FPC kindred with a younger onset (age less than 50). Rulyak *et al*[62] in another study found smoking as a significant risk factor in FPC cohort, especially among males and those under age 50. This factor increases the cancer risk by 2.0-3.7 times and lowers the onset age by 10 years. A risk assessment software tool, PancPRO, has been generated and is available for calculating the risk for individuals with familial pancreatic cancer (http://www4.utsouthwestern.edu/breasthealth/cagene/default.asp)[35].

Screening modalities and the current screening programs: Many of the screening programs have used additional investigational biomarker to complement imaging tests to identify the early lesions. A commonly used marker, CA19-9, is neither sensitive nor specific independently for early PDAC or precursor detection. Kim *et al*[63] in their study found only 0.9% positive-predictive value using the standard cut-off value (37 U/mL). Other biomarkers investigated recently include MIC-1, CEACAM-1, SPan1, DUPAN, Alpha4GNT, and PAM4, but none is validated for routine clinical use[64]. In another approach, elevated fasting-glucose level has been demonstrated to be associated with sporadic PDAC[65] and is currently utilized by an European registry in high-risk people with mutational analysis of pancreatic juice along with *p16* promoter methylation status.

Several international screening programs exist for PDAC in high-risk individuals. "Cancer of the Pancreas Screening Study" (CAPS study), led by John Hopkins University, is the largest screening program that involves 24 American Centers of Excellence. To date, three studies, CAPS 1, CAPS 2 and CAPS 3, have been completed (Table 2).

Table 2 Results of screening programs for pancreatic cancer in high-risk groups *n* (%)

Study	CAPS1	CAPS2	CAPS3	U of Washington	FaPaCa	Dutch Study
Diagnostic Yield	2 (5.3)	8 (10)	92 (43)	10 (13)	1 (1.3)	10 (23)

Positive finding of abnormal imaging such as mass (solid, cyst) or abnormal duct in the cases. CAPS: Cancer of the Pancreas Screening Study; FaPaCa: Familial Pancreatic Cancer Study.

In the CAPS 1, thirty-eight patients including 31 from a kindred with > 3 affected with PDAC, 6 with 2 affected relatives, and 1 patient with Peutz-Jeghers syndrome (PJS) were studied. Screening protocol with EUS revealed six pancreatic masses: 1 invasive PDAC, 1 benign IPMN, 2 serous cystadenomas, and 2 non-neoplastic lesions. The yield of screening was 5.3% in this study[66]. In the CAPS 2, seventy-eight high-risk patients were studied[67]. In 8 patients, the screening found pancreatic neoplasia, confirmed by surgery or FNA: 6 benign IPMNs, 1 IPMN that progressed to invasive PDAC, and another had high-grade pancreatic intraepithelial neoplasia (PanIN-3). The CAPS 3 was a multicenter prospective cohort study involving annual EUS, MRI screening with assays of DNA and protein markers in serum and pancreatic juice. Over 200 patients were enrolled over a three-year period. The study results on the detection modality comparison demonstrated that the EUS has the highest rate of detection of early neoplastic changes in up to 42.6% of the asymptomatic high-risk group[68].

In another study from the University of Washington, high-risk familial cohorts were screened with EUS, beginning 10 years prior to the earliest index PDAC case. If EUS was normal, they underwent a repeat EUS in 2-3 years. With abnormal EUS findings, they were referred for ERCP and if abnormalities were noted, patients were offered surgical intervention[69]. Among 75 screened subjects, 15 had gone to surgery for abnormal EUS and ERCP findings. All surgical cases revealed premalignant lesions: PanIN-3 in 10 and PanIN-2 in five cases[70]. The study gave a yield of 13% (10 out of 75) for detecting PanIN-3 lesions. A single patient developed unresectable PDAC during the surveillance.

In a German PDAC screening program (FaPaCa), 76 patients were followed using annual EUS, MRCP, and laboratory assays (*CDKN2a* and *BRCA2* genetic analysis, CA19-9 and CEA). Any appreciable lesion was evaluated with EUS. With an abnormal finding, the patient underwent surgical exploration and if malignancy was detected, total pancreatectomy was performed. In 10 cases, lesions were seen on EUS as compared to only seven detected by MR scan. Out of the seven MRCP-positive cases, six underwent resections and the histology showed one PanIN-3, one PanIN-2, one PanIN-1, and three with other benign lesions. This resulted in a diagnostic yield of 1.3% for PanIN-3 detection[71]. Another study from the Netherlands in 44 high-risk subjects demonstrated a 7% detection rate for asymptomatic PDAC and a 16% for premalignant lesions[72].

The International CAPS Consortium have recently met and reported a suggested guideline for current PDAC screening based on the risk[73]. A consensus (⩾ 75% agreement by the participants) was reached that the following groups should be offered screening (only to individuals who are surgical candidate): (1) FDRs of the cancer patients from a familial pancreatic cancer cohort with at least two affected FDRs; (2) patients with Peutz-Jeghers syndrome; and (3) *p16*, *BRCA2* and hereditary non-polyposis colorectal cancer mutation carriers with at least single affected FDR. The initial screening should include EUS and/or MRI. However, consensus was not reached on the beginning and the end age of screening/surveillance and the interval of the examination. Their conclusions also included requirement for further studies, and the clinical management should occur at high-volume centers with multidisciplinary teams.

FUTURE OF PANCREATIC CANCER SCREENING

Current screening programs have demonstrated that the EUS evaluation can detect premalignant lesions and early cancers in certain small subset of high-risk groups. However, as the overwhelming majority of PDAC cases involve patients who develop the disease sporadically without a recognized genetic abnormality, the application of this modality for PDAC detection screening is very limited for the general adult population.

Select population based approach

Identification of a higher-PDAC-risk group: As the prevalence of PDAC in the general United States population over the age 55 is approximately 68 per 100000, a candidate discriminant test with a specificity of 98% and a sensitivity of 100% would generate 1999 false-positive test results and 68 true-positives[74]. Thus, relying on a single determinant for distinguishing the PDAC early-stage cases from the general population would necessitate a highly accurate test with a specificity of greater than 99%. More practical approach, then, would be to begin with a subset of population with a higher prevalence, and in conjunction with novel surrogate markers to curtail the at-risk subset, we could begin to identify the

group with significantly increased PDAC risk for whom the endoscopic/imaging-based screening strategy could be applied.

An initial approach in selection of the screening population is to utilize selective clinical parameters that could be used to curtail the subset of the general population at increased PDAC risk. For instance, based on the epidemiological evidence, such clinical parameters include hyperglycemia or diabetes, which are noted in 50%-80% of pancreatic cancer patients[75-79]. Though not encompassing all PDAC patients, this subset includes a much larger proportion of PDAC patients for whom we may select further for screening. Similarly, patients with a history of chronic pancreatitis or obesity are reported to have increased PDAC risk during their lifetime[80-85]. Recent findings from molecular biology and animal studies investigating effects of diet-induced obesity in a PDAC mouse model demonstrated increased occurrence of pancreatic inflammation and accelerated pancreatic neoplastic changes, supporting the association of obesity and pancreatic inflammation and PDAC risks[86,87]. Considering that millions are being diagnosed with diabetes or glucose intolerance, chronic pancreatitis, or obesity annually in comparison to PDAC, however, further refinement of the screening patient group is critically needed to justify for developing a larger scale screening protocol.

Translational research - application of metabolomics approach

Initially established as a key methodology in the field of inborn metabolic errors and toxicology, metabolomics have developed over the years to examine a much wider array of low-molecular-weight products or intermediates within the biological state of a cell, tissue, organ, or organism. A metabolome represents a physiological readout of the biochemical state in an individual's body compartment, and provides the functional terminal signals of the genome and proteome, reflecting more closely the current phenotypic state of an individual in response to the environmental stimuli[88]. Thus, metabolomics data has considerable potential in elucidating cancer-development risks, with its additional capacity for providing temporal molecular information to the ongoing changes originating from genetic PDAC risk alone.

With the recent advancement in the technology and resumed interest in the cancer-associated metabolic abnormality[89,90], application of metabolomics in the cancer field has attracted more attention[91]. Cancer-related metabolic reprogramming, Warburg effect, has been known since nearly a century ago in association with various solid tumors including PDAC[92], as cancer cells undergo energetically inefficient glycolysis even in the presence of oxygen in the environment (aerobic glycolysis)[93]. A number of common cancer mutations including Akt1, HIF (hypoxia-inducible factor), and p53 have been shown to support the Warburg effect through glycolysis and down-regulation of metabolite flux through the Krebs cycle[94-101]. In PDAC, increased phosphorylation or activation of Akt1 has also been reported (illuminating on the importance of enzyme functionality)[102] as well as involvement of HIF1 in the tumor growth *via* effects on glycolytic process[103,104] and membrane-bound glycoprotein (MUC17) regulation[105] - reflective of activation of metabolic pathways. Further evidences of loss-of-function genetic mutations in key mitochondrial metabolic enzymes such as succinate dehydrogenase and fumarate hydratase, isocitrate dehydrogenase, phosphoglycerate dehydrogenase support carcinogenesis and the Warburg effect[106-110]. Other important alternative pathways in cancer metabolism such as glutaminolysis and pyruvate kinase isoform suppression have been shown to accumulate respective upstream intermediates and reduction of associated end products such as NADPH, ribose-5-phosphate and nucleic acids[111-116]. As such, various groups have reported metabolomics biomarker applications for different cancers[117,118].

As a major organ involved in metabolic regulation in a healthy individual, pancreatic disorder such as malignancy is anticipated to influence the normal metabolism, presenting further rationale and interest in elucidating the implication of malignant transformation and PDAC development. Proteomic analysis of the pancreatic cancer cells demonstrated alteration in proteins involved in metabolic pathways including increased expression of glycolytic and reduced Krebs cycle enzymes, and accumulation of key proteins involved in glutamine metabolism, in support of Warburg effect. These in turn play significant role in nucleotide and amino acid biosynthesis required for sustaining the proliferating cancer cells[119]. Applications of sensitive mass spectrometric techniques in metabolomics study of PDAC detection biomarkers have led to identification of a set of small molecules or metabolites (or biochemical intermediates) that are potent discriminants of developing PDAC and the controls (See Figure 1 as an example of metabolomics based analysis, allowing segregation of PDAC from benign cases). Recent reports from our group as well as others have demonstrated that specific candidate metabolites consisting of amino acids, bile acids, and a number of lipids and fatty acids - suspected to be reflective of tumor proliferation as well as many systemic response yet to be determined - were identified as potential discriminant for blood-based PDAC biomarkers[120-123]. As a further supporting data, elucidation of lipids and fatty acids as discriminant factors from PDAC and benign lesions from the cancer tissue and adjacent normal tissue has been reported recently[124].

By virtue of simultaneously depicting the multiple

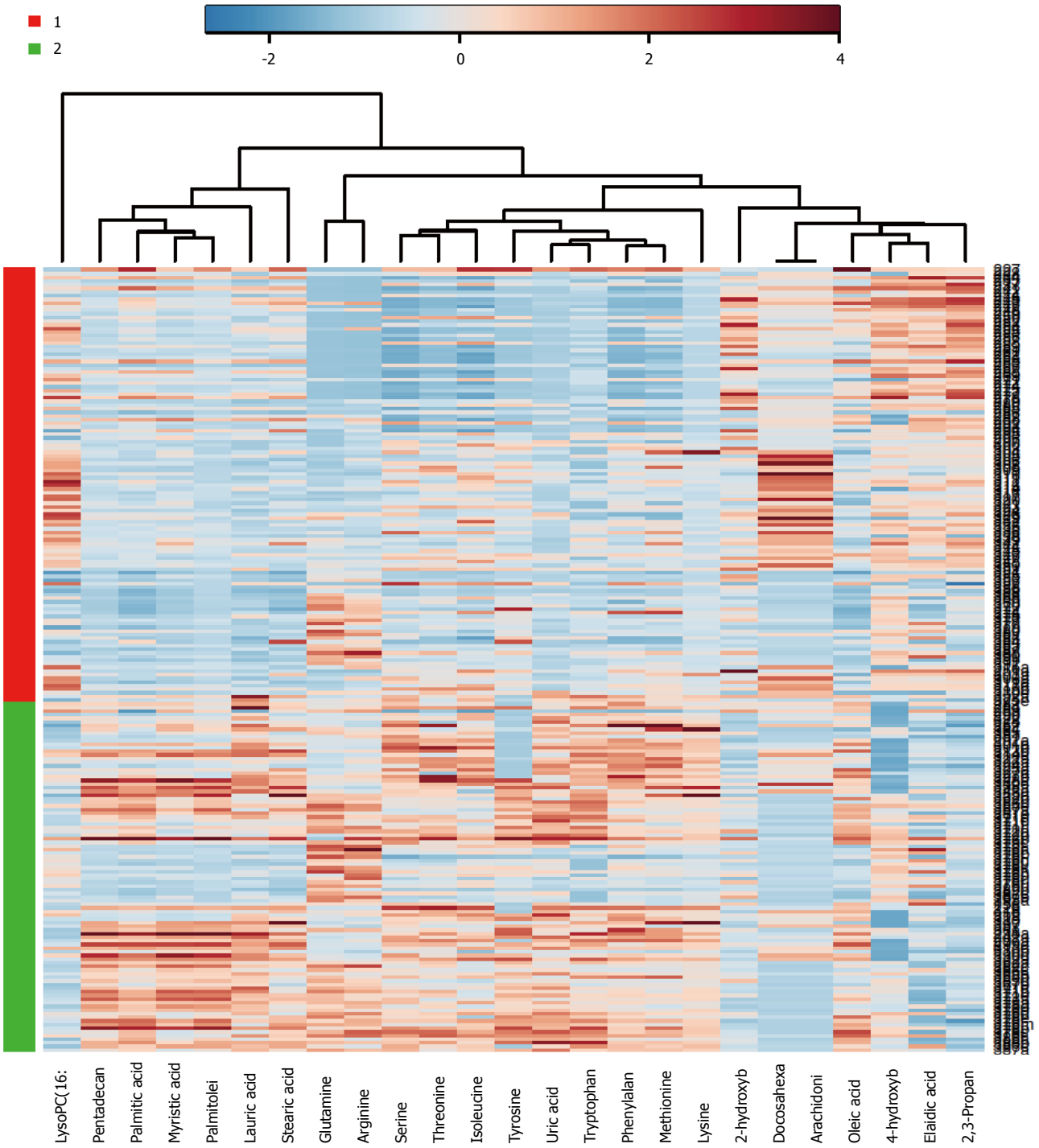

Figure 1 Example of metabolomics based analysis, allowing segregation of pancreatic ductal adenocarcinoma from benign cases. Heat map illustration of discriminant capability of a metabolite set derived from gas chromatography and liquid chromatography/mass spectrometry plasma metabolomics dataset comparing pancreatic ductal adenocarcinoma patients (Red or group 1; *n* = 110) and benign pancreas (includes benign cysts, chronic pancreatitis, and normal pancreas) (Green or group 2; *n* = 90). Metabolites are plotted on x-axis, and the cases on the y-axis. Blue color indicates data points with a value smaller than the median of the respective metabolite and the red indicates higher values. This candidate set of metabolites enabled the segregation of the two groups.

metabolite levels, metabolomics approach reveals various biochemical pathways that are uniquely involved in malignant conditions and has led to findings such as abnormalities of glycine and its mitochondrial biosynthetic pathway, as a potential therapeutic target in certain cancers[125]. Moreover, in combination with other systems biology approaches such as transcriptomics and proteomics, further refinement in characterization of cancer development and therapeutic targets as well as identification of potential biomarkers could be realized for PDAC. Since many enzymes in a metabolic network determine metabolites' level and nonlinear quantitative relationship from the genes to the proteome and me-

tabolome levels exist, a metabolome cannot be easily decomposed to a specific single marker, which will designate the cancer state[126]. Thus, in order to delineate a pathological state such as PDAC, multiple metabolomic features might be required for accurate depiction of a developing cancer. Future studies are anticipated to incorporate cancer systems' biological knowledge, including metabolomics, for optimal designation of PDAC biomarkers, which would be utilized in conjunction with a clinical-parameter-derived population subset for establishing the PDAC screening population. Subsequently, further validation studies for the PDAC biomarkers need to be performed.

CONCLUSION

Current imaging-based detection and diagnostic methods for PDAC is effectively providing answers to clinical questions raised for patients with signs or symptoms of suspected pancreatic lesions. However, the endoscopic/imaging-based screening schemes are currently limited in applications to early PDAC detection in asymptomatic patients, aside from a small group of known genetically high-risk groups. There is a high demand for developing a method of selecting distinct subsets among the general population for implementing the endoscopic/imaging screening test effectively. Application of combinations of clinical risk parameters/factors with the developing molecular biomarkers from translational science such as metabolomics analysis brings hopes of providing us with early PDAC detection markers, and developing effective early detection screening scheme for the patients in the near future.

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