Individualized metabolic profiling stratifies pancreatic and biliary tract cancer: a useful tool for innovative screening programs and predictive strategies in healthcare

Jun Hwa Lee 1, Seung Eun Yu 1, Kyung-Hee Kim 1,2, Myung Hyun Yu 1, In-Hye Jeong 1, Jae Youl Cho 3, Sang-Jae Park 4, Woo Jin Lee 4, Sung-Sik Han 4, Tae Hyun Kim 4, Eun Kyung Hong 4, Sang Myung Woo 1,4,5, Byong Chul Yoo 1,5

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Abstract

Background Pancreatic cancer (PC) and biliary tract cancer (BTC) are highly aggressive cancers, characterized by their rarity, difficulty in diagnosis, and overall poor prognosis. Diagnosis of PC and BTC is complex and is made using a combination of appropriate clinical suspicion, imaging and endoscopic techniques, and cytopathological examination. However, the late-stage detection and poor prognosis of this tumor have led to an urgent need for biomarkers for early and/or predictive diagnosis and improved personalized treatments.

Working hypothesis There are two hypotheses for focusing on low-mass metabolites in the blood. First, valuable information can be obtained from the masses and relative amounts of such metabolites, which present as low-mass ions (LMIs) in mass spectra. Second, metabolic profiling of individuals may provide important information regarding biological changes in disease states that is useful for the early diagnosis of PC and BTC.

Materials and methods To assess whether profiling metabolites in serum can serve as a non-invasive screening tool for PC and BTC, 320 serum samples were obtained from patients with PC (n = 51), BTC (n = 39), colorectal cancer (CRC) (n = 100), and ovarian cancer (OVC) (n = 30), and from healthy control subjects (control) (n = 100). We obtained information on the relative amounts of metabolites, as LMIs, via triple time-of-flight mass spectrometry. All data were analyzed according to the peak area ratios of discriminative LMIs.

Results and conclusions The levels of the 14 discriminative LMIs were higher in the PC and BTC groups than in the control, CRC and OVC groups, but only two LMIs discriminated between PC and BTC: lysophosphatidylcholine (LysoPC) (16:0) and LysoPC(20:4). The levels of these two LysoPCs were also slightly lower in the PC/BTC/CRC/OVC groups compared with the control group. Taken together, the data showed that metabolic profiling can precisely denote the status of cancer, and, thus, could be useful for screening. This study not only details efficient methods to identify discriminative LMIs for cancer screening but also

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Jun Hwa Lee, Seung Eun Yu and Kyung-Hee Kim contributed equally to this work.

1 Biomarker Branch, Research Institute, National Cancer Center, Goyang 10408, Republic of Korea
2 Omics Core Laboratory, Research Institute, National Cancer Center, Goyang 10408, Republic of Korea
3 Department of Genetic Engineering, Sungkyunkwan University, Suwon 16419, Republic of Korea
4 Center for Liver Cancer, Hospital, National Cancer Center, Goyang 10408, Republic of Korea
5 Department of Cancer Biomedical Science, Graduate School of Cancer Science and Policy, National Cancer Center, Goyang 10408, Republic of Korea
Introduction
Pancreatic cancer (PC) and biliary tract cancer (BTC) are highly aggressive cancers, for which the mortality rates closely parallel the incidence rates [1]. Most PC and BTC cases are not accompanied by clinical symptoms until the disease reaches an advanced stage [2, 3]. A minority of PC and BTC patients present with surgically resectable disease, but the relapse rate is high [4, 5]. PC is the fifth-most deadly cancer, and only approximately 8% of patients with PC survive for 5 years; thus, it has the worst survival rate among all 22 common cancers [6, 7]. Meanwhile, the overall 5-year survival time of advanced BTC is less than 1 year [8]. BTCs are generally divided into intrahepatic cholangiocarcinomas, perihilar or extrahepatic cholangiocarcinomas, and gallbladder tumors [9]. The incidence of cholangiocarcinoma remains significantly higher (by up to 40-fold) in China and Korea than in Western countries, and, thus, poses a significant public health problem [10]. Therefore, diagnosing and classifying PC and BTC at the early stage is urgently needed to increase the likelihood of cure.

Concerning early detection of PC or BTC, imaging studies and biomarkers have both been used in the clinic. Imaging modalities, such as endoscopic ultrasound, computed tomography, and magnetic resonance imaging have difficulty in differentiating non-malignant and malignant tissue [11, 12]. In addition, the high cost of these procedures limits their use in the follow-up of asymptomatic cases [13]. The most well-validated and useful biomarker for PC and BTC is carbohydrate antigen (CA19-9); however, CA19-9 is not recommended for general screening because it is upregulated in other inflammatory conditions such as chronic pancreatitis and cholangitis [14, 15]. Other molecular markers of PC and BTC have also been used, such as circulating tumor cells, epigenetic markers, and microRNAs. Liquid biopsy with circulating tumor DNA (ctDNA) is an emerging technology to detect actionable alterations [16]. A recent study found that low-level PIK3CA mutations can be detected in serum using ctDNA, indicating the usefulness of ctDNA to detect cancer-derived mutations in metastatic BTC [17]. KRAS mutations have been detected by digital polymerase chain reaction in ctDNA [2], and it has been suggested that serum miRNA is more useful than CA19-9 for diagnosis of PC and BTC [18]. Recently, methylation-on-beads technology, which can detect methylation changes in DNA circulating in serum, showed potential for PC diagnosis [19–21]. Several novel biomarkers for early diagnosis of PC and BTC have been suggested over the last few years; however, no molecular biomarkers are currently suitable for clinical use.

Recently, several metabolites have been reported as potential biomarkers for various cancers [20]. Some new metabolites have been validated for diagnostic purposes; choline was consistently elevated in breast cancer biopsy samples, for example, compared to levels in normal tissue, and the diagnostic accuracy using this marker was 100% [22]. Increased citrate and decreased spermine levels in prostatic fluid showed potential utility in screening for prostate cancer [23, 24]. Furthermore, a metabolomics-based urine screening test to detect adenomatous polyps has been reported [25]. However, only a few metabolic studies have screened for PC and BTC. In the present metabolic profiling study, we enrolled not only patients with PC and BTC but also those with other types of cancer such as colorectal cancer (CRC), and ovarian cancer (OVC) as a positive control. This was intended to rule out common metabolic factors in cancers such as fibrinogen peptide alpha chain and to select more reliable metabolic candidates relevant to PC or BTC.

Scheme 1 summarizes our research strategy for the metabolic profiling of PC and BTC patients based on serum analysis for predictive diagnosis, targeted prevention, and personalized treatment. Here, we report the metabolic profiles and discuss their clinical utility.

Materials and methods
Study population
In total, 320 serum samples were collected from healthy individuals (control) and patients with PC, BTC, CRC, and OVC (Table 1 and Supporting Information Table 1). Eligible control subjects were selected from individuals in the cancer screening cohort, who were subjected to routine health examinations at the Center for Cancer Prevention and Detection of the National Cancer Center, Korea, between 2001 and 2017. In total, 320 healthy subjects were included. Control patients were matched for age, sex, and date of admission or visit. The PC and BTC groups included high-risk individuals.
Serum extraction for metabolite profiling

Fifty microliters of serum were added to 1 mL water. After vortexing, 2 mL MeOH and 0.9 mL dichloromethane were added. After vortexing and placing on ice for 30 min, 1 mL water and 0.9 mL dichloromethane were added, and the mixture was centrifuged (1500 rpm, 10 min, room temperature). The supernatant was dried under an N₂ stream and subjected to MS analysis.

Serum metabolite analysis

Dried samples were reconstituted in 0.1% (v/v) formic acid and subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a Nexera X2 system (Shimadzu, Tokyo, Japan) coupled to a triple time-of-flight (TOF) 5600+ system (Sciex, Tokyo, Japan) equipped at the front end with a DuoSpray ion source (Sciex). For ultra-high-performance LC, the samples were loaded onto Atlantis T3 sentry guard cartridges (3 µm; 2.1 × 10 mm; Waters, Milford, MA, USA), and separation proceeded via an Atlantis T3 column (3 µm, 2.1 × 100 mm; Waters). The MS system was set to perform one full scan (50 to 1200 m/z, mass-to-charge ratio) followed by MS/MS of the ten most-abundant parent ions (mass tolerance, 50 mDa; collision energy, 35%).

Individual LMIs that enabled discrimination of the PC and BTC groups and the control, CRC, and OVC groups

Lists of LC-MS peaks (.peaks files) were created from a corresponding file (.wiff) for every sample using MarkerView software (Sciex). The parameters for this process were as follows: minimum retention time (RT), 0.00 min; subtraction offset, 10 scans; subtraction multiplication factor, 1.3; noise threshold, 10; minimum spectral peak width, 10 ppm; and minimum RT peak width, 5 scans. Next, a table of peaks was created by importing the .peaks files into the MarkerView software, for all samples simultaneously, using the following parameters: RT tolerance, 0.01 min; mass tolerance, 10.0 ppm; intensity threshold, 10; maximum number of peaks, 20,000; and area reporting using the “area integrated from the raw data, not from original peak finding.” The table of peaks contains data on the mass value (m/z), RT (min), and peak area.

The data in the peak table were converted to logarithms. A peak area of 0 was set to 1 because \( \log_{10}(0) \) is not defined and \( \log_{10}(1) \) is zero again. LMIs having outstanding discriminatory ability (i.e., distinguishing PC and BTC from control,
providing the observed

the Human Metabolome Database (HMDB), the compounds

Information Table 4 were evaluated by an independent sample

Differences in means between any two groups in Supporting

Statistical analyses

sitions within a specified mass tolerance of a given mass value

Finder tool (SCIEX) to determine probable elemental compo-

The MS and MS/MS spectra were analyzed by the Formula

Identification of metabolite ions

The MS and MS/MS spectra were analyzed by the Formula

Statistical analyses

Differences in means between any two groups in Supporting

As with the PC vs. BTC groups, no single LMI showed per-

LMI pairs that enabled discrimination of the PC

and BTC groups, and between the PC and BTC groups

and PC and BTC high-risk groups

To discriminate the PC and BTC groups, dual-ion methods

based on the ratio of logarithmic peak areas, or the difference

between peaks, were devised. Mathematically, the latter is the

logarithm of the ratio of peak areas, whereas the former is the

ratio of the logarithm of peak areas. The dual-ion methods

were executed as follows: 1) $\text{S}_2\text{C}_2$ combinations were arranged

as a list. Each pair of LMIs on the list was examined twice.

One of the two LMIs in each pair was arbitrarily chosen as the

numerator (or minuend) LMI initially, and was then used as

the denominator (or subtrahend) LMI. 2) All LMI pairs in the

list were investigated in sequence. The ratio of the common

logarithm of the two LMIs, or their difference, was calculated

as a discriminant score for all samples. 3) Thresholds were
determined with an increment of 0.01 such that the sum of

the sensitivity and specificity was highest. When more than

one threshold showed the same discrimination performance, the

thresholds were averaged. Furthermore, in cases of perfect
discrimination, discrimination ability was given by the differ-

ence between the maximum and minimum thresholds. 2) A

few discriminative LMIs were determined comparing the dis-

crimination performance of all $N$ LMIs.

LMI pairs that enabled discrimination of the PC

and BTC groups, and between the PC and BTC groups

and PC and BTC high-risk groups

The peak table consisted of 6724 LMIs (Supporting

Information Table 2). The single-ion method identified 14
discriminative LMIs (Fig. 1), each showing perfect discrimi-
nation between the PC and BTC groups and the control, CRC

and OVC groups. Table 2 shows the discrimination perfor-
mance of the LMIs in terms of the difference between the

maximum and minimum thresholds, and Fisher’s discriminant

ratio values. The logarithmic peaks of all 14 LMIs were higher

in the PC and BTC groups than in the control, CRC and OVC

groups. No LMIs that showed perfect discrimination were

higher in the control, CRC and OVC groups than in the PC

and BTC groups.

LMI pairs that enabled discrimination of the PC

and BTC groups

The dual-ion methods revealed five discriminative LMI

pairs (Fig. 2), each showing a combined sensitivity/
specificity > 90%. Table 3 shows their discrimination per-
formance. The first three pairs achieved their high performance

based on the ratio of the logarithm of peak areas, while the

remaining two pairs achieved their performance based on the logarithm of the ratio of peak areas. A mass

ion of 472.2419 m/z at an RT of 16.37 min (Supporting

Information Fig. 1a). Its logarithmic peaks were higher in

the BTC group than in the PC group.

No single LMI showed perfect discrimination between the PC

and BTC groups. The highest performance attained by a sin-
gle LMI was a sensitivity of 80.39% (10 false-negatives) and

a specificity of 94.87% (2 false-positives), achieved by a mass

ion of 271.1904 m/z at an RT of 16.37 min (Supporting

Information Table 2). The single-ion method identified 14
discriminative LMIs (Fig. 1), each showing perfect discrimi-
nation between the PC and BTC groups and the control, CRC

and OVC groups. Table 2 shows the discrimination perfor-
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LMI pairs that enabled discrimination of the PC

and BTC groups and the PC and BTC high-risk groups

As with the PC vs. BTC groups, no single LMI showed per-
flect discrimination between the PC and BTC groups and the

LMI pairs that enabled discrimination of the PC

and BTC groups and the PC and BTC high-risk groups

Individual LMIs that enabled discrimination of the PC

and BTC groups and the control, CRC and OVC groups

Results

as with the PC vs. BTC groups, no single LMI showed per-
flect discrimination between the PC and BTC groups and the
Fig. 1 Individual LMIs discriminating the PC and BTC groups from the control, CRC and OVC groups. The mass peak areas of the selected LMIs were converted to logarithms. The 14 discriminative LMIs independently showed perfect discrimination. Horizontal lines denote the maximum and minimum thresholds. The results should be validated using a large number of new samples and including more LMIs with the next highest performance. LMI, low-mass ion; PC, pancreatic cancer; BTC, biliary tract cancer; CRC, colorectal cancer; OVC, ovarian cancer; HRG, high-risk group

Table 2 Discrimination performance of individual discriminative LMIs (PC/BTC vs. control/CRC/OVC groups)

| Mass ion  | Separating threshold | Fisher’s discriminant ratio |
|-----------|----------------------|----------------------------|
| Mass value (m/z) | Retention time (min) | Maximum | Minimum | Difference |
| 283.2018  | 10.17                | 4.27    | 3.62    | 0.65      | 53.50   |
| 305.1860  | 10.17                | 4.03    | 3.92    | 0.11      | 16.95   |
| 394.2633  | 10.80                | 4.95    | 4.14    | 0.81      | 39.89   |
| 505.3401  | 11.03                | 4.82    | 4.20    | 0.62      | 18.78   |
| 505.3405  | 10.93                | 4.28    | 4.25    | 0.03      | 8.40    |
| 527.3245  | 11.39                | 4.46    | 3.64    | 0.82      | 48.98   |
| 527.3246  | 11.03                | 4.66    | 4.56    | 0.10      | 10.38   |
| 550.3229  | 11.56                | 4.57    | 4.33    | 0.24      | 26.70   |
| 555.2872  | 11.56                | 3.99    | 3.85    | 0.14      | 26.65   |
| 594.3589  | 11.74                | 4.46    | 3.54    | 0.92      | 41.05   |
| 599.3159  | 11.76                | 4.15    | 4.07    | 0.08      | 17.40   |
| 616.3988  | 11.18                | 4.50    | 3.84    | 0.66      | 31.55   |
| 638.3749  | 11.89                | 4.54    | 3.59    | 0.95      | 56.24   |
| 643.3331  | 11.89                | 4.17    | 3.80    | 0.37      | 35.64   |

LMI, low-mass ion; PC, pancreatic cancer; BTC, biliary tract cancer; CRC, colorectal cancer; OVC, ovarian cancer
PC and BTC high-risk groups. The highest performance was a sensitivity of 68.52% (17 false-negatives) and a specificity of 97.22% (1 false-positive), achieved by a mass ion of 1030.6515 m/z at an RT of 11.15 min (Supporting Information Fig. 1b). Its logarithmic peaks were higher in the PC and BTC groups than in the PC and BTC high-risk groups.

The dual-ion methods revealed eight discriminative LMI pairs (Fig. 3), each showing a combined sensitivity / specificity > 90%. Table 4 shows their discrimination performance. The first five pairs achieved their performance based on the ratio of the logarithm of peak areas, and the remaining eight pairs achieved their performance based on the logarithm of the ratio of peak areas. The latter contained the former.

Overall, the logarithmic peak areas of the numerator or minuend LMIs were higher in the PC group than in the BTC group and the reverse was true for denominator or subtrahend LMIs. LMI, low-mass ion; PC, pancreatic cancer; BTC, biliary tract cancer; HRG, high-risk group

Candidate metabolites for individual LMIs and LMI pairs

Supporting Information Table 3 shows candidate metabolites for LMIs. Nine LMIs were not matched with any metabolites in the HMDB and, in many cases, the mass information of single LMIs did not reduce the number of candidate metabolites. However, two LMIs (496.3 m/z at an RT of 18.9 min and 544.3 m/z at an RT of 18.3 min), lysophosphatidylcholine (LysoPC) (16:0) and LysoPC(20:4(5Z,8Z,11Z,14Z)), identified according to their MS/MS patterns in the analyses of the sera of BTC patients, could discriminate between PC and BTC (Figs. 2 and 4, Table 3). The relative amounts of LysoPC(16:0) and LysoPC(20:4(5Z,8Z,11Z,14Z)) were significantly lower in the PC, BTC, CRC, and OVC groups than

| Table 3 | Discrimination performance of discriminative LMI pairs (PC versus BTC) |
|---------|--------------------------------------------------|
| Mass value (m/z) | Retention time (min) | Mass value (m/z) | Retention time (min) | Sensitivity | Specificity |
| Numerator LMI | Denominator LMI |
| 496.3405 | 18.73 | 472.2419 | 10.18 | 90.20% | 92.31% |
| 497.3403 | 18.73 | 472.2419 | 10.18 | 90.20% | 92.31% |
| 544.3365 | 18.15 | 472.2419 | 10.18 | 90.20% | 92.31% |
| Minuend LMI | Subtrahend LMI |
| 498.3499 | 15.23 | 472.2419 | 10.18 | 92.16% | 92.31% |
| 544.3384 | 14.98 | 472.2419 | 10.18 | 90.20% | 92.31% |

LMI low-mass ion, PC pancreatic cancer, BTC biliary tract cancer
in the control group (Fig. 5, Supporting Information Table 4). LMI alone did not allow discrimination of the cancer and control groups (Fig. 5). However, statistical analyses confirmed the differential levels of the two metabolites in the PC/BTC vs. control/CRC/OVC and PC and BTC high-risk groups. The horizontal line denotes the corresponding discrimination threshold. Overall, the logarithmic peak areas of the numerator or minuend LMIs were higher in the cancer group than in the high-risk group and the reverse was true for denominator or subtrahend LMIs. The same five LMI pairs were selected using the relative ratio or difference. LMI; low-mass ion, PC, pancreatic cancer; BTC, biliary tract cancer; HRG, high-risk group

**Discussion**

The rapid development of mass analysis for metabolomics facilitates discovery of reliable cancer-screening biomarkers. Such information will enable predictive, preventive, and personalized medicine (PPPM) for cancer patients [26–28].

**Table 4** Discrimination performance of discriminative LMI pairs (PC/BTC versus PC/BTC HRG)

| Mass value (m/z) | Retention time (min) | Mass value (m/z) | Retention time (min) | Sensitivity  | Specificity |
|-----------------|----------------------|-----------------|----------------------|--------------|-------------|
| **Numerator LMI** |                      | **Denominator LMI** |                      |              |             |
| 756.3643        | 10.02                | 657.6682        | 10.32                | 94.44%       | 91.67%      |
| 380.7359        | 7.03                 | 181.0714        | 7.73                 | 90.74%       | 91.67%      |
| 381.7330        | 6.94                 | 181.0714        | 7.73                 | 90.74%       | 91.67%      |
| 712.9321        | 8.55                 | 657.6682        | 10.32                | 90.74%       | 91.67%      |
| 756.5703        | 10.01                | 657.6682        | 10.32                | 90.74%       | 91.67%      |
| **Minuend LMI** |                      | **Subtrahend LMI** |                      |              |             |
| 630.3031        | 10.01                | 657.6682        | 10.32                | 92.59%       | 91.67%      |
| 756.1579        | 9.99                 | 657.6682        | 10.32                | 92.59%       | 91.67%      |
| 756.3643        | 10.02                | 657.6682        | 10.32                | 92.59%       | 91.67%      |
| 380.7359        | 7.03                 | 181.0714        | 7.73                 | 90.74%       | 91.67%      |
| 381.7330        | 6.94                 | 181.0714        | 7.73                 | 90.74%       | 91.67%      |
| 712.9321        | 8.55                 | 657.6682        | 10.32                | 90.74%       | 91.67%      |
| 756.1579        | 9.99                 | 657.3503        | 10.33                | 90.74%       | 91.67%      |
| 756.5703        | 10.01                | 657.6682        | 10.32                | 90.74%       | 91.67%      |

LMI low-mass ion, PC, pancreatic cancer; BTC, biliary tract cancer; HRG, high risk group
Recently, we analyzed metabolites detected as LMIs by mass spectrometry (MS) and reported the metabolic profiles of cancer patients based on serum analyses. These metabolic profiles were useful for predicting, diagnosing, and predicting the prognosis of cancer [29–31]. In the present study, we obtained metabolic profiles based on MS analysis of serum from PC and BTC patients, and found that the profiles showed potential for diagnosing cancer.

Fourteen discriminative LMIs were identified, and each showed perfect discrimination between the PC and BTC groups. Two LMIs (496.3 m/z, retention time (RT) of 18.9 min a and 544.3 m/z, RT of 18.3 min b) were identified as LysoPC(16:0) and LysoPC(20:4(5Z,8Z,11Z,14Z)) according to the tandem mass spectrometry (MS/MS) patterns of serum samples of BTC patients.

**Fig. 4** LMIs discriminating between the PC and BTC groups. Two LMIs (496.3 m/z, retention time (RT) of 18.9 min a and 544.3 m/z, RT of 18.3 min b) were identified as LysoPC(16:0) and LysoPC(20:4(5Z,8Z,11Z,14Z)) according to the tandem mass spectrometry (MS/MS) patterns of serum samples of BTC patients.

**Fig. 5** Amounts of LysoPC(16:0) and LysoPC(20:4(5Z,8Z,11Z,14Z)) in the serum of PC and BTC patients. The amounts of the two metabolites (unit: logarithm of the mass peak area) were slightly lower in the PC, BTC, CRC and OVC groups than in the control group, and no single metabolite could discriminate between the cancer groups and the control group.
groups and the control, CRC, and OVC groups (Fig. 1 and Table 2). The logarithmic peaks of all 14 LMIs were higher in the PC and BTC groups (Fig. 1). When the data in the peak table were normalized according to the “total area sums” method, two additional individual LMIs (527.3218 m/z, 10.95 min RT and 749.4577 m/z, 11.33 min RT, (Supporting Information Fig. 2A, C) showed perfect discrimination between the PC and BTC groups and the control, CRC and OVC groups. The normalization yielded the same total peak areas for each sample in the peak table, via multiplication by a scaling factor. However, they yielded the next highest (15th and 16th) discrimination performance with the unnormalized peak table (Supporting Information Fig. 2B, D). Because the normalization process made a minor difference, it was not considered in this article.

No single LMI discriminated between the PC and BTC groups, but five LMI pairs were discriminative (Fig. 2 and Table 3). These LMI pairs showed a combined sensitivity/specificity > 90%. The mass ion of 472.2419 m/z at an RT of 10.18 min was used as the denominator or subtrahend LMI, and may be an important metabolite for discriminating between PC and BTC. The HMDB suggested two metabolites, hydroxydesmethyl doxepin glucuronide and (E)-2-hydroxydoxepin glucuronide, as candidates for LMI with 472.2419 m/z (Supporting Information Table 2). Both are metabolites of the psychotropic agent doxepin. Other endogenous metabolites should be sought that respond to the LMI of 472.2419 m/z. Similar to the situation for discriminating between the PC and BTC groups, no single LMI discriminated between the PC and BTC and PC and BTC high risk groups. However, eight LMI pairs were discriminative (Fig. 3 and Table 4) and showed high sensitivity and specificity.

Metabolic profiling provided useful information regarding screening for PC and BTC, and also showed a shortage in the identification of LMIs selected. Two LMIs (496.3 m/z at an RT of 18.9 min and 544.3 m/z at an RT of 18.3 min), identified as LysoPC(16:0) and LysoPC(20:4(5Z,8Z,11Z,14Z)), discriminated between PC and BTC, Figs. 2 and 4, Table 3. However, the relative amounts of LysoPC(16:0) and LysoPC(20:4(5Z,8Z,11Z,14Z)) were also slightly decreased in the PC/BTC/CRC/OVC groups compared with those in the control group (Fig. 5).

LysoPC is a product of the phosphatidylcholine hydrolysis precipitated by phospholipase A activity; in tumor tissue, LysoPC is usually generated by saturated PC after the accumulation of liposomes, and has been shown to activate cells from several lineages [32, 33]. Higher LysoPC levels were associated with lower risks of breast, prostate, and colorectal cancers [34], response to chemoradiotherapy in esophageal squamous cell carcinoma [35], and reduced melanoma cell adhesion and metastasis [32, 36]. However, decreased levels of LysoPC in blood or tissue precede the diagnosis of cancer by several years. Decreased LysoPC levels have been reported in the blood and tissue samples of patients with many types of cancer [30, 37, 38], e.g., blood samples of colorectal [39–41] and cervical cancer patients [40] and tissue samples of gastric [42], prostate [43], and liver [44] cancer patients. Considering these results across many types of cancer, metabolic changes in lipid metabolism may drive tumorigenesis.

Recent studies have shown that LysoPC can cause cholangiocyte senescence, which potentially contributes to the pathogenesis of BTC [45]. Furthermore, LysoPC can inhibit cholangiocyte apoptosis by inducing COX-2 expression via a Raf-1-dependent mechanism, and such anti-apoptotic effects might be important in biliary tract carcinogenesis in patients with compromised pancreaticobiliary ductal junctions [46]. Similar to other types of cancer, we found a reduced level of LysoPC in the serum of BTC patients [47], as well as significantly decreased levels of LysoPC in the bile of these patients compared with those with benign biliary tract disease [10]. These findings strongly imply that LysoPC may be useful for BTC diagnosis and prognosis [47, 48].

Conclusions and expert recommendations

Targeted metabolic profiling uses a mixture of standard metabolites and so allows identification and quantification of metabolites in samples exactly matched to standard metabolites. Targeted metabolic profiling provides information on metabolites of interest, whereas non-targeted metabolic profiling harvests information on the mass-to-charge ratio (m/z) and relative amounts of metabolites in a sample. Compared to targeted metabolic profiling, non-targeted metabolic profiling is limited in its ability to identify metabolites. In non-targeted metabolic profiling, metabolites are present as LMIs in mass spectra, the m/z of each of which can match multiple metabolites. For identification of LMIs by non-targeted metabolomics, candidate metabolites must be listed in HMDB using only their m/z, and the MS/MS patterns of the LMIs must be compared with those of candidate metabolites obtained from commercial sources. Therefore, unlike targeted metabolomics, identification of metabolites is laborious as it depends on the m/z. Furthermore, if standard metabolites are not commercially available, they must be synthesized. Therefore, two types of metabolic profiling can be performed, depending on the goal of our research. Our data indicate that non-targeted metabolic profiling based on blood samples can be done using information on the mass and relative amounts of LMIs. Metabolic profiling can precisely denote the status of diseases such as cancer and, thus, can be used for cancer screening. The present study not only described efficient methods for selecting discriminative (between PC and BTC) LMIs for the purposes of cancer screening but also provided an example of non-targeted metabolic profiling for screening these diseases. Information on all individual metabolites...
obtained in this retrospective, non-targeted metabolic profiling will facilitate validation of our results in a prospective study involving a large number of patients. No screening test has been shown to lower the risk of dying from PC and BTC. Two metabolites [LysoPC(16:0), LysoPC(20:4)] have potential utility for distinguishing PC from BTC when combined with other, previously identified proteins or metabolic biomarkers for predictive preventive personalized medicine to identify individuals at high risk for PC and BTC.

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Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Ethical approval All the patients were informed about the purposes of the study and consequently have signed their “consent of the patient”. All investigations conformed to the principles outlined in the Declaration of Helsinki and were performed with permission by the responsible Ethics Committee of National Cancer Center, Republic of Korea (IRB Numbers: NCC2015-0173, NCC2015-0248, NCC2018-0005).

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