Plasma lipid profile comparison of five different cancers by nanoflow ultrahigh performance liquid chromatography-tandem mass spectrometry

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Abstract
A comprehensive lipidomic analysis at the molecular level using nanoflow ultrahigh performance liquid chromatography-electrospray ionization-tandem mass spectrometry (nUHPLC-ESI-MS/MS) was performed to elucidate the lipid profiles of patient blood samples from five commonly found cancers (liver, lung, gastric, colorectal, and thyroid), which were then compared with the lipid profiles of healthy controls. From a total of 335 lipids identified and quantified, 50 high abundance lipids showing significant changes (>2-fold and \( p < 0.01 \)) in at least one of the five cancers (vs. controls) were analysed. Lipid species were found to be significantly associated with more than one type of cancer; the numbers of lipid species found as significantly changed in all five, four, three, two, and one type of cancer were 1, 8, 8, 15, and 17, respectively. Among these, the high abundance phosphatidylethanolamine species, including lysophosphatidylethanolamine and PE plasmalogen, was significantly low in four cancer types, but was high in thyroid cancer. Receiver operating characteristic analysis resulted in the selection of lipids specific to each cancer: liver (four phosphatidylinositols and diacylglycerol 16:1_18:0), gastric (phosphatidylcholine 34:2, 36:3, and 36:4, and lysophosphatidic acid 18:2), lung (lysophosphatidylinositol 18:0, sphingomyelin d18:1/20:0, and triacylglyceride 50:1 and 54:4), and thyroid (lysophosphatidylinositol 18:0 and 18:1). Our results provide a basis for future validation of cancer-specific lipid markers with high diagnostic ability.

1. Introduction

Cancer is the leading cause of death and a major public issue in most countries because of the increasing healthcare budget. The incidence of cancer is increasing every year, and the predicted number of cases of new cancers by 2025 will be over 20 million
because of population growth and aging [1,2]. Although cancer diagnoses and therapies have been advancing rapidly, accurate diagnosis and prognosis of early-stage cancers are key in fighting cancer. Patients with the same cancer exhibit various phenotypes due to heterogeneous events, including genetic background, metabolism, motility, and environment [3]. Therefore, it is very difficult to accurately determine a patient’s status for personalized therapy. Trials to develop specific biomarkers at the molecular level through proteomic, metabolomic, and lipidomic approaches have been performed to overcome this limitation [3–7].

Lipids play an essential role in not only the formation of membrane structures and energy storage but also in signal transduction, cell proliferation, and apoptosis [8,9]. Lipids can also serve as secondary messengers or hormones in signalling or functional processes of cells [10]. It has been reported that cancer cells show alterations in lipid metabolism, which may influence numerous cellular functions, and lipid composition changes are found in a number of diseases [10–15]. Several lipidomic approaches have been developed to discover potential lipid biomarkers of cancers [3]. Most of these studies were achieved by using specific cells or tissues, such as lung [16,17], breast [18,19], prostate [20], colorectal [21,22], pancreatic [23], and gastric cancers [24]. Few studies have investigated urinary or serum lipids from patients with breast [25], prostate [26], and ovarian cancers [27].

Lipids are classified into eight different categories: fatty acyls, sterol lipids, glycolipids, phospholipids, prenol lipids, sphingolipids, polyketides, and saccharolipids [28]. Lipid analysis requires comprehensive state-of-the-art analytical approaches due to the complicated nature of lipid molecular structures from the variety of different polar head groups, length and unsaturation degree of fatty acyl chains, and substitutions with glycols. While several analytical methods have been utilized for lipid analysis, mass spectrometry (MS) has become a powerful tool for the accurate determination of lipid structures with high sensitivity. Liquid chromatography (LC) with electrospray ionization tandem MS (ESI-MS/MS) has enabled lipid analysis with simultaneous separation and identification of complicated lipid mixtures in an intact state with significant reduction of ion suppression effects [29–31]. Recently, the resolution and speed of lipid separation have been greatly improved by employing ultra-high performance LC (UHPLC) [12,32]. The use of nano-flow UHPLC (nUHPLC-ESI-MS/MS) has facilitated high speed, targeted lipid quantification with only a few micrograms of lipid sample for the analysis of the effect of exercise on skeletal muscle tissue of diabetic rats, for analysis of internal organ tissues of p53 knock-out mice, and urinary exosome from patients with prostate cancer [7,33–35].

In this study, a comprehensive lipidomic comparison was performed with plasma samples from patients diagnosed with five different, but high incidence cancers (liver, gastric, lung, colorectal, and thyroid cancers). Instead of analysing the samples of a specific cancer in separate batches of experiments, this study analysed plasma samples from different patients simultaneously for determination of plasma lipid pattern differences among five different cancer types compared to a healthy control group. A total of 335 lipids were structurally identified by non-targeted analysis using nUHPLC-ESI-MS/MS followed by targeted quantification based on selected reaction monitoring (SRM). Differences in lipid profiles among the five cancers were elucidated with few cancer-specific lipids, as well as few lipid species, which were significantly associated with multiple cancer types.

2. Experimental

2.1. Materials and reagents

A total of 38 lipid standards (Avanti Polar Lipids Inc., Alabaster, AL, USA) were utilized to optimize the nUPLC-ESI-MS/MS run conditions: lysophosphatidylcholine (LPC) 16:0, LPC 17:0, phosphatidylcholine (PC) 12:0/12:0, PC 13:0/13:0, PC 18:0/18:1, PC-plasmalogen (PCp) 18:0/22:6, lysophosphatidylethanolamine (LPE) 17:1, LPE 18:0, phosphatidylethanolamine (PE) 14:0/14:0, PE 16:0/16:0, PE 17:0/17:0, PE plasmalogen (PEp) 18:0/18:1, PEp 18:0/22:6, lysophosphatic acid (LPA) 17:0, LPA 18:0, phosphaticid acid (PA) 17:0/17:0, PA 18:0/18:0, lysophosphatidylglycerol (LPG) 12:0, LPG 17:1, LPG 18:0, phosphatidyglycerol (PG) 15/15:0, PG 18:0/18:0, lysophosphatidylinositol (MPI) 20:4, LPI 17:1, phosphatidylcholine (PC) 16:0/18:2, PI 17:0/20:4, sphingomyelin (SM) d18:0/12:0, SM d18:0/16:0, SM d18:0/17:0, Ceramide (Cer) d18:1/14:0, Cer d18:1/17:0, sulfatide (SulfoHexCer) d18:1/17:0, monohexosylceramide (HexCer) d18:1/17:0, HexCer d18:1/18:0, diacylglycerol (DG) 17:0/17:0-D5, DG 16:0/18:1, triacylglycerol (TG) 17:0/17:1/17:0-D5, and TG 18:0/18:1/18:1. The standard lipids containing odd-numbered acyl chain were used as internal standards added to the plasma lipid extract for SRM quantitation. HPLC grade solvents (H2O, CH3CN, CH3OH, isopropyl alcohol (IPA), methyl-tert-butylo ether (MTBE)) were used (Avantor Performance Materials, Center Valley, PA, USA). NH4HCO2, NH4OH, and CHCl3 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Silica capillary tubes used to prepare the capillary LC columns had inner diameters of 20, 50, and 100 μm and had identical outer diameters of 360 μm (Polymicro Technology, LLC, Phoenix, AZ, USA). Capillary column packing materials consisted of 1.7 μm ethylene bridged hybrid (HE) particles, which were unpacked from an ACQUITY UPLC BEH C18 column (2.1 mm × 100 mm, 130 Å) (Waters, Milford, MA, USA) and Watchers ODS-P C-18 particles (3 μm and 100 Å) (Isu Industry Corp., Seoul, Korea) to form a self-assembled frit of 0.5 cm at the column tip prior to packing with the 1.7 μm HE particles.

2.2. Human plasma samples

A total of 104 human blood plasma samples, including 20 healthy controls, were examined. Control samples (age = 50.2 ± 10.9 years) were provided by the Wonkwang University Hospital Biobank (Iksan, Korea), a member of the Korea Bioresources Network (KBN). Plasma samples from patients diagnosed with five different cancers were provided from the Biobank of Severance Hospital at Yonsei University (Seoul, Korea). This study was approved by Institutional Review Board of Severance Hospital and conducted in accordance with the current version of the Declaration of Helsinki. Only male samples were utilized in this study to exclude possible hormonal influences on the regulation of blood lipid metabolites [36]. The number of patient samples (average age) is 21 (56.1 ± 10.6 years) for liver cancer, 20 (58.5 ± 13.1 years) for gastric cancer, 17 (61.1 ± 9.6 years) for lung cancer, 16 (58.9 ± 10.9 years) for colorectal cancer, and 10 (46.9 ± 19.9 years) for thyroid cancer. Information on the developmental stage of cancer for each sample is listed in Table 1. Plasma samples were stored at −80°C until used.

2.3. Lipid extraction

Plasma lipid extraction was performed following a modified MTBE/MeOH method [37,38] of which recovery of lipid extraction was reported to be about 82–98% for 17 different lipid classes. A 100 μL aliquot of each plasma sample was dried in a Bondirol MCFD 8508 freeze dryer vacuum centrifuge (iShinBioBase, Yangju, Korea) and concentrated. The lyophilized lipid sample was mixed with 300 μL of methanol on an ice bath and incubated for 10 min. Following incubation, 1000 μL of methyl-tert-butylo ether (MTBE) was added to the dissolved lipids and the mixture was vortexed for 1 h. MS-grade water (250 μL) was added to the mixture, vortexed
Lipids were weighed, reconstituted in CHCl₃:CH₃OH (1:9, v/v) at a MilliWrap PTFE membrane (Millipore, Burlington, MA, USA) to avoid lipid evaporation and was dried in a vacuum centrifuge. Dried lipids were weighed, reconstituted in CHCl₃:CH₃OH (1:9, v/v) at a concentration of 15 μg mL⁻¹, and stored at −80 °C. For nUHPLC-ESI-MS/MS analysis, the frozen lipid extract sample was diluted to 5 μg mL⁻¹ in H₂O:CH₃OH (2:8, v/v).

2.4. nUHPLC-ESI-MS/MS of lipids

Lipid analysis was conducted by the two stages: non-targeted identification of lipids from plasma sample at first and targeted SRM quantitation of identified lipids for the individual samples in the second stage. Two sets of nUHPLC-ESI-MS/MS systems were employed in this study: A Dionex Ultimate 3000 RSLCnano System coupled with a LTQ Velos ion trap mass spectrometer (Thermo Scientific, San Jose, CA, USA) for the nontargeted identification of the plasma lipidome and a model nanoACQUITY UHPLC system from Waters(Milford, MA, USA) equipped with a TSQ Vantage triple-stage quadrupole MS system (Thermo Scientific) for the targeted quantitation of lipids. In both systems, the same capillary column prepared in our laboratory was utilized. The analytical column was prepared with a fused silica capillary (100 μm inner diameter and 360 μm outer diameter) in which the tip of the capillary was pulled into a sharp needle by a flame for the self-emitter for ESI. The first 0.5 cm portion at the needle tip of the column was packed with Watchers™ 3 μm ODS-P C18 particles to form a self-assembled frit and the remaining length (6.5 cm) of the column was packed with 1.7 μm XBridge BEH C18 130 Å particles (Waters) under nitrogen gas at 1000 psi. The capillary column was connected to a capillary tube from the UHPLC pump via a PEEK microcross (IDEX, Oak Harbor, WA, USA) and the additional two ports were linked with a platinum wire for ESI voltage and a pressure capillary (20 μm inner diameter and 360 μm outer diameter) to split the pump flow for providing nano-flow. The pressure capillary tube was ended with an on/off switch valve to vent or block the flow. Mobile phase solutions were (9:1, v/v) H₂O:CH₃CN for A and (2:2:6, v/v/v) CH₃OH:CH₃CN:IPA for B. Both were added with a mixed ionization modifier, 5 mM NH₄HCO₂ and 0.05% NH₄OH, available for both positive and negative ion mode of MS analysis. The elution gradient used for non-targeted lipid identification was longer than that used for targeted quantitation and the same run condition was applied in both positive and negative ion modes. The lipid sample was loaded with 100% mobile phase A at 550 nL min⁻¹ for 14 min with the vent valve closed. After sample loading, mobile phase B was increased from 75% to 80% for 4 min and maintained at 80% for 8 min at a pump flow rate of 7 μL min⁻¹ with the vent valve on so that the final flow rate to the capillary column should be 300 nL min⁻¹. It was then increased to 90% for 18 min and finally to 100% for 5 min. Mobile phase B was decreased to 0% and the column was reconditioned for 5 min.

A 3 kV ESI voltage was used at both positive and negative ion modes. For collision-induced dissociation (CID) experiments, 40% of normalized collision energy was applied. The m/z range for the precursor scan was from 250 to 1100. Identification of lipid structures was performed by LiPilot, a computer software program that determines lipid structure from CID spectra, developed in our laboratory [39].

The gradient method used for targeted quantitation with the SRM method was aimed to achieve a high-speed separation. Sample loading was made at 700 nL min⁻¹ for 9 min with mobile phase A. Gradient elution began with the increase of the mobile phase B to 50% for 1 min, rapidly increased to 80% for 10 min, and further to 100% for 5 min. It was then decreased to 0% and the column was reconditioned for 5 min. Detection of lipid ions by SRM quantitation was made with the polarity switching mode, which operated positive and negative ion modes alternatively in a single run, using a scan width m/2 of 1.0, scan time of 0.001 s, and ESI voltage of 3 kV. For SRM quantitation of targeted lipids, each lipid species was programmed for detection during a time interval (retention time ± 1 min) instead of scanning all lipids simultaneously and the amount of each species was measured from the peak area of a precursor ion and its specific product ion (quantifier ion) for quantification. The types of precursor ions and product ions of each lipid class are listed in Table S1, along with collision energy value specific to each lipid class. Prior to quantitation, 1 pmol each of the 17 lipid standards (with odd-numbered fatty acyl chains) was added to each plasma lipid extract sample. In the positive ion mode the polarity switching mode, lipid classes LPC, PC, LPE, PEp, SM, Cer, HexCer, SulfOHexCer, DG, and TG were detected. Classes LPG, PG, LPI, PI, LPA, and PA were detected in the negative ion mode. Statistical evaluations were made with the one-way analysis of variance (ANOVA) test and logistic regression using SPSS software (version 24.0, IBM Corp., Armonk, NY, USA), and principal component analysis (PCA) using Minitab 17 statistical software (State College, PA, USA).

2.5. Method validation

Validation of the nUHPLC-ESI-MS/MS method for lipid quantification was accomplished with a plasma sample spiked with a set of lipid standards with varying injection amounts into the nUHPLC system (0.0025–1 pmol). Separation of lipid standards was achieved under the same run condition utilized in Fig. S2 of Supplementary Material, showing ultrahigh performance separation of the homemade nUHPLC column employed in this study: 19.8 ± 4.2 s for the average peak width (based on 4σ) and 0.4 ± 0.2% for average...
Relative Standard Deviation (RSD) in retention time of all lipid standards, and 408,772 for the calculated plate number of Sulfo-HexCer d18:1/17:0. The limit of detection (LOD) with the limit of quantitation (LOQ) was calculated from the calibration curve established from the peak area of standard lipids based on the signal-to-noise ratio (S/N) = 3 and 10, respectively, as LOD = 3 x (standard deviation of y-intercept)/slope of calibration curve. LOD values (S/N = 3) were in the range of 0.010 (LPC 16:0)-0.057 pmol (HexCer d18:1/12:0) and LOQ values (S/N = 10) were 0.032–0.191 pmol which were based on the S/N = 3 and 10, respectively, from the calibration curves established within the peak area of six different standard lipids at five different concentrations; LPC 16:0, PC 12:0/12:0, PC 18:0/22:6, HexCer d18:1/12:0, TG 17:0/17:1/17:0 D5, and LPG 18:0. Calculated data are in Table S2 of Supplementary Material.

3. Results

3.1. Lipid identification and quantification

For non-targeted lipid identification, a lipid extract sample from a pooled plasma mixture (mixed with an equal aliquot from individual samples) in each sample group was analysed under the same run conditions used for the separation of lipid standards in Supplementary Fig. S1. Extracted ion chromatograms obtained from each sample group are shown in Fig. S2, obtained in both positive ion and negative ion modes. A total of 335 lipids were identified with their molecular structures from all sample groups. Among them, 219 lipids were quantified using nUHPLC-ESI-MS/MS based on the SRM method. Quantification of lipid species in the classes of PC, PE, DG, and TG was made at the level of total carbon number of acyl chains and double bonds because SRM quantification of these species can not differentiate geometrical isomers. The quantified 219 lipids are listed in Table S3 with the calculated results expressed by the corrected peak area (ratio of peak area of an individual lipid molecule to that of an internal standard (1 pmol) specific to each lipid class) and peak area ratio in comparison to that of the control. Table S3 represents the average data (n = 3) obtained from individual samples in each sample group. Isomeric structures of PC, PE, DG, and TG are listed in Table S4.

3.2. Lipid alterations in five different cancer groups

Alterations in overall lipid levels between each cancer type are illustrated with the Volcano plots of 219 lipid species (−log10 (p value) vs. log2 (fold change)) in Fig. 1. While a considerable number of lipid species were significantly (>2-fold with p < 0.01) increased and decreased (upper right and upper left, respectively) in the patient’s plasma samples of both liver and thyroid cancers, most lipids showed significant changes in gastric, lung, and colorectal cancers, which were largely decreased in comparison to those of the control. The overall differences in plasma lipid profiles among the five cancer groups and the control group were further visualized with a Principle component analysis (PCA) plot using lipid species showing >3-fold difference and p < 0.01 (Fig. 2). Each data point represents the overall lipid profile of an individual patient. Data points from each cancer group are clustered together, apart from other cancer groups and separated from the control group, supporting that lipid profiles are distinctly altered upon the development of cancer regardless of its type and differentiated upon the type of cancer. It also shows that data points in the control and thyroid cancer groups are relatively scattered while those of

![Fig. 1](https://example.com/fig1.png) Volcano plots of 219 lipid species representing log_{10}(p value) vs. log_{2} (fold change) in (a) liver, (b) gastric, (c) lung, (d) colorectal, and (e) thyroid cancers in comparison to the control group.
the other four cancer groups were well clustered, supporting that individual variations in the control and the thyroid cancer group were larger than those in the four other cancer groups.

Lipid profiles of the five cancer types can be further compared at the individual molecular levels using a heat map plotted with significantly changed lipids (≥3-fold and p < 0.01) in Fig. 3. Selected LPC species increased in liver and thyroid cancers, as well as in gastric cancer patients, and LPI and PI levels increased in thyroid and liver cancers, respectively. However, most lipid classes, including PC, LPE, PE, and PEP, appeared to decrease in the development of cancer, except in the thyroid cancer group. Among the lipid species in Fig. 3, the molecular structure of PG 22:6/22:6 determined from its MS and MS/MS spectra are attached in Fig. S3 to support the identification of its molecular structure. PG 22:6/22:6 is presumably a bis(monoacylglycero)phosphate, which is a structural isomer of PG with each acyl chain attached to its two glycerol units, although the isomeric structures were not resolved in this study.

For a closer look at the individual lipid profile changes, the following criteria were applied to select few lipid species: high abundance species in each class with significant changes ≥2-fold and p < 0.05 in one of the cancer groups compared to the control (Table S3). High abundance species was defined as a lipid with a relative abundance higher than 100%/number of lipids within the class. Fifty-one lipid species were selected and listed with peak area ratio versus control (Table 2). Total change in the level of each lipid class, along with the individual variation in each sample group are shown with stacked bar graphs in Fig. 4, which were plotted using the corrected peak area of high abundance lipid species marked with acyl chain structures (listed in Table 2), along with the total area of low abundance species marked as "low". Number at the right of each bar graph is the relative amount compared to the control group as 1.00. Six lipid classes (LPC, PE, PEP, LPI, PI, and TG) with significant changes in only one of the cancer groups are shown. The same results were represented with pie-charts in Fig. S4, too. Two high abundant LPC species (16:0 and 18:2) and total LPC amounts were increased in liver and gastric cancers ≥4-fold. Total amounts of PE and PEP were ≥2-fold decreased in all cancer groups, except thyroid cancer. A high abundance PE species (38:4, 38:3, and 40:6) were significantly (p < 0.01) decreased >3-fold in the thyroid cancer group as well. Prominent changes were observed in LPI and PI classes, with the overall LPI amount decreased in liver and gastric cancers, but increased in lung (2.29-fold) and thyroid cancers (5.92-fold). While LPI class was decreased in liver and gastric cancers, PI was increased in these groups, especially in liver cancer (2.62-fold), which may indicate a correlation between LPI and PI. Moreover, low abundance PI species (18:0/22:6), based on the composition in the control group, increased 4.2-fold in liver cancer and its compositional distribution increased too as a high abundance PI species in liver, gastric, lung, and colorectal cancer groups. TG was increased in liver cancer ≥2-fold, but decreased in gastric cancer, and not significantly changed in the remaining three cancer groups. Changes in the remaining lipid classes (PG, LPE, Cer, SulfoHexCer, PC, SM, LPA, DG, and PA) are shown in Fig. S5.
The fold changes of high abundance lipid species with significant differences (>2-fold, \( p < 0.05 \)), sorted by lipid, appeared to associate with multiple cancers (Fig. 5). The open bar represents increased fold and the filled bar represents decreased fold. PI 18:1/18:0 was the only high abundance lipid with a significant change in all five cancer types examined in this study. It was increased only in liver cancer and decreased in the other four cancer types. The five PEs and the three PEPs (Fig. 5b) were decreased significantly in four cancers. LPC 18:2, LPE 18:1, 3 PEs, 3 PEP, and 1 PG (Fig. 5c) were changed in three cancers, liver, lung, and colorectal. Lipid groups in Fig. 5d and e shows lipids associated with dual and single cancer types, respectively. Although lipid species showing significant changes in multiple cancers are not direct candidate molecules to distinguish a specific cancer, they can serve as the general indicators of the pathogenic status and be utilized in combination with a candidate species unique to each cancer. In liver cancer, DG 12:1_18:0 was significantly decreased (7.69-fold), while three PI species, PA 16:0/22:6, and DG 16:1_18:0 were significantly increased, as well as PI 18:1/18:0. Four unique species (LPA 18:2, PC 34:2, 36:3, and 36:4) in gastric cancer were decreased. The lung cancer group was distinguished by decreases in PA 18:1/18:1 and SM 18:1/20:0 and increases in TG 50:1 and 54:4. The thyroid cancer group had significant increases in LPI 18:0 (6.19-fold) and 18:1 (4.09-fold).

For the lipid species showing a unique change in each cancer, including those found in multiple cancers, the receiver operating characteristic (ROC) analysis was accomplished and ROC curves, along with area under curve (AUC) values are plotted for the three PI’s (16:0/18:2, 16:0/20:4, and 18:0/20:3) and DG 16:1/18:0 for liver cancer, as well as two TGs (50:1 and 54:4) for lung cancer (Fig. 6). Candidate lipid species with AUC values > 0.800 were selected and listed in Table 3 by those unique to a specific cancer and to multiple cancers. ROC curves of selected lipid species for other three cancers are in Fig. 5f.
4. Discussion

A recent study reported that PE and PEp species were significantly decreased in patient serum samples of hepatocellular carcinoma and suggested that the two PEp species (16:0p/20:4 and 18:1p/22:5) can be utilized as biomarkers [40]. In our study, PE 16:0p/20:4 was significantly decreased in liver cancer as well, but PE 18:1p/22:5 was not detected, possibly due to low abundance. However, PEs and PEps were decreased in most cancer samples examined in this study, except thyroid cancer. Functions of plasmalogens are not clearly known, but they were reported to be involved in reducing damage from reactive oxygen species in cancer cells [41]. Moreover, several cancers, including colon, prostate, lung, and breast cancers, were reported to be associated with low levels of PEp in serum [40], which was found in our study. Serum sphingolipids in hepatocellular carcinoma, examined by Xu et al., showed decreases in several SM species and about a 2-fold increase of HexCer d18:1/20:0 [42], which are similar to our results.

LPC is known to have a pro-inflammatory function caused by pathological activity and is considered to be associated with cancer metastasis [43,44]. Earlier studies on serum lipids from patients with lung cancer showed significant increases in most LPC levels [45,46], however our study showed an increase for most LPCs in lung cancer, but no statistically significant differences. SM d18:1/22:0 was reported as a candidate marker, showing a significant decrease in the serum of squamous cell lung cancer patients [47], similar to our results (peak area ratio of 0.41 ± 0.08 with p < 0.01). However, in our study, SM d18:1/22:0 was also significantly decreased in thyroid cancer, therefore it is not unique to lung cancer, but may be an indicator for multiple cancers. As described above, PE and PEp were found to be significantly decreased in lung cancer in this study. An earlier work on plasma lipids of patients with squamous carcinoma and adenocarcinoma lung cancers showed that five PEp (38:3, 38:5, 40:1p/20:4, and 18:1p/20:4) were significantly decreased, and TG 54:4 was increased [48], matching our results.

In colorectal cancer, most LPCs were found to be decreased in this study, but their differences were not statistically significant. However, several studies reported that serum or plasma levels of most LPC species were significantly decreased in colorectal cancer by moderate (<2-fold) [49,50] or large (2–3-fold) degrees [51,52], with significant differences, but molecular chain structures of the candidate LPCs from the various studies were somewhat different from each other, except a few commonly found species. The poor statistical comparison of LPC levels in our study may originate from the relatively low number of samples (16 colorectal cancer patients) compared to the reported works. PE and PEp species were significantly lower in colorectal cancer, as observed in liver, gastric, and lung cancers. However, the degree of their decreases in colorectal cancer appeared to be even larger than that of the other three cancers (Fig. 4). Seven PEp species in colorectal cancer were significantly decreased (>5-fold) in our study. Among them, PE 18:1p/20:2 was proposed as a candidate marker of colorectal cancer (2-fold decrease) [51].

Plasma lipid profiles of thyroid cancer were somewhat different from those of the other four cancers in this study. Two high abundant LPEs (18:1 and 18:2) were increased by nearly 3-fold, although they exhibited decreases (2–5-fold) in other cancers. PE
16:1p/22:6 was increased about 2-fold, but their levels in the other four cancers were decreased or not changed. SM d18:1/22:0 has also been reported as decreased by about 2-fold in serum samples of thyroid tumours [14], similar to our results. However, this SM species was significantly decreased in lung cancer samples in this study as well.

5. Conclusions

This study showed a comprehensive lipidomic comparison of patient plasma samples from five different cancers (vs. healthy controls) at the molecular levels by using nUHPLC-ESI-MS/MS, resulting in 50 high-abundance lipid species with significant changes (>2-fold, \( p < 0.05 \)) in at least one of the five cancers. Simultaneous analysis of different cancer samples provided an overview of variations in lipid profiles, which cannot be obtained from individual analysis of each disease. This study revealed that a considerable number of lipids were significantly changed simultaneously in at least two or more types of cancers, however, few high abundance species were unique to each specific cancer.

Investigation of the lipid changes in different cancers revealed that high abundance LPE, PE, and PEp species were commonly decreased in all cancers, except thyroid cancer which had several species (18:1, 18:2, and 16:1p/22:6) increased instead. While PI 18:1/18:0 was a significantly changed high abundance lipid in all cancers, it increased only in liver cancer and decreased in the other four cancers. Therefore it can be utilized as a candidate molecule specific to liver cancer, as well as other unique lipids: three PI's (16:0/18:2, 16:0/20:4, and 18:0/20:3) and DG 12:1_18:0, all showing AUC value >0.800 (Table 3). Gastric cancer can be uniquely distinguished by decreases in two PCs (36:3 and 36:4) and LPA 18:2 with a very high AUC value (>0.9), however, these species had tendencies to decrease in other cancers, although their fold changes were less than 2-fold. Lung cancer showed a unique decrease in SM d18:1/20:0 and discrete increases in two TG's (50:1 and 54:4), and two PE's (38:3 and 18:1p/20:4) were capable of differentiating lung cancer, although they exhibited changes in multiple cancers. Significant increases (4−6-fold) in two LPIs (18:0 and 18:1) were unique in differentiating thyroid cancer, along with LPE 18:1 and LPE 18:2, which showed a decrease in both liver and gastric cancers.

The present study demonstrates that discovery of lipid biomarkers in various cancers can be accomplished with systematic investigations of lipid profiles in combination with other related diseases. We also elucidated the overall lipidomic profiles in five most commonly found cancers, which can be the basis of future studies in establishing cancer-specific lipid markers with a high diagnostic ability. Additional studies with a large number of patient samples should be conducted for further validation with candidate lipid species.

Fig. 5. Significant fold changes of each lipid species compared with the control group (>2 folds, \( p < 0.01 \)) in the different types of cancer. Open bars represent an increased fold and filled bars for the decreased fold.
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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2019.02.021.

References


