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ORIGINAL RESEARCH

Mediation Analysis Reveals Potential Biological Mechanism of Ascites Influencing Recurrence in Patients with Epithelial Ovarian Cancer

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Objective: Ascites, an accumulation of peritoneal fluid, is associated with poor prognosis of certain cancers. The potential mechanism that ascites worsens prognosis has not been well understood. Lipids have been reported to correlate with the prognosis of patients with epithelial ovarian cancer (EOC). Therefore, we aimed here to investigate whether lipids mediate the effect of ascites on the recurrence of EOC.

Methods: We collected the demographic and pathological data of 437 previously untreated patients with EOC to investigate the influence of ascites on recurrence. To identify the mechanism that mediates the potential influence of ascites on recurrence, we used ultra-performance liquid chromatography coupled with mass spectrometry (UPLC-MS) to determine the plasma lipid profiles of 53 patients with EOC. We used mediation analysis to evaluate if lipids mediated the effects of ascites on the recurrence of EOC.

Results: Patients with ascites had a poorer prognosis, which was associated with higher levels of carbohydrate antigen-CA125 (CA125) and FIGO stage. We identified six different lipid metabolites that were associated with ascites and recurrence. Mediation analysis revealed that the lipids LysoPC(P-15:0), PC(P-34:4), and PC(38:6) may mediate the effects of ascites on recurrence.

Conclusion: Our findings suggest that LysoPC(P-15:0), PC(P-34:4), and PC(38:6) mediate the effect of ascites on the prognosis of patients with EOC. We believe therefore that it is reasonable to consider metabolic interventions targeting the metabolism of LysoPC(P-15:0), PC(P-34:4), and PC(38:6) as a palliative treatment for patients with EOC with ascites. Further studies of more patients will be required to validate our findings.

Keywords: ascites, recurrence, mediation analysis, lipids, epithelial ovarian cancer

Introduction

Ascites is an accumulation of peritoneal fluid, which is associated with poor prognosis of certain cancers.[1] For example, ascites is associated with shorter progression-free survival of patients with ovarian cancer.[2] One study that aimed to identify biomarkers in ascites fluid to predict prognosis of OC found that tumor necrosis factor (TNF)-α and interleukin-6 in patients with EOC with ascites are associated with worse progression-free survival.[3] However, this study and others did not attempt to identify the potential mechanism through which ascites influences prognosis. The pathogenesis and progression of certain cancers is associated with dysregulated lipid metabolism.[4] It is of interest to explore whether lipids mediate the effect of ascites on the prognosis of EOC.

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The use of the methodology of mediation to assess the importance of signaling pathways and their mechanisms of action has dramatically expanded over the past decade.[5] For example, we conducted a mediation analysis to identify Platelet-derived Growth Factor-D expression mediates the effect of differentiated degree on prognosis in EOC.[6] Clinical research typically involves the analysis of numerous variables. However, this standard method fails to disentangle mechanisms underlying the associations between explanatory variables and outcomes, although investigators are sometimes interested in identifying the underlying mechanisms that mediate pathophysiology. For example, evidence indicates that corticosteroids are effective for reducing the mortality rate of patients with acute respiratory distress syndrome (ARDS), which is partly mediated by suppression of the inflammatory response. In this scenario, corticosteroids represent the exposure, mortality is the outcome, and the inflammatory response is the mediator. The interactions between corticosteroids and ARDS are mediated via the inflammatory response.[7] Corticosteroids suppress the inflammatory response, which reduces mortality. These promising findings may explain, at least in part, the effects of exposure on outcomes.

Lipids, which are major components of cell membranes, are required for cell differentiation, cell signaling, and energy storage.[8] Moreover, the pathogenesis of prostate, pancreatic, and ovarian cancers is associated with dysregulated lipid metabolism[9,10] and our previous lipidomics studies indicate that lower lipids levels are associated with worse prognosis.[4] Therefore, we hypothesized that the metabolism of plasma lipids mediates the association between ascites and tumor recurrence. Here we use mediation analysis to assess the role and extent of lipid-metabolite mediation between ascites and the prognosis of patients with EOC.

Materials and Methods

Study Population

This prospective study was performed at the Affiliated Tumor Hospital of Harbin Medical University and approved by the institutional review board at the Ethics Committee of Harbin Medical University and was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent. 437 EOC patients were enrolled and the data were put in Supplementary Material. In our study, data on ascites and blood samples were collected from patients at their first hospital admission who had not received any treatment. Among these patients, some had already developed ascites on arrival. After conventional treatments, all patients were followed up for their recurrence information. Therefore, ascites was formed before cancer recurrence in this study. We prospectively collected the plasma from patients in 2009 and matched age (± 5 years) according to recurrence data to determine lipid profiles. Recurrence was defined as the duration of the period from the first day of treatment to the detection of tumor progression including distant recurrence or pelvic recurrence within 3 years. Ascites was identified by a clinical imaging specialist using magnetic resonance imaging or ultrasonography.

Inclusion and Exclusion Criteria

Inclusion criteria were as follows: newly diagnosed, untreated patients with primary EOC; measurable malignant ascites with recovery from any toxicity; age >18 years; no active infection; and no serious damage to liver or kidneys. Exclusion criteria were as follows: uncorrectable obstruction or intestinal dysfunction, free flow of fluid prevented by significant adhesions, significant medical or psychiatric disorders or both.

Lipidomics and Treatment

Anticoagulant dipotassium EDTA tubes were used to collect venous blood samples acquired from fasting patients. These samples were maintained at room temperature within 30 mins and then centrifuged at 1000 g for 10 mins within 1 hr of collection. The supernatants were extracted and then stored at–80°C. Samples (5μL) were injected into a Kinetex Core-shell Silica C18 (2.1 mm×50 mm), 1.3 μL column (40°C) and analyzed using a UPLC system (Waters, Milford, USA). Solvent A, acetonitrile/isopropanol 10/90 (v/v), and solvent B, acetonitrile/deionized water 60/40 (v/v), served as the mobile phase delivered at 0.26 mL/min. A linear mobile phase gradient was as follows: 10% A, held for 1 min, 1–8 mins, 8–18 mins, and 18–20 mins; increased to 30% A, 75% A and 97% A, respectively; 20–24 mins, maintained at 97% A; 24–25 mins, decreased to 10% A, 25–26.4 mins, maintained at 10% A. After each analytical running, we used 1% A as the mobile phase for 0.1 min with equilibration for 1 min. Samples were randomly sampled to minimize analytical variation. Data acquisition was performed using an Agilent 6520-QTOF equipped with an electrospray ionization source. Conditions were as follows: capillary voltage 4.0 kV; desolvation gas flow,10 L/min; 330°C, and nitrogen gas. We collected the centroid data

from 50 m/z to 1000 m/z in full-scan mode. The raw data were converted into *mz* data format using MassHunter Qualitative Analysis Software (Agilent Technologies), and the XCMS package of the R platform was used to process these files. Parameters are described in our previous studies.[4,11] We used the R package CAMERA for annotating isotope peaks, adducts, and fragments in the peak lists and excluded the isotopic peaks before conducting statistical analysis.

Mediation Analysis

We performed a mediation analysis using a hypothetical model (Figure 1). Total effect (TE) was defined as the effect of ascites on recurrence; direct effect (DE) as the effect of ascites on recurrence after removing the effects of lipids on recurrence; and indirect effect (IE) as ascites on recurrence associated with by lipids. We utilized causal mediation analysis to estimate the mediation effect of lipids according to the Cox proportional hazard model.[12] The proportion of each lipid mediating the effect of ascites on recurrence was calculated by[13].

$$Prop.\ Mediated\ (average) = \log(IE)/(\log(IE) + \log(DE))$$

Statistical Analysis and Clinical Correlation

Patients' demographic and pathological characteristics are presented as counts and percentages. We use the chi-square test to evaluate the significant of differences between clinical characteristics of between patients with or without ascites. We further identified the biomarkers associated with both ascites and recurrence according to partial least squares discriminant analysis (PLS-DA) and univariate p value with the cutoff values of variable important in projection (VIP) >1 and p <0.05. The mediation analysis of the model parameter estimation was performed according to Vanderweele et al.[12] Statistical analysis was performed using the R platform.

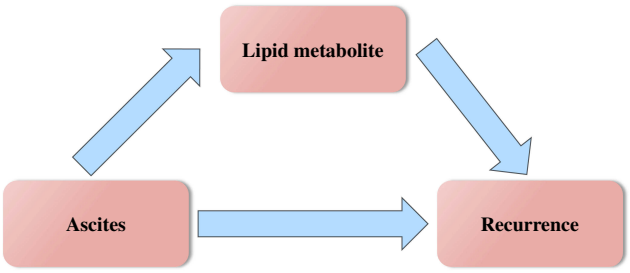

Figure 1 Directed acyclic graph (DAG) illustrating mediation process.

Results

The flow chart of the study was shown in Figure S3.

Demographic and Clinic Pathological Characteristics of Patients

Among the 437 patients with EOC treated from July 2005 to April 2013, 249 (57.0%) had ascites and 188 (43.0%) did not. Patients' demographic and pathological characteristics are displayed in Table 1. There were no significant differences between the two sets of patients with respect to age and degree of tumor differentiation. The CA125 levels of 99.2% patients with ascites were >35 U/mL, which were significantly higher compared with those without ascites. Moreover, the prognoses of the two groups were significantly different.

Identification of Lipids Associated with Ascites and Recurrence

The PLS-DA score revealed a significant difference between non-recurrence without ascites and recurrence with ascites (Figure 2). PLS and univariate analysis were used to select the lipids potentially associated with ascites and recurrence. We detected 14 lipid species that were considered candidate biomarkers of the diagnosis of ascites and 10 for predicting recurrence. The detailed properties of these lipids are presented in Tables S1 and S2 and in Figures S1 and S2.

Lipids Mediate the Effects of Ascites on Recurrence

We identified six lipids that correlated with ascites and recurrence to test for the mediating effect of the association between malignant ascites and prognosis (Table 2). For each lipid metabolite, we fitted mediator models and found that three lipid metabolites mediated the relationship between ascites and prognosis when the IE confidence interval did not include the value 1 (Table 3). The three metabolites, respectively, explained 34.9%, 39.5%, and 52.9% of the prognosis of EOC associated with malignant ascites. The data for LysoPC(P-15:0) serve as an example that explains the results. The risk ratio of ascites upon recurrence TE was 2.04; the effect of ascites on survival was significantly mediated through LysoPC(P-15:0); and IE was 1.32, accounting for 39.5% of TE. The risk ratio of ascites on recurrence DE, without considering LysoPC(P-15:0), was 1.54. We found that the three lipids metabolites identified

Table 1 Patients' Demographic and Pathological Characteristics

Characteristics		Without Ascites (%) N=188	With Ascites (%) N=249	Total (%) N=437	*P*
Age, y	<50 ≥50	58 (30.9) 130 (69.1)	80 (32.1) 169 (67.9)	138 (31.6) 299 (68.4)	0.8568
FIGO stage	I+II III+IV	48 (25.5) 140 (74.5)	22 (8.8) 227 (91.2)	70 (16.0) 367 (84.0)	<0.001
Differentiated degree	G1/G2 G3 unknown	52 (27.7) 133 (70.7) 3 (1.6)	58 (23.3) 186 (74.7) 5 (2.0)	110(25.2) 319 (73.0) 8 (1.8)	0.3642
Histological type	serous other unknown	92 (48.9) 92 (48.9) 4 (2.1)	151 (60.6) 90 (36.1) 8 (3.2)	243 (55.6) 182 (41.6)	0.0259
CA125, U/mL	<35 ≥35	15 (8.0) 173 (92.0)	2 (0.8) 247 (99.2)	17 (3.9) 420 (96.1)	0.0003
Recurrence	No Yes	128 (68.1) 60 (31.9)	137 (55.0) 112 (45.0)	265 (60.6) 172 (39.4)	0.0076

Abbreviations: G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated.

above mediated the effect of ascites on patients' prognoses when CI did not include 1. Thus, the lipid metabolites associated with ascites influenced patients' prognoses.

Discussion

EOC is the most lethal gynecologic malignancy. Ascites, an accumulation of peritoneal fluid that is present in one-third of patients with EOC, is linked to poor prognosis,[14] which is consistent with our present findings (Table 1). Ascites correlates with the immune system[14] and numerous studies show that the status of the host immune system correlates with improved prognosis of EOC through tumor-infiltrating lymphocytes that control the growth of cancers.[15,16] Ascites of patients with EOC inhibits the immune system by suppressing local T cell proliferation, which is mediated by dysregulated lipid metabolism in malignant ascites. Lipid mediators modulate the immune response to cancers.[17,18]

Disorders of lipid metabolism occur in certain cancers, leading to different ratios of intermediates.[19] For example, unsaturated lipids serve as a metabolic marker as well as a target of therapy to kill OC stem cells.[9] LysoPC regulates the invasiveness of cancer cells, inflammation, cell proliferation, and serves as a substrate of lysophospholipase D that converts LysoPC to LPA during cancer progression.[20] The level of LPC, which is reduced in the peripheral circulation

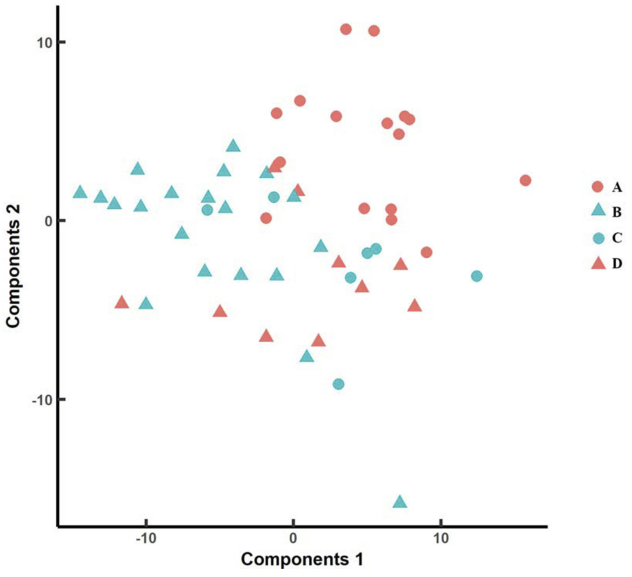

Figure 2 PLS-DA score plot for ascites and recurrence. (**A**) non-recurrence without ascites; (**B**) recurrence with ascites; (**C**) non-recurrence with ascites; (**D**) recurrence without ascites.

Table 2 Lipids Associated with Ascites and Recurrence

ID	Lipid	MZ	rt (min)	ppm
1	LysoPC(P-15:0)	466.3296	4.54	0.77
2	LysoPC(O-16:0)	482.3592	2.91	2.6
3	LysoPC(22:6)	568.3387	2.08	1.96
4	PC(P-34:4)	738.5461	13.25	3.9
5	PE(P-40:6)	776.5617	16.16	3.55
6	PC(38:6)	806.5688	13.98	0.87

Abbreviations: MZ, mass-to-charge ratio; rt (min), retention time; VIP, variable important in projection.

Table 3 Direct, Indirect, and Total Effects of Ascites on Recurrence Incidence Mediated Through Lipid Metabolites

Lipids	P_{IE}	IE [95% CI]	DE[95% CI]	TE[95% CI]	Proportion Mediated (%)
LysoPC(P-15:0)	0.0102	1.32[1.07 1.64]	1.54[0.68 3.50]	2.04[0.92 4.53]	39.5
PC(P-34:4)	0.0041	1.65[1.17 2.33]	1.56[0.71 3.43]	2.58[1.14 5.83]	52.9
PC(38:6)	0.0039	1.28[1.08 1.51]	1.58[0.70 3.53]	2.01[0.91 4.44]	34.9

of patients with liver cirrhosis,[21] regulates inflammatory pathways.[22,23] The levels of LPCs 16:0, 18:0, and 15:0 are significantly lower compared with those of patients without ascites.[24] Here we show that LPCs 16:0, 15:0, 22:6 were reduced in patients with ascites compared with those without. Mediation analysis shows that LPC15:0 mediated the relationship between ascites and recurrence of EOC. Ascites may lead to recurrence through variations in the levels of LPCs. Mediation analysis shows further that LysoPC(P-15:0), PC (P-34:4), and PC(38:6) mediated the relationship between ascites and prognosis.

Here we show that the levels of PCs were lower in EOC patients who experienced recurrence and those with ascites. Altered PC metabolism occurs in breast and prostate cancer cell lines, which may provide choline-based imaging approaches to improve diagnosis and identify new therapeutic targets of EOC.[25] Further, individual surfactant PC species modify macrophage differentiation, suggesting their differential effects on innate and adaptive immune functions.[26]

We believe it is reasonable to consider the use of interventions targeting the metabolism of LysoPC(P-15:0), PC (P-34:4), and PC(38:6) as a palliative treatment for patients with ascites. There is evidence in support of the use of similar strategies. For example, lipid metabolism has been suggested to serve as a target for treating brain cancer,[27] and targeting lipid metabolism using a cocktail of metabolic inhibitors has been shown to eradicate peritoneal metastases formed by ovarian cancer cells.[28] In addition, PC metabolism in cancer cells may allow the identification of novel biomarkers of tumor progression and the discovery of new targeted anticancer therapies. Phosphatidylcholine-specific phospholipase C (PC-PLC) deactivation could serve as a means to promote breast cancer cell differentiation and possibly enhance the effectiveness of antitumor treatments.[29] Up-regulation of PC-PLC has been seen in EOC compared with non-cancer counterparts. Use of a PC-PLC inhibitor, alone or combined with other cancer therapeutics, may, therefore, provide a powerful method to selectively interfere with aberrant pathways responsible for the proliferation and invasiveness of EOC cells.[30] Phospholipase D that catalyzes the hydrolysis of lysophosphatidyl choline (LPC) to generate the bioactive lipid lysophosphatidic acid (LPA). Autotaxin, an extracellular phospholipase D, has been implicated in many pathological processes relevant to cancer development. Intraperitoneal administration of an autotaxin inhibitor has been shown to benefit patients with ovarian cancer.[31]

In conclusion, our study indicates that poor prognosis, higher levels of CA125 and FIGO stage were significantly associated with ascites of patients with EOC. We identified metabolites that were related to ascites or prognosis. We used a causal inference method to explore the mechanism of the effect of ascites to worsen prognosis and to identify lipid metabolites that mediated these interactions. We show further that ascites may induce changes in lipid metabolites in vivo associated with poor prognosis. We believe it is reasonable to conclude, therefore, that the use of metabolic intervention will serve as an effective palliative treatment for patients with ascites. However, further studies are required to support this possibility. Most patients with cancer have ascites before they exhibit symptoms. Therefore, the detection of ascites may provide useful information for the diagnosis of EOC. Moreover, the potential role of ascites in the outcomes of patients with EOC requires further research.

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Author Contributions

Kang Li, Yan Hou, and Chunyan Yang made substantial contributions in the conception of the research idea, study design and all the authors listed have made substantial contribution in acquisition, analysis and interpretation of data for this work. All the authors contributed substantially to drafting and critical revision of the manuscript for intellectual content. All the authors have read and approved the updated current version submitted to the journal, and agree to review and approve the final version

to be published. Including the corresponding authors listed above, all the authors consent to be accountable for all the aspects of the work and agree to satisfactorily investigate and resolve all questions related to the accuracy or integrity of any part of the research work.

Disclosure

The authors declare no conflicts of interest in this work.

References

1. Ayantunde A, Parsons S. Pattern and prognostic factors in patients with malignant ascites: a retrospective study. *Ann Oncol*. 2007;18:945–949. doi:10.1093/annonc/mdl499
2. Szender JB, Emmons T, Belliotti S, et al. Impact of ascites volume on clinical outcomes in ovarian cancer: a cohort study. *Gynecol Oncol*. 2017;146:491–497. doi:10.1016/j.ygyno.2017.06.008
3. Kolomeyevskaya N, Eng KH, Khan AN, et al. Cytokine profiling of ascites at primary surgery identifies an interaction of tumor necrosis factor-alpha and interleukin-6 in predicting reduced progression-free survival in epithelial ovarian cancer. *Gynecol Oncol*. 2015;138:352–357. doi:10.1016/j.ygyno.2015.05.009
4. Li J, Xie H, Li A, et al. Distinct plasma lipids profiles of recurrent ovarian cancer by liquid chromatography-mass spectrometry. *Oncotarget*. 2017;8:46834–46845. doi:10.18632/oncotarget.11603
5. VanderWeele TJ. Mediation analysis: a practitioner's guide. *Annu Rev Public Health*. 2016;37:17–32. doi:10.1146/annurev-publhealth-032315-021402
6. Yang C, Zhang M, Cai Y, et al. Platelet-derived growth factor-D expression mediates the effect of differentiated degree on prognosis in epithelial ovarian cancer. *J Cell Biochem*. 2019;120(5):6920–5.
7. Zhang Z, Chen L, Ni H. The effectiveness of Corticosteroids on mortality in patients with acute respiratory distress syndrome or acute lung injury: a secondary analysis. *Sci Rep*. 2015;5:17654.
8. Huang C, Freter C. Lipid metabolism, apoptosis and cancer therapy. *Int J Mol Sci*. 2015;16:924–949. doi:10.3390/ijms16010924
9. Li J, Condello S, Thomes-Pepin J, et al. Lipid desaturation is a metabolic marker and therapeutic target of ovarian cancer stem cells. *Cell Stem Cell*. 2017;20:303–14.e5. doi:10.1016/j.stem.2016.11.004
10. Esser-von Bieren J. Immune-regulation and -functions of eicosanoid lipid mediators. *Biol Chem*. 2017;398:1177–1191. doi:10.1515/hsz-2017-0146
11. Hou Y, Li J, Xie H, et al. Differential plasma lipids profiling and lipid signatures as biomarkers in the early diagnosis of ovarian carcinoma using UPLC-MS. *Metabolomics*. 2015;12:18.
12. VanderWeele TJ. Causal mediation analysis with survival data. *Epidemiology*. 2011;22:582–585. doi:10.1097/EDE.0b013e31821db37e
13. Dudley WN, Benuzillo JG, Carrico MS. SPSS and SAS programming for the testing of mediation models. *Nurs Res*. 2004;53:59–62. doi:10.1097/00006199-200401000-00009
14. Kampan NC, Madondo MT, Mcnally OM, et al. Interleukin 6 present in inflammatory ascites from advanced epithelial ovarian cancer patients promotes tumor necrosis factor receptor 2-expressing regulatory T cells. *Front Immunol*. 2017;8. doi:10.3389/fimmu.2017.01482.
15. Hwang W-T, Adams SF, Tahirovic E, et al. Prognostic significance of tumor-infiltrating T cells in ovarian cancer: a meta-analysis. *Gynecol Oncol*. 2012;124:192–198. doi:10.1016/j.ygyno.2011.09.039
16. Wouters MCA, Komdeur FL, Workel HH, et al. Treatment regimen, surgical outcome, and T-cell differentiation influence prognostic benefit of tumor-infiltrating lymphocytes in high-grade serous ovarian cancer. *Clin Cancer Res*. 2016;22:714–724. doi:10.1158/1078-0432.CCR-15-1617
17. Kachler K, Bailer M, Heim L, et al. Enhanced acid sphingomyelinase activity drives immune evasion and tumor growth in non-small cell lung carcinoma. *Cancer Res*. 2017;77:5963–5976. doi:10.1158/0008-5472.CAN-16-3313
18. Shimizu T. Lipid mediators in health and disease: enzymes and receptors as therapeutic targets for the regulation of immunity and inflammation. *Annu Rev Pharmacol Toxicol*. 2009;49:123–150. doi:10.1146/annurev.pharmtox.011008.145616
19. Wefers C, Duiveman-de Boer T, Zusterzeel PLM, et al. Different lipid regulation in ovarian cancer: inhibition of the immune system. *Int J Mol Sci*. 2018;19:273. doi:10.3390/ijms19010273
20. Barber MN, Risis S, Yang C, et al. Plasma lysophosphatidylcholine levels are reduced in obesity and type 2 diabetes. *PLoS One*. 2012;7:e41456. doi:10.1371/journal.pone.0041456
21. Zhou L, Ding L, Yin P, et al. Serum metabolic profiling study of hepatocellular carcinoma infected with hepatitis B or hepatitis C virus by using liquid chromatography-mass spectrometry. *J Proteome Res*. 2012;11:5433–5442. doi:10.1021/pr300683a
22. Schmitz G, Ruebsaamen K. Metabolism and atherogenic disease association of lysophosphatidylcholine. *Atherosclerosis*. 2010;208:10–18. doi:10.1016/j.atherosclerosis.2009.05.029
23. Matsumoto T, Kobayashi T, Kamata K. Role of lysophosphatidylcholine (LPC) in atherosclerosis. *Curr Med Chem*. 2007;14:3209–3220. doi:10.2174/092986707782793899
24. Krautbauer S, Eisinger K, Wiest R, et al. Systemic saturated lysophosphatidylcholine is associated with hepatic function in patients with liver cirrhosis. *Prostaglandins Other Lipid Mediat*. 2016;124:27–33. doi:10.1016/j.prostaglandins.2016.06.001
25. Iorio E, Ricci A, Bagnoli M. Activation of phosphatidylcholine cycle enzymes in human epithelial ovarian cancer cells. *Cancer Res*. 2010;70:2126–2135. doi:10.1158/0008-5472.CAN-09-3833
26. Gille C, Spring B, Bernhard W, et al. Differential effect of surfactant and its saturated phosphatidylcholines on human blood macrophages. *J Lipid Res*. 2007;48:307–317. doi:10.1194/jlr.M600451-JLR200
27. Prasanna P, Thibault A, Liu L, et al. Lipid metabolism as a target for brain cancer therapy: synergistic activity of lovastatin and sodium phenylacetate against human glioma cells. *J Neurochem*. 1996;66:710–716. doi:10.1046/j.1471-4159.1996.66020710.x
28. Chen RR, Yung MMH, Xuan Y, et al. Targeting of lipid metabolism with a metabolic inhibitor cocktail eradicates peritoneal metastases in ovarian cancer cells. *Commun Biol*. 2019;2:1–15. doi:10.1038/s42003-019-0508-1
29. Abalsamo L, Spadaro F, Bozzuto G, et al. Inhibition of phosphatidylcholine-specific phospholipase C results in loss of mesenchymal traits in metastatic breast cancer cells. *Breast Cancer Res*. 2012;14:R50. doi:10.1186/bcr3151
30. Spadaro F, Ramoni C, Mezzanzanica D, et al. Phosphatidylcholine-specific phospholipase C activation in epithelial ovarian cancer cells. *Cancer Res*. 2008;68:6541–6549. doi:10.1158/0008-5472.CAN-07-6763
31. Fisher N, Edwards M, Hemming R, et al. Synthesis and activity of a novel autotaxin inhibitor-icodextrin conjugate. *J Med Chem*. 2018;61:7942–7951. doi:10.1021/acs.jmedchem.8b00935