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Proteomic and lipidomic analysis of low-density lipoprotein identifies potential biomarkers of early estrogen receptor-positive breast cancer

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Abstract

Estrogen receptor (ER)-positive breast cancer (BC) is a prevalent and fatal cancer among women, and there is a need to identify molecules involved in the disease pathophysiology which could also serve as biomarkers for early detection. Detection of cancer markers in whole plasma produces excessive information, and identifying important markers involved in cancer progression is challenging. We identified a BC-specific low-density lipoprotein (LDL) particle isolated by ultracentrifugation from the plasma of ER-positive BC patients. This LDL has an aberrant proteome and lipidome, significantly different from that of LDL from healthy women, including a high association with the pro-tumor chemokines CXCL4 and CXCL7, and an enrichment with the lipid subclasses phosphatidylethanolamine, ceramide, triglycerides, lysophosphatidylcholine, phosphatidylserine, phosphatidic acid, and sphingomyelin. In contrast, phosphatidylinositol species were significantly less abundant in LDL from tumor patients than in control. Moreover, BC-associated LDL has a distinct effect on macrophage phenotype, inducing an increased gene expression of IL1β, IL8 and CD206 and decreased gene expression of TNFα, a gene signature characteristic of tumor-associated macrophages (TAMs). This suggests that this formerly unrecognized form of LDL may represent LDL particles that are recruited by the tumor microenvironment to support tumor progression by inducing discrete subsets of TAMs. In conclusion, these data offer BC-associated LDL as an early biomarker detection platform for ER-positive BC. Furthermore, LDL-associated proteins and lipids that promote BC progression may also serve in the future as novel targets for BC therapies.

Keywords Low density lipoprotein, Breast cancer, Proteomics, Lipidomics, Macrophages

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Introduction

Breast cancer (BC) is the most prevalent and fatal cancer affecting women worldwide [1], however we still lack sufficient tools to detect and halt its progression at an early stage. Approximately 80% of BC cases are hormone-responsive BC, characterized by estrogen receptor (ER) expression in BC cells. Despite initial responsiveness to accepted therapeutic modalities, significant portions of high-stage BC patients eventually develop resistance or disease recurrence [2], underscoring the urgency for a deeper understanding of the biology of BC and its tumor microenvironment (TME). Identifying circulating protein and lipid biomarkers is a promising approach for detecting BC in the earliest and most treatable stages of the disease [3, 4, 5]. Plasma is suitable for high throughput methods for biomarker detection because it is easily obtained, is routinely used to diagnose many diseases, and has rich proteome and metabolome. However, due to the huge dynamic range in molecular concentration and the uncertainty as to the cellular and tissue source of the marker identified in plasma, there is great difficulty in the analysis of plasma biomarkers in BC and in understanding their specific role and significance [3]. Moreover, the use of plasma biomarkers to indicate disease is limited by fundamental technical and biological challenges because biomarkers are frequently found in low levels in circulation [6] and can be rapidly degraded both in vivo and ex vivo [7]. Therefore, there is a need to develop more accurate and sensitive methods to identify specific markers present in the early stages of BC, which may be vital for disease progression. Once identified, these markers will expand our knowledge of BC cancer biology and could be used for early detection of BC and as targets for therapies that would prevent disease progression.

Changes in the protein composition of lipoproteins or the proteins associated with lipoproteins were found to be associated with the risk of metabolic disease. For example, high-density lipoprotein (HDL) carries a unique cargo of proteins in humans with clinically significant cardiovascular disease [8], and lipoproteins from atherosclerosis patients were found to be rich in amyloid A [9]. However, the role of lipoprotein-associated proteins on the initiation or progression of cancer is unknown, and to date, there are no studies on the protein composition of lipoproteins in cancer. Recently, highly sensitive methods allowing lipidomic investigation of specific lipoproteins have been developed [10, 11]. Changes in the lipid composition of lipoproteins were found to be associated with the risk of metabolic disease. For example, specific low-density lipoprotein (LDL) lipid composition patterns were identified in clinical states like atherosclerosis and renal failure [12, 13]. Nevertheless, no study has been undertaken investigating lipoprotein lipid composition in cancer and its role in tumor initiation or progression.

The relationship between LDL cholesterol levels and BC incidence and progression has yielded inconsistent findings [14]. Similarly, several studies showed that treatment with statins, a commonly-used drug to lower LDL cholesterol plasma levels, can positively affect BC incidence and outcome [15], while other studies did not find such an association [16]. Altogether, these data imply multifaceted effects of LDL on BC beyond LDL particle quantity or cholesterol content.

We compared the protein and lipid composition of LDL from plasma samples of early-stage BC patients and healthy controls. LDL particles have a relatively long half-life of about three days [17]. This may allow LDL to concentrate and stabilize biomarkers that are otherwise too scarce or unstable to be detected early in the neoplastic process. Indeed, by utilizing proteomic and lipidomic profiling, we found potential tumor-promoting proteins and lipids in LDL from the plasma of patients with early-stage BC. Furthermore, this LDL induced a discrete gene expression pattern in human macrophages characteristic of cancer-promoting tumor-associated macrophages (TAMs).

Materials and methods

Clinical samples

Each blood sample from a BC patient was taken right before a surgical procedure which included lumpectomy with diagnostic biopsies of breast masses and sentinel lymph nodes. There was no evidence of metastatic disease to distant organs, and none of them received neoadjuvant chemotherapy or hormonotherapy before surgery. Unless stated otherwise, there was no lymphatic spread in any of the patients. Samples were reserved in the tissue bank of Sheba Medical Center (SMC). Control blood samples were taken from healthy women who underwent a medical survey at SMC. These blood samples were preserved in the central laboratory of the medical center. Fasting blood samples were taken in both groups. All samples were collected and used following institutional review board approval and in line with informed consent forms. LDL was isolated using either ultracentrifugation (UC), fast protein liquid chromatography (FPLC), or a combination of short (2 h) UC followed by FPLC as previously described [8, 9, 18, 19].

LDL-associated proteins analysis

LDL underwent proteomic analysis on a Q exactive HF orbitrap LC-MS/MS (Thermo scientific). Label-free quantification (LFQ) intensity values were logarithmized (Log2). Only proteins present in all samples in at least one of the groups were kept for the statistical analysis. Signals that were initially zero were imputed with fixed low value. In order to identify significantly-changing proteins between C and T groups, two-sided t-test was

used. A t-test was performed with an FDR value of 0.05 and S0 = 0.5. Hierarchical clustering of t-test significant proteins was performed on logarithmized LFQ values and z-score normalization of the data, using Euclidean distances between averages. LDL from independent validation cohorts which included plasma samples of BC patients or healthy women and matched whole plasma was analyzed by ELISA kits for detection of CXCL4 (ab189573, Abcam) and CXCL7 (ab100613, Abcam).

Lipid profiling by LC/MS

LDL samples were subjected to lipid profiling at the Metabolic Profiling Unit at the Weizmann Institute of Science. LDL was first lyophilized and lipids were extracted with a methanol: methyl tert-butyl ether (MTBE) mixture [20], containing the following internal standards: 0.1 μg ml^{-1} of phosphatidylcholine (17:0/17:0) (Avanti), 0.4 μg ml^{-1} of phosphatidylethanolamine (17:0/17:0, 0.15 nmol ml^{-1} of ceramide/sphingoid Internal Standard Mixture II (Avanti, LM6005), 0.0267 μg/mL d5-TG Internal Standard Mixture I (Avanti, LM6000) and 0.1 μg ml^{-1} palmitic acid-13 C (Sigma, 605573). Post-extraction, the organic phase, containing lipids, was analyzed using a Waters ACQUITY I class UPLC system coupled to a mass spectrometer (Thermo Exactive Plus Orbitrap) operated in switching positive and negative ionization modes. Chromatographic conditions were described by Malitsky et al. [20] with minor modifications. Briefly, the chromatographic separation was performed on an ACQUITY UPLC BEH C8 column (2.1 × 100 mm, i.d., 1.7 μm; Waters Corp., MA, United States). Mobile phase A consisted of 45% water (UPLC grade) with 1% 1 M NH_4Ac, 0.1% acetic acid, and 55% acetonitrile: isopropanol (7:3) with 1% 1 M NH_4Ac, 0.1% acetic acid (mobile phase B). The column was maintained at 40 °C, and the mobile phase flow rate was 0.4 ml min^{-1}. Mobile phase A was run for 1 min at 100%; then, it was gradually reduced to 25% at 12 min, then decreased to 0% at 16 min. Then, mobile phase B was run at 100% for 21 min, and mobile phase A was set to 100% at 21.5 min. Finally, a column was equilibrated at 100% mobile phase A for 25 min.

Lipid species identification and relative normalization

Orbitrap data was analyzed using LipidSearch™ software (Thermo Fisher Scientific). The putative identification of lipids was validated by comparing it to a homemade library that contains lipids produced by various organisms and on the correlation between retention time (RT) and carbon chain length and degree of unsaturation. Relative levels of lipids were normalized to the internal standards and the amount of tissue used for analysis.

Macrophage gene expression

Primary human monocytes were isolated from human peripheral blood mononuclear cells pooled from three healthy donors using CD14 microbeads (Miltenyi biotech). Cells were differentiated with 20ng/ml M-CSF for 7 days and then treated for 24 h with 50 μg/ml LDL isolated from the plasma of healthy women or BC patients. RNA was extracted from lysed cells, reverse-transcribed to cDNA and analyzed for relative gene expression by real-time PCR.

Statistics

In order to minimize batch effects and avoid multiple adjustments, sample preparation was performed in one batch using the same stock solutions. In addition, all measurements that were later to be compared were performed jointly. Finally, for human plasmas used in this study, all participants were matched to avoid significant differences between groups.

Results

Choice of LDL isolation method

We decided to perform proteomic and lipidomic analyses of LDL from healthy women and BC patients to identify distinct protein and lipid composition patterns in BC-associated LDL (Fig. 1A). But first, we wished to define the optimal method to isolate plasma LDL for these assays. Previously, lipoproteins for proteomic or lipidomic analyses have been isolated by either long (16 h) ultracentrifugation (UC) [8, 9, 12, 13], fast protein liquid chromatography (FPLC) [10, 18] or a combination of short (2 h) UC followed by FPLC [19], so we compared the three methods. To isolate LDL, we used a plasma pool from blood taken from healthy women who underwent a medical survey at SMC. Proteomic analysis showed that apolipoproteins, predicted to be the highest-abundant in LDL, were detected with higher intensity in LDL isolated by UC as compared to LDL isolated by FPLC (Fig. 1B). Moreover, ApoB, which is the most abundant protein in LDL, was the highest-intensity protein detected in LDL isolated by UC and not by FPLC or the combined method (Fig. 1C). Together, these findings supported the use of UC as the optimal method to isolate LDL in our next proteomic and lipidomic analyses.

Characterization of BC-associated protein content

Next, we collected plasma samples from early-stage ER-positive BC patients and matched healthy women. All available characteristics of participants are presented in table S1. We isolated LDL from samples by UC, and using proteomic analysis, we identified 234 LDL-associated proteins from which 18 were more abundant in the BC group (Fig. 2A). Of these proteins, the two most

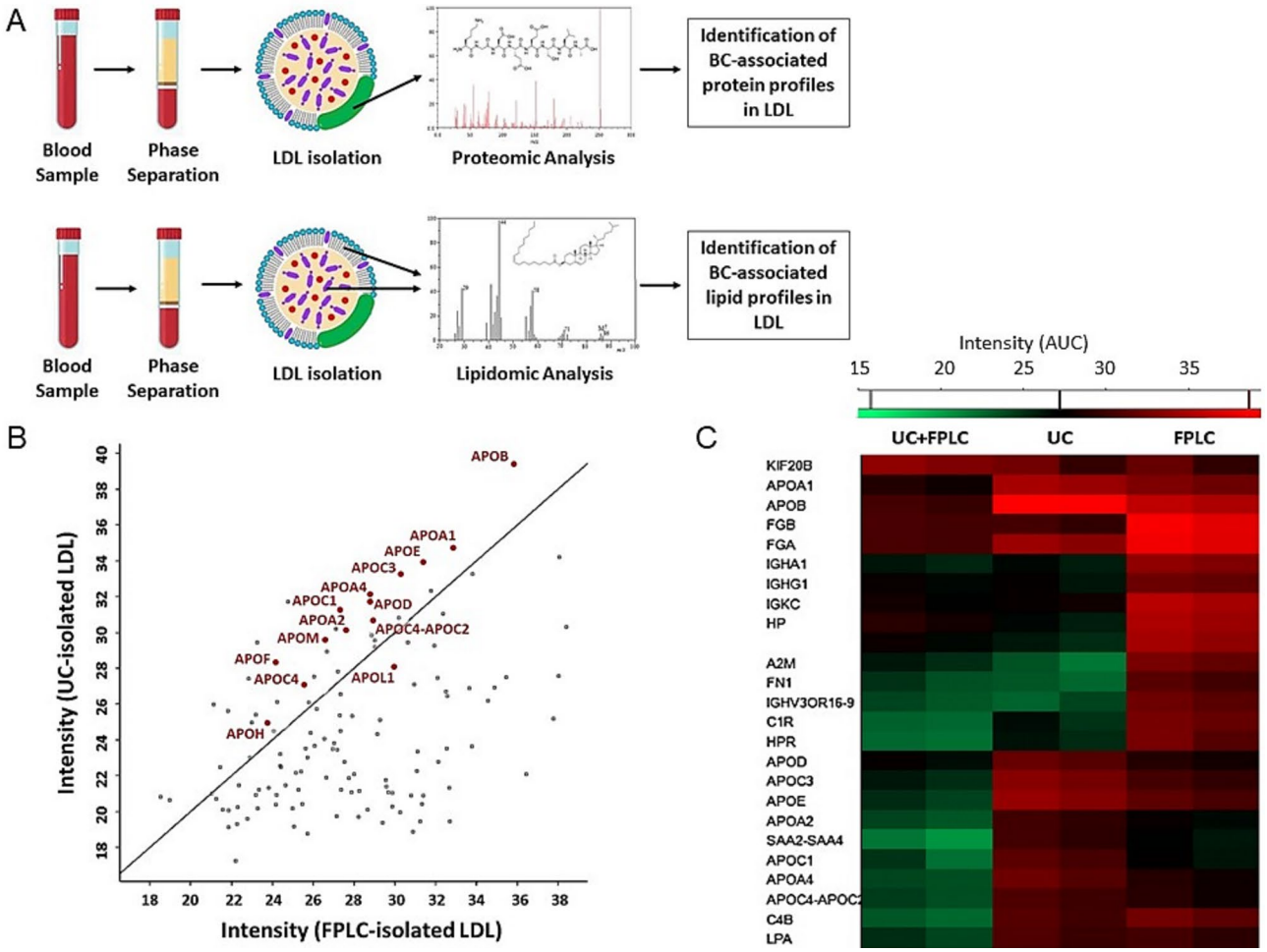

Fig. 1 Methodological scheme and comparison of LDL isolation methods. **A**) Methodological scheme for LDL isolation and analysis by proteomic and lipidomic profiling. **B**) Intensity of proteins detected by proteomic analysis of LDL isolated by UC or FPLC. LDL was isolated from healthy women by either long UC (X-axis) or FPLC (Y-axis). Highlighted in red are apolipoproteins. All but one apolipoprotein show higher detection intensity in LDL isolated by UC compared to LDL isolated by FPLC. Intensity is presented on a log2 scale. **C**) Heat-map comparing three methods of LDL isolation. LDL was isolated from the plasma of a healthy woman by either one of three methods: Left lane - short 2 h UC followed by FPLC; Middle lane– long 16 h UC alone; Right lane–FPLC alone. The isolated LDL was subjected to proteomic analysis on a Q exactive HF orbitrap LC-MS/MS (Thermo Scientific). APOB shows the predicted highest intensity only in proteomics of LDL isolated by long UC

significant ones were CXC motif ligands 4 (CXCL4) and 7 (CXCL7).

We next validated our proteomic results for CXCL4 and CXCL7 by using ELISA analysis on independent validation cohorts comprised of new sets of plasma samples from early-stage ER-positive BC patients and matched healthy women. All other medical conditions and medications, including statins and other cholesterol-lowering medications, were not significantly different between the two groups. All available characteristics of participants are presented in table S2.

In whole plasma, cancer patients showed a 3.10-fold increase in CXCL4 compared to controls and this fold increased to 4.24 when LDL-bound CXCL4 was measured (Fig. 2B, upper panel). The level of CXCL7 was increased in plasma by a fold of 3.59 relative to control. However, this fold markedly increased to 36.21 when LDL-bound CXCL7 was measured (Fig. 2B, lower panel). We further investigated whether LDL in the plasma of triple-negative BC (TNBC) patients is also enriched with CXCL4. Unlike in ER-positive BC, there was no significant increase in CXCL4 neither in plasma nor in LDL of TNBC patients as compared to healthy women (Fig. S1). In conclusion, CXCL4 and CXCL7 are specifically enriched in LDL of ER-positive BC patients.

Characterization of BC-associated lipid content

For lipidomic analysis, we collected plasma samples from early-stage ER-positive BC patients and matched healthy women. All available characteristics of participants are presented in table S3. We isolated LDL from plasma samples by UC and subjected it to lipidomic analysis. We

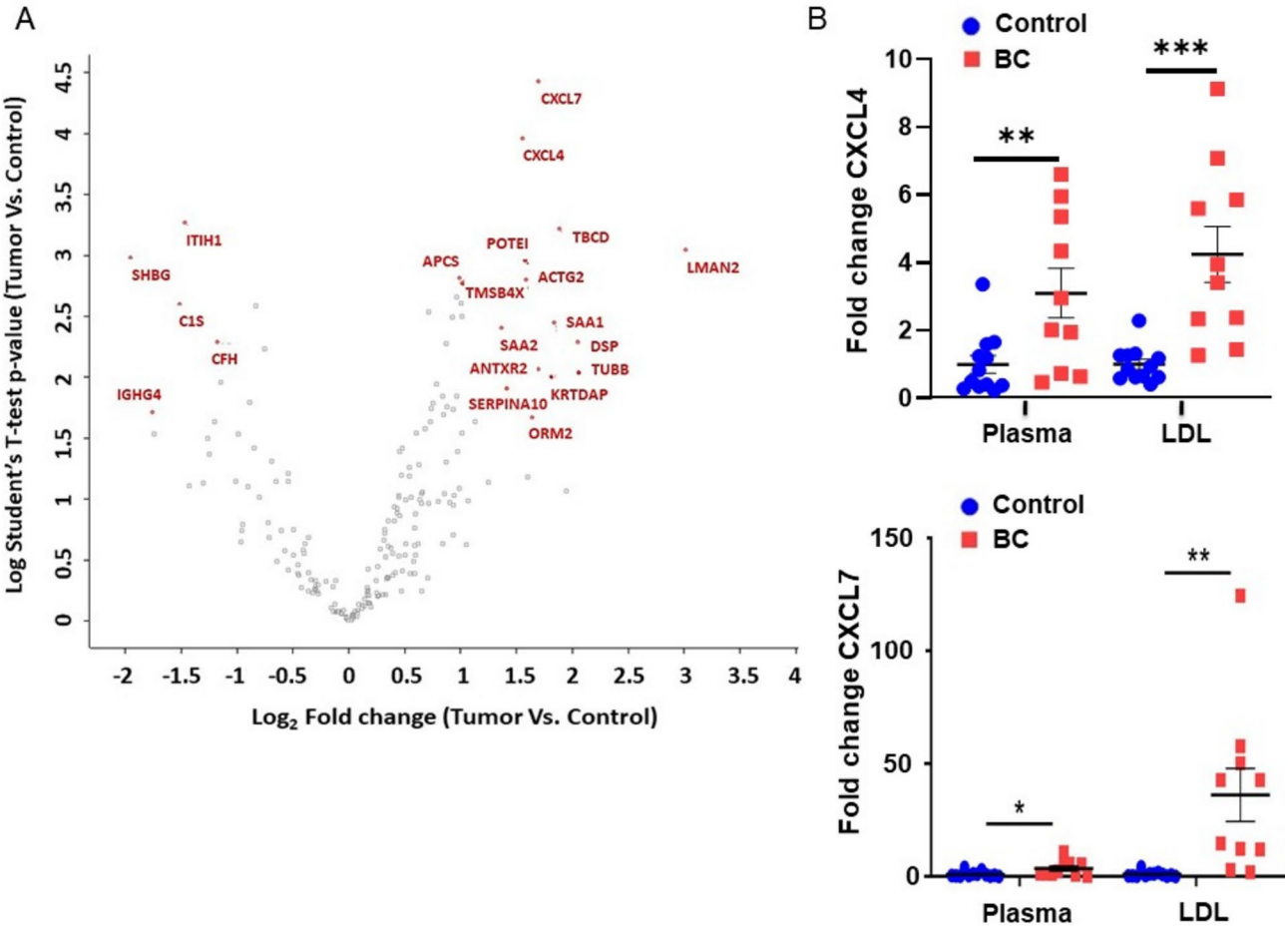

Fig. 2 Analysis of LDL-associated proteins from BC patients and healthy controls. **A**) Pattern of proteins enrichment on BC-associated LDL. LDL was isolated from plasma samples of BC patients or healthy women, and underwent proteomic analysis on a Q exactive HF orbitrap LC-MS/MS (Thermo scientific). Intensity values were logarithmized (Log2). Only proteins present in at least 10 / 40 samples were kept for statistical analysis. Signals that were originally 0 were imputed with fixed low value. Significantly changing proteins are marked red. Proteins are ordered by abundance relative to control (X-axis) and by difference significance (Y-axis). **B**) LDL of BC patients is enriched with CXCL4 and CXCL7. LDL and corresponding whole plasma samples from independent validation cohorts of healthy women and BC patients were analyzed by ELISA for measurement of CXCL4 or CXCL7 in BC patients as compared to healthy controls. Statistical significance was calculated using two-sided t-test and SEM. * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$)

identified 512 different LDL-associated lipid species that belonged to 22 distinct lipid classes. To determine the general difference between lipid signatures of tumor and control samples, we used principal component analysis (PCA), which demonstrated distinct patterns in lipid levels of the two groups (Fig. 3A).

Several potential marker lipid species were found to be significantly more abundant in LDL from breast tumor patients than in healthy women, including specific species of phosphatidylethanolamine (PE), ceramide, triglyceride (TG), lysophosphatidylcholine (LPC), and phosphatidylserine (PS). Interestingly, all lipid species of phosphatidylinositol (PI) were found to be significantly less abundant in BC-associated LDL than in control LDL (Fig. 3B). To gain insight into the pattern of lipid abundance in BC-associated LDL on the lipid class level, we grouped all lipid species into main lipid classes (Fig. 3C). When grouped, the lipid classes that were more abundant in the LDL from tumor patients were PE, ceramide, TG, LPC, PS, phosphatidic acid (PA) and sphingomyelin (SM). In contrast, PI was significantly less abundant in LDL from tumor patients than in control.

Macrophage phenotype

Lipids are crucial players in the function and maturation of TAMs [21], while LDL is the key effector of macrophages in atherogenesis and can promote macrophage maturation and polarization [22, 23]. We thus sought to study the effect of BC-associated LDL on macrophage phenotype. We collected plasma samples from early-stage ER-positive BC patients and healthy women. All other medical conditions and medications, including statins and other cholesterol-lowering medications, were not significantly different between the two groups. All

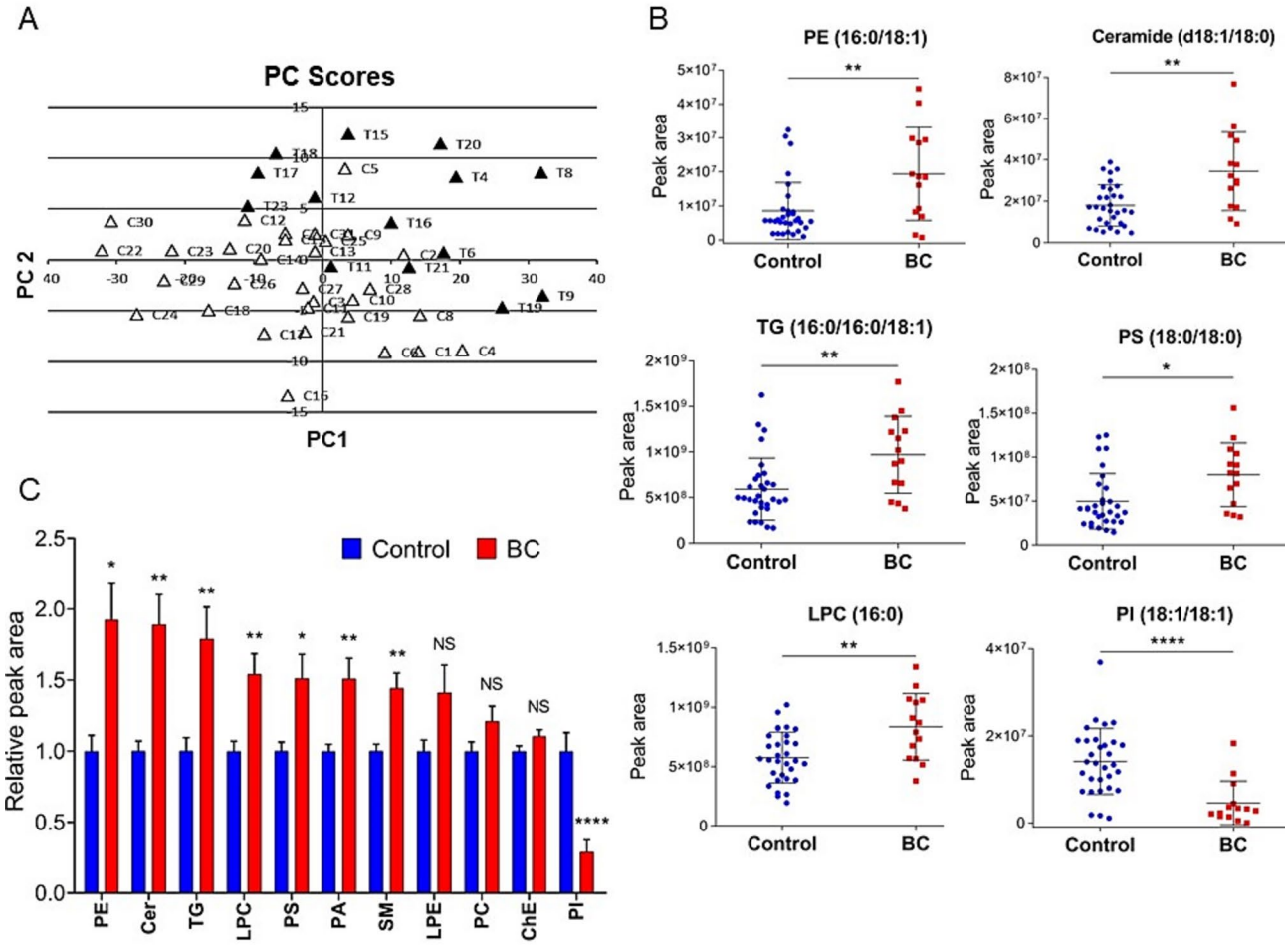

Fig. 3 Lipidomic analysis of LDL from breast tumor and control patients. **A**) PCA of lipids in tumor (T) and control (**C**) samples. LDL was isolated by UC from plasma of breast cancer patients or healthy women and underwent lipidomic analysis using Waters ACQUITY I class UPLC system coupled to a mass spectrometer (Thermo Exactive Plus Orbitrap). **B**) Comparison of representative lipids between tumor and control samples. To identify significant differences, a two-sided t-test was used. PE- phosphatidylethanolamine, TG - triglyceride, LPC– lysophosphatidylcholine, PS - phosphatidylserine, PC– phosphatidylcholine, PI– phosphatidylinositol, * ($p < 0.05$), ** ($p < 0.01$), **** ($p < 0.0001$). **C**) Comparison of main lipid classes between tumor and control samples. For each class, average folds of all lipid species within the class were averaged and presented as relative peak area. To identify significant differences, a two-sided t-test was used. PE- phosphatidylethanolamine, Cer– ceramide, PA– phosphatidic acid, LPC– lysophosphatidylcholine, LPE– lysophosphatidylethanolamine, SM- sphingomyelin, PC– phosphatidylcholine, ChE– cholesterol ester, PI– phosphatidylinositol, NS– non significant, * ($p < 0.05$), ** ($p < 0.01$), **** ($p < 0.0001$)

available characteristics of participants are presented in Table S4. We isolated LDL from samples by UC and used it to treat macrophages differentiated from human primary monocytes (pooled from healthy donors), and then analyzed the expression of key genes by real-time PCR. Unlike LDL from healthy subjects, LDL from BC patients induced a significant gene expression of IL-1β, IL-8, and CD206 and decreased TNFα gene expression (Fig. 4). Altogether, these data demonstrate that BC-associated LDL can induce specific transcriptional programs in macrophages and suggest a functional role of this LDL in polarizing macrophages into a specific tumor-supporting TAM subset in the TME.

Discussion

Mammography is considered the gold standard for early detection of BC [24]. However, it has several limitations, such as false positive and false negative diagnoses, excessive biopsies, irradiation linked to mammography application, and sub-optimal tumor detection rates, as in the case of high-dense breast tissue. Altogether, these limitations increase awareness among experts regarding the need to develop complementary screening tools for early BC detection [24].

Proteins and lipids from whole plasma may serve as disease markers [4, 5, 25, 26], but they tell us little about the origin and significance of these molecules in cancer progression. In this study, we identified unique LDL protein and lipid composition patterns in early breast tumor

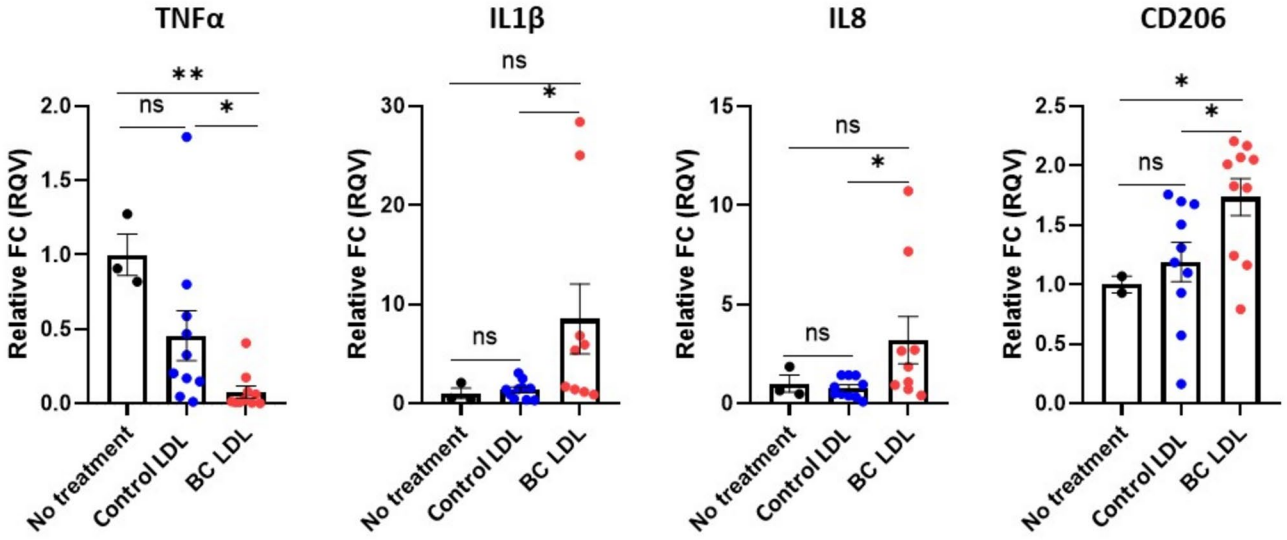

Fig. 4 LDL from BC patients induces a distinct macrophage polarization pattern. Primary human monocytes pooled from three healthy donors were differentiated with 20ng/ml M-CSF for 7 days and then treated for 24 h with 50 µg/ml LDL isolated from plasma of healthy women ($n = 10$) or BC patients ($n = 10$). Relative gene expression was measured by real-time PCR. P value was calculated using two-tailed Student's t-test. ns– not significant, * ($p < 0.05$), ** ($p < 0.01$)

patients. Unlike biomarkers from whole plasma, lipoprotein-associated biomarkers may provide more concise and specific information from early tumors. Moreover, proteins and lipids found in our study may additionally point to a mechanistic role of those molecules in the crosstalk between LDL and the tumor.

We found CXCL4 and CXCL7 to be the most significant proteins enriching BC-associated LDL. These chemokines are secreted by activated platelets or immune cells and exert diverse immune-modulatory effects, including determination of macrophage phenotypes [27, 28, 29, 30]. Both are potential BC biomarkers [31, 32], and can promote its progression by suppressing anti-tumor immunity [33, 34]. Importantly, CXCL4 and CXCL7 were previously associated with LDL in a proteomic analysis [35] and CXCL4 was shown to bind LDL in-vitro and promote its uptake by macrophages [36]. However, the significance of endogenous CXCL4-LDL or CXCL7-LDL complexes and their relation to cancer are unknown. At least some of CXCL4 effects on immune cells are mediated not through its cognate receptor, but rather by binding to membrane-bound proteoglycan [30, 33, 37], much like LDL [38]. Moreover, both LDL and CXCL4 can activate immune cells through endosomal toll-like receptor 8 (TLR8) [39, 40], suggesting a simultaneous cellular entry. Of note, in studies demonstrating CXCL4 effect on immune cells (both in-vitro and in-vivo), concentrations of 1,000 times the physiological plasma levels of CXCL4 were used [28, 33]. One likely explanation is the short half-life of CXCL4 in plasma of several minutes [41]. As the half-life of LDL in plasma is about three days17], association with LDL is likely to significantly enhance CXCL4 stability. Taken together, these data suggest that LDL and CXCL4 may function as a complex that plays an important role in tumor biology through immune regulation of key TME components.

Several lipid classes we found more abundant in BC-associated LDL are known to play a role in BC promotion. TG levels in plasma are correlative with BC initiation and progression [42, 43], suggesting TGs may participate in fueling BC cells. We found TG to be significantly increased in BC-associated LDL. This raises the possibility that BC-associated LDL promotes the tumor through increased TG delivery to fuel cancer cell metabolism. Another lipid we found to be increased in BC-associated LDL is PS. PS is an essential component of bilayer cell membranes and usually is present in the inner leaflet [44]. In the TME, PS exposure on the external leaflet is significantly increased on the surface of breast tumor cells, which have innate immunosuppressive properties and facilitate tumor growth and metastasis [45]. PS in BC cells was found to support tumor cell proliferation and tumor-promoting inflammation [46] and to limit anti-tumor functions of immune cells [47]. Moreover, a PS-targeting antibody was shown to inhibit the progression of BC in mice [48]. PA, also increased in LDL of breast tumor patients, was previously found to enhance the migration of metastatic human BC cells [49].

Several lipid classes found in our study to be relatively abundant in BC-associated LDL were previously reported to enhance LDL entry into various cell types. LDL rich in these lipids may be more accessible to BC cells or other cells in the TME, which supports the tumor more efficiently. Glycosylated PE (Glc-PtdEtn), the major LDL

lipid glycation product, promotes LDL uptake and ChE and TG accumulation by macrophages [50]. Ceramide, which we found more abundant in LDL from BC patients, contributes to the transcytosis of oxidized LDL across endothelial cells [51]. Lysophospholipids are lyso-type metabolites of phospholipids and are generated from the hydrolysis of phospholipids by phospholipase A2. LPC, a lysophospholipid we found to be over-represented in BC-associated LDL, potentiates the mitogenic activity of modified LDL on human macrophages [52]. Association of PS with LDL increases LDL uptake and ChE deposition in macrophages [53]. Moreover, PS also facilitates the intracellular delivery of cholesterol supplied from LDL [54]. PA, like PS and glycated PE, was also shown to increase LDL uptake by macrophages [55]. Altogether, these data suggest that LDL enriched with specific lipid classes may be more accessible to BC cells or other cells in the TME to support the tumor.

The phosphatidylinositol-3-Kinase (PI3K) signaling pathway is activated in BC and is associated with cell survival [56]. We found that unlike other phospholipid classes, species of PI, the initial substrate of this pathway, are significantly less abundant in BC-associated LDL. A possible effect of this reduced PI content in LDL on the PI3K pathway activation in BC cells should be studied in future works.

When applied to human primary macrophages, BC-associated LDL induced gene expression patterns that may characterize TAMs. In the TME, TAMs are notably prevalent, constituting up to 50% of the tumor mass in most cancer types [57]. They support cancer by fostering cancer cell stemness, altering energy metabolism, promoting metastasis, inducing angiogenesis, and suppressing immune cell activity [57]. Similar to macrophages treated with BC-associated LDL in the present study, TAMs are characterized by the expression of surface markers like CD206 and by the production of pro-tumor cytokines like IL-1β and IL-8 [58, 59], as well as reduced production of anti-tumor cytokines like TNFα [60]. Thus, BC-associated LDL may support the tumor by fostering the maturation and function of TAMs.

Our study has several limitations. First, sample sizes are relatively small. Consequently, more studies characterizing BC-associated LDL protein and lipid content in larger patient cohorts are needed. Moreover, our data demonstrating the effect of BC-associated LDL on the expression of key genes in macrophages is preliminary. Gene expression patterns of macrophages or other cells (like BC cells and normal mammary cells) treated with BC-associated LDL should be expanded to produce unbiased expression profiles using RNA-seq analyses. Functional assays investigating more phenotypes of BC-associated LDL-treated macrophages, like secreted cytokine profiling and phagocytic potential are also warranted in future studies. Second, while obesity and overweight are associated with BC risk [61], data acquisition in our study was retrospective, and not all available medical records of study participants included calculations of body mass index. However, no participant included in this study had a diagnosis of obesity or overweight in her medical records. Future studies will have to be designed with accurate anthropometric matching between BC patients and control individuals. Finally, our study focused on the prevalent ER-positive BC subtype. Other clinically important BC subtypes, like TNBC tumors or tumors with a mutational background like BRCA mutations, were not fully investigated in our analysis, and the protein and lipid composition of LDL in these BC subtypes should be addressed in future studies.

In conclusion, this study has two important implications: First, LDL could serve as a biomarker for early cancer detection, and second, LDL-associated proteins and lipids mediating LDL uptake by BC cells or other cells in the TME may also serve in the future as novel targets for cancer therapies.

Abbreviations

LDL	Low-density lipoprotein
VLDL	Very low-density lipoprotein
HDL	High-density lipoprotein
UC	Ultracentrifugation
FPLC	Fast protein liquid chromatography
HPLC	High-performance liquid chromatography
CXCL4	CXC motif ligands 4
CXCL7	CXC motif ligands 7
PE	Phosphatidylethanolamine, TG: triglyceride
LPC	Lysophosphatidylcholine
PS	Phosphatidylserine
PI	Phosphatidylinositol
PA	Phosphatidic acid
SM	Sphingomyelin
LPE	Lysophosphatidylethanolamine
PC	Phosphatidylcholine
ChE	Cholesterol ester

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40170-025-00390-6.

Supplementary Material 1

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Author contributions

R.K., Y.A. M. and R.T. initiated the study and conceived the project, designed experiments, interpreted results, and wrote the manuscript. A.S., G.S.L. and L.M. supported technical assistance to all experiments. E.G.S. and T.G. performed proteomic analysis. S.M. and M.I. performed lipidomic analysis. H.C., Y.K., A.S., A.H., A.L. and D.H. supported project administration and provided expert comments.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This clinical study occurred at the SMC and complied with recognized ethical guidelines. The study protocol was approved by the SMC Research Subject Review Board (Israeli Ministry of Health identifier: 202120045), and written informed consent was obtained from all participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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