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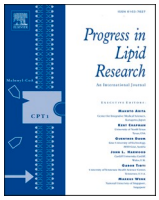

Lipid dysregulation in triple negative breast cancer: Insights from mass spectrometry-based approaches

Xiaoyue Huang [a], Ahmed Ali [a,*], Dounia E.I. Yachioui [a], Sylvia E. Le Dévédec [b,*], Thomas Hankemeier [a]

[a] Metabolomics and Analytics Center, Leiden Academic Centre for Drug Research, Leiden University, 2333 CC Leiden, The Netherlands
[b] Division of Cell Systems and Drug Safety, Leiden Academic Centre for Drug Research, Leiden University, 2333 CC Leiden, The Netherlands

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ABSTRACT

Triple negative breast cancer (TNBC) has the worst prognosis among breast cancers due to its aggressive nature and the absence of targeted treatments. Development of novel anti-cancer drugs for TNBC faces challenges stemming from its heterogeneity and high potential for metastasis. Metabolomics can be a useful technology in finding novel therapeutic targets and probing the heterogeneity of TNBC. Metabolomics has been enabled by advancements in mass spectrometry (MS)-based platforms that facilitated comprehensive profiling of TNBC metabolism. This review provides an overview of metabolomic changes in TNBC with emphasis on lipid alterations, and describes the key MS analytical techniques, providing the necessary background for examining the role of lipids in TNBC development.

1. Introduction

Triple negative breast cancer (TNBC) accounts for 15–20 % of all breast cancer cases worldwide and is responsible for approximately 40 % of breast cancer related deaths [1,2]. TNBC is a subtype of breast cancer that is characterized by its lack of estrogen and progesterone receptors (ER, PR), and low human epidermal growth factor receptor 2 (HER2) protein expression. It has the highest metastasis ratio and the lowest overall survival ratio compared to ER/PR/HER2 positive breast cancer subtypes [3]. TNBC is a highly heterogeneous subtype of breast cancer that lacks specific therapeutic targets, making chemotherapy the primary treatment option [4–6]. Therefore, it is crucial to explore the heterogeneity of TNBC to discover novel drug targets for its treatment. This is typically done in using three types of models: cell line models, three-dimensional culture models such as patient-derived organoids in vitro, and animal models like patient-derived xenografts in vivo [7]. Each of these models, and particularly their combination, can significantly enhance our understanding of TNBC, especially considering the limited availability of clinical samples. However, extensive molecular characterization of said models is needed to reveal novel drug targets for TNBC treatment.

Mass spectrometry has emerged as the method of choice for revealing the biochemical changes in these models in search of novel drug targets for TNBC treatment [8,9]. MS, with its high-throughput and high-resolution capabilities, enables the identification and quantification of metabolites associated with TNBC progression. Using MS, metabolic profiling has already revealed significant differences between TNBC and ER-positive breast cancer. For instance, metabolites such as sarcosine and 2-hydroxyglutarate have been found at higher levels in TNBC tumors [10], and TNBC patients exhibited elevated levels of glutathione and glutamine compared to those with ER positive breast cancer [11]. While metabolic alterations in glucose and amino acid metabolism in TNBC have been specifically reviewed [12,13], membrane lipids such as glycerophospholipids and sphingolipids have only recently been taken as key contributors to cancer progression. Therefore, identifying lipid alterations that emerge and accumulate during disease progression is essential for more effective TNBC treatments. Nevertheless, studying these metabolic and lipidomic alterations is still a work in progress, with many new methods being developed to help identify features associated with aggressive disease phenotypes.

To address this gap, this review aims to reveal lipid dysregulation specific to TNBC progression which can be used as novel biomarkers or drug targets. This will be achieved by first discussing the MS methods that can be used in metabolomic studies of TNBC. Then, the heterogenous metabolic profiles and lipid alterations in TNBC will be summarized, and finally, lipid alterations that are potentially related to TNBC

* Corresponding authors.
E-mail addresses: ali@lacdr.leidenuniv.nl (A. Ali), s.e.ledevedec@lacdr.leidenuniv.nl (S.E. Le Dévédec).

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metastasis will be critically reviewed.

2. MS technologies for metabolic profiling of TNBC

MS is the leading technique used for metabolic profiling due to its sensitivity, selectivity, and metabolic coverage [14,15]. The choice of the MS technique is critical, as each method has unique advantages and limitations that determine its suitability for addressing specific biological questions. To address this, this section will highlight the various MS techniques available for investigating the metabolic heterogeneity of TNBC across different models (Fig. 1). (See Table 1.)

2.1. MS techniques in TNBC study

MS-based methods have been extensively used to study TNBC metabolism and heterogeneity, providing valuable insights into promising TNBC therapeutic targets [16,17]. LC-MS has been the most widely used method to analyze metabolites and lipids markers in TNBC patients [15,16] due to its high sensitivity and selectivity. It has also been used to classify TNBC into three metabolic subtypes with distinct prognoses [18]. Despite its high selectivity, LC-MS cannot elucidate the molecular structure of some compounds, such as unsaturated lipids, due to isotopic interference. To identify the structure of such compounds, ion mobility mass spectroscopy (IM-MS) can be used. IM-MS is a gas-phase electrophoretic technique that allows for the separation and identification of ions based on the size, shape and charge [19]. IM-MS was used to study the isomeric glycans in sphingolipids, revealing potential glycosylation signatures in TNBC subtypes [20]. Another drawback of analytical LC methods is that they are not ideal for analyzing samples with limited volume. In such cases, techniques such as micro-flow and nano-flow LC can be used. Micro-flow LC-MS has been successfully employed in TNBC metabolomic studies [21], while nano-flow LC-MS commonly used in proteomics, has demonstrated utility in inhibitor studies [22]. For even greater sensitivity, CE-MS is a more suitable approach. CE-MS can be used for profiling polar and charged metabolites to explore markers for TNBC, such as amino acids and energy metabolites, while it is generally less effective for separating non-polar or complex mixtures of compounds compared to LC [23,24]. However, CE-MS is sensitive to technical factors such as capillary alignment and system stability, which may present challenges for intra-patient studies. Both CE-MS and LC-MS are excellent techniques to study inter- and intrapatient heterogeneity due to their high biochemical resolution and sensitivity. However, they are not suitable to investigate higher levels of heterogeneity such as intra-tumor and cellular heterogeneity due to their poor spatial resolution (Fig. 1).

To investigate intra-tumor heterogeneity, techniques such as MS imaging can be used. Two widely used MS imaging techniques in metabolomics are matrix-assisted laser desorption/ionization MS (MALDI-MS) and secondary ion MS (SIMS). Both techniques are complementary, particularly in terms of spatial resolution, and throughput [25]. Advantages of MALDI-MS compared to SIMS include higher sensitivity and throughput [26]. MALDI-MS imaging has been applied by Chughtai et al. for the visualization of phosphatidylcholine and sphingomyelin species in different microenvironmental regions of an MDA-MB-231 TNBC xenograft model, highlighting the intra-tumor heterogeneity in TNBC [27]. One limitation of MALDI-MS is its relatively low spatial resolution due to matrix crystal size and the laser beam diameter [28]. SIMS imaging provides higher spatial resolution due to the highly focused ion beam. SIMS has been widely applied in breast cancer research to study metabolites composition within specific tumor regions [29], across xenograft models [30], among cell lines of different subtypes [8], and at single-cell level [31]. While SIMS provides the highest spatial resolution, reaching nanometer scale, it does so at the expense of throughput. Furthermore, both MALDI and SIMS require extensive sample preparation, and typically operate under vacuum conditions. This can significantly alter the cellular metabolism. In experiments where this is a major limitation, desorption electrospray ionization MS (DESI-MS) can be used. DESI-MS is an ambient ionization technique with minimal sample preparation, and is used for tissue imaging in TNBC [32]. By correlating lipid intensities with spatial distributions, Calligaris et al. applied DESI-MS to discriminate breast tumor margins from normal tissues [33]. Another valuable approach for studying tissue heterogeneity is laser micro-dissection (LMD), which allows isolation of specific tissue regions, single cells, or even subcellular

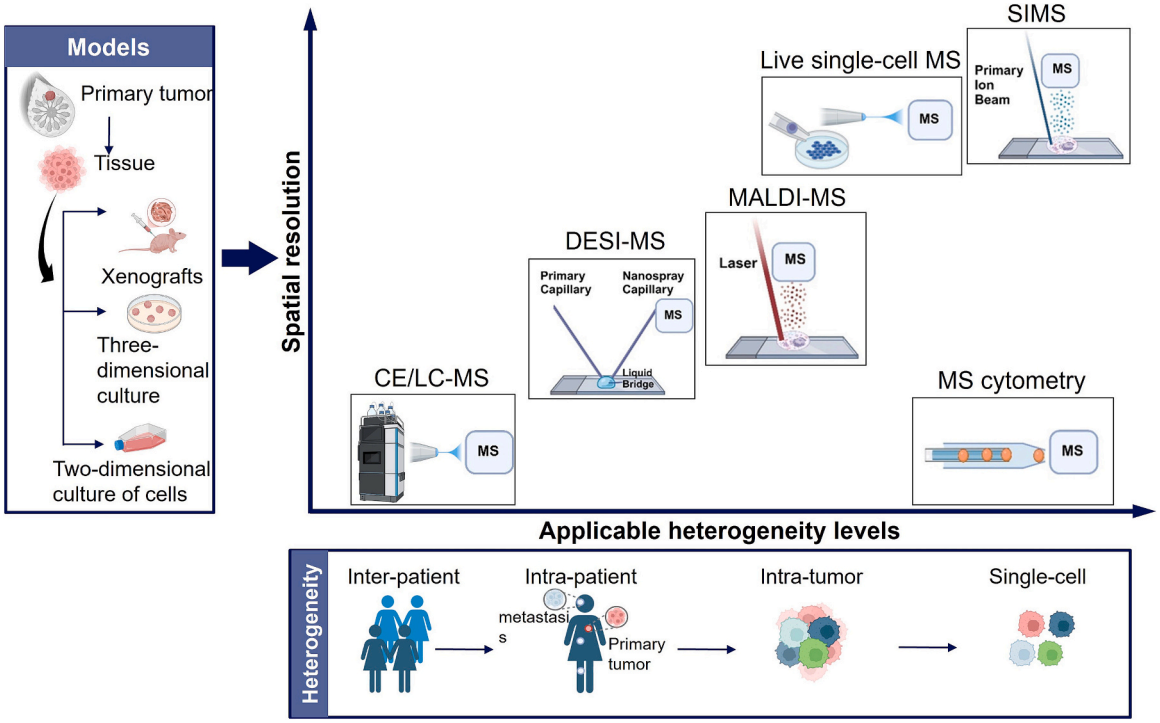

Fig. 1. Schematics for different models, mass spectrometry techniques, and their application across various heterogeneity levels in TNBC study.

Table 1
A summary of the discussed methods in TNBC study.

Methods	Models	Heterogeneity levels	Spatial resolution	Sensitivity	Coverage in TNBC study
LC-MS	Clinical sample (tissue [15], plasma [16]); Cell lines, xenograft models, organoid [18]	Inter-patient, intra-patient	No spatial resolution (Bulk analysis)	High	594 annotated polar metabolites, 1944 annotated lipids [15]; 110 lipids [16]; 594 metabolites [18]
CE-MS	Clinical sample (biopsy tissue [23]; plasma [24])	Inter-patient	No spatial resolution (Bulk analysis)	High	116 energy metabolites [23]; 147 metabolites [24]
MALDI-MS	xenograft [27]	Intra-patient, intra-tumor	Moderate [25] (micro-scale)	High	metabolites and glycerophospholipid species [27]
SIMS	cell lines [8], xenograft [30]	Intra-tumor ([8,30]) single-cell [31]	High [25] (Nano-scale)	Moderate-low	30 lipids [8]; choline metabolites [30]
DESI	Clinical sample (tissue) [32,33]	Intra-patient, intra-tumor	Moderate (Micro- to millimeter-scale)	High	76 metabolites [32]; 15 assigned lipid features [33]
MS cytometry	Cell lines [35]	Single-cell	Moderate (Micro-scale)	High-moderate	61 annotated PC features [35]
Live single-cell MS	Cell lines [39]	Single-cell	Moderate (Micro-scale)	Moderate	175 assigned metabolites [39]

structures. When coupled with MS, this technique enables detailed molecular analysis. In summary, MS imaging is a valuable tool that enables the spatial analysis of metabolites in complicated matrices, enhancing the understanding of intra-tumor heterogeneity [34]. However, the individual characteristics of each MS method should be kept in mind when designing experiments, especially in terms of spatial resolution, throughput, and disruption to the microenvironment.

In bulk metabolic analyses, it is challenging to determine whether the observed features are broadly regulated across the entire population or driven by specific subpopulations. Therefore, single-cell metabolomics is essential to reveal metabolic heterogeneity within tumors. One of the highest throughput single-cell MS methods is MS cytometry which was used for identifying differences among breast cancer cell lines [35,36]. However, cytometry often requires extensive cell manipulation, which can change the cell morphology, metabolome, and the microenvironment [37]. MS imaging techniques such as MALDI-MS and SIMS do possess single-cell resolution, however, they also increase the risk of metabolite perturbations [38]. To address this, capillary-based live single-cell sampling was developed, enabling minimal disruption to the microenvironment. This method has successfully differentiated breast cancer subtypes at the single-cell level [39] and can be integrated with techniques like LC-MS to study metabolic changes in TNBC [40]. Live single-cell MS further advances this approach by sampling individual cells in an incubator under physiological conditions (5 % CO_2, 37 °C) [41]. However, throughput is largely limited by the manual sampling process.

2.2. Normalization in metabolomics using MS

MS-based techniques are often used to detect and quantify metabolites simultaneously. However, in practice, metabolomics data can inherently exhibit variations derived from sample preparation and instrumental analysis, thus making it incorrect to directly compare samples between varying time points, concentration levels, cell lines or patients [42]. To reduce these discrepancies and improve data quality, normalization is a crucial step in metabolomics.

Normalization techniques such as dry mass measurements, cell count, protein, and DNA content are traditionally used in metabolomics. However, they may not be optimal in every scenario. For example, dry sample mass is suitable for bulk sample analysis but may become less accurate with small cell quantities, and the drying process can affect the metabolic profile [43,44]. Similarly, cell counting through trypsinization or imaging can be difficult with clustered cells or 3D cultures [45]. The organic solvents used for cell quenching can hinder accurate cell counting [46]. Consequently, when sample volume is limited, protein normalization can be used by measuring total protein content with colorimetric assays such as bicinchoninic acid (BCA). However, this method has been shown to have a large error, poor correlation with cell numbers, and low protein recovery due to the extraction solvents used [47,48]. Another normalization technique that can be used in volume-limited samples is DNA concentration-based normalization. The main advantage of DNA-based normalization that the concentration can be obtained from the same cell population used in the metabolomic analysis [47]. This method was shown to be consistent and reliable across different cell lines and may offer more reliable normalization compared to protein-based methods [47].

Using internal standards and quality control (QC) samples is particularly useful in MS-based metabolomics, where sample size and heterogeneity may pose challenges. Internal standards allow for a more accurate quantification of metabolites by compensating for any variations in sample preparation or instrumental conditions. Biological QC samples, which are representative of the overall sample population, help identify outliers or deviations in the analysis process, ensuring data reliability [49]. In targeted experiments, metabolites intensities or peak areas can be normalized against known internal standards. For example, region-specific standards or pixel-wise normalization strategies improve accuracy for MS imaging quantification [50]. In untargeted experiments, especially when sample volume is limited, such as in single-cell analysis, statistical normalization methods like median or probabilistic quotient normalization can be used to ensure consistency across different tissue regions and experimental runs. In an attempt to compare the effects of different normalization methods, lipidome analysis was performed on a total of 52 breast cancer cell lines, concluding that selecting an appropriate normalization strategy is crucial in lipidomics to balance improving replicate similarity and minimizing artifacts [51]. Thus, normalization methods should be tailored to the specific experimental design, especially when working with samples of limited volume or when high spatial resolution is required for detailed metabolic profiling.

3. Metabolic heterogeneity in TNBC

The aforementioned techniques have been used to investigate TNBC heterogeneity which impacts clinical outcomes [52]. TNBC heterogeneity exists at various levels, namely, subtype-specific, cell line-specific, and on the single-cell level. In this section, the levels of heterogeneity of TNBC will be discussed, with a specific emphasis on metabolism since it is the most representative of the cellular phenotype.

Lehmann et al. first classified the molecular subtyping of TNBC based on gene expression profiling in 2011. These subtypes include luminal androgen receptor (LAR), basal-like, immunomodulatory (IM), mesenchymal and mesenchymal stem-like subtypes [6]. Afterwards, several molecular TNBC subtypes have been identified [53,54], but they are still not confirmed clinically. In 2019, Jiang et al. classified four TNBC

subtypes based on transcriptomic data from 360 primary TNBC samples [55], including LAR, IM, basal-like and immune-suppressed (BLIS), and mesenchymal-like (MES) subtype (Fig. 2). In 2021, based on the 465 TNBCs multi-omics database, Gong et al. classified TNBC into distinct metabolic-pathway-based subtypes (MPSs) which was further validated using untargeted metabolomic analysis of 72 samples [18]. This study demonstrated metabolic dysregulation and heterogeneity in TNBC. One metabolic subtype MPS1, in which 70 % cases belonging to LAR subtype, showed significant alterations in lipid metabolism, whereas the other metabotype MPS2, which is mostly BLIS subtype, exhibited lower glucose levels and a greater reliance on glycolysis. This study also categorized 32 TNBC cell lines into MPS subtypes, aligning them with the molecular subtypes previously defined by Lehmann et al. To promote the findings of potential therapeutic markers, the follow-up study by Xiao et al. conducted a comprehensive metabolome profiling on 330 TNBC tumors and 149 paired normal breast tissues by LC-MS and LC-MS/MS. This study systematically linked TNBC metabolome to genomics and classified TNBC into three distinct metabolomics subtypes: C1, C2, and C3 (Fig. 2). The authors also found that targeting sphingosine-1-phosphate (S1P), which is the tumor-promoting intermediate of the ceramide pathway, using SPHK1 inhibitors has shown potential in inhibiting LAR TNBC tumors. Additionally, inhibiting *N*-acetyl-aspartyl-glutamate formation showed promise as a therapeutic target for BLIS TNBC tumors [15]. When comparing with MPSs, the MPS1 subtype showed a strong overlap with the C1 subtype, while the MPS2 and MPS3 subtypes were interlaced with the C2 and C3 subtypes. These findings underscore the growing understanding of TNBC heterogeneity through metabolic and molecular subtyping, highlighting the potential for tailored therapeutic strategies targeting specific metabolic pathways. However, further integration of metabolomic and other multi-omics data with clinical outcomes is essential to translate these insights into effective treatments.

Metabolomic profiling across different breast cancer cell lines has further demonstrated cell line-specific metabolic heterogeneity. One untargeted metabolomic profiling of intracellular extracts across 18 breast cell lines including ER/PR positive, HER2 positive and TNBC cell lines, reported that cell lines can be clustered into two or more groups with different metabolic phenotypes, providing valuable metabolic information for cell line classification [56]. A targeted cellular lipidomic profiling using Triple TOF MS/MS further highlighted notable cell line-specific differences. In five malignant TNBC cell lines (MDA-MB-231, SUM159PT, Hs578T, MDA-MB-468, SUM149PT), intracellular levels of phosphatidylglycerol (PG) were lower compared to controls which are normal human breast cells (MCF-10 A, MCF-12 A). Among these, three claudin-low TNBC cell lines (MDA-MB-231, SUM159PT, Hs578T) exhibited more consistent lipid phenotypes, with elevated levels of phosphatidylserine (PS), phosphatidylcholine (PC), sphingomyelin (SM), and cholesterol ester (CE) species, compared to controls. Interestingly, the SUM149PT profile formed a distinct cluster from the other TNBC cell lines, further underscoring metabolic variability [57]. This study clearly demonstrated dysregulated glycerophospholipid metabolism in TNBC cell lines. However, caution is necessary when comparing metabolomic data across cell lines, as factors such as growth conditions, media composition, cell confluency, cell morphology, and passage number can confound metabolic profiles, potentially influencing the results and interpretations [51]. Moreover, future studies should explore metabotypes in more in vivo-relevant models, such as xenografts.

Additionally, the analysis of the metabolome at the single-cell level has recently gained attention as a promising approach for biomarker discovery [58]. PC and phosphatidylethanolamine (PE) are frequently detected metabolites in single cells due to their high abundance in membrane lipids. For example, PCs such as PC(32:1) and PC(34:1) have been demonstrated to be different in concentrations across subtypes of breast cancer cell lines, while the level of PE(35:0) was the highest in TNBC MDA-MB-468 single cells [39]. A variety of PCs such as PC(34:1) and PC(36:2) from single MDA-MB-231 cells in a direct-infusion lipidomic study were detected and four subtypes of breast cancer cells were classified by structural information such as the location of double bonds [59]. It is possible to tell variations of metabolites of single cells between different subtypes using multiple microextraction methods and the specific information provided by MS2. Despite its infancy, single-cell metabolomic studies show promise in revealing subtypes of TNBC and novel potential drug targets. However, there are still some technical limitations that have to be overcome first, mainly, throughput, quantitation performance and sampling without altering the microenvironment.

In summary, metabolic heterogeneity in TNBC has been observed across different tumor subtypes, cell lines, and even at the single-cell level. This heterogeneity has been increasingly mapped using advancements in MS techniques, with lipid alterations emerging as promising markers and therapeutic targets for TNBC. The following

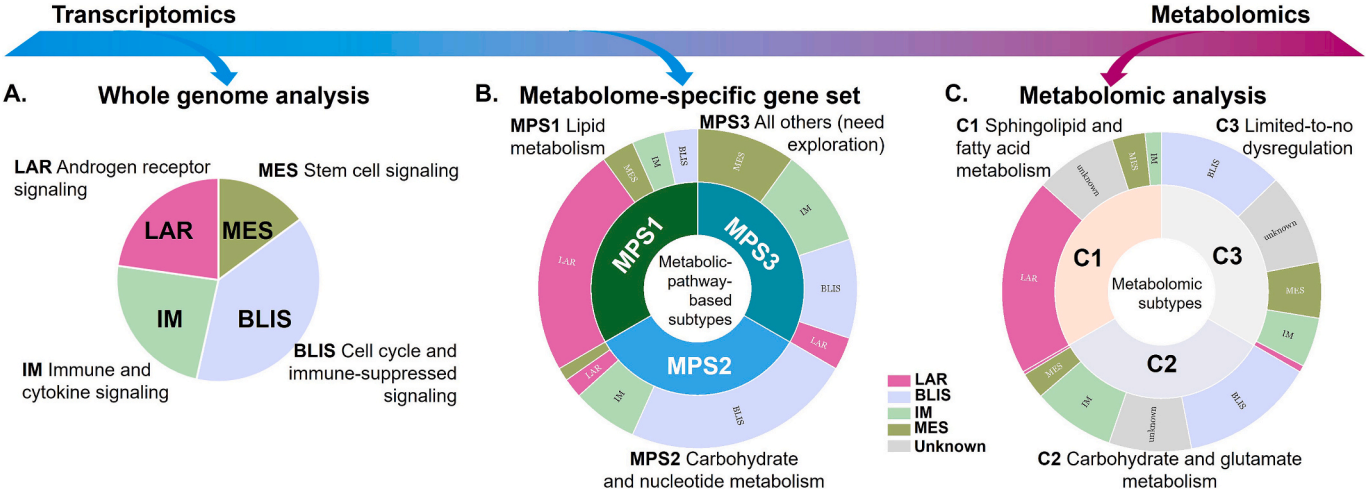

Fig. 2. Representative TNBC subtypes based on transcriptomics and metabolomics. (A) Distribution of mRNA subtypes in TNBCs, and subtype-specific features obtained from paper [55], LAR in red, BLIS in blue, IM in light green, MES in dark green; (B) Overlay of metabolic-pathway-based subtypes (MPSs, inner ring) with TNBC molecular subtypes in A) obtained from paper [18]; (C) Overlay of metabolomic subtypes (C1/C2/C3, inner ring) with TNBC molecular subtypes in A) obtained from paper [15]. Unknown in the outer ring is in grey. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

section will therefore focus on lipid dysregulation in TNBC.

4. Lipid dysregulation in TNBC

Lipids can be classified into five main classes: glycerolipids, glycerophospholipids, sphingolipids, sterols and fatty acids [60]. To understand the role of lipids in TNBC progression, this section will delve into the specific lipid metabolic profile in TNBC, both in clinical samples and models.

4.1. Glycerophospholipids

Changes in the lipid metabolism significantly contribute to malignancy and cancer progression [61,62]. This phenomenon is particularly evident in TNBC, where alterations have been reported across various lipid classes, including glycerophospholipids, glycerolipids, and sphingolipids [63–65]. Glycerophospholipids, the predominant structural lipids in eukaryotic cellular membranes, are often upregulated in TNBC, correlating with oncogenesis and tumor progression [64,66]. The dysregulation of glycerophospholipids levels or fractions can reflect changes in membrane structure and function. One example is a recent study on lipid metabolism in metastatic TNBC tumors relative to primary tumors, which found the abundance of saturated and monounsaturated fatty acid-containing glycerophospholipids was significantly decreased, while the abundance of polyunsaturated-containing glycerophospholipids was significantly higher [67]. Phosphatidylcholine (PC), for instance, has been linked to cell proliferation, motility, invasion and differentiation [68]. A higher level of PC(32:1) has been detected in recurrent TNBC tissues compared to non-recurrent tissues using MALDI-MS [69]. Elevated levels of PC(34:1) and PC(32:0) were exhibited in breast cancer tissues compared to adjacent normal tissues, with specific increases of PC(30:0) and PC(32:1) noted in TNBC compared to ER and HER2 subtypes [70]. Notably, a clear trend of increased abundance of PC $\geq$ C-40 was found in TNBC cell lines (MDA-MB-231 and MDA-MB-436) compared to other breast cancer subtypes [65]. Additionally, another study compared the lipidomic profile of ER negative cells (MDA-MB-231), normal breast cells (MCF-10 A) and ER positive (MCF-7) cells [71]. It revealed that eight PCs were significantly upregulated in MDA-MB-231 cells compared to MCF-10 A. However, when compared to MCF-7, only PC(38:4) showed a significantly higher abundance [71]. It has been hypothesized that cancer cells with elevated levels of ester-linked glycerophospholipids (e.g., PC, PE), tend to show heightened alkylglycerone phosphate synthase (AGPS) expression. For example, AGPS is highly expressed in aggressive breast cancer cells (MDA-MB-231) compared to less aggressive cells (MCF-7) [72]. Single-cell metabolomics studies further identified PCs with saturated fatty acid or monounsaturated fatty acid side chains, such as PC(30:0), PC(32:1) and PC(34:1), as significant components among breast cancer cell lines (BT474, MCF-7, MDA-MB-468 and SK-BR-3 cells), suggesting the potential of these key PCs as TNBC progression markers [39]. Collectively, these mentioned findings have shown a similar trend of PC regulation in TNBC tissue samples and cell line research. Phosphatidylethanolamine (PE), another abundant glycerophospholipid, plays an important role in biological processes such as cell division, cell cycle and apoptosis [73,74]. In the mentioned study, the abundance of PE(38:0) and ether-PEs were higher in MDA-MB-231 cells compared to MCF-7 [65]. Another study demonstrated that several PEs, including PE(28:0), PE(32:2), and PE(34:2), were upregulated while a few PEs were downregulated in MDA-MB-231 cells compared to MCF-7 cells, indicating the inconsistent direction of PEs dysregulation [64]. Both PC and PE are synthesized by the CDP-choline and CDP-ethanolamine Kennedy pathways, respectively [75], and PE can be converted to PC by PE *N*-methyltransferase [76]. Given the high abundance of PC and PE, the ratio of PC/PE is frequently used to study membrane function and metabolite alterations, such as in MDA-MB-468 cells [77,78]. Both PC and PE can produce PS, which is also one of the main glycerophospholipids in cell membrane [74]. In TNBC, PS plays a key role in metastasis, making it a potential therapeutic target [79].

Glycerophospholipids can be hydrolyzed to produce signaling molecules such as lysophospholipids, including lysophosphatidylcholine (LPC) and lysophosphatidylethanolamine (LPE) [66]. Among these, LPE has been shown to play a role in enforcing mitochondrial fission and migration capacity through a reactive oxygen species (ROS)-dependent mechanism in TNBC, acting as a key signaling lipid [80]. Specifically, LPE(18:1) and LPE(14:0) can activate intracellular Ca^{2+} signaling in MDA-MB-231 cells to induce cell proliferation [81]. Upregulation of LPE (18:0) and LPE(18:1) were detected in MDA-MB-231 cells compared to ER positive MCF-7 cells [64]. Additionally, these two LPE species have also been identified as significant components in breast cancer progression when comparing patients to healthy controls [82]. In serum samples of TNBC patients, elevated levels of LPE(18:1), LPE(18:2), and LPE(22:5) were detected compared to healthy controls [83]. In contrast, inconsistent trends were noted in LPC, PS, and PI species when comparing MDA-MB-231 cells to MCF-7 cells (Table 3). Lysophosphatidic acid (LPA) is a bioactive lipid mediator with significant roles in cancer research. It can be derived from phosphatidic acid or converted from LPC through the lysophospholipase D activity of autotaxin [84]. LPA has been found to promote stem cell expansion in TNBC by activating the Ca^{2+} pathway [85]. These lysophospholipids are derived from their corresponding glycerophospholipids by phospholipase A1/2 (PLA1/2) [81,86–88]. On the other hand, lysophospholipids can be converted to glycerophospholipids in Lands' cycle by glycerophospholipid remodeling enzyme lysophospholipid acyltransferases [89]. Thus the PC/LPC and PE/LPE ratios are vital for evaluating enzyme activity and potentially better indicators of disease progression than individual glycerophospholipid or lysophospholipid levels. Although these ratios have been reported as effective markers for various diseases [90,91], their potential ratios in TNBC still needs further exploration.

Overall, glycerophospholipids have shown their levels are associated with TNBC progression and it highlights the need for further research to speed up their clinical application.

4.2. Glycerolipids

For glycerolipids, triglycerides (TGs) stored in lipid droplets provide fatty acids for beta oxidation, producing large amounts of energy [92]. A significantly higher abundance of TG species with two or three unsaturated fatty acyl chains, such as TG(48:2), TG(50:2), TG(50:3), and TG (54:3), was reported in MCF-7 cells comparing to TNBC cells (MDA-MB-231 and MDA-MB-436) [65]. However, higher TG levels were detected in TNBC plasma compared to other breast cancer subtypes [93]. This suggests the importance of studying metabolite expression in both cells and plasma samples to reveal its function. The PC/TG ratio has been linked to cancer cell proliferation, as seen in luminal breast cancer tissue, but more research is needed to understand its role in cancer progression [94]. Diacylglycerol(DG) plays a role in cell proliferation, differentiation, survival, and exhibits important functions in membrane composition and energy storage [95,96]. DG acts as a hub between lipid metabolism and intracellular signaling, and can be formed through various enzymatic pathways, with many of these reactions being reversible [97]. Notably, DG is both a precursor to TG by DG acyltransferase and a product of TG hydrolysis [98]. Therefore, the DG/TG ratio can monitor this conversion, and is especially suitable for TG-rich samples like those from TNBC [99]. Studies have shown significant upregulation of DG(32:0) and DG(34:0) in TNBC cell lines compared to normal breast cells [65]. However, DG levels exhibited a trend of downregulation in TNBC plasma compared to non-TNBC [100].

4.3. Sphingolipids

Sphingolipids, including sphingomyelins (SMs) and ceramides

(Cers), have been extensively studied in breast cancer development. SMs have shown their potential as markers in cell line studies; for instance, SM(32:1) was significantly down-regulated in the TNBC cell line MDA-MB-231, and SM(34:2) was significantly up-regulated in another aggressive TNBC cell line MDA-MB-436 compared to normal breast cell MCF-10 A [65]. SM is first hydrolysed to Cer, which can be degraded to sphingosine, and finally, sphingosine can be phosphorylated to form the messenger lipid sphingosine-1-phosphate (S1P). S1P is a well-characterized marker involved in cell proliferation and metastasis in breast cancer [101]. Beyond serving as an intracellular second messenger, S1P binds to specific receptors on the cell surface to modulate cell proliferation and migration. High expression of extracellular S1P has been found to be positively correlated with TNBC metastasis [102]. Notably, higher SM levels and decreased sphingomyelinases activity were linked to better prognosis in TNBC patients, whereas higher Cer level in TNBC patients was linked to worse prognosis [63]. Cer levels, especially Cer(18:1/24:0), exhibited upregulated level in TNBC tissues compared to luminal ones [103]. Higher levels of Cer(36:1), Cer (38:1), and Cer(44:2) were reported in plasma of TNBC patients compared to non-TNBC by LC-MS/MS, suggesting the long-chain Cers may promote TNBC proliferation [100]. Both SM and Cer levels were found to be upregulated in breast cancer tumors [104]. Besides, the upregulation of Cer levels was associated with the dysfunction of the sphingolipid signaling pathway in breast cancer tumors [105]. Notably, Cer is taken as a crucial component for sphingolipids. Cer can be synthesized to SM by SM synthase, and SM can be converted back to Cer by sphingomyelinase [106]. Thus, the SM/Cer ratio can reflect the relative change of SM and Cer as well as sphingomyelinases activity and is sensitive to lipolytic activity [107]. Considering Cer phosphorylation can produce ceramide-1-phosphate (C1P) by Cer kinase (CerK), and CerK upregulation promotes proliferation and migration in TNBC cells, C1P/Cer ratio may be a valuable marker for these activities [108]. Future studies are needed to further investigate the role of sphingolipids for clinical applications. Furthermore, lipid metabolism-related genes and enzymes also play an important role in lipid metabolism of breast cancer. These changes are summarized in Table 2 and discussed in detail in other manuscripts [12,109,110].

Collectively, in TNBC, lipid metabolism alterations impact several biological functions, including energy storage, signaling, and structural components of cell membranes, to influence the phenotypes of TNBC such as cell migration and growth contributing to TNBC progression. The notable lipid markers that exhibit significant differences in TNBC compared to corresponding controls clinically and in vitro were summarized (Table 3). Recent studies have shown that metabolites of the same species often exhibit similar changes. However, it is also common for certain metabolites within the same species to display inconsistent levels. For instance, in TNBC plasma, PC(32:1) and PC(34:1) are upregulated, while PC(40:2) is downregulated [118]. Additionally, the dysregulation trend between patient-derived samples and cell line studies varies, posing a challenge to the exploration of metabolic markers in a stable way. This inconsistency highlights the need for further research to better understand the metabolic alterations and to identify reliable targets for study.

5. The lipidome and its impact on TNBC metastasis

Metastasis is the major cause of death in breast cancer [119]. Only recently, it is increasingly becoming clear that metastatic cells acquire metabolic features to be able to survive and grow in new environment [120]. To expand on this, in this section we describe the metabolic changes observed when comparing metastatic samples to healthy or non-metastatic ones. We further discuss metabolic alterations during epithelial-mesenchymal transition (EMT) and the differences between leader and follower cells in collective migration.

Table 2
Recent research findings of altered lipid metabolism genes and enzymes in TNBC.

Lipid-related genes or enzymes	Highlights in TNBC	References
FASN	FASN inhibition has anticancer effects both in sensitive cells and in chemoresistant cells.	[111]
NFYAv1 gene	upregulates the transcription of essential lipogenic enzymes ACACA and FASN to enhance the malignant behavior of TNBC.	[112]
FABP4	mediates fatty acid metabolism, thereby influencing TNBC progression.	[113]
monoacylglycerol lipase (MAGL)	controls the intracellular release of fatty acids. Increased levels of MAGL levels in aggressive BC cells (MDA-MB-231) compared to a nonaggressive model (MCF-7).	[114]
Alkylglycerone phosphate synthase (AGPS)	the critical enzyme for ether lipid synthesis; highly expressed across aggressive breast (MDA-MB-231) compared with less aggressive cancer cells (MCF-7); elevated in TNBC tumors compared to normal tissue.	[72]
ACLY mRNA	less in TNBC compared to HER2 subtype.	[115]
apolipoprotein E (ApoE) receptor	proliferation and invasiveness potentials in HER2-enriched and TNBC cell lines.	[116]
Bile acid biosynthesis-related genes (HSD3B7, AMACR, CYP39A1, CYP46A1, CYP7A1, and CYP8B1); FAO-related genes (EHHADH and HSD17B4)	showed essentiality for TNBC growth.	[117]

5.1. Lipid dysregulation in metastatic cells

Given the need of nutrients to move and grow, lipid metabolism alterations play a crucial role in cancer metastasis. In terms of lipid level dysregulation, higher levels of polyunsaturated lipids (i.e., PE(38:4), PE (P-38:5), PE(36:4)) were detected in BT-20 and MDA-MB-231 cells compared to MCF-7 by an in situ detection of membrane lipid profiles by MALDI-Fourier transform ion cyclotron resonance (MALDI-FTICR) MS [121]. In this mentioned study, the level of PI(36:1) in MDA-MB-231 relative to MCF-7 was increased [121]. PIs are located on the intracellular side of the membrane and participate in regulating membrane traffic and cell signaling [122,123]. In another study, a decreased level of PI(18:0/18:1) was detected in MDA-MB-231 cells, while the level of PI (18:0/20:4) was increased, compared to the MCF-7 cell line [71].

Higher concentration of PS, except for being the structural component of membranes, also plays a role in cancer metastasis. For example, PS(22:0/0:0) is linked to a higher chance of metastasis and death rate among TNBC patients [83]. PS(18:0/20:4) was found to be increased in metastatic breast cancer cells MDA-MB-231 relative to less-metastatic breast cancer cells MCF-7 [71]. Among TNBC metastatic cell lines, an untargeted metabolic profiling found that the levels of sphingosine decreased, whereas sphingomyelins increased in low metastatic potential cell lines (BT-549 and HCC1143) compared to high metastatic potential cell lines (MDA-MB-231, MDA-MB-436 and MDA-MB-468) [124].

In metastatic TNBC tumors compared to primary tumors, 71 lipids were significantly increased while 70 were decreased. The significantly increased lipids included PC and PE, such as PC(16:0/20:4), PC(18:0/20:4), PC(18:1/20:4), PE(16:0/20:4), PE(18:0/20:4), and PE(18:1/

Table 3
List of the discussed notable lipid alterations involved in TNBC by MS techniques.

Lipid species	Lipid markers associated with TNBC progression	Subjects compared	Overall trend in TNBC	Techniques	Ref.
PC	PC(32:1), PC (30:0)	breast cancer subtypes (TNBC tissue samples vs ER and HER2 breast cancer)	↑	MALDI-MS	[70]
	PC(32:1)	recurrent TNBC tissues vs non-recurrent tissues	↑	MALDI-MS	[69]
	PC ≥ C-40	more aggressive vs less aggressive cell lines (MDA-MB-231 and MDA-MB-436 vs non-TNBC cells)	↑	UPLC-QTOF-MS	[65]
	PC(16:1/18:1), PC(18:1/18:1), PC(18:0/20:4); PC(18:0/20:4)	more aggressive vs less aggressive cell lines (MDA-MB-231 cells vs MCF-10 A; MDA-MB-231 cells vs MCF-7)	↑	Direct infusion mass spectrometry	[71]
	PC(30:0), PC (32:1), PC(34:1)	Single cells (MDA-MB-468 vs BT474, MCF-7, SK-BR-3 single cells)	↑	ESI-Q Exacitve Orbitrap mass spectrometer	[39]
	25 PE targets including PE (32:2), PE (34:2), PE (38:5), PE(38:6)	TNBC vs non-TNBC and healthy controls (Plasma)	No significant difference	LC-MS/MS	[100]
PE	PE(28:0), PE (32:2), PE (34:2), PE (34:3), PE (36:6), PE (38:5), PE(38:6)	more aggressive vs less aggressive cell lines (MDA-MB-231 vs MCF-7)	↑	LC-MS	[64]
	PE(20:3/P-18:1), PE(18:4/P-18:0), PE (20:5/P-18:0), PE(P-18:1/22:5), PE(P-18:0/16:0), PE (P-18:1/20:5), PE(P-18:1/22:6), PE(P-18:1/18:0), PE (P-18:0/14:0)		↓		
	PE(18:1/18:1)	more aggressive vs less aggressive cell lines (MDA-MB-231 vs MCF-7/MCF-10 A)	↓	Direct infusion mass spectrometry	[71]
	PE(38:4), PE(P-38:5), PE(36:4)	more aggressive vs less aggressive cell lines (BT-20, MDA-MB-231 vs MCF-7)	↑	MALDI-FTICR MS	[121]
LPE	LPE(18:1), LPE (18:2), LPE (22:5)	TNBC vs healthy controls (serum)	↑	UHPLC-QTOF MS	[83]
	LPE(18:0), LPE (18:1)	more aggressive vs less aggressive cell lines (MDA-MB-231 vs MCF-7)	↑	LC-MS	[64]
LPC	LPC(14:0), LPC (16:2), LPC(O-18:1)	TNBC Tissue vs healthy controls	↓	LC-MS/MS	[100]
	LPC(15:0), LPC (18:0), LPC (20:1), LPC (20:2), LPC (20:4), LPC (22:5)	TNBC Serum vs healthy controls	↓	UHPLC-QTOF MS	[83]
	LPC(16:1), LPC (18:3), LPC (18:4)		↑		
PS	PS(14:0/20:0), PS(20:3/21:0), PS(22:0/17:2)	TNBC Serum vs healthy controls	↓	UHPLC-QTOF MS	[83]
	PS(19:0/0:0), PS (O-18:0/0:0), PS (O-20:0/20:2)		↑		
	PS(16:1/18:1), PS(16:0/18:1), PS (16:0/18:0), PS(18:1/18:2), PS(18:0/18:1), PS(18:0/18:2), PS(18:0/20:4), PS(18:0/22:4), PS(18:0/22:6)	more aggressive vs less aggressive cell lines (MDA-MB-231 vs MCF-7)	↑	Direct infusion mass spectrometry	[71]
PI	PI(18:3/22:1), PI(22:0/16:1)	TNBC Serum vs healthy controls	↑	UHPLC-QTOF MS	[83]
	PI(22:0/18:0)		↓		
	PI(36:1)	more aggressive vs less aggressive cell lines (MDA-MB-231 vs MCF-7)	↑	MALDI-FTICR MS	[121]
	PI(18:0/18:1)	more aggressive vs less	↓	Direct infusion	[71]
	PI(18:0/20:4)		↑		

(continued on next page)

Table 3 (*continued*)

Lipid species	Lipid markers associated with TNBC progression	Subjects compared	Overall trend in TNBC	Techniques	Ref.
		aggressive cell lines (MDA-MB-231 vs MCF-7)		mass spectrometry	
	Sphingomyelins levels	better vs worse disease-free survival in TNBC patients	↑	LC-MS	[63]
	SM(34:1)	more aggressive vs less aggressive cell lines (MDA-MB-231 vs MDA-MB-361)	↓	MALDI-FTICR MS	[121]
SM	SM(32:1)		↓		
	SM(34:2)	more aggressive vs less aggressive cell lines (MDA-MB-231 vs MCF-7; MDA-MB-436 vs MCF-7)	↑	UPLC-QTOF-MS	[65]
	Sphingosine	low metastatic potential cell lines (BT-549 and HCC1143) vs high metastatic potential cell lines (MDA-MB-231, MDA-MB-436 and MDA-MB-468)	↓	UPLC-MS/MS	[120]
	Ceramides levels in the untargeted platform	better vs worse disease-free survival in TNBC patients	↓	LC-MS	[63]
	Cer(36:1), Cer(42:2), Cer(43:1), Cer(44:2), Cer(42:3), Cer(38:1)	TNBC plasma vs healthy controls	↑	LC-MS/MS	[100]
	Cer(18:1/24:0), Cer(18:1/18:0), Cer(18:1/18:1)	breast cancer subtypes (TNBC tumor vs luminal tumor)	↑		
Cer	Cer(18:1/16:0), Cer(18:1/18:1)		↓	LC-MS	[103]
	Cer(22:0/24:1), Cer(16:0/24:1), Cer(22:0/24:1)	TNBC tumor vs healthy tissue	↑		
	Cer(18:0/24:0), Cer(18:0/26:1)	more aggressive vs less aggressive cell lines (MDA-MB-231 vs MCF-7) TNBC	↑	LC-MS	[64]
	TG(45:0), TG(54:3), TG(56:3), TG(58:9)	Plasma vs non-TNBC/ healthy controls	No significant difference	LC-MS/MS	[100]
TG	TG(48:2), TG(50:2), TG(50:3), TG(52:2), TG(52:3), TG(54:2), TG(54:3)	more aggressive vs less aggressive cell lines (MDA-MB-231 and MDA-MB-436 vs MCF-7)	↓	UPLC-QTOF-MS	[65]
	1) DG(34:2); 2) DG(34:0); 3) DG(32:0), DG(34:1), DG(34:2), DG(36:1), DG(36:2), DG(38:4)	1) TNBC Plasma vs non-TNBC; 2) TNBC Plasma vs HER2+; 3) TNBC vs healthy controls	↓	LC-MS/MS	[100]
DG	DG(32:0), DG(34:0)	aggressive vs healthy cell lines (MDA-MB-231 and MDA-MB-436 vs MCF-10 A)	↑	UPLC-QTOF-MS	[65]

20:4) [67]. These findings suggest that lipid metabolism alterations are characteristic of metastasis and may serve as potential biomarkers.

The key regulators of lipid metabolism have been well reviewed such as CD36 and fatty acid synthase (FASN) [120,125]. Recent research has identified PNPLA8 as a driver of migration and a promoter of glycerophospholipid remodeling [57]. In this systematic lipidomic study among TNBC cell lines, six glycerophospholipid species, PE(40:4), PE(40:5), PE(40:6), PE(O-40:0), PE(O-42:2), and PC(O-36:5), and one sphingolipid species, SM(38:0;4), were positively correlated with PNPLA8 protein levels. In contrast, two other sphingolipid species, SM(38:2;2) and SM(36:2;3), as well as one glycerophospholipid, PG(O-38:0), were negatively correlated [57]. Metastatic tumors have been reported to upregulate acyl-CoA synthetase long-chain family member 4 (ACSL4). ACSL4 activates polyunsaturated fatty acids into their CoA derivatives, resulting in alterations in membrane glycerophospholipid composition. This glycerophospholipid remodeling induced lipid raft localization and integrin β1 activation, which promote TNBC metastasis [67].

Additionally, a byproduct of lipid metabolism is the generation of ROS, which are critical for maintaining cellular redox homeostasis. Disruptions in redox balance can potentially lead to cancer progression and other pathological conditions [126]. High ROS levels have been

associated with the aggressive TNBC cell lines [127]. However, increased ROS induced by mitochondrial fission in TNBC cell line inhibited cell migration [80]. Similarly, inhibition of ROS coupled with stimulation of glucose oxidative metabolism may be an efficient strategy to block metastasis [128]. In addition, inhibiting the production of hypoxia-induced mitochondrial ROS has been shown to reverse EMT in the TNBC cell line MDA-MB-468, and reduce MDA-MB-231 cell migration and invasion [129,130].

These findings underscore the complexity and significance of lipid metabolism in TNBC metastasis.

5.2. *Lipid dysregulation in EMT and in collective migration*

EMT is a dynamic process and considered as a significant step in tumor cell invasion cascade because it marks epithelial cells undergo a transition from a collective to a single-cell disseminated pattern [131]. It was reported that EMT can promote cancer cell motility and invasion [132]. Epithelial-like cells are characterized by strong cell-to-cell junctions, whereas mesenchymal-like cells typically exhibit reduced cell-to-cell adhesion, accompanied by increased migratory and invasive capabilities (Fig. 3A). Metabolic differences between epithelial-like and mesenchymal-like cells underscore their distinct characteristics. Alterations in lipid levels have been linked to the metastatic potential of the tumor and the EMT process in multiple studies. For example, researchers identified 130 lipids that differ between epithelial-like and mesenchymal-like cells [133]. Following the EMT transition, PC and PE were significantly increased in mesenchymal cells compared to epithelial cells [133]. LPE is derived from PE through the partial hydrolysis of PE and the removal of a fatty acid group [87,88]. LPE plays an important role in lipogenesis and lipolysis through mitogen-activated protein

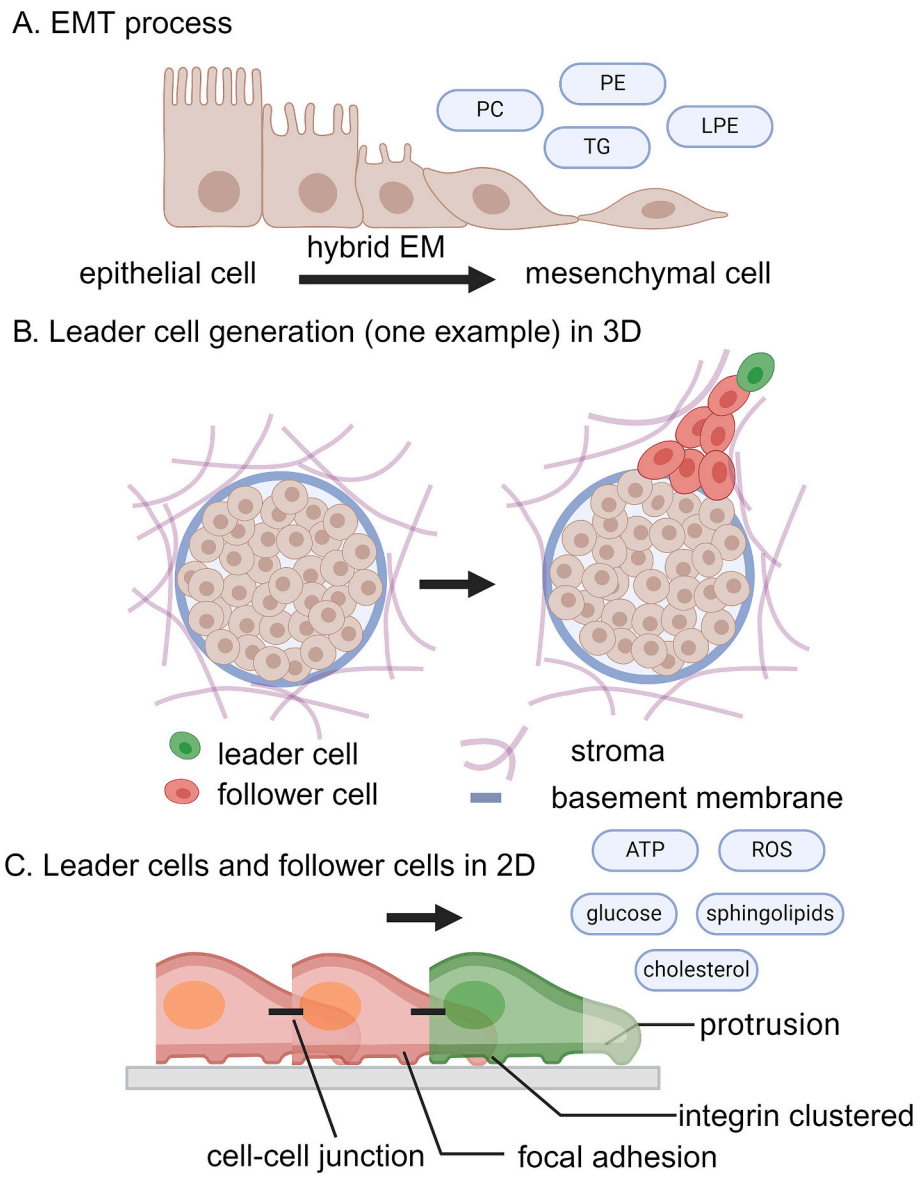

Fig. 3. The EMT process and the behavior of leader cells in three-dimensional (3D) and two-dimensional (2D) environments in cell migration. (A) EMT process demonstrates the transition of epithelial cells through an epithelial-mesenchymal hybrid state to mesenchymal state, which is associated with lipid alterations, such as PC, PE, TG and LPE. (B) In a 3D environment, the leader cell (green) emerges at the invasive front, guiding the follower cells (red) through the stroma and basement membrane, suggesting a role in directed migration and invasion. (C) In a 2D environment, leader cells (green) show protrusions forward, forming integrin clusters, and follower cells (red) exhibit focal adhesions and cell-cell junctions. Metabolites such as ATP, ROS, glucose, sphingolipid, and cholesterol are involved in the activities of leader cells and follower cells. (By BioRender: AS278ZQMLC). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

kinase (MAPK) / extracellular signal-regulated kinase 1/2 (ERK1/2) pathway, downstream of the intracellular Ca^{2+} increase [81,134,135]. It was reported that LPE(18:1) can activate of ERK1/2 in MDA-MB-231 cells to induce EMT along with lysophosphatidic acid [81]. Also, the knockout of β-catenin has shown a specific role in regulating the lipid metabolism in breast cancer mesenchymal cells [136]. In this study, mesenchymal cells showed extreme upregulation of TG and involved enzymes such as the diacylglycerol acyltransferase-1, whereas higher levels of monounsaturated fatty acids were detected in breast cancer epithelial cells, including TNBC [136]. In TNBC, EMT can be promoted by many inducers such as TGF-β [137] and TNF-α [138]. Thus, exploring metabolic changes related with EMT inducers is vital. Lipid metabolism plays an important role in EMT process. However, it is still unclear whether these lipids changes may be the driver of EMT or are a result. Some studies have shown that EMT is not required for breast cancer metastasis, possibly due to its molecular complexity [139,140]. Investigating the metabolism of leader cells may help clarify these controversies.

In the process of metastasis, migration can occur either individually or collectively, with collective migration playing a predominant role. Cancer cells exhibit collective migration in the form of strands or clusters [131]. At the tip of the migratory cell collectives are termed as 'leader cells' [141], which guide the movement and coordinate with follower cells. In three-dimensional (3D) migration, leader cells generate the path by penetrating through extracellular matrix (ECM), mimicking the complex in vivo tumor microenvironment [141,142] (Fig. 3B). Two-dimensional (2D) migration occurs across flat surfaces, providing simpler models to study cell movement. Leader cells typically undergo morphological changes such as the formation of protrusions and focal adhesions [141]. Protrusion of leader cells, the outward pushing of the membrane by actin filaments in the direction of migration, is the first step of cell migration [131,143] (Fig. 3C). This morphological changes in the cell membrane are also accompanied by lipidomic profile alterations, particularly in lipid rafts. Lipid rafts, which are cholesterol and sphingolipids-enriched membrane nanodomains [144], are concentrated in the protrusions of breast cancer cells and promote signal transduction and membrane motility [145]. Filopodia, often observed in leader cells, promote adhesion to the ECM through asymmetric cholesterol accumulation at the tips of the filopodia [146]. Therefore, depletion of cholesterol can be a suitable avenue of research in future drug development, with recent studies showing that its depletion causes disruption of lipid rafts and inhibition of TNBC adhesion and invasion in MDA-MB-231 and MDA-MB-468 cell lines [147]. In 3D cell migration, integrins and proteases are necessary for cell-ECM interaction. Cancer cell migration involves integrin signaling to induce focal contacts with ECM. Integrins belonging to cell adhesion molecule family, are clustered in the cell membrane and facilitate cell migration through cell-matrix interactions [148]. It was reported the integrins interacted with sphingolipids GM2, promoting cancer cell migration in an integrin-dependent manner [149]. Proteases mainly function by degrading the nearby ECM and modulating tumor environment, and have been reported to promote EMT [150]. Among the protease families, matrix metalloproteinase-9 (MMP-9) was found to be upregulated in leader cells by biosensors in 3D breast cancer invasion assays [151]. Additionally, lipid metabolism related to MMP, including cholesterol, has been reviewed [152]. Leader cells were reported to exhibit increased expression of mesenchymal proteins such as vimentin and N-cadherin compared to follower cells, suggesting a partial leader cell EMT [153]. However, the exact changes in the metabolome are yet to be studied.

The typical characteristic of the leader cells is an altered energy generation profile. Many studies revealed that glycolytic ATP production and glucose metabolism play a pivotal role in regulating the rearrangement of leader and follower cells, indirectly promoting invasion [154–157]. When the invasion through tissue becomes challenging, often due to the increased density of the tissue, leader cells adapt by consuming more glucose and increasing energy production, and leader cells may transition to follower cells if the ATP/ADP ratio drops too low [154]. This showcases yet another facet of phenotypic plasticity, wherein cells demonstrate the ability to switch between follower and leader roles. These leader cells are promising candidates for novel anti-metastasis therapy. However, most studies reviewing metabolic changes in metastasis have focused on only a few nutrients, such as energy metabolites (ATP/ADP, NADH/NAD+ ratios, ROS) [142], due to technology limitations in the metabolomics of leader cells. Consequently, metabolic changes in leader cells still require extensive exploration.

6. Conclusions and perspectives

Metabolomics has provided valuable insights into TNBC heterogeneity, offering sensitive and nuanced analyses. By identifying metabolic differences between more aggressive and less aggressive cancer cells, researchers can gain insights that aid in cancer diagnosis and prognosis. Furthermore, clarifying the metabolic diversity underlying TNBC progression and metastasis, researchers can improve the prediction of treatment responses and develop more effective therapeutic strategies. MS-based metabolomics has made crucial contributions to TNBC study, however, challenges remain in metabolite extraction, identification, and data normalization, as well as false positive discovery of metabolite biomarker [158]. On the other hand, single-cell metabolomics is very attractive for dissecting cellular heterogeneity, it faces obstacles such as metabolite quantification and identification, as well as quality control [58]. Finally, integrating metabolomics with other omics technologies such as transcriptomics and proteomics, will provide a comprehensive view of the molecular intricacies driving TNBC progression, paving the way for the development of targeted therapeutic strategies.

6.1. Footnote

In Table 3, lipids are annotated with either sum formulas or with resolved structures at fatty acyl level according to the shorthand notation for lipid structures proposed by Liebisch et al. [159]. In sum formulas for *glycero*(phospho)lipids, such as PC(34:1), the total number of carbons followed by double bonds for all fatty acyl chains are indicated. For sphingolipids, the sum formula, e.g., Cer(42:2), specifies the total number of carbons in the long-chain base and fatty acyl moiety, followed by the total number of double bonds in both components. In resolved structures, individual fatty acyl chains or the long-chain base are separately defined, such as PI(18:0/20:4) or Cer(18:1/24:0).

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CRediT authorship contribution statement

Xiaoyue Huang: Writing – review & editing, Writing – original draft, Visualization, Methodology, Conceptualization. **Ahmed Ali:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Dounia E.I. Yachioui:** Writing – original draft. **Sylvia E. Le Dévédec:** Writing – review & editing, Supervision, Conceptualization. **Thomas Hankemeier:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors have no conflict of interest to declare.

Data availability

No data was used for the research described in the article.

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