

# Metabolomics: a promising diagnostic and therapeutic implement for breast cancer

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**Abstract:** Breast cancer (BC) is the most commonly diagnosed cancer among women and the leading cause of cancer death. Despite the advent of numerous diagnosis and treatment methods in recent years, this heterogeneous disease still presents great challenges in early diagnosis, curative treatments and prognosis monitoring. Thus, finding promising early diagnostic biomarkers and therapeutic targets and approaches is meaningful. Metabolomics, which focuses on the analysis of metabolites that change during metabolism, can reveal even a subtle abnormal change in an individual. In recent decades, the exploration of cancer-related metabolomics has increased. Metabolites can be promising biomarkers for the screening, response evaluation and prognosis of BC. In this review, we summarized the workflow of metabolomics, described metabolite signatures based on molecular subtype as well as reclassification and then discussed the application of metabolomics in the early diagnosis, monitoring and prognosis of BC to offer new insights for clinicians in breast cancer diagnosis and treatment.

**Keywords:** diagnosis, biomarkers, treatment, metabolites

## Background

Metabolomics (metabonomics), different from genomics and proteomics, focuses on metabolites with a molecular weight <1500 Da, such as glycolipids and polysaccharides, during metabolism at a precise time and condition to reflect the endogenous responses of metabolites to internal and external factors.<sup>1</sup> Roger Williams described the concept of “metabolic profile” for the first time in the late 1940s and proposed that individuals had their own “metabolic profile” characterized by their body fluids.<sup>2</sup> In the 1960s and 1970s, scientists started to utilize metabolites as biomarkers to illustrate the relationship between metabolites and diseases with advancing technology.<sup>3,4</sup> Recently, metabolomics has provided a new avenue in cancer research because the chemical entity can reflect the cellular state and microenvironment of cancer.

Metabolomics is a more convenient, less damaged and easier measuring choice than traditional detection methods that may be invasive or radiant in detecting diseases.<sup>5</sup> In addition, metabolomics samples can be obtained in a variety of ways, and the types of metabolomics are also extensive, such as blood, urine, saliva and tissue.<sup>6–9</sup> Metabolites extracted from different clinical samples may reveal abnormal situations or diseases in the body.

Being the most commonly diagnosed cancer among women, breast cancer (BC) is the leading cause of cancer death, followed by colorectal cancer and lung cancer.<sup>10</sup> Moreover, BC remains difficult to completely cure due to its heterogeneity. The

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diagnosis of BC with existing means of inspection is still not sufficiently timely; therefore, patients usually miss the optimal opportunity for treatment, leading to poor prognosis. The expression of many specific metabolites might be changed during the occurrence and development of cancers.<sup>11</sup> Thus, the detection and monitoring of cancer would be possible with promising metabolic biomarkers.<sup>12,13</sup> This review summarizes the current advances of BC in metabolomics and provides a reference for the diagnosis, therapy and monitoring of BC in clinical practices.

## The workflow of metabolomics

### Metabolomic analysis platforms

In general, the procedure for metabolomics can be concluded in sample preparation, component separation, detection and identification, data analysis and interpretation.<sup>5,14</sup> According to the objective and purpose of the research, metabolomics strategies can be divided into four levels:<sup>15</sup> (1) Metabolite target analysis focuses exclusively on one or several components. This strategy is mainly used for screening purposes and for analyses that need extreme sensitivity. (2) Metabolic profiling is a quantitative analysis focused on a smaller number of pre-defined metabolites. (3) Metabolic fingerprinting is a high-throughput and rapid qualitative strategy that analyses samples without isolation. Metabolic fingerprinting is suitable for diagnostic usage and clinical routines. and (4) Metabolomics is a qualitative and quantitative strategy that determines all metabolic components in a specific biological sample under defined conditions.<sup>15</sup> Briefly, metabolomics strategies can be divided into targeted and untargeted approaches, and the main distinction is that the targeted approach is focused on specific metabolites or pathways.<sup>5</sup>

The representative detection methods of metabolomics include mass spectrometry (MS), which is used to identify and quantify metabolites following the optional separation by various methods, such as gas chromatography (GC), liquid chromatography (LC) or capillary electrophoresis (CE). Nuclear magnetic resonance (NMR) spectroscopy is a non-destructive and non-selective detection technique that can work independently.<sup>16,17</sup> Additionally, there are many derivative technologies that perform better under a specific condition, such as ultra-performance liquid chromatography (UPLC)-MS, which is suitable for pharmaceutical research.<sup>18</sup> Choosing a suitable platform is required for different purposes and sample types. MS is characterized by extremely high sensitivity that can detect low

concentration metabolites. The liquid chromatography and mass spectrometry (LC/MS) combination has the advantage of analysing and determining non-volatile, polar and thermolabile compounds, such as proteins, peptides and polymers, while the gas chromatography and mass spectrometry (GC/MS) combination is mainly used for more volatile and less polar substances.<sup>19,20</sup> The combination of capillary electrophoresis and mass spectrometry is good for analysing highly polar and charged metabolites in complex biological samples.<sup>19</sup> NMR, as mentioned above, is a non-destructive and unbiased detection technique for dynamic detection in real-time.<sup>17</sup> However, the low sensitivity of NMR makes it hard to detect low-abundance metabolites and the signal overlap hampers the identification and qualification of metabolites.<sup>21</sup>

### Data processing and identification

Data processing and identification is the most difficult and complex step in metabolomics studies. Before analysis, the data acquired from instruments should undergo pre-processing, normalization and pre-treatment to eliminate unwanted variation and allow quantitative comparison.<sup>22-24</sup> Simple univariate analyses cannot meet the requirements of the complex interplay between metabolites, thus multivariate statistical methods are necessary.<sup>25</sup> The multivariate statistical methods can be broadly divided into unsupervised methods for which the analysis has no predefined values or groups and supervised methods for which the analysis of the data was based on pre-established groups or variables.<sup>20,26</sup> Unsupervised methods include principal component analysis (PCA)<sup>27</sup> and hierarchical cluster analysis (HCA).<sup>28</sup> Supervised methods include partial least squares (PLS), soft independent modelling of class analogy (SIMCA), partial least squares discriminant analysis (PLS-DA), orthogonal PLS-DA (OPLS-DA) and random forest regression analysis.<sup>27</sup> Generally, an unsupervised method is first used to classify unknown samples and find the different components. Then, based on the results, a supervised method is introduced to screen possible biomarkers and finally interpret the results to meaningful parameters.<sup>26</sup>

### Factors influencing metabolomics raw data

Since the whole body participates in the metabolic process and each cell releases its own metabolites, many factors can influence the results of metabolomics raw data<sup>29</sup> such as, gender,<sup>30</sup> drug usage<sup>3</sup> and dietary habits.<sup>31</sup> African-American or

Caucasian women have shown different metabolic profiles of triple-negative breast cancer (TNBC).<sup>32</sup> These differences suggest that racial differences may alter the metabolites of BC. The screening of salivary volatiles of putative BC revealed that Portuguese samples and Indian samples possessed discrepancies.<sup>9</sup> Moreover, the metabolite patterns of the pre-menopausal period differed from those of the postmenopausal period.<sup>33</sup> Aside from race and age, the outcome of metabolite screening was also influenced by the sampling process. For example, the time in vacuum storage before freezing had a minimal effect on the exploration of histological, transcriptomic and proteomic profiles but markedly affected metabolite profiles. Additionally, the sample freezing time also influenced the metabolite outcome.<sup>34,35</sup>

## Molecular signatures of BC based on metabolomics

### The metabolic signatures of basic BC subtypes

BC can be classified into four subtypes, including Luminal A, Luminal B, HER-2-enriched and triple-negative breast cancer, based on the status of the hormone receptors, such as oestrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2), and the expression of ki-67.<sup>36,37</sup> In addition, four basic molecular subtypes can be identified by metabolomics. Eight metabolites, including carnitine, lysophosphatidylcholine (20:4), proline, alanine, lysophosphatidylcholine (16:1), glycochenodeoxycholic acid, valine and 2-octenedioic acid, were selected as a panel to identify HER2-negative or HER2-positive patients and ER-positive or ER-negative patients. Compared to HER2-negative patients, HER2-positive patients showed elevated levels of carnitine, lysophosphatidylcholine (20:4), proline, valine, and 2-octenedioic acid. The ER-positive patients, compared with ER-negative patients, showed an elevated level of glycochenodeoxycholic acid and a decreased level of alanine, lysophosphatidylcholine (16:1), valine and 2-octenedioic acid.<sup>38</sup> Further, ER-positive subtype revealed an elevated level of glutamine biosynthesis and glutamine secretion compared with those of the ER-negative subtype, and the ER-negative subtype showed remarkably higher serine metabolism and glutamine utilization than those of the ER-positive subtype.<sup>39</sup> In addition, higher beta-alanine accumulation can be found in ER-negative BC, while 4-aminobutyrate aminotransferase expression was decreased in the ER-positive subtype.<sup>40</sup> In addition, another group

reported that the luminal B subgroup preferentially used fatty acids as an energy substrate, while HER-2 and basal-like subgroups revealed obvious alterations in glucose or glutamine metabolism.<sup>41</sup> The epithelial mesenchymal transition (EMT) is an important phenotype that indicates a more aggressive behaviour of cancer cells.<sup>42</sup> compared the less aggressive breast cancer cell line MCF-7 with the most aggressive breast cancer cell line MDA-MB-231 by using MALDI-TOF/TOF mass spectrometry and NMR and found differences in the lipidomic profiles between these cells. MDA-MB-231 presented lower de novo lipid synthesis and higher triacylglycerol (TAG) metabolism than did MCF7 cells, which could be a signature to differentiate basal-like breast cancer from luminal breast cancer.<sup>42</sup> Determining the degree of malignancy based on metabolite patterns may provide clinicians with more accurate personalized treatments (Table 1).

### Reclassification of BC based on metabolomics

The clinical response of treatment in BC can vary, even in the same subtype, owing to the cancer heterogeneity; thus, a more specific classification is needed.<sup>41</sup> A study introduced stable isotopic <sup>13</sup>C substrates to track the metabolism pathway of glucose, nucleotides and amino acids in BT474 and MCF-7 cell lines and MDA-MB-468 and MDA-MB-231 cell lines. The metabolites showed significant differences in the same subtype and similarity across the subtypes. Cell lines with the same hormone receptor status have different energy utilization patterns.<sup>43</sup> Borgan et al analysed tissue samples combining gene expression microarrays and high-resolution magic angle spinning magnetic resonance spectroscopy (HR MAS MRS). These authors found that luminal A can be separated into three subgroups, A1-A3. The results also showed significant differences in glucose signals, a-H amino acid signals, alanine signals, lipid residue signals, and signals from myo-inositol among these three subtypes. Additionally, these subgroups showed great differences among biological processes and molecular functions by a GO terms enrichment analysis.<sup>44</sup> BC molecular subtypes classified by metabolomics can help make personalized treatment decisions. In another study, the Luminal A subtype was classified into three distinct metabolic clusters (1–3) based on their metabolite patterns, which were mainly different in phospholipid metabolism, glycolytic activity and glutaminolysis. Different levels of metabolites in these sub-clusters were associated with different tumour

Table 1 Molecular subtypes of BC based on metabolomics

| Category                       | Biomarker  | Sample                   | Alteration               | Ref            |
|--------------------------------|--|--------------------------|--------------------------|----------------|
| Subtype                        |  |                          |                          |                |
| HER2-positive<br>HER2-negative | Carnitine, lysophosphatidylcholine (20:4), proline, valine, and 2-octenedioic acid   | plasma                   | up<br>-                  | 38             |
| ER-positive                    | Glycochenodeoxycholic acid<br>Alanine, lysophosphatidylcholine (16:1), valine and 2-octenedioic acid.<br>Glutamine biosynthesis and glutamine secretion<br>4-aminobutyrate aminotransferase expression | plasma<br>cell<br>tissue | up<br>down<br>up<br>down | 38<br>39<br>40 |
| ER-negative                    | Serine metabolism and glutamine utilization<br>Beta-alanine  | cell<br>Tissue           | up<br>Up                 | 39<br>40       |
| Triple-negative                | De novo lipid synthesis<br>Metabolism of triacylglycerols (TAGs)   | Cell                     | Down<br>Up               | 42             |
| Reclassification               |  |                          |                          |                |
| Breast cancer cells            |  | Cell                     |                          | 43             |
| MDA-MB-468 and MCF7            | <sup>13</sup> C-aspartate production from [ <sup>13</sup> C]-glucose or [ <sup>13</sup> C]-glutamine lactate (media)   |                          | Low<br>High              |                |
| BT474 and MDA-MB-231           | Lactate (media)<br><sup>13</sup> C-aspartate production from [ <sup>13</sup> C]-glucose or [ <sup>13</sup> C]-glutamine  |                          | Low<br>High              |                |
| BT474 and MDA-MB-468           | <sup>13</sup> C-glycine production from media [ <sup>13</sup> C]-glucose   |                          | High                     |                |
| MCF-7 and MDA-MB-231           | <sup>13</sup> C-glycine production from media [ <sup>13</sup> C]-glucose   |                          | Low                      |                |
| Luminal A                      |  | Tissue                   |                          | 44             |
| A1                             | Lipid residues<br>a-H amino acid and myo-inositol  |                          | High<br>Low              |                |
| A2                             | Glucose<br>alanine and lactate   |                          | Low<br>High              |                |
| A3                             | a-H amino acid   |                          | High                     |                |

(Continued)

Table 1 (Continued).

| Category  | Biomarker   | Sample | Alteration  | Ref |
|-----------|---|--------|-------------|-----|
| Luminal A |   | Tissue |             | 45  |
| Cluster 1 | Glycerophosphocholine, phosphocholine and lactate glucose |        | High<br>Low |     |
| Cluster 2 | Glucose lactate and alanine                               |        | High<br>Low |     |
| Cluster 3 | Lactate and alanine glucose                               |        | High<br>Low |     |

Abbreviations: HER2, human epidermal growth factor receptor 2; ER, estrogen receptor.

biological behaviours and the prognosis of BC.<sup>45</sup> Reclassifying the molecular subtypes based on metabolite patterns may facilitate clinicians to make better clinical decisions (Table 1).

## The application of metabolomics in the clinical practice of BC

### Metabolomics serves as a risk filter for BC

The metabolite levels may serve as an indicator for people who were likely candidates for cancer to take prevention measures.<sup>46</sup> The results showed that women who have higher levels of valine, lysine, arginine, glutamine, creatine, creatinine and glucose or lower levels of lipoproteins, lipids, glycoproteins, acetone, glycerol-derived compounds and unsaturated lipids were at higher risk of developing BC.<sup>47</sup> Body weight has previously been regarded as an independent risk factor for postmenopausal BC. A previous study reported that weight loss and energy restriction reduce BC risk.<sup>8</sup> The relationship of body weight and BC was revealed by metabolites. The results showed that postmenopausal BMI is associated with increased circulating levels of parent oestrogens and the decreased methylation of catechol oestrogen metabolites, which were correlated with higher BC risk. This finding illustrated that endogenous oestrogens/oestrogen metabolites probably serve as indicators to evaluate the BMI-associated risk of BC.<sup>48</sup> Another group found that 16 $\alpha$ -hydroxy-DHEA-3-sulfate and 3-methylglutaryl-carnitine (BMI-associated risk metabolites) were independently associated with invasive BC.<sup>49</sup> Diet was another important factor that can influence the incidence of various cancers, including BC. Greek-Cypriot women have a decreased risk of BC because of their Mediterranean dietary habit.<sup>31</sup> Playdon et al explored the circulating diet-related metabolites related to BC and found that the concentration of metabolites was associated with alcohol. Simultaneously, these authors found that animal fats associated with ER-positive BC and metabolites related to vitamin E can be protective factors.<sup>50</sup> This finding offered a potential pre-diagnostic method of diet-related exposure that contributed to BC risk.

### Metabolomics serves as a diagnostic detector of BC

The change in metabolites could be detected in an earlier stage, even if the abnormality was subtle. Even a subtle abnormality in the body can be increased through transcriptional, translational and molecular interactions, and



metabolites are the terminal products of biochemical reactions throughout the body. Therefore, metabolomics can be a promising early biomarker of BC.<sup>51</sup>

### Blood-derived sample

A dried blood spot-based direct infusion MS metabolomic analysis was used to find a quick way to diagnose BC, which targets 23 amino acids and 26 acylcarnitines. Twenty-one BC-related metabolites were differently changed in the blood, and a diagnostic panel was generated (piperamide, asparagine, proline, tetradecenoylcarnitine/palmitoylcarnitine, phenylalanine/tyrosine, and glycine/alanine). This panel showed a sensitivity of 92.2% and a specificity of 84.4% when used to test another set of BC and non-BC samples.<sup>52</sup> The level of amino acid metabolites was also altered in the blood.<sup>53</sup> In addition, the plasma samples of primary BC patients show lower levels of many amino acids.<sup>54</sup> The serum amino acid and organic acid profiles of BC patients, benign patients and controls also showed significant differences. The most meaningful finding is the elevated levels of taurine and glutamic acid and the decreased levels of taurine and glutamic acid between BC patients and controls.<sup>55</sup> Tryptophan exhibited a significantly higher level in the serum samples of malignant tumours than in those of benign tumours.<sup>56</sup> A differential analysis of the metabolomics profiles among invasive ductal carcinoma (IDC) patients in control and benign groups revealed that the combination of tryptophan, tyrosine, and creatine may serve as a convenient filter for both control and benign IDC subjects.<sup>57</sup> Another group reported the 9-cisRA showed a prominent decrease from normal controls with localized BC to metastatic BC based on the analysis of 46 clinical serum and urine samples.<sup>58</sup> Oestrogen was regarded as a dual effector of BC.<sup>59</sup> Lysophosphatidylcholines and phosphatidylcholines were found to be significantly elevated in the oestrogen-induced group after comparing the serum metabolites profiles of an oestrogen-induced BC model with those of the control group through ultra-high performance liquid chromatography (UHPLC)-time-of-flight tandem mass spectrometer (QTOF-MS/MS).<sup>60</sup> In summary, the metabolite patterns in blood might be a promising method to differentiate BC patients from putative healthy individuals.

### Urine-derived sample

A group analysed the urine samples of healthy controls and BC patients among Hispanic women based on LC-MS or GC-MS and found that dimethylheptanoylcarnitine and

succinic acid were significantly changed. Then, the combination of these two metabolites was evaluated through receiver operating characteristic analysis with a sensitivity of 93% and a specificity of 86%.<sup>61</sup> Nam et al combined tissue transcriptomics and urine metabolomics and selected nine metabolic pathways based on the breast cancer gene expression signatures. Finally, four metabolic biomarkers (homovanillate, 4-hydroxyphenylacetate, 5-hydroxyindoleacetate and urea) were found to be changed between normal and abnormal samples.<sup>62</sup> These studies demonstrated that the analysis of the metabolites in urine was a promising biomarker at least for an early diagnosis of BC.

### Salivary-derived sample

Saliva is a convenient and noninvasive sample. Salivary metabolite analysis and identification specific to BC showed cancer-specific changes in salivary metabolites but still need further confirmation.<sup>63</sup> Another group, lysophosphatidylcholine (18:1), lysophosphatidylcholine (22:6) and monoacylglycerol (0:0/14:0/0:0), was upregulated by analysing saliva samples from BC patients and healthy controls, and their AUC values were 0.920, 0.920 and 0.929, respectively.<sup>64</sup> Takayama et al established a diagnostic method to detect BC patients based on the ratios of polyamines and their acetylated forms in saliva by a UPLC-MS/MS analysis. In this research, eight polyamines or their acetylated forms (cadaverine, spermine, spermidine, N1-acetyl-spermine, N1-acetyl-spermidine and N8-acetyl-spermidine) were illustrated to be strongly correlated with cancer patients.<sup>65</sup> In short, salivary samples can also distinguish BC patients from healthy individuals by metabolite analysis.

### Other available samples

Nipple aspirate fluids (NAF) can reveal the total ductal/alveolar breast tree condition.<sup>66</sup> A previous group introduced an untargeted metabolomics analysis of breast ductal fluid from both affected breasts and unaffected unilateral breast cancer based on ultra-performance liquid chromatography coupled with a quadrupole time-of-flight (UPLC-QTOF) mass spectrometer. The LASSO regression selected 21 metabolites after adjusting for race, menopausal status, smoking, grade and TNM stage, and this panel had an AUC of 0.956.<sup>67</sup> Breath volatile organic compounds can also be a potential sample for the early diagnosis of breast cancer.<sup>68</sup> The metabolites, including 2-pentanone, 2-heptanone, 3-methyl-3-buten-1-ol, ethyl

acetate, ethyl propanoate and 2-methyl butanoate, can only be detected in BC cell lines by analysing volatile organic compounds from the headspace of BC cells and normal human mammary epithelial cells.<sup>69</sup> Triple negative breast cancer (TNBC) has the same histological characteristics as germline BRCA1-associated tumours, while widely known mutations in BRCA-associated tumours. Metabolomics analysis performed on triple-negative hereditary breast cancer patients with BRCA1 mutations showed lower levels of adenine, N6-methyladenosine and 1-methyladenosine than did those without BRCA1 mutations. Therefore, these metabolites may be potential BC biomarkers associated with BRCA1 mutations.<sup>70</sup>

### Metabolomics serves as a treatment evaluator and monitor of BC

The pharmacokinetics and pharmacodynamics revealed by metabolomics can act as guides to evaluate the efficiency and side effect of BC treatment.<sup>71</sup> Ewens et al used a mouse model to examine the efficacy of doxorubicin plus interleukin-2 chemioimmunotherapy against BC and then analysed serum samples by NMR. The results showed that mice that responded to the treatment took on a unique NMR spectral profile with the high expression of chemicals.<sup>72</sup> This finding indicated that metabolomics may predict the patients response to treatment to determine the best prescription for individuals. Another group reported that the polymer-conjugated form of doxorubicin showed a more prominent increase in apoptosis, less glycolysis and a decreased level of phospholipids via evaluating the biochemical alterations induced by doxorubicin and its N-(2-hydroxypropyl) methacrylamide copolymer-conjugated form.<sup>73</sup> Another group analysed the metabolomics profile of trastuzumab-paclitaxel neo-adjuvant therapy and identified a good response in HER-2 positive BC and further revealed a higher level of circulating spermidine and a lower level of tryptophan in these patients.<sup>74</sup> Another group identified 24 biomarkers and found the pathways of protein and cysteine synthesis, glutamine metabolism and the ammonia cycle were related to drug resistance by using MCF-7/ADR and its parental MCF-7/S cells with ultra-performance liquid chromatography-tandem mass spectrometry.<sup>75</sup> Moreover, the ratios of GSH/GSSG, ATP/ADP and ATP/AMP were prominently higher in MCF-7/ADR cells compared to MCF-7/S cells.<sup>76</sup> Statins are considered to have antitumour potential in recent years.<sup>77</sup> The proteomics showed that the lovastatin

regulates specific proteins related to cell proliferation, and lovastatin exposure referred to the induction of oxidative stress and the suppression of glycolytic, Krebs cycle or lipid biosynthesis.<sup>78</sup> In addition, the possibilities of early recurrence may also be revealed by metabolite profiles.<sup>79</sup> Baseline lactate, alanine, and body fat were all correlated with weight gain, which is a risk factor related to recurrence in patients receiving chemotherapy. In addition, the patients who respond to chemotherapy can also be revealed by the lactate level.<sup>80</sup>

Radiotherapy is also an indispensable part of BC treatment. The serum of 115 female patients who received loco-regional radiotherapy (RT) post-surgery showed a lower concentration of glycolysis, citric acid cycle and amino acid metabolism, especially serine, leucine and isoleucine, before radiotherapy and after surgery; however, this phenomenon was reversed completely or partially after radiotherapy. These changes in metabolites indicated that residual micro-metastatic diseases had been cleared by radiotherapy and thus be an evaluator of its efficacy. However, it is uncertain whether the delay in the post-surgery effect or the concomitant treatment with chemotherapy or hormone therapy can influence the reverse in the concentration of metabolites.<sup>81</sup>

As one of the most famous anti-neoplastic agents, cisplatin has been widely used for treating tumours.<sup>82</sup> The cysteine-cysteine, 3-hydroxy-butyrate based on GC/MS analysis and 3-acylcarnitines and phosphatidylethanolamine with C18:2-C18:2 based on LC/MS analysis were significantly different in the early stage of cisplatin-induced nephrotoxicity, and these metabolites can be detected earlier than the traditional biomarkers of acute kidney injury, such as BUN and creatinine.<sup>83</sup> Psychoneurologic (PN) symptom means that patients undergoing chemotherapy may experience pain, fatigue and depression. A previous group analysed changes in the global and targeted metabolomics of the tryptophan pathway before and after chemotherapy compared with the changes in PN symptoms pre-chemotherapy and post-chemotherapy in 19 early-stage BC samples. Global metabolites showed higher concentrations of acetyl-l-alanine and indoxyl sulfate and lower levels of 5-oxo-l-proline post-chemotherapy. Moreover, the targeted analysis revealed higher kynurenine levels and kynurenine/tryptophan ratios.<sup>84</sup> Another group found that pre-treatment concentrations of histidine, phenylalanine, and threonine may be the candidate biomarkers of the severity of PN after chemotherapy.<sup>85</sup> Metabolomics can be a complementary

approach that offers information on the drug responses and side effects throughout the body.<sup>86</sup>

## Metabolomics serves as a prognostic predictor of BC

The glycolysis process in malignant tumour cells is still active despite sufficient oxygen. The phenomenon that cancer cells incline to aerobic glycolysis rather than the more efficient oxidative phosphorylation pathway called the “Warburg effect”.<sup>87</sup> An elevated level of ketones and lactates can accelerate cancer growth and metastasis, which may be promising biomarkers to predict poor clinical outcomes in BC patients.<sup>88</sup> Neu5Ac (N-acetylneuraminic acid) was found to be the most obvious different glucose metabolite in TNBC cell lines— malignant (BPLER) and less malignant (HMLER). Further exploration showed that the overexpression of CMAS in HMLER promoted the invasive ability of the cells, and the knock-down of CMAS in BPLER prominently destroyed the invasiveness of the cells.<sup>89</sup>

Overweight is a risk factor for BC recurrence.<sup>90</sup> Notably, 27-hydroxycholesterol, an oxysterol metabolite of cholesterol, was supposed to contribute to the initiation of distal metastasis.<sup>91</sup> In highly invasive cell lines (MDA-MB-231; BT-549), membrane phospholipids, including phosphatidylglycerol (PG), phosphatidic acid (PA), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylcholine (PC) and phosphatidylinositol (PI), were supposed to be the most increased metabolites, while sphingomyelin (SM), PE (O-18:0), fatty acids (FA), glycerophosphoglycerophosphoglycerols (CL) and three unknown lipids were identified as the most decreased metabolites. The high level of PG, which can affect the invasive ability, was supposed to correlate with mitochondrial dysfunction, and the low level of SM may be associated with decreased ceramide, which may affect the apoptosis process.<sup>92</sup> The metabolic and lipidomic profiling of MCF-10A, MCF-7 and MDA-MB-231 BC cell lines showed a panel comprising xanthine, glucose-6-phosphate, mannose-6-phosphate, guanine, and adenine, which may be a prognostic marker for BC metastasis, and lipidomic profiling further revealed that the phospholipid levels of metastatic groups were higher than those in the normal cell.<sup>93</sup> A pilot study showed that the serum samples from 20 invasive ductal carcinoma patients and healthy controls differentially expressed 15 metabolites, mainly concentrating on the sphingolipid metabolism pathway, phospholipid metabolism pathway and fatty acid  $\beta$ -oxidation pathway.<sup>94</sup>

The common metastatic sites of BC include the lungs, brain, bone and liver.<sup>95</sup> A previous group analysed plasma along with pulmonary metastasis progression by inoculating 4T1 metastatic BC cells in mice by LC-MS/MS-based targeted metabolomics and lipidomics and found that alterations in the arginase (ARG) pathway could imply early stage cancer metastasis and be a possible target for therapy in the future.<sup>96</sup> Alterations in glycolysis and the signalling and structure of lipids could be alternative biomarkers for aggressive cancer and late phase metastasis.<sup>96</sup> A research group found that creatinine and allantoin decreased significantly and trimethylamine, trimethylamine-N-oxide, creatine +phosphocreatine and taurine were all more abundant in the early phase when analysing urine metabolites of BALB/c mice by (1)H NMR spectroscopy.<sup>97</sup> In addition, an elevation in pro-metastatic arachidonic acid and a reduction in anti-metastatic lysophosphatidylcholines can be identified during bone metastasis.<sup>98</sup>

The survival outcome can be evaluated by metabolomics. Metabolites, including glucose, glycine, serine and acetate, are significantly associated with patient survival.<sup>99</sup> Low concentrations of Z-4OHam and Z-endoxifen refer to the poor survival outcome of patients.<sup>100</sup> Thus, the outcomes of the curative effects of patients may be evaluated directly through metabolomics.

## Conclusions and future expectations

Metabolism alteration in tumours are mainly focused on glucose, lipid, amino acids, glutamine,<sup>41</sup> purine and pyrimidine.<sup>70</sup> Glucose metabolism in BC is usually refers to the “Warburg effect”, which illustrates that cancer glucose utilization, even with adequate oxygen, would incline to the glycolysis.<sup>87</sup> Lipid metabolism as an energy source is connected to membrane fluidity and the adhesion of tumour cells, which is related to the metastasis ability of tumour cells.<sup>91,98</sup> Elevations in amino acid, purine and pyrimidine metabolism are mainly caused by the high requirement of substrates for the biosynthesis of BC cells, indicating the active proliferation of cancer cells.<sup>93</sup> Glutamine metabolism alterations in several cancers has garnered attention because it provided carbon and nitrogen sources for cancer cell and ATP biosynthesis. In short, glutamine not only acts as a biosynthesis substrate promoting cancer growth but also offers energy resources.<sup>101–103</sup> Recent metabolomics research is summarized in Table 2. We found that studies that refer to the same metabolism pathway do not exactly show the same results, which may be attributed to the different choices of technologies, samples and data



**Table 2** The application of metabolomics in the risk factors, diagnosis, treatment and prognosis of breast cancer

| Application | Biomarker  | Sample           | Pathway  | Alteration   | Ref |
|-------------|--|------------------|--|--|-----|
| Risk factor | Dehydroepiandrosterone sulfate; other androgens, estrogens, ethyl-glucuronide; a-hydroxyisovalerate, caprate (10:0), a medium-chain SFA, 10-undecenoate (11:n-1), an odd-carbon MUFA, 2-hydroxyoctanoate, a hydroxy fatty acid; g-CEHC; Vitamin E (a-tocopherol was inversely associated, whereas d-tocopherol and g-CEHC) | Serum            | Androgen pathway, estrogen pathway.  | Up   | 50  |
|             | Valine, lysine, arginine, glutamine, creatine, creatinine, glucose lipoproteins, lipids, glycoproteins, acetone, glycerol-derived compounds, unsaturated lipids  | Plasma           | Amino acids metabolism, glucose metabolism, glutamine metabolism, lipid metabolism   | Up<br>Down   | 47  |
|             | Methylation of catechol estrogen Parent estrogens  | Serum            | Estrogen metabolism  | Down<br>Up   | 48  |
|             | 16a-hydroxy-DHEA-3-sulfate, 3-methylglutaryl carnitine, allo-isoleucine, 2-methylbutyryl carnitine   | Serum            | Steroid hormone metabolism, branched chain amino acid metabolism                     | Up   | 49  |
|             | Lysophosphatidylcholines, phosphatidylcholines   | Serum            | Phospholipid metabolism  | Up   | 60  |
|             | Tryptophan<br>Tyrosine<br>Creatine   | Serum and tissue | Amino acids metabolism   | Down (serum)<br>Down (serum); up (tissue)<br>Down (serum); up (tissue) | 57  |
| Diagnosis   | Adenine, N6-methyladenosine, 1-methyladenosine   | Plasma           | Purine metabolism, nucleotide metabolism   | Down (BRAC1 mutated)   | 70  |
|             | Lysophosphatidylcholine (18:1), lysophosphatidylcholine (22:6), monoacylglycerol   | Saliva           | Phospholipid catabolism, glycerolipids catabolism                                    | Up   | 64  |
|             | Glutamine  | Cell             | Glutamine metabolism   | Down   | 105 |
|             | Homovanillic acid, 4-hydroxyphenylacetate, 5-hydroxyindoleacetate<br>Urea  | Urine            | Tyrosine metabolism, tryptophan metabolism, pyrimidine metabolism, purine metabolism | Down<br>Up   | 62  |
|             |  |                  |  |  |     |

(Continued)

Table 2 (Continued).

| Application | Biomarker  | Sample      | Pathway   | Alteration       | Ref |  |
|-------------|--|-------------|---|------------------|-----|--|
| Treatment   | 3-hydroxy-butyrate<br>AC 14:0, AC 18:1, and AC 18:2<br>Cysteine—cystine<br>Phosphatidylethanolamine 18:2 | Plasma      | Lipid metabolism  | Up<br>Down       | 83  |  |
|             |  | Serum       | Energy metabolism, amino acid metabolism<br>Tryptophan/kynurenine pathways      | Up<br>Down<br>Up | 84  |  |
|             |  | Whole blood | Amino acids metabolism  | Down             | 85  |  |
|             |  | Cell        | Glutathione biosynthesis  | Down             | 106 |  |
|             |  | Serum       | Amino acids metabolism  | Up               | 81  |  |
|             |  | Cell        | Glutamine metabolism, glucose metabolism (glycolysis/oxidative phosphorylation) | Up               | 76  |  |
|             |  | Serum       | Spermidine and tryptophan metabolic pathways                                    | Up<br>Down       | 74  |  |
|             |  |             |   |                  |     |  |
|             |  |             |   |                  |     |  |
|             |  |             |   |                  |     |  |

(Continued)

Table 2 (Continued).

| Application              | Biomarker   | Sample  | Pathway  | Alteration | Ref |
|--------------------------|---|---|--|------------|-----|
| Prognosis and metastasis | Xanthine, glucose-6-phosphate, mannose-6-phosphate  | Cell  | Purine metabolism, glucose metabolism, Purine metabolism           | Down       | 93  |
|                          | Guanine, adenine  |   |  | Up         |     |
|                          | 9cisRA  | Serum, urine                                    | Retinol metabolism   | Down       | 58  |
|                          | Cytidine monophosphate  | Cell  | Glucose metabolism (sialic acid metabolism)                        | Down       | 107 |
|                          | Cytidine monophosphate-sialic acid, sialic acid   |   |  | Up         |     |
|                          | Putrescine, asymmetric dimethylarginine, total phosphatidylcholines, diester-bound phospholipid fraction, lysophospholipids | Plasma  | Arginine, energy metabolism, lipid metabolism                      | Up         | 96  |
|                          | Prometastatic arachidonic acid  | Serum   | Lipid metabolism   | Up         | 98  |
|                          | Antimetastatic lysophosphatidylcholines   |   |  | Down       |     |
|                          | 27-hydroxycholesterol   | Plasma  | Lipid metabolism   | Up         | 91  |
|                          | Creatinine and allantoin  | Urine   | Uric acid metabolism, creatine metabolism, phospholipid metabolism | Down       | 97  |
|                          | Trimethylamine, trimethylamine-N-oxide, creatine+phosphocreatine and taurine  |   |  | Up         |     |
|                          | Glucose, glycine, serine, acetate   | Tissue  | Glucose metabolism, amino acids metabolism                         | Down       | 99  |
|                          | Z-4-hydroxy-tamoxifen, Z-endoxifen  | Serum   | Tamoxifen metabolism   | Down       | 100 |
| Phenylalanine, glutamate | Serum   | Phenylalanine metabolism, glutamine metabolism, | Up   | 79         |     |
| Ketones and lactates     | Cell  | Glucose metabolism                              | Up   | 88         |     |
| N-acetylneuraminic acid  | Cell  | Glucose metabolism (sialic acid metabolism)     | Up   | 89         |     |

**Abbreviations:** BC, breast cancer; MS, mass spectrometry; GC, gas chromatography; LC, liquid chromatography; CE, capillary electrophoresis; NMR, nuclear magnetic resonance; UPLC, ultra performance liquid chromatography; PCA, principal component analysis; HCA, hierarchical cluster analysis; PLS, partial least squares; SIMCA, soft independent modeling of class analogy; PLS-DA, partial least squares discriminant analysis; OPLS-DA, orthogonal PLS-DA; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2; BMI, body mass index; IDC, invasive ductal carcinoma; LBC, localized breast cancers; MT, metastatic breast cancers; NC, normal controls; QTOF-MS/MS, liquid chromatography (UHPLC)-time-of-flight tandem mass spectrometer; LPCs, lysophosphatidylcholines; PCs, phosphatidylcholines; NAF, nipple aspirate fluids; UPLC-QTOF, ultra-performance liquid chromatography coupled with a quadrupole time-of-flight; TNBC, triple negative breast cancer; LESA-MS, liquid extraction surface analysis with mass spectrometry; MDR, multidrug resistance; RT, radiotherapy; PN, psychoneurologic; Neu5Ac, N-acetylneuraminic acid; CMAS, N-acetylneuraminic acid synthetase; CMP, cytidine monophosphate; HFD, high-fat diet; 27HC, 27-hydroxycholesterol; MALDI-MSI, matrix-assisted laser desorption/ionization mass spectrometry imaging; GC-MS, chromatography mass spectrometry; DI-MS, direct infusion mass spectrometry; Z-4OHtam, Z-4-hydroxy-tamoxifen; GC/MS, gas chromatography mass spectrometry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; ARG, arginase; PG, phosphatidylglycerol; PA, phosphatidic acid; PE, phosphatidylethanolamine; PS, phosphatidylserine; PC, phosphatidylcholine; PI, phosphatidylinositol; SM, sphingomyelin; FA, fatty acids; CL, glycerophosphoglycerophosphoglycerols; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; CEHC, carboxyethyl hydrochroman; DHEA, dehydroepiandrosterone; AC, acylcarnitines; GSH, glutathione; GSSG, oxidized glutathione; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; 9cisRA, 9-cis-retinoic acid.

processing methods. The requirement of analysing metabolomics is relatively high due to short times and unbiased small metabolite analyses, and data processing and pattern recognition are still immature. Another marked challenge of metabolomics is the heterogeneity of the tumour. Despite these challenges, metabolic pathways may be potential targets for drug therapy, and metabolomics in cancer have provided new insights to diagnose and treat patients. Metabolomics makes the further classification of breast cancer possible so that patients can receive more specific personal treatment options. The different metabolites in cancer have been revealed as biomarkers for the diagnosis, treatment and monitoring of BC.<sup>104</sup> Therefore, the application of metabolomics in BC is promising, despite the temporary limitations.

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## Author contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

## Disclosure

The authors report no conflicts of interest in this work.

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