Serum amino acids quantification by plasmonic colloidosome-coupled MALDI-TOF MS for triple-negative breast cancer diagnosis

Xinyue Han, Dandan Li, Shurong Wang, Yuxiang Lin, Yun Liu, Ling Lin, Liang Qiao

ARTICLE INFO

Keywords:
- Plasmonic colloidosome
- MALDI-TOF MS
- Triple-negative breast cancer
- Amino acid quantification
- Liquid biopsy

ABSTRACT

Triple-negative breast cancer (TNBC) is characterized with high diffusion, metastasis and recurrence. The early treatment and early diagnosis of TNBC are highly important for the survival of TNBC patients. Nevertheless, traditional methods for TNBC diagnosis, i.e. imaging examinations and tissue biopsy, cannot achieve early diagnosis, are invasive and associated with the risk of arousing tumor spreading. Herein, we developed colloidosomes-coupled matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to quantify free serum amino acids for the diagnosis of TNBC. Gold nanoparticles (AuNPs) were used to form water-in-oil colloidosomes and to encapsulate deproteinated serum for MALDI-TOF MS analysis. With small sample spot size (200–300 μm) and densely packed AuNPs monolayer, the dried sample spot from colloidosomes can facilitate MALDI-TOF MS analysis of small molecule metabolites with high sensitivity, high reproducibility and good quantification performance. We used the method to quantify free amino acids in human serum. A cohort of 30 TNBC patients, 30 breast lump patients and 30 healthy controls were recruited for the study. It was found that the concentrations of free amino acids in TNBC patients were significantly lower than that of healthy controls, and the concentrations were also significantly different from that of breast lump patients. Based on the quantities of serum free amino acids, we have built a machine learning-based classification model to differentiate TNBC patients from the controls, including healthy controls and breast lump patients, and the sensitivity, specificity and accuracy were 95%, 100% and 97%, respectively. The assay consumes less than 1 μL serum per analysis, and takes only minutes to analyze a sample. With the advantages of low cost, low sample consumption, high throughput in analysis and high accuracy in identification, the non-invasive liquid biopsy method is promising to be applied to clinical diagnosis of TNBC.

1. Introduction

Breast cancer is the most frequent malignancy affecting women’s health worldwide. In females from Easten Asia, breast cancer is the most common cancer, accounting for 43.3% of all new female cancer cases [1]. Triple-negative breast cancer (TNBC) accounts for about 10.0%–20.4% of all breast cancers and occurs more frequently in younger women [2]. Compared with other subtypes of breast cancer, TNBC with estrogen receptor (ER), human epidermal growth factor receptor 2 (HER2) and progesterone receptor (PR) negative is characterized with high heterogeneity, poor prognosis and lack of precise therapeutic targets. TNBC is highly invasive and is likely to metastasize to organs such as lung, liver and bone, which brings difficulties to tumor treatment and leads to low overall survival rates [3]. The clinical therapy strategies against TNBC include surgery and chemotherapy, which are only effective in the early stage [4]. Therefore, the early diagnosis and treatment of TNBC is of urgent need for improving the survival rate of patients.

To date, TNBC is usually diagnosed by imaging examinations and tissue biopsy, which cannot achieve early diagnosis, are invasive and associated with the risk of arousing tumor spreading [5,6]. Liquid biopsy based on tumor markers is an emerging technique with the advantages of non-invasive, simple operation, low cost and high throughput in analysis. Recent studies have shown that many cancers are characterized with metabolic disorders, and amino acids (AAs) played an important role in the metabolism of organisms and in the construction of proteins, indicating the significance of amino acids analysis in disease diagnosis [7].

* Corresponding author.
** Corresponding author.

E-mail addresses: linglin@fudan.edu.cn (L. Lin), liang_qiao@fudan.edu.cn (L. Qiao).
Regulation of plasma branched-chain AAs was reported to be associated with an increased risk of pancreatic cancer [8], and the detection of small metabolites, including AAs, in body fluids has attracted significant concerns for the diagnosis of TNBC [9,10]. Brantley et al. has used liquid chromatography tandem mass spectrometry (LC-MS/MS) for the identification of 307 metabolites including phenylalanine, tyrosine and tryptophan in plasma, wherein the correlation of amino acid regulation with breast cancer development including TNBC was demonstrated [11]. The LC-MS/MS based on triple quadrupole mass spectrometer is considered as the gold standard for amino acids quantification, which however needs time-consuming sample pretreatment, complicated operations, long analysis time per sample and is at high cost, limiting its wide application in clinical diagnosis.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has aroused attention for its application in clinical diagnosis with the unique advantages of convenient operation and high throughput in sample analysis. It has been used in clinical labs to identify pathogenic bacteria [12] and detect single nucleotide polymorphisms [13]. During the last years, MALDI-TOF MS based metabolic profiling from body fluids has attracted significant interests for various diseases diagnosis [14,15]. However, there are two main limitations of the traditional MALDI-TOF MS for metabolic profiling. First, the traditional organic matrices, such as alpha-cyano-4-hydroxycinnamic acid (CHCA) and 2,5-dihydroxybenzoic acid (DHB), show strong peaks by themselves in the low molecular weight (LMW) region (<500 Da), interfering the detection of metabolites [16]. Second, due to the ‘sweet spot’ effect, the traditional MALDI process constitutes a stochastic system, which however needs time-consuming sample pretreatment, complicated operations, low analysis time per sample and is at high cost, limiting its wide application in clinical diagnosis.

Inorganic nanomaterials-assisted laser desorption ionization has been developed to overcome the limitations of MALDI-TOF MS in the analysis of small molecule metabolites. Nanomaterials with rough surface and small pore size can efficiently enhance the desorption and ionization of metabolites and eliminate background interferences from matrix [18,19]. In addition, changing the elemental composition can result in significant difference in the matrix phase transition and the interaction between the analyte and the matrix under laser irradiation for sensitive metabolites detection [20,21]. Compared to organic matrix, inorganic nanomaterials also can facilitate the uniform distribution of analytes, and hence reducing the ‘sweet spot’ effect. In 2020, we developed a plasmogic gold nanoparticles (AuNPs) colloidosomes coupled MALDI-TOF MS to detect the metabolism of ampicillin by drug resistant bacteria β-lactamases producing Escherichia coli, wherein good quantification performance was obtained [22].

Herein, we proposed self-assembled colloidosomes formed by modified gold nanoparticles (AuNPs) to assist serum free AA detection for TNBC diagnosis (Scheme 1). The modification of 1H,1H,2H,2H-perfluorodecanoelel (PFDT) with rich sulphhydril groups made AuNPs lipophilic and hydrophobic, so that colloidosome could be easily formed when analytes in aqueous phase was added to the PFDT-AuNPs in oil phase with shaking. The colloidosomes with diameter of few hundred microns encapsulating analytes were then deposited on a steel target plate for MALDI-TOF MS analysis. We used the colloidosomes coupled MALDI-TOF MS to quantify 10 free amino acids in human serum using stable isotope labelled amino acids as internal standards for calibration. Ninety clinical sera samples, including 30 for TNBC patients, 30 for breast lump patients, and 30 for healthy controls, were analyzed by the method, and it was demonstrated that TNBC patients can be differentiated from the controls, including healthy controls and breast lump patients, based on the absolute serum amino acids level, and a significant decrease of free amino acids level in serum in the TNBC patients and breast lump patients compared to healthy controls was observed. Considering the facility and low cost of the method as well as the high accuracy in TNBC identification, it is promising to be applied for clinical diagnosis of TNBC patients.

2. Experimental section

2.1. Chemicals and instrumentation

Trifluoroacetic acid (TFA, 99%), α-cyano-4-hydroxycinnamic acid (CHCA, 98%), cyclohexane (99.5%), t-serine (99%), t-aspartic acid (98%), cell free amino acid mixture,15N (98%, 767972-1 EA) and 1H,1H,2H,2H-perfluorodecanoelel (PFDT, 97%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HAuCl4⋅4H2O (99.9%), acetonitrile (99.9%), ethanol (99.7%) and methanol (99.8%) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). tri-arginine (99%), l-valine (99%) and sodium citrate tribasic dihydrate (99%) were purchased from J&K Scientific (Beijing, China). Novec™ 7500 Engineered Fluid (HFE-7500) was purchased from Minnesota Mining and Manufacturing Corporation (Maplewood, MN, USA). Deionized (DI) water (18.2 MΩ cm) used in all experiments was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). SEM images were collected on a

![Scheme 1. Schematic illustration of self-assembled plasmogic colloidosomes coupled MALDI-TOF MS quantification of human serum amino acids for TNBC diagnosis.](image-url)
Zeiss Gemini 300 scanning electronic microscopy (Carl Zeiss AG, Oberkochen, Germany). MALDI-TOF MS analysis was performed with a Bruker MicroFlex LRF mass spectrometer (Bruker, Bremen, Germany). Optical microscopic image was obtained with inverted microscope (Cewe Optoelectronic Technology Ltd, Shanghai, China).

2.2. Clinical sample collection and ethical statement

All of the serum samples used in this study were obtained from Fujian Medical University Union Hospital, with the approval of the Ethics Committee of Fujian Medical University Union Hospital (Fujian, China). Written informed consent was provided by all participants.

Cases were randomly selected from 30 TNBC patients, 30 breast lump patients, while an age matched control group was randomly selected from 30 healthy individuals. Patients were selected upon their first hospital visit, who did not undergo any neoadjuvant chemotherapy or radiotherapy prior to surgery. TNBC patients were diagnosed with ER, PR, and HER2 negative, and their overall tumor grade was determined by tubule formation, pleomorphism and mitoses observed via postoperative hematoxylin and eosin (H&E) staining. All grade scoring was performed by two experienced pathologists independently. Clinical information, including age, histological grade, Ki-67 labeling index, tumor number, diameter of tumor and status of axillary lymph node metastasis, was collected. As for breast lump patients, two common types were included in this study, fibroadenoma and intraductal papillomas. All participants with diabetes, cardiovascular diseases, or other diseases that would affect the metabolic profiles were excluded from the study. Healthy donors with a cancer history or showing any sign of cancer based on physical exam, laboratory tests or imaging tests including a computerized tomography (CT) scan, bone scan, magnetic resonance imaging (MRI), positron emission tomography (PET) scan, ultrasound and X-ray were excluded from the control group. Detailed information on these subjects is summarized in Table S1.

2.3. Metabolites extraction for MALDI-TOF MS analysis

The collected serum sample was immediately subjected to metabolites extraction. 200 μL serum was added to 800 μL of methanol/acetonitrile (1:1, v/v). The mixture was kept for 30 s with vortex, and 2 min under 4 °C with sonication. Then, the mixture was centrifuged for 30 min under −20 °C to precipitate proteins. Afterwards, the mixture was centrifuged at 4 °C to collect the supernatant which was then dried under nitrogen. Finally, the obtained substance was redissolved in 150 μL methanol/acetonitrile/water (1:1:2, v/v/v) and centrifuged to collect the supernatant for subsequent analysis.

2.4. Synthesis of PFDT modified gold nanoparticles

AuNPs were synthesized using the method reported by Frens et al. [23] In brief, 150 mL aqueous solution of 1% HAuCl4 were prepared and flask at 130 °C with constant stirring. Subsequently, 3.9 mL of 1% sodium citrate solution was quickly added, and the mixture was boiled for another 6 min till the color turning wine-red. The obtained AuNPs were then washed with water and ethanol in turn to remove the excessive citrate. After that, 4 mL ethanol and 15 μL PFDT were added to the resulting AuNPs, and the mixture was stirred continuously for 16 h to modify the AuNPs. The obtained PFDT-AuNPs were washed with ethanol 3 times and with cyclohexane once. Finally, the precipitate was dispersed in HFE with the final concentration of PFDT-AuNPs at 40 μM and stored avoid light. The stored PFDT-AuNPs should be sonicated for 15 min before usage.

2.5. Formation of colloidosome encapsulated with analytes

One μL of serum metabolites was added into 200 μL of the PFDT-AuNPs HFE solution and oscillated sufficiently to form colloidosomes of the analytes in aqueous phase encapsulated by PFDT-AuNPs. Diameter of colloidosomes were found in the range of 200–300 μm, and the volume could be calculated as 4–14 μL. Therefore, dozens of colloidosomes could be fabricated by shaking with 1 μL of sample which was obtained from ~1.3 μL of serum.

2.6. MALDI-TOF MS analysis

One μL of the CHCA matrix (10 mg mL−1 in acetonitrile/water/TFA [50:47.5:2.5, v/v/v]) was deposited on a sample well of a steel target plate of MALDI-TOF MS and dried under ambient condition. Then, the obtained colloidosomes were transferred to the target plate coated with CHCA and dried. All analyses were performed on a MALDI-TOF MS under linear positive mode. The mass scan range was set as m/z 80 to 220, and other instrumental parameters were set as 40% laser intensity, laser attenuator with 35% offset and 40% range, accumulation from 1000 laser shots, 10.5 × detector gain, and 120 ns delayed extraction time. Three colloidosomes were analyzed for each sample.

2.7. Statistical analysis of MALDI-TOF MS data

The raw data of MALDI-TOF MS were converted to text files using Bruker Daltonics flexAnalysis (Bruker, Bremen, Germany). Then statistics-sensitive non-linear iterative peak-clipping (SNIP) baseline correction, Savitzky–Golay smoothing and square root transformation were applied to the data using the R packages MALDIquant and MALDIquantForeign [24], from which the m/z values and intensity of peaks were obtained. The parameters were set as follows: the signal-to-noise ratio of peak detection was 4, the half window size was 20 and peaks were removed with the binPeaks function with a tolerance of 0.005.

The calculated concentrations of AAs were subjected to statistical analyses using MetaboAnalyst 5.0 (McGill University, Montreal, Canada, https://www.metaboanalyst.ca/) [25], including principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), sparse PLS-DA (sPLS-DA), orthogonal projections to latent structures discriminant analysis (OPLS-DA), hierarchical clustering (HCA) and receiver operating characteristic (ROC) curve analysis. Python 3.10 (Python Software Foundation, Delaware, USA) was employed to construct the confusion matrix according to actual values and predicted values from the ROC curve.

3. Results and discussion

3.1. Characterization of AuNPs and self-assembled colloidosomes

AuNPs with uniform particle size were synthesized according to the Frens method through sodium citrate reduction [19]. The synthesized AuNPs were then modified with PFDT to enable the self-assembling of colloidosomes at the water/oil interface. The diameters of colloidosomes obtained were in the range of 200–300 μm (Fig. 1a and b). As shown in the SEM image (Fig. 1c), the surface of the colloidosomes was formed by the homogeneous PFDT-AuNPs with particle size of 13–15 nm (Fig. 1d). The AuNPs were densely packed as a monolayer with little crack, and hence the dried colloidosome on the MALDI target plate can uniformly cover the analytes to guarantee good performance in quantification and reproducibility. It should be noted that the PFDT-AuNPs showed distinct hydrophobicity compared with AuNPs before modification. After dispersing the nanoparticles in HFE, colloidosomes could be quickly formed after violent oscillation, which ensured the independence and stability of each individual colloidosomes-formed sample spots. The dried colloidosomes showed a size close to the laser beam size (~100 μm). In such a case, it is easy to consume all the samples in the colloidosomes by laser shooting, which can benefit high sample usage efficiency, low limit of detection and high accuracy in quantification.
3.2. Quantitative detection of AAs by the colloidosomes coupled MALDI-TOF MS

To demonstrate the performance of the colloidosomes coupled MALDI-TOF MS in the quantitative analysis of AAs, a mixture of 20 kinds of 15N-labelled amino acids (cell free amino acid mixture-15N, 100 times diluted, Table S2) were analyzed by the method in comparison to the typical MALDI-TOF MS using only CHCA matrix. As shown in Fig. 2a, the number of effective peaks of the 15N-amino acids as well as the signal intensity increased under the same instrumental settings when using the colloidosomes coupled MALDI-TOF MS compared to the typical MALDI-TOF MS, demonstrating a superior sensitivity of our method in the analysis of amino acids. The enhanced sensitivity is benefited on one hand from the concentration effect of the sample preparation method, wherein all samples in a colloidosome were loaded on a spot with the size of only 200 to 300 μm, and on the other hand from the AuNPs enhanced laser desorption and ionization of analytes. In our previous study, we have demonstrated that a tightly packed AuNPs monolayer is favorable for the localized heat generation and confinement on the AuNPs surface, thus facilitating the desorption/ionization of analytes [26].

Limit of detection of the 20 AAs by the method was assessed using the mixture of the 20 kinds of 15N-labelled AAs by serial dilution. The result showed that the detection limits of all AAs were lower than 10 μM, and that the detection of limits of His and Arg were even lower than 500 nM (Fig. 2b). His and Arg are classified as basic amino acids, of which the side chains contain basic groups that can be easily protonated, hence exhibited lower limit of detection than the other AAs. Considering the extreme low sample volume per colloidosome, i.e. ~10 nL, the absolute amounts of sample consumption per analysis were lower than 100 femole for all the 20 AAs and lower than 5 femole for His and Arg. The mass differences between 15N-Ile and 15N-Leu, 15N-Asn and 15N-Asp, as well as 15N-Lys, 15N-Gln and 15N-Glu were less than 500 ppm, which can hardly be distinguished by MALDI-TOF MS, therefore we considered the total concentration of these AAs to represent their detection limits.

Reproducibility performance of the colloidosomes coupled MALDI-TOF MS were then evaluated using AAs (200 μM final concentration, Arg, Ser, Asp and Val) mixed with the corresponding 15N labelled AAs (100 μM 15N-Arg, 350 μM 15N-Ser, 600 μM 15N-Asp and 400 μM 15N-Val) as internal standard. Arg, Ser, Asp and Val were chosen because they are typical basic, neutral polar, acidic and nonpolar AAs. The results confirmed that the relative signal intensities (I_{AA}/I_{IS}) of the target AAs in seven colloidosomes with different sizes were highly consistent and the corresponding RSD were all below 3% (Fig. S1). In addition, the relative intensity of mass spectrometry signal sampled from different regions of one colloidosome were also highly stable (RSD <1%, Fig. S2). We also compared the performance of the colloidosomes coupled MALDI-TOF MS to typical MALDI-TOF MS using CHCA matrix to analyze 200 μM serine mixed with 350 μM 15N-serine. As shown in Fig. 2c, the RSD of I_{AA}/I_{IS} was 4.0% (n = 7) using typical MALDI-TOF MS, and the RSD of I_{AA}/I_{IS} was 1.1% (n = 7) using the colloidosome coupled MALDI-TOF MS. All the results demonstrated the superior reproducibility of the method in AAs analysis.

To further assess the quantification performance of the method for AAs, we prepared a series of solutions containing Ser with concentrations from 1 to 200 μM and 350 μM 15N-Ser as internal standard. As shown in Fig. 2d, a linear correlation between the relative intensity (I_{ser}/I_{IS}) and the relative concentration of Ser and 15N-Ser was obtained (y = 1.044x + 0.0323, R^2 = 0.9965, n = 3) using the colloidosomes coupled MALDI-TOF MS. In contrast, when typical MALDI-TOF MS using CHCA matrix was used, the linear coefficient was not as good (y = 1.234x + 0.1009, R^2
The slope coefficient was much larger than 1 and the intercept was much larger than 0, both indicating strong background interference to the quantification. The results showed that the colloidosomes coupled MALDI-TOF MS can be used for absolute quantification of AAs, and can provide a performance much better than the typical MALDI-TOF MS.

### 3.3. Quantification of free amino acids in serum of TNBC patients, breast lump patients and healthy control

With the superior performance in AAs quantitative detection, the colloidosomes coupled MALDI-TOF MS was further used to quantify free AAs in human serum. A serum sample from a healthy volunteer was selected to demonstrate the feasibility of the method dealing with clinical samples. After deproteinization, the serum sample was mixed with 15N-AAs mixture (10 times diluted) as internal standards at a volume ratio of 9:1. Then, 1 μL of the mixture was dropped into the HFE solution of PFDT-AuNPs, and the colloidosomes obtained by oscillation were analyzed by MALDI-TOF MS. Some of the 20 AAs (Ile/Leu, Asn/Asp and Lys/Gln/Glu) cannot be resolved by the MALDI-TOF MS; while some (Gly, Cys and Met) were interfered by the background peaks of CHCA. Therefore, only the remaining 10 AAs (Ala, Ser, Pro, Val, Thr, His, Phe, Arg, Tyr, Trp) were quantified by the colloidosomes coupled MALDI-TOF MS. Fig. S3 shows the relative intensity (r.I.) of the 10 AAs from the serum sample and the corresponding 15N-AAs internal standards. Based on the $I_{AA}/I_{IS}$ and the concentration of internal standard, the concentration of the 10 target AAs can be calculated. Table S3 shows the concentrations (mean ± SD) of serum free AAs from 30 healthy individuals detected by the colloidosomes coupled MALDI-TOF MS, which are all close to the reference concentrations of AAs in healthy adults [27]. Such a result indicated that the colloidosomes coupled MALDI-TOF MS can accurately quantify AAs in serum.

Then, we applied the method to quantify the 10 AAs in the serum of 30 TNBC patients, 30 breast lump patients and the 30 healthy controls. Demographic information of the participants is shown in Table S1. Statistical analysis and machine learning methods were applied to analyze the quantities of the 10 AAs in the cohort of TNBC patients and controls. As shown in Fig. 3a, healthy control (pink) and TNBC patients (blue) can be grouped into two sets with little overlap by the unsupervised machine learning method PCA based on the AA contents of each sample, indicating that the colloidosomes coupled MALDI-TOF MS-based AAs quantification method can distinguish the two groups of people. However, the TNBC patients significantly overlapped with the breast lump patients (green) by PCA, demonstrating the high similarity of the two groups of people in serum AAs quantities. The result of HCA also revealed an apparent separation between healthy people and patients with breast lump or TNBC (Fig. S4), with the AA contents obviously down-regulated in the patients with breast lump or TNBC. We further adopted the
supervised machine learning methods of PLS-DA, sPLS-DA and OPLS-DA to analyze the dataset. PLS-DA also showed a clear distinction between healthy individuals and TNBC patients, while sPLS-DA and OPLS-DA effectively distinguished all three types of samples (Fig. 3b–c, Fig. S5). sPLS-DA can select the most predictive or discriminative features in the data to classify the sample, and hence can achieve more efficient separation of the TNBC patients from the breast lump patients [28]. Considering that the sample number (90) in the study was much larger than the feature number (10), a supervised machine learning model is not likely to introduce overfitting.

Volcano plots show the fold change (FC) of mean concentration and t-test p-value of each AA between 2 groups. As shown in Fig. 3d–e, between the healthy control and TNBC groups (Control-Tumor) and between the healthy control and breast lump groups (Control-Lump), 4 AAs (Arg, Tyr, Trp, Phe) showed significant and strong down-regulation (p-value < 0.05 and FC > 1.5), while the other target AAs show also significant down regulation but with small FC (p-value < 0.05 and FC < 1.5). Between the TNBC and breast lump groups (Lump-Tumor), all the target AAs except His showed statistically significant changes although the fold change was diminutive (p-value < 0.05 and FC < 1.5, Fig. 3f). Four AAs (Phe, Tyr, Ser, Arg) were more abundant in the TNBC group, while 6 AAs (Pro, Trp, Val, Thr, Ala, His) were more abundant in the breast lump group.

To explore more precisely the difference of each AA among the three groups, box plot with t-test p-value of each target AA is shown in Fig. 4. Compared to healthy controls, both TNBC and breast lump patients show significant down regulation of AAs. The concentrations of AAs between TNBC and breast lump were closer, but still mostly with significant regulation except Trp. Previous reports also confirmed the relevance of AA content change with breast cancer. The consumption and utilization of AAs were considered essential for the proliferation and maintenance of tumor cells [29,30]. Dysregulation of the metabolism of branched-chain amino acids (BCAAs) including leucine (Leu), valine (Val), and isoleucine (Ile) has been reported to be associated with specific cancer phenotypes. BCAAs can inhibit the growth and metastasis of tumor [31], thereby changes in the content of BCAAs can often reflect the systemic changes in cancer patients compared to healthy controls [32]. Jasbi et al. have used a targeted LC-MS/MS method for metabolic profiling to compare plasma metabolomics between breast cancer patients and healthy individuals. The results showed that plasma metabolites of Arg, Pro and Trp decreased in breast cancer patients [33]. Huang et al. developed a novel computer simulation method to analyze pathway-based metabolomics, revealing the characteristics of metabolic pathways of Ala, Asp and Glu [34]. The work also identified the down-regulation of Ser and Thr in the serum of breast cancer patients. Our result was well in consistent with the previous reports. In addition, the growth of breast lump was also triggered by unusual cell proliferation requiring utilization of amino acids, which could be considered as an intermediate state between breast cancer and normal tissue, and hence the down-regulation of serum AAs in the breast lump group was also observed. All the results from our work as well as the others demonstrated that the quantification of multiple AAs in
serum can be used for the diagnosis of breast cancer as a non-invasive and facile method. We have also compared the concentrations of 10 AAs in the cohort of TNBC patients with different histological grades and lymph node status as shown in Fig. S6. It was found that most AAs did not show significant difference in contents among TNBC patients with different histological grades and lymph node status. Nevertheless, since there were only 30 TNBC patients, further studies should be adopted to investigate the possible difference of AAs contents of TNBC patients with different histological grades and lymph node status.

3.4. Diagnosis of TNBC based on the serum AAs quantities

We further verified the potential of the method for TNBC diagnosis by employing the quantities of 10 free AAs in serum. Two third samples from each group were selected as the training data, while the rest were used as the test data. PLS-DA with cross validation was used to build the classification models on the training data. As shown in Fig. 5a, ROC analysis with cross-validation on the training data of 20 healthy controls and 20 TNBC patients showed an area under the curve (AUC) value of 0.999, demonstrating a high confidence of the method in differentiating TNBC patients from healthy controls. By applying the model to the test data set of 10 healthy controls and 10 TNBC patients, only one sample was mis-classified, and the sensitivity, specificity and accuracy of the classification model for identifying healthy controls and TNBC patients were 91%, 100% and 95%, respectively (Fig. 5b).

In clinical diagnosis, it is more challenging to differentiate breast lump from breast cancer patients. Therefore, we further combine the healthy controls and the breast lump patients as the control group compared to the TNBC patients. Forty controls and 20 TNBC patients were involved in the training set, and the ROC analysis with cross-validation on the training dataset using classification models built by PLS-DA showed an AUC value of 0.953, Fig. 5c. With the test dataset, the sensitivity, specificity and accuracy of the classification model for distinguishing TNBC patients from healthy controls and breast lump patients were 95%, 100% and 97%, respectively (Fig. 5d). All the results indicated that the quantification of AAs in serum by the colloidosomes coupled MALDI-TOF MS is promising for the diagnosis of TNBC.
future, it is promising to apply the method to the detection of many different small molecule metabolites in various body fluids. With the advantages of low sample consumption (only ~10 nL per analysis), low cost, high throughput, high sensitivity and good quantification performance, the colloidosomes coupled MALDI-TOF MS is a promising technique for liquid biopsy and may be used for the diagnosis of different diseases in the future.

Credit author statement

Xinyue Han: Investigation, Methodology, Writing - Original draft preparation. Dandan Li: Formal Analysis. Shurong Wang: Investigation. Yuxiang Lin: Resources. Yun Liu: Writing - Revising and Editing. Ling Lin: Conceptualization, Writing - Review & Editing, Supervision. Liang Qiao: Conceptualization, Writing - Review & Editing, Supervision, Funding Acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data associated with this study are available in the main text or the supplementary materials.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (NSFC, 22022401, 22074022, and 21934001), and the Ministry of Science and Technology of China (2020YFF0304502).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mtbio.2022.100486.

References

[1] H. Sung, J. Ferlay, R.L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, F. Bray, Global cancer statistics 2020: globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA A Cancer J. Clin. 71 (3) (2021) 209–249, https://doi.org/10.3322/caac.21660.
