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ORIGINAL ARTICLE

Quantitative proteome and lysine succinylome analyses provide insights into metabolic regulation in breast cancer

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Abstract

Background Breast cancer, the most common invasive cancer and cause of cancer-related death in women worldwide, is a multifactorial, complex disease, and many molecular players and mechanisms underlying the complexity of its clinical behavior remain unknown.

Methods To explore the molecular features of breast cancer, quantitative proteome and succinylome analyses in breast cancer were extensively studied using quantitative proteomics techniques, anti-succinyl lysine antibody-based affinity enrichment, and high-resolution LC–MS/MS analysis.

Results Our study is the first to detect the regulation of lysine succinylation in breast cancer progression. We identified a novel mechanism by which the pentose phosphate pathway and the endoplasmic reticulum protein processing pathway might be regulated via lysine succinylation in their core enzymes.

Conclusions These results expand our understanding of tumorigenesis mechanisms and provide a basis for further characterization of the pathophysiological roles in breast cancer progression, laying a foundation for innovative and novel breast cancer drugs and therapies.

Keywords Breast cancer · Proteomics · Lysine succinylation · Quantitative analysis · Bioinformatics analysis

Introduction

Breast cancer currently has the highest incidence rate among malignant tumors in women worldwide, and its disease etiology has also been a hot topic [1]. Breast cancer is a multifactorial, complex disease, and many factors play vital roles in its pathogenesis, including environmental, genetic, and lifestyle influences [2]. Studies on all of these factors will help better understand the pathogenesis of breast cancer. As direct contributors to major bodily functions, proteins play decisive roles in the development and progression of many physiological processes and diseases [3]. Thus, proteomics has an irreplaceable role in cancer therapy and precision medicine, which has gained increasing attention [4]. With recent advances in markable biotechnology, mass spectrometry (MS) serves as the mainstream current and future proteomic analysis method, allowing the possibility to study the characteristics and mechanisms of human cancers in a high-throughput manner with high resolution [5, 6]. MS allows not only the quantitative examination of large numbers of proteins in complicated protein mixtures but also the identification of protein signatures or biomarkers for diseases [4, 7].

The ultimate function of a protein is closely related to its modification. Protein post-translational modifications (PTMs) have also been shown to play important roles in regulating protein functions, with specific PTMs on certain substrate residues diversifying and regulating the cellular proteome [8, 9]. Currently, acetylation and phosphorylation are widely reported to be involved in tumorigenesis, and a variety of drugs have been used in the clinic [10]. Lysine succinylation, a novel PTM, participates in energy

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metabolism and signaling pathways and closely cross-talks with acetylation [11]. As an evolutionarily conserved modification existing in multiple species, lysine succinylation may affect various cellular functions, including carbon and fatty acid metabolism [12]. Therefore, lysine succinylation may play significant roles in the regulation of cellular metabolism.

Currently, studies on proteomics and PTMs, including phosphorylation, acetylation, and methylation, in breast cancer are continually emerging with marked developments in proteomic technologies. However, no studies on lysine succinylation in breast cancer have been reported. In addition, though they highlight the potential of the use of proteomic technology to elucidate clinically relevant cancer signatures, the existing studies lack comparisons with adjacent normal tissue. More importantly, analysis of para-carcinoma tissue would provide more sufficient and specific proteomic information as a control and more accurate cancer tissue-specific results. Hence, we performed comparative quantitative proteome and lysine succinylome analyses of breast cancer tissue and adjacent tissues in the same patient to minimize individual differences, which can sufficiently reveal the mechanism of this disease.

In this study, we carried out quantitative proteome and lysine succinylome analyses in breast cancer. A series of bioinformatics analyses were conducted to explore the molecular mechanisms of breast cancer genesis and progression, wherein changes in the protein proteome and succinylation levels may be involved. Therefore, this study may increase our understanding of cancer biology and provide a method to screen novel biomarkers and targets for breast cancer diagnostics and treatments. Our study is the first to detect the regulation of lysine succinylation in breast cancer progression, which should be further investigated for clinical applications.

Materials and methods

Sample preparation

Three pairs of human breast cancer tissue samples were collected at the time of surgery at the no. 1 Hospital of Anhui Medical University and deposited in liquid nitrogen. All samples were obtained from the patients who provided informed consent based on the Declaration of Helsinki. The three patients had not been previously subjected to preoperative radiotherapy, chemotherapy, or any other therapy (tissues were sectioned for hematoxylin and eosin staining and evaluated by two pathologists to confirm and classify the histopathological samples as breast cancer tissue or normal tissue). A summary of basic clinical information regarding the three patients is shown in Supplementary Table 1.

Protein extraction, tryptic digestion, TMT labeling, HPLC fractionation, and lysine succinylated peptide affinity enrichment

All tissue samples were first ground using liquid nitrogen and then suspended in ice-cold lysis reagent [8 M urea, 10 mM dithiothreitol (DTT), 50 mM nicotinamide (NAM), 3 μM trichostatin A (TSA) and 1% protease inhibitor cocktail]. The remaining debris was removed by centrifugation at 20,000*g* for 10 min at 4 °C, and protein concentrations were determined using a 2-D Quant kit. Afterward, the proteins were precipitated with 20% trichloroacetic acid (TCA) overnight at 4 °C, and the resulting precipitates were desalted 3 times with ice-cold acetone. Dried protein pellets were re-suspended in 100 mM triethylammonium bicarbonate (TEAB) and digested with trypsin at an enzyme-to-substrate ratio of 1:50 for 12 h at 37 °C. Then, the peptides were reduced with DTT and alkylated with iodoacetamide (IAA) in the dark. To ensure complete digestion, a second digestion was conducted by adding trypsin at an enzyme-to-substrate ratio of 1:100 for 4 h at 37 °C.

Then, tandem mass tag (TMT)-6 plex labeling was performed for global proteome and lysine succinylome quantification. Peptides were reconstituted in 0.5 M TEAB and processed using a 6-plex TMT kit. Tumor and normal tissues were labeled with TMT-126, TMT-127, TMT-128, TMT-129, TMT-130, and TMT-131, and each sample was then fractionated by high pH reverse-phase high performance liquid chromatography (HPLC) using an Agilent 300 Extend C18 column (5 μm particles, 4.6 mm ID, 250 mm length). In brief, the peptides were first separated on a 2–60% acetonitrile gradient in 10 mM ammonium bicarbonate (pH 10) over 80 min into 80 fractions. Then, the peptides were combined into 18 fractions and dried by vacuum centrifugation.

To enrich succinylated lysine (Ksucc) peptides, tryptic peptides dissolved in NETN buffer (100 mM NaCl, 1 mM EDTA, 50 mM Tris–HCl, 0.5% NP-40, pH 8.0) were incubated with pre-washed antibody beads (PTM Biolabs) at 4 °C overnight with gentle shaking. The beads were washed four times with NETN buffer and twice with ddH2O. Bound peptides were eluted with 0.1% trifluoracetic acid (TFA) and dried under a vacuum.

LC–MS/MS analysis

Peptides were dissolved in 0.1% formic acid (FA) and directly loaded onto a reversed-phase pre-column (Acclaim PepMap 100, Thermo Fisher Scientific), and peptide separation was performed using a reversed-phase analytical

column (Acclaim PepMap RSLC, Thermo Fisher Scientific). The gradient comprised an increase in solvent B from 6 to 22% (0.1% FA in 98% acetonitrile, ACN) over 24 min, from 22 to 36% over 8 min, a climb to 80% over 4 min and then a hold at 80% for the final 4 min at a constant flow rate of 280 nl/min on an EASY-nLC 1000 ultraperformance liquid chromatography (UPLC) system. The resulting peptides were analyzed using a Q Exactive™ Plus Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific). Peptides were subjected to a nanospray ionization (NSI) source followed by tandem mass spectrometry (MS/MS) on a Q Exactive™ Plus MS (Thermo) coupled to the UPLC online. Intact peptides were detected in the Orbitrap at a resolution of 70,000. Peptides were selected for MS/MS using a normalized collision energy (NCE) setting of 30, and ion fragments were detected in the Orbitrap at a resolution of 17,500. A data-dependent procedure that alternated between one MS scan followed by 20 MS/MS scans was applied to the top 20 precursor ions above a threshold ion count of 2E4 in the MS survey scan with a 30.0 s dynamic exclusion. A 2.0 kV electrospray voltage was applied. Automatic gain control (AGC) was used to prevent overfilling of the ion trap, and MS/MS spectra were generated from 5E4 accumulated ions. For MS scans, the *m*/*z* scan range was 350–1800.

Database search

MS/MS data were processed using MaxQuant with an integrated Andromeda search engine (v.1.4.1.2). Tandem mass spectra were searched against the SwissProt Human database concatenated with a reverse decoy database. Trypsin/P was specified as the cleavage enzyme, allowing up to 4 missing cleavages, 5 modifications per peptide, and 5 charges. The mass error was set to 10 ppm for precursor ions and 0.02 Da for fragment ions. Carbamidomethylation on cysteine was specified as a fixed modification, and oxidation on methionine and succinylation on lysine were specified as variable modifications. False discovery rate (FDR) thresholds for proteins, peptides, and modification sites were specified at 1%. The minimum peptide length was set to 7. For the quantification method, TMT-6 plex was selected. All other parameters in MaxQuant were set to the default values. The site localization probability was set as > 0.75.

Bioinformatics analysis

Protein functional annotations, enrichment, enrichment-based clustering, motifs, and other analyses were used in our study. Detailed descriptions of these analyses are described in the Supplementary Information. When performing bioinformatics analyses, P values < 0.05 were considered significant.

Results

Identification of quantified proteins in breast cancer tissues at the proteomic level

In this work, quantitative proteomic analysis of tumor and adjacent normal tissues from breast cancer patients was performed using a combination of TMT labeling, basic HPLC fractionation, and LC–MS/MS analysis (Fig. 1a). In total, 3237 proteins were identified in breast cancer and matched normal tissues, among which 141 differentially expressed proteins were obtained with a threshold fold-change > 1.5 and $P < 0.05$ (Supplementary Table 2). We plotted the results according to the quantitative differences in the proteins in each group (Fig. 1c–e and Supplementary Table 3). We used these three pairs as biological replicates for total proteomic analysis, aiming to find global differentially expressed proteins. The results were highly reproducible, indicating strong consistency and correlation among the three biological replicates (Fig. 1b).

To reveal the nature of the differentially expressed proteins, gene ontology (GO) annotation analysis was conducted according to biological processes, cellular components, and molecular functions (Fig. 2 and Supplementary Table 4). Molecular function analysis indicated that 48% of the differentially expressed proteins were related to binding. Cellular component-based classification analysis showed that cell- and organelle-related proteins were the main proteins represented, while other cellular component-related proteins were detected in relatively less amounts. Ontology analysis of biological processes demonstrated that cellular processes and single-organism process-related proteins were the two largest groups.

GO enrichment was further performed to investigate the biological functions of the altered proteins in breast cancer tissue (Fig. 3 and Supplementary Table 5). As shown in Fig. 3, intermediate filament (IF)-related proteins, such as desmoplakin (P15924), were significantly enriched in cellular components. The markedly enriched molecular functions were structural molecular activity and structural constituents of cytoskeleton proteins, including plakophilin-1 (P13835) and KRT1 (P04264). In the ontology analysis of biological process, most of these markedly enriched terms, such as keratinocyte differentiation, were related to skin epidermis development, including KRT10 (P13645).

To elucidate the functions of the differentially expressed proteins, GO enrichment-based clustering analysis (Fig. 4 and Supplementary Table 6) was performed. In the biological processes category (Fig. 4a), differentially quantified proteins were significantly enriched in biosynthetic processes, such as nucleobase-containing compounds and aromatic compound biosynthetic processes with high T/C

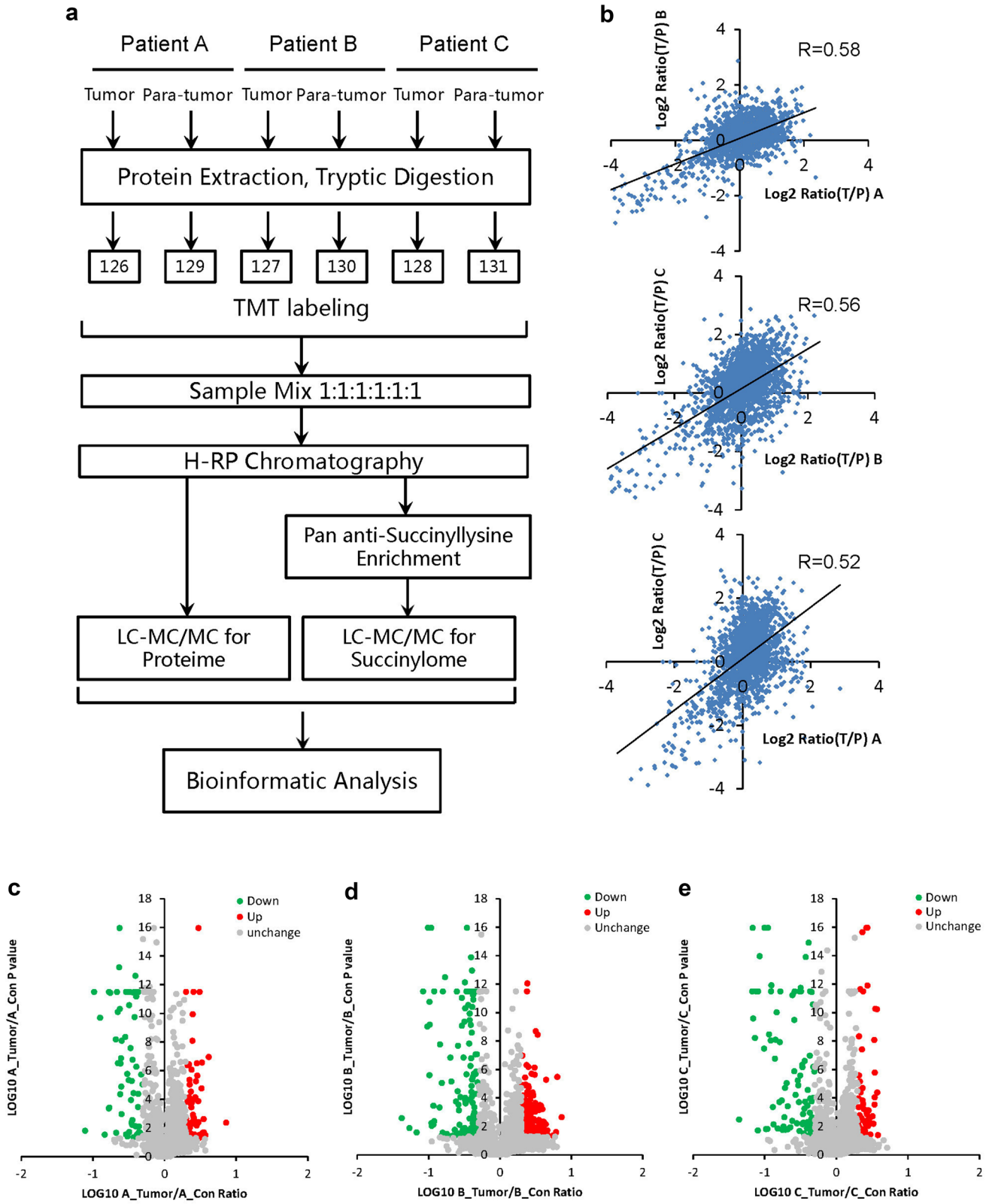

Fig. 1 The workflow for quantitative proteomic and lysine succinylation analysis in three pairs of breast cancer tissues and matched normal tissues (**a**). Correlation analysis among three biological replicates (**b**). Volcano plot of significantly up-regulated or down-regulated proteins in three patients [patient A (**c**), patient B (**d**), patient C (**e**)]

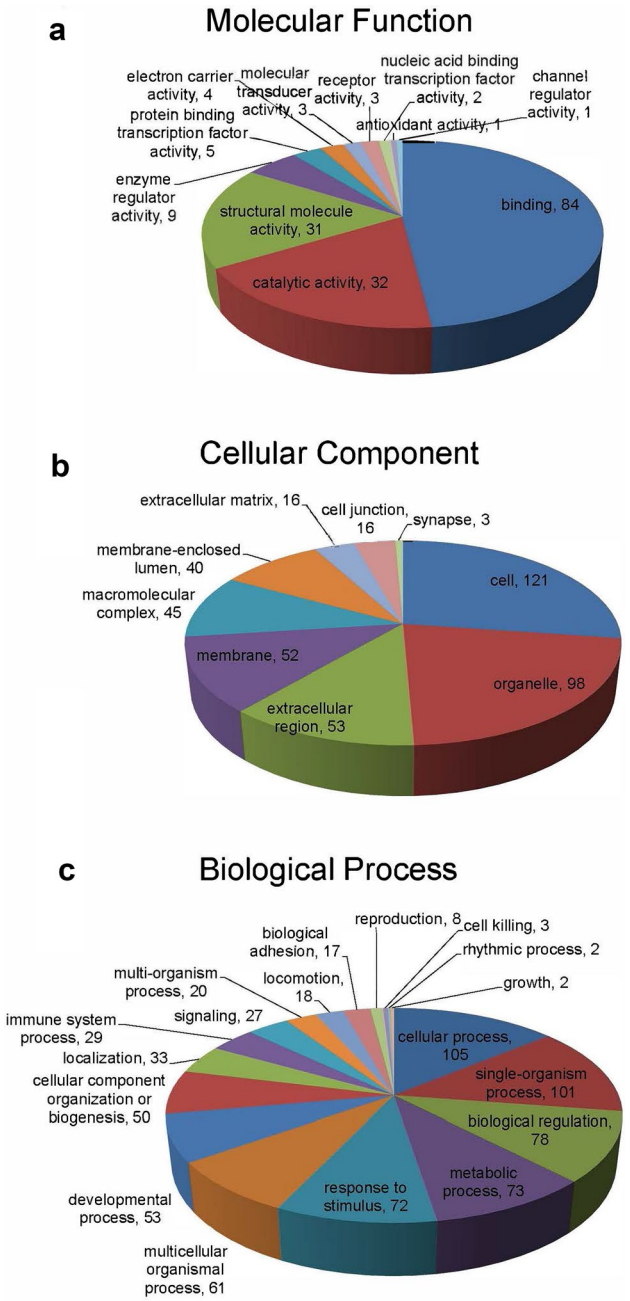

Fig. 2 GO classification of the identified differentially expressed proteins in the breast cancer proteome according to the biological process (**a**), molecular function (**b**), and cellular compartment (**c**) categories. Numbers refer to assigned proteins in each category with a threshold fold-change > 1.5 and $P < 0.05$

ratios, while the processes were related to IF proteins with low T/C ratios. For the cellular components (Fig. 4b), the expression levels of proteins associated with membrane-enclosed lumen and organelle lumen were increased, while extracellular matrix and IF proteins were expressed at low levels. Enrichment analysis of molecular functions (Fig. 4c) showed that proteins involved in RNA binding were enriched toward high T/C ratios, while structural molecular activity and structural cytoskeleton constituents were enriched toward low T/C ratios in breast cancer tissue.

Determining the succinylation levels of quantified proteins in breast cancer tissues

In addition, we also conducted quantitative lysine succinylome analysis in breast cancer tissues. Altogether, 291 lysine succinylation sites in 195 proteins were identified, of which 102 up-regulated lysine succinylation sites on 88 proteins and 93 down-regulated sites on 68 proteins showed altered succinylation levels with a threshold fold-change > 2.0 and a P value < 0.05 (Supplementary Table 7).

To characterize the possible specific sequence motifs surrounding succinylated lysine residues in breast cancer tissues, we generated a sequence logo at positions surrounding the succinylation site. Four significantly enriched motifs were obtained from all the identified succinylation sites, KsuccP, Ksucc*E, Ksucc*D, and KsuccD (Ksucc represents a succinylated lysine, and * represents a random amino acid residue, Fig. 5a). To determine whether specific amino acids are adjacent to succinylated lysines, we examined amino acid sequences flanking the succinylation sites using a heat map (Fig. 5b). Proline (P) was overrepresented at the +1 position, while glutamic acid (E) was overrepresented at the +2 position of the succinylation sites. Aspartic acid (D) also appeared in the −1 to +3 positions with high frequency. Noticeably, serine (S) appeared to be unwelcome surrounding the succinylation sites, as its frequency of occurrence was obviously lower than those of other amino acids both upstream and downstream of the succinylation sites.

Furthermore, to investigate the differentially expressed Ksucc proteins involved in breast cancer tissue pathways, Kyoto Encyclopedia of Genes and Genome (KEGG) pathway analysis was conducted. As patient C exhibited larger differences than patients A and B, all succinylation was significantly down-regulated; therefore, we analyzed the pentose phosphate pathway (PPP; Fig. 6) and the ER protein processing pathway (Fig. 7) in patients A and B via bioinformatics. The succinylation levels of the two patients showed a similar trend in both pathways, indicating that succinylation may play a significant role in regulating the PPP and the ER protein processing pathway. As shown in Fig. 6, the succinylation of transketolase (TK), a key enzyme in the PPP pathway that acts as a catalyst, was significantly up-regulated. In addition, the succinylated expression levels of binding protein (BiP), glucose-regulated protein 94 (GRP94), protein disulfide isomerases (PDI), calreticulin (CRT), and B cell receptor-associated protein 31 (Bap31), associated with the ER protein processing pathway, were obviously increased.

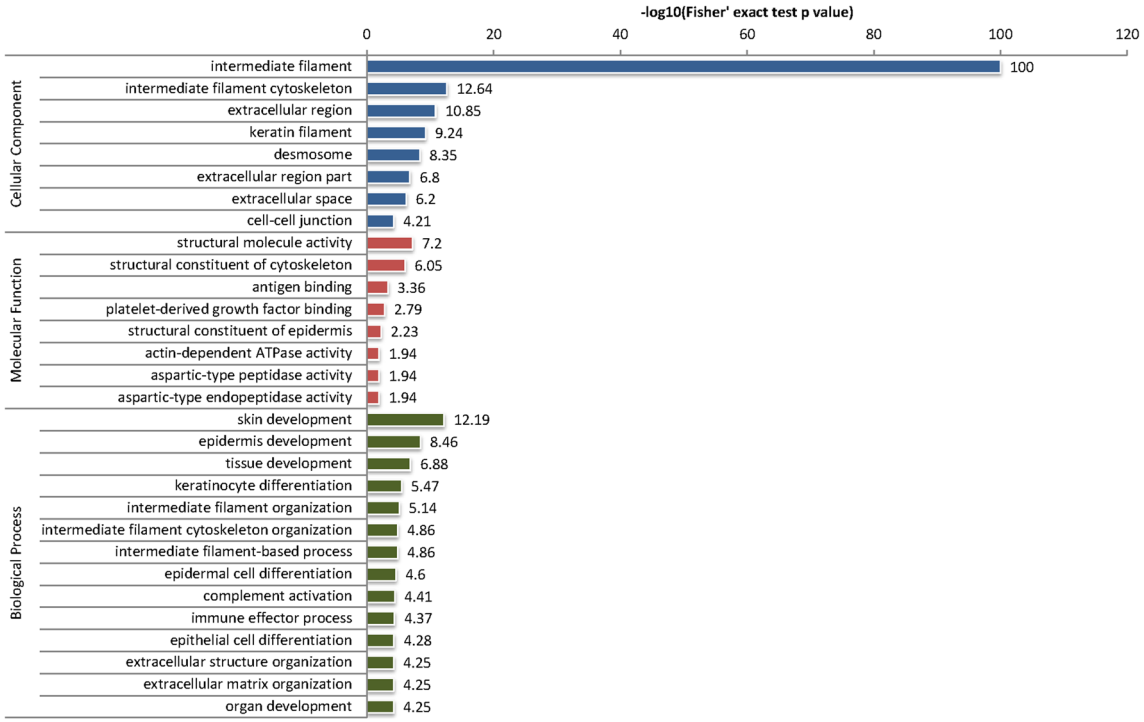

Fig. 3 Enrichment analysis of differentially expressed proteins at the proteome level in breast tumors compared with adjacent normal tissues with a threshold fold-change > 1.5 and $P < 0.05$. GO enrichment analysis was performed on the ontology of cellular components, molecular functions, and biological processes

Discussion

Identification of quantified proteins at the proteomic level in breast cancer tissues

In the present study, the differentially expressed proteins identified in breast cancer tissues showed a wide range of functions. Bioinformatics analysis of functional classifications, GO enrichment and GO enrichment-based clustering analysis identified differentially expressed proteins with very similar distributions of biological processes, cellular components, and molecular functions at the global proteomic level.

In the clustering analysis (Fig. 4a, b), many cytoskeleton-related processes were found to be markedly enriched with down-regulated proteins, especially proteins related to IFs, which were classified by our data as mostly coming from the keratin family, including K10, K14, and K17. IF proteins constitute an extensive cytoskeletal network whose protein constituents are encoded by a large family of genes expressed in a tissue- and differentiation state-specific manner [13]. Keratins are a family of epithelial-specific IF proteins that can be classified as type I or type II and are arranged in heterotypic pairs [14]. The expression of different keratins is usually dependent upon the epithelial cell type and differentiation stage, making keratins effective diagnostic markers for determining tumor origin [15].

Recent studies have demonstrated that many types of keratins are specifically expressed in human tumors and participate in cell proliferation and invasion [16]. Furthermore, keratins participate in many intracellular signal transduction pathways [17]. Previous studies showed that abnormal keratin protein expression is associated with the AKT/mTOR signaling pathway in cancer, which is often activated in invasive tumors, improving the possibility that some keratin-mediated AKT proteins play important roles in the occurrence of epithelial tumors [17, 18]. In addition, the expression of keratin in different tissues is correlated with tumor formation and tumor cell invasive potential. K10 is generally expressed in keratinocytes after mitosis, which can inhibit the proliferation of keratinocytes and cell cycle progression and reduce skin tumorigenesis [19]. K14 in airway epithelial cells may induce squamous metaplasia, atypical hyperplasia, precancerous carcinoma in situ lesions and invasive carcinoma [20]. K17 promotes epithelial proliferation and tumor growth by polarizing the immune response in skin [21]. Therefore, studying the association between tumors and keratin will aid in tumor diagnosis and treatment.

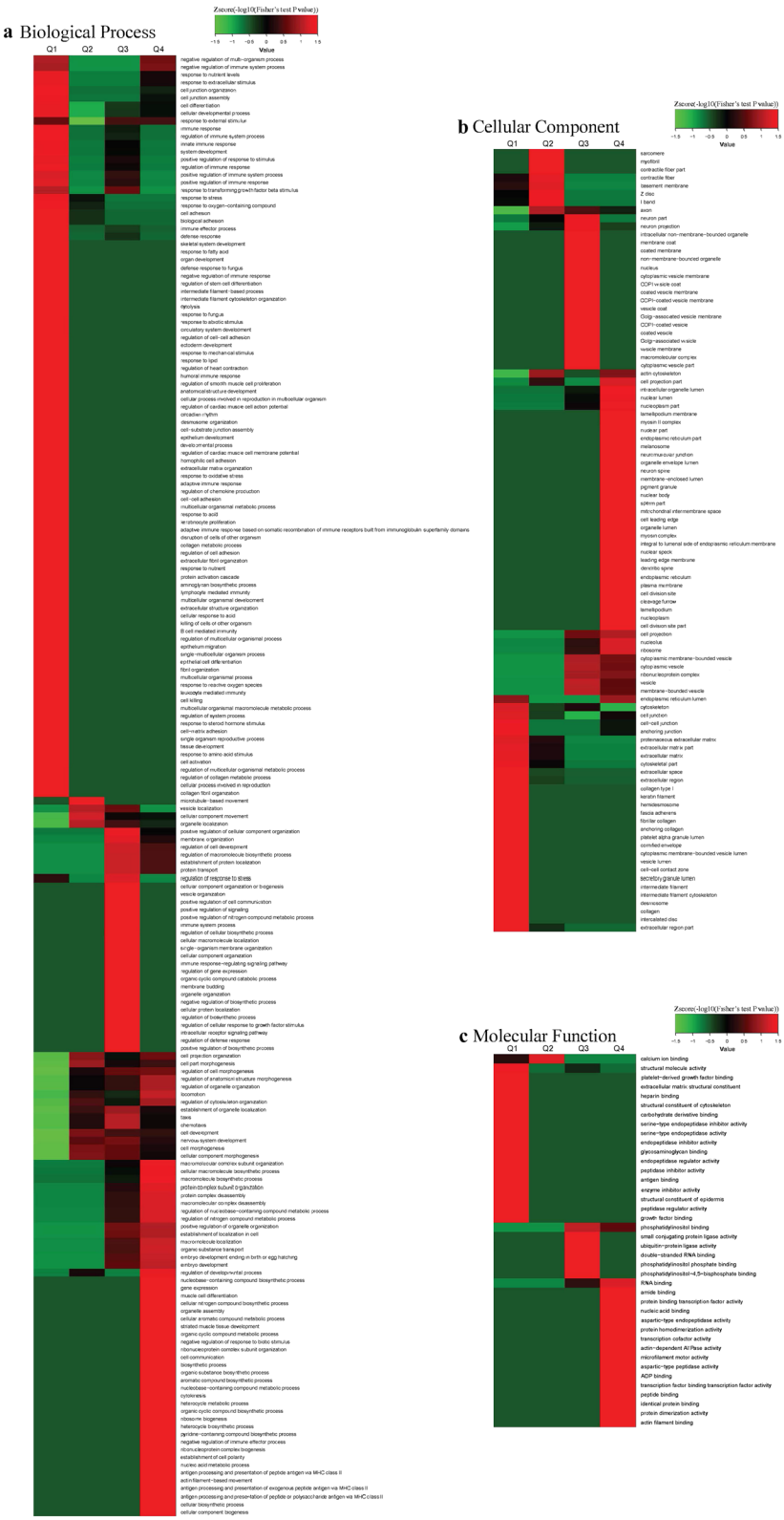

Fig. 4 Enrichment and clustering analysis of the quantitative protein datasets from breast cancer patients based on the biological process (**a**), molecular function (**b**), and cellular compartment (**c**) categories. All the quantified proteins were divided into four quantiles (Q1–Q4), with a threshold fold-change > 1.5 and $P < 0.05$ according to T/C ratios (T: tumor, C: control; Q1: < 1/1.5, Q2: 1/1.5–1/1.3, Q3: 1.3–1.5, Q4: > 1.5)

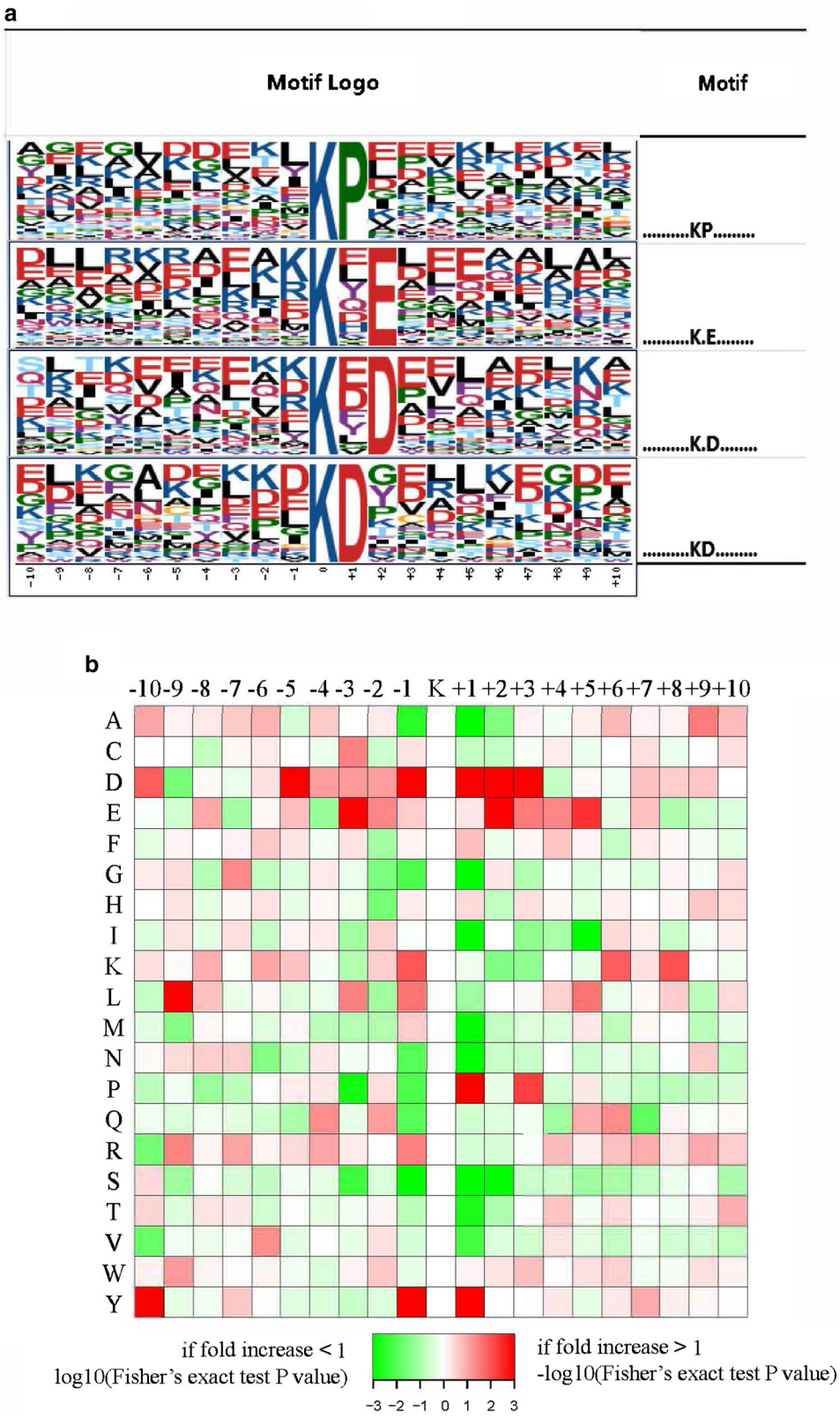

Fig. 5 Properties of succinylated peptides. Succinylation motifs and conservation of succinylation sites (**a**). Heat map of the amino acid composition of the succinylated sites (**b**). All identified 291 succinylation sites were used for the analysis, and $P < 0.05$ was considered significant for this analysis

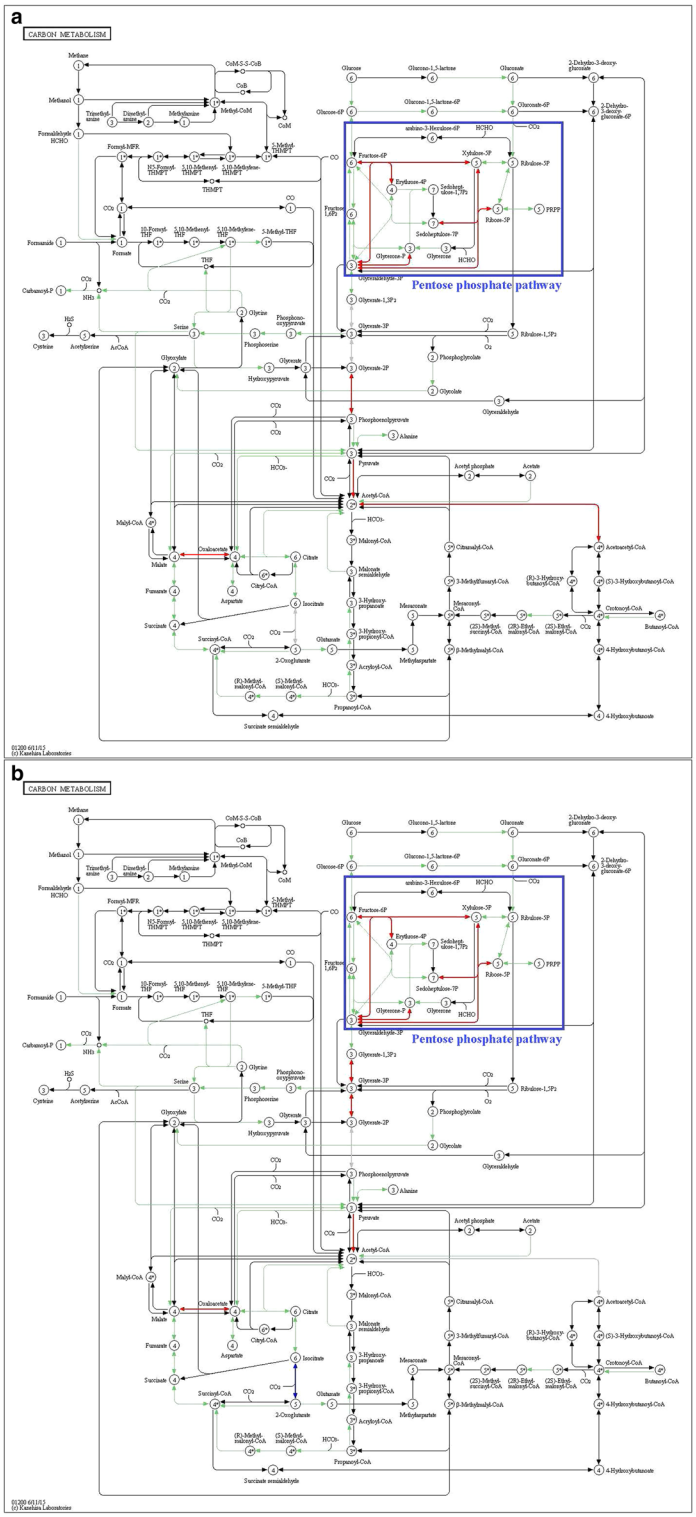

Fig. 6 Pentose phosphate pathway (PPP) of patient A (**a**) and patient B (**b**). Arrows represent enzymes that act as a catalyst for adjacent metabolites. Red arrows indicate succinylation of enzymes identified as up-regulated; green arrows indicate succinylation of enzymes identified as showing a significant change; gray arrows indicate no significant change in our study

Determining the succinylation levels of quantified proteins in breast cancer tissues

Lysine succinylation is a new PTM identified in histone proteins that impacts diverse metabolic pathways [22]. Several studies suggest that lysine succinylation regulates metabolism, yet the details of these regulations remain elusive. We selected an important glucose metabolic process, the PPP, and the ER protein processing pathway, which are known to be significantly regulated in cancer cells and can

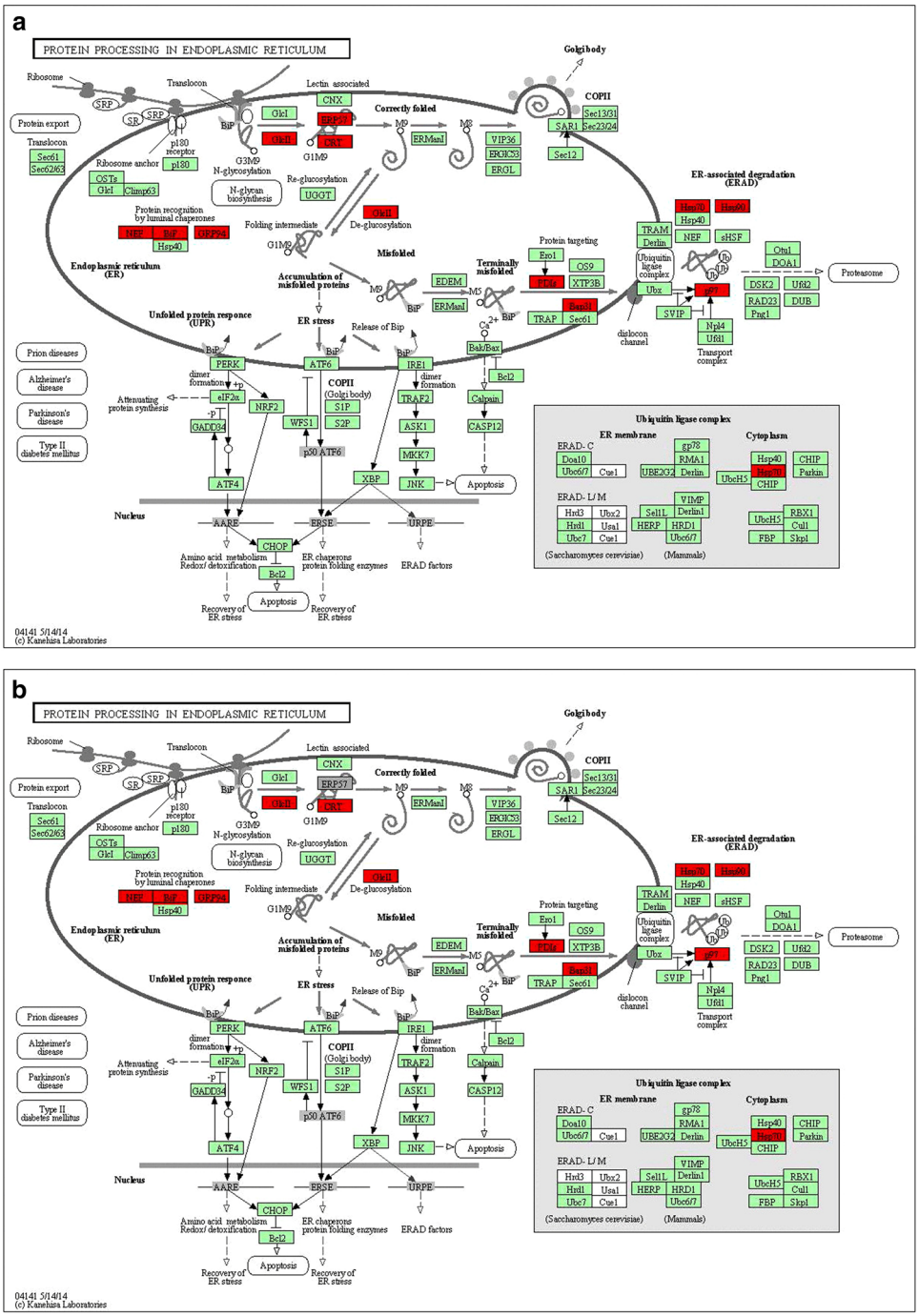

Fig. 7 Putative endoplasmic reticulum protein processing pathway of patient A (**a**) and patient B (**b**). A putative endoplasmic reticulum protein processing pathway was constructed based on KEGG mapping. Red squares indicate proteins identified as up-regulated proteins; green squares indicate proteins identified as differentially expressed proteins; white circles indicate proteins that were not identified as differentially expressed in our study

be detected when changes in Ksucc levels follow specific patterns.

According to the basic clinical information (Supplementary Table 1), the expression of Ki67 in patient C (40–50%) was higher than that in patients A (5%) and B (10–15%). Many studies have shown that Ki67 expression is closely related to the development of various kinds of tumors. Clinically, the Ki67 index is usually detected by immunohistochemistry to reflect the proliferative activity of normal and diseased tissues, which may help identify benign and malignant diseases [23, 24]. In addition, Ki67, which is expressed in all active cell cycle stages, acts as a proliferation marker for tumor cells. Furthermore, its location in the nucleus is complex and specific and changes with the cell cycle [25]. Here, we deduced significant differences between the succinylation levels of patient C versus those of patients A and B that correlated with the expression of Ki67, suggesting that Ki67 expression may be more likely to cause differences in modification levels and not protein expression levels.

Succinylation was recently discovered to be a conserved type of lysine post-translational modification found in many eukaryotic organisms. Several studies have suggested that lysine succinylation is involved in energy metabolism. Here, we selected an important glucose metabolic process, the PPP, that is known to be significantly regulated in cancer cells and determined whether the changes in succinylation levels in this pathway follow specific patterns. The PPP is a branch of the glycolysis pathway. Glycolytic pathways are enhanced when aerobic respiration is inhibited; the PPP is also activated, and NADPH levels increase to meet the demands for fast growth in cancer cells [26]. The relative concentration of succinylation in the PPP was determined; most of the associated reactions are reversible, indicating that succinylation can control the PPP equilibrium. Proteins in the PPP show greater increases in succinylation in later stages of cancers, indicating that activation of lysine succinylation may result in opposite changes in protein expression. The TCA cycle generates and consumes succinyl-CoA, an intermediate substrate for lysine succinylation, and the PPP uses the intermediate component from glycolysis to generate the reducing substrate NADPH, which is essential for the oxidative stress response [26, 27]. Therefore, we speculate that the balance between succinyl-CoA generation and consumption in metabolic pathways might contribute to this sophisticated regulation of succinylation and protein expression in tumor-activated glucose metabolic tuning. In our study, the relative concentrations of succinylated proteins were found to be up-regulated, especially that of TK, the key enzyme in the non-oxidative PPP process. Adjacent metabolites can be converted to one another during the catalysis of TK. For example, TK can transfer two carbon atoms from D-xylulose-5P to D-ribose-5P, forming sedoheptulose-7P and glyceraldehyde-3P. TK can also transfer two carbon atoms from D-xylulose-5P to erythrose-4P, forming fructose-6P and glyceraldehyde-3P [28]. Both reactions are reversible, indicating that succinylation can control the pentose phosphate pathway equilibrium. Many studies have confirmed that TK can affect cell proliferation by regulating PPP metabolites [29]. Other research has indicated that TK may also be associated with cell migration, invasion and drug resistance, involving transcriptional regulation, signal transduction, and protein modification [30]. In contrast, transketolase (TK) is the key rate-limiting enzyme in the non-oxidative branch of the PPP of carbohydrate transformation. TK belongs to the group of thiamine diphosphate (TPP)-dependent enzymes, and TPP is the coenzyme of TK [31]. Lysine succinylation is a post-translational protein modification that requires short-chain acyl-coenzyme metabolites. The substrate for succinylation is the succinyl group derived from succinyl-CoA [32]. The addition of a succinyl group causes a greater change in the charge of the lysine target residue (from + 1 to − 1), which will in turn promote more substantial transformation of the chemical properties of the target protein with protein post-transcriptional modifications. Therefore, we speculate that succinylation can significantly alter the ability of TK to bind to transient multienzyme complexes and affect metabolic functions [33]. TK is considered a potential biomarker for cancer diagnosis and therapy.

In addition, the expression levels of the identified succinylated proteins were obviously increased in the ER pathway, suggesting that succinylation may play an important role in ER protein processing. The ER is the cellular compartment in which secretory proteins are synthesized and folded [34]. In brief, ER stress is defined as an imbalance between the protein folding capacity of the ER and the protein load, resulting in the accumulation of misfolded proteins [35]. Based on our findings, we speculate that the entire ER protein processing pathway might be disrupted in breast cancer tissue due to the abnormal expression of ER-associated proteins. ER chaperones consist of numerous soluble molecular chaperones and folding enzymes, such as BiP, GRP94, PDI, and CRT. BiP belongs to the HSP70 family and is a core molecular chaperone that binds the hydrophobic regions of unfolded proteins and facilitates folding [36]. Studies have identified increased BiP expression as an independent prognostic marker for predicting poor outcomes in cancer [37, 38]. GRP94 is an HSP90 family chaperone associated with the regulation of protein machinery and the modulation of endoplasmic reticulum homeostasis whose important roles in stem cell development and invasion of various cancers have been demonstrated [39]. CRT is the major calcium-binding chaperone protein, and previous reports have suggested a close relationship between the cell-surface expression of CRT and apoptosis [40]. PDI acts as a folding enzyme that coordinates the oxidation of cysteine residues of nascent proteins and helps proteins form correct disulfide bonds. The subsets of proteins from this pathway and other altered pathways should be further investigated first for technical validation and then for clinical applications.

In conclusion, our study is the first to detect the regulation of lysine succinylation in breast cancer progression. We identified a novel mechanism by which the PPP and the ER protein processing pathway might be regulated via lysine succinylation in their core enzymes. These results not only confer a novel perspective for elucidating the mechanism underlying breast cancer genesis and development but also potentially underlie innovation for new drugs and therapies to cure breast cancer. In addition, the differentially expressed proteins identified herein could be potential diagnostic biomarkers and/or therapeutic targets for breast cancer treatment.

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Compliance with ethical standards

Conflict of interest The authors have declared that no competing interest exists.

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