Research Paper

Identification of Nucleobindin-2 as a Potential Biomarker for Breast Cancer Metastasis Using iTRAQ-based Quantitative Proteomic Analysis

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Abstract

Metastasis is a lethal step in the progression of breast cancer. None of the metastasis-associated biomarkers identified up to now has a definite prognostic value in breast cancer patients. This study was designed to identify biomarkers for breast cancer metastasis and predictors of the prognosis of breast cancer patients. The differentially expressed proteins between 23 paired primary breast tumor and metastatic lymph nodes were identified by quantitative iTRAQ proteomic analysis. Immunohistochemistry was applied to locate and assess the expression of NUCB2 in paired primary breast tumor and metastatic lymph node tissues (n = 106). The relationship between NUCB2 expression and the clinicopathological characteristics of breast cancer patients (n = 189) were analyzed by χ^2 test. Kaplan-Meier analysis and Cox hazard regression analysis were utilized to investigate the relationship between its expression and prognosis of breast cancer patients. The iTRAQ proteomic results showed that 4,837 confidential proteins were identified, 643 of which were differentially expressed in the primary breast cancer tissues and the paired metastatic lymph nodes. NUCB2 protein was found decreased in paired metastatic lymph nodes (P = 0.000), with the positive expression rate being 82% in primary breast cancer tissues and 47% in paired metastatic lymph nodes, respectively. According to Kaplan-Meier analysis, the overall survival time of patients with positive expression of NUCB2 protein were shorter than those with negative NUCB2 expression (P = 0.004). Cox regression model suggested that NUCB2 was a risk factor of breast cancer patients (P = 0.045, RR = 1.854). We conclude that NUCB2 can be used as a potential biomarker for breast cancer metastasis and a prognostic predictor of breast cancer patients.

Key words: Breast cancer, Metastasis, Prognosis, iTRAQ, Nucleobindin-2.

Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females worldwide, with an estimated 1.67 million new cases and 521,900 deaths in 2012. In developing countries, breast cancer incidence and mortality are rising, which have exceeded developed countries [1]. Metastasis is considered as a lethal step in the progression of breast cancer. Several risk factors have been identified to be related to breast cancer metastasis, such as tumor size, nodal status,

histological grade, vascular invasion, etc. In addition, the expression of estrogen receptor (ER) and progesterone receptor (PR) and the amplification of HER2/c-erbB2 are closely linked with breast cancer metastasis, which provides the guidance to the adjuvant therapy for breast cancer [2]. Other molecules, such as miR-185-5p [3], cadherin-5 [4], Ku86 [5], and transforming growth factor β (TGF-β), are also associated with breast cancer metastasis. In addition, epithelial-to-mesenchymal transition (EMT) [6-8], circulating tumor cells [9], and the tumor microenvironment [10, 11] also contribute to the metastatic spread of breast cancer. While how these molecules and/or processes contribute to breast cancer metastasis is still not clearly understood, none of these has a definite prognostic value as a biomarker in breast cancer patients.

In recent years, innovations in high-throughput proteomic profiling approaches have allowed for highly sensitive, accurate, and quantitative identification of altered proteins in multiple samples at the same time. Isobaric tags for relative and absolute quantitation (iTRAQ) has been used successfully for the characterization of protein bioindicators of diverse effects [12]. Our goal in this study is to explore the differentially expressed proteins in matched primary breast tumor and metastatic lymph node tissue samples by the iTRAQ technique coupled with LC-MS/MS, aiming to identify reliable biomarkers for the prediction of breast cancer metastasis and prognosis.

Materials and Methods

Study subjects and clinical data

A total of 23 paired female fresh primary breast tumor and metastatic lymph node tissues were collected and used for iTRAQ followed by LC-MS/MS. All of these patients were diagnosed as breast cancer and received surgeries without any pre-operative chemotherapy or radiotherapy in Hunan Cancer Hospital & The Affiliated Cancer Hospital of Xiangya School of Medicine of Center South University from January to May, 2014. Histological type was confirmed by two pathologists in the Department of Pathology of Hunan Cancer Hospital. Each tissue sample was divided into two parts, one part used for routine pathological examination and the other stored in liquid nitrogen for iTRAQ analysis.

In addition, 106 paraffin-embedded tissue samples for immunohistochemistry were obtained from female patients with metastatic breast cancer who had received surgeries in Hunan Cancer Hospital between 1996 and 2008. These were paired

primary breast tumors and lymph node metastases (*n* = 106). All of these patients had no pre-operative chemotherapy or radiotherapy and the pathological parameters of the patients together with patients without lymph node metastasis (*n* = 83) were summarized in **Table 1**. This study was approved by the Research Ethics Committee of Hunan Cancer Hospital, China and informed consent was obtained from all of the patients. The experiments performed in this study comply with the current laws of China relating to the use of human subjects.

Table 1. Clinicopathological characteristics of breast cancer patients

Parameters	Numbers
Median age (years, range)	45 (30-73)
Median follow up period for survivors (months, range)	80 (8-210)
Histological type, n (%)	
Ductal	154 (81.5)
Lobular	35 (18.5)
Histological grade, n (%)	
I & II	33 (17.5)
III	156 (82.5)
Nodal status, n (%)	
Negative	83 (43.9)
Positive	106 (56.1)
Tumor size (d, cm), n (%)	
d ≤ 2	56 (29.6)
2 < d ≤ 5	112 (59.3)
d > 5	20 (10.6)
Unknown	1 (0.5)
Clinical stage, n (%)	
I	29 (15.3)
II	100 (52.9)
III	59 (31.2)
Unknown	1 (0.5)
ER, n (%)	
Negative	90 (47.6)
Positive	99 (52.4)
PR, n (%)	
Negative	78 (41.3)
Positive	111 (58.7)
C-erbB-2, n (%)	
Negative	57 (30.2)
Positive	122 (64.6)
Unknown	10 (5.3)
Menstrual history, n (%)	
Premenopause	123 (65.1)
Postmenopause	66 (34.9)
Modus operandi, n (%)	
Radical mastectomy	82 (43.4)
Modified radical mastectomy	100 (52.9)
Other type	7 (3.7)

iTRAQ proteomics

The iTRAQ kit was purchased from AB Sciex (USA) and proteomic analysis performed essentially as described in the manufacturer's instructions. The prepared lysates were treated with 4 µl reducing reagent for 1 h at 60°C and then blocked by 2 µl Cysteine blocking reagent for 10 min at room

temperature. After centrifugation, the supernatant was collected and incubated with trypsin and tetraethylammonium bromide (TEAB) overnight at 37°C. The next day, the prepared sample and iTRAQ reagents were mixed together. Afterwards, two-dimensional LC-MS/MS was used to analyze the labeled samples and Proteinpilot 4.0 (AB Sciex) protein retrieval software was used to retrieve the separated peptides. This process was repeated for three times and the average was accepted as the final results. A greater than 1.5-fold change in expression was considered different between the primary breast tumor tissue and the paired metastatic lymph node tissue. This research was assisted by the FitGene BioTechnology proteomics platform (http://www. fitgene.com).

Immunohistochemistry

Paraffin sections were baked in oven at 60°C for 2 h, and then dewaxed in two changes of xylene for 10 min each, followed by hydration in graded ethanol for 5 min each. For antigen retrieval, the slides were immerged into citric acid retrieval solution and heated in a microwave and cooled down at room temperature. Endogenous peroxidase activity was quenched by 3% H_2O_2 for 15 min. After blocking of nonspecific binding, the sections were incubated with the NUCB2 antibody (1:200, Abcam, UK) at 4°C overnight. Next, the sections were incubated with biotinylated secondary antibody followed by incubation with the streptavidin-peroxidase conjugate and color development using the DAB/H_2O_2 system. A negative control was included by replacing the primary antibody with PBS.

The immunostaining was evaluated by two independent experienced pathologists. The results of the two reviewers were compared and any discrepant scores were re-examined by both pathologists to reach a consensus score. A total immunostaining score (TIS) was calculated as the product of a proportion score and an intensity score. The proportion score described the estimated fraction of positive-stained tumor cells (0: 0-4%; 1: 5-25%; 2: 26–50%; 3: 51–75%; 4: >75%). The intensity score represented the estimated staining intensity (0: no or marginal staining; 1: weak; 2: moderate; 3: strong). The TIS ranged from 0 to 12. We defined 0 as negative, 1-4 as weak positive, 5-8 as moderate positive, and 9-12 as strong positive.

Statistical analysis

The difference of NUCB2 expression between paired primary breast tumor and metastatic lymph node was analyzed with McNemar test. In addition, the difference of NUCB2 expression between every other two different groups of patients with breast disease was evaluated with χ^2 test. The relationship between NUCB2 expression and clinicopathologic characteristics was examined with χ^2 test. The survival rate was calculated with the Kaplan-Meier method and examined with log-rank test. Multivariate Cox hazard analysis was used to analyze the role of NUCB2 in breast cancer prognosis.
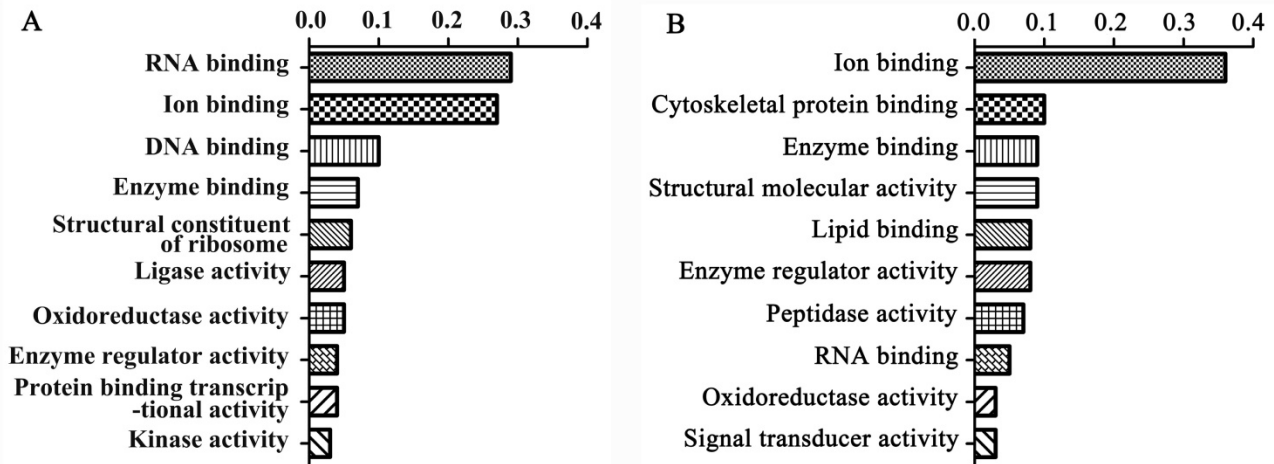
Results

Differentially expressed proteins between primary breast tumor and metastatic lymph node tissues

We first employed the iTRAQ technique to explore the differentially expressed proteins between 23 paired primary breast tumor and metastatic lymph node tissues. 4,837 confidential proteins were identified with a confidence interval (CI) ≥ 95%. Among these, 643 were differently expressed proteins, including 402 up-regulated and 241 down-regulated proteins in metastatic lymph nodes compared with the primary breast tumors. Gene ontology (GO) analysis revealed that the up-regulated proteins could be divided into 39 functional groups. The top ten up-regulated functional groups included RNA binding (29%), ion binding (27%), DNA binding (10%), enzyme binding (7%), structural constituent of ribosome (6%), ligase activity (5%), oxide reductase activity (5%), enzyme regulator activity (4%), protein binding transcriptional activity (4%), and kinase activity (3%) (**Figure 1A**). The down-regulated proteins could be divided into 31 functional groups. The top ten down-regulated functional groups included ion binding (36%), cytoskeletal protein binding (10%), enzyme binding (9%), structural molecular activity (9%), lipid binding (8%), enzyme regulator activity (8%), peptidase activity (7%), RNA binding (5%), oxide reductase activity (5%), and signal transducer activity (3%) (**Figure 1B**). The proteins up-regulated in the metastatic lymph nodes compared with the primary breast tumors were summarized in **Table 2**.

NUCB2 is differentially expressed in lymph node metastasis in breast cancer

The expression of NUCB2, which is one of the most up-regulated proteins associated with DNA binding, was 1.92-fold higher in metastatic lymph node tissues than primary breast tumor tissues. According to GO analysis, NUCB2 is located in various compartments of the cell and its molecular functions include ion binding and DNA binding (http://www.uniprot.org/uniprot/P80303). The basic biological information about NUCB2 was summarized in **Table 3**.

Figure 1. Functional groups of differentially expressed proteins between the primary breast tumors and metastatic lymph nodes determined by GO analysis. The top ten functional groups of proteins up-regulated (A) or down-regulated (B) in metastatic lymph nodes compared with primary breast tumors are shown.

**Table 2.** Up-regulated proteins in metastatic lymph nodes compared to primary tumors

Protein name	Accession number	Sequence coverage (%)	Molecular weight	Isoelectric point	Ratio
Transcriptional repressor protein YY1	sp|P25490|TYY1_HUMAN	20.05	44,713	6.22	2.31
Golgin subfamily B member 1	sp|Q14789|GOGB1_HUMAN	38.45	376,019	4.66	1.93
Dual specificity protein phosphatase 23	sp|Q9BVJ7|DUS23_HUMAN	63.33	16,588	8.15	1.93
U1 small nuclear ribonucleoprotein C	sp|P09234|RU1C_HUMAN	30.19	17,394	10.14	1.92
Ribosome biogenesis protein BRX1 homolog	sp|Q8TDN6|BRX1_HUMAN	28.05	41,401	10.60	1.92
Pescadillo homolog	tr|B3KXD6|B3KXD6_HUMAN	50.33	51,464	4.87	1.92
WD repeat-containing protein 70	tr|D6RIW8|D6RIW8_HUMAN	27.39	43,240	4.69	1.92
Translocation protein SEC62	sp|Q99442|SEC62_HUMAN	31.58	45,862	7.09	1.92
28S ribosomal protein S23, mitochondrial	sp|Q9Y3D9|RT23_HUMAN	56.84	21,771	9.28	1.92
Nucleobindin-2	sp|P80303|NUCB2_HUMAN	46.9	50,196	4.78	1.92
Complement component 1 Q subcomponent-binding protein, mitochondrial	sp|Q07021|C1QBP_HUMAN	57.45	31,362	4.47	1.92
WD40 repeat-containing protein SMU1	sp|Q2TAY7|SMU1_HUMAN	31.77	57,544	7.15	1.92
Chromatin target of PRMT1 protein	sp|Q9Y3Y2|CHTOP_HUMAN	67.74	26,397	12.74	1.91
Copper chaperone for superoxide dismutase	tr|J3KNF4|J3KNF4_HUMAN	38.43	27,086	5.76	1.91
Isoform 2 of Treacle protein	sp|Q13428-2|TCOF_HUMAN	27.85	144,314	9.78	1.91
Eukaryotic translation initiation factor 5B	sp|O60841|IF2P_HUMAN	35.98	138,827	5.15	1.89
DDRGK domain-containing protein 1	sp|Q96HY6|DDRGK_HUMAN	50.32	35,611	4.81	1.89
28S ribosomal protein S22, mitochondrial	sp|P82650|RT22_HUMAN	47.49	41,280	7.85	1.89
CAP-Gly domain-containing linker protein 1	tr|J3KP58|J3KP58_HUMAN	38.75	148,129	4.99	1.89
SAFB-like transcription modulator	sp|Q9NWH9|SLTM_HUMAN	31.24	117,148	7.93	1.89
Pentatricopeptide repeat domain-containing protein 3, mitochondrial	sp|Q96EY7|PTCD3_HUMAN	25.54	78,550	6.37	1.88
Ubiquitin-like modifier-activating enzyme 1	sp|P22314|UBA1_HUMAN	57.94	117,849	5.50	1.88
Isoform 3 of UPF0696 protein C11orf68	sp|Q9H3H3-3|CK068_HUMAN	46.08	31,517	6.25	1.88
Small nuclear ribonucleoprotein F	sp|P62306|RUXF_HUMAN	66.28	9,725	4.34	1.87
Isoform 3 of Cullin-4B	sp|Q13620-3|CUL4B_HUMAN	41.84	84,017	6.74	1.87
Epithelial cell adhesion molecule	sp|P16422|EPCAM_HUMAN	41.72	34,932	7.40	1.85
26S protease regulatory subunit 8	sp|P62195|PRS8_HUMAN	81.77	45,626	7.48	1.78
Protein S100-A8	sp|P05109|S10A8_HUMAN	67.74	10,835	6.98	1.72
Isoform B of Nucleoporin SEH1	sp|Q96EE3-1|SEH1_HUMAN	45.84	46,578	7.87	1.68
FAS-associated death domain protein	sp|Q13158|FADD_HUMAN	45.19	23,279	5.40	1.51

We further examined the expression of NUCB2 in primary breast tumors and paired metastatic lymph node tissues. NUCB2 staining was found predominantly in the cytoplasm of breast epithelial cells. The majority of the tissues examined showed weak staining of NUCB2 (TIS ≤ 4). NUCB2 expression was lower in metastatic lymph node than primary breast tumor tissues ($P < 0.001$). (**Figure 2**). The positive expression rate of NUCB2 was 82.1% (87/106) in primary breast tumors and 47.2% (50/106) in metastatic lymph nodes, respectively. The expression of NUCB2 in primary breast tumors and metastatic lymph nodes was summarized in **Table 4**. The results show that NUCB2 expression was down-regulated in metastatic lymph node tissues compared with primary breast tumors.

Table 3. Basic biological information about NUCB2

Name	Access number	Molecular weight	Isoelectric point	Cellular compartment	Molecular function
Nucleobinding-2	sp | P80303 | NUCB2-HUM	50,196	4.80	Nucleus	Ion binding
				Cytoplasm	DNA binding
				Cytosol	
				Extracellular region	
				Plasma membrane	
				Golgi apparatus	
				Endoplasmic reticulum	
				Nuclear envelope	
				Extracellular space (secreted)	
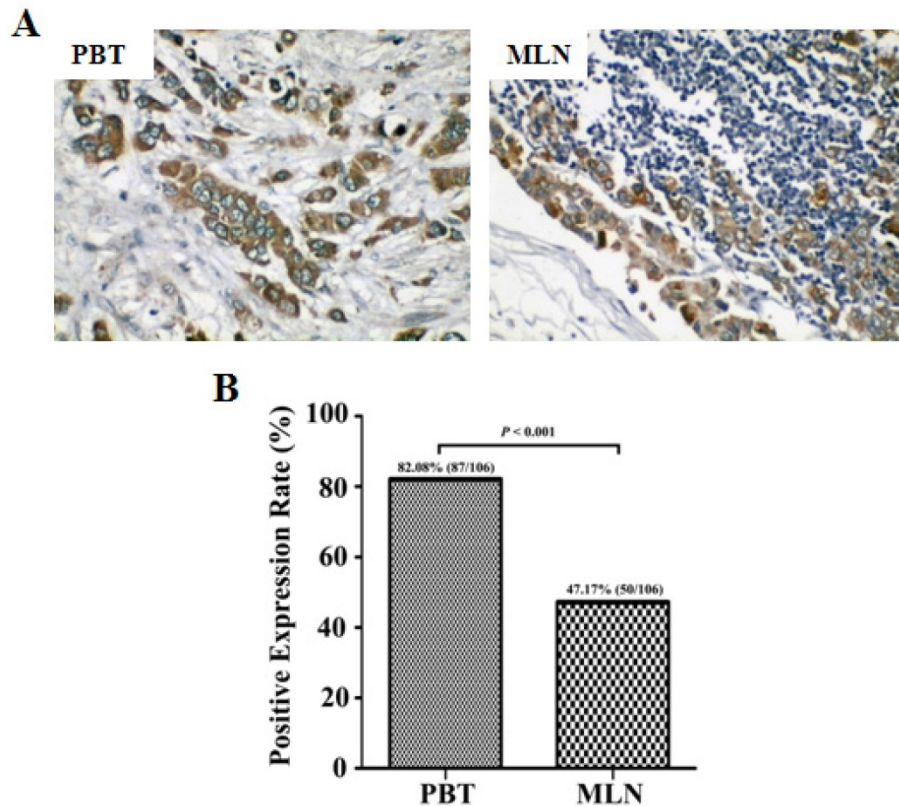
Figure 2. Expression of NUCB2 protein in primary breast tumors and metastatic lymph node tissues. The paraffin-embedded tissue sections were immunohistochemically stained for NUCB2 expression and statistically evaluated by the χ^2 test. (A) Representative immunohistochemical images showing the predominantly cytoplasmic staining of NUCB2 in breast tumor samples. Original magnification: 200×. (B) Summary of the positive expression rate of NUCB2 in breast tumor samples. PBT, primary breast tumor; MLN, metastatic lymph node.

Correlation between NUCB2 expression and clinicopathological parameters

The correlation between NUCB2 expression and the clinicopathological parameters of breast cancer patients was analyzed by χ^2. NUCB2 expression in breast tumors was significantly correlated with the extent of nodal invasion ($P = 0.000$), poor clinical stage ($P = 0.001$), and ER positivity ($P = 0.020$) (**Table 5**). On the other hand, no association was found between positive expression of NUCB2 and age, histological type, histological grade, tumor size, PR, HER2/c-erbB-2, menstrual history, or modus operandi. Kaplan-Meier analysis revealed that the overall survival time of patients with positive NUCB2 expression was significantly shorter than those with negative NUCB2 expression ($P = 0.004$, **Figure 3**). In addition, Cox regression analysis showed that among all the risk factors examined, only high expression of NUCB2 ($P = 0.025$, RR = 2.065) was a risk factor for short overall survival of breast cancer patients (**Table 6**). This means that high positive expression of NUCB2 is indeed associated with short overall survival time of breast cancer patients. Meanwhile, positive expression of NUCB2 in primary breast tumor is an independent risk factor for poor prognosis of breast cancer patients regardless of age, histological type, histological grade, nodal status, tumor size, clinical stage, ER, PR, HER2/c-erb-B2, menstrual history, and modus operandi.

Discussion

Approximately 10-15% of patients with breast cancer have an aggressive disease and develop distant metastases within 3 years after the initial detection of the primary tumor. As it is not possible to accurately predict the risk of metastasis development in individual patients, 80% of the patients received adjuvant chemotherapy, among which 40% relapse and ultimately die of metastatic breast cancer [2]. Thus, we need to identify effective biomarkers or establish metastatic models to predict the occurrence of breast cancer metastasis to provide a better treatment for these patients. At present, many researches are focused on the different proteins of the primary tumor between breast cancer patients with or without lymph node metastases [13], or exploring the different proteins between cell lines with different metastatic potential [14-17]. However, no comparison has been made between the primary site and the metastatic site in patients with metastatic breast cancer.

Table 4. Expression of NUCB2 in primary breast tumors and metastatic lymph nodes

| | NUCB2 expression | | | | | |
| | TIS (0) | | TIS (1-4) | | TIS (5-8) | |
	n	%	n	%	n	%
PBT	19	17.9	73	68.9	14	13.2
MLN	56	52.8	47	44.3	3	2.8

TIS, total immunostaining score; PBT, primary breast tumor; MLN, metastatic lymph node.
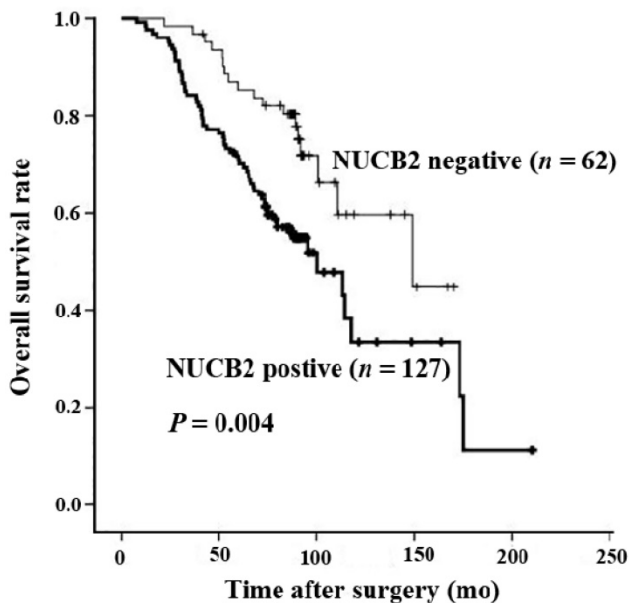
Figure 3. Kaplan-Meier survival curves of patients with positive and negative NUCB2 expression. The overall survival rate of breast cancer patients that were positive or negative for NUCB2 expression was calculated with the Kaplan-Meier method and examined with log-rank test. There was significant difference between the two groups (log-rank *P* = 0.004).

Table 5. Relationship between NUCB2 expression and clinicopathological parameters in breast cancer patients

| Characteristics | No. of patients | NUCB2 expression | | *P* value* |
		No	Yes	
Age at diagnosis (years)				0.304
< 50	112	40	72	
≥50	77	22	55	
Histological type				0.555
Ductal	154	52	102	
Lobular	35	10	25	
Histological grade				0.632
I- II	33	12	21	
III	156	50	106	
Nodal status				0.000
Negative	83	43	40	
Positive	106	19	87	
Tumor size (d, cm)				0.277
d ≤ 2	56	22	34	
2 < d ≤ 5	112	36	76	
d > 5	20	4	16	
Unknown #	1			
Clinical stage				0.001
I	29	15	14	
II	100	38	62	
III	59	9	50	
Unknown #	1			
ER				0.020
Negative	90	37	53	
Positive	99	25	74	
PR				0.165
Negative	78	30	48	
Positive	111	32	79	
C-erbB-2				0.095
Negative	57	23	34	
Positive	122	34	88	
Unknown #	10			
Menstrual history				0.592
Premenopause	123	42	81	
Postmenopause	66	20	46	
Modus operandi				0.442
Radical mastectomy	82	29	53	
Modified radical mastectomy	100	30	70	
Other type #	7			

* χ^2 test.
# Unknown and other type cases are excluded from *P*-value calculation.

Table 6. Cox regression analysis of risk factors for short overall survival time of breast cancer patients

Variables	B	SE	Wald	Sig.	Exp (B) (95% CI)
Age	-0. 005	0. 020	0. 066	0. 797	0. 995 (0.956-1.035)
Histological type	0. 423	0. 298	2. 017	0. 156	1. 526 (0.852-2.735)
Histological grade	0. 654	0. 450	2. 056	0. 152	1. 906 (0.789-4.602)
Nodal status	0. 338	0. 269	1. 575	0. 209	1. 402 (0.827-2.375)
Tumor size	0. 093	0. 235	0. 156	0. 693	1. 097 (0.692-1.738)
Clinical stage	0. 140	0. 413	0. 115	0. 734	1. 151 (0.512-2.587)
Estrogen Receptor	-0. 218	0. 334	0. 428	0. 513	0. 804 (0.418-1.546)
Progesterone Receptor	-0. 430	0. 342	1. 579	0. 209	0. 651 (0.333-1.272)
C-erbB-2	-0. 70	0. 279	0. 062	0. 803	0. 933 (0.540-1.612)
Menstrual history	0. 153	0. 406	0. 141	0. 707	1. 165 (0.526-2.581)
Modus operandi	0. 281	0. 203	1. 921	0. 166	1. 324 (0.890-1.969)
NUCB2 expression	0. 725	0. 323	5. 027	0. 025*	2. 065 (1.096-3.891)

95% CI: 95% confidence interval
*$P < 0.05$

In the present study, we employed the iTRAQ proteomic technique to screen the differentially expressed proteins between primary breast tumor and metastatic lymph node tissues. The initial search identified 4,837 confidential proteins and 643 differentially expressed proteins. Compared with primary breast tumors, 402 proteins were up-regulated and 241 proteins down-regulated in metastatic lymph nodes. The majority of these proteins are associated with RNA/DNA binding, enzyme binding and cytoskeleton protein binding, etc. These proteins are involved in lamellipodia formation [18, 19], angiogenesis [20, 21], circulating tumor cells [22], EMT [23], and tumor microenvironment [24] and may affect cell motility [25], cell proliferation [26, 27], invasion, migration, and metastasis [28, 29]. At the same time, several inflammatory factors were identified, such as complement C1r and complement component C8 gamma chain (C8G) etc. This might be an indication of a complex background including inflammatory response in the development of breast cancer metastasis [30, 31].

NUCB2 is a calcium-binding protein composed of 396 aa residues with a 24-aa signal peptide [23]. The human NUCB2 gene spans 55 kb and contains 14 exons and 13 introns [32]. It is highly conserved across mammalian and non-mammalian vertebrates, suggesting its physiological importance [33]. It is ubiquitously expressed in normal tissues, such as lymphoid tissue, pancreas, gut, and adipose [34, 35]. NUCB2 appears to play an important role in regulating energy homeostasis and food intake [36]. Adipose tissue plays important role in energy homeostasis and NUCB2 protein expression was significantly increased in high-fat-fed mice [37]. Li *et al.* reported that in high fat diet induced obese mice, gastric mTOR signaling and NUCB2 were increased [38]. Moreover, the activity of gastric mTOR is linked to the regulation of gastric NUCB2. A relation between obesity and ovarian cancer mortality was established and the N-terminal fragment of NUCB2 can inhibit the proliferation of human ovarian epithelial carcinoma HO-8910 cells through inducing apoptosis via the mTOR and RhoA/ROCK signaling pathway [39].

In addition, NUCB2 was also reported to be related to the development and progression of renal clear cell carcinoma [40] and prostate cancer [41-43]. In breast cancer, it has a positive association with lymph node metastasis and the ER status of the patients. Results of siRNA transfection experiments showed that NUCB2 was involved in cell proliferation, migration, and invasion in both ER-positive MCF-7 and ER-negative SK-BR-3 cells [44]. In our study, we analyzed the correlation between NUCB2 expression and clinicopathological features in 189 breast cancer patients. We demonstrated that NUCB2 has a significant positive correlation with the extent of nodal invasion, poorly clinical stage, and ER positivity. Obesity causes an increase in local estrogen production, which has a close correlation with breast cancer [45]. Suzuki *et al.* [23] demonstrated that NUCB2 was up-regulated by estrogen and our study showed that there was a positive correlation between NUCB2 expression and the ER status. Moreover, we found that breast cancer patients with high NUCB2 expression had a significantly poorer overall survival. Cox hazard regression analysis showed that NUCB2 expression and clinical stage were associated with overall survival of breast cancer. All these observations suggest that NUCB2 plays an important role in breast cancer progression and may be a potential prognostic biomarker for poor survival of breast cancer patients. Compared with the recently described serum biomarkers for breast cancer metastasis such as Y-box binding protein 1 (YB-1) [46] and HER2 [47], the immunohistochemical biomarker NUCB2 offers the advantages of increased sensitivity and higher specificity, because immunohisto-chemistry can reveal a biomarker change at an earlier stage and in a more specific location than a serum biomarker.

In summary, several differentially expressed proteins involved in breast cancer metastasis were identified by iTRAQ. NUCB2 was one of the highest expressed proteins in the paired primary breast tumors and metastatic lymph nodes and showed positive correlation with poor survival of breast cancer patients. NUCB2 has the potential to be used as an effective and novel biomarker for the prognostic prediction of breast cancer.

Acknowledgements

Competing Interests

The authors have declared that no competing interest exists.

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