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Original Article

High expression of GLUT1 and GLUT3 correlate with neoadjuvant chemotherapy ineffectiveness breast cancer patients

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Abstract: Background: Researches have proven that cancer cells are at accelerated glucose metabolism than benign tumor cells. In order to survive and proliferation, more glucose transport into malignant tumor cells to sustain its viability. The glucose transporters (GLUTs) are responsible for this transportation activity. The purpose of this study was to compare the expression level of GLUT1 or GLUT3 in the different clinical response groups of breast cancer neoadjuvant chemotherapy (NAC) patients. Methods: Expression of Glucose transporter 1 (GLUT1) and Glucose transporter 3 (GLUT3) were analyzed by immunohistochemistry (IHC) on the 49 untreated breast cancer specimens by core needle biopsy, GLUTs expression diversity were assessed in different NAC response groups. Results: GLUT1 expression was significant higher in progressive disease (PD) group and stable disease (SD) group than in partial response (PR) group; GLUT3 expression was significant higher in PD group than in SD group and PR group. Conclusion: Both GLUT1 and GLUT3 expression tended to be higher in poor NAC effectiveness breast cancer patients, GLUT1 and GLUT3 may suggest predictive value in breast cancer NAC effectiveness.

Keywords: Glucose transporter 1 (GLUT1), glucose transporter 3 (GLUT3), breast cancer, neoadjuvant chemotherapy (NAC), immunohistochemistry (IHC)

Introduction

Since Warburg explored the glucose metabolic feature of malignant tumor [1], great improvements has been made concerning to cancer glucose metabolism [2]. Cancer cells use extra glucose as its energy resources to sustain malignant proliferation even in the poorly oxygen condition [3], this phenomenon is considered the cause of the cancer's hallmarks [4], which is closely related to oncogenesis and promote cancer develop.

GLUTs are a cluster of proteins which can facilitate glucose enter into tumor cells, thus providing nutrition for the living of tumor cells and connected with elevated tumor metabolism [5]. Studies shown over-expression of GLUTs is closely related with tumor malignancy and unfavorable prognosis to cancer patients [6-12].

Breast cancer has becoming a leading threat to woman's life worldwide. NAC is a widely used form of therapy which could down-stage the tumor thus offer a chance for breast conserving operations, furthermore, NAC could predict the effectiveness of a certain chemotherapy regimen for subsequent treatment and NAC response have been proven to be related to the prognosis and survival [13].

AS a heterogeneous disease, the prognosis of breast cancer depending on many factors [14, 15]. This study focus on the expression of two GLUTs, GLUT1 and GLUT3, in the newly diagnosed breast cancer patients, and trying to elucidate whether connections exist between NAC effectiveness and GLUT1/GLUT3 expression.

Materials and methods

Patients' selection

All the included study subjects were diagnosed and treated from Jinling Hospital Breast Surgery

Center, Medical School of Nanjing University. The inclusion criteria were as follows: Newly pathologic diagnosed breast cancer patients by core needle biopsy; No distant metastasis confirmed by head magnetic resonance, Chest and abdomen CT and bone scan; No history of malignant tumors or hereditary breast cancer; No history of chemotherapy, radiotherapy and endocrinotherapy. By searching electronic me-dical record system for breast cancer patients from June 2011 to June 2015, 441 female breast cancer patients were treated in our center, then we included 114 neodjuvant chemotherapy BC patients, in order to analysis, we finally included 49 clinicopathologic data complete patients. All the 49 patients received 2-4 cycle (every 3 weeks) TE (docetaxel and epirubicin) or TEC (docetaxel, epirubicin and cyclophosphamide) neoadjuvant chemotherapy regimens before surgery.

Data collection

Breast ultrasound were implemented before each cycle of NAC to assess the size of the target tumor, maximum diameter of the lesions were documented to evaluate the chemotherapeutic efficacy according to Response Evaluation Criteria in Solid Tumors (RECIST version 1.1) [16] tumor response are expressed as complete response (CR): disappearances of all targeted lesions; Partial response (PR): more than 30% decrease of diameters of target lesions; Progressive disease (PD): at least a 20% increase in the size of the lesions; stable disease (SD): neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD. Pathology information of tumor sizes and lymph node status were analyzed by pathology department after surgery for the tumor staging according to the American joint committee on cancer staging manual (AJCC 7th edition). ER, PR, Her-2 and Ki-67 status were routinely analyzed by pathology department for the classify of breast cancer subtype according to 2013 St Galen international expert consensus [17] Her-2 status were considered positive when immunostaining was 3+ and negative when 0-1+, further evolution were implemented by FISH analysis for Her-2 amplification when IHC analysis of 2+. Baseline demographics and anthropometric data as well as tumor grade were reviewed for analysis.

Immunohistochemistry

Breast carcinoma Pathological sections of the 49 patients were collected from pathology department of Jinling Hospital for IHC analysis of GLUT1 and GLUT3. The determination of GLUT1 and GLUT3 expression were performed on 5-μm sections from formalin-fixed, paraffin-embedded core needle aspiration biopsy tissue. Polyclonal rabbit anti-GLUT1 and anti-GLUT3 antibodies were purchased from Abcam company (Cambridge, USA) through Beijing Zhongshan Golden Bridge Biotechnology Company.

The sections were deparaffinized and rehydrated by a series of xylene and ethanol. Antigen retrieval was performed by boiling in EDTA PH 9.0 for 15 minutes. Endogenous peroxidase activity was blocked by 25 minutes incubation in 3% H2O2 in phosphate buffer. The primary antibodies to GLUT1 and GLUT3 were incubated overnight. Next, sections were incubated with the secondary antibody (HRP anti-rabbit) for 50 minutes. Then, sections were incubated with DAB substrate for 10 minutes. Subsequently sections were counterstained with haematoxylin, dehydrated in graded ethanol and xylene and coverslipped. Image pro-plus 6.0 software was used for evaluate the integrated optical density (IOD) in digitized picture. 3 photos were taken in the randomly chosen fields of each section stained by GLUT1 antibody and GLUT3 antibody for the analysis of IOD value. mean value was recorded as the final IOD value of each GLUT1 and GLUT3 section.

Statistical analysis

This study aims to evaluate the relationship between GLUT1/GLUT3 expression and the NAC effectiveness. Logarithmic transformation was used when continuous variable did not meet normal distribution, Data from all quantitative assays were expressed as means ± SD. Pearson's chi-square test were used to compare the difference in categorical variables, Partition of chi-square was used to evaluate the differences between groups, Bonferroni correction was used to revise the significant level. Student's T test was used to compare the difference in continuous variables. Statistical comparison between more than two different groups was performed using one-way ANOVA followed by SNK-q test. Two sided *P*-values <0.05 were considered as statistic significant differ-

Table 1. Clinicopathologic characteristics in 49 breast cancer subjects

Age, years, mean ± SD	48.9 ± 1.4
Menopausal, n (%)	
Premenopausal	28 (57%)
Postmenopausal	21 (43%)
Tumor size, n (%)	
T1	21 (43%)
T2	24 (49%)
T3	3 (6%)
T4	1 (2%)
Lymph node status, n (%)	
N0	16 (33%)
N1	10 (20%)
N2	14 (29%)
N3	9 (18%)
Histological grade, n (%)	
G1	0
G2	19 (39%)
G2~G3	19 (39%)
G3	8 (16%)
unknown	17 (35%)
Histological types, n (%)	
IDC	5 (10%)
other	46 (94%)
AJCC stage, n (%)	
IA	3 (6%)
IIA	7 (14%)
IIB	10 (21%)
IIIA	7 (14%)
IIIC	9 (18%)
ER, n (%)	
Positive	14 (29%)
Negative	9 (18%)
PR, n (%)	
Positive	33 (67%)
Negative	16 (33%)
HER-2, n (%)	
Positive	27(55%)
Negative	22 (45%)
Ki-67	21 (43%)
≤14%	14 (29%)
>14%	35 (71%)
Subtype, n (%)	
lumina A	8 (16%)
lumina B	24 (49%)
Her-2 over expressing	10 (21%)
TNBC	7 (14%)

ence. SPSS (version 21) software for windows was used for analysis.

Results

The clinicopathologic characteristics of the included subjects are listed in **Table 1**. the mean age of the breast cancer patients was 48.9 years (range, 30-69 years), most women were premenopausal (57%). Invasive ductal carcinoma (94%) were the major Histological type and the hormone receptor tended to be positive, T1 and T2 tumor comprised 92% of the included patients. 71% tissue samples were Ki-67 >14%. About 2/3 patients were Lymph node metastasis.

Based on the RECIST criteria, 10 (20%) patients were defined as PR, 11 (22%) patients were defined as SD, and 28 (56%) patients were defined as PD. No CR patient. The clinicopathologic information of PR, SD and PD groups were compared in the **Table 2**. As illustrated in the **Table 2**, we concluded that the tumor size of the three groups were different, after adjusted significant level by Bonferroni correction (0.05/3) and perform multiple comparisons between groups, tumor sizes were significant larger in PD group than PR and SD group, while tumor sizes in PR and SD group did not meet significant difference (Showed in **Table 3**). AS to tumor subtype, we didn't find significant difference after multiple comparisons were performed although initial comparison exist difference (Showed in **Table 4**).

The expression of GLUT1 and GLUT3 was measured by the integrated optical density (IOD). The IOD value is positive correlated with the expression intensity of GLUT1 and GLUT3 proteins.

The expression of GLUT1 and GLUT3 was observed in all of the included cases. **Figure 1A-D** are representative pathological image of GLUTs IHC staining. **Figure 1A** and **1C** shows high GLUT1 and GLUT3 IHC staining respectively. **Figure 1B** and **Figure 1D** shows low GLUT1 and GLUT3 IHC staining respectively. The mean value of expression intensity of GLUT1 and GLUT3 in PR, SD and PD groups was summarized in **Table 5**. After one-way ANOVA, we found significant difference exist in the 3 groups mentioned above in the GLUT1 and GLUT3 expres-

Table 2. Comparison of clinicopathologic characteristics in PR, SD and PD groups

Parameter	PR (n=10)	SD (n=11)	PD (n=28)	*P*
Age ($\bar{x}$ ± s)	45.0 ± 10.2	50.5 ± 8.6	49.7 ± 10	0.361
Menopausal				
Premenopausal	9	5	14	0.059
Postmenopausal	1	6	14	
Tumor size				
<T2	9	8	4	<0.001
≥T2	1	3	24	
HER-2				
Positive	5	2	14	0.189
Negative	5	9	14	
Ki-67				
≤14%	3	4	7	0.833
>14%	7	7	21	
Subtype				
Lumina	8	10	14	0.03
Her-2+TNBC	2	1	14	

Table 3. Multiple comparisons subjected to tumor size

	multiple comparisons between groups								
	PR	SD	*P*	SD	PD	*P*	PR	PD	*P*
<T2	9	8	0.586	8	4	0.001	9	4	<0.001
≥T2	1	3		3	24		1	24	

Significant level was revised by Bonferroni correction: 0.05/3=0.0167.

Table 4. Multiple comparisons subjected to tumor subtype

	multiple comparisons between groups								
	PR	SD	*P*	SD	PD	*P*	PR	PD	*P*
Lumina	8	10	0.586	10	14	0.028	8	14	0.143
Her-2+TNBC	2	1		1	14		2	14	

Significant level was revised by Bonferroni correction: 0.05/3=0.0167.

sion level. We then used SNK-q test in order to conduct multiple comparison, we concluded that GLUT1 expression was significant lower in Partial Response (PR) group than in Stable Disease (SD) group and Progressive Disease (PD) group (Showed in **Table 6A**), GLUT3 expression was significant lower in Partial Response (PR) group and Stable Disease (SD) group than in Progressive Disease (PD) group (Showed in **Table 6B**).

Discussion

14 glucose transporter subtypes had been identified so far [5]. GLUT1-5 were considered most closely related with glucose transportation [7] and the distributions are different under Physiologi-cal conditions: GLUT1 represents the most ubiquitously expressed isoform [18], GLUT2 were mainly detected in the liver, kidney, and intestine [19]. GLUT3 dominant expression in the brain in various species [20]. GLUT4 is related with glucose uptake of skeletal and muscle [21]. GLUT5 is a fructose transporter and responsible for the uptake of fructose from the small intestine [22].

GLUT overexpression is reported to be associated with an increased risk of different types of cancer, including lung, breast, colorectal, and ovary cancers [23]. GLUT1 and GLUT3 overexpression could link cancer have been extensively investigated and discussed. A study by Krzeslak A demonstrated that both GLUT1 and GLUT3 were highly expressed in endometrial and breast cancers [9]. furthermore, higher levels of GLUT1 and GLUT3 expression are associated with increased malignant potential and poor prognosis in cancers. Grover-McKay M's in vitro study demonstrated a strong and direct association between GLUT1 expression and breast cancer cell invasiveness by in situ immunohistochemical staining [6]. Jang S. M's findings indicated that GLUT1 expression was higher in malignant transformation in breast cancer and was correlated with higher histological grade, larger tumor size, absence of estrogen receptor, absence of progesterone receptor, and triple-negative phenotype. GLUT1 expression was also an independent prognostic factor of poorer overall survival and disease-free survival [24]. Masin M' s research provided evidence that GLUT3 is strongly up-regulated during epithelial-mesenchymal transition and contributes to glucose uptake in lung tumor cells [12]. Similar results have been observed by Kocdor M. A that GLUT-3 expression is increased in Estrogen-induced breast carcinogenesis [25]. It was reported that GLUT1 and GLUT3 expression is upregulated by hypoxia-inducible factor-1α (HIF-1α) which is induced by low oxygen conditions and found in high levels in malignant solid tumors [26, 27].

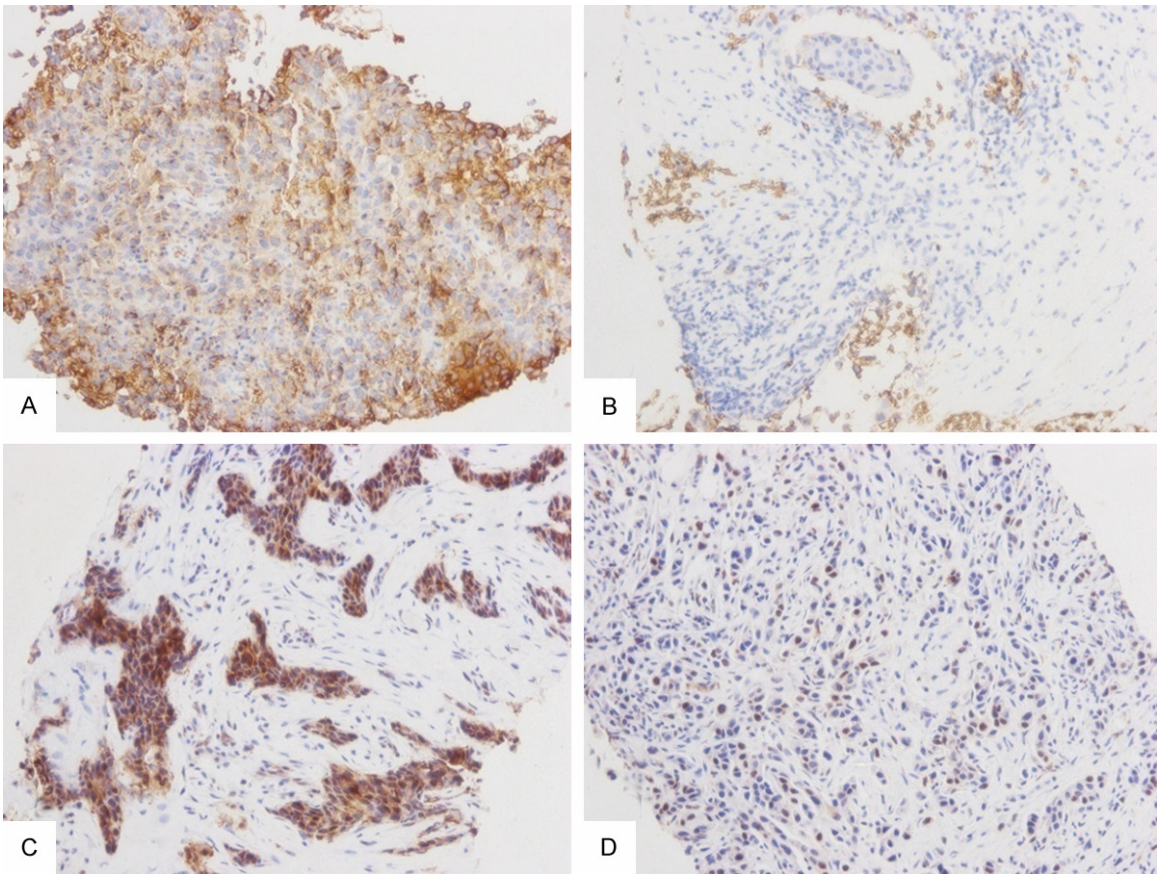

Figure 1. Representative photomicrographs of glucose transporter 1 (GLUT1) and glucose transporter 3 (GLUT3) immunostaining in invasive ductal carcinomas (×200). A. GLUT1 high expression (IOD=46851.43), B. GLUT1 low expression (IOD=4053.245), C. GLUT3 high expression (IOD=26017.62), D. GLUT3 low expression (IOD=609.5662).

Table 5. Comparison of GLUT1 and GLUT3 protein expression in PR, SD and PD group

	GLUT expression (IOD)	
GROUP	GLUT1	GLUT3
PR (n=10)	2.36 ± 0.42	2.83 ± 0.82
SD (n=11)	3.51 ± 0.22	2.89 ± 0.39
PD (n=28)	3.72 ± 0.58	3.44 ± 0.53
F-value	28.135	6.157
p-value	<0.001	0.004

In the present study we investigated the expression of GLUT1 and GLUT3 in 49 breast cancer core needle aspiration tissues, and evaluated the correlations between GLUT1 and GLUT3 expression and patient NAC effectiveness. Of the 49 breast cancer patients after systemic treatment with surgery and chemotherapy, with the mean age of 48.9 years which showed the

Table 6. GLUT1 and GLUT3 expression difference between groups by multiple comparisons

A. Multiple comparison in GLUT1 level by IOD			
Group	N	Subset for alpha = 0.05	
		1	2
PR	10	2.3562	
SD	11		3.5092
PD	28		3.7153
Sig		1.000	0.290
B. Multiple comparison in GLUT3 level by IOD			
Group	N	Subset for alpha = 0.05	
		1	2
PR	10	2.8315	
SD	11	2.8917	
PD	28		3.4433
Sig		0.789	1.000

similar characters of China [28]. Three categories of response was determined according to RECIST guideline in this study, the primary target lesions was larger in PD group than in PR and SD groups. This result is consistent with a former study showing that Breast cancer NAC effectiveness negatively correlate with tumor size, tumor size was independent predictors of car [29]. After characterizing the immunohistochemical (IHC) expression of Glut-1 and GLUT3 in patients with each groups we found that Glut-1 expression was significantly higher in PD and SD group than in PR group; besides, GLUT3 expression was significantly higher in PD group than in SD and PR group. These results suggest elevated expression of GLUT1 and GLUT3 trend With the decrease of the effect of breast cancer NAC. Cancer cells need more oxygen for rapid proliferation, under the hypoxic conditions, a transcription factor named Hypoxia inducible factor (HIF-1) that controls the cellular adaptation of transformed cells to low oxygen is activated, GLUTs expression are transcriptionally increased by HIF-1 following hypoxic conditions, larger amounts of glucose can transport into cancer cells to expanding the tumor mass [26]. this may be the reason high expression of GLUT1 and GLUT3 reduces the effectiveness of NAC of breast cancer patients. We speculate that GLUT1 and GLUT3 may serve as predicted value for breast cancer NAC effectiveness [30].

To conclude, our results provide evidence that high expression of GLUT1 and GLUT3 are related with poor NAC effectiveness in breast cancer patients, which provide orientation for breast cancers treatment. Further research is needed by combining chemotherapy and GLUT1/GLUT3 targeted therapy to promote breast cancer therapeutic efficacy. GLUT1 and GLUT3 may serve as a therapeutic target in the treatment of breast cancer.

Disclosure of conflict of interest

None.

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