Positive correlation between expression level of mitochondrial serine hydroxymethyltransferase and breast cancer grade

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Abstract: Metabolic reprogramming plays an essential role in supporting the survival and proliferation of cancer cells. Serine hydroxymethyltransferase (SHMT) directs serine to the metabolism of one-carbon unit and the synthesis of thymidilate as a key factor in this metabolic shift. Although the mitochondrial isoform of SHMT (SHMT2) has been proven to be a crucial factor in the serine/glycine metabolism in several cancer cell types, the expression pattern of SHMT2 and the correlation of expression level of SHMT2 and other clinicopathological parameters in clinical breast cancer remain to be explored. In this research, 76 breast cancer patients who underwent modified radical mastectomy were enrolled for immunohistochemical analysis of the expression level of SHMT2 in their cancerous breast tissues for comparison with that in matching, distant noncancerous tissues. The results showed that SHMT2 was not expressed in the distant noncancerous cells. In contrast, SHMT2 protein could be stained in all breast cancer samples at varying degrees. Higher level of SHMT2 was expressed in grade III breast cancer cells than those in grade I–II (P<0.05). In conclusion, SHMT2 was highly expressed in breast cancer cells, and the expression level of SHMT2 was positively correlated with breast cancer grade, suggesting that SHMT2 could be a target for anticancer therapies.

Keywords: SHMT2, breast cancer, histological grading, predictive biomarkers

Introduction

Breast cancer is the most common malignant tumor in women, with a rapidly increasing incidence in recent years. Despite advancements in early detection and treatment of breast cancer, it is still the leading cause of death among all cancer-related deaths in women worldwide. Clinically, tumor stage and histological grade have been useful for evaluating and predicting breast cancer progression. And different tumor markers, such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor-2 (HER2), could lead to different responses to clinical treatment and different prognoses. Early detection, which could guide therapy, is still the key to survival of patients. Thus, identification and evaluation of novel tumor markers will be helpful for early detection of breast cancer and the development of novel therapeutic targets for treatment of breast cancer patients.

At present, increasing evidences have implicated the essential role of metabolic reprogramming in supporting the survival and proliferation of cancer cells. Particularly, it is recently been confirmed that hyperactivation of serine/glycine biosynthetic pathway drives tumorigenesis. The serine synthesis pathway utilizes the glycolytic intermediate glyceraldehyde-3-phosphate, which is catalyzed to yield serine by phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase 1 (PSAT1), and
phosphoserine phosphatase (PSPH).16,17,21 And, serine can be
transformed into glycine by serine hydroxymethyltransferase
(SHMT).22,23 Although the mitochondrial isoform of SHMT
(SHMT2) has been shown to be a crucial factor for the
serine/glycine metabolism of several cancer cell types,24–27
the expression pattern of SHMT2 and the correlation of
expression level of SHMT2 and other clinicopathological
parameters in clinical breast cancer remain to be explored.

In this study, we evaluate the expression level of SHMT2
protein in breast cancer cells and distant normal tissue samples
by immunohistochemical streptavidin peroxidase-conjugated
(SP) method in order to figure out whether this protein is
clinically associated with breast cancer. Furthermore, the
correlation between the expression level of SHMT2 and
clinicopathological parameters from breast cancer patients
was demonstrated.

Materials and methods
Materials
Fresh biopsy specimens of breast cancer tissue and normal
breast tissue from the incisal margin were collected from
76 patients with breast cancer who underwent radical surgery at
Ningbo First hospital. None of the patients, aged 32–73 years
(mean age, 49), had received any chemotherapy, radiotherapy,
or other adjuvant therapy before the operation. This study was
approved by The Ethics Committee of Ningbo First hospital
and all patients provided informed consent. Fifty-six specimens
were confirmed pathologically as infiltrating lobular carcinoma
(ILC), and the other 20 specimens were invasive ductal carci-

toma (IDC). Tumors were diagnosed and classified according
to the American Joint Committee on Cancer-breast cancer the

tumor node metastasis (TNM) staging system
28 and the World
Health Organization breast cancer histology classifications.29

Immunohistochemical streptavidin
peroxidase-conjugated method
(SP method)
All fresh specimens were fixed with formalin and embedded
in paraffin according to the standard protocol. Tissue sec-
tions were deparaffinized and rehydrated routinely and then
subjected to antigen retrieval by placing slides in 1× citrate
buffer for 15 minutes at 100°C in a microwave oven. After
treatment with 3% H2O2 for 30 minutes, the sections were
incubated with 20% normal serum for 50 minutes and then
with the primary antibody overnight at 4°C. The primary
antibodies were ER (ab32063, Abcam, Cambridge, UK), PR
(ab32085, Abcam), HER2 (ab8282, Abcam), and SHMT2
(ab180786, Abcam). On the following day, the sections
were washed with PBS thrice and then processed using an
ultrasensitive TM S-P kit (Maixin Biotechnology, Fuzhou,
People’s Republic of China). After the washes in PBS, the
color reaction was conducted using a 3,3′-diaminobenzidine
kit (Maixin Biotechnology). The sections were counter-
stained with hematoxylin and covered with a coverslip.

The stained tissue sections were reviewed and scored
independently by two pathologists (Dr Jian Wang and Ming
Li). The percentage of SHMT2 positive cells was rated as
follows: –, ≤5% positive tumor cells; +, 5%–30% positive
cells; ++, 30%–55% positive cells; and ++++, >55% positive
cells. ER and PR positivity was defined as strong nuclear
staining in at least 3/8 of the tumor cells reviewed. HER2/neu
positivity was defined as strong (3+) membranous staining in
at least 10% of tumor cells, whereas scores of 0 to 2+ were
regarded as negative.

Statistical analysis
Data were analyzed by SPSS 19.0 statistical software (IBM
Corporation, Armonk, NY, USA). Measurement data were
analyzed by Student’s t-test, while categorical data were
analyzed by the chi-square test. P<0.05 was considered as
significant.

Results
Differential expression level of SHMT2
protein in breast cancer and its distant
noncancerous tissues
We first detected expression level of SHMT2 protein by
immunohistochemical staining in breast cancer cells and the
matching distant normal tissue samples from 76 patients of
which 56 were ILC and 20 were IDC. The results of SHMT2

Table 1 The proportion of SHMT2 positive samples in breast cancer

<table>
<thead>
<tr>
<th>Class</th>
<th>Number</th>
<th>SHMT2 grade (number/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infiltrating lobular carcinoma</td>
<td>56</td>
<td>0/0.00 7/12.50 28/50.00 21/37.50</td>
</tr>
<tr>
<td>Invasive ductal carcinoma</td>
<td>20</td>
<td>0/0.00 12/57.20 8/38.10 0/0.00</td>
</tr>
<tr>
<td>Total</td>
<td>76</td>
<td>0/0.00 19/25.00 36/47.40 21/27.60</td>
</tr>
</tbody>
</table>

Notes: The percentage of SHMT2 positive cells was rated as follows: –, ≤5% positive tumor cells; +, 5%–30% positive cells; ++, 30%–55% positive cells; and ++++, >55% positive cells.

Abbreviation: SHMT2, mitochondrial serine hydroxymethyltransferase.
Figure 1 Expression of SHMT2 in breast cancer and its distant noncancerous tissues.

Notes: Breast cancer samples were of two types—Infiltrating lobular carcinoma and Invasive ductal carcinoma. The percentage of SHMT2 positive cells was rated as follows: −, ≤5% positive tumor cells (A, B); +, 5–20% positive cells (C, D); ++, 20–55% positive cells (E, F); and ++++, >55% positive cells (G). Bar: 200 μm.

Abbreviation: SHMT2, mitochondrial serine hydroxymethyltransferase.
staining were scored as none (−), weak (+), moderate (++), and strong (+++) according to the assessment of two independent pathologists. No SHMT2 protein was expressed in the distant noncancerous cells (Table 1, Figure 1A and B). In contrast, SHMT2 protein was stained in all breast cancer samples to varying degrees (Table 1, Figure 1C–G). In most of ILC cases, SHMT2 protein was expressed moderately or strongly (+++), while in all of IDC cases, SHMT2 protein was expressed weakly or moderately (+, 12/20, 57.20%; ++, 8/20, 38.10%). Overall, the results demonstrated that SHMT2 is overexpressed in breast cancer cells.

**Correlation between expression level of SHMT2 and clinicopathological parameters**

We then conducted an association analysis between the expression level of SHMT2 protein and clinicopathological parameters from breast cancer patients (Table 2). The results showed that more SHMT2 was expressed in grade III breast cancer than grade I–II (P<0.05). It suggested that the expression level of SHMT2 was positively correlated with breast cancer grade. However, there was no association between expression level of SHMT2 and other clinicopathological parameters, such as age, tumor size, TNM stage, lymph node status, vascular invasion status, and biomarkers (ER, PR, HER2) (Table 2).

**Discussion**

In this study, we detected the expression level of SHMT2 protein in breast cancer and distant normal tissue samples in different cancer types, including ILC and IDC which account for 88% of breast cancer.\(^3\) And, we found that SHMT2 is overexpressed in all of the breast cancer samples. Our results are consistent with some previously published work: first, Jain et al\(^1\) found that SHMT2 was commonly overexpressed in several types of cancer cells, especially in rapidly proliferating cells; second, Paone et al\(^2\) showed that the expression level of SHMT2 was increased in the lung cancer tissue by an average of 2.22-fold. As we know, serine and glycine provide the essential precursors for the biosynthesis of proteins, nucleic acids, and lipids and also are required for the maintenance of cellular redox state. SHMT2 is a key protein in this pathway, and its main function is to catalyze the interconversion of serine and glycine and to generate one-carbon units from serine, which are exported as folate into the cytosol to support one-carbon metabolism.\(^3\) Therefore, SHMT2 should have a pivotal role in proliferating cells, including cancer cells.

### Table 2 Association between SHMT2 expression and clinicopathological factors from breast cancer patients

<table>
<thead>
<tr>
<th>Clinicopathological features</th>
<th>Variable</th>
<th>N (%)</th>
<th>The proportion of SHMT2 positive cells (mean ± SD)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>≥60</td>
<td>7 (9.2)</td>
<td>0.453±0.235</td>
<td>0.945</td>
</tr>
<tr>
<td></td>
<td>&lt;60</td>
<td>69 (90.8)</td>
<td>0.402±0.264</td>
<td></td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td>≤2.00</td>
<td>32 (42.1)</td>
<td>0.378±0.239</td>
<td>0.756</td>
</tr>
<tr>
<td></td>
<td>2.01–5.00</td>
<td>35 (46.1)</td>
<td>0.442±0.256</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥5.00</td>
<td>9 (11.8)</td>
<td>0.374±0.331</td>
<td></td>
</tr>
<tr>
<td>Histology grade</td>
<td>I</td>
<td>4 (5.3)</td>
<td>0.245±0.098</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>37 (48.7)</td>
<td>0.309±0.104</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>35 (46.1)</td>
<td>0.528±0.115</td>
<td></td>
</tr>
<tr>
<td>TNM stage</td>
<td>I</td>
<td>10 (13.2)</td>
<td>0.368±0.330</td>
<td>0.858</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>57 (75)</td>
<td>0.424±0.389</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>7 (9.2)</td>
<td>0.357±0.268</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>2 (2.6)</td>
<td>0.300±0.157</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>Yes</td>
<td>46 (60.5)</td>
<td>0.468±0.245</td>
<td>0.482</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>30 (39.5)</td>
<td>0.367±0.325</td>
<td></td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>Yes</td>
<td>53 (69.7)</td>
<td>0.415±0.329</td>
<td>0.528</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>23 (30.3)</td>
<td>0.309±0.267</td>
<td></td>
</tr>
<tr>
<td>ER</td>
<td>+</td>
<td>62 (81.6)</td>
<td>0.309±0.189</td>
<td>0.329</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>14 (18.4)</td>
<td>0.408±0.278</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>+</td>
<td>55 (72.4)</td>
<td>0.339±0.267</td>
<td>0.209</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>21 (27.6)</td>
<td>0.456±0.328</td>
<td></td>
</tr>
<tr>
<td>HER2</td>
<td>+</td>
<td>47 (61.8)</td>
<td>0.378±0.216</td>
<td>0.398</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>29 (38.2)</td>
<td>0.429±0.319</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor-2; SHMT2, mitochondrial serine hydroxymethyltransferase; SD, standard deviation; TNM, tumor node metastasis.
SHMT2 is overexpressed in all of the breast cancer samples, SHMT2 could serve as a diagnostic marker in the future.

We also conducted an association analysis between the expression level of SHMT2 protein and clinicopathological parameters from breast cancer patients. We found a positive association between the expression level of SHMT2 and breast cancer grade. We found that higher level of SHMT2 was expressed in high histological grade breast cancer compared with low grade tumors. The histological grading system in breast cancer is based on differentiation of tumor cells, which is an important factor in predicting prognosis of breast cancer patients and tumor aggressiveness. Thus, we speculate that breast cancer with higher expression level of SHMT2 in the high histological grade might be more likely to recur and/or have a worse prognosis. In fact, Jain et al. found that higher expression level of genes in mitochondrial glycine biosynthetic pathway was associated with greater mortality in breast cancer patients. And recently, Lee et al. and Antonov et al. reported that elevated expression level of SHMT2 was found to be associated with worse prognosis in human cancer. It supported that the development of molecular therapies focused on SHMT2 or components of glycine biosynthetic pathway. Besides that, based on our clinical experiences, malignancy of breast cancer is closely associated with tumor size, TNM stage, lymph node metastasis, vascular invasion, and expression of other biomarkers (such as ER, PR, and HER2). However, we failed to find any statistical significance between the expression level of SHMT2 and these prognostic factors. This indicates that SHMT2 functions and whether SHMT2 could serve as a prognostic marker or not warrant further investigation in breast cancer.

Conclusion
In conclusion, we found that SHMT2 was overexpressed in breast cancer cells, and the expression level of SHMT2 is positively correlated with breast cancer grade. We suggest that SHMT2 could be a target for anticancer therapies, and identification of selective SHMT2 inhibitors could be an innovative and successful approach.

Disclosure
The author reports no conflicts of interest in this work.

References


