GSTT1, GSTP1, and GSTM1 genetic variants are associated with survival in previously untreated metastatic breast cancer

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ABSTRACT

Purpose: The polymorphisms in genes including GSTM1, GSTP1 and GSTT1 have been found to predict development and therapeutic efficacy in various malignancies. Breast cancer is one of the most common cancers among women. In this study, we evaluated the prognostic value of three functional polymorphisms of GSTs in patients with previously untreated metastatic breast cancer (MBC).

Patients and Methods: The genotype of GSTT1, GSTP1, and GSTM1 in 170 patients with previously untreated MBC from one single center were assessed via PCR-based RFLP methods. The prognostic of polymorphisms on overall survival (OS) was examined using the Kaplan-Meier estimates and Cox proportional hazard ratio (HR) regression analyses.

Results: The null genotypes of GSTT1 and GSTM1 were significantly correlated to poor OS compared with the present genotypes, respectively. After adjusting for clinic-pathologic factors, GSTT1 and GSTM1 genetic variants were still significantly associated with OS (HR, 1.92; 95% CI, 1.26-2.91 and HR, 1.53; 95% CI, 1.05-2.23). GSTT1 and GSTM1 were independent survival predictors and GSTP1 was not associated with overall survival of previous untreated MBC.

Conclusion: This exploratory analysis suggests that in addition to clinic-pathologic factors, the genetic variants in GSTT1 and GSTM1 might be predictive of survival outcome in patients with previously untreated MBC.

INTRODUCTION

Breast cancer is a heterogeneous disease and the most common cancer among women with a drastically increasing rate in China [1]. Approximately 6% of women were initially diagnosed with metastatic breast cancer (MBC) and about 20% of patients would develop to MBC at an early stage [2]. Despite of significant improvements in the treatment of MBC during the last decade, it still remains an incurable disease with a median overall survival of 18-30 months [3]. Current therapy decision of MBC relies on clinical features, histological factors and well-defined biomarkers [4]. Effective chemotherapy drugs used in the treatment of various malignant tumors always result in drug resistance and toxicity. It was reported that many genetic polymorphisms were involved in metabolism enzyme function, drug resistance, toxicity and efficiency of chemotherapy [5–11]. Hunting for genetic markers to improve clinical outcome of MBC patients becomes a big challenge.

Glutathione S-transferase (GST) enzymes play an indispensable role in detoxifying chemotherapy drugs. They detoxify products of oxidation or alkylating drugs by directly combining to reactive compounds or drugs...
Table 2).

metastatic site significantly influenced patient prognosis
analysis, age, RFI, number of metastatic sites, and type of
sites, and type of metastatic site. According to univariate
previous adjuvant treatment, RFI, number of metastatic
characteristics of patients
Comparison of survival according to baseline
cancer patients have the potential
to be developed as novel biomarkers for diagnosis and
prognosis of MBC patients.

RESULTS
Patient characteristics and clinical outcomes
The distribution of demographic, treatment
cancer patients were verified to be consistent
with the HWE. Interestingly, the GSTT1, GSTM1
differences such as type 2 diabetes
defined, and so on [16–18]. In
addition, GSTs genetic variants have been reported to be
involved in fluorouracil and platinum-based chemotherapy
of various metastatic or advanced cancers, such as acute
tumor, non-small cell
cancer and prostate cancer [19–21].

For potential prognostic value, genetic
cancer patients with MBC.
Moreover, genetic polymorphism can be easily detected
and applied to clinical application. Because genetic
polymorphism is found to be strongly associated with
chemotherapy efficacy and prognosis of breast cancer,
it can be used to establish a refined model to predict
prognosis of this disease [22]. Therefore, we performed
a study in patients with previously untreated MBC to
assess the impact of GSTM1 null/present, GSTT1 null/
present, and GSTP1 rs1695 polymorphisms on the
survival. The present study demonstrates that these genetic
polymorphisms in MBC cancer patients have the potential
to be developed as novel biomarkers for diagnosis and
prognosis of MBC patients.

Effects of SNPs on OS
The allelic frequencies for multiple genes’ variants
are summarized in Table 3. All observed genotype
frequencies in patients were verified to be consistent
with the HWE. Interestingly, the GSTT1, GSTM1
polymorphisms were significantly associated with patient
survival. As shown in Table 3, patients with the present
genotypes of GSTT1 and GSTM1 had 6.2 and 8.1 months
longer survival (median OS, 23.4 and 28.2 months; 95% CI,
18.7-28.1 and 18.8-37.6 months, respectively) than
those with the null genotypes (median OS, 17.2 and
20.1 months; 95% CI, 14.9-19.5 and 17.0-23.2 months,
respectively; P = 0.003 and 0.046 for log-rank test; Figures
1 and 2). But GSTP1 rs1695 was not found associated
with overall survival of previously untreated MBC in our
study.

Multivariate analysis
In the multivariate Cox proportional hazards model,
after adjustment for age, menstruation status, molecular
polymorphism was targeted to ER, PR and HER2 have provided
type, previous adjuvant treatment, or number of
metastatic sites, the prognostic significance of GSTT1,
GSTM1 polymorphisms, RFI and type of metastatic site
still existed. The hazard ratios (HRs) of patients with
GSTM1 null genotype, GSTT1 null genotype, RFI > 2
ter metastasis on OS were 1.92 (95% CI, 1.26-2.91), 1.53 (95% CI, 1.05-2.23), 0.56 (95% CI, 0.38-
0.84) and 1.68 (95% CI, 1.11-2.55), respectively (Table 2).

DISCUSSION
As an incurable disease, MBC need systemic
treatments which include chemotherapy, endocrine
therapy, molecular therapy and immunotherapy. Some
clinical characteristics are fundamental for therapy
decision, such as lymph node metastasis, hormone
receptors status, human epidermal growth factor receptor
2 (HER2) expression and types of metastatic site [23].
Molecular targeting therapies and immunotherapy have
shown important and potential status in recent years for
their remarkable effect and lower toxicity compared with
the traditional chemotherapies. The discovery and use
of agents targeted to ER, PR and HER2 have provided
clinician with effective therapies. However, drug-
resistance remains a crucial obstacle to tackle [24]. What
is more, the potential of biomarker-based treatments
improving target therapies, emphasized the requirement to
find molecular markers involved in pathogenesis of breast
cancer, which are the prognostic factors of therapeutic
response and survival [25].

A number of prognostic factors have been shown
to significantly predict the survival of patients with
metastatic disease. These mainly include adjuvant
chemotherapy, RFI, dominant metastatic site, menopausal
| Characteristics | N (%) |
|-----------------|-------|
| **Age at MBC diagnosis (years)** | |
| Median | 50.0 |
| Range | 25.0-74.0 |
| ≥ 60 | 28 (16.5) |
| 40-59 | 113 (66.5) |
| < 40 | 29 (17.1) |
| **Menstruation status** | |
| Post-menopausal | 71 (41.8) |
| Pre-menopausal | 99 (58.2) |
| **Molecular subtype** | |
| Luminal A | 36 (21.2) |
| Luminal B (HER-2 negative) | 12 (7.1) |
| HER-2 positive | 18 (10.6) |
| Triple-negative | 98 (57.6) |
| Unknown | 6 (3.5) |
| **Adjuvant therapy** | |
| No | 18 (10.6) |
| Only CT (± RT) | 119 (70.0) |
| CT + HT (± RT) | 33 (19.4) |
| **Relapse-free interval** | |
| Median (months) | 15.2 |
| ≤ 2 years | 119 (70.0) |
| > 2 years | 51 (30.0) |
| **No. of metastatic sites** | |
| 1 | 69 (40.6) |
| 2 | 45 (26.5) |
| ≥3 | 56 (32.9) |
| **Metastatic site** | |
| Liver | 50 (29.4) |
| Lung | 75 (44.1) |
| Brain | 6 (3.5) |
| Lymph node | 108 (63.5) |
| Bone | 45 (26.5) |
| Chest wall | 32 (18.8) |
| Others | 38 (22.4) |
| **Type of metastatic site** | |
| Non-visceral | 51 (30.0) |
| Visceral | 119 (70.0) |

Abbreviation: MBC, metastatic breast cancer; CT, chemotherapy; RT, radiotherapy; HT, hormone therapy; No., number.

* Number determined at MBC diagnosis includes metastasis to more than one site
status, receptor status, and multiple organ involvement [26]. In terms of the metastatic site, visceral like liver diffusion was reported to be a predictor of undesirable survival while non-visceral metastatic including only metastatic in bony skeleton or a single bone lesion can be considered as an indolent disease [27, 28]. Genetic polymorphisms involving in drug metabolism, DNA repair and apoptosis could alter the efficacy of chemotherapeutic regimens, and hence have effects on cancer progression.

In the present study, we examined the association of GSTs genetic polymorphisms and patient survival in a cohort of 170 patients with previously untreated MBC. We found that the null genotypes of GSTT1 and GSTM1 significantly contributed to poorer OS compared with
After adjusting for clinic-pathologic factors, the two genetic variants were still significantly associated with OS, showing that these polymorphisms were independent survival predictors. Additionally, RFI and type of metastatic site were also independently associated with OS of MBC patients in the cohort.

The glutathione-S-transferases (GSTs) make up a family of multifunctional enzymes with detoxification ability on electrophilic compounds [29]. In some previous clinical studies, the higher levels of GST enzymes in tumors are considered to reduce responses to chemotherapy and associated with a poorer survival in patients with carcinomas of the breast [30], stomach [31], esophagus [32], ovary [33, 34] and head and neck [35]. It has been identified that independent gene deletion are unable to express an active protein at both GSTM1 and GSTT1 [36, 37]. One might expect that a null genotype of enzyme would increase response to chemotherapy and improve clinical outcome [13]. Recently, Jian et al. [38] examined GST genotypes in 244 advanced non-small cell lung carcinoma patients, they found the null GSTM1 and the GG genotype of GSTP1 IIe105Val were correlated improved overall survival. Tatjana I. Djukic et al. [39] found patients with increased level of GSTT1 enzymes has the shorter mean life expectancy compared to null GSTT1. Our results are in accordance with previous reports that null genotypes of GSTT1 and GSTM1 significantly contribute to poorer OS compared with the present genotypes in MBC, which is quite different from the results in EBC setting [46, 47], but in accordance with the findings in other metastatic cancer types [40–44]. This may be partly because reduced GST activity leads to increased glutathione levels and elevated glutathione reduced the capability of DNA to bind to cytotoxic drugs such like platinum compounds [42, 50, 51], DNA-reactive metabolites of anthracyclines, and various alkylating agents. These most commonly used chemotherapeutic agents in the treatment of breast carcinoma, are substrates for GST-mediated glutathione conjugation [52, 53], more than that, the glutathione can protect DNA from damage and adduct formation by coupling [54]. That is why these enzymes are susceptible to chemotherapy. The function of GSTs extends beyond detoxification and chemosensitivity, as they have been found to play a critical role in kinase signaling [55, 56]. The function of cell signaling controlled will provide novel therapeutic targets of new drugs. It will provide the possibility to develop antagonists or agonists aiming at signaling pathway and exert positive biological effect. Additional, acquiring of GSTs genotyping from

| Genotype  | No. | Median (mo) | P  | HR | 95% CI  |
|-----------|-----|-------------|----|----|--------|
| GSTT1     |     |             |    |    |        |
| Present   | 131 | 23.4        | 1.00 | 1.00 | 1.00   |
| Null      | 39  | 17.2        | 0.003 | 1.84 | 1.22-2.76 |
| GSTM1     |     |             |    |    |        |
| Present   | 77  | 28.2        | 1.00 | 1.00 | 1.00   |
| Null      | 93  | 20.1        | 0.046 | 1.43 | 1.00-2.04 |
| GSTP1 (rs1695) |     |             |    |    |        |
| AA        | 116 | 20.4        | 1.00 | 1.00 | 1.00   |
| AG        | 50  | 22.9        | 0.768 | 1.06 | 0.72-1.57 |
| GG        | 4   | 50.0        | 0.292 | 0.47 | 0.12-1.91 |
| AG+GG     | 54  | 24.3        | 0.992 | 1.00 | 0.68-1.46 |

Abbreviation: No., number; mo, month; OS, overall survival; HR, hazard ratio; CI, confidence interval; NC, not calculated.

The present genotypes, respectively. After adjusting for clinic-pathologic factors, the two genetic variants were still significantly associated with OS, showing that these polymorphisms were independent survival predictors. Additionally, RFI and type of metastatic site were also independently associated with OS of MBC patients in the cohort.

Thus, this is the first study to find the null genotypes of GSTT1 and GSTM1 significantly contribute to poorer OS compared with the present genotypes in MBC, which is quite different from the results in EBC setting [46, 47], but in accordance with the findings in other metastatic cancer types [40–44]. This may be partly because reduced GST activity leads to increased glutathione levels and elevated glutathione reduced the capability of DNA to bind to cytotoxic drugs such like platinum compounds [42, 50, 51], DNA-reactive metabolites of anthracyclines, and various alkylating agents. These most commonly used chemotherapeutic agents in the treatment of breast carcinoma, are substrates for GST-mediated glutathione conjugation [52, 53], more than that, the glutathione can protect DNA from damage and adduct formation by coupling [54]. That is why these enzymes are susceptible to chemotherapy. The function of GSTs extends beyond detoxification and chemosensitivity, as they have been found to play a critical role in kinase signaling [55, 56]. The function of cell signaling controlled will provide novel therapeutic targets of new drugs. It will provide the possibility to develop antagonists or agonists aiming at signaling pathway and exert positive biological effect. Additional, acquiring of GSTs genotyping from
Figure 1: Kaplan–Meier curve demonstrating the overall survival (OS) of genotypes of GSTT1. The median OS was 23.4 months (95% CI: 18.7-28.1) in present genotypes of GSTT1 and 17.2 months (95% CI: 14.9-19.5) in null genotypes of GSTT1; p=0.003.

Figure 2: Kaplan–Meier curve demonstrating the overall survival (OS) of genotypes of GSTM1. The median OS was 28.2 months (95% CI: 18.8-37.6) in present genotypes of GSTM1 and 20.1 months (95% CI: 17.0-23.2) in null genotypes of GSTM1; p=0.046.
blood samples leads to personalized modality and better effectiveness. Therefore, the variants of GSTT1 and GSTM1 could be a novel and helpful predictive factor to identify specific MBC patients who may benefit from signaling pathway. As our study is the inclusion of untreated MBC, the identified patients may acquire higher response and lower toxicity as they accept targeting therapies earlier, at the same time, spare those patients unlikely to benefit from needless therapies and toxicity.

Several limitations of this study should be noted. The sample size of our study is relative moderate. And further research is necessary to choose patients according to genetic characteristics and find the optimized targeted treatment or tailored chemotherapy for patients with null genotypes of GSTT1 and GSTM1. Results of the presented study should be validated in prospective studies. And, due to possible ethnic differences, our results should be further verified in different ethnic populations to acquire more accurate and solid conclusions in the future.

In conclusion, we have reported for the first time that there were significant differences in the OS among previously untreated MBC patients with different GSTT1 and GSTM1 genotypes. Our results suggest that in addition to clinic-pathologic factors, genetic variants in GSTs might be suggestive factors in untreated MBC patients and further research is warranted.

MATERIALS AND METHODS

Patients

From March 2002 through November 2011, a total of 170 patients from Fudan University Shanghai Cancer Center (FUSCC) with previously untreated MBC were enrolled. Criteria for inclusion were as follows: female gender with histologically confirmed invasive ductal carcinoma, age of 18 to 70, with Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1, with adequate liver, renal function, and adequate bone marrow function. Exclusion criteria included: pretreatment of metastatic disease; more than one primary malignancy (except carcinoma in situ of the cervix or basal cell carcinoma of the skin with proper treatment); other serious complications/comorbidities that might affect survival.

Baseline information of these patients was collected and all specimens (blood samples) were obtained before treatment aiming at the metastatic disease. Classification of molecular subtypes and the clinic-pathology were based on the 2013 St. Gallen consensus [57]. Survival information was collected from hospital medical records and/or the follow-ups every 3 months. Each patient provided signed informed consent of using their DNA and clinical data. The study was approved by the Institutional Review Board of FUSCC.

SNP genotyping

DNA was collected from 5-mL blood sample from each patient. The polymorphisms of multiple genes including GSTM1 null/present, GSTT1 null/present, and GSTP1 rs1695 were performed by PCR-based RFLP methods, then applied DNA sequencing of the PCR products to further confirm the genotypes [58]. To make sure the accuracy of method and total reproducibility, 15% random samples were genotyped repeat by different people.

Statistical analysis

For each polymorphism, Pearson χ² test was applied to test the Hardy-Weinberg equilibrium (HWE). OS was calculated from diagnosis of MBC to death. Survival distributions were analyzed by the Kaplan-Meier method and log-rank test was used to compare the survival analyses. Multivariate Cox proportional hazards models were applied to evaluate the effect of prognostic and clinical factors on OS, including age, molecular subtype, menstruation status, previous adjuvant treatment, relapse-free interval (RFI), number of metastatic sites, and type of metastatic site. Statistical significance was set at a level of 0.05 and all the statistical analyses were conducted using the SPSS software package (version 17.0).

Abbreviations

GSTM1: glutathione S-transferase theta; GSTP1: glutathione S-transferase pi; GSTT1: glutathione S-transferase mu; MBC: metastatic breast cancer; OS: overall survival; HR: hazard ratio; GST: glutathione S-transferase; T2DM: type 2 diabetes mellitus; RFI: relapse-free interval; ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2; FUSCC: Fudan University Shanghai Cancer Center; ECOG: Eastern Cooperative Oncology Group.

Author contributions

Conception and design: JZ, YW, XH and BW; Collection and assembly of data: JZ and YW; Data analysis and interpretation: JZ, YW and ZS; Pathological slides reviewing: JZ, YW, JC and LW; Manuscript writing: JZ, YW and ZW; Final approval of manuscript: JZ, YW, XH, BW, LW, SZ, JC and ZW.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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