Association of *SIPA1* 545 C > T polymorphism with survival in Chinese women with metastatic breast cancer

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Abstract It has been demonstrated that single nucleotide polymorphisms (SNPs) of *SIPA1* (signal-induced proliferation associated gene 1) are associated with metastatic efficiency in both human and rodents. The purpose of this study was to determine whether *SIPA1* 545 C > T polymorphism was associated with overall survival in patients with metastatic breast cancer. In this study, *SIPA1* 545 C > T polymorphism was detected in 185 metastatic breast cancer patients using polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP). Survival curves for patients with *SIPA1* 545 C > T polymorphism was compared using the Kaplan-Meier method with log-rank tests. We found that *SIPA1* 545 C > T polymorphism was significantly associated with survival in 185 patients with metastatic breast cancer. Patients with *SIPA1* 545 T/T genotype had a significantly worse overall survival (OS) than did patients with C/T or C/C genotype (50.0% vs. 62.9%, \( P = 0.042 \)). Moreover, in multivariate analysis, as compared with the C/C or C/T genotype, the T/T genotype remained an independent unfavorable prognostic marker of OS in this cohort (hazard ratio [HR] = 2.16; 95% CI = 1.12–4.15; \( P = 0.022 \)). Our findings indicate that metastatic breast cancer patients with *SIPA1* 545 T/T genotype have a poorer survival compared to patients with C/C or C/T genotype.

Keywords  *SIPA1*; polymorphism; metastatic breast cancer; survival

Introduction

Breast cancer is one of the most common cancers in women worldwide. Although the prognosis is good for patients with early-stage breast cancer, it is dramatically poor for patients with metastatic breast cancer [1]. Metastatic breast cancer is a biologically heterogeneous disease, and some metastatic breast cancer patients may have relatively longer survival than others [2]. Therefore, it is of the great importance to predict the prognosis of metastatic breast cancer patients in the clinical setting.

*SIPA1* (signal-induced proliferation-associated gene 1), located on human chromosome 11q13.3 [3], was cloned by Hattari in 1995 [4]. It was the first identified as a potential cancer metastasis modulator in the mouse model. The N-terminal domain of SIPA1 has GTPase activating protein (GAP) activity, which is specific for RapGTPases (RapGTP).

Rap is a member of the superfamily of Ras-related proteins, and is involved in cell proliferation, morphology and adhesion. SIPA1 negatively regulates Rap via its RapGAP activity, which catalyzes the hydrolysis of active RapGTP into inactive RapGDP. Recently, it has been demonstrated that the expression of SIPA1 is correlated with cellular metastatic capacity [5–10].

One polymorphism in exon 1 of *SIPA1* generates a 545 C to T transition, leading to phenylalanine (Phe, F) 182 to serine (Ser, S) amino acid substitution [11]. In the current study,
SIPA1 545 C > T polymorphism was determined in 185 metastatic breast cancer patients using PCR-restriction fragment length polymorphism assay (PCR-RFLP). We aimed to investigate the association between this polymorphism and survival in this cohort.

Materials and methods

Study subjects

192 metastatic breast cancer patients were recruited at the Breast Center, Peking University Cancer Hospital, from March 2003 to December 2007. Of these, four patients lost the follow-up, and three specimens failed to obtain the PCR products because of poor-quality DNA when detecting SIPA1 545 C > T polymorphism. Thus, a total of 185 patients were available for SIPA1 545 C > T polymorphism. The age of patients ranged from 23 to 76 years, with a median of 52 years, and the median follow-up is 25 months (range: 4 to 85 months). This study was approved by the Research and Ethical Committee of Peking University Cancer Hospital.

DNA extraction and genotyping

Genomic DNA was obtained from peripheral blood lymphocytes of each patient using phenol-chloroform extraction. SIPA1 polymorphism was detected by using a PCR-RFLP technique. Forward primer 5′-CCAGCTCGACCTGCTG-3′ and reverse primer 5′-GATGGACACGGCCGTTT-3′ were used for detection of SIPA1 545 C > T, as previously described by Crawford et al. [11]. PCR was performed in 20 μl reaction mixture containing 100 ng of genomic DNA template, 2 μl 10 × PCR buffer, 0.8 mmol/L dNTP, 2.5 mmol/L MgCl2, 0.5 μmol/L primers, and 1 unit AmpliTaq DNA polymerase (Promega, USA). The reaction condition employed was initial denaturation at 94°C for 2 min, followed by 35 step cycles of denaturation at 94°C for 30 s, annealing 57°C for 45 s, and extension at 72°C for 30 s followed by a terminal extension time of 10 min. 15 μl of PCR product was digested with Bsm I restriction endonuclease for SIPA1 545 C > T (New England Biolabs Inc.) for 2 h at 37°C. The digestion products were then resolved on a 2.5% agarose gel containing ethidium bromide. The homozygous SIPA1 545 C/C genotype was identified by one band (128 bp), the homozygous SIPA1 545 T/T genotype produced two bands (78 bp and 50 bp), and heterozygous SIPA1 545 C/T genotype displayed three bands (128 bp, 78 bp and 50 bp).

Statistical analysis

The correlation between the genotype variants and clinicopathological characteristics was determined using Pearson’s χ² test. Overall survival (OS) was defined as the time from date of metastasis to the last point of follow-up, or to the date of death. Survival curves were derived from Kaplan-Meier estimates and the curves were compared by log-rank tests. A Cox regression model was applied to determine whether a factor was independent predictor of overall survival in multivariate analysis. All statistical tests were two-sided, and P values less than 0.05 were considered as statistically significant. The statistical analyses were performed using SPSS 16.0 software.

Results

Association between SIPA1 545C > T polymorphism and clinicopathological characteristics

The frequency of SIPA1 545C > T polymorphism was as follows: 44.9% (83 of 185) of the patients were homozygous for C/C genotype, 44.3% (82 of 185) were heterozygous for C/T and 10.8% (20 of 185) were homozygous for T/T genotype. This polymorphism did not deviate from the Hardy-Weinberg equilibrium (χ² = 0.001, P = 0.97).

SIPA1 545C > T polymorphism was not statistically significantly associated with the age, menopausal status, estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2) status and triple negative status (absence of the ER, PgR, HER2) (P > 0.05, Table 1).

The association between the SIPA1 545C > T polymorphism and overall survival

SIPA1 545C > T polymorphism was significantly associated with the overall survival in 185 metastatic breast cancer patients. Patients with T/T genotype had a significantly poorer overall survival compared to patients with C/C or C/T genotypes (2-year overall survival rate, 50.0% vs. 62.9%, P = 0.042) (Table 2, Fig. 1).

In multivariate analysis, as compared with the C/C or C/T genotype, the T/T genotype remained an independent unfavorable prognostic marker of overall survival after adjusting for age, menopausal status, HER2 status, ER or PgR status and metastatic site in this cohort of 185 patients (hazard ratio [HR] = 2.16; 95% CI = 1.12–4.15; P = 0.022) (Table 3).

Discussion

In the present study, we showed that SIPA1 545C > T polymorphism was significantly associated with survival in