Association of SENPs single-nucleotide polymorphism and breast cancer in Chinese population

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Abstract
SUMO-specific Cysteine Proteases (SENPs) have involvement in the initiation and progression of human cancers. In the present study, we evaluated the association of SENPs polymorphism with susceptibility as well as clinicopathologic features and patients’ response of breast cancer (BC) in a Chinese population.

We genotyped SENP1 (rs61918808), SENP2 (rs6762208), and SENP7 (rs61697963) by sequencing in a case–control study including 210 BC patients and 225 healthy volunteers. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to assume the association strength.

No significant association was found between polymorphism of the 3 SENPs and BC susceptibility. However, SENP1 rs61918808 (C>T) and SENP7 rs61697963 (A>C) was associated with HER-2 expression (P<.05). SENP2 rs6762208(C>A) was correlated with increasing risk of lymph node metastases (P<.05). Among the patients who received neoadjuvant chemotherapy, T allele and TT genotype of SENP1 rs61918808 were less likely to achieve pCR (P<.05).

We first reported SENPs variants were not associated with BC risk in Chinese population, but presented specific effect on clinicopathological features of BC. Moreover, SENP1 rs61918808 may be a predictor for the clinical response in local advanced BC patients who received neoadjuvant chemotherapy.

Abbreviations: BC = breast cancer, CI = confidence interval, ER = estrogen receptor, HER-2 = human epidermal growth factor receptor-2, HER-2 = human epidermal growth factor receptor 2, OR = odds ratio, pCR = pathological complete response, PR = progesterone receptor.

Keywords: breast cancer, cancer susceptibility, SENPs, SUMO-specific Cysteine Proteases, variant

1. Introduction
Posttranslational modifications (PTMs) including methylation, phosphorylation, acetylation, glycosylation, ubiquitination, and SUMOylation play important roles in the normal biological function of the protein. Among these, SUMOylation is a particularly interesting PTM involved in control of various cellular processes, including cell growth, survival and apoptosis, gene expression, intracellular transport, and protein stability.[1,2]

SUMOylation requires an E1-activating enzyme, E2-conjugating enzyme (UBC9), and E3-ligating enzymes.[3] SUMO proteins are activated by the activating enzyme E1 and transferred to the conjugating enzyme UBC9. E3-ligating enzymes then promote the transfer of SUMO from E2 to the substrate, which helps the modification to be more efficient.[4] SUMOylation of target proteins results in formation of isopeptide bond between the C-terminal glycine residue of SUMO and the e-amino group of lysine residue in the target proteins.[5] SUMOylation pathway is required to maintain the basal breast cancer (BC) subtype. Disruption of the sumoylation pathway by knockdown of sumoylation enzymes, mutation of the SUMO-target lysine of TFAP2A, or treatment with sumoylation inhibitors induced a basal to luminal transition.[5] SUMOylation is highly dynamic process that can be reversed by a family of SUMO-specific proteases (SENP5). At present, 6 SENP isoforms have been identified in humans, namely, SENP1, 2, 3, 5, 6, and 7, which have different substrate specificities and subcellular localizations.[6,7] SENP1 processes SUMO-1 in preference to SUMO-2, but shows weak activity for SUMO-3.[8,9] SENP2 processes SUMO-2 more efficiently than SUMO-1, but processes SUMO-3 almost as poorly as SENP1, SENP6, and SENP7 have excellent deconjugating activity for polySUMO-2/3 chains.[10] Emerging studies have demonstrated that overexpression of various SENP isoforms is involved in the tumorsogenesis. SENP1, for example, is unregulated in thyroid oncocyte tumor, prostate cancer, and...
Genomic DNA Miniprep Kit (Beijing, China) according to the manufacturer’s directions. The polymorphisms of SENP1 rs61918808, SENP2 rs6762208, and SENP7 rs61697963 were genotyped using the Sequenom MassARRAY technology platform with the complete iPLEX Gold Reagent Set (Sequenom, CA) in the conditions recommended by the manufacturer. Assay data were analyzed using Sequenom TYPER software (version 4.0). The primers were designed by ADS software 2.0 (Agena Bioscience, CA). The primer sequences used for genotyping were listed in Table 1.

### Peripheral blood samples were collected from each subject into a test tube containing EDTA as anticoagulant. Genomic DNA was isolated from the whole blood using the BioTECH Blood Genomic DNA Miniprep Kit (Beijing, China) according to the manufacturer’s directions. The polymorphisms of SENP1 rs61918808, SENP2 rs6762208, and SENP7 rs61697963 were genotyped using the Sequenom MassARRAY technology platform with the complete iPLEX Gold Reagent Set (Sequenom, CA) in the conditions recommended by the manufacturer. Assay data were analyzed using Sequenom TYPER software (version 4.0). The primers were designed by ADS software 2.0 (Agena Bioscience, CA). The primer sequences used for genotyping were listed in Table 1.

### RESULTS

#### Population Characteristics

A total of 435 age-matched subjects (210 BC cases and 225 healthy controls) were genotyped to explore the association of BC susceptibility and SENP1 rs61918808, SENP2 rs6762208, and SENP7 rs61697963 polymorphisms in this study. Distributions of clinical characteristics between BC cases and healthy controls were shown in Table 2. No statistically significant difference was observed between the 2 groups in age, age at menarche, age at menopause, menopausal status, number of pregnancies, number of abortions, breast-feeding history, and family history of BC. All tests were 2-sided and a P < .05 was considered to be statistically significant.

#### Allelic frequencies and genotype distribution of SENP1 rs61918808, SENP2 rs6762208, and SENP7 rs61697963

The frequency distributions of alleles and genotypes of SENP1 rs61918808, SENP2 rs6762208, and SENP7 rs61697963 for BC cases and controls were shown in Table 3. The genotype frequency distributions of the 3 studied SNPs did not deviate from Hardy–Weinberg equilibrium in the controls (P = .677 for rs61918808, P = .871 for rs6762208, and P = .837 for rs61697963).
Logistic regression analysis for associations between selected SNPs and risk of Breast Cancer.

Table 3

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| Genotype or allele | Breast cancer patients (n=210) | Controls (n=225) | OR (95% CI) | P   |
|--------------------|-------------------------------|-----------------|-------------|-----|
|                    | Number | Frequency | Number | Frequency |               |              |
| SENP1 (rs61918808) |         |           |         |           |               |              |
| CC                 | 158     | 0.752     | 150    | 0.667     | 1             |              |
| CT                 | 46      | 0.219     | 66     | 0.293     | 0.693 (0.382–1.225) | 0.226 |
| TT                 | 6       | 0.029     | 9      | 0.040     | 0.526 (0.214–2.241) | 0.385 |
| CT+TT              | 52      | 0.248     | 75     | 0.333     | 0.665 (0.370–1.166) | 0.154 |
| C                  | 362     | 0.862     | 366    | 0.813     | 1             |              |
| T                  | 58      | 0.138     | 84     | 0.187     | 0.694 (0.425–1.134) | 0.144 |
| SENP2 (rs6762208)  |         |           |         |           |               |              |
| CC                 | 122     | 0.581     | 118    | 0.524     | 1             |              |
| CA                 | 68      | 0.324     | 83     | 0.369     | 0.794 (0.460–1.370) | 0.406 |
| AA                 | 20      | 0.095     | 24     | 0.107     | 0.773 (0.325–1.837) | 0.560 |
| CA+AA              | 88      | 0.419     | 107    | 0.476     | 0.789 (0.475–1.309) | 0.359 |
| C                  | 312     | 0.743     | 320    | 0.711     | 1             |              |
| A                  | 108     | 0.257     | 130    | 0.289     | 0.803 (0.535–1.205) | 0.289 |
| SENP7 (rs61697963) |         |           |         |           |               |              |
| AA                 | 58      | 0.276     | 69     | 0.307     | 1             |              |
| AC                 | 118     | 0.562     | 109    | 0.484     | 1.247 (0.697–2.231) | 0.456 |
| CC                 | 34      | 0.162     | 47     | 0.209     | 0.887 (0.417–1.909) | 0.757 |
| AC+CC              | 152     | 0.729     | 156    | 0.693     | 1.141 (0.656–1.986) | 0.640 |
| A                  | 234     | 0.557     | 247    | 0.549     | 1.000          |              |
| C                  | 186     | 0.443     | 203    | 0.451     | 0.977 (0.681–1.402) | 0.901 |
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Table 2

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| Characteristics | BC cases (n=210), n (%) | Healthy controls (n=225), n (%) | P     |
|----------------|-------------------------|---------------------------------|-------|
| Age (mean±SD)  | 51.94±10.66             | 52.09±11.99                     | 0.589 |
| Age at menopause | 12.91±1.28             | 12.54±1.27                      | 0.673 |
| No. of pregnancy | ≤2: 117 (55.71)         | 126 (56)                        | 0.110 |
| No. of abortion | >2: 93 (44.29)          | 99 (44)                         |       |
| Breast-feeding | No: 18 (8.57)           | 19 (8.44)                       | 0.521 |
| Family history of cancer | No: 192 (91.43) | 206 (91.56) |       |
| PR status      | 0.521                   |                                 |       |
| ER status      | 0.521                   |                                 |       |
| HER-2 status   | 0.521                   |                                 |       |
| Metastases in lymph nodes | Yes: 11 (5.23) | 13 (5.77) |       |
| Healthy controls (n=225), n (%) | | | |
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3.3. Association between SENP polymorphisms and BC risk

To define whether there was a statistically significant increased risk of BC susceptibility in terms of the SENP1 rs61918808, SENP2 rs6762208, and SENP7 rs61697963 genotypes, we carried out logistic regression analysis. As shown in Table 3, no significant associations between these polymorphic variants and the risk for developing BC were found (for SENP1 rs61918808 T vs C: OR: 0.694, 95% CI, 0.425–1.134, P=0.144; CT vs CC: OR: 0.693, 95% CI, 0.382–1.225, P=0.226; TT vs CC: OR: 0.526, 95% CI, 0.124–2.241, P=0.358; CT + TT vs CC: OR: 0.665, 95% CI, 0.379–1.166, P=0.154. For SENP2 rs6762208 A vs C: OR: 0.803, 95% CI, 0.535–1.205, P=0.289; CA vs AA: OR: 0.794, 95% CI, 0.460–1.370, P=0.406; AA vs CC: OR: 0.773, 95% CI, 0.325–1.837, P=0.560; CA + AA vs CC: OR: 0.789, 95% CI, 0.475–1.309, P=0.359. For SENP7 rs61697963 C vs A: OR: 0.977, 95% CI, 0.681–1.402, P=0.901; AC vs AA: OR: 1.247, 95% CI, 0.697–2.231, P=0.456; CC vs AA: OR: 0.887, 95% CI, 0.417–1.890, P=0.757; AC + CC vs AA: OR: 1.141, 95% CI, 0.656–1.986, P=0.640.

3.4. Clinical Parameters of BC patients and SENP polymorphism

Next, we further investigated the correlations of SENP1 rs61918808, SENP2 rs6762208, and SENP7 rs61697963 polymorphism with clinical parameters of BC patients.
Table 4
The genotype and allele frequency and odds ratios (OR) of SENP1 rs61918808 and SENP7 rs61697963 polymorphism in groups of patients with breast cancer with positive and negative HER-2 status.

| Genotype or allele | HER-2 positive (n=120) | HER-2 negative (n=90) | OR (95% CI) | P |
|--------------------|------------------------|-----------------------|-------------|---|
| SENP1 (rs61918808) |                        |                       |             |   |
| CC                 | 98                     | 0.817                 | 60          | 0.667 | 1 |
| CT                 | 20                     | 0.167                 | 26          | 0.289 | 0.416 (0.154–1.126) | .084 |
| TT                 | 2                      | 0.017                 | 4           | 0.044 | 0.207 (0.017–2.542) | .219 |
| CT+TT              | 22                     | 0.183                 | 30          | 0.333 | 0.383 (0.148–0.889) | .037 |
| C                  | 86                     | 0.700                 | 56          | 0.657 | 1 |
| T                  | 24                     | 0.100                 | 34          | 0.189 | 0.486 (0.219–1.079) | .076 |
| SENP7 (rs61697963) |                        |                       |             |   |
| AA                 | 24                     | 0.200                 | 34          | 0.378 | 1 |
| AC                 | 76                     | 0.633                 | 42          | 0.467 | 2.830 (1.104–7.256) | .030 |
| CC                 | 20                     | 0.167                 | 14          | 0.156 | 2.657 (0.721–9.800) | .142 |
| AC+CC              | 96                     | 0.800                 | 56          | 0.622 | 2.755 (1.113–6.818) | .028 |
| A                  | 124                    | 0.517                 | 110         | 0.611 | 1 |
| C                  | 116                    | 0.483                 | 70          | 0.389 | 1.503 (0.831–2.717) | .178 |

OR=odds ratio, CI=confidence interval. HER-2=human epidermal growth factor receptor-2.
italic values are statistically significant.

3.5. Associations between SENPs polymorphism and pathological complete response (pCR)
In our study, 64 BC patients received epirubicin followed by taxanes based on neoadjuvant chemotherapy (80 mg/m² of epirubicin, 800 mg of cyclophosphamide and 80 mg/m² of docetaxel on the first day, with 21 days a cycle). As shown in Table 6, the frequency of CC, CT, and TT genotype of SENP1 rs61918808 was 50.0%, 32.0%, and 18.0%. pCR was achieved in 24 cases (37.5%), with CC genotype 15 cases (46.9%), CT genotype 7 cases (35%), and TT allele 2 cases (16.7%). Patients with T allele and TT genotype of SENP1 rs61918808 were less likely to achieve pCR. Multivariate analysis showed the SNP rs61918808 was significant predictive factor of clinical response (T vs C, OR 0.276, 95% CI, 0.190–0.400, P = .001; CC vs TT, OR 0.214 95% CI, 0.118–0.398, P = .021).

4. Discussion
As an important protein modification, SUMOylation plays a wide range role to promote the initiation and progression of human cancers.[21,22] SENPs can act as de-conjugating or maturation/processing enzymes. The roles of SENPs have been widely reported in cancer, but epidemiological studies evaluating tumor susceptibility conferred by genetic polymorphisms have not been extensively assessed.[19] Indeed, to the best of our knowledge, this is the first study to investigate the role of SENP1 rs61918808, SENP2 rs6762208, and SENP7 rs61697963 in BC susceptibility and patient prognosis. No statistically significant association emerged between the 3 SENPs polymorphism and BC risk. However, our results showed that CT + TT genotype of

Table 5
The genotype and allele frequency and odds ratios (OR) of SENP2 rs6762208 polymorphism in groups of patients with breast cancer with positive and negative lymph node status.

| Genotype or allele | Lymph node positive (n=134) | Lymph node negative (n=76) | OR (95% CI) | P |
|--------------------|-----------------------------|----------------------------|-------------|---|
| SENP2 (rs6762208)  |                            |                            |             |   |
| CC                 | 66                          | 0.493                      | 56          | 0.737 | 1 |
| CA                 | 50                          | 0.373                      | 18          | 0.237 | 2.438 (0.939–6.331) | .067 |
| AA                 | 18                          | 0.134                      | 2           | 0.026 | 3.223 (2.086–5.563) | .024 |
| CA+AA              | 68                          | 0.507                      | 20          | 0.263 | 3.218 (2.312–7.899) | .011 |
| C                  | 182                         | 0.679                      | 130         | 0.855 | 1 |
| A                  | 86                          | 0.321                      | 22          | 0.145 | 2.870 (1.370–4.010) | .005 |

Data in italic are statistically significant.
OR=odds ratio, CI=confidence interval.
SENPI rs61918808 correlated with the lack of HER-2 expression (OR 0.383, 95% CI, 0.148–0.689). SENPI was highly expressed in triple-negative BC (TNBC) tissues.[31] The absence of SENPI significantly suppressed the proliferation and invasion of TNBC cells.[23] Moreover, clinical data showed that SENPI was positively associated with lymph node metastasis and TNM stage of pancreatic cancer. Silencing of SENPI impaired pancreatic cancer cell growth, migration, and invasion. Knockdown of SENPI downregulated the expression of MMP-9, suggesting that SENPI played an important role in PDAC progression and metastasis.[14] Further studies showed that SENPI-silencing sensitizes lung cancer cells to radiation and enhanced IR-induced lung cancer cell cycle arrest at the G0/G1 stage. In addition, SENPI inhibition significantly upregulated IR-induced γ-H2AX expression, which was positively associated with tumor radiosensitivity.[24,25] It has been studied a single nonconsensus nuclear localization signal was presented in N terminus of SENPI, the mutation of which results in pronounced cytoplasmatic accumulation in contrast to the nuclear accumulation of the parental protein.[24] Studied by us, polymorphic site in SENPI rs61918808 (C>T) is located in the 5-UTR region, closed to the transcription start position, and therefore may affect transcriptional activity and proteolytic activity of SENPI. All these results demonstrate that SENPI may be a potential drug target for cancer treatment. Notably, our study found SENPI rs61918808 may be a predictor for the clinical response in local advanced BC patients who received neoadjuvant chemotherapy.

In the case of SENPI rs6762208 polymorphism, we did not observe association of rs6762208 with the risk of BC. Of note, increased risk of lymph node metastases was observed in patients with allele A (OR 2.870, 95% CI, 1.370–6.250) and the genotypes AA (OR 3.223, 95% CI, 2.068–5.563) and CA + AA (OR 3.218, 95% CI, 2.312–7.898). In contrast to our findings, Mirecka et al found higher risk of BC for carriers of the A allele of rs6762208 polymorphism in Poland population, yet the genotype CC and the allele C decrease a risk of BC. Moreover, they observed that the AA genotype correlated with the lack of estrogen receptor.[19] Rational explanation for the significant difference may be attributed to differences in the genetic background of studied population and genotyping techniques as well as random errors. For example, the frequency of allele mutation is related to different ethnic populations. In this study, we found that the A allele frequency of SENPI rs6762208 polymorphism was 0.289 in our healthy Chinese women subjects. In the study conducted by Mirecka et al, the frequency of A allele was 0.39 in Polish population. Unfortunately, unlike other polymorphisms, no data related the allele frequency of SENPI rs6762208 polymorphism recorded in international HapMap Project. In spite of this, the results of present study provide referable data of allele frequency rs6762208 for the HapMap Project and other researchers.

SENPI7 are specialized chain-editing enzymes.[10,27] Multiple SENPI7 isoforms have differences in cellular and histological localization and biological function. For example, SENPI7S, which is located in the cytosol, is highly expressed in normal mammary epithelia, yet decreases approximately 50% in precancerous ductal carcinoma in situ (DCIS) and is lost in multiple BC subtypes and invasive carcinoma. Nevertheless, SENPI7 protein is elevated in invasive carcinoma.[30] Previously, it was showed that SENPI7 is required for chromatin relaxation in response to DNA damage, for homologous recombination repair and for cellular resistance to DNA-damaging agents.[19] It was also demonstrated that targeted knockdown of the SENPI7 gene transcript NM_001077203.2 alters Wnt-activated β-catenin signaling in a sarcoma cell line.[30] In the present study, no association between rs61697963 polymorphism and BC susceptibility was observed. However, we found patients with genotype AC (OR 2.380, 95% CI, 1.104–7.256) and AC + CC (OR 2.755, 95% CI, 1.113–6.818) had more HER-2 expression, which is an important prognostic marker of BC.[31]

Several potential limitations of the present study are as follows: first, inherent choice and information bias might exist because it was a hospital-based case–control study, and BC cases and healthy controls were from a singer center. Therefore, it is crucial to verify results of our case-control study in population-based prospective study in the future. Second, the sample size of the present study was not large enough, which may impact on the precision and accuracy of statistical analysis, especially for statistical analyses of subgroups which are stratified by clinicopathological features. Prospective case-control studies with larger sample size should be performed to verify the association between SENPI1 rs61918808, SENPI2 rs6762208, and SENPI7 rs61697963 polymorphism and BC risk.

In conclusion, SENPI1 rs61918808, SENPI2 rs6762208, and SENPI7 rs61697963 variants have not played any major role in genetic susceptibility to breast carcinogenesis within the Chinese population, but present specific effect on clinicopathological features of BC. Moreover, SENPI1 rs61918808 may be a predictor for the clinical response in local advanced BC patients who received neoadjuvant chemotherapy.

Author contributions

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