Absence of Mutations in the Wilms’ Tumor Gene WT1 in Primary Breast Cancer

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Background: It was recently demonstrated that the WT1 gene was overexpressed in primary breast cancer and that the high expression levels of WT1 mRNA significantly correlated with poor prognosis. However, it remained undetermined whether or not the WT1 gene expressed in breast cancer had mutations.

Methods: Breast cancer tissues were obtained from 36 patients with breast cancer. WT1 genomic DNA was PCR-amplified and examined for mutations by direct sequencing.

Results: The sequencing analysis showed the absence of mutations through the whole 10 exons of the WT1 gene in the 36 cases of primary breast cancer. Two different single nucleotide polymorphisms (SNP) without an amino acid change (Pro42, C to T in exon 1, and/or Arg300, A to G in exon 7) were detected in the WT1 gene in 31 (86%) of the 36 cases examined.

Conclusion: The results indicate that the wild-type WT1 gene plays an important role in the tumorigenesis of primary breast cancer.

Key words: Wilms’ tumor gene – WT1 – breast cancer – mutation

INTRODUCTION

The WT1 gene was originally isolated as a tumor-suppressor gene that was inactivated in a subset of Wilms’ tumors and mutated in the germline of children with genetic predisposition to this kidney neoplasm of childhood (1–3). The WT1 gene encodes a zinc finger transcription factor that regulates transcription of growth factor (PDGF-A chain, CSF-1 and IGF-II) (4–6) and growth factor receptor (IGF-IR) (7) genes and other genes (RAR-α, c-myc and bcl-2) (8,9).

The WT1 gene was expressed in cancer cells derived from various kinds of cancers (10–12) and overexpressed in primary leukemia (13), lung cancer (14), bone and soft-tissue sarcoma (15), head and neck squamous cell carcinoma (HNSCC) (16) and thyroid cancer (17). Growth of WT1-expressing cancer cells was inhibited by the treatment with WT1 antisense oligomers (11,18,19). Therefore, we proposed that the WT1 gene plays an oncogenic role rather than tumor-suppressor gene function in the tumorigenesis of various types of cancers (20). It was recently demonstrated that the WT1 gene was over-expressed in primary breast cancer (21,22) and that the high expression levels of WT1 mRNA significantly correlated with poor prognosis (22). These findings strongly indicated that the WT1 gene plays an important role in the tumorigenesis of primary breast cancer. However, it remains undetermined whether or not the WT1 gene expressed in breast cancer has mutations.

In the present study, we examined 36 patients with breast cancer who expressed the WT1 mRNA and demonstrate absence of mutations in the WT1 gene in all of the cases examined.

PATIENTS AND METHODS

PATIENTS AND SAMPLE TISSUES

Breast cancer tissues were obtained with informed consent from 36 patients with breast cancer at Osaka University Hospital. The expression of the WT1 mRNA was detected in all of these tissues by real-time RT-PCR in our previous study (22). WT1 expression was scored as overexpressed or unchanged, according to the cut-off level which corresponded to mean ± SD of the WT1 mRNA expression levels in normal breast tissues (Table 1). Clinicopathological features of the patients are shown in Table 1.
Genomic DNA was isolated from frozen breast cancer tissues using a standard technique, dissolved in distilled water and stored at 4°C until use. WT1 genomic DNA was analyzed for mutations as described previously (16). For amplification of exons 2–10 of the WT1 gene, 0.2 µg of genomic DNA was added to the PCR buffer (100 mM Tris–HCl, pH 8.3, 500 mM KCl and 3 mM MgCl₂) containing 250 µM of each dNTP, 1.25 U of Ex Taq polymerase (Takara, Shiga, Japan), 0.5 µM for-

| Patient No. | Age (years) | Gender | Histology* | WT1 mRNA expression† | WT1 SNP
|-------------|-------------|--------|------------|---------------------|--------
| 1           | 48          | F      | 1          | Overexpressed       | T/T A/G
| 2           | 47          | F      | 1          | Unchanged           | T/T A/G
| 3           | 31          | F      | 1          | Unchanged           | T/T G/G
| 4           | 49          | F      | 1          | Unchanged           | C/C A/G
| 5           | 73          | F      | 2          | Overexpressed       | C/C A/G
| 6           | 47          | F      | 2          | Overexpressed       | T/T A/A
| 7           | 53          | F      | 2          | Overexpressed       | C/C A/A
| 8           | 50          | F      | 2          | Overexpressed       | C/T A/G
| 9           | 47          | F      | 2          | Overexpressed       | C/T A/G
| 10          | 47          | F      | 2          | Overexpressed       | T/T G/G
| 11          | 48          | F      | 2          | Overexpressed       | T/T G/G
| 12          | 64          | F      | 2          | Overexpressed       | C/T A/A
| 13          | 47          | F      | 2          | Overexpressed       | T/T G/G
| 14          | 30          | F      | 2          | Overexpressed       | T/T G/G
| 15          | 40          | F      | 2          | Overexpressed       | C/T A/G
| 16          | 54          | F      | 2          | Overexpressed       | T/T G/G
| 17          | 51          | F      | 2          | Unchanged           | T/T G/G
| 18          | 73          | F      | 2          | Unchanged           | T/T A/G
| 19          | 54          | F      | 2          | Unchanged           | C/T A/G
| 20          | 61          | F      | 2          | Unchanged           | C/C A/A
| 21          | 50          | F      | 2          | Unchanged           | T/T G/G
| 22          | 57          | F      | 2          | Unchanged           | T/T G/G
| 23          | 49          | F      | 2          | Unchanged           | T/T G/G
| 24          | 51          | F      | 2          | Unchanged           | C/T A/G
| 25          | 66          | F      | 2          | Unchanged           | C/C A/A
| 26          | 54          | F      | 2          | Unchanged           | C/C A/G
| 27          | 47          | F      | 2          | Unchanged           | C/C A/A
| 28          | 57          | F      | 2          | Unchanged           | T/T A/G
| 29          | 55          | F      | 2          | Unchanged           | C/C G/G
| 30          | 48          | F      | 2          | Unchanged           | C/T A/G
| 31          | 65          | F      | 2          | Unchanged           | T/T A/G
| 32          | 58          | F      | 2          | Unchanged           | C/C A/A
| 33          | 72          | F      | 2          | Unchanged           | C/C A/G
| 34          | 71          | F      | 2          | Unchanged           | C/C G/G
| 35          | 44          | F      | 2          | Unchanged           | T/T G/G
| 36          | 56          | F      | 2          | Unchanged           | C/C G/G

*1. Non-invasive cancer; 2, invasive cancer. †WT1 mRNA expression levels were determined by real-time RT-PCR in the previous study (22). WT1 expression was scored as overexpressed or unchanged according to the cut-off level which corresponded to mean ± SD of the WT1 mRNA expression levels in normal breast tissues.

**Table 1. Clinical features of patients with breast cancer**

| Patient No. | Age (years) | Gender | Histology* | WT1 mRNA expression† | WT1 SNP
|-------------|-------------|--------|------------|---------------------|--------
| 1           | 48          | F      | 1          | Overexpressed       | T/T A/G
| 2           | 47          | F      | 1          | Unchanged           | T/T A/G
| 3           | 31          | F      | 1          | Unchanged           | T/T G/G
| 4           | 49          | F      | 1          | Unchanged           | C/C A/G
| 5           | 73          | F      | 2          | Overexpressed       | C/C A/G
| 6           | 47          | F      | 2          | Overexpressed       | T/T A/A
| 7           | 53          | F      | 2          | Overexpressed       | C/C A/A
| 8           | 50          | F      | 2          | Overexpressed       | C/T A/G
| 9           | 47          | F      | 2          | Overexpressed       | C/T A/G
| 10          | 47          | F      | 2          | Overexpressed       | T/T G/G
| 11          | 48          | F      | 2          | Overexpressed       | T/T G/G
| 12          | 64          | F      | 2          | Overexpressed       | C/T A/A
| 13          | 47          | F      | 2          | Overexpressed       | T/T G/G
| 14          | 30          | F      | 2          | Overexpressed       | T/T G/G
| 15          | 40          | F      | 2          | Overexpressed       | C/T A/G
| 16          | 54          | F      | 2          | Overexpressed       | T/T G/G
| 17          | 51          | F      | 2          | Unchanged           | T/T G/G
| 18          | 73          | F      | 2          | Unchanged           | T/T A/G
| 19          | 54          | F      | 2          | Unchanged           | C/T A/G
| 20          | 61          | F      | 2          | Unchanged           | C/C A/A
| 21          | 50          | F      | 2          | Unchanged           | T/T G/G
| 22          | 57          | F      | 2          | Unchanged           | T/T G/G
| 23          | 49          | F      | 2          | Unchanged           | T/T G/G
| 24          | 51          | F      | 2          | Unchanged           | C/T A/G
| 25          | 66          | F      | 2          | Unchanged           | C/C A/A
| 26          | 54          | F      | 2          | Unchanged           | C/C A/G
| 27          | 47          | F      | 2          | Unchanged           | C/C A/A
| 28          | 57          | F      | 2          | Unchanged           | T/T A/G
| 29          | 55          | F      | 2          | Unchanged           | C/C G/G
| 30          | 48          | F      | 2          | Unchanged           | C/T A/G
| 31          | 65          | F      | 2          | Unchanged           | T/T A/G
| 32          | 58          | F      | 2          | Unchanged           | C/C A/A
| 33          | 72          | F      | 2          | Unchanged           | C/C A/G
| 34          | 71          | F      | 2          | Unchanged           | C/C G/G
| 35          | 44          | F      | 2          | Unchanged           | T/T G/G
| 36          | 56          | F      | 2          | Unchanged           | C/C G/G

*1. Non-invasive cancer; 2, invasive cancer. †WT1 mRNA expression levels were determined by real-time RT-PCR in the previous study (22). WT1 expression was scored as overexpressed or unchanged according to the cut-off level which corresponded to mean ± SD of the WT1 mRNA expression levels in normal breast tissues.
**RESULTS**

To determine whether or not the WT1 gene expressed in breast cancer had mutations, the WT1 genomic DNA from 36 breast cancers that expressed the WT1 mRNA was PCR-amplified and examined for mutations by direct sequencing. The sequencing analysis showed the absence of mutations in the 10 exons of the WT1 gene in all of the 36 different cases of primary breast cancer (data not shown).

Two different single nucleotide polymorphisms (SNP) without an amino acid change (Pro42, C to T in exon 1 (NCBI dbSNP 1799925) and/or Arg300, A to G in exon 7 (NCBI dbSNP 16754)) were detected in the WT1 gene in 31 (86%) of the 36 cases examined. SNP (Pro42, C to T in exon 1) was detected in two of these 31 cases. SNP (Arg300, A to G in exon 7) was detected in another seven cases. Both of the SNP were detected in the remaining 22 cases (Table 1).

**DISCUSSION**

The WT1 gene was originally isolated as a tumor-suppressor gene responsible for Wilms’ tumor, a kidney neoplasm of childhood. However, we have hypothesized that the WT1 gene played an oncogenic role in the tumorigenesis of various types of cancers on the basis of the following findings (20): (a) the WT1 gene was overexpressed in leukemia (13), lung cancer (14), bone and soft-tissue sarcoma (15), HNSCC (16) and thyroid cancer (17); (b) high expression levels of WT1 mRNA significantly correlated with poor prognosis in leukemia (13) and with high tumor stage in HNSCC (16) and testicular germ-cell tumors (24); (c) growth of WT1-expressing cancer cells was inhibited by the treatment with WT1 antisense oligomers (11,18,19); and (d) constitutive expression of WT1 blocked differentiation and instead induced proliferation in response to granulocyte colony-stimulating factor (G-CSF) in 32D cl3 myeloid progenitor cells (25) and normal myeloid progenitor cells (26). In breast cancer, it was recently demonstrated that the WT1 gene was overexpressed in primary breast cancer (21,22) and that the high expression levels of WT1 mRNA significantly correlated with poor prognosis (22). These results indicated that the overexpressed WT1 gene played an important role in the tumorigenesis of breast cancer. Therefore, it is important to determine whether or not the WT1 gene expressed in breast cancer has mutations. In the present study, we demonstrated the absence of mutations through all 10 exons of the WT1 gene in 36 breast cancers that expressed the WT1 mRNA. Moreover, Zapata-Benavides et al. (27) showed that WT1 protein expression levels increased when proliferation of breast cancer cells was stimulated by 17β-estradiol, but decreased when inhibited by tamoxifen or all-trans-retinoic acid (ATRA) and that growth of breast cancer cells was inhibited by the WT1 antisense oligomer treatment (27). These results and our present results indicate that the overexpressed, wild-type WT1 gene plays an important role in the tumorigenesis of human breast cancer.

**Acknowledgment**

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**Table 2. Primers used for amplification and sequencing of exons of WT1 genomic DNA**

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References


