Correlation between WT1 Expression and Cell Proliferation in Endometrial Cancer

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Abstract. Background: The Wilms’ tumor gene WT1 is overexpressed in endometrial cancer. Although recent studies have revealed that WT1 is a new prognostic factor, it remains unclear whether WT1 plays a pathophysiological role including cell proliferation. Patients and Methods: A series of 70 endometrial cancer patients who had undergone a curative resection was studied by immunohistochemistry to determine the correlation between WT1 expression and cell proliferation (proliferating cell nuclear antigen; PCNA). Results: WT1 expression was observed in 64 cases (91%). WT1 expression was associated with advanced FIGO stage (p=0.0228), myometrial invasion (p=0.0114) and high-grade histological differentiation (p=0.0004), indicating up-regulation of WT1 expression with tumor progression. A positive correlation between PCNA labeling index and score of WT1 expression was observed (p=0.0081, ρ=0.319). Conclusion: These results showed that WT1 might regulate cell proliferation in endometrial cancer.

Endometrial cancer is the most common gynecological malignancy in the United States. In Japan, it is the second most common gynecological cancer, but its frequency has dramatically increased in the last decade. Although there are well-established surgical and chemotherapeutic treatments for endometrial cancer, the need for molecular-target therapy has increased, especially for recurrent disease that has acquired radio- or chemoresistance; thus, there exists a need for a better understanding of the molecular pathways of endometrial carcinogenesis.

The Wilms’ tumor gene WT1 was isolated as a gene responsible for a childhood renal neoplasm, Wilms’ tumor (1, 2). This gene encodes a zinc finger transcription factor and plays an important role in cell growth and differentiation (3, 4). Although WT1 gene was initially categorized at first as a tumor-suppressor gene, it was recently demonstrated that the wild-type WT1 gene exhibited an oncogenic rather than a tumor-suppressor function in many kinds of malignancies (5). For example, WT1 gene is highly expressed in hematological malignancies and solid tumors, including endometrial cancer (6, 7).

Moreover, in vitro and in vivo studies revealed that WT1 was associated with cell proliferation in malignant melanoma (8, 9), breast cancer (10, 11), myeloid leukemia cells (12), glial tumors (13) and epithelial ovarian tumors (14). However, it remained unclear whether WT1 affected cell proliferation in endometrial cancer.

Therefore, in the present study, we immunohistochemically analyzed the expression of WT1 protein in 70 cases of primary endometrial cancer to study the relationship between WT1 expression and cell proliferation (proliferating cell nuclear antigen; PCNA) in endometrial cancer patients.

Patients and Methods

Patients. This study included 70 primary endometrial cancer patients who had been consecutively admitted, treated and followed-up by the Department of Obstetrics and Gynecology, Kanazawa University Hospital from January 1995 to December 2002. None of the patients had received any pre-surgical treatment and all had undergone a total abdominal or radical hysterectomy plus bilateral salpingo-oophorectomy. At the time of laparotomy, peritoneal fluid samples were obtained for cytological testing. Systemic pelvic lymphadenectomy was...
performed in 51 (72.9%) patients. Paraortic lymph node sampling was performed in two patients because of visible or palpable enlarged lymph nodes. All patients were classified by the International Federation of Gynecology and Obstetrics (FIGO) surgical staging system (1988). No patient had remaining macroscopic tumors or known distant metastasis immediately after surgery. High-risk patients (e.g. those with deep myometrial invasion, cervical involvement, special histology, or peritoneal cytology) underwent external radiotherapy and/or six cycles of chemotherapy (paclitaxel: 180 mg/m², carboplatin: according to cytology) underwent external radiotherapy and/or six cycles of chemotherapy (paclitaxel: 180 mg/m², carboplatin: according to cytology).

Patient characteristics. The average patient age at the time of surgery was 57.3 years (range, 26-78 years); 22 patients had premenopausal status, 4 perimenopausal and 44 postmenopausal. The mean patient preoperative body mass index (BMI) was 24.0 (range, 16.9-32.9).

WT1 expression in endometrial cancer. WT1 expression was positive exclusively in cancer cells in 64 cases (91%). The mean score of WT1 expression was 4.114±1.749, and median value was 4. Typical WT1 expression in endometrial cancer cells is shown in Figure 1a. A majority of the positive cases showed diffuse or granular staining in the cytoplasm. The staining of WT1 was heterogeneous in advanced tumors and WT1 was frequently located at the invasion front of the tumor. The association between WT1 expression and clinicopathological variables is shown in Table I.

WT1 overexpression was associated with advanced FIGO stage (p=0.0228), myometrial invasion (p=0.0114) and high-grade histological differentiation (p=0.0004), indicating up-regulation of WT1 expression with tumor progression in this study. Additionally, WT1 expression was stronger in postmenopausal patients (p=0.0281).

PCNA labeling index (PCNA-LI) in endometrial cancer. The mean score of PCNA-LI was 56.1±30.9 and the median value was 4. Typical PCNA expression in endometrial cancer cells is shown in Figure 1b. The association between PCNA-LI and clinicopathological variables is shown in Table II. PCNA-LI was significantly higher in elderly and postmenopausal patients (p=0.0050 and p=0.0314, respectively).

WT1 and PCNA. PCNA-LI was significantly higher in the strong expression WT1 group (final score: 5-7) than the weak expression WT1 group (final score: 0-4) (Mann-Whitney U-test: p=0.041). Moreover, considering WT1 expression scores as continuous variables, a strong association was found between WT1 expression and the PCNA-LI (p=0.0081, n=70, ρ=0.319) using the Spearman rank-correlation coefficient (Figure 2).

Discussion

Recent studies have shown that WT1 influences disease progression and prognosis in various types of cancer. In endometrial cancer, Dupont et al. reported that WT1/p53 double positivity were negative prognostic indicators using univariate analysis (15). Chiusa et al. showed that elevated
Figure 1. Immunohistochemical staining of endometrial cancer with WT1 expression and PCNA (original magnification: a, ×200; b, ×200).

a. WT1 expression of endometrial cancer as indicated by asterisks showed diffuse or granular staining in the cytoplasm; b. PCNA within endometrial cancer cells as indicated by arrows.
levels of WT1 in leukemia were associated with poor prognosis following standard chemotherapy treatment (16). We have also revealed that cytoplasmic expression of WT1 may provide additional prognostic information for endometrial cancer patients (7). Moreover, the present study demonstrated that WT1 overexpression was associated with advanced FIGO stage, myometrial invasion and high-grade histological differentiation.

WT1 protein is vital to cell proliferation and as such serves as a prognostic factor. Wagner et al. pointed out that WT1 protein and PCNA were co-localized in malignant melanoma (8). Hashiba et al. found a significant correlation between WT1 protein expression score and mindbomb homolog 1 (MIB-1) staining index showing cell proliferation activity (13). We also found that WT1 protein expression was significantly associated with PCNA expression of cell proliferation. Our results are congruent with previous reports of other types of cancer.

Other research may clarify the mechanism by which WT1 can do this. Zepata-Benavides et al. showed that WT1 protein might be involved in breast cancer proliferation by regulating cyclinD1 protein levels (11). Rong et al. reported that WT1 promoted cell proliferation in the presence of activated signal transducers and activators of transcription (STAT3) (17). Han et al. demonstrated that the exogenous expression of WT1 in

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>WT1 expression score</th>
<th>p-Value</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
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<tr>
<td>&lt;65 (n=43)</td>
<td>4.00 [2.00] (3.860±1.684)</td>
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<tr>
<td>≥65 (n=27)</td>
<td>5.175 [4.519±1.305]</td>
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<td>FIGO stage</td>
<td></td>
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<tr>
<td>I (n=52)</td>
<td>4.00 [2.00] (3.827±1.757)</td>
<td>0.0228</td>
</tr>
<tr>
<td>II, III, IV (n=27)</td>
<td>5.00 [4.944±1.474]</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
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<td></td>
</tr>
<tr>
<td>Negative (n=65)</td>
<td>4.00 [2.00] (4.108±1.795)</td>
<td>&gt;0.9999</td>
</tr>
<tr>
<td>Positive (n=5)</td>
<td>5.00 [2.00] (4.200±1.095)</td>
<td></td>
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<tr>
<td>Myometrial invasion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a, b (n=53)</td>
<td>4.00 [2.25] (3.792±1.758)</td>
<td>0.0114</td>
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<tr>
<td>c (n=17)</td>
<td>5.00 [5.118±1.317]</td>
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<td>Histopathology-degree of differentiation</td>
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<td>Grade 1 (n=38)</td>
<td>4.00 [2.00] (3.447±1.655)</td>
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<td>Grade 2, 3 (n=32)</td>
<td>5.00 [2.00] (4.906±1.532)</td>
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<td>Menopause</td>
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<td>Peri, pre (n=26)</td>
<td>4.00 [3.00] (3.500±2.005)</td>
<td>0.0281</td>
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<tr>
<td>Post (n=44)</td>
<td>5.00 [2.00] (4.477±1.486)</td>
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<td>Body mass index</td>
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<tr>
<td>&lt;25 (n=45)</td>
<td>4.00 [1.00] (4.178±1.300)</td>
<td>0.7012</td>
</tr>
<tr>
<td>≥25 (n=25)</td>
<td>5.00 [2.00] (4.000±1.683)</td>
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IQR*: Interquartile range; SD**: standard deviation.

The human breast cancer cell lines MDA-MB-468 and MCF-7, and in human leukemic K562 cells can activate the c-Myc promoter and stimulate cellular proliferation (18). Yamazaki et al. and Ito et al. found that loss of WT1 was associated with
decreased growth of leukemic cells and rapid induction of apoptosis (19, 20). Mayo et al. found that stable overexpression of WT1 led to increased endogenous Bcl-2 protein in the rhabdoid tumor cell line G401 (21). These results are worth reflecting on when considering future cancer treatment strategies targeting WT1.

In conclusion, results of the present study showed that WT1 might regulate cell proliferation in endometrial cancer.

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References


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