# WT1 Expression Correlates with Angiogenesis in Endometrial Cancer Tissue

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**Abstract.** *Background: No direct comparison has been made of the relationship between the expression of Wilms' tumor gene WT1 within tumor cells and angiogenesis in vivo. Materials 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, angiogenesis (proliferation of endothelial cell adhesion molecule-1, PECAM-1/ CD31) and angiogenic growth factor (vascular endothelial growth factor, VEGF). Results: A strong association was found between WT1 expression score and mean vascular density ($p<0.001$, $n=70$, $\varrho=0.568$). Immunohistochemistry of serial sections revealed that WT1 and VEGF were co-expressed in the same area of endometrial cancer tissue. Conclusion: Tumor-produced WT1, which may regulate the expression of VEGF, is found to be associated with induction of angiogenesis 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, the need for molecular-target therapy has increased, especially for recurrent disease that has acquired radio- or chemoresistance. A better understanding of the molecular pathways of endometrial carcinogenesis is thus.

The Wilms' tumor gene *WT1* has been isolated and identified as a gene responsible for a childhood renal neoplasm, Wilms' tumor (1-3). This gene encodes a zinc finger transcription factor and plays important roles in cell growth and differentiation (4, 5). Although *WT1* was first categorized as a tumor-suppressing gene, it was recently demonstrated that the wild-type *WT1* possessed an oncogenic rather than tumor-suppressing function in many kinds of malignancies (6). *WT1* is highly expressed in hematological malignancies and solid tumors, including endometrial cancer (7, 8).

In addition, recent studies have reported correlations between WT1 and neovascularization in histogenetics, normal genitourinary development, cardiac malformation and tumor angiogenesis (9-11). Angiogenesis is an important step for tumor growth in the transition from a small cluster of malignant cells to a visible macroscopic tumor capable of spreading to other organs *via* the vasculature (12, 13). The seminal *in vitro* study by Cash *et al*. revealed that the DNA binding domain of WT1 within tumor cells plays an essential role in the transcriptional regulation of vascular epithelial growth factor (VEGF), which is an important factor in inducing tumor angiogenesis (14). Although the potential for angiogenesis associated with tumor-produced WT1 has been suggested, few reports are available on the effect of WT1 as an angiogenic inducer in the intratumoral microenvironment of human solid tumors.

To date, no direct comparison has been made of the relationship of WT1 expression within tumor cells and angiogenesis in terms of the mechanism of tumor progression and metastasis. To address these questions, our work aimed to investigate the role of WT1 within tumor cells in angiogenesis by assessing proliferation of endothelial cell

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*Key Words:* Wilms' tumor gene, *WT1*, angiogenesis, immunohistochemistry.

Table I. *Characterization of the antibodies used in this study.*

| Antibody (Manufacture) | Species | Dilution | Incubation time (temperature) | Epitope retrieval |
|---|---|---|---|---|
| WT1 (DakoCytomation Inc. USA) | Mouse IgG | 1:100 | Overnight (4˚C) | Microwave |
| CD31 (DakoCytomation Inc. USA) | Mouse IgG | 1:1 | 1 hour (room temperature) | Microwave |
| VEGF (Thermo Fisher Scientific Inc. USA) | Mouse IgG | 1:100 | 2 hours (room temperature) | Microwave |

adhesion molecule-1 (PECAM-1, CD31) in surgically-removed endometrial carcinomas.

## Materials and Methods

*Patients*. This study included 70 primary endometrial carcinoma patients 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 salphingo-oophorectomy. At the time of laparotomy, peritoneal fluid samples were obtained for cytological testing. Systemic pelvic lymphadenectomy was performed in 51 (73%) patients. Paraaortic lymph node sampling was performed in two patients because of visible or palpable enlarged lymph nodes. All patients were classified by the FIGO surgical staging system (1988). No patient had remaining macroscopic tumors or known distant metastasis immediately following 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/m$^2$, carboplatin: according to Chatelut's formula [area under the curve, AUC=5 mg min/ml]) as postoperative adjuvant therapy. All treatments and clinical research were conducted with written informed consent.

*Immunohistochemistry*. WT1, CD31 and VEGF were evaluated in serial sections stained with appropriate antibodies. Table I shows the primary antibodies, their sources, dilutions, incubation times and epitope retrieval. Formalin-fixed, paraffin-embedded slides (5 μm-thick) were deparaffinized and rehydrated in graded alcohols. Endogenous peroxidase activity was quenched by dipping in 3% hydrogen peroxide for 30 minutes. Staining was performed by avidin-biotin complex (ABC) technique, using SAB-PO kit (Nichirei Co.,Tokyo, Japan). Color development was carried out with the peroxidase substrate 3-amino-9-ethylcarbazole (AEC) against WT1, CD31 and VEGF. All slides were counterstained with Mayer's hematoxylin. Sections without primary antibodies, as well as those with non-immunized mouse serum, served as negative controls.

*Evaluation of staining*. For evaluation of WT1 expression, staining intensity was scored as 0 (negative), 1 (weak), 2 (medium), and 3 (strong). The extent of staining was scored as 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%) and 4 (76-100%) according to the percentage of positive staining area in relation to the whole carcinoma area. The sum of the intensity and extent score was used as the final staining score (0-7) for WT1. Tumors having a final staining score of ≥5 were considered to exhibit strong expression.

In assessment of tumor microvessels, the densest vascular areas were identified by scanning tumor sections at low magnification (×40). After identification of the densest vascularization, a vessel count was performed at ×400 magnification. The average vessel number in 5 fields was expressed as the microvessel density (MVD).

Evaluation and counting were conducted by two observers (S.D. and S.O.) who were unaware of any details regarding the patients' background.

*Statistical analysis*. Chi-square test for 2×2 tables was used to compare the categorical data. The difference in continuous variables between groups was compared using Mann-Whitney *U*-test. Relations between continuous variables were investigated by means of Spearman's rank correlation coefficient test. A $p$-value of $<0.05$ was considered to indicate statistical significance. All statistical analyses were performed using the statistical package StatView version 5.0 for Macintosh (Abacus Concepts, Berkeley, CA, USA).

## Results

*Characteristics of patients*. The patients' average age at time of surgery was 57.3 years (range, 26-78, years). Patients with endometrial cancer included: 22 of premenopausal status, 4 of perimenopausal status and 44 of postmenopausal status. The patients' mean preoperative body mass index (BMI) was 24.0 (range, 16.9-32.9).

*WT1 expression*. WT1 expression was positive exclusively in cancer cells in 64 cases (91%). The expression of WT1 was strong (final staining score of 5-7) in 31 patients (44%) and weak (final staining score of 0-4) in 39 patients (56%). Typical WT1 expression in endometrial cancer cells is shown in Figure 1A. The majority of positive cases showed diffuse or granular staining in the cytoplasm. 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 II.

WT1 overexpression was associated with advanced FIGO stage ($p$=0.0266), myometrial invasion ($p$=0.0477) and high-grade histological differentiation ($p$=0.0049), indicating up-regulation of WT1 expression with tumor progression in this study.

*CD31 expression*. CD31-positive endothelial cells lining microvessels within tumors were detected in 58 out of 70 patients (Figure 2). MVD varied from 0 to 66.8, with a median of 13.6 and mean of 20.0 (standard deviation, 20.1). MVD was significantly higher in tumors of advanced FIGO

Table II. *WT1 expression and clinicopathological characteristics*.

| Variable | WT1 expression | | *P*-value ($\chi^2$ test) |
|---|---|---|---|
| | Strong (n=31) | Weak (n=39) | |
| Age (years) | | | |
| <60 (n=43) | 16 | 27 | |
| ≥60 (n=27) | 15 | 12 | 0.1325 |
| FIGO stage | | | |
| I (n=52) | 19 | 33 | |
| II, III, IV (n=18) | 12 | 6 | 0.0266 |
| Lymph node metastasis | | | |
| Negative (n=65) | 28 | 37 | |
| Positive (n=5) | 3 | 2 | 0.4629 |
| Depth (myometrial invasion) | | | |
| a (n=17) | 4 | 13 | |
| b, c (b, n=36; c, n=17) | 27 | 26 | 0.0477 |
| Histopathology, degree of differentiation | | | |
| Grade 1 (n=38) | 11 | 27 | |
| Grade 2, 3 (n=32) | 20 | 12 | 0.0049 |
| Menopause | | | |
| Peri, pre (n=26) 8 18 | | | |
| Post (n=44) 23 21 0.0801 | | | |
| Body mass index | | | |
| <25 (n=45) | 19 | 26 | |
| ≥25 (n=25) | 12 | 13 | 0.6410 |

Table III. *Microvessel density and clinicopathological characteristics*.

| Variable | Microvessel density mean±SD (median) | *P*-value (Mann-Whitney *U*-test) |
|---|---|---|
| Age (years) | | |
| <60 (n=43) | 19.3±19.9 (13.6) | |
| ≥60 (n=27) | 21.3±20.8 (13.6) | 0.8135 |
| FIGO stage | | |
| I (n=52) | 16.9±18.3 (10.9) | |
| II, III, IV (n=18) | 29.2±23.0 (19.6) | 0.0191 |
| Lymph node metastasis | | |
| Negative (n=65) | 19.9±20.0 (13.6) | |
| Positive (n=5) | 21.8±24.8 (10.8) | 0.8999 |
| Depth (myometrial invasion) | | |
| a (n=17) | 9.3±14.5 (4.2) | |
| b, c (b, n=36; c, n=17) | 23.4±20.6 (17.8) | 0.0120 |
| Histopathology, degree of differentiation | | |
| Grade 1 (n=38) | 18.2±19.2 (13.6) | |
| Grade 2, 3 (n=32) | 23.3±21.8 (13.6) | 0.0019 |
| Menopause | | |
| Peri, pre (n=26) | 12.6±14.7 (6.0) | |
| Post (n=44) | 28.8±22.3 (19.4) | 0.5223 |
| Body mass index | | |
| <25 (n=45) | 17.1±18.2 (13.9) | |
| ≥25 (n=25) | 21.7±21.2 (12.2) | 0.4388 |

stage ($p$=0.0191), with myometrial invasion ($p$=0.0120) and of high-grade histological differentiation ($p$=0.0019) (Table III). The other clinicopathological characteristics were not associated with MVD.

*Association between WT1 expression and CD31*. CD31 in the strong-expression WT1 group was significantly higher than in the weak-expression WT1 group, by Mann-Whitney *U*-test ($p$<0.001) (data not shown). Moreover, considering WT1 expression scores as continuous variables, a strong association was found between WT1 expression and CD31 expression ($p$<0.001, n=70, ϱ=0.568) using Spearman's rank-correlation coefficient (Figure 3).

*VEGF expression in WT1-positive cancer cells*. Typical VEGF expression within tumor cells is shown in Figure 1B. Immunohistochemistry of serial sections revealed that VEGF and WT1 were co-expressed in the same area of endometrial cancer tissue.

## Discussion

Recent studies showed that tumor-produced WT1 influenced tumor progression in various types of cancer. Yamamoto *et al*. found that WT1 protein might be an accelerator of the progression of ovarian serous adenocarcinoma (15). Sera *et al*. concluded that overexpressed WT1 was associated with tumor growth, and resulted in a worsening prognosis of hepatocellular carcinoma (16). Hylander *et al*. demonstrated that patients with WT1-positive tumors had a higher grade ($p$=0.006) and advanced stage ($p$=0.002) of epithelial ovarian cancer (17). Miyoshi *et al*. found that tumors >2 cm also showed a trend toward an increase ($p$=0.09) in *WT1* mRNA levels as compared with tumors <2 cm in breast cancer (18). In the present study, we found that WT1 overexpression was associated with advanced FIGO stage, myometrial invasion and high-grade histological differentiation, indicating up-regulation of *WT1* expression with tumor progression. Our results are congruent with previous reports of the other types of cancer.

Tumor development and progression are inherently dependent on the process of angiogenesis. Ozalp *et al*. found that microvessel density (MVD) as assessed by factor VIII antigen was correlated with high surgical stage ($p$<0.001), cervical involvement ($p$=0.01), adnexal involvement ($p$=0.04), lymphovascular space involvement ($p$=0.02), pelvic and paraaortic lymph node metastasis ($p$<0.001), and positive peritoneal cytology ($p$<0.001) in endometrial carcinoma (19). Salvesen *et al*. found that MVD as assessed by CD105/endoglin antigen was significantly correlated with MVD as assessed by factor VIII antigen (linear regression

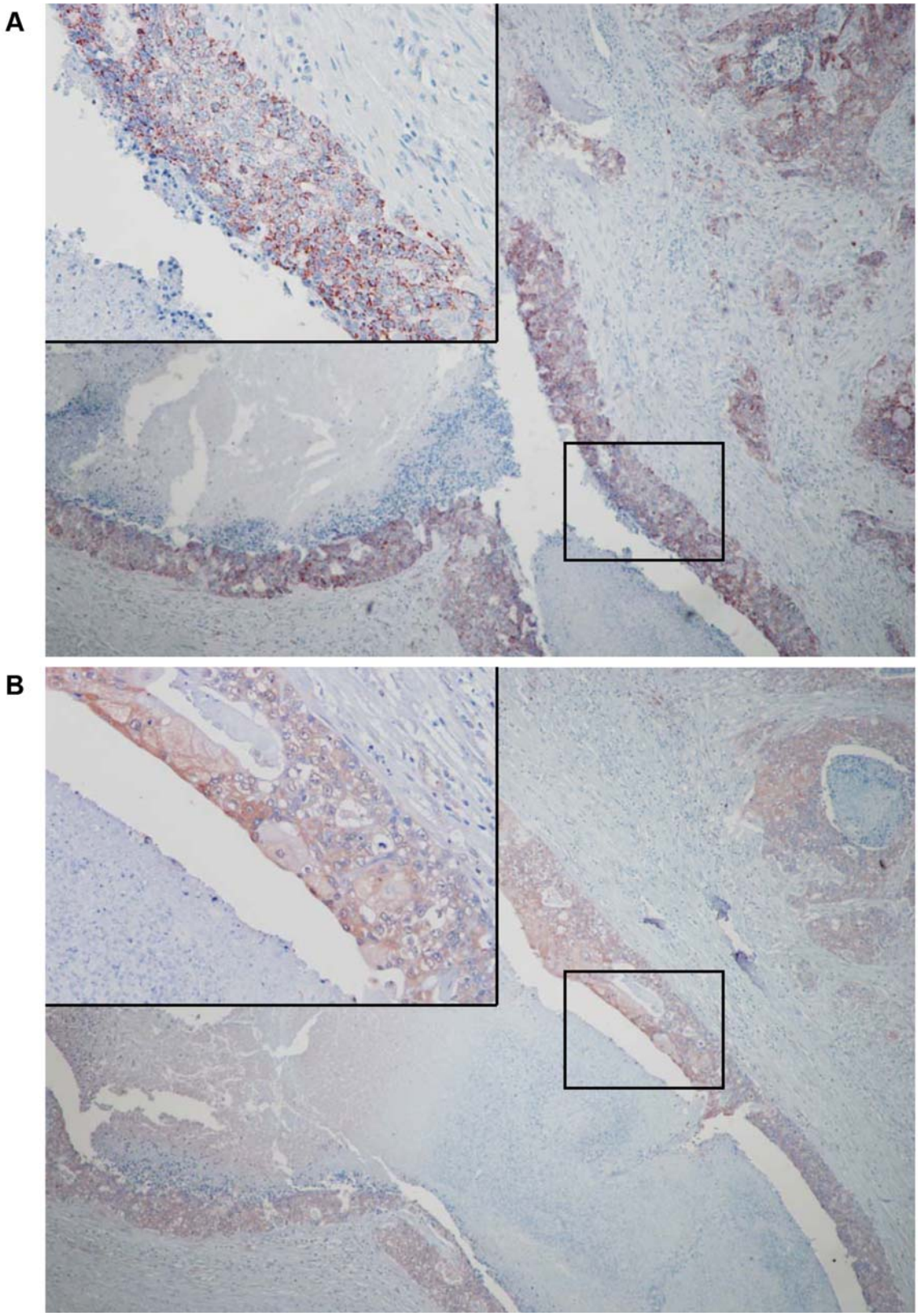


Figure 1. *Representative section of endometrial cancer with immunohistochemical staining of A: WT1 (×40; inset ×200) and B: VEGF (×40; inset ×200). Strong cytoplasmic staining of WT1 and VEGF is observable in the same area of serial sections.*

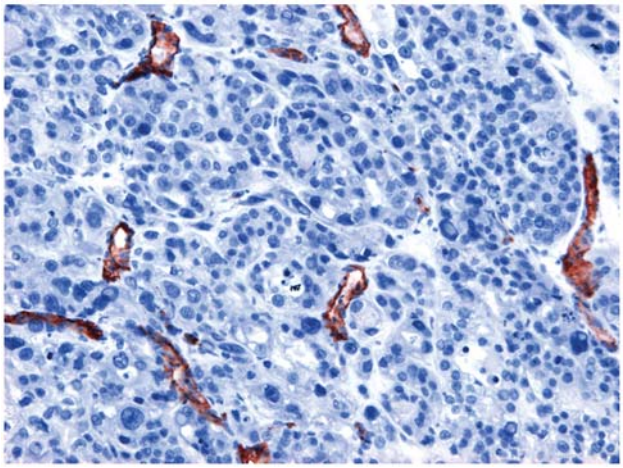

Figure 2. *Representative section of endometrial tumor with immunohistochemical staining of CD31 (×100).*

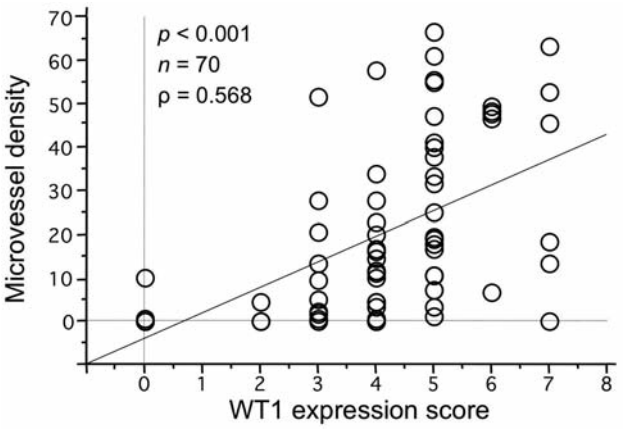


Figure 3. *A positive correlation between microvessel density and WT1 staining score was observed using Spearman's rank correlation coefficient.*

0.32, $p$=0.001), and that CD105/endoglin-MVD is significantly associated with FIGO stage ($p$=0.03), tumor cell proliferation estimated by the expression of Ki-67 ($p$=0.007) in endometrial cancer (20). Ueda *et al*. demonstrated that intratumoral MVD (immunohistochemically using anti-CD34 antibody) was well correlated with histological type ($p$=0.0415), depth of myometrial invasion ($p$=0.0176), endometrial invasion ($p$=0.0354) and pelvic lymph node metastasis ($p$=0.0354) in uterine cervical cancer (21). Kamat *et al*. showed that high expression of MVD as assessed by CD31 expression was associated with high-stage and grade 3 tumors ($p$=0.03 and 0.04, respectively) in endometrial cancer (22). In the present study, we found that MVD as assessed by CD31 expression was associated with advanced FIGO stage, myometrial invasion and high-grade histological differentiation. Furthermore, a strong association was found between WT1 staining score and MVD using Spearman's rank correlation coefficient. These results suggest that WT1 may regulate tumor progression and angiogenesis in endometrial cancer.

As a key mediator of angiogenesis, VEGF is tightly regulated at both the transcriptional and post-transcriptional levels. VEGF regulation is complex, since it is up-regulated by hypoxia, growth factors, steroid hormones and transcription factors including WT1 (23). Cash *et al*. showed that WT1 had both transcriptional and post-transcriptional effects on *VEGF* mRNA levels in prostate cell lines (14). Hanson *et al*. found that methyltrienolamine (androgen analog, R1881) increased the transcriptional activation of the *VEGF* promoter by WT1 in LNCaP prostate cancer cells (24). Overall, these reports suggest that WT1 plays an essential role in the transcriptional regulation of *VEGF* in cancer cells. On the other hand, WT1 is essential for kidney development (25-27) and is co-expressed with VEGF in normal kidney cells and in some Wilms' tumors (27-30). Therefore, for both developmental and cancer studies, it is important to elucidate the mechanisms whereby WT1 regulates VEGF and thereby, angiogenesis. In the present study, we also found that WT1 and VEGF were co-expressed in the same area of endometrial cancer tissue. This result suggests that WT1 may regulate expression of VEGF.

These results imply that WT1 plays an important role in endometrial cancer-associated angiogenesis, probably *via* induction of angiogenesis by VEGF. To the best of our knowledge, this may be the first report to demonstrate the positive role of WT1 in endometrial cancer-associated angiogenesis and may prove of great benefit in finding a rational approach to endometrial cancer therapy.

Recently, anti-angiogenic therapy has begun to show promise as an effective treatment strategy for many types of solid tumor. WT1 is also a target for cancer immunotherapy, and this study suggests that a WT1 peptide vaccine therapy might be effective not only as cancer immunotherapy but also as anti-angiogenesis therapy.

In conclusion, tumor-produced WT1, which may regulate the expression of VEGF, is associated with the induction of angiogenesis in endometrial cancer.

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## References


1 Call KM, Glaser T, Ito CY, Buckler AJ, Pelletier J, Haber DA, Rose EA, Kral A, Yeger H, Lewis WH, Jones C and Housman DE: Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. Cell *60*: 509-520, 1990.

2 Gessler M, Poustka A, Cavenee W, Neve RL, Orkin SH and Bruns GA: Homozygous deletion in Wilms tumors of a zinc-finger gene identified by chromosome jumping. Nature *343*: 774-778, 1990.

3 Rivera MN and Haber DA: Wilms' tumour: connecting tumorigenesis and organ development in the kidney. Nat Rev Cancer *5*: 699-712, 2005.

4 Sugiyama H: Wilms' tumor gene WT1: its oncogenic function and clinical application. Int J Hematol *73*: 177-187, 2001.

5 Oka Y, Tsuboi A, Kawakami M, Elisseeva OA, Nakajima H, Udaka K, Kawase I, Oji Y and Sugiyama H: Development of WT1 peptide cancer vaccine against hematopoietic malignancies and solid cancers. Curr Med Chem *13*: 2345-2352, 2006.

6 Yang L, Han Y, Suarez Saiz F and Minden MD: A tumor suppressor and oncogene: the WT1 story. Leukemia *21*: 868-876, 2007.

7 Nakatsuka S, Oji Y, Horiuchi T, Kanda T, Kitagawa M, Takeuchi T, Kawano K, Kuwae Y, Yamauchi A, Okamura M, Kitamura Y, Oka Y, Kawase I, Sugiyama H and Aozasa K: Immunohistochemical detection of WT1 protein in a variety of cancer cells. Mod Pathol *19*: 804-814, 2006.

8 Ohno S, Dohi S, Ohno Y, Kyo S, Sugiyama H, Suzuki N and Inoue M: Immunohistochemical detection of WT1 protein in endometrial cancer. Anticancer Res *29*: 1691-1695, 2009.

9 Wagner N, Michiels JF, Schedl A and Wagner KD: The Wilms' tumour suppressor WT1 is involved in endothelial cell proliferation and migration: expression in tumour vessels *in vivo*. Oncogene *27*: 3662-3672, 2008.

10 Lee SB and Haber DA: Wilms tumor and the *WT1* gene. Exp Cell Res *264*: 74-99, 2001.

11 van Loo PF, Mahtab EA, Wisse LJ, Hou J, Grosveld F, Suske G, Philipsen S and Gittenberger-de Groot AC: Transcription factor *Sp3* knockout mice display serious cardiac malformation. Mol Cell Biol *27*: 8571-8582, 2007.

12 Semenza GL: Angiogenesis in ischemic and neoplastic disorder. Annu Rev Med *54*: 17-28, 2003.

13 Cohen MM Jr.: Vascular update: morphogenesis, tumors, malformations, and molecular dimensions. Am J Med Genet A *140*: 2013-2038, 2006.

14 Cash J, Korchnak A, Gorman J, Tandon Y and Fraizer G: *VEGF* transcription and mRNA stability are altered by WT1 not DDS(R384W) expression in LNCaP cells. Oncol Rep *17*: 1413-1419, 2007.

15 Yamamoto S, Tsuda H, Kita T, Maekawa K, Fujii K, Kudoh K, Furuya K, Tamai S, Inazawa J and Matsubara O: Clinicopathological significance of WT1 expression in ovarian cancer: a possible accelerator of tumor progression in serous adenocarcinoma. Virchows Arch *451*: 27-35, 2007.

16 Sera T, Hiasa Y, Mashiba T, Tokumoto Y, Hirooka M, Konishi I, Matsuura B, Michitaka K, Udaka K and Onji M: Wilms' tumour 1 gene expression is increased in hepatocellular carcinoma and associated with poor prognosis. Eur J Cancer *44*: 600-608, 2008.

17 Hylander B, Repasky E, Shrikant P, Intengan M, Beck A, Driscoll D, Singhal P, Lele S and Odunsi K: Expression of Wilms tumor gene (*WT1*) in epithelial ovarian cancer. Gynecol Oncol *101*: 12-17, 2006.

18 Miyoshi Y, Ando A, Egawa C, Taguchi T, Tamaki Y, Tamaki H, Sugiyama H and Noguchi S: High expression of Wilms' tumor suppressor gene predicts poor prognosis in breast cancer patients. Clin Cancer Res *8*: 1167-1171, 2002.

19 Ozalp S, Yalcin OT, Acikalin M, Tanir HM, Oner U and Akkoyunlu A: Microvessel density (MVD) as a prognosticator in endometrial carcinoma. Eur J Gynaecol Oncol *24*: 305-308, 2003.

20 Salvesen HB, Gulluoglu MG, Stefansson I and Akslen LA: Significance of CD 105 expression for tumour angiogenesis and prognosis in endometrial carcinomas. APMIS *111*: 1011-1018, 2003.

21 Ueda M, Terai Y, Kumagai K, Ueki K, Okamoto Y and Ueki M: Correlation between tumor angiogenesis and expression of thymidine phosphorylase, and patient outcome in uterine cervical carcinoma. Hum Pathol *30*: 1389-1394, 1999.

22 Kamat AA, Merritt WM, Coffey D, Lin YG, Patel PR, Broaddus R, Nugent E, Han LY, Landen CN Jr, Spannuth WA, Lu C, Coleman RL, Gershenson DM and Sood AK: Clinical and biological significance of vascular endothelial growth factor in endometrial cancer. Clin Cancer Res *13*: 7487-7495, 2007.

23 Jo'sko J, Mazurek M: Transcription factors having impact on vascular endothelial growth factor (VEGF) gene expression in angiogenesis. Med Sci Monit *10*: RA89-RA98, 2004.

24 Hanson J, Gorman J, Reese J and Fraizer G: Regulation of vascular endothelial growth factor, *VEGF*, gene promoter by the tumor suppressor, WT1. Front Biosci *12*: 2279-2290, 2007.

25 Hammes A, Guo JK, Lutsch G, Leheste JR, Landrock D, Ziegler U, Gubler MC and Schedl A: Two splice variants of the Wilms' tumor 1 gene have distinct functions during sex determination and nephron formation. Cell *106*: 319-329, 2001.

26 Kreidberg JA, Sariola H, Loring JM, Maeda M, Pelletier J, Housman D and Jaenisch R: WT-1 is required for early kidney development. Cell *74*: 679-691, 1993.

27 Armstrong JF, Pritchard-Jones K, Bickmore WA, Hastie ND and Bard JB: The expression of the Wilms' tumour gene, *WT1*, in the developing mammalian embryo. Mech Dev *40*: 85-97, 1993.

28 Gao X, Chen X, Taglienti M, Rumballe B, Little MH and Kreidberg JA: Angioblast-mesenchyme induction of early kidney development is mediated by WT1 and VEGFA. Development *132*: 5437-5449, 2005.

29 Karth J, Ferrer FA, Perlman E, Hanrahan C, Simons JW, Gearhart JP and Rodriguez R: Coexpression of hypoxia-inducible factor 1-alpha and vascular endothelial growth factor in Wilms' tumor. J Pediatr Surg *35*: 1749-1753, 2000.

30 Baudry D, Faussillon M, Cabanis MO, Rigolet M, Zucker JM, Patte C, Sarnacki S, Boccon-Gibod L, Junien C and Jeanpierre C: Changes in *WT1* splicing are associated with a specific gene expression profile in Wilms' tumour. Oncogene *21*: 5566-5573, 2002.


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