

Overexpression of PTEN in ovarian cancer cells suppresses i.p. dissemination and extends survival in mice

Yuji Takei,¹ Yasushi Saga,¹ Hiroaki Mizukami,² Takeshi Takayama,¹ Michitaka Ohwada,¹ Keiya Ozawa,² and Mitsuaki Suzuki¹

¹Department of Obstetrics and Gynecology and ²Division of Genetic Therapeutics, Center for Molecular Medicine, School of Medicine, Jichi Medical University, Tochigi, Japan

Abstract

The main mode of progression of ovarian cancer is peritoneal dissemination, and its inhibition may lead to improved outcome. Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) reportedly inhibits the proliferation, migration, and invasion of cancer cells. The purpose of this study is to explore the possibility of PTEN gene therapy for ovarian cancer. We transfected the ovarian cancer cell line SHIN-3 [vascular endothelial growth factor (VEGF)–hypersecretory cell line] with PTEN or luciferase (LUC)–expressing plasmid. After selection, PTEN-overexpressing cells (SHIN-3/PTEN) and control cells (SHIN-3/LUC) were obtained. SHIN-3/PTEN implanted s.c. into nude mice was examined for the change in tumor diameter and the number of new blood vessels. Mice with peritoneally disseminated tumors created by i.p. inoculation of the same cells were examined for changes in body weight and abdominal circumference and for survival time. The growth of s.c. SHIN-3/PTEN was significantly lower than that of control ($P < 0.001$). Compared with controls, mice with i.p. inoculated SHIN-3/PTEN showed significantly smaller increases in the body weight and abdominal circumference ($P < 0.01$) and a significantly longer survival time ($P < 0.05$). VEGF concentration in the supernatant of SHIN-3/PTEN was about half that of controls ($P < 0.05$). The number of new blood vessels in SHIN-3/PTEN was significantly smaller than that in controls ($P < 0.001$).

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Requests for reprints: Yuji Takei, Department of Obstetrics and Gynecology, School of Medicine, Jichi Medical University, 3311-1, Yakushiji, Shimotsuke, Tochigi 329-0498, Japan. Phone: 81-285-58-7376; Fax: 81-285-44-8505. E-mail: ytakei@jichi.ac.jp

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Overexpression of PTEN suppressed tumor growth and peritoneal dissemination of VEGF-hypersecretory ovarian cancer cells and prolonged the survival time of the mice with peritoneal disseminated tumor. PTEN gene therapy could have therapeutic potential for ovarian cancer and exerts some of this effect by inhibiting angiogenesis. [Mol Cancer Ther 2008;7(3):704–11]

Introduction

Ovarian cancer is detected as an advanced disease with peritoneal dissemination in more than half of all cases (1) and has the highest mortality rate of all gynecologic cancers (1, 2). Even after optimal treatment, more than half of the patients recur and eventually die (3, 4). The most frequent mode of recurrence is peritoneal dissemination with massive accumulation of ascitic fluid. Therapy with conventional anticancer agents has already reached a limit, necessitating a new therapeutic strategy to improve outcome.

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is a cancer suppressor gene located on chromosome 10q23 (5, 6) and functions to inhibit cell proliferation, migration, and invasion (7). PTEN is deleted or mutated in many tumors such as glioblastoma (8), melanoma (9), lymphoid malignancy (10), breast cancer (11), endometrial cancer (12), and prostate cancer (13); thus, we speculate that inactivation of PTEN plays an important role in tumorigenicity.

Unlike the above cancers, fewer cases of ovarian cancer had PTEN mutation, and the role of PTEN in ovarian cancer is considered to be small (14, 15). When we transfected the PTEN gene to the ovarian cancer cell lines without PTEN mutation, interestingly, migration of the cells was inhibited, and their sensitivity to anticancer drugs was increased (16, 17). Thus, in this study, we induced overexpression of the PTEN gene in SHIN-3, an ovarian cancer cell line without PTEN gene mutation, and investigated the tumor growth-inhibitory effect of PTEN in mice inoculated with the cancer cells.

Materials and Methods

Cell Lines and Plasmids

The human ovarian serous cystadenocarcinoma cell lines SHIN-3 (18) and HRA (19) were cultured in DMEM (Life Technologies) supplemented with 10% heat-inactivated FCS, 2 mmol/L L-glutamine, 100 units penicillin, and 100 µg/mL streptomycin at 37°C under 5% CO₂. SHIN-3 is a vascular endothelial growth factor (VEGF)–hypersecretory cell line, and HRA is a VEGF-hyposecretory cell line. The experiments below were done mainly using the SHIN-3 cell

line. The construction of PTEN-expressing or luciferase (LUC)-expressing plasmids and the preparation of PTEN gene-transfected SHIN-3 cells (SHIN-3/PTEN) and LUC gene-transfected SHIN-3 cells (SHIN-3/LUC) were done as described previously (17). We have already confirmed that the transfection of cancer cells with the LUC gene does not influence the proliferation, migration, or invasion of cancer cells (20, 21) and does not influence the sensitivity to anticancer agents or radiation (17, 22) and that SHIN-3/PTEN and SHIN-3/LUC do not differ in the degree of cell proliferation *in vitro* (17).

S.c. Tumor Growth

Female 4- to 5-week-old BALB/c nude mice (Japan Clea Laboratories) were used in the experiment. SHIN-3/PTEN or SHIN-3/LUC cells (5×10^6) were s.c. transplanted into the backs of the mice and tumor size was measured with a micrometer caliper twice weekly. The tumor volume was calculated by the formula: volume = (short diameter)² × (long diameter) × 0.5 (23). This experiment was also done with the HRA cell line. All of the animal experiments were done under the guidelines of Jichi Medical University.

Peritoneal Dissemination and Ascites Accumulation

SHIN-3/PTEN or SHIN-3/LUC cells (5×10^6) were inoculated into mice i.p. 21 days later. The mice were sacrificed with diethyl ether. We measured the abdominal circumference and body weight of mice on the assumption that both reflected a combination of the degree of peritoneal dissemination and the amount of ascitic fluid (24). We measured the abdominal circumference and body weight of mice immediately before the i.p. inoculation of cancer cells, and later at the time of sacrifice, and obtained the value (Δ) by subtracting the former from the latter.

Survival Rate

SHIN-3/PTEN or SHIN-3/LUC cells (5×10^6) were inoculated into mice i.p., and the mice were monitored twice daily until they died of massive ascites. Survival rates were calculated using the Kaplan-Meier method. This experiment was also done with the HRA cell line.

VEGF Quantitation in Cell Culture Supernatants

SHIN-3, SHIN-3/LUC, and SHIN-3/PTEN were inoculated into 10-cm dishes and cultured in 10% serum-supplemented DMEM. When the cells grew to ~80% confluence, the culture supernatant was replaced with serum-free culture medium. Twenty-four hours later, the culture supernatant was recovered. The concentration of VEGF in the supernatant was determined by ELISA system (R&D Systems) according to the manufacturer's instructions.

Measurement of Serum VEGF and Ascitic VEGF

SHIN-3/LUC ($n = 5$) or SHIN-3/PTEN ($n = 5$) was s.c. injected into the backs of mice. Blood was collected from the tail vein 21 days after inoculation, and the serum VEGF level was measured by ELISA (R&D Systems).

SHIN-3/LUC ($n = 10$) or SHIN-3/PTEN ($n = 10$) was i.p. inoculated into mice. Blood was collected from the tail vein on days 14 and 21 after cell inoculation from five animals each in the SHIN-3/LUC and SHIN-3/PTEN groups. The mice were sacrificed just after collecting the blood,

and 500 μ L PBS was immediately injected into the abdominal cavity. The abdomen was opened, ascites containing PBS were collected, and the ascitic VEGF level was measured as described above.

Angiogenesis in S.c. Tumor

On the 21st day after the s.c. transplantation of 5×10^6 SHIN-3/PTEN or SHIN-3/LUC cells into the back, the mice (five mice each) were sacrificed and the s.c. tumors were excised. After fixation of the tumors in 4% paraformaldehyde, frozen sections were cut, and the endogenous peroxidases were blocked with 3% H₂O₂. The sections were incubated overnight at 4°C with a 1:50 dilution of anti-CD31 antibody (PharMingen) as the primary antibody recognizing vascular endothelial cells then reacted with the secondary antibody, that is, peroxidase-conjugated anti-rat antibody (Simple Stain Mouse MAX-PO, Rat; Nichirei) at room temperature for 30 min, followed by color development with diaminobenzidine. The number of newly formed vessels was counted under a light microscope at $\times 100$ magnification. A single section was prepared per mouse in five animals per group, and new blood vessels were counted in the five sections and averaged.

Immunohistochemical Staining and Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End Labeling Assay

SHIN-3/LUC or SHIN-3/PTEN was s.c. injected into the backs of mice (five mice each). The animals were sacrificed 21 days after inoculation, and s.c. tumors were excised. The tumors were fixed in 4% formalin, and paraffin-embedded sections were prepared. Antigen enhancement was done by heating the sections at 121°C in sodium citrate buffer (0.01 mol/L; pH 6.0) for 5 min, and endogenous peroxidase was blocked with 3% H₂O₂. The sections were then reacted with specific antibodies, that is, anti-cleaved caspase-3 (Asp¹⁷⁵) antibody (1:100 dilution; Cell Signaling Technology) and anti-MIB antibody (1:20 dilution; Immunotech) at 4°C overnight, respectively. Then, the sections were reacted with secondary antibody, that is, the LSAB+System-HRP kit (DakoCytomation) according to the manufacturer's instructions, followed by color development with diaminobenzidine. In the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay, antigen was enhanced by treatment with 0.002% proteinase for 15 min, endogenous peroxidase was blocked with 3% H₂O₂, and the assay was done using the in-site Apoptosis Detection Kit (TAKARA Biomedicals) following the manufacturer's instructions. Cleaved caspase-3-positive cells were counted in five randomly selected visual fields at $\times 400$ magnification, and the means of the five sections were calculated. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling-positive cells were counted by the same procedure. Ki-67-positive cells per 1,000 cancer cells were counted in randomly selected visual fields at $\times 400$ magnification, and the means of the five sections were calculated.

Statistical Analysis

Intergroup differences were tested for significance using Student's *t* test. Survival rates were analyzed by the

generalized Wilcoxon and log-rank tests. *P* values less than 0.05 were considered significant.

Results

S.c. Tumor Growth

The growth of SHIN-3/PTEN s.c. tumor was markedly inhibited compared with that of the control (SHIN-3/LUC; Fig. 1A). The size of s.c. tumors on the 37th day after the transplantation of SHIN-3/PTEN was significantly smaller than that of the control (166 ± 161 versus $1,579 \pm 642$ mm³; *P* < 0.001). In contrast, no significant difference was noted in the tumor growth between the HRA/PTEN and control (HRA/LUC; Fig. 1B).

Peritoneal Dissemination and Ascites Accumulation

Figure 2 shows the effects of PTEN overexpression on the peritoneal dissemination of ovarian cancer cells. Figure 2A shows the i.p. appearance of a mouse on the 21st day after the i.p. inoculation of SHIN-3/PTEN or SHIN-3/LUC. In control mice, the abdomen was markedly distended with massive hemorrhagic ascites, whereas in SHIN-3/PTEN mice the abdominal distension and ascites were clearly suppressed. Figure 2B shows the Δ abdominal circumference and Δ body weight in mice. The Δ abdominal circumference and Δ body weight were significantly smaller in the SHIN-3/PTEN-inoculated mice than in the control mice (5 ± 2 versus 21 ± 6 mm; *P* < 0.01; 2.6 ± 0.5 versus 8.8 ± 2.2 g; *P* < 0.01). Figure 2C shows the i.p. appearance after peritoneal incision. In the control mice, many peritoneally disseminated tumors (arrows) were observed, whereas in the SHIN-3/PTEN mice peritoneal dissemination was clearly suppressed. Thus, overexpression of the PTEN gene markedly suppressed the peritoneal dissemination of ovarian cancer and the production of ascitic fluid.

Survival Rate

The survival of mice was monitored after inoculating tumor cells i.p. The following findings were noted on comparison of the SHIN-3/PTEN and SHIN-3/LUC (control). In the SHIN-3/LUC group, the accumulation of ascitic fluid became prominent from the 14th day after inoculation, and all mice died by the 42nd day. In contrast, in the SHIN-3/PTEN group, ascites accumulation was suppressed, resulting in a significantly longer survival (*P* < 0.001; Fig. 3). In contrast, no significant difference was noted in the survival rate between the HRA/PTEN and HRA/LUC (control; data not shown).

Detection of VEGF in Cell Culture Supernatants

The concentrations of VEGF in the culture supernatant of SHIN-3, SHIN-3/LUC, and SHIN-3/PTEN were $2,300 \pm 400$, $2,500 \pm 200$, and $1,300 \pm 100$ pg/mL, respectively (Fig. 4). These findings suggested that PTEN exerts its antiangiogenic effect partially by inhibiting VEGF expression.

Serum VEGF Concentration and Ascitic VEGF Concentration

The serum VEGF level was lower than the detection limit in both SHIN-3/PTEN and SHIN-3/LUC groups. As

shown in Fig. 5, the ascitic VEGF level on day 14 in the SHIN-3/PTEN group was 2.2 ± 0.7 ng/mL, significantly lower than that (14.9 ± 3.9 ng/mL) in the SHIN-3/LUC group (*P* < 0.01). Similarly, the ascitic VEGF level on day 21 (8.5 ± 0.9 ng/mL) was significantly lower in the SHIN-3/PTEN group than in the SHIN-3/LUC group (13.7 ± 3.2 ng/mL; *P* < 0.05). However, the level was significantly increased on day 21, compared with that on day 14, in the SHIN-3/PTEN group (*P* < 0.001). Ascites was not collected after day 21; hence, the ascitic VEGF level was not measured.

Angiogenesis in S.c. Tumor

Typical immunohistochemistry of tumors with anti-CD31 antibody is shown in Fig. 6A, and the numbers of new

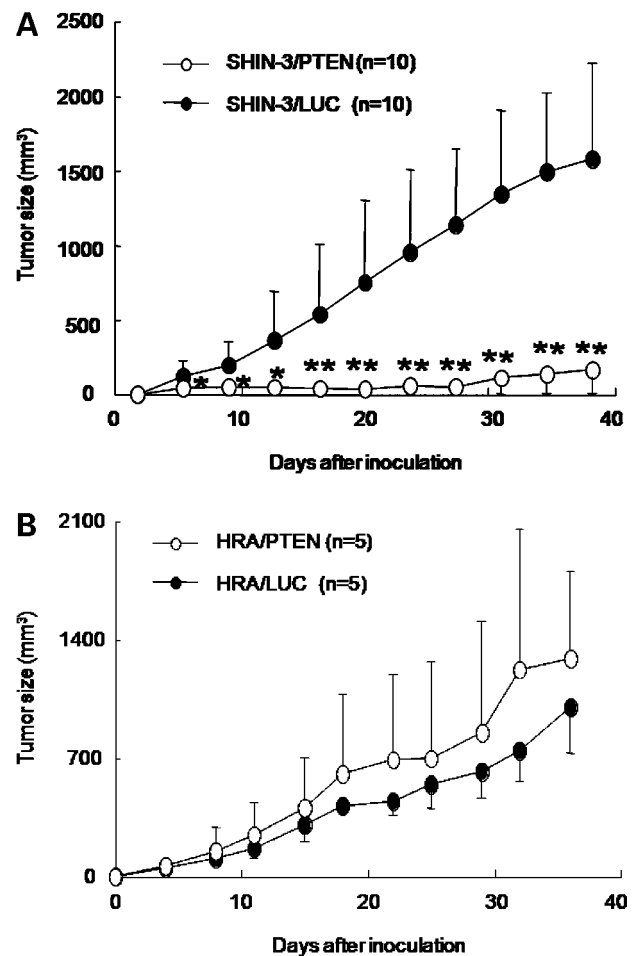


Figure 1. *In vivo* tumor growth curves. **A**, tumor growth curves of SHIN-3/PTEN (○) and SHIN-3/LUC (●). Tumor cells were s.c. injected into the backs of mice and measured twice weekly. S.c. tumor growth was markedly suppressed in SHIN-3/PTEN. The tumor size of SHIN-3/PTEN at 37 d after inoculation was 166 ± 161 mm³, which was significantly smaller than SHIN-3/LUC ($1,579 \pm 642$ mm³; *P* < 0.001). *, *P* < 0.05; **, *P* < 0.001. **B**, tumor growth curves of HRA/PTEN (○) and HRA/LUC (●). No significant difference was noted between the two groups. Mean \pm SD.

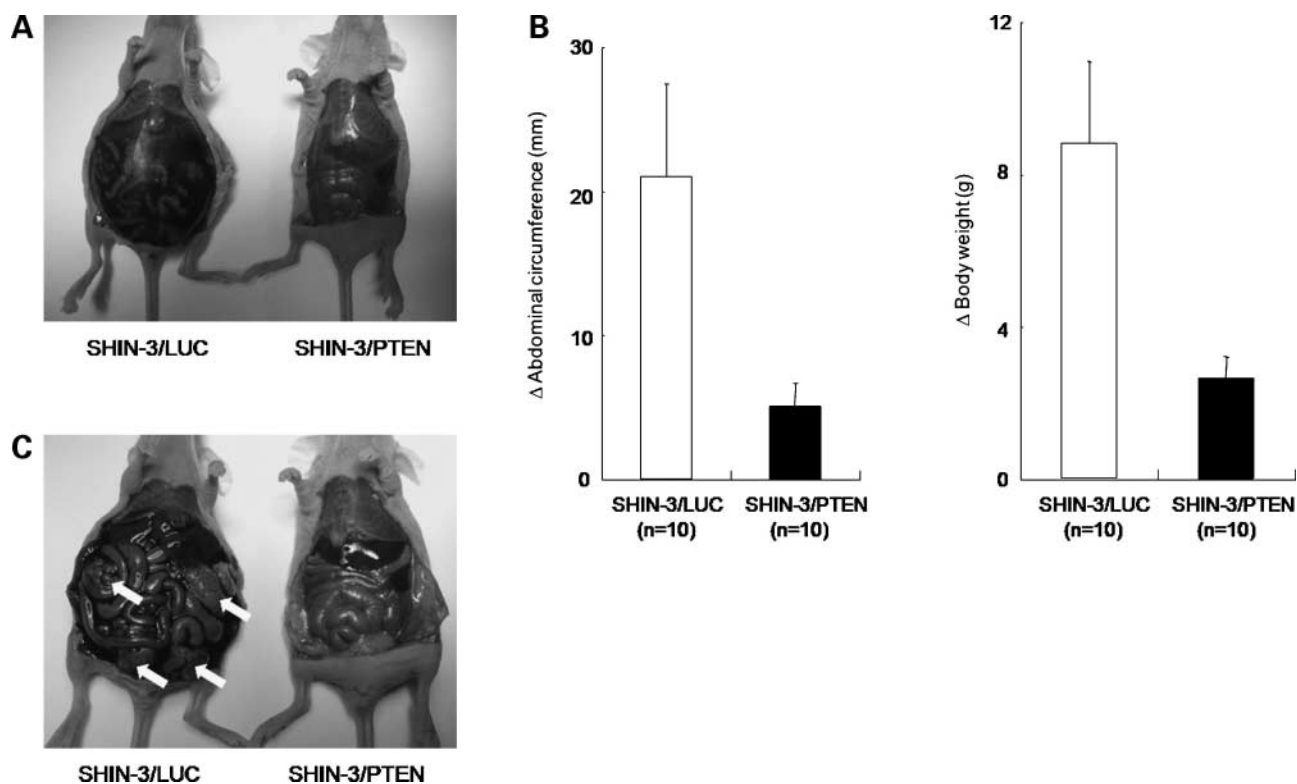


Figure 2. Peritoneal dissemination and ascites accumulation after i.p. inoculation of SHIN-3/PTEN or SHIN-3/LUC. **A**, i.p. appearance of a mouse on the 21st day after the i.p. inoculation of SHIN-3/PTEN or SHIN-3/LUC. In the SHIN-3/LUC-inoculated mouse, the abdomen was markedly distended with massive hemorrhagic ascites, whereas in the SHIN-3/PTEN-inoculated mouse the abdominal distension and ascites were clearly suppressed. **B**, changes in mouse abdominal circumference (*left*) and mouse body weight (*right*). The abdominal circumference and body weight were smaller in the SHIN-3/PTEN-inoculated mice than in the control mice [5 ± 2 versus 21 ± 6 mm; $P < 0.01$ (*left*); 2.6 ± 0.5 versus 8.8 ± 2.2 g; $P < 0.01$ (*right*)]. Mean \pm SD. **C**, intra-abdominal appearance after peritoneal incision. Arrows, lesions of peritoneal dissemination. In the SHIN-3/LUC-inoculated mice, many peritoneally disseminated lesions were observed, whereas in the SHIN-3/PTEN-inoculated mice peritoneal dissemination was clearly suppressed.

blood vessels are summarized in Fig. 6B. The number of new blood vessels was significantly smaller in SHIN-3/PTEN than in the control (4.0 ± 1.0 versus 12.4 ± 2.6 ; $P < 0.001$).

Immunohistochemical Staining and Terminal Deoxynucleotidyl Transferase – Mediated dUTP Nick End Labeling Assay

Cleaved caspase-3-positive cells (apoptotic cells) were few both in the SHIN-3/PTEN and SHIN-3/LUC (control) showing no difference, that is, 7.8 ± 2.0 and 4.8 ± 2.2 cells per five fields of $\times 400$ magnification, respectively. Similarly, no significant difference was noted in the number of apoptotic cells between the two groups on terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay (3.4 ± 0.9 versus 2.2 ± 1.1 cells per five fields of $\times 400$ magnification).

As for Ki-67, 597 ± 47 cells/1,000 cells were positive in the SHIN-3/PTEN group and 771 ± 61 cells/1,000 cells in the SHIN-3/LUC (control) group, showing that significantly fewer cells were positive in the SHIN-3/PTEN group ($P < 0.001$; Supplementary Fig. S1).³

Discussion

This *in vivo* study using PTEN gene-transfected ovarian cancer cell SHIN-3 (VEGF-hypersecretory cell line; ref. 25) showed that PTEN suppressed s.c. tumor growth, peritoneal dissemination, and ascitic fluid production and confirmed that PTEN inhibited the production of VEGF by cancer cells, thereby inhibiting angiogenesis.

In vivo studies using the PTEN gene have reported results for bladder cancer (26) and melanoma (27) but not for ovarian cancer. Moreover, previous work has addressed the inhibition of s.c. tumor growth and pulmonary metastasis but not of peritoneal dissemination. This article is the first to report the effects of PTEN on the peritoneal dissemination of ovarian cancer.

Recent studies have reported that PTEN inhibits the production of VEGF, an angiogenesis factor (26–29). In this

³ Supplementary material for this article is available at Molecular Cancer Therapeutics Online (<http://mct.aacrjournals.org/>).

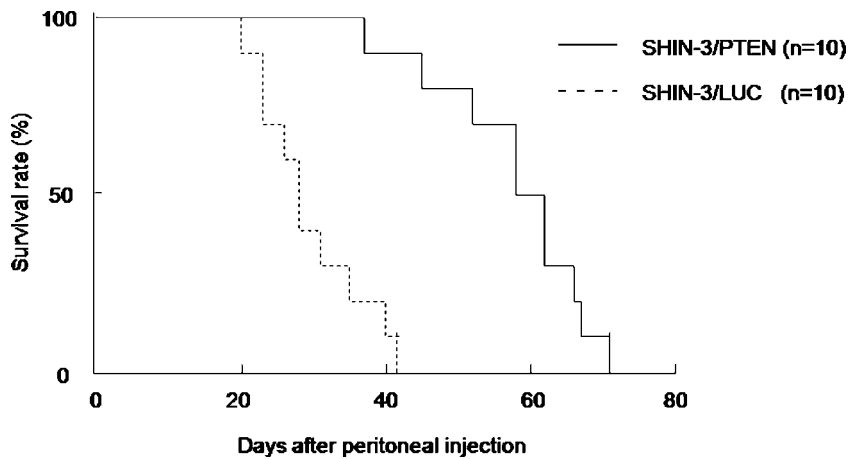


Figure 3. Kaplan-Meier survival curves of mice i.p. injected with SHIN-3/PTEN or SHIN-3/LUC. The survival of mice injected with SHIN-3/PTEN was significantly longer than that of mice injected with SHIN-3/LUC ($P < 0.001$, generalized Wilcoxon and log-rank tests).

study, we also confirmed that the PTEN gene inhibited the production of VEGF by ovarian cancer cells (SHIN-3). Because SHIN-3 is a VEGF-hypersecretory cell line, we speculate that the marked growth-inhibitory effect of PTEN on s.c. implanted ovarian cancer cells is mediated by VEGF inhibition. In the experiment with a VEGF-hyposecretory cell line HRA (HRA/PTEN), PTEN did not suppress s.c. tumor growth in mice or prolong their survival time. These findings could reflect that PTEN suppresses tumor growth by inhibiting VEGF.

Interestingly, the proliferation of SHIN-3/PTEN s.c. tumors was inhibited immediately after s.c. implantation. Tumor growth inhibition mediated by angiogenesis inhibition often occurs after the tumor has reached a certain size (25). Therefore, it is possible that the tumor growth-inhibitory effect of PTEN overexpression is by a mechanism other than angiogenesis inhibition. In addition to inhibiting angiogenesis, PTEN reportedly inhibits the phosphorylation of focal adhesion kinase (FAK) and thereby suppresses cell proliferation, migration, and invasion (7). The results of this study may partially reflect this direct effect of PTEN on cancer cells. PTEN is also known to inhibit phosphorylation of AKT (30), suggesting the involvement of an AKT-mediated mechanism. However, introduction of the PTEN gene into SHIN-3 cells did not inhibit AKT phosphorylation in our previous study (17), suggesting that AKT involvement in the findings observed in SHIN-3 in this study is small. PTEN may act via an alteration of other genes in SHIN-3 cells. Matsushima-Nishiu et al. reported that the introduction of the PTEN gene into endometrial carcinoma cells induced alterations in genes involved in cell proliferation and apoptosis (31). The introduction of the PTEN gene may also have induced alterations of genes other than AKT and led to the findings in our study.

Neither cleaved caspase-3-positive cells nor terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling-positive cells were present in s.c. SHIN-3/PTEN tumor. The following reason is considered for the absence

of apoptotic cells: PTEN does not induce apoptosis by directly affecting cancer cells but makes the induction of cancer cell apoptosis easier in the presence of a concomitant anticancer drug (32). Thus, introduction of the PTEN gene alone may not have increased apoptotic cells.

PTEN markedly inhibited ascitic fluid production as well as peritoneal dissemination. In addition to being an angiogenesis factor, VEGF has vascular permeability-enhancing activity; therefore, it is also called vascular permeability factor (33, 34). Thus, PTEN can inhibit not only the growth of a primary tumor and peritoneal disseminated tumor but also the production of ascitic fluid. We speculate that prolongation of survival of mice with peritoneal dissemination, due to PTEN overexpression, is the overall result of inhibition of tumor growth, peritoneal dissemination, and ascitic fluid production.

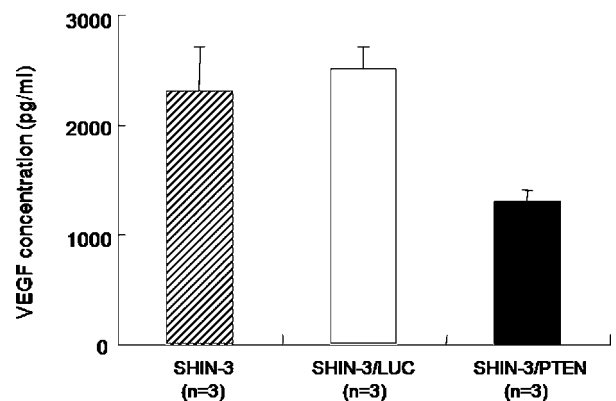


Figure 4. VEGF concentration in cell culture supernatant. SHIN-3, SHIN-3/LUC, and SHIN-3/PTEN were inoculated into 10-cm dishes and cultured in serum-free medium for 24 h. The concentration of VEGF in the supernatant of SHIN-3/PTEN was $1,300 \pm 100$ pg/mL, which was significantly lower ($P < 0.05$) than that in the supernatant of SHIN-3 ($2,300 \pm 400$ pg/mL) and that in the supernatant of SHIN-3/LUC ($2,500 \pm 200$ pg/mL). Mean \pm SD.

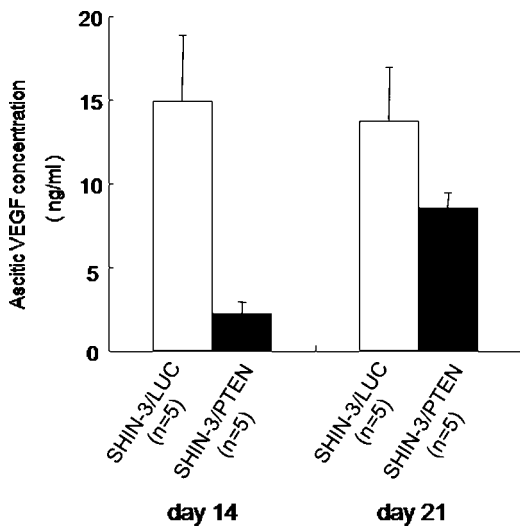


Figure 5. Ascitic VEGF concentration in mice with peritoneal dissemination. Mice were i.p. inoculated with SHIN-3/PTEN or SHIN-3/LUC and sacrificed after 14 or 21 days. The ascitic VEGF level was 2.2 ± 0.7 ng/mL on day 14 in the SHIN-3/PTEN group, significantly lower than that (14.9 ± 3.9 ng/mL) in the SHIN-3/LUC group ($P < 0.01$). Similarly, the ascitic VEGF level on day 21 was significantly lower in the SHIN-3/PTEN group (8.5 ± 0.9 ng/mL) than in the SHIN-3/LUC group (13.7 ± 3.2 ng/mL; $P < 0.05$). In the SHIN-3/PTEN group, the ascitic VEGF level on day 21 was significantly higher than that on day 14 ($P < 0.001$). Mean \pm SD.

However, all mice i.p. inoculated with SHIN-3/PTEN finally died by day 71. The ascitic VEGF level increased with time after i.p. inoculation of SHIN-3/PTEN. The effect of PTEN may have slowly decreased with time, with which the ascitic VEGF level may have increased. In mice s.c. inoculated with SHIN-3/PTEN, the effect of PTEN

decreased with time, and the s.c. tumor grew from about day 50. In mice i.p. inoculated with SHIN-3/PTEN, ascites accumulation became marked from about day 50 (peritoneal disseminated tumor may also have enlarged), and mice died after day 50. To test the above hypothesis, measurements of the ascitic VEGF level and PTEN expression level in peritoneal disseminated tumor are necessary. Unfortunately, we could not confirm the hypothesis because we did not collect ascites or tumor after day 50. This will remain to be investigated.

Epithelial ovarian cancer originates from the ovarian surface epithelium, and the surface epithelium is considered to have the same origin as the peritoneum. Accordingly, epithelial ovarian cancer may exhibit high-level tropism with the peritoneum. The environment of i.p. inoculated ovarian cancer cells may be more suitable for their colonization and development than that of s.c. inoculation. Thus, the effect of PTEN on peritoneal disseminated tumor may have not been as strong as that on s.c. tumor, and VEGF may also have been more markedly secreted by peritoneal disseminated tumor than by s.c. tumor.

VEGF was simultaneously injected i.p. with i.p. SHIN-3/PTEN inoculation. Survival was shortened in the SHIN-3/PTEN + VEGF group compared with the SHIN-3/PTEN group (without concomitant VEGF; median survival, 51 versus 59 days, respectively), but the action of PTEN could not be completely cancelled out by concomitant VEGF administration. One reason may be the short half-life of VEGF, and another may be i.p. administration of exogenous VEGF, which may not have elevated the intratumor VEGF concentration to a sufficient level. We previously did a tumor growth inhibition experiment with drugs that block VEGF action and SHIN-3 cells and succeeded in inhibiting SHIN-3 cell tumor growth using a

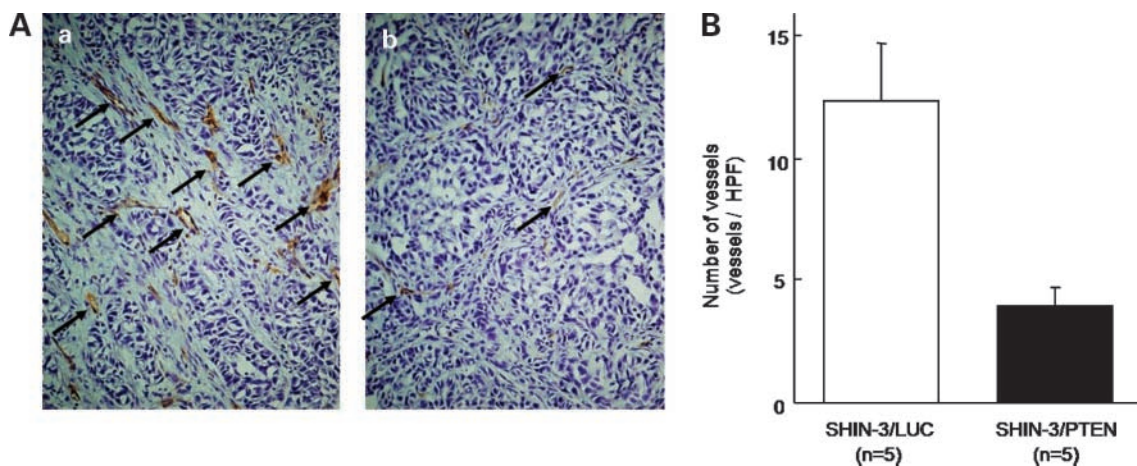


Figure 6. Immunostaining of s.c. tumors of SHIN-3/PTEN and SHIN-3/LUC with anti-CD31 antibody. **A**, endothelial cells of newly formed vessels (arrow) were stained dark brown. In the SHIN-3/PTEN tumors, the number of newly formed vessels was clearly small. (a) SHIN-3/LUC; (b) SHIN-3/PTEN. **B**, numbers of new blood vessels in SHIN-3/PTEN and SHIN-3/LUC s.c. tumors on the 21st day after inoculation. The number of new blood vessels in SHIN-3/PTEN tumors (4.0 ± 1.0 /HPF) was significantly smaller than that in SHIN-3/LUC tumors (12.4 ± 2.6 /HPF; $P < 0.001$). Mean \pm SD.

molecular-targeted agent acting as an inhibitor of VEGF receptor, SU6668 (35). Molecular-targeted agents, such as SU6668, may be used in VEGF-targeted ovarian cancer therapy in the future, in addition to the use of PTEN.

VEGF-targeted molecular therapy has been reported to use the anti-human VEGF monoclonal antibody bevacizumab (36, 37) or soluble VEGF receptor-1 (sFlt-1; refs. 38, 39). Although PTEN gene therapy is a VEGF-targeted therapy, PTEN also has a direct effect on cancer cells through the inhibition of FAK phosphorylation. Because PTEN is not a secretory protein, the clinical application of PTEN gene therapy requires the direct transfection of cancer cells with the gene. Therefore, adenovirus vectors with high transfection efficiency are most suitable. However, there are limits to gene transfection efficiency; therefore, the clinical application of gene therapy assumes PTEN gene therapy in combination with conventional anticancer agents. PTEN inhibits the phosphatidylinositol 3-kinase/AKT pathway downstream of FAK and induces apoptosis of cancer cells by anticancer agents (30, 32). Studies have reported that transfection of cancer cells with the PTEN gene increases sensitivity to irinotecan, doxorubicin, paclitaxel, and other anticancer agents (17, 40, 41). Therefore, PTEN gene therapy in combination with these anticancer agents may be effective.

In summary, the PTEN gene inhibited not only s.c. tumor growth but also peritoneal dissemination and ascites accumulation and prolonged the survival time of mice inoculated with VEGF-hypersecretory ovarian cancer cells. This study also suggested the involvement of PTEN-induced suppression of VEGF production in one of the main mechanisms. PTEN gene therapy may become a new therapeutic strategy for ovarian cancer, which progresses mainly by peritoneal dissemination.

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