Functional Expression of the Angiotensin II Type 1 Receptor in Human Ovarian Carcinoma Cells and Its Blockade Therapy Resulting in Suppression of Tumor Invasion, Angiogenesis, and Peritoneal Dissemination

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Abstract

Purpose: Angiotensin II is a bioactive peptide of the renin-angiotensin system, acting not only as a vasoconstrictor but also as a growth promoter via angiotensin II type 1 receptors (AT1R). The present study examined AT1R expression in human ovarian carcinoma and attempted to determine whether AT1R blocker could suppress the tumor progression.

Experimental Design: Expression of AT1R, vascular endothelial growth factor (VEGF), and CD34 was immunohistochemically analyzed in ovarian tumor tissues (n = 99). Effects of AT1R blocker on invasive potential and VEGF secretion in ovarian cancer cells were examined in vitro. Effects of AT1R blocker in vivo were evaluated in a mouse model of peritoneal carcinomatosis.

Results: AT1R was expressed in 57 of 67 (85%) invasive ovarian adenocarcinomas and 12 of 18 (66%) borderline malignant tumors but in only 2 of 14 (14%) benign cystadenomas. In invasive carcinomas, VEGF expression intensity and intratumor microvessel density were significantly higher in cases that were strongly positive for AT1R (n = 37) compared with those in cases weakly positive (n = 20) or negative (n = 10) for AT1R. Angiotensin II significantly enhanced the invasive potential and VEGF secretion in AT1R-positive SKOV-3 ovarian cancer cells, both of which were completely inhibited by the AT1R blocker candesartan. Administration of candesartan into SKOV-3-transplanted athymic mice resulted in the reduction of peritoneal dissemination, decreased ascitic VEGF concentration, and suppression of tumor angiogenesis.

Conclusions: AT1R is functionally expressed in ovarian carcinoma and involved in tumor progression and angiogenesis. AT1R blockade therapy may become a novel and promising strategy for ovarian cancer treatment.

Despite significant advances in diagnosis and treatment, ovarian carcinoma remains the leading cause of death from female genital malignancies (1). More than half of the patients present with advanced and metastatic disease, and the common tumor progression pathway in ovarian carcinomas is peritoneal dissemination with progressive accumulation of ascites. Therefore, considerable attention has been focused on the mechanisms responsible for this characteristic progression pathway in ovarian cancer, and novel strategies in addition to conventional surgery and chemotherapy need to be developed.

Angiotensin II, a multifunctional bioactive octapeptide of the renin-angiotensin system (RAS), plays a fundamental role as a vasoconstrictor in controlling cardiovascular function and renal homeostasis. Angiotensin II also acts as a potent growth factor not only for vascular smooth muscle cells (2) but also for certain cancer cell lines (3, 4). In addition, angiotensin II stimulates cell migration (5) and induces angiogenesis via upregulation of vascular endothelial growth factor (VEGF; refs. 6–8). Recent studies at our laboratory showed that angiotensin II stimulates cell growth, invasion, or VEGF secretion in gynecologic cancer cells in vitro, including cervical cancer (9, 10), endometrial cancer (11), and choriocarcinoma (12). These cellular effects of angiotensin II are mostly mediated through specific G-protein-coupled angiotensin II type 1 receptors (AT1R).

Recently, the concept of a localized RAS in the female reproductive organs has evolved (13, 14). Especially, the ovarian RAS is strongly involved in reproductive physiology (15, 16). We found previously that activated RAS exists in human uterine endometrium, ovary, and placenta under both physiologic and pathologic conditions (12, 17–19). However, activation of the local RAS was also shown under neoplastic conditions in certain tumor tissues (20–24). In this tumor-related RAS, angiotensin II is generated mostly from...
angiotensin I by angiotensin-converting enzyme (ACE), and AT$_1$R expression is generally up-regulated. Based on these findings, recent studies showed that ACE inhibitors had antitumor activity through reduction of local angiotensin II levels in various animal models (22, 25–28). These results prompted us to hypothesize that AT$_1$R blockers, like ACE inhibitors, could also inhibit tumor growth or metastasis in vivo via blockade of the RAS. However, there have been very few studies on the tumor-suppressive potential of AT$_1$R blocker using mouse models (23, 29–31), and the antitumor activity of AT$_1$R blocker against human cancer in vitro and in vivo remains to be determined.

The present study examined expression of AT$_1$R in human epithelial ovarian tumors to determine whether AT$_1$R expression is up-regulated with progression from benign to malignant phenotypes and whether it is correlated with angiogenic factors. Furthermore, we assessed whether AT$_1$R blocker could suppress the progression of ovarian cancer in vitro and in vivo and evaluated the potential of this drug as a novel targeted therapy.

Fig. 1. Immunohistochemical expression of AT$_1$R in human ovarian cancer tissues. A and B, serous adenocarcinoma; C, mucinous adenocarcinoma; D, endometrioid adenocarcinoma; E, clear cell adenocarcinoma; F, normal ovary (surface epithelium indicated by arrows); G, negative control (anti-AT$_1$R antibody replaced with normal rabbit IgG); H, positive control (placental trophoblastic tissue). Original magnification, ×100 (A–E and G) and ×200 (F and H).
Materials and Methods

Reagents and antibodies. Human angiotensin II was purchased from the Peptide Institute (Osaka, Japan). AT_1R antagonist, candesartan (CV-11974), was generously donated by Takeda Chemical Industries (Osaka, Japan). AT_2R antagonist, PD123319, was obtained from Sigma Chemical Co. (St. Louis, MO). Rabbit anti-human AT_1R polyclonal antibody (306) and anti-human VEGF polyclonal antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal antibody against human CD34, a marker of endothelial cells, was obtained from Immunotech (Marseilles, France). Anti-mouse CD34 antibody was purchased from Hycult Biotechnology (Uden, the Netherlands).

Tissue samples. Human epithelial ovarian tumor tissues, including benign cystadenoma (n = 14), borderline malignant tumors (n = 18), and invasive adenocarcinoma (n = 67), were obtained from patients who underwent surgical treatment at Nagoya University Hospital. All tissue samples were fixed in 10% formalin and embedded in paraffin. The histologic cell types were assigned according to the criteria of the WHO classification. The use of tissues was approved by the Institutional Review Board of Nagoya University Graduate School of Medicine and by individual patients.

Immunohistochemistry. Formalin-fixed, paraffin-embedded tissue sections were cut at a thickness of 4 μm. For heat-induced epitope retrieval, deparaffinized sections in 0.01 mol/L citrate buffer were treated at 90°C for 5 minutes using a microwave oven. Immunohistochemical staining was done using the avidin-biotin immunoperoxidase technique (Histofine SAB-PO kit, Nichirei, Tokyo, Japan). Sections were incubated at room temperature for 2 hours with primary antibody (anti-AT_1R at 1:100 dilution, anti-VEGF at 1:200, and anti-CD34 at 1:40). The sections were incubated for 30 minutes with biotinylated second antibody, then incubated for 15 minutes with horseradish peroxidase–conjugated streptavidin, and finally treated with 3-amino-9-ethylcarbazole (for AT_1R) or 3,3'-diaminobenzidine tetrahydrochloride (for VEGF and CD34) in 0.01% H_2O_2 for 10 minutes. The slides were counterstained with Meyer's hematoxylin. As a negative control, the primary antibody was replaced with normal rabbit IgG or mouse IgG at an appropriate dilution. Immunostaining intensity for AT_1R and VEGF was scored semiquantitatively based on the percentage positivity of stained cells on a three-tiered scale as follows: –, negative (no positive cells); +, focally or weakly positive (<50% positive cells); and ++, diffusely or strongly positive (>50% positive cells). Scoring was done twice independently by two investigators.

Fig. 2. Immunohistochemical expression of VEGF (A and B) and CD34 (C and D) in ovarian cancer tissues. VEGF was expressed in tumor cells, whereas CD34-positive microvessels were clearly detected in tumor stroma. A and C, serous adenocarcinoma; B and D, clear cell adenocarcinoma. Original magnification, ×100 (A–D).

Table 1. Immunohistochemical expression of AT_1R in human epithelial ovarian tumors (n = 99)

<table>
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<th>AT_1R expression level, n (%)</th>
<th>No. cases</th>
<th>AT_1R expression</th>
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<tr>
<td>-</td>
<td>12 (86)</td>
<td>2 (14) 0 (0)</td>
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Benign cystadenomas 14 12 (86) 2 (14) 0 (0)
Serous 7 6 (86) 1 (14) 0 (0)
Mucinous 7 6 (86) 1 (14) 0 (0)
Borderline malignant tumors 18 6 (33) 8 (44) 4 (22)
Serous 9 2 (22) 4 (44) 3 (33)
Mucinous 9 4 (44) 4 (44) 1 (11)
Invasive adenocarcinomas 67 10 (15) 20 (30) 37 (55)
Serous 22 3 (14) 6 (27) 13 (59)
Mucinous 12 1 (8) 4 (33) 7 (58)
Endometrioid 14 3 (21) 5 (38) 6 (43)
Clear cell 19 3 (16) 5 (26) 11 (58)
expression scores for AT1R and VEGF. The average number of microvessels of the three different areas was recorded as the MVD for each case.

**Cell line and culture.** Three human ovarian cancer (serous adenocarcinoma) cell lines were used. SKOV-3 was generously donated by Memorial Sloan-Kettering Cancer Center (New York, NY). HRA was kindly provided by Prof. M. Kikuchi (National Defense Medical College, Saitama, Japan). NOS2 was established at our laboratory. All cell lines were maintained in RPMI 1640 (Sigma Chemical) supplemented with 10% FCS (Sigma Chemical) and penicillin/streptomycin and then incubated at 37°C in a humidified atmosphere of 5% CO2.

**Reverse transcription-PCR.** Total RNA was extracted from cells with the RNeasy kit (Qiagen, Hilden, Germany). RNA (1 μg) was used for reverse transcription using a GeneAmp RNA PCR kit (Perkin-Elmer Co., Norwalk, CT). Reverse transcription products (1 μL solution) were applied to PCR as described previously (11). The sense and antisense primers for glyceraldehyde-3-phosphate dehydrogenase were 5'-TGACATCGAAGGTGGAAGAGTGGGAGTTGCTG-3' and 5'-GGCAGCAGGAAAATGATAAGATTGACACAG-3', respectively (12). The sense and antisense primers for VEGF were 5'-GCTGTTGACATCTTCCAG-GAGTACC-3' and 5'-GCACTGAAAGCTGAGTGGTGGC-3', respectively (33). The sense and antisense primers for β-actin were 5'-GGCTTACAGCTTACCCCAA-3' and 5'-AGAGATGTCACAGTTCACAG-3', respectively. The sense and antisense primers for glyceraldehyde-3-phosphate dehydrogenase were 5'-ATGCTGAAGGTCCCTGTTGACCAAGATTTGCC-3' and 5'-GCTACCGAGGTGAGCTGGTGGTGGC-3', respectively. PCR products were separated by electrophoresis on 2% agarose gel and visualized by ethidium bromide staining.

**Immunoblot analysis.** Immunoblotting analysis for AT1R expression was done as described previously (12). Cells were lysed in a lysis buffer consisting of 20 mmol/L Tris-HCl (pH 7.5), 2 mmol/L EDTA, 150 mmol/L NaCl, 1% Triton X-100, and protease inhibitor mixture. After centrifugation at 15,000 × g for 30 minutes, the supernatant was obtained. Protein extract (30 μg) was separated by 10% SDS-PAGE, transferred onto nitrocellulose membranes, and immunoblotted with anti-AT1R antibody. Immunoreactive proteins were stained using an enhanced chemiluminescence detection system (Amersham, Arlington Heights, IL).

**In vitro cell invasion assay.** Cell invasion was evaluated using 24-well Matrigel invasion chambers (Becton Dickinson, Franklin Lakes, NJ). Cells were suspended in the upper chamber at a density of 4 × 104 cells in 200 μL medium with various concentrations of angiotensin II. The lower chamber contained 750 μL RPMI 1640 supplemented with 10% FCS as a chemotactic agent. After incubation for 16 hours, the remaining tumor cells on the upper surface of the filters were removed by wiping with cotton swabs, and invading cells on the lower surface were stained using May-Grünewald Giemsa. The number of cells on the lower surface of the filters was counted under a microscope. Data were obtained from three individual experiments in triplicate.

**ELISA for vascular endothelial growth factor.** Cells were cultured for 48 hours in serum-free medium in the absence or presence of various concentrations of angiotensin II. The conditioned medium was collected and centrifuged at 15,000 × g for 10 minutes, and the VEGF concentration in the supernatant was assayed using VEGF ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions.

**In vivo studies using a mouse model of peritoneal carcinomatosis.** An in vivo model of peritoneal carcinomatosis of ovarian cancer was used as described previously (34, 35). Six-week-old female athymic mice (BALB/c, nu/nu) were obtained from Chubu Kagaku (Nagoya, Japan) and maintained in a pathogen-free environment. SKOV-3 cells (1 × 107 per 1.0 mL medium per mouse) were injected i.p. into nude mice (n = 5/group) on day 0. Beginning on day 1, i.p. administration of candesartan (10 or 100 mg/kg in body weight) or vehicle (control group) was done daily for 25 days. On day 26, the mice were sacrificed and evaluated for the formation of i.p. tumor dissemination. All tumors were resected from mice, and total tumor weight in each mouse was measured. Some tumors were fixed in 10% formalin for subsequent immunohistochemistry. Ascitic fluid in each mouse was also collected to measure VEGF concentrations.

**Statistical analysis.** Each experiment was done in triplicate, and the results are presented as the mean ± SD of three independent experiments. ANOVA with Bonferroni corrections was applied to compare the difference between experimental groups, and P < 0.05 was considered significant. Nonparametric Kruskal-Wallis test and Spearman’s correlation test were done to compare VEGF staining scores.

**Results**

**Immunohistochemical expression of angiotensin II receptors in human ovarian tumor tissues.** First, we examined AT1R expression in human epithelial ovarian tumor tissues by
immunohistochemical staining. AT1R was expressed in all four histologic types of ovarian carcinoma and was localized both on the membrane and in the cytoplasm of tumor cells (Fig. 1A-E). In contrast, AT1R was not expressed on the surface epithelium of the normal ovary (Fig. 1F). AT1R immunoreactivity was not detected in negative control experiments (Fig. 1G), although it was clearly detected in placental trophoblastic tissues used as a positive control (Fig. 1H) as described previously (12).

AT1R expression levels were immunohistochemically analyzed in a total of 99 ovarian tumors (Table 1). AT1R was weakly expressed in only 2 of 14 (14%) benign cystadenomas, although it was expressed in 12 of 18 (66%) borderline malignant tumors and strongly positive staining was observed in only 4 (22%) cases. In contrast, AT1R was expressed in 57 of 67 (85%) invasive adenocarcinomas and more than half of these (55%) were strongly positive. In addition, AT1R expression was not dependent on the histopathologic subtype. These results indicate that AT1R expression is markedly up-regulated with progression from benign to malignant phenotypes of epithelial ovarian tumors, and AT1R is present in the majority of invasive ovarian carcinoma.

Relation between angiotensin II receptors and vascular endothelial growth factor or microvessel density.

To clarify whether AT1R is associated with tumor angiogenesis, we examined expression of VEGF and CD34 in the same series of ovarian cancer tissues (n = 67) by immunohistochemical staining. AT1R was expressed in all four histologic types of ovarian carcinoma and was localized both on the membrane and in the cytoplasm of tumor cells (Fig. 1A-E). In contrast, AT1R was not expressed on the surface epithelium of the normal ovary (Fig. 1F). AT1R immunoreactivity was not detected in negative control experiments (Fig. 1G), although it was clearly detected in placental trophoblastic tissues used as a positive control (Fig. 1H) as described previously (12).

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To clarify whether AT1R is associated with tumor angiogenesis, we examined expression of VEGF and CD34 in the same series of ovarian cancer tissues (n = 67) by immunohistochemical staining. VEGF was localized mainly in tumor cells (Fig. 2A and B) and expressed in 49 of 67 (73%) ovarian carcinomas, of which 25 were weakly positive and 24 were strongly positive.
CD34-positive microvessels were present in tumor stroma (Fig. 2C and D), and MVD ranged from 20 to 150 (mean, 75.2). Interestingly, VEGF expression intensity was positively correlated with AT1R expression intensity (Fig. 3A). Furthermore, MVD was significantly higher in cases strongly positive for AT1R compared with those in cases weakly positive or negative for AT1R (Fig. 3B). These results suggest that AT1R expression may correlate with VEGF expression and angiogenesis of ovarian carcinoma.

**Effects of angiotensin II and angiotensin II receptor blocker on invasive potential of ovarian cancer cells in vitro.** Based on the results of immunohistochemistry for AT1R expression in clinical tissue samples, we examined the effects of angiotensin II and AT1R blocker on invasiveness of ovarian cancer cells in vitro. Of three ovarian cancer cell lines used in this study, SKOV-3 and NOS2 clearly expressed AT1R, whereas HRA showed almost no expression on reverse transcription-PCR (Fig. 4A) and immunoblot analyses (Fig. 4B). Angiotensin II significantly enhanced the invasive potential at 10−8 to 10−7 mol/L in AT1R-positive SKOV-3 cells but not in AT1R-negative HRA cells (Fig. 4C). This enhancement of invasive activity by angiotensin II in SKOV-3 cells was completely inhibited by AT1R blocker candesartan but not by AT1R blocker PD123319 (Fig. 4D). There was no significant change in invasive activity when cells were treated with candesartan in the absence of angiotensin II. These results indicate that candesartan inhibits angiotensin II–induced ovarian cancer cell invasion via blockade of AT1R.

**Effects of angiotensin II and angiotensin II receptor blocker on vascular endothelial growth factor expression and secretion in vitro.** Next, we tested the effect of AT1R blocker on VEGF induction by angiotensin II in vitro. Reverse transcription-PCR analysis showed three main splicing variants of VEGF mRNA (VEGF 121, 165, and 189; VEGF 121 was dominant) in SKOV-3 cells, consistent with findings reported previously (36). Angiotensin II at 10−8 to 10−7 mol/L markedly up-regulated expression of all three splicing variants (Fig. 5A). Angiotensin II also significantly enhanced VEGF secretion from SKOV-3 cells (Fig. 5B) but not from HRA cells (data not shown). Angiotensin II–mediated VEGF release from SKOV-3 cells was significantly inhibited by the AT1R blocker candesartan and decreased to the basal level when treated with 10−8 mol/L candesartan (Fig. 5B).

**Treatment of ovarian cancer with angiotensin II receptor blocker in vivo using a mouse model of peritoneal carcinomatosis.** To determine whether AT1R blocker could inhibit ovarian cancer progression and angiogenesis in vivo, we used SKOV-3-transplanted (i.p.) nude mice as a model of peritoneal carcinomatosis. As shown in Fig. 6A, control mice receiving vehicle alone developed abdominal swelling with massive ascites and cachexia on day 26. In contrast, administration (i.p.) of candesartan at a dose of 10 or 100 mg/kg/d for 25 days resulted in a marked reduction of ascites accumulation and i.p. tumor dissemination. Total weight of disseminated tumors in candesartan-treated groups was significantly reduced compared with those in the control group (Fig. 6B). In addition, ascitic VEGF levels in the candesartan (100 mg/kg)–treated group was significantly low compared with those in the control group (Fig. 6C).

I.p. tumors disseminated on the mesenterium were resected from each mouse and histologically and immunohistochemically analyzed. As shown in Fig. 6D, tumors presented solid and trabecular patterns of growth with high cellularity in the control mice. In contrast, a decrease in tumor cellularity with an increase in stromal fibrous tissue was observed in candesartan-treated mice. Furthermore, intratumor neovascularization was dramatically reduced in candesartan-treated mice. Intratumor MVD was dose dependently reduced by candesartan treatment (Fig. 6E). These results show that AT1R blocker suppressed tumor dissemination and neovascularization in a mouse model in vivo.

**Discussion**

Recent studies showed that local RAS exists in various malignant tumor tissues and suggested that the main effector peptide angiotensin II could act as a key factor for tumor growth and angiogenesis via AT1R (20, 22, 23). Therefore, considerable attention has been focused on the development of RAS blockade therapy as a new strategy for cancer treatment. In the present study, we showed, for the first time, the expression of AT1R in human ovarian tumors using a large number of clinical samples and the efficacy of its blockade therapy against ovarian cancer progression.

Our findings showed that AT1R expression was dramatically up-regulated with progression from benign to malignant phenotypes of the ovarian tumors. Indeed, AT1R was expressed in 85% of invasive ovarian carcinomas and was not dependent on the histopathologic subtype. In addition to AT1R, ACE, an enzyme converting angiotensin I to angiotensin II, was also detected in tumor stroma of ovarian cancer in our preliminary immunohistochemical studies (data not shown). These data provide the first evidence supporting the existence of the local RAS in ovarian cancer and suggest that angiotensin II is locally generated by ACE in the tumor stroma and may play functional roles via binding to the AT1R expressed on the tumor cells. Although AT1R expression was shown in other gynecologic cancers (10–12) as well as in several human malignant tumors, including those of the breast (20), skin (21), and prostate (31), the frequency of its expression in ovarian cancer is very high. In addition, AT1R was not detected on surface epithelium of the normal ovary. These findings might be of great advantage for the clinical application of the AT1R-targeting therapy.

It is of interest that ovarian carcinoma strongly positive for AT1R showed high VEGF expression and high intratumor MVD. VEGF is a main angiogenic factor in ovarian cancer, and over-expression of VEGF significantly correlated with poor prognosis (37–39). MVD was also reported to be a useful prognostic factor of ovarian cancer (40), although the relationship between MVD and patient survival or between MVD and VEGF expression remains controversial (39, 41–43). Our immunohistochemical analysis suggested that AT1R was associated with tumor angiogenesis in ovarian cancer; however, further studies are required to elucidate whether AT1R correlates with patient survival.

Our in vitro studies showed that angiotensin II significantly enhanced the invasive potential of AT1R-positive SKOV-3 cells. This invasion-stimulatory effect of angiotensin II dose-dependently increased at lower concentrations and reached the maximum level at 10−8 mol/L, although it dose-dependently decreased at more than 10−7 mol/L. These results are well consistent with previous reports showing the biphasic behavior of angiotensin II or thrombin on cell migration and invasion (5, 10, 44). Furthermore, we showed that AT1R blocker candesartan completely inhibited the angiotensin II–induced cell invasion of SKOV-3. These findings suggest that
angiotensin II–mediated tumor cell invasion involves the activation of AT1R; thus, selective AT1R blockade therapy could efficiently inhibit the invasiveness of AT1R-expressing tumor cells under conditions exposed to angiotensin II.

In parallel with enhancing invasive activity, angiotensin II up-regulated VEGF mRNA expression in SKOV-3 cells as shown by reverse transcription-PCR. Angiotensin II also stimulated VEGF secretion, which was inhibited to the basal levels by addition of candesartan. These findings indicate that angiotensin II induces VEGF in ovarian cancer cells via AT1R. It is well known that hypoxic conditions induce VEGF expression via hypoxia-inducible factor-1. Interestingly, Richard et al. reported recently that G-protein-coupled receptor agonists, such as angiotensin II, can induce hypoxia-inducible factor-1 more strongly than hypoxia, which results in strong induction of VEGF expression (45, 46). In support of these findings, Mukhopadhyay et al. showed that several human cancer cell lines, including ovarian cancer, produced high levels of VEGF.
even in normoxia, suggesting multiple regulatory pathways of VEGF expression in tumors (47). Taken together, it is suggested that the angiotensin II-AT1R system may control hypoxia-independent (but VEGF-dependent) angiogenic signals in ovarian cancer.

Finally, the potential for clinical application of RAS blockade therapy is discussed. Several studies showed that ACE inhibitors suppressed tumor angiogenesis and had antitumor activity in various animal models (22, 25–28). However, angiotensin II is synthesized not only by ACE but also by other enzymes (48). Therefore, the use of ACE inhibitor alone could not completely block the angiotensin II–mediated effects on tumor cells in vivo. Because the tumor-stimulatory effects of angiotensin II on ovarian cancer cells are mediated through AT1R, selective AT1R blockade may be more advantageous than ACE inhibition. Recently, Egami et al. showed that angiogenesis in murine melanoma was reduced in AT1R-deficient mice, and the tumor growth in wild-type mice was suppressed by the AT1R blocker candesartan (23). To date, only three additional studies have shown that candesartan inhibited in vivo tumor growth and metastasis of murine renal cancer, sarcoma, and human prostate cancer in experimental models (29–31). In these studies, candesartan was orally given at doses ranging from 2.5 to 100 mg/kg/d, because prior toxicity tests showed that doses of up to 300 mg/kg/d were not toxic and had no excessive hypotensive effect in rats (49). In the present study, we showed that i.p. administration of candesartan at doses of 10 to 100 mg/kg/d markedly suppressed peritoneal dissemination and neovascularization of ovarian cancer. Furthermore, candesartan also significantly reduced ascitic VEGF levels with decreased ascites accumulation, which may be due to decreasing vascular permeability. Although the doses of candesartan used in the present study are relatively high and cannot directly be applied to clinical studies, our data strongly suggest a clinical potential

**Fig. 6. Continued**

D, microscopic appearance (top) and immunohistochemical detection of CD34-positive microvessels (bottom) in the center of the tumors disseminated on the mesenterium in control mice and candesartan (100 mg/kg/d)–treated mice. Note: Neovascularization was dramatically reduced in the candesartan-treated mice. E, effects of candesartan treatment on MVD in the center of the tumors disseminated on the mesenterium. Columns, mean; bars, SD. *, P < 0.05.
of i.p. treatment with AT1R blocker as a tumor-dormancy therapy for advanced ovarian cancer patients.

In summary, we showed here that AT1R is frequently expressed in invasive ovarian carcinomas, and the angiotensin II-AT1R pathway plays a significant role in tumor cell invasion and angiogenesis via up-regulation of VEGF. Furthermore, AT1R blockade therapy significantly inhibited the peritoneal dissemination and angiogenesis of ovarian cancer along with suppression of ascites formation in vivo. Because AT1R blocker has been widely used clinically as an antihypertensive agent without serious side effects, it may also be available as an anticancer agent, and this novel targeted therapy could become a promising strategy for ovarian cancer treatment.

References


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