Vascular Endothelial Growth Factor Immunoneutralization Plus Paclitaxel Markedly Reduces Tumor Burden and Ascites in Athymic Mouse Model of Ovarian Cancer

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Ovarian cancer is characterized by rapid growth of solid intraperitoneal tumors and production of large volumes of ascites. Our previous studies of intraperitoneal ovarian carcinoma in an athymic mouse model demonstrated that a monoclonal antibody (mAb) to human vascular endothelial growth factor (VEGF) could prevent ascites formation. Although ascites was almost completely inhibited, tumor burden was variably reduced. To develop more effective therapy, we assessed the combination of a human VEGF mAb plus paclitaxel. Four groups of female athymic nude mice were inoculated intraperitoneally with OVCAR3 cells. Two weeks after inoculation, one group was treated with a human VEGF mAb intraperitoneally twice weekly plus paclitaxel intraperitoneally three times weekly for 6 weeks. The second group was treated with VEGF mAb alone. The third group was treated with paclitaxel alone. The remaining group was treated with vehicle only. Tumor burden in the VEGF mAb plus paclitaxel and paclitaxel alone groups was reduced by 83.3% and 85.7% and 58.5% and 59.5%, respectively, in two separate experiments, compared to controls. VEGF mAb alone caused no significant decrease in tumor burden, nor did treatment of mice inoculated intraperitoneally with HEY-A8 cells, a non-VEGF-secreting ovarian cell line. Virtually no ascites developed in the combined treatment group or the group treated with VEGF mAb alone. Paclitaxel alone reduced ascites slightly, but not significantly. Morphological studies demonstrated that VEGF immuno-neutralization enhanced paclitaxel-induced apoptosis in these human ovarian cancers. Thus, combination therapy with inhibitors of VEGF plus paclitaxel may be an effective way to markedly reduce tumor growth and ascites in ovarian carcinoma. *(Am J Pathol 2002, 161:1917–1924)*

Ovarian cancer is characterized by rapid growth and spread of solid intraperitoneal tumors and, in some patients, the formation of large volumes of ascites. It is the major cause of death from gynecological malignancy and is the fifth most common cause of death from cancer in American women. Despite improved methods of surgery and chemotherapy, the mortality rates in women with advanced, recurrent, or persistent ovarian cancer have remained largely unchanged for the last 4 decades.[1]

Vascular endothelial growth factor (VEGF) is a dimeric glycoprotein, specific for endothelial cells, which stimulates angiogenesis. It also possesses potent vascular permeability-enhancing activity[2,3] and is also known as vascular permeability factor (VPF). VEGF/VPF induces ascites accumulation, at least in part, by increasing the permeability of diaphragmatic and tumor-associated vasculature.[4] In particular, VEGF/VPF plays an important role in ascites formation associated with ovarian cancer.[5–7] Our previous studies in a model of intraperitoneal ovarian carcinoma in athymic mice inoculated with SKOV3 cells demonstrated that a monoclonal antibody (mAb) to human VEGF can prevent ascites.[6] We also showed that administration of a VEGF mAb could reverse pre-existing ascites in mice inoculated with cells derived from an OVCAR3 cancer cell line, in which ascites develops earlier in the course of the disease than with the SKOV3 cell line.[8] Although ascites was almost completely inhibited, tumor burden was variably reduced.

In an effort to develop more effective forms of therapy for ovarian carcinoma, we sought to develop VEGF mAb-based combination therapy. In the past few years, several chemotherapeutic agents, including paclitaxel (Taxol), *cis*-platin, and etoposide have demonstrated some efficacy in the treatment of ovarian carcinoma.[9–11] However, resistance to these agents usually supervenes.

Paclitaxel, a naturally-occurring diterpenoid originally isolated from the Pacific yew, is the first representative of a class of anti-tumor agents that is now widely used in the treatment of many forms of cancer, including those of the ovary, breast, and lung.[7,12,13] Paclitaxel promotes assembly of microtubules, inhibits tubulin disassembly, and blocks cell cycling at the G_2/M stage.[14] Paclitaxel also inhibits DNA synthesis,[15] releases tumor necrosis factor-α,[15,16] and causes apoptotic cell death in a variety of cancer cell types.[9] Because of its unique mechanism of action and its wide spectrum of activity,[17,18] and because of recent studies in which paclitaxel was used in combination with other agents in other types of neoplasms,[19,20] we elected to test paclitaxel combined with the mAb to VEGF/VPF in our athymic mouse model[6] to assess the extent of inhibition of tumor burden as well as ascites. We explored the possible effects of the interaction between immunoneutralization of VEGF and paclitaxel by assessing tumor burden and ascites volume before, during, and after treatment. In addition, we assessed the effects of treatment on apoptosis. To further assess the central role of VEGF in these processes, we also studied the effects of the VEGF mAb plus paclitaxel in a non-VEGF-expressing ovarian cancer cell line.

Materials and Methods

Materials

Paclitaxel was obtained from Sigma Chemical Co. (St. Louis, MO). A mouse mAb (A4.6.1) directed against human VEGF was used to neutralize VEGF activity *in vivo*. Characterization of this antibody, including its specificity for human VEGF and its ability to inhibit VEGF activity *in vitro* and *in vivo*, as well as its ability to block binding of VEGF to its receptors *in vivo*, has been described previously.[21]

All cell culture reagents were obtained from the Cell Culture Facility, University of California, San Francisco (UCSF).

Experimental Animals

Five- to seven-week-old female athymic immunodeficient mice (Simonsen Laboratories, Gilroy, CA) were delivered to the UCSF Laboratory Animal Resource Center, housed in isolated conditions, fed autoclaved standard pellets and water, and allowed to adapt to their new environment. All protocols involving immunodeficient mice were approved by the Committee on Animal Research, UCSF.

Experimental Design

Experiment 1

Four groups of female athymic nude mice (5 to 7 weeks of age) were inoculated intraperitoneally with OVCAR3 cells ($n = 18$). Two weeks after inoculation, one group ($n = 5$) was treated with the human VEGF mAb plus paclitaxel for 6 weeks. The second group of mice ($n = 5$) was treated with VEGF mAb alone. The third group ($n = 4$) was treated with paclitaxel alone. The remainder ($n = 4$) were treated with the same volume of vehicle (phosphate-buffered saline). The human VEGF mAb (5 μg/g body weight) was administered intraperitoneally twice weekly as in our previous studies.[5] The dose of paclitaxel (20 μg/g body weight), was based on previous studies.[22,23] Administration was twice weekly in the first week and increased to three times weekly for the last 5 weeks. There was no apparent toxicity.

Experiment 2

The design of experiment 2 was similar to that of experiment 1 except that paclitaxel was administrated three times weekly for 6 weeks, while paclitaxel was administrated twice weekly in the first week and increased to three times weekly for the last 5 weeks in experiment 1.

Four groups of female athymic nude mice (5 to 7 weeks of age) were inoculated intraperitoneally with OVCAR3 cells ($n = 49$). Two weeks after inoculation, one group of mice ($n = 12$) was treated with the human VEGF mAb (5 μg/g body weight) twice weekly plus paclitaxel (20 μg/g body weight) three times weekly for 6 weeks. The second group of mice ($n = 13$) was treated with VEGF mAb alone. The third group ($n = 12$) was treated with paclitaxel alone. The remainder ($n = 12$) was treated with the same volume of vehicle.

As an additional control, we inoculated nude mice ($n = 20$) with HEY-A8 ovarian cancer cells, which do not express VEGF mRNA and/or secrete the protein.[24] The protocol for HEY-A8-inoculated mice was the same as for OVCAR3 inoculated mice except that mice inoculated with HEY-A8 were treated for 4 weeks because they developed advanced disease earlier than the mice inoculated with OVCAR3, and did not survive longer than 4 weeks of treatment.

Methods

To prepare cells for inoculation, they were collected from the ascites fluid of athymic mice inoculated with the OVCAR3 line. Ascites fluid was collected and placed in a 4°C refrigerator for 1 to 2 hours. The supernatant was then discarded. The cells were diluted with medium RPMI 1640 supplemented with 2.0 g/L of glucose and 0.3 g/L of L-glutamine, which had been prewarmed in a 37°C incubator. Athymic nude mice (5 to 7 weeks of age) were inoculated intraperitoneally with OVCAR3 cells ($n = 49$; 2×10^6 cells per mouse in 500 μl of RPMI 1640). In addition, 20 athymic nude mice (5 to 7 weeks of age) were inoculated intraperitoneally with HEY-A8 cells (2×10^6 cells per mouse in 500 μl of RPMI 1640).

Abdominal circumference and body weight were measured twice weekly. At the end of the experiment, mice underwent euthanasia with CO_2. The volume of ascites was measured, tumor tissue was excised, weighed, fixed in 4% paraformaldehyde, pH 7.4, at 4°C for 24 hours, and embedded in paraffin. Paraffin sections (5 μm) were used for histochemical analysis.

Light Microscopy and Analysis

Tumor tissue sections from OVCAR3-inoculated mice treated with VEGF mAb plus paclitaxel were examined with a Leica DMRB or Leica Ortholux II photomicroscope at low and high magnifications. Images were collected with a Photonics DEI-470 CCD camera and a RasterOps 24XLTV frame grabber, imported directly into Adobe Photoshop 4.0, and stored on a ZIP external 100 MB drive (Iomega). Photomicrographic plates were composed from the original data in Photoshop, without alteration or manipulation, and annotated with rub-on letters and symbols.

Assessment of Apoptosis

Paraffin sections (5 μm) of cancer tissue from OVCAR3 cell-inoculated mice treated with VEGF mAb plus paclitaxel were used to assess apoptosis. DNA labeling with digoxigenin deoxy-UTP and terminal transferase, followed by immunocytochemical staining with peroxidase-coupled anti-digoxigenin antibody and diaminobenzidine, was performed with the reagents supplied in the Apoptag kit (Intergen, Purchase, NY) according to the manufacturer's instructions, except that Tris was substituted for phosphate in the wash buffer. After light counterstaining with hematoxylin, nuclei that stained brown were scored as positive for apoptosis and those that stained blue were scored as negative. At least five $\times$300 microscopic fields were scored, and the apoptotic index was calculated as the percentage of cells that were scored positive.

Statistics

Results are presented as means $\pm$ SE. Data were analyzed using one-way analysis of variance followed by unpaired Student's *t*-test for comparison between groups. Differences between groups were considered statistically significant at $P < 0.05$. Experiments were performed in duplicate.

Results

We examined the potential interactions occurring between the angiogenesis inhibitor VEGF mAb (A.4.6.1) and paclitaxel, given singly and in combination, in the control of ovarian tumor growth and ascites formation, to assess whether these interactions increase the therapeutic effects of each agent individually.

At postmortem examination, tumors were found on the surface of the peritoneum, intestines, mesentery, and uterus in both treatment and control groups. Eighty-seven percent, 58%, and 20% of the mice in the control, VEGF antibody-treated and paclitaxel-treated groups, respectively, had tumors on the diaphragm and in the hilus of the liver. However, these tumors were not found in the combined VEGF mAb plus paclitaxel treatment groups. There were numerous small bead-like tumors in the VEGF mAb plus paclitaxel-treated mice, which are not commonly found in untreated control mice.
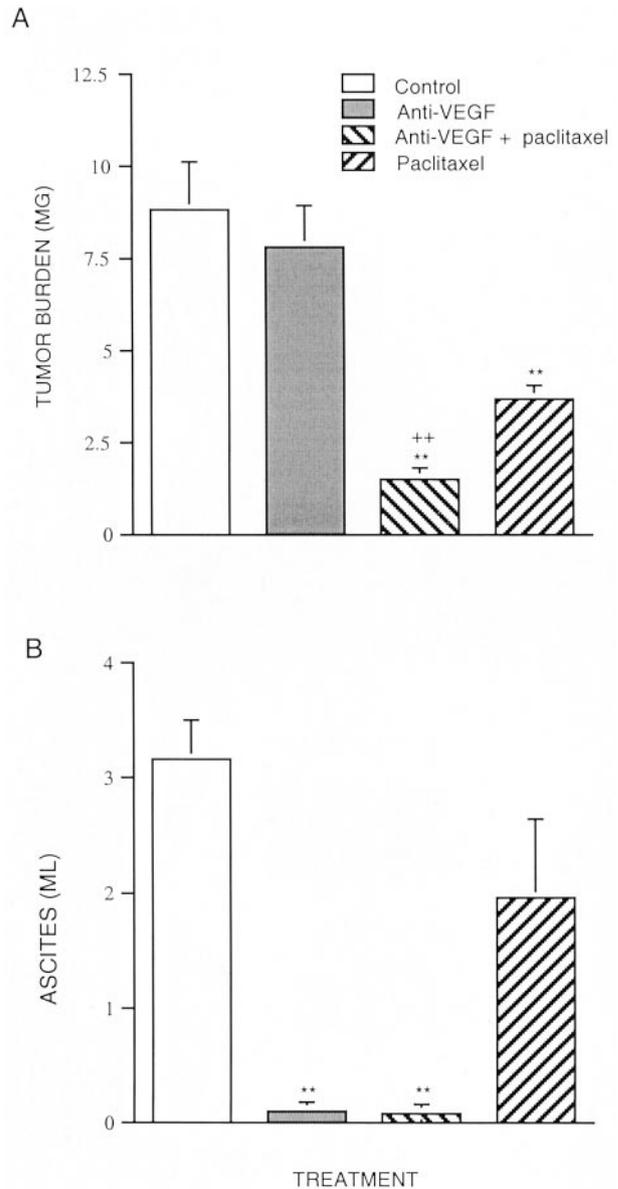

Figure 1. Effects of VEGF mAb plus paclitaxel on tumor burden (**A**) and ascites formation (**B**) in mice inoculated with OVCAR3 cells. Experiment 1. Four groups of athymic immunodeficient mice were used. OVCAR3 cells (2×10^6) were injected as a bolus intraperitoneally in 5- to 7-week-old athymic immunodeficient mice. Treatment was initiated 2 weeks after inoculation. Treatment groups consisted of control (vehicle alone), VEGF mAb alone, VEGF mAb plus paclitaxel, and paclitaxel alone. The VEGF mAb (5 μg/g body weight) was administered intraperitoneally twice weekly for 6 weeks. Administration of paclitaxel (20 μg/g body weight) was twice weekly in the first week and increased to three times weekly for the last 5 weeks. At autopsy, ascites fluid was quantified and tumors were excised and weighed ($n = 15$). Data are expressed as mean $\pm$ SE **. $P < 0.01$ *versus* control; $++$, $P < 0.01$ *versus* paclitaxel.

Experiment 1

The results of this initial study of treatment of VEGF mAb and paclitaxel, singly and in combination, are shown in Figure 1A. The mean tumor burden in the combined VEGF mAb plus paclitaxel-treated group was 1.47 $\pm$ 0.31 g ($n = 4$). (At the beginning of the experiment, there were five mice in this group. One mouse had leakage of ascites fluid because the peritoneum was accidentally torn

with the injection needle 2 weeks before the end of the experiment; this mouse was not included in the analysis.)

The tumor burden in the group treated with paclitaxel alone (3.64 ± 0.42 g) was significantly reduced compared to that of the controls (8.78 ± 1.32 g) ($n = 2$). (At the beginning of the experiment, there were four mice in the control group. However, two died 10 and 22 days, respectively, before the end of the experiment, most likely as a result of the marked tumor burden and ascites.) The tumor burdens in the anti-VEGF plus paclitaxel and paclitaxel alone groups were reduced by 83.3% and 58.5%, respectively, compared to the control group. VEGF mAb alone (7.79 ± 1.33 g) ($n = 4$) had no significant effect on tumor burden compared to the control group.

Figure 1B shows the results of the initial study of the control of ascites formation by the VEGF mAb and paclitaxel treatment, singly and in combination. The mean volume of ascites in the control group was 3.15 ml. In contrast, virtually no ascites developed in the VEGF mAb plus paclitaxel-treated group nor in the group that was treated with VEGF mAb alone. Paclitaxel alone reduced ascites formation slightly, but not significantly.

Experiment 2

As in experiment 1, tumor burden in both the combined VEGF mAb plus paclitaxel ($n = 12$) and paclitaxel alone ($n = 12$) treated groups was significantly reduced compared to the control group (Figure 2A). The tumor burden of the VEGF mAb plus paclitaxel group ($n = 12$) and the group treated with paclitaxel alone ($n = 12$) was reduced by 85.7% (0.529 ± 0.12 g) and 59.5% (150 ± 0.21 g), respectively, compared to the control group (3.71 ± 0.37 g) ($n = 12$). There was no significant difference in tumor burden between the group treated with VEGF mAb alone ($n = 13$) and the control group.

Changes in ascites volume (Figure 2B) also were similar to the initial study. The volume of ascites in mice that did not receive VEGF mAb (control), was 3.6 ml, whereas ascites in the VEGF mAb alone or VEGF mAb plus paclitaxel-treated groups was barely detectable. Again, paclitaxel alone slightly, but not significantly, reduced ascites formation.

As an additional control we inoculated nude mice with HEY-A8 ovarian cancer cells, which do not express VEGF mRNA and do not secrete VEGF.[24] We found that the mice inoculated with HEY-A8 did not produce ascites, and the VEGF mAb did not enhance the inhibitory effect of paclitaxel alone on tumor growth (Figure 3). The tumor burden in the group treated with paclitaxel alone (0.704 ± 0.09 g) ($n = 5$) was significantly reduced (44.3%) compared to the controls (1.26 ± 0.32 g) ($n = 5$). There were no significant differences in tumor burden between the group treated with paclitaxel alone and VEGF-mAb plus paclitaxel (0.678 ± 0.13 g) ($n = 5$) in the mice inoculated with HEY-A8. VEGF alone (1.346 ± 0.18 g) ($n = 5$) had no effect on tumor burden compared to the control group.
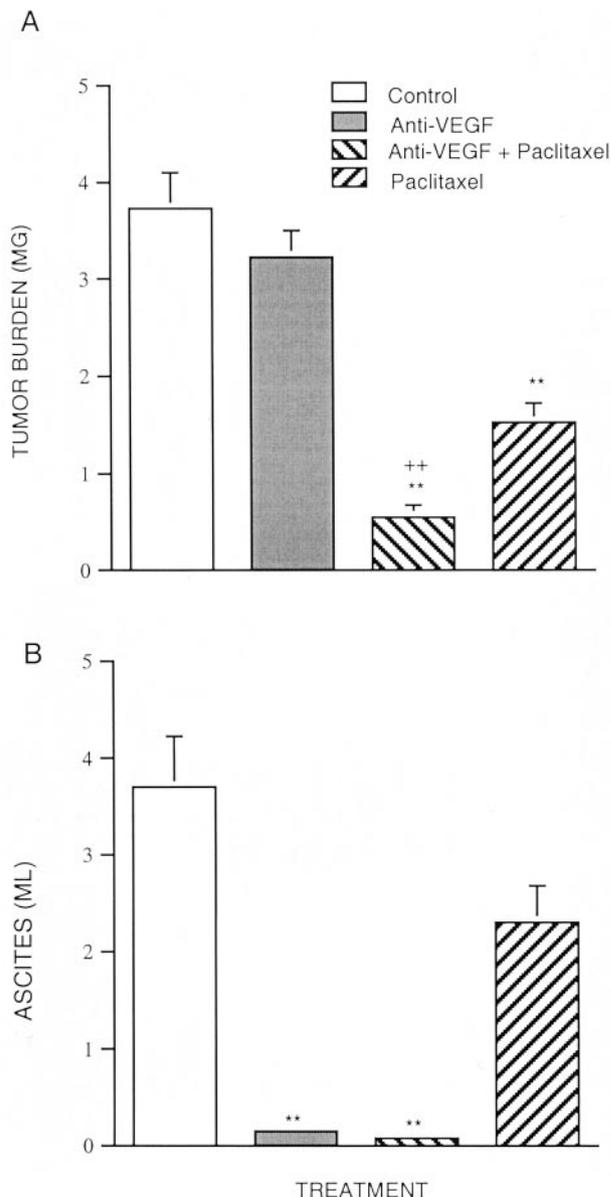

Figure 2. Effects of VEGF mAb plus paclitaxel on tumor burden (**A**) and ascites formation (**B**) in mice inoculated with OVCAR3 cells. Experiment 2. Four groups of athymic immunodeficient mice were used. The OVCAR3 cells (2×10^6) were injected as a bolus intraperitoneally in 5- to 7-week-old athymic immunodeficient mice. Treatment was initiated 2 weeks after inoculation. Treatment groups consist of control (vehicle alone), VEGF mAb alone, VEGF antibody plus paclitaxel, and paclitaxel alone. The VEGF mAb (5 μg/g body weight) was administered intraperitoneally twice weekly for 6 weeks. Administration of paclitaxel (20 μg/g body weight) was three times weekly for 6 weeks. At autopsy, ascites fluid was quantified and tumors were excised and weighed ($n = 49$). Data are expressed as mean ± SE. **, $P < 0.01$ *versus* control; ++, $P < 0.01$ *versus* paclitaxel.

Apoptosis

Figure 4 illustrates apoptotic cells in cancer tissue from OVCAR3-inoculated mice. The brown nuclei (indicated by arrows) indicate cells that underwent apoptosis. Approximately 30% of OVCAR3 cells in the paclitaxel-treated group were apoptotic, whereas more than 50% in the slides from the VEGF mAb plus paclitaxel group were necrotic and apoptotic, with cytoplasmic debris and cal-
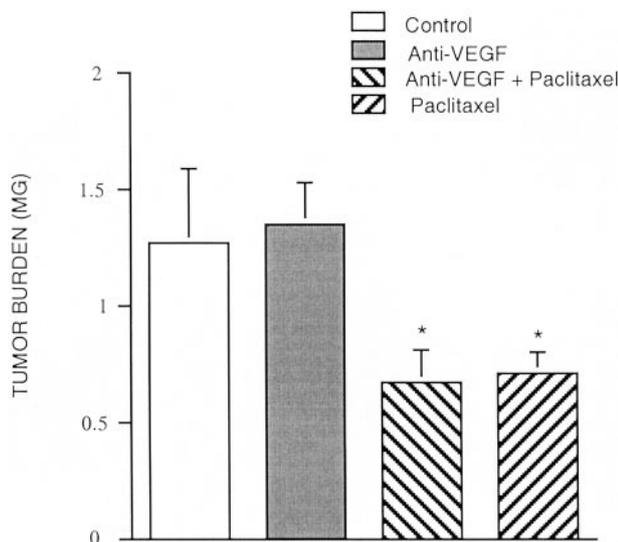
Figure 3. Effects of VEGF mAb plus paclitaxel on tumor growth in mice inoculated with Hey-A8 cells. Four groups of athymic immunodeficient mice were used. Hey-A8 cells (2×10^6) were injected as a bolus intraperitoneally in 5- to 7-week-old athymic immunodeficient mice. Treatments were started 2 weeks after inoculation. Treatment groups consisted of control (vehicle alone), VEGF mAb alone, VEGF mAb plus paclitaxel, and paclitaxel alone. The VEGF mAb (5 μg/g body weight) was administered intraperitoneally twice weekly for 4 weeks. Paclitaxel (20 μg/g body weight) was administered three times weekly for 4 weeks. At autopsy, tumors were excised and weighed ($n = 20$). Data are expressed as mean $\pm$ SE. *, $P < 0.05$ *versus* control.

cification. There were no significant differences between the control group and the group treated with the VEGF mAb alone.

Discussion

Recent studies indicate that the combination of anti-angiogenic agents and conventional chemotherapeutic agents can significantly inhibit tumor growth and metastasis.[25–28] The combination of TNP-470, an angiogenesis inhibitor, and microcyline resulted in marked inhibition of tumor growth in rats bearing implanted 9L-gliosarcomas.[25] Co-administration of TNP-470 and paclitaxel also produced a significant inhibition of growth in a non-small-cell lung cancer cell line.[26] Furthermore, the effects of TNP-470 on liver metastases of human pancreatic carcinoma in nude mice were enhanced by combination with *cis*-platin.[27,28] DC101, a mAb that targets VEGFR-2 (flk-1) in combination with paclitaxel, enhanced apoptosis and inhibited tumorigenesis, angiogenesis, and metastasis in human metastatic transitional cell carcinoma of the bladder in a murine model.[20] Continuous low-dose therapy with vinblastine and DC101 induces sustained xenografts of neuroblastoma regression without overt toxicity.[29] Tumor regression was more dramatic by combining DC101 with microtubule-modulating drugs than with those that damaged DNA.[29] The present data are consistent with these studies and demonstrate that the combination of the human VEGF mAb plus paclitaxel, which markedly inhibited tumor growth, was extremely effective in the treatment of ovarian carcinoma in that it virtually eliminated ascites and reduced tumor burden by >85.7%.

Thus, the effect of paclitaxel on ovarian cancer was markedly enhanced by combination with the VEGF mAb. The anti-metastatic, anti-tumor, and anti-ascites effects of the VEGF mAb plus paclitaxel were markedly greater than those of paclitaxel alone.

The mechanism by which this combination therapy exerts its effect is not clear. A recent study demonstrates that survivin, an inhibitor of apoptosis, plays a role in the maintenance of microtubules within the mitotic spindle and appears to be overexpressed in common cancers but not in corresponding normal adult tissues.[30] VEGF-induced survivin expression is a major drug resistance mechanism downstream of the PI3K/PKB pathway; activation of this pathway may significantly hamper the ability of chemotherapeutic drugs to damage or kill activated endothelial cells.[31] The blockade of VEGF signaling can significantly enhance the efficacy of chemotherapeutic regimens.[29] We have demonstrated that the PI3K inhibitor, LY294002, enhances the effects of paclitaxel on tumor growth and ascites formation and decreases development of resistance to paclitaxel.[32] We also have demonstrated that OVCAR3 cells release high levels of VEGF,[33] which can result in overexpression of survivin. Our use of the VEGF mAb to neutralize VEGF could, in turn, reduce the expression of survivin and decrease the drug resistance that develops with paclitaxel alone.

Paclitaxel inhibits microtubule depolymerization and blockade of cell division in the G_2/M phase of the cell cycle.[14] In addition, paclitaxel can inhibit angiogenesis by suppressing VEGF expression.[34] Thus, tumor growth might be affected not only by direct cytotoxicity but also by inhibition of new vessel formation, and the VEGF mAb could enhance the anti-angiogenic effects of paclitaxel, as well as decreasing development of drug resistance to paclitaxel.[32]

On the other hand, the inhibition of angiogenesis might have led to the death of tumor cells most distal to the established vasculature, thereby decreasing tumor volume and facilitating access of the cytotoxic agents throughout the tumor tissue.[26] When we administered combination therapy to mice that had been inoculated with HEY-A8 ovarian cancer cells, which do not express VEGF mRNA and do not secrete VEGF,[24] we found that the mice did not produce ascites and that the VEGF mAb did not enhance the inhibitory effect of paclitaxel on tumor growth.

The present study indicates that, similar to our previous experiment,[6] the human VEGF mAb markedly inhibits the ascites that develops after intraperitoneal inoculation with OVCAR3 cells. The function-blocking human VEGF mAb, A.4.6.1, neutralized the tumor-derived VEGF activity, blocking access of VEGF to its receptors, specifically inhibiting the activity of tumor-derived VEGF, as it is specific for human VEGF.[21]

The mechanisms by which the VEGF mAb inhibits ascites formation also are not completely understood. Ascites is linked to peritoneal, as well as tumor microvascular hyperpermeability,[22] and several studies have implicated VEGF in ascites formation by increasing vascular permeability.[5,6] Tumor secretion of VEGF is essential for ascites accumulation.[5,35,36] Anti-VEGF targeting
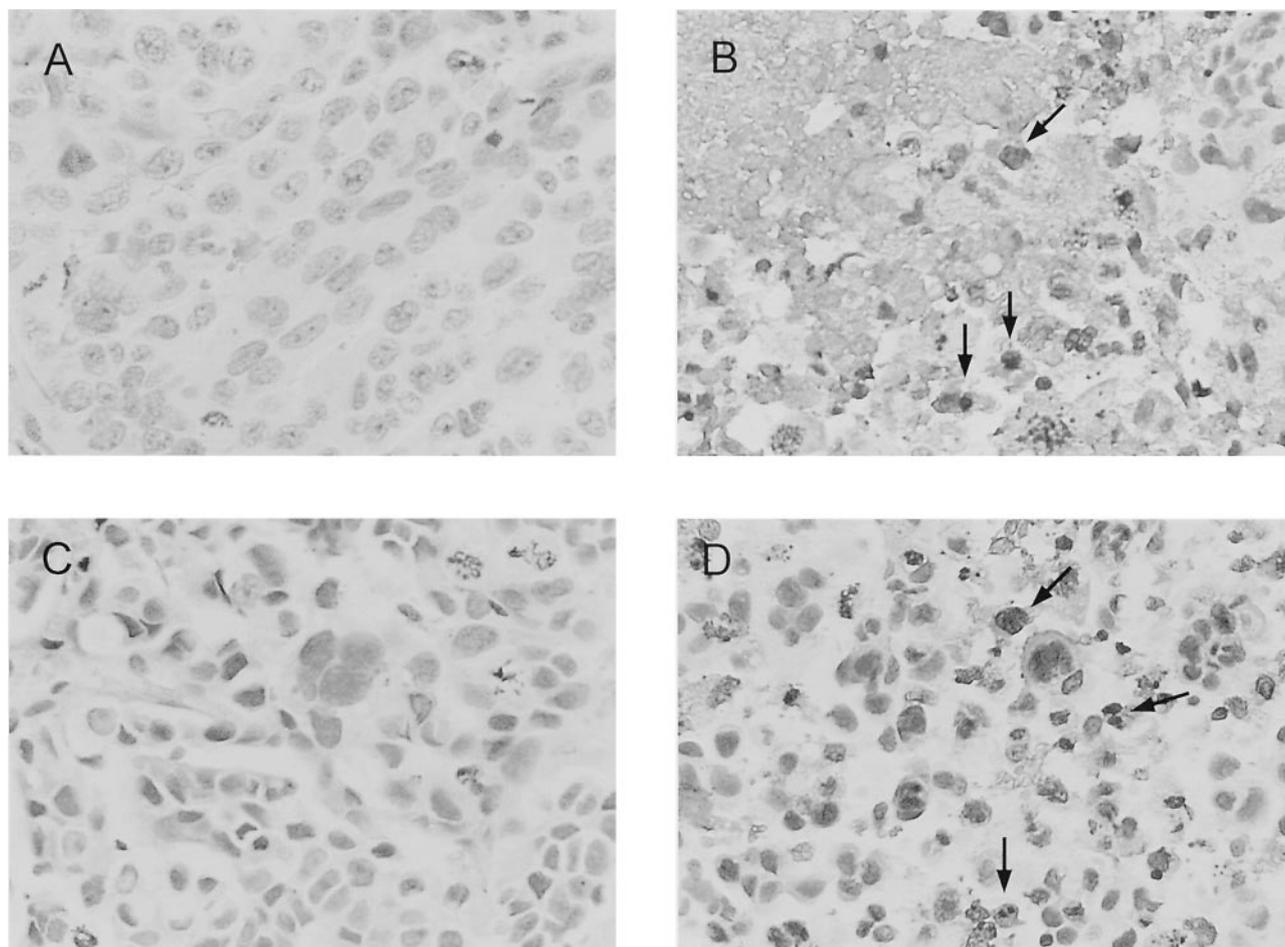
Figure 4. Histological appearance and apoptosis in tumor tissue from OVCAR3-inoculated athymic mice with and without paclitaxel or VEGF mAb plus paclitaxel treatment. **A:** Representative section of tumor from control group. Tumor cells have large, atypical nuclei, prominent nucleoli, a moderate amount of cytoplasm, grow in sheets, and undergo mitosis. **B:** Section of tumor from VEGF mAb plus paclitaxel treatment group. Focus of necrosis and apoptosis with cytoplasmic debris and calcification. A few clusters of viable tumor cells surround this central focus (**bottom right**). Similar foci were scattered throughout tumors removed from the treated animals. The brown-stained cells (**arrows**) indicate apoptosis. **C:** Section of tumor from VEGF mAb-treated group shows tumor cells with large, atypical nuclei. Some cells are swollen, whereas in others the cytoplasm is dense and decreased in amount. No evidence of apoptosis was detected. **D:** Section of tumor from paclitaxel-treated group shows degenerative changes, including decreased nuclear size, hyperchromasia, and smudging of the nuclear chromatin. A few cells are swollen, whereas in others the cytoplasm is dense and decreased in amount. **Arrows** indicate apoptotic cells. Original magnifications, ×300.

agents block ascites formation[7,37,38] and immunoneutralization of VEGF alone in our nude mouse model inhibits ascites.[6] In contrast, inoculation with HEY-A8 cells, which do not express VEGF, was not associated with ascites formation. We have also demonstrated that VEGF release from OVCAR-3 cells modulates endothelial cell monolayer permeability in a dual-chamber permeability assay (L Hu, N Ferrara, and RB Jaffe, submitted). In that study, we used HEY-A8 and OCC1 cells as controls, as neither cell line expresses VEGF, and found that neither the HEY-A8 nor OCC1 cells induced significant increases in monolayer permeability.

Angiogenesis plays a pivotal role in the progression of cancer by permitting tumor growth and facilitating metastasis. The present studies, similar to our previous study using the SKOV3 cell line,[6] indicate that blocking tumor-derived VEGF activity with A.4.6.1 alone does not markedly inhibit intraperitoneal human ovarian tumor growth, although it did decrease subcutaneous SKOV3 cell growth during treatment.

Interestingly, in VEGF mAb-treated mice there were numerous small bead-like tumors, which are not commonly found in untreated control mice. This observation suggests that the inhibition of angiogenesis by the human VEGF antibody, which only inhibits tumor-derived VEGF, led to the inhibition of new vessel development, thereby limiting growth of tumors; cell death occurred in the cells most distant from the established vasculature. Because endogenous VEGF and/or other angiogenic factors may support some tumor growth and spread, tumors near established vasculature remain small and bead-like. Further, Xu and colleagues[39] have suggested that although VEGF/VPF may be the major substance involved in the ascites associated with ovarian carcinoma, interleukin-8, which also is angiogenic, may play a major role in solid tumor growth of ovarian and other[40,41] tumors. A recent report indicated that IL-8 reduced tumorigenicity of human ovarian cancer.[42] VEGF likely is not the only angiogenic factor that can be involved in the maintenance and growth of intraperitoneal carcinomatosis.

Our morphological observations, in agreement with previous studies,[43–45] indicate that paclitaxel induces nuclear pyknosis and fragmentation as well as reduced cytoplasmic volume in tumor cells, indicating apoptosis. Using digoxigenin-UTP and terminal transferase labeling with immunocytochemistry, we have also demonstrated that the VEGF mAb enhanced paclitaxel-induced OVCAR3 cell apoptosis *in vivo*. More than 50% of the cells from the VEGF mAb plus paclitaxel-treated groups underwent necrosis, perhaps because of apoptosis that subsequently can lead to necrosis.[46]

In summary, our studies suggest that combination therapy with an anti-angiogenic agent, such as the VEGF mAb plus paclitaxel, administered intraperitoneally or via another route, may be an effective way to markedly inhibit tumor growth and ascites in ovarian cancer.

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