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A Trial of Anti-Angiogenic Therapy in Gynecologic Cancer

—Review Article—

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Abstract : Angiogenesis, the formation of new blood vessels, is essential for both tumor growth and metastasis. Many gynecologic cancers, especially sarcomas and carcinosarcomas, are highly angiogenic tumors and thus patients with these tumors tend to show an aggressive clinical behavior. Numerous classes of molecules have been implicated in regulating angiogenesis and thus, novel agents that target and counteract biologic mechanisms are now being developed. Anti-angiogenic agents have also been evaluated for gynecologic cancers *in vitro* and *in vivo*. TNP-470, an anti-angiogenic agent (synthetic derivative of the antibiotic fumagillin), showed an inhibition of growth in various types of gynecologic cancers, but only in a limited number of patients with advanced cervical cancer in a clinical trial. Phase Ⅱ studies using Thalidomide in patients with epithelial ovarian cancers or sarcomas are now in progress as clinical trials. In this paper, the recent experimental data and clinical trials performed to evaluate anti-angiogenic therapies for gynecologic cancers are reviewed.

Key words : angiogenesis, anti-angiogenic therapy, TNP-470, vascular endothelial growth factor (VEGF), endometrial cancer, uterine sarcoma, cervical cancer, ovarian cancer

Intoroduction

Many gynecologic malignancies are highly angiogenic and have a weak or poor response to any chemotherapuetic agent currently used and also to radiotherapy. Especially, sarcomas and carcinosarcomas are the most malignant and angiogenic tumors known in the female genital tract.[1] A high frequency of vascular space invasion has been reported as 75% of cases, or almost all primary uterine carcinosarcomas examined, whereas the same frequency was only reported to be 14% in endometrial carcinomas.[2)3)] It is expected that new anti-cancer drugs will allow for more effective treatments than cytotoxic agents, which are also less toxic and can be administered for long-term maintenance.

One important class of agents functions by an anti-angiogenic mechanism, targeting the blood vessel supply of the tumor and inhibiting tumor growth. As a result, anti-angiogenic therapy is one of the promising treatment modalities for gynecologic tumors.

Tumor angiogenesis

Angiogenesis is a critical process in the growth, invasion and metastasis of cancer. The progressive recruitment of blood vessels to the tumor site results in a proliferation and allowed cancer cells access to the vascular system for hematogenous metastasis.[4] Many

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reports have demonstrated that tumor neovascularity, as measured by the intratumoral microvessel density correlated with the prognosis in patients with carcinomas of various organs.[5] Numerous classes of molecules have been implicated in the regulation of angiogenesis. Therefore, in order to stimulate angiogenesis, tumors secrete growth factors that act on endothelial cells, thus resulting in neovasculature support for both tumor expansion and metastasis. Vascular endothelial growth factor (VEGF) is considered to play a key role in the angiogenesis of various types of malignancies including gynecologic tumors.[6] Angiopoietin (Ang) 1 and Ang 2 have been identified as novel angiogenic factors and as ligands of the endothelial cell-specific tyrosine kinase receptor Tie 2.[7] Some encouraging findings regarding the relationship between Angs and gynecologic tumors are expected in the near future. Thirty years ago, Folkman proposed that tumor growth and metastases are angiogenesis dependent and thus suggested that this process may therefore be potential a therapeutic target.[8]

Anti-angiogenic therapy

Most solid-tumor malignancies in various organs remain incurable. Novel agents that target and counteract biologic mechanisms are now being developed. A number of agents have been discovered and developed that aim to inhibit angiogenesis and to convert the tumor to a dormant state. Based on successful experimental or preclinical data, several anti-angiogenic agents alone or in combination with conventional therapies are now undergoing clinical trials (Table).

Ⅰ. TNP-470

The fumagillin analogue TNP-470 is an inhibitor of angiogenesis that has been shown to inhibit endothelial cell proliferation and migration *in vitro*. In animal models, TNP-470 has been shown to be effective in the treatment of a wide variety of tumors and their metastases.[9][10] This agent is one of the first anti-angiogenic compounds to enter

Table Selected clinical trials to evaluate the usefulness of anti-angiogenic agents for gynecologic cancers

Agent	Proposed mechanism	Trial
TNP-470	Inhibition of endothelial cell proliferation and migration	Phase Ⅰ : recurrent/metastatic squamous cell carcinoma of the uterine cervix
Thalidomide	Inhibition of TNF-alpha, bFGF, VEGF production	Phase Ⅱ : advanced ovarian epithelial cancer, combined with or without carboplatin Phase Ⅱ : platinum-resistant ovarian epithelial cancer Phase Ⅱ : sarcoma
Squalamine	Inhibits the sodium-hydrogen exchanger type 3 (NHE3)	Phase Ⅱ : recurrent stage Ⅲ/Ⅳ ovarian cancer, combined with carboplatin
CAI	lnhibitor of calcium influx	Phase Ⅱ : recurrent ovarian, tubal, and peritoneal carcinomas
IM862	Inhibirion of VEGF production activation of NK cells	Phase Ⅱ : stage Ⅲ ovarian, and peritoneal carcinomas, combined with paclitaxel and carboplatin
SU5416	VEGF-receptor inhibitor	Phase Ⅰ : advanced ovarian cancer

VEGF, vascular endothelial growth factor; TNF, tumor necrosis factor; FGF, fibroblast growth factor; NK, natural killer

clinical trials in Kaposi's sarcoma, renal cell carcinoma, brain cancer, breast cancer, cervical cancer and prostate cancer. The inhibition of growth in various types of gynecologic tumors, such as choriocarcinoma, endometrial cancer, ovarian cancer, uterine carcinosarcoma as well as the inhibition of metastasis in an ovarian cancer by TNP-470 was shown *in vitro* and/or *in vivo*.[9)-12)] VEGF secretion in uterine carcinosarcoma or basic FGF secretion in endometrial carcinoma has been demonstrated to be inhibit by TNP-470 *in vitro*.[12)13)] A phase I study of TNP-470 for 18 patients with either recurrent or metastatic squamous cell carcinoma (SCC) of the uterine cervix was reported.[11)] In this clinical trial, although the treatment was well tolerated with reversible dose limiting neurotoxicity (fatigue, vertigo, and ataxia), the effectiveness of TNP-470 for SCC of uterine cervix may need to be further studied. After this trial, one case of complete remission of metastatic cervical cancer with TNP-470 was reported.[14)] However, close monitoring is still required to identify any toxic effects on the normal angiogenic processes in the female reproductive system.

Ⅱ. Thalidomide

Thalidomide is a well-known teratogen with anti-inflammatory and anti-angiogenic activity.[15)] Thalidomide inhibits the processing of mRNA encoding peptide molecules including tumor necrosis factor-alpha (TNF-alpha) and the angiogenic factor VEGF. A case of complete remission on an innovative regimen of docetaxel, gemcitabine, and thalidomide for a patient with a platinum-resistant ovarian yolk sac tumor has been reported.[16)] However, a phase Ⅱ clinical trial with a continuous low dose (100 mg/day) Thalidomide for 19 ovarian cancer patients showed no objective responses.[17)] A phase Ⅱ randomized study of carboplatin with or without thalidomide in patients with advanced ovarian epithelial cancer is now under going a clinical trial in the UK (Protocol ID: EU-99018). Another group is now clinically evaluating the effect of thalidomide treatment in patients with platinum refractory or resistant ovarian epithelial carcinomas (Protocol ID: NCI-G01-1943). In patients with gynecologic sarcomas or carcinosarcomas (mixed mesodermal tumors), the usefulness of thalidomide is now also being evaluated in another clinical trial in the USA (Protocol ID: NCI-314) (http://cancertrials.nci.nih.gov).

Ⅲ. Endostatin and Angiostatin

Endostatin is a cleavage product of collagen XⅧ that inhibits tumor angiogenesis and growth. Angiostatin, an internal fragment of plasminogen, has been shown to inhibit the process of angiogenesis or neovascularization. An experimental study showed that both angiostatin and endostatin inhibited tumor growth in ovarian cancer. The residual tumors obtained from angiostatin- and endostatin-treated animals showed a decreased number of blood vessels, as well as an increased degree of apoptosis in tumor cells. The combined treatment with angiostatin and endostatin showed that endothelial cell proliferation was synergistically inhibited.[18)] Another study of adenoviral vector-mediated delivery of genes encoding endostatin and/or angiostatin proteins for ovarian cancer cell lines demonstrated the downregulation of ascites formation, tumor growth, vascularity, and a prolongation of animal survival after intraperitoneal treatment.[19)]

Ⅳ. Squalamine

Squalamine, an aminosterol antibiotic isolated from the dogfish shark, has been postulated to inhibit neovascularization by selectively inhibiting the sodium-hydrogen antiporter exchanger. A phase Ⅱ study of squalamine lactate and carboplatin in patients with recurrent or refractory stage Ⅲ/Ⅳ ovarian cancer is now undergoing a clinical trial (Protocol ID: NCI-G01-1987).

Ⅴ. CAI

Carboxyamido-triazole (CAI), an inhibitor of Ca (2+) -mediated signal transduction, has been previously shown to inhibit angiogenesis *in vitro* and *in vivo* and to down-regulate the matrix metalloproteinase-2 *in vitro*. A phase Ⅱ study of CAI in patients with refractory or recurrent ovarian epithe-

lial, fallopian tube, or primary peritoneal cancer is now undergoing a clinical trial (Protocol ID : NCI-98-C-0012, NCI-T97-0112)

Ⅵ. IM862

IM862 is a naturally occurring peptide with antiangiogenic properties that inhibits the production of VEGF as well as activating NK cells. A phase Ⅱ randomized study of adjuvant paclitaxel, carboplatin, and IM-862 in patients with optimally resected stage Ⅲ ovarian epithelial or primary peritoneal cancer is now undergoing a clinical trial (Protocol ID : CYTRAN-IM862-302).

Ⅶ. Marimastat

Matrix metalloproteinases (MMPs) have been implicated in the processes of tumor growth, invasion, and metastasis. In addition, MMPs are frequently overexpressed in malignant tumors ; and have been associated with an aggressive malignant phenotype and adverse prognosis in patients with cancer. A number of MMP inhibitors are being developed for the treatment of cancer. Marimastat and batimastat are potent broad-spectrum inhibitors of all major MMPs and have been shown to either prevent or reduce the spread and growth of a number of different malignant tumors, including ovarian cancer, in numerous animal models.[20]

Ⅷ. SU5416

SU5416 and SU6668 are potent antiangiogenic small-molecule inhibitors of receptor tyrosine kinases, including those of the vascular endothelial growth factor (VEGF receptor-2) and platelet-derived growth factor receptor families. A phase I study of SU5416 in patients with advanced solid tumors, including ovarian cancer, is now undergoing a clinical trial (Protocol ID : NCI-T99-0095).

Ⅸ. Interferon

The anti-angiogenic effects of tamoxifen and interferon-beta were shown in an estrogen-independent ovarian carcinoma cell line.[21]

Ⅹ. Interleukin-12

Vasostatins are N-terminal chromogranin A peptides and have been shown to be present in several endocrine tissues vasoinhibitory activity *in vitro*. Vasostatin inhibits endothelial cell growth and neovascularization, and interleukin-12 (IL-12) targets the tumor vasculature acting through interferon-gamma (IFN-gamma). In combination, these inhibitors have been shown to halt the growth of human ovarian carcinoma.[22]

Angiogenesis is a fundamental process in reproduction and wound healing. Under these conditions, neovascularization is tightly regulated. Unregulated angiogenesis may lead to several angiogenic diseases and it is also thought to be indispensable for solid tumor growth and metastasis. There are many various tumor types in the female genital tract and the patterns of angiogenic factors secreted are known to be equally heterogeneous. As a result, the interactions between tumor cells and endothelial cells must be further studied in each tumor type. Moreover, to define the critical role of angiogenic stimulators as well as angiogenic inhibitors, it is necessary to examine the signal pathway and the transcriptional mechanisms of these factors in gynecologic tumors.

VEGF is perceived to be one of the most important mediators of tumor-associated angiogenesis.[6] Exposure to hypoxia rapidly induces VEGF expression. Although many experimental studies have showed some successful data in anti-angiogenic therapy for several tumor types using anti-VEGF antibody,[23] to the best of our knowledge, no reports have yet evaluated it for any gynecologic tumors. There is no doubt that VEGF is the best-validated target for anti-angiogenic therapies, based on its overwhelming genetic, mechanistic and animal efficacy data. As a result, further studies on gynecologic cancer by using anti-VEGF antibodies or soluble VEGF receptors might be now called for.

Although several clinical trials using anti-angiogenic agents described above are now in progress, the long-term side effects of many anti-angiogenic therapies on normal tissues and physiological angiogenesis remain un-

clear. In addition, the mechanism of anti-angiogenic effect in some of these agents is still unclear. Clinical studies must demonstrate that these agents affect tumor vasculature. In the future, however, improved anti-cancer drugs, refined molecular biologic profiling of gynecologic tumors, and new methods of combining anti-angiogenic agents with cytotoxic drugs may lead to the development of more effective and tolerable therapies for gynecologic cancers.

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