Synergy between Angiostatin and Endostatin: Inhibition of Ovarian Cancer Growth

Yumi Yokoyama, Mohanraj Dhanabal, Arjan W. Griffioen, Vikas P. Sukhatme, and S. Ramakrishnan

Department of Pharmacology [Y. Y., S. R.], Obstetrics and Gynecology, and Comprehensive Cancer Center [S. R.], University of Minnesota, Minneapolis, Minnesota 55455; Renal Division, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215 [M. D., V. P. S.]; and Tumor Angiogenesis Laboratory, Department of Internal Medicine, University Hospital Maastricht, Maastricht, The Netherlands [A. W. G.]

ABSTRACT

Ovarian cancer is the leading cause of mortality among gynecological malignancies. Ovarian cancer growth is angiogenesis-dependent, and an increased production of angiogenic growth factors such as vascular endothelial growth factor is prognostically significant even during early stages of the disease. Therefore, we investigated whether antiangiogenic treatment can be used to inhibit the growth of ovarian cancer in an experimental model system. Mouse angiostatin (kringle 1–4) and endostatin were expressed in yeast. Purified angiostatin and endostatin were then used to treat established ovarian cancers in athymic mice. These studies showed that both angiostatin and endostatin inhibited tumor growth. However, angiostatin treatment was more effective in inhibiting ovarian cancer growth when compared with endostatin in parallel experiments. Residual tumors obtained from angiostatin- and endostatin-treated animals showed decreased number of blood vessels and, as a consequence, increased apoptosis of tumor cells. Subsequently, the efficacy of a combined treatment with angiostatin and endostatin was investigated. In the presence of both angiostatic proteins, endothelial cell proliferation was synergistically inhibited. Similarly, a combination regimen using equal amounts of angiostatin and endostatin showed more than additive effect in tumor growth inhibition when compared with treatment with individual angiostatic protein. These studies demonstrate synergism between two angiostatic molecules and that antiangiogenic therapy can be used to inhibit ovarian cancer growth.

INTRODUCTION

Tumor growth and metastasis require neovascularization, the process by which new blood vessels are formed from preexisting host vasculature (1). Neovascularization is a complex process involving proteolysis of basement membrane, endothelial cell migration, proliferation, and matrix remodeling. Recent studies have shown that several growth factors such as FGFs (2) (acidic FGF, bFGF) (2), VEGF (3), and angiopoietins (4) participate either alone or in combination to coordinate the formation of new blood vessels. Apart from pathological conditions (malignancy, retinopathy), angiogenesis regularly occurs in female reproductive tissues such as ovaries and endometrium. Positive and negative mediators of angiogenesis, probably regulated by hormonal changes, orchestrate the cyclical induction and regression of new blood vessels in ovaries (corpus luteum). At least in

Received 9/29/99; accepted 2/18/00.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Supported in part by grants from the United States Army Medical Research and Material Command, Shirley Ann Sparrow Endowment, Women’s Health Fund, and Gynecological Oncology Group of America (to S. R.) and by a grant from the Dutch Cancer Society (to A. G.).

To whom requests for reprints should be addressed, at 6-120 Jackson Hall, 321 Church Street, S.E., Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455. Phone: (612) 624-1461; Fax: (612) 625-8408; E-mail: sunda001@maroon.tc.umn.edu.

The abbreviations used are: FGF, fibroblast growth factor; bFGF, basic FGF; VEGF, vascular endothelial growth factor; BCE, bovine adrenal gland capillary endothelial cells; HUVE, human umbilical vein endothelial cells; PMSF, phenylmethylsulfonyl fluoride; MTIC, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2,4-tetrathiazolium bromide; CAM, chick chorioallantoic membrane; CPAE, bovine pulmonary artery endothelial cells; FBS, fetal bovine serum.

MATERIALS AND METHODS

Cell Lines. BCEs were obtained from Clonetics, Inc. (San Diego, CA). HUVE cells, passage 2, were kindly provided by Dr. Vercelotti (University of Minnesota, Minneapolis, MN). MA148, a human epithelial ovarian carcinoma cell line, was established at the University of Minnesota from a patient with stage III epithelial ovarian cystadenocarcinoma (34). The BCE and HUVE cells were maintained in endothelial cell growth medium (Clonetics) supplemented with 10 ng/ml human epidermal growth factor, 1 µg/ml hydrocorti-
Purification of Recombinant Angiostatin and Endostatin.

Mouse angiostatin (kringle 1–4) and endostatin have been cloned and expressed in Pichia pastoris by Dhanabal et al. (30). Pichia clones were cultured in baffled shaker flasks and induced by methanol as previously described (35). For large-scale expression, fermentation was used. Culture supernatants from shaker flasks and induced by methanol as previously described (35). For

A window (1 cm diameter, 1 mm thickness) was placed on the CAM. Ten rings were first tested in a CAM assay. Three-day-old fertilized White Leghorn eggs were incubated at 37°C for 4 days with rotating everyday. Confluent BCE and HUVE cells were trypsinized and resuspended in M199 (Life Technologies, Inc.) medium with

Purified materials were dialyzed against PBS (137 mM NaCl, 8.1 mM Na2HPO4, 2.68 mM KCl, 1.47 mM KH2PO4 (pH 7.3)) and stored in aliquots at −70°C.

Endothelial Cell Proliferation Assay. Essentially, the method described by O’Reilly et al. (28) was used. Confluent BCE and HUVE cells were trypsinized and reseeded in M199 (Life Technologies, Inc.) medium with 5% FBS. Cells were then seeded into gelatinized, 96-well culture plates at a density of 5000 cells/well. After 24 h, different concentrations of angiostatin and/or endostatin were added. Twenty minutes later, cultures were treated with 5 ng/ml of bFGF (Life Technologies, Inc.) in the presence of 1 µg/ml heparin. The viability of the control and the treated cells was determined by the MTT assay (Sigma Chemical Co., St. Louis, MO) colorimetric assay (36) after 72 h of incubation.

The ability of mouse endostatin and angiostatin to inhibit angiogenesis in vivo was first tested in a CAM assay. Three-day-old fertilized White Leghorn eggs were incubated at 37°C for 4 days with rotating everyday. A window (1 × 2 cm) was gently cut on day 7. On day 9, sterilized silicon rings (1 cm diameter, 1 mm thickness) were placed on the CAM. Ten micromgrams of endostatin or angiostatin were added inside the rings every day for 3 days. Control CAMs were treated similarly with sterile saline. At the end of the experiment, CAMs were fixed with 10% neutral buffered formalin and photographed using a digital camera.

Tumor Growth Inhibition Studies. Female athymic nude mice (6–8 weeks old) were purchased from the National Cancer Institute and allowed to acclimatize to local conditions for 1 week. Logarithmically growing human ovarian carcinoma cells were harvested by trypsinization and were suspended in fresh medium at a density of 2 × 105 cells/ml. One hundred microliters of the single-cell suspension was then injected s.c. into the flanks of mice. When the tumors became visible (7 days after inoculation), mice were randomized into four groups. One group was injected with mouse endostatin s.c. at a dose of 20 mg/kg/day for 30 days. A second group of mice was treated with angiostatin at the same dose. A third group of mice was treated with a combination of mouse endostatin (20 mg/kg/day) and angiostatin (20 mg/kg/day) to evaluate the effect of combination therapy. A control group of mice (fourth) was treated with sterile PBS under similar conditions. All injections were given s.c. at the neck, which is about 3 cm away from the growing tumor mass. Tumor growth was monitored by periodic caliper measurements. Tumor volume was calculated by the following formula: tumor volume (mm³) = (a × b²)/2, where a = length in mm and b = width in mm.

Statistical significance between control and treated groups was determined by Student’s t test. A minimum of five animals was used in each group and the experiments were repeated at least twice. Data from independent experiments were pooled for statistical analysis.

Determination of Vessel Density and Apoptosis. To determine the effect of antiangiogenic treatments on vessel density and apoptosis, residual tumors were surgically resected and snap frozen. Cryostat sections (4 µm) of tumors were then treated with PBS containing 0.1% BSA and 5% human serum to block nonspecific binding (background). Sections were then incubated with 1:50 dilution of an anti-CD31 (mouse) monoclonal antibody conjugated to phycoerythrin (Sigma). After 1 h incubation at room temperature, sections were washed thoroughly with PBS containing 0.1% BSA and 5% human serum and were then examined under an Olympus (New Hyde Park, NY) BX-60 fluorescence microscope at ×10 magnification. Images were captured by the Metamorph program for analysis. Detection of apoptosis was carried out by using an In Situ Cell Death Detection Kit (Boehringer Mannheim, Indianapolis, IN) following the manufacturer’s protocol. Tumor samples were also fixed in 10% neutral buffered formalin and processed for histochromistry (H&E staining).

RESULTS

Expression and Purification of Recombinant Mouse Angiostatin and Endostatin. The Pichia expression system was used to prepare recombinant mouse angiostatin and endostatin in soluble form (30). Purification of angiostatin and endostatin was carried out by affinity chromatography using lysine and heparin linked to ceramic particles (matrix) respectively. A typical purification run is shown in Fig. 1, A and B. Mouse angiostatin was eluted as a single homogenous peak and contained pure protein with <5% contamination in SDS-PAGE (Fig. 1C).

The apparent molecular weight of the purified angiostatin was about M₉ 42,000 as per the relative mobility on SDS-PAGE under nonreducing conditions. Angiostatin (residues Val₉₋Gly₅₈) encompassing kringle 1–4 (19, 24) has a total of 361 amino acid residues. Peptide composition analysis (MacVector, 4.1.5) predicts a theoretical molecular weight of M₉ 41,100, which is very close to the observed value. Endostatin was eluted from the affinity matrix at 500 mM NaCl as a single peak containing a M₉ 20,000 protein. The preparation of endostatin showed small but detectable levels of dimers in SDS-PAGE by silver staining and Western blotting (data not shown). Typically, angiostatin was expressed in higher quantity than endostatin in shaker flasks. Yields for angiostatin varied between 15 and 20 mg/L. In contrast, endostatin was expressed at a lower level (5–8 mg/L). A batch fermentation run provided about 50–60 mg/L of mouse endostatin from a working volume of 6 L. Purified endostatin was analyzed by mass spectrometry. Endostatin showed a molecular weight of M₉ 19,787. In addition, two smaller peaks corresponding to a molecular weight of M₉ 39,500 and M₉ 59,300 were also observed (Fig. 1D). The higher molecular weight peaks correspond to dimeric and trimeric forms of endostatin. Every batch of endostatin showed a similar profile. However, the proportion of individual component cannot be accurately determined from mass spectrometry (inherent limitation). On the basis of SDS-PAGE, the relative amount of higher molecular weight endostatin was <5%. Each batch of endostatin was analyzed for biological activity. For in vivo studies, a single batch was used for each experiment.

Biological Activity of Recombinant Angiostatin and Endostatin. Antiproliferative activity in vitro and antiangiogenic activity in vivo were used to evaluate the biological activity of the recombinant proteins. Inhibition of endothelial cell proliferation was determined using BCE and HUVE cells. Purified angiostatin and endostatin inhibited HUVE cell proliferation by 50% (IC₅₀) at concentrations of 10 µg/ml and 8.6 µg/ml, respectively. Interestingly, when cultures

2191

Downloaded from cancersres.aacrjournals.org on October 22, 2017. © 2000 American Association for Cancer Research.
Inhibition of Angiogenesis. To study in vivo antiangiogenic activity, endostatin and angiostatin were tested in a CAM assay. This assay system is based on developmental angiogenesis and is used to get an initial indication of angiostatic activity prior to testing in vivo tumor growth models. In a modified CAM assay, 9-day-old fertilized eggs were used. Angiostatin and endostatin were applied directly on the CAM within the confined space of silastic rings. In this assay system, both endostatin and angiostatin inhibited development of new embryonic blood vessels without affecting preexisting vasculature (Fig. 3).

Inhibition of Ovarian Cancer Growth. To test whether mouse angiostatin and mouse endostatin could inhibit ovarian cancer growth, we used the human ovarian carcinoma cell line MA148. This model system has been previously used in our laboratory to determine the effect of anti-VEGF antibodies on tumor angiogenesis and tumor growth (38). MA148 cells were grown s.c. so that changes in tumor growth could be easily monitored. Tumors were first allowed to establish for 7 days. At this time, small palpable tumor nodules could be easily seen under the skin. Mice were then randomized and divided into groups. Angiostatin and endostatin were administered s.c. for a period of 30 days. Two independent experiments were carried out. Data in Fig. 4 show the relative effect of angiostatin and endostatin therapy. Angiostatin was found to statistically inhibit proliferation of endothelial cells.
to inhibit ovarian cancer growth better when compared with endostatin in parallel experiments. For example, after 2 weeks of treatment with angiostatin, a mean tumor volume of 200 mm³ was observed. Under similar conditions, endostatin-treated animals showed a mean tumor volume of 362 mm³, whereas mean tumor volume of control mice was 589 mm³. Tumor growth was significantly reduced during the entire treatment period with angiostatin.

**Effect on Tumor Blood Vessels and Apoptosis.** To evaluate the consequence of antiangiogenic therapy, we examined the residual tumors histologically. Frozen tumor sections were immunohistochemically stained with an endothelial specific antibody against CD31. Immunofluorescence studies showed that angiostatin- or endostatin-treated tumors decreased the density of blood vessels (Fig. 5, A, D, and G). The same frozen sections were also analyzed for changes in the viability of tumor cells using a TUNEL assay (Fig. 5, B, E, H). Serial sections of each tumor were also stained by H&E to assess necrotic changes (Fig. 5, C, F, and I). When compared with control tumor sections, endostatin- and angiostatin-treated tumors showed pronounced increase in apoptosis. Increased incidence of apoptosis coincided with increase in calcification and necrosis of tumor tissue. However, histopathological analysis of normal tissues from the same animals did not show any increase in apoptosis or necrosis (data not shown). Collectively, these results show that antiangiogenic therapy results in reduced tumor angiogenesis leading to apoptotic death of ovarian cancer cells.

**Synergistic Effect of Angiostatin and Endostatin on Ovarian Cancer Growth.** Because endothelial proliferation was inhibited better when angiostatin and endostatin were added together, we investigated in an independent study whether angiostatin treatment can be combined with endostatin to improve antitumor effect. Fig. 6 shows mean tumor volume on day 42. Endostatin and angiostatin alone showed inhibition of tumor growth by 5% and 57%, respectively, in this experiment. However, a combination of angiostatin and endostatin showed better antitumor activity with about 81% inhibition of tumor growth. Table 1 summarizes relative tumor volume of control and treated groups on three different time points. Combination therapy showed more than additive effect on tumor growth inhibition. On day 36, there was 1.34-fold improvement in antitumor activity in the combination group when compared with the expected additive effect. At this time point, endostatin alone inhibited tumor growth by 20% (fractional tumor volume, 0.797 mm³) when compared with the control group. With time, there was a progressive improvement in antitumor activity. On day 42, angiostatin and endostatin combination group showed a 2-fold higher inhibition of tumor growth over additive effect (expected fractional tumor volume). In the present study, both angiostatic proteins were given together in a fixed schedule and dose. Therefore, the observed synergism can be further improved by modulating dosage and frequency of administration based on pharmacokinetics, distribution, and bioavailability.

**DISCUSSION**

Recombinant forms of angiostatin and endostatin have been expressed in prokaryotic and eukaryotic cells (23, 28, 30). Bacterial expression systems have often resulted in insoluble proteins necessitating a refolding protocol. However, insoluble endostatin has been shown to be effective in vivo. Slow release of endostatin from the insoluble suspension coupled with proper refolding in vivo is suggested to be responsible for the potent inhibition of angiogenesis and tumor growth (28). Solubility problems can be avoided by expressing endostatin in other host cells such as yeast. Heterologous expression of mammalian proteins can sometimes result in altered post-translational modifications and heterogeneity at the termini. Indeed, amino terminal heterogeneity has also been observed in yeast-derived endostatin (39). In one instance, host (yeast) cells were genetically altered to reduce proteolytic heterogeneity in the carboxyl terminus of...
endostatin (40). Sometimes processing of the termini can affect the biological activity of recombinant proteins. Proteolytic cleavage between histidine (H3) and glutamine (Q4) residues at the amino terminus has been observed in mouse endostatin. Such truncation results in the loss of the first three (HTH) residues at the amino terminus. These three residues are involved in tetrahedral complexing with a single atom of Zn$^{2+}$ (39). Zinc binding and dimerization have been implicated in antitumor activity of endostatin. Endostatin preparation used in this study showed a homogeneous, major peak corresponding to a molecular weight of $M_r$ 19,787, which is slightly less than the expected size. Microsequencing of the amino terminus confirmed that the first three amino acid residues, HTH, were proteolytically cleaved in the mouse endostatin. The amino terminus started with a glutamine residue. Absence of the first two histidines (H1 and H3) is expected to affect zinc binding. The endostatin preparation used in the present study showed only a small fraction of dimeric and trimeric proteins by mass spectrometry. Despite the amino terminal processing, endostatin was very effective in inhibiting endothelial cell proliferation in vitro and angiogenesis in vivo (CAM). A recent study by Yamaguchi et al. (41) supports our finding that endostatin activity (in vitro and in vivo) may not be dependent on zinc binding. The endostatin preparation used in the present study showed only a small fraction of dimeric and trimeric proteins by mass spectrometry. Despite the amino terminal processing, endostatin was very effective in inhibiting endothelial cell proliferation in vitro and angiogenesis in vivo (CAM). A recent study by Yamaguchi et al. (41) supports our finding that endostatin activity (in vitro and in vivo) may not be dependent on zinc binding. In this particular study, endostatin was genetically modified to eliminate the Zn$^{2+}$ binding site. Such a construct was still biologically active. Structural features in angiostatin that are important for the antiangiogenic activity are not known. The basic kringle structure itself may be a requirement for angiostatic activity. For example, kringle 5 of plasminogen (42) as well as the kringle 2 of prothrombin (43) are potent inhibitors of endothelial cell proliferation. However, the definitive structure/function correlation has not been established yet. Further mutational studies can identify the regions of importance within the kringle region, which are important for angiostatic activity.

Potency of angiostatic molecules has been found to vary a lot depending on the cell type used. Ji et al. (42) reported that BCE cells are more sensitive than HUVE cells to kringle 5 in a migration assay. Dhanabal et al. (30) reported that CPAE cells are more sensitive than

### Table 1 Combination therapy with angiostatin and endostatin

<table>
<thead>
<tr>
<th>Day$^b$</th>
<th>Endostatin $^c$</th>
<th>Angiostatin $^c$</th>
<th>Expected$^d$</th>
<th>Observed$^d$</th>
<th>Ratio of expected FTV/observed FTV$^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>0.797</td>
<td>0.393</td>
<td>0.313</td>
<td>0.254</td>
<td>1.338</td>
</tr>
<tr>
<td>39</td>
<td>0.804</td>
<td>0.439</td>
<td>0.320</td>
<td>0.199</td>
<td>1.060</td>
</tr>
<tr>
<td>42</td>
<td>0.950</td>
<td>0.432</td>
<td>0.410</td>
<td>0.196</td>
<td>2.092</td>
</tr>
</tbody>
</table>

$^a$ FTV (mean tumor volume experimental)/(mean tumor volume control).

$^b$ Day after tumor cell transplantation.

$^c$ (Mean FTV of endostatin) × (mean FTV of angiostatin).

$^d$ Obtained by dividing the expected FTV by the observed FTV. A ratio of >1 indicates a synergistic effect, and a ratio of <1 indicates a less than additive effect.

---


Fig. 6. Combination effect of mouse angiostatin and endostatin on ovarian tumor growth. Female, athymic mice transplanted with MA148 cells were treated by angiostatin and/or endostatin. Tumor sizes were measured 42 days after inoculation. Statistical significance was determined using Student’s t test. *, $P < 0.05$; **, $P < 0.01$. Error bars indicate SE.
other endothelial cell lines. Therefore, we compared the effect of angiostatin and endostatin on two different endothelial cells, BCE and HUVE cells. BCE cells were much more sensitive to both angiostatin and endostatin. We also tested CPAE and human microvascular endothelial cells. CPAE cells were as sensitive as BCE cells, and mouse microvascular endothelial cells were similar to HUVE cells (data not shown).

Angiostatin and endostatin effectively inhibited developmental angiogenesis in vivo. We used a modified CAM assay in which test solutions are applied directly onto a localized area of the CAM. In this method, the samples stay inside the rings, and new blood vessel formation inside the rings then can be compared with normal vascular surrounding the ring. Another advantage of this method is that the blood vessels can be easily fixed by buffered formalin so that it is possible to cut CAMs out and observe them in detail. Direct application of angiostatin and endostatin expressed in yeast clearly inhibited actively growing blood vessels inside the ring. Vasculature outside the ring was not affected by this treatment.

Although angiostatin and endostatin have been tested in many tumor models, the relative potency has not been established in parallel experiments. Angiostatin is found to be effective against Lewis lung carcinoma at doses ranging from 1 mg/kg (24) and 50 mg/kg (44). Endostatin, on the other hand, is used in the same model in an insoluble form at a dose of 10 and 20 mg/kg (29). It is difficult to compare the relative potency because the rate of release of endostatin from the insoluble form is not determined. In the present study, we compared the relative antitumor effect of angiostatin and endostatin in soluble form against human ovarian carcinomas established in athymic mice. Both reagents were given at a similar dose and schedule. These studies showed that angiostatin was more potent in inhibiting ovarian cancer growth compared with endostatin. It is possible that the absence of zinc-binding residues could have contributed to the low antitumor activity of endostatin. However, endostatin was equally effective as angiostatin in inhibiting endothelial cell proliferation in vitro and developmental angiogenesis in vivo. Other reasons for the differences in antitumor activity could be due to tumor-dependent variations in the microenvironment affecting endothelial sensitivity. For example, it is possible that different types of tumors can secrete distinct sets of growth factors that can modulate the sensitivity of tumor vasculature to angiostatin and endostatin differently. Differences in pharmacokinetics and tissue distribution can also differentially alter bioavailability of angiostatin and endostatin. Angiostatin is expected to have a longer half-life than endostatin. Endostatin, with a molecular weight of $M_t$ 20,000, will be cleared from the circulation rapidly by renal filtration. Apart from the circulatory half-life, endostatin is observed to bind host vasculature, which can restrict its availability at tumor target site (45). Interaction with normal blood vessels can affect tissue distribution and will reduce bioavailability of endostatin. It will be possible to improve the efficacy of angiostatic molecules by (a) pharmacological approaches and (b) structural changes to increase half-life/bioavailability.

Angiostatin and endostatin are believed to act on endothelial cells by different mechanisms. Angiostatin has been recently shown to bind $\alpha_\beta$ subunits of a membrane-bound ATP synthase (46). However, endostatin seems to affect levels of antiapoptotic proteins such as BCL-2 inside the cell (47). These studies suggest that upstream apoptotic signaling cascades of caspases are activated by endostatin treatment. Due to the nonoverlapping nature of the inhibitory pathways, treatment of endothelial cells with a combination of angiostatin and endostatin resulted in synergistic inhibition. Synergy between the two angiostatic molecules was confirmed by isobolographic analysis. Improved antiangiogenic activity was also reflected in vivo when tumor-bearing animals were treated with a combination of equal doses of angiostatin and endostatin. Compared with expected additive effects, a 2-fold increase in antitumor activity was observed when mice were treated with equal doses of angiostatin and endostatin. The observed synergy between the two angiostatic proteins can be further improved by optimizing dosage and schedule of administration. These questions will be addressed in future studies using genetically redesigned second-generation angiostatic molecules. Whereas our current studies suggest a potential use of combination therapy using angiostatic proteins, one could also achieve better antitumor response by combining antiangiogenic therapy with other antitumor therapies (e.g., chemo-radiation). For example, radiation therapy when combined with angiostatin showed potentiation of antitumor activity (25, 48). In another related strategy, antibodies to VEGF were used in combination with radiation therapy to achieve improved antitumor activity (49). Radiation induces elevated expression of VEGF as a survival factor from tumor cells. Therefore, neutralizing VEGF under these conditions resulted in better inhibition of tumor growth. In summary, our studies show for the first time that antiangiogenic therapy can be used to inhibit the growth of ovarian cancer and that angiostatin can synergize with endostatin in inhibiting tumor growth.

REFERENCES


Synergy between Angiostatin and Endostatin: Inhibition of Ovarian Cancer Growth

Yumi Yokoyama, Mohanraj Dhanabal, Arjan W. Griffioen, et al.

Cancer Res 2000;60:2190-2196.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/60/8/2190

Cited articles
This article cites 48 articles, 20 of which you can access for free at:
http://cancerres.aacrjournals.org/content/60/8/2190.full#ref-list-1

Citing articles
This article has been cited by 39 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/60/8/2190.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.