Effect of Antiangiogenic Therapy on Slowly Growing, Poorly Vascularized Tumors in Mice


Background: Angiogenesis is essential for tumor growth and progression. Therefore, inhibition of angiogenesis is being studied as a new anticancer therapy. Because cytotoxic chemotherapy is more effective on rapidly growing tumors than on slowly growing tumors, it has been assumed that antiangiogenic therapy will also be effective only on rapidly growing, highly vascularized tumors. We compared the effects of two angiogenesis inhibitors, TNP-470 and angiostatin, on slowly growing, poorly vascularized and rapidly growing, highly vascularized human tumors in mice. Methods: Slowly growing (RT-4) and rapidly growing (MGH-U1) human bladder carcinoma cell lines were grown in severe combined immunodeficiency mice. Established tumors were treated with one of the two angiogenesis inhibitors. Tumor volumes, vascularization, and proliferation indices were determined. The in vitro effects of TNP-470 and of angiostatin on the proliferation of RT-4 and MGH-U1 cells were also investigated. All statistical tests were two-sided. Results: RT-4 and MGH-U1 tumor growth was statistically significantly inhibited by both angiogenesis inhibitors (P<.001). Both inhibitors decreased the blood vessel density in both tumor types but did not alter the in vivo proliferation indices of the tumors. TNP-470, but not angiostatin, marginally decreased the in vitro proliferation of MGH-U1 cells. Conclusion: Slowly growing, poorly vascularized tumors in animal models respond as well as rapidly growing, highly vascularized tumors to therapy with the angiogenesis inhibitors TNP-470 and angiostatin.

Angiogenesis, the sprouting of new capillaries from pre-existing blood vessels, is essential for tumor growth and progression (1,2). Tumors do not usually start out angiogenic but may exist as microscopic in situ lesions for months to years. Once tumors switch to the angiogenic phenotype, they recruit their own blood supply. Tumor growth, invasion, and metastasis now become possible. Like the primary tumor, metastasis is also angiogenesis dependent. In fact, the risk of future metastasis in human breast cancer and in other tumors is predicted by microvessel density in the primary tumor (3,4).

The dependence of tumor growth on angiogenesis has led to the concept of antiangiogenic therapy for malignant tumors (5,6). Several inhibitors of angiogenesis have been isolated and tested in animals; at least 20 angiogenesis inhibitors are in clinical trials in the United States, and seven have entered phase III trials (7–14). It is envisioned that, as angiogenesis inhibitors are approved, they will be added to conventional chemotherapy, to radiotherapy, and to other anticancer modalities such as immunotherapy. Two or more angiogenesis inhibitors may also be used together.

Certain assumptions that underlie the use of cytotoxic chemotherapeutic agents may not apply to angiogenesis inhibitors. For example, it is known that cytotoxic chemotherapeutic agents are more effective against rapidly growing tumors than against slowly growing, indolent tumors, both in animals and in patients (15). Many oncologists have assumed that angiogenic therapies will behave similarly (16). The assumption is that, because experimental xenografted tumors usually have a higher proliferation index than human cancers (15,16), antiangiogenic therapy will be less effective in human tumors than in animal tumors.

To compare the effect of antiangiogenic therapy against slowly growing, poorly vascularized tumors and against rapidly growing, highly vascularized tumors, we studied two human bladder carcinomas that differ in their growth rates and degree of vascularization. Two different angiogenesis inhibitors were used for this study: TNP-470 and angiostatin. TNP-470, a selective angiogenesis inhibitor, is a synthetic analogue of fumagillin, a low-molecular-weight compound isolated from Aspergillus fumigatus that inhibits angiogenesis and tumor growth (7,17). Angiostatin, a 38-kd cleavage product of plasminogen, is a potent and specific inhibitor of endothelial growth and angiogenesis (8).

Materials and Methods

Cell Lines

RT-4, a slowly growing, well-differentiated, moderately aggressive human bladder carcinoma cell line, was purchased from the American Type Culture Collection (Manassas, VA) (18). MGH-U1, a rapidly growing, poorly differentiated, highly aggressive human bladder carcinoma cell line, was a gift from Dr. W. S. McDougal (Massachusetts General Hospital, Boston) (19). Both cell lines were grown in Falcon tissue culture flasks (Becton Dickinson Labware, Franklin Lakes, NJ) at 37°C in a humidified atmosphere of 10% CO2 and were maintained as monolayer cultures in Dulbecco’s modified Eagle’s medium (DMEM) (JHR Biosciences, Inc. [GIBCO BRL], Rockville, MD) and washed once with medium containing 10% calf serum to remove the trypsin. The cells were resuspended at a concentration of 1 × 106 cells/mL in DMEM without serum and antibiotics. The mice were anesthetized by inhalation of isoflurane (Forane; Baxter, Deerfield, IL). After a small dorsal incision was made, 2 × 106 bladder carcinoma cells were injected subcutaneously and permitted to grow unabated until the tumors reached a size of about 70–200 mm3, accounting for approximately 1% of the mouse body weight. At that time, the mice were randomly assigned to treatment and control groups and antiangiogenic therapy was initiated.


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Two-dimensional measurements were taken weekly with calipers during the treatment period, and tumor volume was calculated with the use of the following formula: tumor volume \( (\text{mm}^3) = a \times b^2 \times 0.52 \), where \( a \) is the longest diameter, \( b \) is the shortest diameter, and 0.52 is a constant to calculate the volume of an ellipsoid. One measurement was taken per time point per mouse. Most measurements were taken by different coauthors, who were blinded to the treatment; however, some measurements were also made by the first author, who was not blinded. Results of the measurements did not differ significantly between investigators. At the end of the experiment, the mice were killed by cervical dislocation. The tumors were harvested by microdissection, fixed in formalin (Buffered Formaldehyde-Fresh: Fisher Scientific, Fair Lawn, NJ), and embedded in paraffin. The tumor response to therapy was expressed as percent inhibition of growth of the treated tumor relative to the control tumor at the end of the experiment.

**Angiogenesis Inhibitors and Antiangiogenic Therapy**

Inhibitors, control proteins, or phosphate-buffered saline (PBS) (Sigma Chemical Co., St. Louis, MO) was subcutaneously injected at sites distal from the established tumors. Two antiangiogenic inhibitors were used for these studies. TNP-470, a gift from Takeda Chemical Industries, Osaka, Japan, was delivered subcutaneously at a concentration of 30 mg/kg in PBS every other day. TNP-470 inhibits endothelial proliferation \textit{in vitro} and inhibits angiogenesis \textit{in vivo}. However, TNP-470 is considered to be a selective inhibitor of endothelial cell proliferation because it also inhibits the \textit{in vitro} proliferation of other cell types, including fibroblasts, smooth muscle cells, and tumor cells, but at concentrations that are several logs higher than those required to inhibit endothelial cells (20–22). The other inhibitor, murine recombinant angiostatin, was delivered subcutaneously at a concentration of 20 mg/kg every other day. Murine angiostatin was produced as a fusion protein with the murine immunoglobulin \( \gamma \)-2a Fc fragment (mFc-m-angiostatin) in a murine myeloma cell line as described previously (14,23). The protein was diluted in PBS, filtered through a Millipore filter, and stored at \(-20^\circ\text{C}\) until used. The purity of the mFc-m-angiostatin ranged between 90% and 95% (data not shown). Control animals received an injection of PBS in volumes equivalent to those used for injection of the inhibitors.

**Immunohistochemistry**

Sections (5 \( \mu \text{m} \)) were cut from paraffin blocks. After deparaffinization and rehydration, the sections were incubated overnight at 4°C with a 1 : 250 dilution in 3% rabbit serum of rat anti-mouse CD31 antibody (PharMingen, San Diego, CA), which specifically recognizes an epitope on the surface of endothelial cells (24). Sections were then treated with biotinylated rabbit anti-rat secondary antibody (Vector Laboratories, Inc., Burlingame, CA) at a dilution of 1 : 200 in 3% rabbit serum. Proliferating cells were detected by staining adjacent sections with the PC10 monoclonal antibody against proliferating cell nuclear antigen (PCNA) (Dako Corp., Carpinteria, CA) at a dilution of 1 : 150. These sections were then incubated with biotinylated goat anti-mouse immunoglobulin G (1 : 400) (Vector Laboratories, Inc.) (25). Staining was detected with tyramide–horseradish peroxidase and visualized with 3-aminobien-9-ethylcarbazole (Vector Laboratories, Inc.), according to the manufacturer’s instructions. The counterstain was Gill’s hematoxylin (Fisher Scientific Co., Pittsburgh, PA). For staining controls, the identical procedures were performed without the primary antibody.

**Determination of Microvascular Density and Quantity of Proliferating Cells**

All tumors used for immunohistochemistry were harvested at the end of the different experiments and after final measurements were taken. The microvessel density was determined by screening the immunohistologically stained (CD31) sections for the areas of highest vascularity, with the use of low magnification (\( \times 100 \)) as described previously (3). The vessels in the areas of most intense neovascularization were counted at 400× (i.e., a field of 0.19 \( \text{mm}^2 \)) (26). Tumor cell proliferation was determined by screening neighboring sections for areas of homogeneous PCNA staining with the use of low magnification (\( \times 100 \)). After these areas were identified, the total number of cells was counted at 400×, the value set to 100%, and the percentage of PCNA-positive cells calculated. The staining controls showed no or weak background staining for CD31 and PCNA reactions. All counts were performed by one of the authors who was blinded to the treatment of the tumors.

**In Vitro Cell Proliferation Assay**

For the \textit{in vitro} tumor cell proliferation assays, 1 \( \times 10^4 \) RT-4 or MGH-U1 cells/well were seeded into 24-well plates (Costar, Corning Inc., Corning, NY) in complete culture medium. After 24 hours, the medium was replaced with complete medium containing mFc-m-angiostatin (1–50 \( \mu \text{g/mL} \)) or TNP-470 (5 \( \mu \text{g/mL} \) to 500 \( \mu \text{g/mL} \)). Control wells were incubated with fresh complete medium containing only the PBS. After 72 hours of continuous incubation with the inhibitors, the cells were dispersed with trypsin and the total cell number was determined with the use of a Z1 Coulter counter (Coulter Corp., Miami, FL). Assays were performed in triplicate. The results of the assays were expressed as percent of the cell number in the untreated controls.

**Statistical Analysis**

Medians and ranges of treated and control tumor volumes are presented. The statistical significance of the differences in tumor growth among tumor-bearing mice treated with TNP-470, mFc-m-angiostatin, or PBS was calculated with the use of the nonparametric Wilcoxon rank-sum test. The results of the counts for vascular density and cell proliferation are expressed as medians with 95% confidence intervals (CIs). The Wilcoxon rank-sum test was used for comparing vessel counts between groups and for comparing \textit{in vivo} and \textit{in vitro} cell proliferation between groups. All statistical tests were two-sided, and differences were considered to be statistically significant when \( P<.05 \).

**RESULTS**

**Tumor Inhibition in SCID Mice**

The effect of antiangiogenic therapy with TNP-470 and angiostatin was compared in two models of human bladder cancer (RT-4 and MGH-U1) in SCID mice. The volume-doubling time for the RT-4 tumors in mice was approximately 15–20 days, while the doubling time for MGH-U1 tumors was 5–7 days. Furthermore, RT-4 tumors demonstrated a lower vascular density than MGH-U1 tumors. All experiments on MGH-U1 tumors were terminated on day 21 after initiation of therapy because, by that time, the volume of the control tumors approached the maximum allowed by university guidelines for animal care (1000 \( \text{mm}^3 \)). Although the RT-4 tumors grew more slowly, these experiments were terminated at the same time for direct comparison.

We first determined the effectiveness of the angiogenesis inhibitors on the rapidly growing MGH-U1 tumors. When the MGH-U1 tumor volumes reached 58–196 \( \text{mm}^3 \), therapy with TNP-470 was initiated at 30 mg/kg every other day (\( n = 12 \), from three experiments), and therapy with mFc-m-angiostatin was initiated at 20 mg/kg per day (\( n = 9 \), from two experiments). Control animals received PBS (\( n = 17 \), from five experiments) injected in volumes equivalent to those used for the angiogenesis inhibitors. After 21 days, the median tumor volume was 999 \( \text{mm}^3 \) (95% CI = 721 to 1182 \( \text{mm}^3 \)) for the PBS-treated control mice, 345 \( \text{mm}^3 \) (95% CI = 210 to 508 \( \text{mm}^3 \)) for the TNP-470-treated mice, and 257 \( \text{mm}^3 \) (95% CI = 124 to 298 \( \text{mm}^3 \)) for the mFc-m-angiostatin-treated mice. TNP-470 inhibited MGH-U1 tumor growth by 65% (\( P<.001 \); Fig. 1, A), and mFc-m-angiostatin inhibited MGH-U1 tumor growth by 74% (\( P<.001 \); Fig. 1, B) when compared with the control tumors.

In a subsequent experiment, we determined the effects of the angiogenesis inhibitors on the slowly growing RT-4 tumors. When the RT-4 tumor volumes reached 20–94 \( \text{mm}^3 \), therapy with TNP-470 was initiated at 30 mg/kg given every other day (\( n = 11 \), from three experiments), and therapy with mFc-m-angiostatin was initiated at 20 mg/kg per day (\( n = 14 \), from three experiments). Control animals received PBS (\( n = 15 \), from six experiments). Although most of the RT-4 tumors used in this
study were smaller than the MGH-U1 tumors, all tumors were established and well vascularized by the time treatment was begun. After 21 days of treatment, the median tumor volume was 134 mm³ (95% CI = 119 to 171 mm³) for PBS-treated control tumors, 55 mm³ (95% CI = 19 to 75 mm³) for TNP-470-treated tumors, and 15 mm³ (95% CI = 14 to 38 mm³) for mFc-m-angiostatin-treated tumors. Compared with the PBS-treated control tumors, TNP-470 inhibited RT-4 tumor growth by 60% (P < .001; Fig. 1, D) and mFc-m-angiostatin inhibited RT-4 tumor growth by 89% (P < .001; Fig. 1, E, and Fig. 2). In one of each of the experiments in which RT-4 tumors were treated with TNP-470 or with mFc-m-angiostatin, the treatment period was extended to 35 days. Prolongation of therapy did not change the results.

To determine whether the slowly growing RT-4 tumors could resume growth after discontinuation of therapy, we performed a crossover experiment. After 35 days of treatment with PBS, when the median tumor volume had reached 248 mm³, therapy with TNP-470 (30 mg/kg given every other day) was initiated and continued for an additional 21 days. Likewise, after an initial 35 days of treatment with TNP-470, when the median tumor volume was 53 mm³, the former TNP-470-treated group was then treated with PBS. After 21 days (day 57 of the crossover experiment), the RT-4 tumors treated previously with PBS and treated subsequently with TNP-470 were decreased in size to a median volume of 173 mm³ (down from 248 mm³). By contrast, the RT-4 tumors treated previously with TNP-470 and treated subsequently with PBS increased in size to a median volume of 193 mm³ (up from 53 mm³). These experiments demonstrate the growth potential of TNP-470-treated RT-4 tumors after the termination of antiangiogenic therapy as well as the efficacy of TNP-470 on larger RT-4 tumors (Fig. 3, A).

Fig. 1. Treatment of xenograft MGH-U1 and RT-4 human bladder cancers with the angiogenesis inhibitors TNP-470 and mFc-m-angiostatin in severe combined immunodeficiency mice. Closed circles represent median tumor volumes, and bars represent ranges. The lines indicate trend lines of tumor volumes. MGH-U1 tumors in mice treated with TNP-470 (n = 12; panel A), mFc-m-angiostatin (n = 9; panel B), or phosphate-buffered saline (n = 17; panel C). RT-4 tumors in mice treated with TNP-470 (n = 11; panel D), mFc-m-angiostatin (n = 14; panel E), or phosphate-buffered saline (n = 15; panel F).

Fig. 2. Treatment of RT-4 xenograft tumors in severe combined immunodeficiency mice with mFc-m-angiostatin. Photographs of the mice were taken 35 days after the initiation of therapy with mFc-m-angiostatin (upper panel) or with phosphate-buffered saline (lower panel).
We also examined the growth potential of mFc-m-angiostatin-treated tumors in mice after the termination of antiangiogenic therapy. Fig. 3, C, shows two mice after 21 days of treatment with mFc-m-angiostatin. The median tumor size at the initiation of therapy was 71.5 mm$^3$ (range = 59–84 mm$^3$). After 21 days of mFc-m-angiostatin treatment, the median tumor volume was 11 mm$^3$ (range = 8–14 mm$^3$).

Fig. 3, D, shows the growth of tumors in the same mice 27 days later after the therapy was terminated. Twenty-seven days after the therapy was terminated, the median tumor volume was 107 mm$^3$ (range = 102–112 mm$^3$). This experiment demonstrates the growth potential of RT-4 tumors after the termination of antiangiogenic therapy with mFc-m-angiostatin.

To exclude the possibility that the mFc portion of the mFc-m-angiostatin was responsible for inhibiting tumor growth, we treated the RT-4 tumors with either the mFc fragment alone (equivalent to 20 mg/kg per day) or PBS (Fig. 3, B). Tumor volumes at the initiation of treatment were between 49 and 58 mm$^3$ (n = 6). After 17 days, there was no statistically significant difference between the two groups (P = .700). Thus, the mFc fragment alone had no effect on tumor growth.

**Microvessel Density and Proliferation in Treated and Control RT-4 and MGH-U1 Tumors**

Because antiangiogenic therapy was effective against both slowly growing and rapidly growing tumors, we determined whether the two angiogenesis inhibitors were directly suppressing angiogenesis or were cytotoxic against the tumors. We quantified microvessel density in the RT-4 and the MGH-U1 tumors by staining microvessel endothelial cells with an antibody to CD31. We analyzed six RT-4 tumors from the PBS-treated and five RT-4 tumors of each of the TNP-470-treated and mFc-m-angiostatin-treated groups. PBS-treated RT-4 control tumors had a median microvessel density of 14 vessels per field (95% CI = 11 to 19) in the area of most intense neovascularization. The TNP-470-treated tumors had a median of eight vessels per field (95% CI = 6 to 11), a statistically significant decrease compared with the control tumors (P = .004). The mFc-m-angiostatin-treated tumors had a median microvessel density of six vessels per field (95% CI = 5 to 11), a statistically significant decrease compared with the control tumors (P = .004). We also analyzed nine MGH-U1 tumors from each of the PBS-treated and the TNP-470-treated groups and five MGH-U1 tumors from the mFc-m-angiostatin-treated groups. PBS-treated MGH-U1 control tumors had a median microvessel density of 66 vessels per field (95% CI = 60 to 88) in the area of most intense neovascularization. The TNP-470-treated MGH-U1 tumors had a median microvessel density of 37 vessels per field (95% CI = 32 to 50). When these tumors were treated with mFc-m-angiostatin, the median microvessel density was 39 vessels per field (95% CI = 35 to 44). Compared with the control MGH-U1 tumors, there was a statistically significant decrease in microvessel density in the TNP-470-treated MGH-U1 tumors (P<.001) and in the mFc-m-angiostatin-treated MGH-U1 tumors (P<.001).

To determine the proliferation indices of the tumor cells in all tumors, we immunostained tumor sections for PCNA, a nuclear antigen that is highly expressed only in dividing cells. By counting the number of positive cells and the total number of cells in a defined area of 0.19 mm$^2$, we determined that the six PBS-treated RT-4 control tumors had a median proliferation index of 15.5% (95% CI = 13.1% to 17.6%). Six TNP-470-treated RT-4 tumors had a median proliferation index of 13.5% (95% CI = 12.9% to 19.7%). Six mFc-m-angiostatin-treated RT-4 tumors had a median proliferation index of 14.2% (95% CI = 10.8% to 18.3%). There was no statistically significant difference in the proliferation indices of the PBS-treated RT-4 control tumors compared with those of the TNP-470-treated tumors (P = .699) or those of the mFc-m-angiostatin-treated RT-4 tumors (P = .394).

The rapidly growing MGH-U1 tumors had a higher proliferation index than the slowly growing RT-4 tumors. Six PBS-treated MGH-U1 control tumors had a median proliferation index of 48.1% (95% CI = 33.0% to 52.4%). Six TNP-470-treated MGH-U1 tumors had a median proliferation index of 44.7% (95% CI = 41.2% to 51.9%). Six mFc-m-
the proliferation indices of the PBS-treated control and inhibitor-treated RT-4 and MGH-U1 tumors. This finding suggests that the reduced tumor size that followed antiangiogenic therapy was not a direct consequence of inhibition of tumor cell proliferation. There was, however, a statistically significant reduction in microvessel density in both tumor models when inhibitor-treated tumors and control tumors were compared, suggesting that the reduction in tumor growth was due mainly to the inhibition of endothelial cells in the tumors and not to a direct effect on the tumor cells themselves. It has been reported that antiangiogenic therapy in other mouse tumor models leads to an increased apoptotic index of tumor cells (27).

**In Vitro** Effects of TNP-470 and mFc-m-Angiostatin on RT-4 and MGH-U1 Cells

Both TNP-470 and mFc-m-angiostatin inhibited tumor growth in vivo. Although in vivo microvessel density was reduced and the proliferation index was unchanged, suggesting that the inhibitors functioned specifically to block angiogenesis, we sought to determine if the inhibitors had direct effects on the proliferation of RT-4 and MGH-U1 cells in vitro. Continuous exposure (72 hours) to TNP-470 had no growth-inhibitory effects on RT-4 cells at any of the concentrations tested (5 pg/mL to 500 ng/mL). At a concentration of 500 ng/mL, proliferation was 112% (95% CI = 100% to 130%; Fig. 4, A) relative to PBS-treated controls. By contrast, TNP-470 (500 ng/mL) inhibited the proliferation of MGH-U1 cells by 38% (95% CI = 36% to 49%; Fig. 4, A) relative to PBS-treated controls. These results suggest that, if the tumor-inhibitory effect of TNP-470 in vivo was due to a direct cytotoxic effect, this drug should be more active in the treatment of MGH-U1 tumors than in the treatment of RT-4 tumors. However, in the animal experiments, TNP-470 had the same inhibitory effect on RT-4 (60% inhibition) as on MGH-U1 (65% inhibition) tumors. This result suggests that TNP-470 has a direct antiangiogenic effect followed by a potentially indirect antitumor effect in vivo. Murine Fc-m-angiostatin, at concentrations up to 50 μg/mL, had no effect on RT-4 or MGH-U1 bladder carcinoma cells in vitro (Fig. 4, B).

**Discussion**

This study was initiated because of the widespread assumption that antiangiogenic therapy may follow a similar pattern as conventional cytotoxic therapy, i.e., high efficacy for rapidly growing tumors but poor efficacy for slowly growing or indolent tumors (16). Also, it has been assumed that only highly vascularized tumors will respond to antiangiogenic therapy (16).

Our results suggest that the assumptions and experience with cytotoxic therapy may not apply to antiangiogenic therapy. The experiments show that antiangiogenic therapy is effective in vivo against both slowly growing, poorly vascularized tumors and rapidly growing, highly vascularized tumors. The clinical implication of this study is that rapidly growing tumors may require somewhat higher doses of antiangiogenic therapy than slowly growing tumors, in contrast with conventional cytotoxic therapy, where slowly growing tumors are generally less responsive than rapidly growing tumors. Because antiangiogenic therapy usually has less toxicity and fewer side effects than conventional cytotoxic therapy, the doses can potentially be increased to match the growth rate of a given tumor.

Angiostatin is considered to be a specific angiogenesis inhibitor, which does not at this writing inhibit proliferation of nonendothelial cells (8), including as shown here, bladder cancer cells. However, TNP-470 is not a specific angiogenesis inhibitor (20–22) and can inhibit nonendothelial cells at a 3–4 log higher concentration than that required to inhibit endothelial cells. We showed that TNP-470 caused a slight inhibition of proliferation of MGH-U1 cells and no inhibition of RT-4 cells. However, both TNP-470 and mFc-m-angiostatin potently inhibited tumor growth in vivo. Furthermore, TNP-470 did not suppress the proliferation index of tumor cells in vivo, providing further support that it was the antiangiogenic activity of TNP-470 that led to the inhibition of tumor growth in vivo.
The total angiogenic activity of a tumor can be considered as the sum of the angiogenesis stimulators it produces together with the inhibitors that it may generate. It should be emphasized that, while microvesSEL density is a good prognostic indicator for the risk of future metastasis or mortality in a wide variety of tumors (3,28), it may not always reflect angiogenic activity following antiangiogenic therapy. The existence of cancers in which there is a substantial decrease in microvesSEL density following antiangiogenic therapy suggests that the antiangiogenic agent is acting to inhibit vessel formation. Nevertheless, the absence of a decrease in microvesSEL density cannot be taken to mean that the agent is not working. If tumor regression coincides with capillary dropout that is equivalent to tumor cell apoptosis, then the mean intercapillary distance and microvesSEL density may remain constant.

An additional clinical implication of these results is that antiangiogenic therapy may not be as dependent on tumor growth rate as is cytotoxic chemotherapy. Antiangiogenic therapy successfully treated both slowly growing and rapidly growing tumors in mice, whether they were poorly or richly vascularized. Because mouse tumors grow more rapidly than human tumors, it has been assumed that antiangiogenic therapy, like cytotoxic chemotherapy, would be less effective in human tumors than in mouse tumors. Our experiments indicate that antiangiogenic therapy may actually be as effective, or more effective, against slowly growing human cancers than the xenograft models would predict.

REFERENCES


NOTES

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