Intravital Microscopy Reveals Novel Antivascular and Antitumor Effects of Endostatin Delivered Locally by Alginate-encapsulated Cells

Tracy-Ann Read, Mohammed Farhadi, Rolf Bjerkvig, Bjørn Reino Olsen, Anne Mari Rokstad, Peter C. Huszthy, and Peter Vajkoczy

Department of Anatomy and Cell Biology, University of Bergen, 5009 Bergen, Norway [A. M. R.]; Department of Neurosurgery, Klinikum Mannheim, 68167 Mannheim, Germany [M. F., P. V.]; Harvard Medical School, Boston, Massachusetts 02155 [B. R. O.]; and Institute of Cancer Research and Molecular Biology, The Norwegian University of Science and Technology, N-7491 Trondheim, Norway [A. M. R.]

ABSTRACT

The current study describes new, antivascular, and antitumor effects of human endostatin. A novel system for continuous, localized delivery of antiangiogenic compounds to brain tumors was used. The delivery system was composed of endostatin-producing 293 cells encapsulated into immuno-isolating sodium alginate. Intravital multifluorescence microscopy was used to assess vascular and antitumor effects of endostatin in C6 glioma spheroids implanted into an ectopic as well as an orthotopic setting. Analysis of total and functional vascular density, microvascular diameters, vessel perfusion, tumor growth, and tumor cell migration were performed repetitively.

Tumor growth was reduced by 35% in treated animals. It was of interest that tumor cell invasion into the surrounding tissue was also inhibited. The total vascular density was reduced by 67.6%, perfusion by 67%, and vessel diameters by 37%. This resulted in a significant reduction in tumor perfusion, although the vessel permeability was not influenced.

We have demonstrated that human endostatin not only reduces total vascular density, as shown previously, but also greatly reduces the functionality and the diameters of the vessels. Furthermore, we show that this therapeutic approach also inhibits tumor cell invasion, thus supporting the hypothesis that tumor angiogenesis and invasion represent two interrelated processes. Finally, this work further confirms the new therapeutic concept using alginate cell-encapsulation technology for the localized delivery of therapeutic compounds to central nervous system malignancies.

INTRODUCTION

Without angiogenesis, most solid tumors, including gliomas, cannot grow to a critical size because of inadequate tissue oxygenation and nutrient supply (1, 2). These tumors are therefore considered strong candidates for antiangiogenic therapy (3). Endostatin is a Mr 20,000 proteolytic fragment of collagen XVIII, which is found in the basement membranes of various tissues, including the brain (4–9).

Endostatin has been shown to inhibit the growth of a number of different ectopic tumors (10–16), induce apoptosis in bovine pulmonary endothelial cells (C-PAE) in vitro (17) by activation of tyrosine kinase (18) and does not compete with fibroblast growth factor-2 or VEGF3 binding (19, 20).

However, the effects of endostatin on tumor microcirculation in vivo has not been thoroughly described because authors tend to report reduction in vessel count only, which does not predict vascular function and oxygenation of the tumor.

We have recently introduced a new therapeutic concept for malignant brain tumors based on the encapsulation of endostatin secreting 293 HEK cells in an immuno-protective device (bioreactor) consisting of sodium alginate (21). Transplantation of such endostatin-secreting bioreactors together with BT4C gliosarcoma cells into the rat brain significantly prolongs animal survival (21).

In the current study, we demonstrate using intravital fluorescence video microscopy that local release of endostatin from alginate bioreactors not only reduces vascular density but also largely reduces vascular diameters and perfusion in C6 gliomas. These findings thus suggest additional antiangiogenic mechanisms of endostatin, which may in part explain the potent antitumor action. For a direct comparison between ectopic and orthotopic tumors, dorsal skin-fold chambers as well as a cranial window model were used. Furthermore, we demonstrate that glioma cell invasion into the surrounding tissue was significantly inhibited, adding a new feature to the antitumor activity of endostatin.

MATERIALS AND METHODS

Cells and Cell Culture. Human fetal kidney 293 cells (293-EBNA) expressing EBNA-1 were obtained from Invitrogen (Carlsbad, CA). The cells were liposome transfected with the episomal expression vector pCEP-Pu containing the gene encoding human endostatin (20). The transfected cells (293-endo) and the C6 rat glioma cells were grown in DMEM (BioWhittaker, Walkersville, MD), supplemented as described previously (20, 21). Both cell lines were grown to confluence in 80-cm2 culture flasks (Nunc, Roskilde, Denmark) and kept at 37°C in a humidified atmosphere with 5% CO2 in air.

Spheroid Cultures. Spheroids were initiated as described elsewhere (22).

Briefly, C6 monolayer cultures were trypsinized and seeded into culture flasks base coated with 0.75% agar noble in DMEM. On the day of implantation, the spheroids were stained with the vital dye DiI (Molecular Probes, Eugene, OR) and kept at 37°C in a humidified atmosphere with 5% CO2 in air.

Fluorescence was measured in optical sections through the alginate using a video microscopy that local release of endostatin from alginate bioreactors not only reduces vascular density but also largely reduces vascular diameters and perfusion in C6 gliomas. These findings thus suggest additional antiangiogenic mechanisms of endostatin, which may in part explain the potent antitumor action. For a direct comparison between ectopic and orthotopic tumors, dorsal skin-fold chambers as well as a cranial window model were used. Furthermore, we demonstrate that glioma cell invasion into the surrounding tissue was significantly inhibited, adding a new feature to the antitumor activity of endostatin.
confocal scanning laser microscope with a krypton-argon laser (Leica TCS-NT, Heidelberg, Germany), using rhodamine and FITC filter optics. Fluorescence was recorded in a plane between 70 and 120 μm inside the beads.

**Western Blotting.** To verify that endostatin was released from the beads, conditioned medium from encapsulated endo-293 cells was collected and used for standard SDS-PAGE Western blotting (Ref. 28; endostatin antibody concentration: 1:1000).

**In Vitro Studies.** To evaluate whether endostatin had any cytotoxic or antiangiogenic effects on the tumor cells, monolayers were exposed to endostatin-secreting alginat beads. C6 cells were seeded into 24-well culture plates (8000 cells/well, 5 parallels at each time point, and 5 time points) and were allowed to adhere before being exposed to either clear alginate beads or beads containing endo-293 or 293-EBNA cells (mock transfected). The cells were counted every second day for 10 days, using a Coulter counter (Coulter Electronics, Ltd., Herpenden Herts, United Kingdom), and growth curves were established.

A similar assay was chosen for studying migration, where C6 spheroids were generated as mentioned above and thereafter allowed to adhere to the plastic surface of 24-well plates (Nunc). The area of migration was studied as described previously (27). At each time point, migrating spheroids were fixed in 4% paraformaldehyde, stained with hematoxylin, and photographed for documentation.

**Animals and Dorsal Skin-Fold Chamber Model.** The animal experiments were approved by the ethics committee for animal research. Athymic nude mice (nu/nu; 18 male/female, 28–32 g) were bred and maintained within a specific-pathogen, germ-free environment. The technique for implantation of the dorsal skin-fold chamber has been described previously (29). Briefly, animals were anesthetized by s.c. injections of 7.5 mg of ketamine hydrochloride and 2.5 mg of xylazine per 100 mg of body weight. Two symmetrical titanium frames flanked the dorsal skin-fold of animals to sandwich the extended double layer of skin and create the dorsal skin-fold chamber, which consisted of one layer of striated muscle, s.c. tissue, and epidermis. An observation window, covered with a glass coverslip, allowed for repeated intravitral microscopic observations. Two days after chamber preparation, the coverslip was temporarily removed, and a single tumor spheroid (200 μm in diameter) and four to six alginate beads (depending on the size) were placed at the surface of the striated skin muscle. Seven mice received alginat beads containing 293-endo cells, and 6 received clear alginate beads (controls). The animals tolerated the skin-fold chambers well and showed no signs of discomfort or changes in sleeping and feeding behavior.

**Cranial Window Preparation.** The procedure for the preparation of the cranial window was similar to that described in previous reports (34). The heads of the animals were fixed in a rodent stereotactic frame (David Kopf Instruments, Tujunga, CA). The skin on top of the frontal and parietal skull was cut, and the underlying peristomeum was scraped off to the temporal crests. Using an electrical high-speed drill with a burr tip size of 0.5 mm, a circular bone flap (0.7–1 cm in diameter) was created and freed from the underlying dura and sagittal sinus with the aid of a modified microdissector. The dura was removed with Iris microscissors, avoiding any damage to the sinus and bridging veins. Finally, the glioma spheroid (200 μm in diameter) and alginate bioreactors were directly placed onto either hemisphere, and the window was sealed with a glass coverslip adhered to the bone using a histocompatible glue.

**Experimental Protocol.** Intravitral multifluorescence microscopic studies of glioma growth, angiogenesis, and microcirculation were performed on days 3, 6, 10, 14, and 18 after implantation in cranial windows. The newly formed microvasculature within the fluorescent glioma mass (intratumorally) and at the glioma periphery (peritumorally), i.e., outside the tumor and next to the tumor edge, were assessed separately. Vascular measurements included newly formed tumor microvessels only, which can be clearly distinguished from the autochthonous host striated muscle and cerebral microvessels by their chaotic arrangement and heterogeneous diameters (35, 36). In addition, it is possible to evaluate whether the vessels are in the tumor or in the normal tissue simply by changing the filters. Vascular densities were measured in four to six regions of interest per animal and per observation time point. Microvascular diameters as well as hemodynamic parameters were determined by analyzing 5–10 microvessels per region of interest. At the end of the in vivo experiments (14 and 18 days after implantation), the animals were sacrificed with an overdose of ketamine/xylazine, and the skinfold chamber preparations and brains were processed for histological and immunohistochemical analysis.

**Intravitral Multifluorescence Video Microscopy.** Intravitral multifluorescence video microscopy (epi-illumination) was performed using a modified Axioptech Vario microscope with a blue (450–490 nm) and green (520–570 nm) filter block (22). Observations were made using ×3.2 short distance, ×10 long distance, and ×20 water immersion working objectives (all from Zeiss, Oberkochen, Germany), resulting in magnifications of ×50, ×200, and ×400, respectively. The glioma cells and microvasculature were visualized by means of a low-light level charge-coupled device video camera (CF8/1 FMC; Kappa, Gleichen, Germany; Ref. 22). Microscopic images were recorded using an S-VHS video system (Panasonic, Munich, Germany) for off-line analysis. Dil labeling of glioma cells allowed for a precise delineation of the spheroid from the surrounding, unaffected host tissue as well as identification of individual tumor cells applying green light epi-illumination (22). By contrast evaluation with 2% FITC-conjugated dextran (0.1 ml of FITC-dextran 150, i.v. via tail vein: M, 150,000; Sigma Chemical Co., St. Louis, MO) and use of the blue light epi-illumination, angiogenic sprouts, individual microvessels, and, finally, the glioma microvasculature was visualized.

**Analyzed Parameters.** Quantitative analysis of intravitral microscopic observations was performed by a computer-assisted image analysis system (33). Tumor growth and tumor cell migration were assessed by measurement of the tissue area (mm²) covered by the fluorescent solid tumor mass and the migrating tumor cells, respectively. Analysis of microcirculatory parameters include the total tumor vascular density (cm⁻¹), which was defined as the length of all newly formed microvessels per area of interest and observation time point, the functional vascular density (cm⁻¹), which was defined as length of RBCs perfused, microvessels per area of interest, and observation time point and microvessel diameters (μm). To assess the permeability of the vessels, we compared intravascular with extravascular fluorescence intensity of multiple individual tumor microvessels in both peritumoral and intratumoral areas and calculated a permeability index as follows: permeability index = intravascular fluorescence/extravascular fluorescence. Finally, the vascular surface is defined as the calculated mean area covered by the tumor vessels per total area of observation. Thus, vascular surface is the product of vessel density (cm⁻²) and vessel diameter (μm; Ref. 36).

**Statistical Analysis.** Quantitative data are given as mean values ± SD. Mean values of microcirculatory data were calculated from the average values in each animal. For analysis of differences between the groups, post-hoc Bonferroni test was used following one-way ANOVA. Results with P < 0.05 were considered significant (*, mean ± SD, P < 0.05 versus control).

**Histology and Immunohistochemistry.** At experiment completion, the dorsal skin-fold chamber preparations and brains were dissected free and frozen in liquid nitrogen for histological and immunohistochemical analyses. The sections were mounted on stubs, embedded in Tissue-Tek (Miles Laboratories, Inc., Naperville, IL), frozen in 2-methylbutane (E. Merck, Darmstadt, Germany), and cooled with liquid N₂.

Serial axial sections (10 and 60 μm) were cut and mounted on slides precoated with gelatin (Sigma Chemical Co.). The sections were stained with Harris H&E and counterstained with Van Gieson elastic tissue as well as identification of individual tumor cells applying green light epi-illumination (22). By contrast evaluation with 2% FITC-conjugated dextran (0.1 ml of FITC-dextran 150, i.v. via tail vein: M, 150,000; Sigma Chemical Co., St. Louis, MO) and use of the blue light epi-illumination, angiogenic sprouts, individual microvessels, and, finally, the glioma microvasculature was visualized.

The sections were analyzed with regard to endostatin distribution and blood vessel density (von Willebrand factor/CD31). The immunostaining was performed according to standard procedures (37). The following antibodies were used (1:100 dilution in DPBS): anti-von Willebrand factor (Dako A/S, Copenhagen, Denmark), anti-CD31 (Becton Dickinson, San Jose, CA), and antihuman endostatin, polyclonal (Chemicon, Temecula, CA). Species-appropriate, FITC-conjugated secondary antibodies were used for primary antibody detection (1:30 dilution in DPBS). Cell nuclei were stained by treating the sections with RNase (Sigma Chemical Co.; 1 mg/ml in DPBS), followed by a short exposure to propidium iodide (Sigma Chemical Co.; 50 μg/ml in DPBS). Finally, the sections were washed in DPBS and mounted with Vectashield (Vector Laboratories, Inc., Burlingame, CA).

All sections were viewed and evaluated using a Leica TCS NT confocal laser scanning microscope with an argon-krypton laser (Leica, Heidelberg, Germany) applying TRITC and FITC filter optics.
RESULTS

Capsule Analysis. The transfected cells were encapsulated in alginate, which resulted in beads (bioreactors) ranging from 400 to 600 μm in diameter, harboring 500–700 cells/capsule. After 3 weeks in culture, the live/dead viability assay showed that the majority of the cells within the beads were viable, as indicated by the green fluorescence emitted by the intracellular esterase-converted calcein. Furthermore, the cells had formed spheroids within the beads, as described previously (Ref. 21; Fig. 1A). Western blots of conditioned medium from endostatin-transfected cells showing that endostatin is released from the encapsulated cells into the culture medium, as indicated by a single band of M_r ~20,000 (20 KDa), when hybridized with antihuman endostatin antibodies.

Early Intravital Microscopy of C6 Glioma Spheroids and Alginate Beads. The bioreactors were well tolerated by both chamber settings (dorsal skin-fold and cranial window), showing no apparent signs of host immune reactions, which are normally characterized by increased leukocyte count/endothelial interaction and edema (Ref. 33; data not shown).

The tumor take rate was 100%, as reported previously (22). On the day of implantation into both settings, the DiI-labeled glioma spheroids showed an even fluorescence, a smooth edge, and no apparent signs of passive displacement of individual cells (Fig. 2, A and D). Three days after implantation in dorsal skin-fold chambers, the spheroids had established microtumors, consisting of a dense core surrounded by detaching/migrating cells, resulting in a fluorescent halo around the spheroid (Fig. 2B). Some tumor cells even migrated beyond this peritumoral area, and the adjacent alginate beads (Fig. 2, G and H). In contrast, the cranial window-established microtumors showed markedly reduced cell density within the core, and more cells
detached and migrated in a radial pattern from the spheroid, suggesting a higher migratory activity of tumor cells on day 3 within the orthotopic setting (Fig. 2, F and I). The view of this area using FITC optics revealed initial tumor-induced angiogenesis in the cranial window setting, characterized by vascular sprouts and a chaotic vascular network (Fig. 2, E and J), as also observed in the skin-fold chambers (Fig. 2C).

**Endostatin-secreting Alginate Bioreactors Inhibit Glioma Growth and Invasion.** Throughout the observation period, tumor growth in dorsal skin-fold chambers was significantly inhibited by endostatin. After 18 days, the tumors were 35% smaller than the control tumors (Fig. 3A). Surprisingly, tumor cell migration into peritumoral areas also appeared to be inhibited (Fig. 3B), although because of large SDs, this trend proved not to be statistically significant. Histological analysis at the end of the observation period, however, confirmed the trend in migration inhibition observed by intravitral microscopy. As illustrated in Fig. 4, the treated tumors show a clear-cut border between the tumor and host tissue (Fig. 4A), whereas the control tumors showed extensive infiltration of glioma cells into the adjacent muscle and s.c. tissue (Fig. 4B). In parallel,

Fig. 3. Local release of endostatin not only inhibits solid C6 glioma growth (A) but also affects glioma cell migration (B). The tissue covered by the microtumor and the migrating cells was analyzed planimetrically off-line using a computer-assisted image analysis system. The mean values are represented; bars, SD. Statistical analysis was performed using ANOVA, followed by unpaired Student’s t test; *, P < 0.05 versus control. E−, endostatin-treated animals; E−−, controls.

Fig. 4. A and B, H&E-stained cryosections of C6 dorsal skin-fold tumors treated with either control alginate bioreactors (A) or encapsulated 293-endo cells (B). There is an obvious difference in the invasion pattern between treated and control animals, where the treated animals have a clear demarcated tumor border and the control tumors show an extensive invasion into the s.c. muscle layer. Insets, an overview of the sections with arrows pointing to the s.c. muscle layers. A and B, ×400. C, an alginate bioreactor releasing endostatin. The sections were stained with anti-human endostatin antibody, revealing free human endostatin in the tumor tissue and endostatin-positive, 293 cells within the beads. D, human endostatin is also located in the s.c. muscle layer, at some distance to the alginate bioreactors, which are not within the plane of this section. C, ×300; D, ×80.
immuno-staining with anti-human endostatin antibody demonstrated that the locally released endostatin was not only located in the near vicinity of the beads but also in the more distant muscle and s.c. layers representing the invasion zone of control tumors (Fig. 4, C and D).

Endostatin-secreting Alginate Bioreactors Affect Glioma-induced Angiogenesis with Regard to Vascular Density, Morphology, and Functionality. Considerable differences in vascular morphology, density, functionality, and diameters were observed in the dorsal skin-fold chambers of endostatin-treated animals as compared with the controls. The control animals showed characteristic tumor-induced angiogenesis consisting of multidirectional microvascular sprouting, originating from host capillaries and postcapillary venules, which formed tortuous interconnected microvascular networks (Fig. 5A). The endostatin-treated animals, however, displayed fewer, less tortuous, and branched vessels, resembling a granulation tissue-like microvasculature rather than tumor-induced angiogenesis (Fig. 5B). Tumor-induced angiogenesis was observed within both the intratumoral and peritumoral zones, although it was predominant in the latter. Consequently, this was also the area in which endostatin had the greatest inhibitory effect on angiogenesis (Fig. 5C and D). At day 6, the total vascular density was already considerably reduced in the treated animals (Fig. 5C), showing >80% reduction in vessel density in the peritumoral areas. Furthermore, the functionality of the vessels in terms of perfusion was also greatly reduced (Fig. 5D), where again the peritumoral areas showed the most severe differences at day 6 with an almost 80% reduction in functional vessel density.

Differences were also seen with regard to vessel diameters in the treated animals (Fig. 6A). On average, the endostatin-treated vessels were 37% smaller in diameter than the controls, in both intra and peritumoral areas (Fig. 6A). In line with the observations regarding reduction in tumor size and total vascular density, the vascular exchange surface of the endostatin-treated tumors was ~50% smaller than the control tumors (Fig. 6B). Of interest, despite the inhibitory effects of endostatin on tumor angiogenesis and tumor microcirculation, microvascular permeability was not affected and remained high for the macromolecular fluorescent marker FITC-dextran (Fig. 6B).

The results from the orthotopic experiments using the cranial window as implantation site showed a tendency similar to that observed in the dorsal skinfold chambers with regard to the inhibitory effects of endostatin on total and functional vessel densities, as well as on the vascular exchange surface, tumor size, and vessel diameter, although the latter two parameters failed to reach statistical significance (Table 1).

In Vitro Assay. The in vitro proliferation and migration assays showed no inhibitory effects of endostatin alone on the tumor cells (Fig. 7).
DISCUSSION

Numerous studies on the antiangiogenic effects of endostatins on in vitro and in vivo angiogenesis exist, where a variety of assays and forms of endostatin (i.e., recombinant human and mouse, produced by both pro- and eukaryotic cells) have been used, resulting in somewhat conflicting data regarding the functional effects of endostatin (38, 39).

It has, for example, been suggested that human endostatin does not inhibit murine or bovine endothelial cell migration/proliferation in vitro (39, 40) and that circulating human endostatin has no antiangiogenic activity at all (41). In contrast to this, it has also been reported that human endostatin inhibits VEGF-induced human umbilical vein endothelial cell migration in vitro (20) and inhibits the growth of several different rodent tumor models in vivo (21, 42, 43). In the current study, we show that localized delivery of recombinant human endostatin from encapsulated 293-endo cells not only inhibits solid tumor growth but also affects invasion of tumor cell into the surrounding tissue. Furthermore, we show for the first time that endostatin not only affects the tumor vascular density but also greatly reduces vessel functionality, diameters, and hence microvascular perfusion in both an ectopic and orthotopic setting.

The concept of cell encapsulation and consequent implantation in the central nervous system for the treatment of brain tumors is novel, and we have shown recently that an experimental rat intra-cerebral gliosarcoma model (BT4C) can be treated with endostatin-secreting bioreactors, resulting in prolonged survival of the animals (21).

The results presented herein show that this method of sustained localized therapy is not only applicable within the brain parenchyma but also on the cerebral cortex and in s.c. and muscle tissue. Both skin-fold and cranial window preparations seemed to tolerate the bioreactors (data not shown), showing no apparent signs of immune reaction in forms of increased leukocyte count/endothelial interactions or edema (data not shown). Furthermore, the physiological angiogenic response to the grafts was not inhibited by recombinant human endostatin; in fact, these vessels grew around and over the bioreactors (data not shown), suggesting that the antiangiogenic response is tumor specific. This is essential in predicting long-term viability of the encapsulated 293-endo cells because inhibition of physiological angiogenesis would also affect nutrient supply to the bioreactors.

One of the most interesting findings in this study was that the tumor cell migration/invasion pattern was different in the dorsal skin-fold chambers of treated animals (Fig. 3). This is in contrast to the in vitro proliferation and migration assays, which did not reveal inhibitory effects of endostatin (Fig. 7). When the skin-fold chambers were immunostained for human endostatin, the protein was found to be located as expected, in the immediate vicinity of the alginate bioreactors (Fig. 4C). However, distant deposits were also observed in the muscle and s.c. layers of the skin, into which the tumor cells did not invade (Fig. 4D).

In addition, we report that after 18 days of treatment, >67% of the newly formed vessels were not functional (i.e., no RBC flow) and that the vessel diameters were also reduced by 37%, the latter of which is a hallmark for tumor vessel regression after VEGF neutralization (51). These vascular effects of endostatin are previously unreported and

### Table 1 Quantitative analysis of intravital microscopic observations in the cranial window preparations

<table>
<thead>
<tr>
<th></th>
<th>E+ a</th>
<th>SD</th>
<th>E−</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size (mm²)</td>
<td>28.59</td>
<td>9.78</td>
<td>36.20</td>
<td>13.42</td>
</tr>
<tr>
<td>FVD (cm²/cm²)</td>
<td>45.50</td>
<td>2.04</td>
<td>54.73</td>
<td>9.03</td>
</tr>
<tr>
<td>Diameter</td>
<td>12.29</td>
<td>1.07</td>
<td>1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>VS (%)</td>
<td>3.53</td>
<td>20.65</td>
<td>6.39</td>
<td>0.06</td>
</tr>
<tr>
<td>Permeability index</td>
<td>0.04</td>
<td>1.10</td>
<td>0.06</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* E+, endostatin positive; E−, endostatin negative; NS, not significant; FVD, total vascular density; VS, vascular surface.

![Fig. 7. Endostatin has no detectable effect on tumor cell migration in vitro. A, hematoxylin-stained glioma spheroids in a migration assay show no significant difference in the area of migration between endostatin-treated spheroids (E+) and controls (E−). B, quantitative analyses of area of migration in treated (E+) and control spheroids (E−). ×20. Bars, SD.](image)
becomes clear that endostatin has the greatest antitumor effects from end effects. In fact, if one compares the dorsal skin-fold tumors, it period may not be enough for endostatin to induce significant antitu- weeks). Given the aggressiveness of this tumor, this short growth they quickly grow to a critical size with respect to chamber size (2 weeks). than the dorsal skin-fold implants, and because the window is smaller, spheroids implanted into the cranial window grow substantially faster longed survival of rats bearing BT4C brain tumors (21). The glioma reason for this is not clear, because we have shown previously that between endostatin-treated and control cranial window tumors. The However, we failed to show statistical significance in tumor size effects were comparable between the ectopic and orthotopic model. In this study, we treated gliomas implanted in both dorsal skin-fold INTRAVITAL MICROSCOPIC STUDY

REFERENCES

TOTAL VASCULAR DENSITY, AS DEMONSTRATED PREVIOUSLY, BUT IT ALSO AFFECTS FUNCTIONAL VASCULAR DENSITY, DIAMETER, AND MORPHOLOGY, RESULTING IN A LARGE REDUCTION OF VASCULAR SURFACE AND TUMOR PERFUSION.

In conclusion, this study shows that not only does endostatin affect
Intravitral Microscopy Reveals Novel Antivascular and Antitumor Effects of Endostatin Delivered Locally by Alginate-encapsulated Cells

Tracy-Ann Read, Mohammed Farhadi, Rolf Bjerkvig, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/18/6830

Cited articles
This article cites 53 articles, 19 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/18/6830.full.html#ref-list-1

Citing articles
This article has been cited by 5 HighWire-hosted articles. Access the articles at:
/content/61/18/6830.full.html#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.