Effect of Tumor Host Microenvironment on Photodynamic Therapy in a Rat Prostate Tumor Model

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ABSTRACT

**Purpose:** Tumor host microenvironment plays an important role in tumor growth, metastasis, and response to cancer therapy. In this study, the influence of tumor host environment on tumor pathophysiology, photosensitizer distribution, and photodynamic therapy (PDT) treatment effect was examined in the metastatic at lymph node and lung (MatLyLu) rat prostate tumor.

**Experimental Design:** MatLyLu tumors implanted in different host environment [i.e., orthotopically (in the prostate) or s.c.] were compared for difference in vessel density, average vessel size, vascular permeability, tumor vascular endothelial growth factor production, and tumor oxygenation. Uptake of photosensitizer verteporfin in tumors in both sites was determined by fluorescence microscopy. To compare tumor response to PDT, both orthotopic and s.c. MatLyLu tumors were given the same doses of verteporfin and laser light treatment, and PDT-induced tumor necrotic area was measured histologically.

**Results:** Orthotopic MatLyLu tumors were found to grow faster, have higher vessel density and more permeable vasculature, have higher vascular endothelial growth factor protein levels, and have lower tumor hypoxic fraction than the s.c. tumors. Uptake of photosensitizer verteporfin in the orthotopic tumor was higher than in the s.c. tumors at 15 minutes after injection (1 mg/kg, i.v.), and became similar at 3 hours after injection. For the vascular targeting PDT treatment (0.25 mg/kg verteporfin, 50 J/cm² at 50 mW/cm², 15 minutes drugelight interval), there was no significant difference in PDT-induced tumor necrotic area between the orthotopic and s.c. tumors, with 85% to 90% necrosis in both types of tumors. However, tumor necrosis induced by the cellular targeting PDT (1 mg/kg verteporfin, 50 J/cm² at 50 mW/cm², 3 hours druglight interval) was significantly different in the orthotopic (64%) versus the s.c. (29%) tumors.

**Conclusions:** Tumor host environment can significantly affect photosensitizer verteporfin distribution and PDT treatment effect. VerteporfinPDT regimen targeting tumor cells is more sensitive to such influence than the vascular targeting PDT. Our study showed the importance of tumor host environment in determining tumor physiologic properties and tumor response to PDT. To obtain clinically relevant information, orthotopic tumor model should be used in the experimental studies.

INTRODUCTION

Photodynamic therapy (PDT) has become an established cancer treatment that involves the interaction of a photosensitizer and light in an oxygenated environment (1). Upon activation by light of a specific wavelength, the photosensitizing compound, localized in the target cell/tissue, reacts with molecular oxygen, and generates cytotoxic reactive oxygen species, leading to cell/tissue damage. PDT has been clinically applied for the treatment of cancers such as cancers in the digestive and respiratory tracts, skin cancer, head and neck cancer, cervical cancer, and bladder cancer (2).

A growing number of studies suggest that PDT can also be a promising treatment for prostate cancer (3, 4). Studies on the optical characteristics of human and animal prostate tissue/cancer indicate that it is possible to provide entire prostate treatment with optical fibers (5–9). The effectiveness of PDT in prostate tumor management has been shown in various rat and mouse tumor models (4, 10–15). However, most of these studies were conducted on prostate tumors induced in the subcutaneous (s.c.) space (ectopic) and only two PDT studies on the orthotopic prostate tumor models have been reported (4, 10). This is presumably because the s.c. tumor is easy to produce and convenient to study. However, accumulating evidence shows that tumor host microenvironment affects tumor gene expression and has a significant effect on tumor physiologic characteristics such as growth pattern, vascular function, angiogenesis, and metastasis (16–20). Evaluation of cancer therapies in a nonphysiologically relevant tumor model can hamper the translation of experimental results to the clinical application because tumors grown in different physiologic environments might react differently to the same treatment. Indeed, it has been reported that the same tumor grown in different organ environments show distinct difference in the delivery and therapeutic effect of anticancer agents (21). PDT involves a complex interaction among light, photosensitizer, oxygen, and tumor tissue. Any changes in photosensitizer distribution, tissue oxygen delivery,
or tissue optical properties may affect the final PDT outcome. It is not yet known whether implanting prostate tumor in different host microenvironments would influence these PDT variables and lead to changes in tumor response to PDT. Considering the fact that most PDT studies on prostate cancer were done in a physiologically nonrepresentative host environment, it is necessary to determine the influence of tumor host microenvironment on photosensitizer uptake, tissue oxygenation, and PDT response.

The Dunning R-3327 rat model system is the most widely used animal prostate cancer model (22, 23). This tumor system consists of many sublines varying widely in androgen response, tumorigenicity, and metastatic potential. The metastatic at lymph node and lung (MatLyLu) subline is an androgen-independent, fast-growing, and highly metastatic Dunning tumor line (23). MatLyLu tumor model is very commonly used in assessing the therapeutic effect of cancer therapies. However, there is no report comparing tumor vascular variables, vascular function, and oxygenation status between the MatLyLu tumors implanted in the orthotopic (prostate) and s.c. sites. This information is not only important for PDT, but also necessary for other therapies such as radiation and chemotherapy because they all depend on a functional vasculature for drug and oxygen delivery.

The objectives of this study are (1) to evaluate the influence of tumor host microenvironment (prostatic versus s.c. sites) on the MatLyLu tumor physiologic variables including tumor vessel size, vessel density, permeability, vascular endothelial growth factor (VEGF) production, and tumor oxygen; and (2) to determine the effect of tumor host microenvironment on photosensitizer verteporfin distribution and the PDT treatment outcome.

**MATERIALS AND METHODS**

**Photosensitizer.** Verteporfin (lipid-formulated benzoporphyrin derivative monoaacid ring A) was obtained from QLT, Inc. (Vancouver, Canada). A stock saline solution of verteporfin was reconstituted according to the manufacturer’s instructions and stored at 4°C in the dark. It was injected i.v. to the animals at the dose of 0.25 mg/kg at 15 minutes or 1.0 mg/kg at 3 hours before light irradiation.

**Animals and Tumor Models.** Male Copenhagen rats (6-8 weeks old), obtained from Charles River Laboratories (Wilmington, MA), were used throughout the study. All animal procedures were carried out according to protocols approved by the Dartmouth College Animal Care and Use Committee. The R3327-MatLyLu Dunning prostate tumor is an androgen-independent carcinoma, syngeneic to the Copenhagen rats, and highly metastatic to both lung and lymph nodes (23). R3327-MatLyLu prostate cancer cells were cultured in RPMI 1640 with glutamine (Mediatech, Herndon, VA) supplemented with 10% fetal bovine serum (HyClone, Logan, UT) and 100 units/mL penicillin-streptomycin (Mediatech).

The s.c. MatLyLu tumors were induced by injecting approximately 1 × 10⁷ MatLyLu cells (suspended in 0.05 mL PBS) s.c. into the shaved rat flank. Tumor growth was documented regularly by external measurements with calipers. Tumors were used for the experiment at 9 to 12 days after inoculation, with a surface diameter of 7 to 9 mm and a thickness of 2 to 4 mm. The orthotopic MatLyLu rat prostate cancer model was reproduced as previously described (10). Briefly, animals were anesthetized with an injection (i.p.) of ketamine (90 mg/kg) and xylazine (9 mg/kg). After anesthesia, a 2-cm longitudinal incision was made on the lower abdomen just above the pubic bone, pulling the bladder aside to expose the prostate. The tumors were induced by injecting 1 × 10⁷ MatLyLu cells (in 0.05 mL PBS) into the prostate ventral lobe. The injection site was cleaned with 70% ethanol to kill any possible residual tumor cells and the incision was closed aseptically. Tumors were used for experiment at 7 to 8 days after implantation when reaching a size of 6 to 9 mm in diameter. Both s.c. and orthotopic tumors had similar size (6-9 mm in diameter) and observed no spontaneous necrosis at the time of PDT treatment.

**Analysis of Functional Tumor Vasculature.** Tumor-bearing animals were i.v. injected with a carboxycyanine dye DiOC₃(3) (Molecular Probes, Eugene, OR), a marker of functional vasculature, at a dose of 1 mg/kg, 1 minute before sacrificing the animals (24). The tumor tissue was harvested, embedded in the Tissue Tek medium and snap-frozen in liquid nitrogen-cooled isopentane. Cryosections with thickness of 10 µm were taken from the tumor and imaged with a Nikon Diaphot-TMD fluorescence microscope (excitation, 480/20 nm; emission, 540/40 nm) equipped with Qimaging Micropublisher Imaging System (Burnaby, BC, Canada), which includes a high resolution CCD digital camera and the image acquisition software. To quantify the functional vessel density and the average vessel size in the s.c. versus the orthotopic tumor, five to seven microscopic fields under a 10× objective lens (with an area of 1 mm²) were randomly taken from each section. Three sections were examined for each tumor and 10 animals were included in each of the s.c. and orthotopic tumor groups, resulting in a large sampling of the tumor vasculature patterns. The fluorescence images (682 × 512 pixels, 1 pixel = 1.69 µm), captured with the same exposure conditions, were analyzed with NIH ImageJ software with an automatic threshold setting to generate the functional vessel count and average vessel size.

**Assessment of Tumor Vessel Permeability.** The permeability of tumor blood vessels in the orthotopic and s.c. MatLyLu tumors was assessed by the Evans blue extravasation assay (25). Evans blue (10 mg/mL in PBS) was injected at a dose of 10 mg/kg (i.v.) to the tumor-bearing animals. Tumors were excised at 15 minutes, 1, 3, and 24 hours after the administration. Before harvesting the tumor tissue, animals were perfused with 50 mL physiologic saline to remove the Evans blue dye in the circulation. Tumors were weighed, minced, and extracted with formamide (1 mL per 100 mg tumor tissue) for 72 hours. The solutions were centrifuged and the absorbance of Evans blue in the supernatant was measured at 620 nm by a spectrophotometer. The absorbance data were all normalized to the highest value obtained to generate relative results for comparison between tumors in different sites. At least three animals were included in each group.

**Measurement of Tumor Oxygenation.** Tumor oxygen partial pressure (pO₂) distribution was measured by using an Eppendorf polarographic oxygen electrode (Eppendorf, Hamburg, Germany; ref. 26). Before and between measurements, the 300-µm electrode probe was calibrated in physiologic saline by flushing alternatively with air (100% oxygenated) and nitrogen (0% oxygenated). Tumor-bearing animals were anesthetized...
with inhalation of 1.5% isoflurane mixed with air. For the s.c. tumor, a small cut was made on the rat skin to facilitate the probe insertion. An abdominal incision was made to expose the orthotopic tumors. The oxygen electrode was first inserted into the tumor. After the \( pO_2 \) value became stable, the probe was set to advance automatically through the tissue in a 0.7-mm increment, followed by a 0.3-mm backward step before measurement. The probe track length was predetermined by the tumor size and three diagonal tracks were made through each tumor. From the \( pO_2 \) values obtained for each tumor, the median and mean \( pO_2 \) values and the percentage of readings <5 mm Hg were calculated. The values of each group and the SD were obtained based on the data of each tumor in the group. To show the \( pO_2 \) distribution, a histogram was generated by pooling all the values from animals in each group and displaying the percentage of \( pO_2 \) values in each \( pO_2 \) range category.

**VEGF-A ELISA Assay.** Proteins were extracted from the orthotopic and s.c. MatLyLu tumors. Briefly, frozen tumors were pulverized into a powder in a tissue homogenizer and thawed in 1 mL/mg lysis buffer containing protease inhibitors (1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 10 mg/mL PMSF, 100 mmol/L sodium orthovanadate, and protease inhibitors cocktail). Extracts were incubated on ice for 30 minutes with vortexing every 5 minutes followed by centrifuging at 10,000 \( \times \) g for 10 minutes at 4°C. The supernatant was transferred and centrifuged again. Protein samples were stored at \( \sim 80°C \) until ELISA assay. A rat VEGF DuoSet ELISA development system (R&D Systems, Minneapolis, MN) was used, according to the manufacturer’s instruction, to quantify rat VEGF levels in tumor extracts. Results were normalized to protein concentrations. Four tumors were included in each group.

**Photosensitizer Distribution and Uptake.** Tissue distribution of verteporfin was studied by fluorescence microscopy at 15 minutes and 3 hours following drug administration (i.v., 1 mg/kg). To visualize the perfused blood vessels, perfusion marker DiOC(3) was injected (i.v.) at a dose of 1 mg/kg, 1 minute before excising the tumor tissue (24). Cryosections (10 \( \mu \)m) were cut from the tumor and the same microscopic fields (with an objective lens of 10 \( \times \)) were imaged for both verteporfin (excitation, 425/40 nm; emission, 700/30 nm) and perfusion marker DiOC(3) (excitation, 480/20 nm; emission, 540/40 nm) with a Nikon Diaphot-TMD fluorescence microscope. To quantify verteporfin fluorescence intensity in the s.c. and orthotopic tumors, five to seven microscopic fields (with an area of 1 mm\(^2\)) were randomly taken from each section. Three sections were examined for each tumor and 5 or 6 animals were included in each group. The intensity of the fluorescence image (682 \( \times \) 512 pixels, 1 pixel = 1.69 \( \mu \)m) was measured with NIH Imagej software. With the same exposure setting, background images were also taken from control tumors, which were injected only with DiOC(3). The average intensity of these background images was subtracted from the values of verteporfin fluorescence intensity.

**PDT Treatment and Tumor Histologic Analysis.** A diode laser system (Applied Optronics, Newport, CT) with 690 nm wavelength was used throughout this study. The light was delivered through an optical fiber (140-\( \mu \)m core diameter) and irradiated the tumor surface over a 1-cm-diameter beam spot using a fiber optic collimator. The MatLyLu tumors in both sites were exposed to an incident fluence rate of 50 mW/cm\(^2\) for 1,000 seconds (50 J/cm\(^2\)), as measured by a Thorlabs optical power meter (Thorlabs, Inc., North Newton, NJ). Animals were anesthetized with an injection (i.p.) of ketamine (90 mg/kg) and xylazine (9 mg/kg) and placed on a heating pad, maintained at 37°C, throughout the treatments. For the orthotopic MatLyLu tumors, abdominal surgery was made to expose the tumor in the prostate for light treatment. To ensure the delivery of the same light dose (50 J/cm\(^2\)), skin over the s.c. MatLyLu tumor was surgically retracted during the light treatment and sutured after treatment. The same operation was done on all control tumors. PDT-induced tumor necrosis was measured histologically at 2 days after treatment (27). Briefly, H&E staining tumor images were digitally recorded using an Olympus BX50 microscope with an Olympus C3030 CCD camera. An objective of 1.25\( \times \) was used to cover the whole tumor section. The whole tumor area was outlined on the computer screen using the freehand drawing tool and measured with NIH Imagej software. Tumor necrotic area was outlined and measured in a similar way. Tumor outlines were confirmed by visual inspection of tissue slides under 10\( \times \) or 40\( \times \) magnification. All these measurements were corroborated by a pathologist (P.J.H.).

**Statistical Analysis.** The values of vessel density and average vessel size were transformed to log scale for correlation analysis and probability estimation, which was calculated by the number of occurrence over the total sample size. Standard unpaired \( t \) test was used to compare the statistical significance of vessel density, vessel size, and VEGF release between different groups. Statistical analysis of vascular permeability and percentage of \( pO_2 \) values was done by nonparametric Wilcoxon test. Analysis of fluorescence intensity and percentage of tumor necrosis among different groups was done by nonparametric ANOVA test (Kruskal-Wallis test). Statistical significance was accepted at \( P < 0.05 \).

**RESULTS**

**Effect of Tumor Host Microenvironment on MatLyLu Tumor Growth and Histology.** The MatLyLu tumor growth in the prostatic and s.c. environment was compared by implanting the same amount of (1 \( \times \) 10\(^5\)) cells in both sites. Tumors were excised and weighed at 14 days after implantation. The average tumor weight was 2.84 \( \pm \) 0.77 g (SD, 5 tumors) and 6.58 \( \pm \) 1.86 g (SD, 5 tumors) for the s.c. and orthotopic MatLyLu tumors, respectively. H&E staining of tumor sections revealed that both s.c. and orthotopic MatLyLu tumors were poorly differentiated adenocarcinomas (Fig. 1). Quite some mitotic nuclei throughout the entire tumor sections indicated that the tumor had a high growth rate. Infiltration of macrophage and lymphocytes were often observed, especially in the orthotopic tumor. No significant difference in tumor cell morphology was found between tumors in different sites.

**Effect of Tumor Host Microenvironment on MatLyLu Tumor Vessel Density and Vessel Size.** The functional vasculature of the MatLyLu tumors was stained by i.v. injecting a fluorescent perfusion marker DiOC(3) and the representative images are shown in Fig. 2. The number of functional blood vessels and average vessel size in each fluorescence microscopic field (10 \( \times \), with an area of 1 mm\(^2\))...
was analyzed with NIH ImageJ using an automatic threshold. As shown in Fig. 3A, substantial heterogeneity in tumor vessel size and density is observed in both orthotopic and s.c. MatLyLu tumors. A slight but statistically significant inverse correlation between the vessel density and the average vessel size was established in both cases. The correlation coefficient was $-0.20$ and $-0.31$ for the orthotopic and s.c. tumor, respectively. The averaged probability distributions of tumor vessel density and average vessel size in the orthotopic and s.c. MatLyLu tumors, generated from a summation of 209 orthotopic and 191 s.c. tumor images, are shown in Fig. 3B. It was found that the orthotopic MatLyLu tumors tended to have a higher vessel density but smaller vessel size than the s.c. ones ($P < 0.01$). The orthotopic tumor had on average 85 functional vessels per mm$^2$ and the average vessel size was 443 $\mu m^2$, whereas the average vessel density in the s.c. tumor was 73 per mm$^2$ and the average vessel size was 511 $\mu m^2$.

**Effect of Tumor Host Microenvironment on MatLyLu Tumor Vascular Permeability.** Vascular permeability in the MatLyLu tumors implanted in orthotopic and s.c. sites was assessed by Evans blue tumor uptake (Fig. 4). Uptake of Evans blue dye was significantly higher in the orthotopic tumor than the s.c. one at 15 minutes after injection ($P < 0.01$). No significant difference in tumor Evans blue level was found at 1 hour after administration ($P > 0.05$) and by 24 hours after injection, the same amount of Evans blue dye was detected in the orthotopic and s.c. tumors.

**Effect of Tumor Host Microenvironment on MatLyLu Tumor VEGF Level.** VEGF protein levels in the orthotopic and s.c. MatLyLu tumors were determined by a rat VEGF ELISA kit. As shown in Fig. 5, VEGF level in the orthotopic tumors was more than twice higher than in the s.c. tumor.

**Effect of Tumor Host Microenvironment on MatLyLu Tumor Oxygen.** Tumor oxygenation was assessed with Eppendorf measurements and the resulting histograms of assembled data are shown in Fig. 6. The figure shows results from 198 points for the orthotopic and 365 points for the s.c. tumors. The averaged mean $pO_2$, median $pO_2$, and the percentage of values $<5$ mm Hg from all tumors in each group together with SD are summarized in Table 1. Whereas the mean and median

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**Fig. 1** H&E staining of orthotopic and s.c. MatLyLu tumors in male Copenhagen rats (original magnification 40×). Tumor cells in both sites show no significant difference in cell morphology and are present as poorly differentiated adenocarcinoma. Note many mitotic nuclei (white arrows) in both tumors and prostate glandular tissue (*) is also visible in the orthotopic tumor. Black arrows, some blood vessels.

**Fig. 2** Fluorescence images of verteporfin in the orthotopic and s.c. MatLyLu tumors at 15 minutes or 3 hours after injection (1 mg/kg, i.v.). The perfusion marker DiOC$_7$(3) (1 mg/kg, i.v.) was injected 1 minute before animal sacrifice to visualize the functional blood vessels. The same microscopic field (objective lens of 10×) was imaged for both DiOC$_7$(3) (green) and verteporfin (red) by using different filter set. Bar, 100 $\mu m$. 

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values are not significantly different between the tumors at two different sites, the percentage <5 mm Hg (hypoxic values) is significantly different, with 11.8 ± 3.9 mm Hg in the orthotopic and 30.1 ± 29.5 mm Hg in the s.c. case, suggesting fewer hypoxic pockets in the orthotopic tumor.

**Effect of Tumor Host Microenvironment on Verteporfin Uptake.** To begin assessing the effectiveness of PDT with verteporfin in the orthotopic and s.c. MatLyLu tumors, the uptake of verteporfin was first quantified by fluorescence microscopy. Representative fluorescence images of verteporfin and corresponding perfusion marker DiOC7(3) images are shown in Fig. 2. The distribution of verteporfin in the s.c. tumor at 15 minutes after administration was almost identical to the perfusion marker, indicating a typical intravascular localization (28). However, verteporfin distribution in the orthotopic tumor

**Fig. 3 A.** A dot plot showing an inverse correlation between vascular density (per mm²) and average vessel size (µm²) in both orthotopic and s.c. MatLyLu tumors. **B.** Probability estimation of tumor vessel density and average vessel size in the orthotopic (white) and s.c. (black) MatLyLu tumors. Functional tumor vasculature was stained by injecting a perfusion marker DiOC7(3) (1 mg/kg, i.v.) to the tumor-bearing animal. Tumor vessel density and vessel size data were generated by analyzing 209 orthotopic and 191 s.c. tumor images with NIH ImageJ software.

**Fig. 4** Difference in tumor vascular permeability to Evans blue dye between the s.c. and orthotopic MatLyLu tumors. Tumors were excised at different time points after i.v. injection of Evans blue dye (10 mg/kg in PBS). The uptake of Evans blue dye was measured by spectrophotometry after tissue extraction with formamide. Each group includes 3 to 5 tumors. Bars, SD.

**Fig. 5** Difference in tumor VEGF release between the orthotopic and s.c. MatLyLu tumors. Total tumor proteins were extracted from tumors in both sites and VEGF level was quantified by ELISA assay. Four tumors were included in each group. Bars, SD.

**Fig. 6** Difference in tumor oxygenation profile between the orthotopic and s.c. MatLyLu tumors. Tumor oxygenation was measured by an Eppendorf polarographic oxygen electrode, described in the MATERIALS AND METHODS. The histogram was generated by pooling all the measurements from tumors in the s.c. (7 tumors) and orthotopic (5 tumors) tumor groups.
orthotopic and s.c. tumors. Verteoporphyrin fluorescence was generally observed throughout the entire tumor section in both orthotopic and s.c. tumors at 3 hours after injection. Verteoporphyrin fluorescence intensity was quantified by analyzing 75 to 100 microscopic images from 5 or 6 animals in each group and the results are shown in Fig. 7 as a boxplot to illustrate the variance (distribution heterogeneity), mean, and median values. Nonparametric ANOVA test indicated that the fluorescence intensity from the orthotopic tumor samples was significantly higher ($P < 0.05$) than from the s.c. tumor samples at 15 minutes after injection. However, no significant difference in verteporfin fluorescence intensity was found at 3 hours after injection.

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>MatLyLu tumors</th>
<th>S.c. MatLyLu tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean $pO_2 \pm SD$ (mm Hg)</td>
<td>20.8 ± 4.6</td>
<td>19.8 ± 9.5</td>
</tr>
<tr>
<td>Median $pO_2 \pm SD$ (mm Hg)</td>
<td>20.8 ± 3.0</td>
<td>16.9 ± 11.6</td>
</tr>
<tr>
<td>Percentage of values $&lt;5$ mm Hg ± SD</td>
<td>11.8 ± 3.9</td>
<td>30.1 ± 29.5</td>
</tr>
<tr>
<td>No. values</td>
<td>198 ($n = 5$)</td>
<td>365 ($n = 7$)</td>
</tr>
</tbody>
</table>

* $P < 0.05$, significant difference between the orthotopic and s.c. MatLyLu tumors.

**DISCUSSION**

A number of studies have shown that the photosensitizer uptake and oxygen level can vary among different tumor models, resulting in varied tumor response to PDT treatment. However, few have systematically examined whether the same tumor line can vary on these variables under different host microenvironment and how the changes of these variables will affect the final PDT outcome. This issue becomes even more important when considering that most current PDT studies are done on tumors grown in the ectopic tumor environments (such as s.c. space) rather than in their favorable orthotopic organ environment. For the first time, we studied the effect of tumor host environment on the uptake of photosensitizer verteporfin and PDT response in the R3327-MatLyLu tumors, a well-characterized and widely used prostate tumor model (23).

One striking difference between the orthotopic and s.c. MatLyLu tumor is the growth potential. It was found that tumors in the prostate weighed more than twice than tumors in the s.c. space at 14 days after implantation. This result, consistent with the previous studies (29, 30), shows that the prostate environment is more favorable for the MatLyLu tumor growth. The increased tumor growth in the orthotopic environment is likely related to its high vessel density, which can provide a good supply of nutrients and oxygen to the tumor tissue. Since s.c. MatLyLu tumors have a lower vascular density than the orthotopic ones, the intervascular space at 14 days after injection might be larger, which leads to regions of low oxygenation due to limited oxygen diffusion distances (31). Tumor oxygenation measured by the
Eppendorf electrode confirmed that the s.c. MatLyLu tumor had a higher fraction of hypoxic area (pO2 < 5 mm Hg) than the orthotopic tumor (Table 1).

The difference in vessel density between the s.c. and orthotopic MatLyLu tumor can be partially explained by the difference in the VEGF level in the tumor tissue. To maintain a sustained growth, tumors need to develop new vasculature to fulfill the requirements for oxygen and nutrients. This angiogenic process is often triggered by the release of proangiogenic factors such as VEGF and basic fibroblast growth factor (32). VEGF is the most potent proangiogenic factor detected in various tumors (33). We found in this study that the VEGF level in the orthotopic MatLyLu tumor was about 2.5-fold greater than in the s.c. tumor. This is in agreement with the higher vascular density (Fig. 3) and vessel permeability (Fig. 4) observed in the orthotopic tumor vasculature, suggesting a stronger angiogenic stimulus in the orthotopic environment (16, 34). Higher expression of other angiogenic factors has also been reported in some human prostate tumor lines orthotopically implanted in the mouse prostate (19, 20, 35). Thus, prostate cancer cells grew in the orthotopic site displayed much higher growth potential than cells in the ectopic (s.c.) host environment.

A functional vascular network is not only necessary for tumor growth but also crucial for the delivery of therapeutic agents. The vascular property (increased vessel density and vascular permeability) shown in the orthotopic tumor may facilitate the diffusion of photosensitizer out of vasculature into the tumor tissue. The diffusion of verteporfin in the orthotopic tumor seemed to be faster than in the s.c. tumor (Fig. 2). Using mathematical simulation, our earlier paper indicated that the effective diffusion coefficient for verteporfin in the tumor tissue was about twice as high for the orthotopic tumor as for the s.c. tumor (36). The faster drug diffusion rate observed in the orthotopic tumor led to a higher verteporfin uptake in the tumor tissue at 15 minutes after injection (Fig. 7). Leakage of photosensitizer into the tumor tissue at a high rate could affect the treatment by supplying more drug to the tissue during the time of treatment. This would only be a major factor when the diffusion rate is high and the treatment time is long and especially when the photobleaching of the drug is a dominant factor during treatment. At present, it is not clear whether this is the case in verteporfin-PDT treatment of the MatLyLu tumors, but this will be analyzed in the future study.

PDT-induced tumor necrosis was measured to compare the tumor response in different sites. We attempted to isolate the direct tumor cellular effect and the secondary vascular effect by using the drug-light interval at either 15 minutes or 3 hours. In previous studies, we have shown that these two regimens can determine where the damage largely occurs (28, 37). It is interesting to find that the vascular targeting approach (15 minutes drug-light interval) yielded similar tissue damage (near 85-90%) for both types of tumor (Table 2), although there was a significant difference in photosensitizer uptake and tumor hypoxic area. This result suggests that vascular targeting PDT is not sensitive to tumor microenvironment issues related to photosensitizer and oxygen delivery since the vessels may have an adequate supply of both. A recent study shows that vascular targeting PDT can even circumvent the multidrug resistance in cancer cells for an improved cancer therapy (38). These results suggest that vascular targeting PDT can be used to compensate the conventional cancer therapy such as radiation and chemotherapy whose efficacy critically depends on tumor microenvironment and cancer cell phenotype.

However, the cellular targeting PDT treatment (3 hours drug-light interval) did show strong dependence on tumor microenvironment. Significant difference in tissue damage was observed in the s.c. versus the orthotopic tumors. Photosensitizer uptake apparently is not a major cause for this difference since the drug level was found to be similar at the same time of PDT treatment. The difference in tumor response to the cellular targeting PDT is likely due to different tumor vasculature and oxygenation status observed in the orthotopic and s.c. tumor microenvironment. It has been shown that microregional differences in vascular density affect oxygen delivery, and that a larger intercapillary spacing pattern can decrease PDT efficacy due to the limitation of oxygen delivery, thereby reducing the amount of singlet oxygen produced in the tissue (39, 40). Thus, the lower vascular density and higher hypoxic fractions observed in the s.c. MatLyLu tumors might be the major reason for the limited tumor response. Moreover, the larger intercapillary distance may also limit the diffusion of photosensitizer during light treatment, and if photobleaching of the drug is significant, this effect could decrease the overall efficacy of treatment as well. Further study of tumor oxygenation and drug distribution heterogeneity immediately following PDT would be required to assess how the differences in pretreatment drug distribution and oxygenation distribution will determine the differences in treatment outcome.

In summary, we show in the present study that the R3327-MatLyLu tumors, grown orthotopically in the prostate and s.c., display difference in tumor vasculature, oxygenation and the level of VEGF production. The difference in tumor pathophysiological properties induced by tumor host environment affects photosensitizer uptake and response to PDT. Cellular targeting PDT (long drug-light interval) was found to be vulnerable to the influence of tumor organ environment, whereas the vascular targeting approach (short drug-light interval) was more resistant to such variation. Our study emphasizes the importance of using orthotopic tumor model in preclinical evaluation of cancer treatments.

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