Quantification of Tumor Vascularity
With Contrast-Enhanced Sonography
Correlation With Magnetic Resonance Imaging
and Fluorodeoxyglucose Autoradiography in
an Implanted Tumor

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Objective. To correlate the quantitated tumor vascularity of implanted murine tumors as depicted by contrast-enhanced sonography with estimates made with magnetic resonance imaging and with estimates of the percentage of viable (metabolically active) tumor as depicted by fluorodeoxyglucose autoradiography. Methods. Implanted tumors in 10 mice were imaged with contrast-enhanced sonography, magnetic resonance imaging, and fluorodeoxyglucose autoradiography. Tumor vascularity was estimated with each modality and compared with the percentage of viable tumor. Results. Quantitated estimates of tumor vascularity with contrast-enhanced sonography closely correlated \( r = 0.95 \) with estimates made by magnetic resonance imaging and with the percentage of viable tumor \( r = 0.93 \) as depicted by fluorodeoxyglucose autoradiography. Conclusions. Contrast-enhanced sonography accurately depicts tumor vascularity in these implanted tumors. Tumor vascularity correlated with the amount of metabolically active tumor. Key words: contrast-enhanced sonography; fluorodeoxyglucose autoradiography; magnetic resonance imaging; tumor vascularity.

Angiogenesis is a central component of tumor growth and proliferation.1 Tumors lacking vascularity enter a dormant state with areas of apoptosis (programmed cell death).2 Microscopically, tumor cell proliferation has been shown to correlate with increased vascularity.3 Macroscopically, tumor growth is dependent on the development and maintenance of a vascular network. The correlation of tumor vascularity with its viability and function is complex. This study is based on the supposition that contrast-enhanced sonography and magnetic resonance imaging (MRI) can depict tumor vascularity, which, in turn, reflects the percentage of the tumor that is viable (metabolically active) as depicted by fluorodeoxyglucose (FDG) autoradiography.

Recently, novel therapeutic agents have been developed, which affect tumor growth and spread on the basis of their ability to target tumor angiogenesis.4 Tumor cells of dormant metastases exhibit increased apoptosis in response to antiantigenic therapy.5 To assess the tumor
response, the likelihood of malignancy, or both, a diagnostic modality must accurately depict changes in tumor vascularity and function. Accordingly, this study assessed the accuracy of contrast-enhanced sonography to depict tumor vascularity relative to standards of MRI and FDG autoradiography. This study also attempted to assess the correlation of tumor vascularity with its metabolic function and viability. We also investigated the use of fractal mathematics for the quantification of tumor vascularity depicted by contrast-enhanced sonography.

Materials and Methods

Ten mice with implanted Madison lung tumors, which ranged from 17 to 20 mm in average dimension, were studied. To have adequate vascular access for the infusion of a contrast agent, catheters were surgically placed within the jugular veins of the mice. Tiny amounts (0.01–0.03 mL) of a microbubble contrast agent (Definity [perflutren]; Bristol-Myers Squibb Company, North Billerica, MA) were infused into the catheter through a 27-gauge needle followed by a 0.02-mL saline flush. A CL15-7 linear array transducer connected to an HDI 5000 scanner (Philips Medical Systems, Bothell, WA) was held over the tumor after gel was applied to the skin overlying the tumor. Harmonic imaging with a low mechanical index (0.1) was used to obtain the greatest vascularity, followed by high mechanical index (1.0) settings to image the actual vessels. A static image depicting the greatest area of contrast enhancement was sent offline to a computer for quantification. Vascularity was quantitated by a specifically designed program for weighted pixel quantification (HDI-Lab).

With the box-counting technique, fractal mathematics was used to determine the fractal dimension of tumor vascularity. This unitless value reflected the repetition of the vessel branching patterns. Images obtained with sonography, MRI, and FDG autoradiography were stored digitally and exported for analysis on a workstation. This technique involved offline analysis of static contrast-enhanced sonograms by a specifically designed software program (Image J software; National Institutes of Health, Bethesda, MD). The box-counting technique involved analysis of the vessels within a specific area of interest as the area of interest was incrementally decreased and plotted as a log/log function. Fractal analysis was performed on the sonograms because the contrast is intravascular rather than both an intravascular and extravascular distribution on gadolinium-enhanced MRI.

Magnetic resonance imaging was performed using a small-bore 4.7-T scanner before and after intravenous injection of gadolinium. Longitudinal relaxation time–weighted gradient echo images (repetition time, 80 milliseconds; echo time, 6 milliseconds; matrix, 256 × 128 pixels) were obtained. The mice were restrained in a fixation device during the scan acquisition.

With sonography and MRI, contrast enhancement of the tumor vascularity was depicted by digitizing the precontrast and postcontrast images and subtracting the precontrast image from the postcontrast image. The subtracted image was then coded in red and quantified as the fractional blood volume. This parameter was quantitated as the percentage of the tumor that had vascularity, as described using power Doppler sonography by Rubin et al. Because vessels were interrogated at various angles, it was not possible to normalize the angle of interrogation. Power-weighted pixel density measurements were used, and because the tumor was superficial, these values did not take into account attenuation.

Fluorodeoxyglucose autoradiography was performed after a 300-µCi injection of 18-FDG. The mice were sacrificed 45 minutes after injection. The tumor was excised and sectioned into 30-µm slices. These were placed on Kodak Min-R mammography film (Eastman Kodak Co, Rochester, NY). The film was processed and then digitized. The percentage of the tumor that had function was quantitated, as well as the relative amount of function as shown by the intensity (darkness) of the signal. This differs from the power-weighted pixel quantification used for contrast-enhanced sonography and MRI because FDG is also metabolized by viable tumor cells.

The study protocol was approved by the Institutional Animal Care and Use Committee of the Institutional Review Board.

Results

Among the 10 subjects tested, interpretable images were obtained for 7. Reasons for failure included the inability to immobilize the mice for a sufficient time for MRI and failed injections due to obstructions of the catheters implanted in the
jugular veins of 2 subjects. On contrast-enhanced sonograms, the pixel-weighted enhancement of the tumors averaged 19% and ranged from 5% to 30%. On MRI, this value averaged 19% with a range of 10% to 28%. On FDG, the percentage of viable tumor averaged 66% and ranged from 50% to 78%. On contrast-enhanced sonography, the fractal dimensions ranged from 1.59 to 1.79 with an average of 1.68.

There was a high degree of correlation with contrast-enhanced sonography and MRI ($r = 0.95$) and FDG autoradiography ($r = 0.93$) in the different tumors. Figures 1 and 2 are representative images that show the correlation of contrast-enhanced sonography with MRI (Figure 1) and FDG autoradiography (Figure 2). Figures 3 and 4 show the correlation of contrast-enhanced sonography, MRI, and FDG autoradiography as performed on the same subject.

**Discussion**

The purpose of this study was to determine whether metabolic imaging by use of FDG autoradiography correlated with blood flow as depicted by contrast-enhanced sonography and MRI. We found a direct correlation between increased metabolic activity and areas of increased blood flow (Figures 3 and 4). These data are supported by prior studies that indicated areas of cell proliferation correlating with increased vascularity as depicted by color Doppler sonography. Moreover, studies of antiangiogenic drugs have shown that reduction in tumor vascularity is associated with dormancy in tumor cells and that this process could be detected on color Doppler sonography. Taken together, these studies indicate that studies of tumor blood flow and viability can be used to correlate tumor blood vessel response to therapy.

Our results further indicate that contrast-enhanced sonography is an accurate, noninvasive means for quantification of tumor vascularity. It is particularly accurate in distinguishing areas of viable tissue from necrotic tumors. The power-weighted pixel density depicted by contrast-enhanced sonography also seemed to correlate with the relative metabolic activity. Although the actual values obtained regarding tumor vascularity depicted by contrast-enhanced sonography and MRI differed, this was considered a reflection of the greater relative values observed before and after contrast.
enhancement with MRI compared with sonography. The higher values obtained with FDG autoradiography may reflect artifactual “blooming” of the 18-FDG on the Min-R film. It also probably reflects the fact that FDG imaged both the vessels and metabolically active tumors. When we compared our most recent result with previous studies, contrast-enhanced sonography also seemed to be more accurate and reproducible than color Doppler sonography. By varying the mechanical index, contrast-enhanced sonography has the advantage of showing reperfusion after intentional disruption of the microbubbles with a high mechanical index.

The use of fractal mathematics in quantification of differences in benign versus malignant microvascularity was suggested by Schoenfeld et al. in an editorial describing differences in vascular networks in normal and neoplastic ovaries. As opposed to the orderly branching pattern seen in normal ovaries, tumors had numerous arteriovenous shunts and showed nonhierarchical branching.

Kurjak et al. also mentioned the potential of fractal mathematics to distinguish branching patterns in benign versus malignant ovarian lesions. Because we did not have a benign tumor to study, we could only attempt to quantify the fractal dimensions of the implanted tumors. Our results indicated that there was a fairly narrow range for the fractal dimensions (1.68 ± 0.9), indicating that the branching patterns in the implanted tumors were relatively consistent between specimens. Admittedly, further comparison between benign and malignant microvascularity needs to be performed before a definite conclusion about the predictability and use of the fractal dimension is determined.

Several studies have reported excellent correlation of sonographic quantification of tumor vascularity on color Doppler sonography and contrast-enhanced sonography with the microvascularity seen on vessel staining. However, this study correlated the tumor microvascularity depicted by contrast-enhanced sonography with that shown on other imaging modalities, including MRI and FDG autoradiography. It should be noted that FDG values were greater than those obtained with sonography or MRI because FDG is also a function of metabolically active tumors. Fluorodeoxyglucose autoradiography could be performed only after excision of the tumor and subsequent microsectioning and, therefore, cannot be used serially as a means to determine tumor response. Magnetic resonance imaging was found to be accurate only when the subject was immobilized for the relatively long periods required for the various pulse sequences (=15 minutes). Similarly, unrestricted movements of the subject may hamper the use of FDG positron emission tomography.

The correlation of tumor vascularity with tumor viability and function warrants further investigation. It is clear that contrast-enhanced sonography shows areas of tumor necrosis. Whether it can show subtle changes in tumor metabolism related to response remains to be determined. Another area that deserves further investigation is the correlation of contrast kinetics with tumor function and response. Is there a correlation between changes in uptake with vessel permeability, and is this affected with treatment?

It is clear from a practical sense that contrast-enhanced sonography provides an excellent means for assessing tumor vascularity, at least in these superficial tumors. Admittedly, deeper tumors may be more difficult to image. However, even with these limitations, contrast-enhanced sonography seems to be an accurate modality for serial assessment of tumor vascularity as an indication of tumor response. Additional studies, such as one reported before and after angiogenesis inhibitors were administered, need to be performed to determine the most accurate parameter for assessing tumor response. These studies should provide a basis for the use of contrast-enhanced sonography for determining tumor response in humans who are treated with various antiangiogenic therapies.

References


