Tumor Angiogenesis and Interstitial Hypertension

Yves Boucher, Michael Leunig, and Rakesh K. Jain

ABSTRACT

Due to the high permeability of tumor vessels to fluids and plasma proteins, the microvascular pressure (MVP) is the principal driving force for interstitial hypertension in solid tumors; as a result, hydrostatic pressures between the microvascular and interstitial space are close to equilibrium. Based on these observations, we hypothesized that the tumor interstitial fluid pressure (IFP) should increase following the onset of angiogenesis. To this end, the relationship between IFP and tumor neovascularization was determined in the human colon adenocarcinoma (LS174T) and the murine carcinoma (MCaIV) implanted in a transparent dorsal skin fold chamber in severe combined immunodeficient mice. Three stages in the development of the tumor neovasculature were characterized by intravital microscopy. Stage I tumors were avascular, stage II was characterized by vascular sprouts and loops, and in stage III, the tumor vasculature was completely developed and blood flow was obvious. The IFP was measured with micropipettes and a servo-null system. For both tumor types, the IFP in stage I tumors was close to 0 mm Hg, and IFP increased significantly from one stage to the next. To further confirm that interstitial hypertension was associated with the development of the tumor vasculature, IFP was measured in LS174T spheroids. The mean pressure in spheroids was 0.2 ± 0.3 mm Hg. In stage III tumors, the IFP was compared to the MVP. In MCaIV, the MVP was comparable to the IFP; however, in LS174T the MVP was significantly higher than the IFP. In conclusion, the results demonstrate that avascular tumors have atmospheric pressures and that tumor interstitial hypertension is associated with the development of the neovasculature.

INTRODUCTION

The elevated IFP is a general characteristic of experimental and human solid tumors (1–3). Theoretical studies have identified elevated IFP as a significant cause in the poor delivery of large therapeutic agents (e.g., monoclonal antibodies and genes) to solid tumors (4, 5). The potential of using tumor interstitial hypertension as a prognostic indicator during cytotoxic therapy or to distinguish between malignant and benign lesions is being evaluated (1, 6, 7). We have shown theoretically and experimentally that the MVP is nearly equal to the IFP; thus, the MVP represents the principal driving force for interstitial hypertension in solid tumors (4, 8–10). A significant implication of the transmission of the MVP to the interstitial space of tumors is that the IFP should begin to rise following the onset of angiogenesis. During the prevascular stage of primary tumor and micrometastasis development, nutrients are provided to neoplastic cells by pre-existing vessels. Further tumor growth is dependent on the formation of new blood vessels from the surrounding vasculature by angiogenesis (11, 12). To determine the relationship between the IFP and tumor neovascularization, tumors were implanted in a transparent dorsal skin fold chamber in SCID mice.

MATERIALS AND METHODS

Transparent Tumor Model. The human colon adenocarcinoma (LS174T) and murine mammary carcinoma (MCaIV) were implanted on the striated skin muscle in the dorsal skin fold chamber of SCID mice. The tumor chambers were prepared as described previously (13). In brief, a double layer of skin was sandwiched between two titanium frames. A layer of skin with a diameter of 15 mm was removed, and a coverslip attached to the titanium frame was placed in contact with the remaining layer, composed of skeletal muscle, s.c. tissue, and the epidermis. Two to 3 days after the chamber preparation, 200,000 LS174T cells or MCaIV chunks (~1 × 1 × 0.5 mm) were deposited on the skeletal muscle, and the preparation was sealed with a coverslip covered with a thin transparent plastic wrap, thus minimizing the adherence of the tumor to the coverslip, which was removed for measuring IFP. In a previous study, we have shown that in tumors with a completely developed vasculature (corresponding to stage III in the present study), the IFP was not modified by the removal of the coverslip (13). The tumor IFP measured from the skin side before removing the coverslip was similar to measurements made after removing the coverslip.

Tumor Spheroids. LS174T spheroids were grown in a gel of collagen type I (Vitrogen; Collagen Corp., Palo Alto, CA). Type I collagen gel was prepared at 4°C by mixing eight parts of Vitrogen, one part of 10× minimal essential medium, and 1 part 10× sodium bicarbonate buffer. A cell inoculum (250 cells/ml) was added to the collagen and poured into a Petri dish; the preparation formed a gel within 30 min at 25°C. Culture medium was added and changed every 2 days.

Pressure Measurements. The development of the tumor neovascularization was characterized from day 2 to 18 after tumor implantation with an intravital microscope (Axioplan; Zeiss, Oberkochen, Germany). For the measurement of IFP and MVP, the animals were anesthetized with Ketamine/Xylazine (90/10 mg/kg body weight, s.c.) and placed on a temperature-regulated heating pad, and body temperature was maintained between 36°C and 37°C. The left carotid artery was cannulated with PE 20 tubing to monitor the mean arterial blood pressure. After the removal of the coverslip, a lactated Ringers solution (35°C–37°C) was superfused on the tumor, and the IFP was measured with micropipettes and a servo-null system (IPM, San Diego, CA), as described previously (8, 14). Micropipettes with tip diameters varying between 2 and 4 μm were introduced in the central regions of tumors or in microvessels with a graded micromanipulator (MP4; IPM) under stereomicroscopic guidance (SMZ-8; Nikon, Tokyo, Japan). Each IFP measurement was monitored for at least 10 s. The average value for one tumor was obtained from 4 to 10 measurements. The MVP was measured in vessels with diameters between 30 and 100 μm. To verify that the tip of the micropipette was in the lumen of the tumor vessels, fast green (0.5%) was infused via the micropipette. MVP measurements were considered valid when the fast green disappeared rapidly with blood flow following the infusion. MVP was compared to the IFP measured at a distance ≥0.3 mm from the tumor surface. Following the completion of the measurements, the dorsal skin fold chamber was removed and placed in a fixative solution. After 2 to 3 days, the tumor was cut in three parts, and the tumor thickness was measured with a graded graticule placed in the objective of a stereomicroscope.

The IFP was measured in spheroids of 0.6–0.8 mm in diameter. The tip of the micropipettes was introduced in the center of the spheroids, and two to three measurements per spheroid were obtained.

Data Analysis. The data is presented as the mean ± SD. The data were analyzed with the Student’s t test or by ANOVA. For ANOVA with significant
RESULTS AND DISCUSSION

Three stages of tumor neovascularization were identified by intravital microscopy. In stage I, on day 3 and 4 after tumor implantation the tumor was avascular, and the underlying and peripheral normal vessels were dilated (Fig. 1a). Stage II (days 6 and 7) was characterized by vascular sprouts and loops that were evident in the tumor periphery and appeared as dark spots in the center of the tumor (Fig. 1b). In stage III (days 10—17), the tumors were fully vascularized, and blood flow was obvious (Fig. 1c). The distinction between the three stages was based on the morphological characteristics given above. For both tumor types, no significant difference in mean tumor thickness was found between stages I and II; the tumor thickness varied between 0.16 and 0.45 mm. In stage III tumors, the tumor thickness varied between 0.6 and 2.0 mm, and the mean thickness was 1.1 ± 0.3 mm in MCaIV tumors and 1.2 ± 0.5 mm in LS174T tumors.

The mean arterial blood pressure was 83.0 ±13.0 mm Hg. Similar to previous studies (15), the mean IFP in the skin was −1.0 mm Hg (n = 4). For both tumors, the IFP data are shown in Fig. 2. In stage I tumors, the mean IFP was close to atmospheric pressure, and IFP increased significantly from one stage to another. The data suggest that the IFP increase is associated with the formation of new blood vessels. The increase in IFP between stage I and II cannot be explained by a change in tumor size since the thickness of the tumor in both stages was comparable.

To further demonstrate that neovascularization is essential for the increase in tumor IFP, IFP was measured in LS174T spheroids with diameters between 0.6 and 0.8 mm (n = 5). The IFP in four spheroids varied between −0.1 to 0.1 mm Hg, and in another spheroid, the IFP was 0.7 mm Hg. The mean IFP in LS174T spheroids was 0.2 ± 0.3 mm Hg. For comparison, in LS174T tumors with a thickness of 0.6 to 0.8 mm, the IFP varied between 2.0 and 3.0 mm Hg.

In stage III tumors, the MVP was measured in vessels with diameters between 30 and 100 μm and compared to the IFP in the tumor center. The comparison between the MVP in superficial vessels and the IFP in the center of the tumor is based on the assumption that the MVP of superficial and central vessels is similar (8). The measurement of IFP as a function of radial position demonstrated that the IFP was high in the center and generally decreased at a distance of 0.3 mm or less from the tumor surface. For MCaIV tumors, the mean MVP and IFP were similar, whereas for the LS174T tumors, the MVP was significantly higher than the IFP (P < 0.02; Fig. 3). In all of the LS174T tumors (n = 6), the MVP was higher than the IFP by at least 1.0 to 4.0 mm Hg. Furthermore, all of the single values of MVP (n = 14) varied between 5.0 and 7.0 mm Hg, whereas except for one value of 6.0 mm Hg, all of the values of IFP (n = 32) varied between 2.0 and 5.0 mm Hg. Similar to the results with MCaIV tumors, we have shown in two other studies that the MVP and the IFP were similar (8). In tumors, the oncotic pressure of plasma and interstitial fluid are at or close to equilibrium. The equilibration of hydrostatic and oncotic pressures between the microvascular and interstitial space has been associated with the high vascular permeability and hydraulic conductivity of the wall of tumor vessels as well as the absence of functional lymphatics in tumors (4, 5, 8). In LS174T tumors, the hydrostatic pressure gradient between tumor vessels and the interstitial space could be due to a relatively lower permeability to plasma proteins and fluids, or an increased fluid flow through the interstitial space.

The highest IFP measured in this study was 9.0 mm Hg in a MCaIV tumor, which is significantly lower than the IFPs of 30 to 100 mm Hg
measured in some human tumors in situ (1, 16–18). Since the IFP is determined by the MVP, significant differences in the organization of the vascular network or vascular resistance could explain these differences in IFP. The relatively low MVPs and IFPs of the newly formed tumor vessels depend most likely on their origin and connection with post-capillary venules and the small veins of the host circulation (19, 20). The pressure in post-capillary venules and small veins of the skeletal muscle and s.c. tissue can be as low as 8 to 10 mm Hg (21). If newly formed vessels originated from terminal arterioles (22) or if the tumor vessels can connect with the arterial supply of the host (23, 24), one would expect the MVPs and IFPs to be significantly higher (14, 18). The higher IFPs could also be due to the increase in the vascular resistance of the venous vessels draining the tumor (8).

In conclusion, the present data strongly suggest that the development of the tumor neovascularature is a prerequisite for the elevated IFP. The finding that IFP is also elevated in very small tumors suggests that the delivery of large anticancer agents to micrometastasis could be impeded by interstitial hypertension.

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