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BIOLOGY CONTRIBUTION

INTERSTITIAL FLUID PRESSURE AND VASCULARITY OF INTRADERMAL AND INTRAMUSCULAR HUMAN TUMOR XENOGRAFTS

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Purpose: **High interstitial fluid pressure (IFP) in tumors has been shown to be associated with poor prognosis. Mechanisms underlying the intertumor heterogeneity in IFP were investigated in this study.**

Methods and Materials: **A-07 melanoma xenografts were transplanted intradermally or intramuscularly in BALB/c *nu/nu* mice. IFP was measured in the center of the tumors with a Millar catheter. Tumor blood perfusion and extracellular volume fraction were assessed by dynamic contrast–enhanced magnetic resonance imaging (DCE-MRI). The necrotic fraction, vascular density, and vessel diameters of the tumors were determined by image analysis of histological preparations.**

Results: **Significant intertumor heterogeneity in IFP, blood perfusion, and microvascular morphology was observed whether the tumors were transplanted intradermally or intramuscularly. High IFP was mainly a consequence of high resistance to blood flow caused by low vessel diameters in either transplantation site. IFP decreased with increasing blood perfusion in intradermal tumors and increased with increasing blood perfusion in intramuscular tumors, mainly because the morphology of the tumor microvasculature differed systematically between the two tumor models.**

Conclusion: **The potential of DCE-MRI as a noninvasive method for assessing the IFP of tumors may be limited because any relationship between IFP and blood perfusion may differ with the tumor growth site.** **© 2011 Elsevier Inc.**

Interstitial fluid pressure, Blood perfusion, Vascular morphology, Transplantation site, Dynamic contrast-enhanced magnetic resonance imaging.

INTRODUCTION

Malignant solid tumors generally develop a higher interstitial fluid pressure (IFP) than the surrounding normal tissue (1–3). Clinical and experimental studies have provided significant evidence that high IFP in tumors is an important therapeutic problem (3–9). First, high tumor IFP has been shown to cause heterogeneous uptake of chemical therapeutic agents, leading to resistance to chemotherapy, immunotherapy, and some forms of gene therapy (3, 4). Second, the IFP of the primary tumor has been shown to be an independent prognostic parameter for patients with locally advanced cervical carcinoma treated with radiation therapy alone (5, 6). In these studies, the patients with high tumor IFP showed increased probability of developing both local and distant recurrences, and high tumor IFP was associated with poor disease-free survival independent of conventional prognostic factors such as tumor size, stage, and lymph node status. Third, high tumor IFP has been shown to be associated with increased incidence of pulmonary and lymph node metastases as well as poor radiocurability in human melanoma xenografts (7–9). These studies showed that high IFP may be linked to poor radiocurability through hypoxia-dependent (8) as well as hypoxia-independent (9) mechanisms.

Elevated IFP in malignant tumors is a consequence of severe microvascular, lymphatic, and interstitial abnormalities (1, 2). Briefly, tumors develop elevated IFP because they show high resistance to blood flow, low resistance to transcapillary fluid flow, and impaired lymphatic drainage. The microvascular hydrostatic pressure is the principal driving force for the elevated IFP in tumors (10). Fluid is forced from the microvasculature into the interstitium where it accumulates, distends the extracellular matrix, and causes interstitial hypertension. In experimental tumors, the IFP is relatively uniform throughout the tissue except close to the surface, where it drops precipitously to normal tissue values (7, 11). The central IFP is nearly equal to the microvascular hydrostatic pressure in most experimental tumors because the ratio of transmural hydraulic conductivity to interstitial hydraulic conductivity is high (10).

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The numeric value of the central IFP differs substantially among individual experimental and human tumors, even among equal-sized tumors of the same experimental line transplanted to the same site (2, 12). Although the mechnisms leading to interstitial hypertension in tumors have been identified, the mechanisms underlying the intertumor heterogeneity in IFP are not well understood. Theoretical studies have suggested that differences in IFP among tumors can be attributed mainly to differences in microvascular hydrostatic pressure, caused by differences in microvascular architecture and resistance to blood flow, rather than differences in transvascular and interstitial permeability, particularly in transplantable tumors with IFP values in the upper range (13, 14). Experimental studies investigating the validity of this suggestion are sparse. In fact, a few attempts to establish correlations between IFP and tumor vascularity have provided negative results. Thus, no correlation was found between IFP and blood vessel density or vascular perfusion in ME-180 cervical carcinoma xenografts (12) and between IFP and blood perfusion in R-18 melanoma xenografts (15).

Noninvasive diagnostic strategies for assessing the IFP and therapeutic strategies for reducing the IFP of tumors are highly needed (2). Identification of the principal cause of the intertumor variability in IFP may benefit the development of such strategies. The primary aim of the study reported here was to identify the mechanisms underlying the intertumor heterogeneity in IFP in A-07 human melanoma xenografts. A-07 tumors show high permeability to transvascular fluid flow, suggesting that the IFP is nearly equal to the microvascular hydrostatic pressure (16). Therefore, we hypothesized that the intertumor variability in IFP was mainly a consequence of differences in tumor vascularity and to a lesser degree a consequence of interstitial differences. To test this hypothesis, we measured tumor blood perfusion and extracellular volume fraction by dynamic contrast–enhanced magnetic resonance imaging (DCE-MRI) and tumor necrotic fraction, vascular density, and vessel diameters by image analysis of histological preparations. Because it has been reported that intramuscular tumors may show particularly high IFP values (12), A-07 tumors transplanted intramuscularly as well as intradermally were included in the study.

METHODS AND MATERIALS

Tumors

A-07 human melanoma xenografts growing in adult female BALB/c *nu/nu* mice were used as tumor models. Tumors were initiated from cells cultured in RPMI-1640 medium supplemented with 13% bovine calf serum, 250 mg/L penicillin, and 50 mg/L streptomycin. Approximately 3.5×10^5 cells in 10 μl of Hanks' balanced salt solution were inoculated intradermally or intramuscularly in the leg. Tumors with volumes ranging from 160 to 610 mm^3 were included in experiments. IFP measurements and DCE-MRI were performed with mice anesthetized with fentanyl citrate (Janssen Pharmaceutica, Beerse, Belgium), fluanisone (Janssen Pharmaceutica), and midazolam (Hoffmann-La Roche, Basel, Switzerland) in doses of 0.63 mg/kg, 20 mg/kg, and 10 mg/kg, respectively. Animal care and experimental procedures were carried out in accordance with the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Marketing, and Education (New York Academy of Sciences, New York, NY).

IFP measurements

IFP was measured in the center of the tumors by using a Millar SPC 320 catheter equipped with a 2F Mikro-Tip tranceducer with diameter 0.66 mm (Millar Instruments, Houston, TX) (17). The catheter was connected to a computer via a Millar TC-510 control unit and a model 13-66150-50 preamplifier (Gould Instruments, Cleveland, OH). Data acquisition was carried out by using LabVIEW software (National Instruments, Austin, TX).

DCE-MRI

DCE-MRI was carried out as described earlier (18). Briefly, Gd-DTPA (Schering, Berlin, Germany) was administered in a bolus dose of 0.3 mmol/kg. T_1-weighted images (TR = 200 ms, TE = 3.2 ms, and α_{T1} = 80°) were recorded at a spatial resolution of $0.31 \times 0.31 \times 2.0$ mm^3 and a time resolution of 14 s by using a 1.5-T whole-body scanner (Signa; General Electric, Milwaukee, WI) and a slotted tube resonator transceiver coil constructed for mice. Two calibration tubes, one with 0.5 mmol/L Gd-DTPA in 0.9% saline and the other with 0.9% saline only, were placed adjacent to the mice in the coil. The tumors were imaged axially in a single section through the tumor center by using an image matrix of 256×128, a field of view of 8×4 cm^2, and one excitation. Two proton density images (TR = 900 ms, TE = 3.2 ms, and α_{PD} = 20°) and three T_1-weighted images were acquired before Gd-DTPA was administered, and T_1-weighted images were recorded for 15 min after the administration of Gd-DTPA. Gd-DTPA concentrations were calculated from signal intensities by using the method of Hittmair *et al.* (19). The DCE-MRI series were analyzed on a voxel-by-voxel basis by using the arterial input function of Benjaminsen et al. (20) and the modified Kety pharmacokinetic model described by Tofts *et al.* (21):

$$C_t(T) = E \cdot F \cdot \rho \cdot \int_0^T C_a(t) \cdot e^{-E \cdot F \cdot \rho \cdot (1-Hct) \cdot (T-t)/ve} dt,$$

where $C_t(T)$ is the Gd-DTPA concentration in the tumor tissue at time T, E is the initial extraction fraction of Gd-DTPA, F is the blood flow per unit tumor tissue weight, ρ is the density of the tumor tissue (1.0 g/mL), $C_a(t)$ is the arterial input function, Hct is the hematocrit (0.4), and v_e is the fractional distribution volume of Gd-DTPA. Images of $E \cdot F$ and v_e were generated by using the SigmaPlot software (SPSS Science, Chicago, IL). This experimental procedure provides numeric values of $E \cdot F$ [in units of mL/(g · min)] and v_e of melanoma xenografts that are closely related to the absolute values of blood perfusion and extracellular volume fraction (18). The v_e values of voxels in the viable tissue of A-07 tumors have been shown to vary from 0.1 to 0.6 (22). The pharmacokinetic model used here is not valid for necrotic tissue. Our algorithms produce either unphysiologically high v_e values, usually $v_e > 1{,}000$, or unphysiologically low v_e values, usually $v_e < 0.001$, for voxels in necrotic tumor regions, depending on whether the voxels are located in the periphery or in the center of the necroses (18, 22). Median values of $E \cdot F$ and v_e were calculated for the viable tissue of each tumor by excluding all voxels with $v_e > 0.6$ or $v_e < 0.1$.