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Noninvasive Multimodality Imaging of the Tumor Microenvironment: Registered Dynamic Magnetic Resonance Imaging and Positron Emission Tomography Studies of a Preclinical Tumor Model of Tumor Hypoxia[1,2]

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Abstract

In vivo knowledge of the spatial distribution of viable, necrotic, and hypoxic areas can provide prognostic information about the risk of developing metastases and regional radiation sensitivity and may be used potentially for localized dose escalation in radiation treatment. In this study, multimodality *in vivo* magnetic resonance imaging (MRI) and positron emission tomography (PET) imaging using stereotactic fiduciary markers in the Dunning R3327-AT prostate tumor were performed, focusing on the relationship between dynamic contrast-enhanced (DCE) MRI using Magnevist (Gd-DTPA) and dynamic ^{18}F-fluoromisonidazole (^{18}F-Fmiso) PET. The noninvasive measurements were verified using tumor tissue sections stained for hematoxylin/eosin and pimonidazole. To further validate the relationship between ^{18}F-Fmiso and pimonidazole uptake, ^{18}F digital autoradiography was performed on a selected tumor and compared with the corresponding pimonidazole-stained slices. The comparison of Akep values (kep = rate constant of movement of Gd-DTPA between the interstitial space and plasma and A = amplitude in the two-compartment model (Hoffmann U, Brix G, Knopp MV, Hess T and Lorenz WJ (1995). *Magn Reson Med* **33**, 506–514) derived from DCE-MRI studies and from early ^{18}F-Fmiso uptake PET studies showed that tumor vasculature is a major determinant of early ^{18}F-Fmiso uptake. A negative correlation between the spatial map of Akep and the slope map of late (last 1 hour of the dynamic PET scan) ^{18}F-Fmiso uptake was observed. The relationships between DCE-MRI and hematoxylin/eosin slices and between ^{18}F-Fmiso PET and pimonidazole slices confirm the validity of MRI/PET measurements to image the tumor microenvironment and to identify regions of tumor necrosis, hypoxia, and well-perfused tissue.

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Abbreviations: DAR, digital autoradiography; DCE-MRI, dynamic contrast-enhanced magnetic resonance imaging; Fmiso, fluoromisonidazole; FOV, field of view; FWHM, full-width half-maximum; H&E, hematoxylin/eosin; MRI, magnetic resonance imaging; NA, number of averages; NR, number of repetitions; PET, positron emission tomography; ST, slice thickness; TE, echo time; TR, repetition time

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[2]This article refers to supplementary materials, which are designated by Figures W1 and W2 and are available online at www.neoplasia.com.

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Introduction

It is anticipated that the ability to image the tumor microenvironment *in vivo* will provide useful prognostic information including an assessment of factors that influence response to treatment. For example, hypoxia, typically distributed heterogeneously in locally advanced tumors, is known to affect both radiation sensitivity and the development of metastases [1–4]. Tumor hypoxia results from an imbalance between the supply and use of oxygen in tumor tissues. Thus, major determinants of tumor hypoxia include the structure and functionality of tumor vasculature and the degree of angiogenesis [5–8]. The direct measurement of tumor hypoxia usually requires invasive procedures such as the insertion of polarographic electrodes. However, such invasive methods are restricted both spatially and temporally, being limited to a relatively small number of measurements on easily accessible tumors at, typically, a single point in time. Noninvasive imaging offers several advantages, including the feasibility of longitudinal measurements on the same subject, the generation of complete three-dimensional maps of tumor hypoxia, and the potential application to image-guided therapy. Imaging modalities, such as magnetic resonance imaging (MRI), positron emission tomography (PET), electron paramagnetic resonance, and optical imaging, have their own unique advantages. Magnetic resonance imaging provides unique functional and structural information on tumor vasculature and physiology at high spatial resolution. Positron emission tomography can measure sensitively and quantitatively local concentrations of radioactive molecular targets of interest, such as labeled fluoromisonidazole (^{18}F-Fmiso).

^{18}F-Fmiso PET is currently under intense investigation as a method of imaging tumor hypoxia. This is based on the selective bioreduction of ^{18}F-Fmiso in hypoxic tumor regions followed by the binding of its metabolites to macromolecules [9–12]. However, the spatial resolution, based on the full-width half-maximum (FWHM) of the point spread function of the PET activity signal, is relatively coarse, ranging from 1 to 2 mm for dedicated small-animal scanners to 5 to 6 mm for clinical PET scanners. Magnetic resonance techniques provide unique opportunities to obtain noninvasive structural and functional information on tumor vasculature and physiology with anatomical details at finer spatial resolution. Magnetic resonance methods, which include dynamic contrast-enhanced MRI (DCE-MRI) [13,14], blood oxygen level–dependent imaging [15], fluorine-19 MR measurements of oxygen-sensitive compounds [16–18], and the measurement of lactate [19,20], may provide high-spatial resolution functional information to complement other imaging modalities.

In particular, DCE-MRI provides vasculature/perfusion information of the tumor microenvironment [21,22] and, thus, not only offers complementary information to PET hypoxia imaging but also may address the relationship between tumor hypoxia and vasculature. In this article, we focus on the relationship between DCE-MRI studies using Gd-DTPA as the contrast agent, and dynamic ^{18}F-Fmiso PET, in the syngeneic Dunning R3327-AT prostate tumor in rats. The *in vivo* imaging results were validated by *ex vivo* studies featuring staining with hematoxylin/eosin (H&E) (tumor necrosis) and pimonidazole (tumor hypoxia) together with ^{18}F digital autoradiography (DAR; ^{18}F-Fmiso distribution) on tumor tissue sections accurately registered to the corresponding *in vivo* slices. Accurate registration of macroscopic (MRI and PET) and microscopic images (H&E, pimonidazole and ^{18}F DAR) is a unique and important feature of the current study.

Experimental Methods

Animal Preparation

Animal studies were conducted in compliance with protocols approved by the Institutional Animal Care and Use Committee of Memorial Sloan-Kettering Cancer Center (MSKCC). The rat prostate cancer cell line R3327-AT [23] was cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified CO_2 incubator. Cells were harvested on reaching 75% to 80% confluence and suspended in phosphate-buffered saline (PBS) at a final concentration of 2×10^6 cells/0.1 ml. Two to four million R3327-AT cells were injected in the right hind leg of 6- to 8-week-old Copenhagen rats. Tumor volume (V) was calculated as $V = (\pi/6) \times x \times y \times z$, where x, y, and z were the three orthogonal dimensions of the tumor [24]. The experiments were performed over a tumor size range of 500–2500 mm^3. A total of six rats were used. At the start of each experiment, the tail vein was catheterized with a 24G catheter and connected to a three-way stopcock (Stopcock Nylon 3-Way, 420163-4503; Kimble Kontes LLC, NJ), facilitating intravenous (IV) injection of the different agents at various stages of the multimodality imaging experiment. The catheter was kept patent by injecting heparinized saline.

Magnetic Resonance Coil and Fiduciary Marker Assembly

The MRI coil [diameter (D) = 4 cm, Helmholtz configuration] was constructed in two parts (Figure 1*A*) with the upper part of the MRI coil and marker assembly initially detached to facilitate subsequent positioning of the animal. The spatial marker assembly is shown in Figure 1*A* attached to the rest of coil-marker system, and Figure 1*B* shows an enlarged side view of the marker assembly alone (adapted from [25,26]). The marker assembly was composed of two cylindrical disks and one flat plate. The top disk, referred to as the marker holder disk [D = 1.5 cm, thickness (t) = 0.9 cm] had three holes (D = 0.9 mm) for the vertical markers. A bottom histology marker disk (D = 1.5 cm, t = 0.5 cm) with identically aligned holes was separated from the marker holder disk by a flat plate. Separate release screws fastened each disk to the flat plate, which was fixed to the top of the radiofrequency coil. At the end of the imaging experiments, the histology marker disk was detached from the rest of the marker assembly and served as a reference to align the tumor sections with the *in vivo* image slices as described below (procedure detailed in the Preparation of Tumor Cryosections section). The flat plate had one side hole to contain a horizontal marker. An additional holder was placed at the center of the bottom coil with a grooved disk (D = 1 cm, t = 0.3 cm) containing another horizontal marker as shown in Figure 1*A*. The three vertical (forming an oblique triangle with lengths of 0.9, 0.7, and 0.5 cm, respectively) and two leveled horizontal markers (22G catheter; Terumo Surflo I.V. Catheter, Somerset, NJ) were filled with Gd-DTPA–doped and red-colored water, sealed at the ends with Critoseal (Cascade Healthcare, Portland, OR), and placed at each of the designated positions in the marker holders. The three vertical markers were pushed into the 0.9-mm-sized holes through the two separate cylindrical disks (the marker holder and histology marker disks). The three vertical and two horizontal markers appear as the red tubes in Figure 1*A*. The distance between the top and bottom coil was adjustable over a range of 2 to 3 cm to accommodate tumors of various sizes.