Characterization of a Prostate Cancer Xenograft in Orthotopic and Subcutaneous Sites

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Introduction: Prostate cancer is the second most common malignancy in the Western male population. Metastastic prostate cancer is frequently refractory to treatment and over the past decade we have focused on understanding the role of the hypoxia, vascularization and metabolism in invasion and metastasis to identify permissive or preventive microenvironments for invasion and metastasis to occur. Since human malignant cell lines metastasize more readily from relevant orthotopic sites than from anatomically irrelevant heterotopic sites (1), here we have compared hypoxia, vascularization and extracellular pH (pHe) for a human prostate cancer xenograft implanted in these two different sites to obtain further insight into the role of the physiological environment in invasion and metastasis. A stably transfected human prostate cancer cell line expressing green fluoresent protein (GFP) under the control of a hypoxia response element (HRE) was used for these studies. Thus, hypoxia can be determined from the GFP expression in the tumors. MRSI of the extracellular pH (pHe) marker 2-imidazole-1-yl-3-ethoxy-carbonyl propionic acid (IEPA) was used to obtain pHe maps, and MRI with the intravascular contrast agent albumin-GdDTPA provided maps of vascular volume and permeability.

Methods: PC-3 tumors were derived from cells stably transfected with the hypoxia response element of human VEGF-A ligated to the enhanced green fluorescent protein gene (2). The spatial distribution of GFP expression was used as an index of hypoxia. Intact tumor tissue (<1mm^3) was implanted in the prostate of anesthetized male SCID mice. Identically sized pieces were implanted subcutaneously in a separate group of mice for comparison. All the MR acquisitions were performed on a Bruker Biospec 4.7T spectrometer. The mice were anesthetized with a mixture of ketamine and acepromazine. The tail vein was catheterized before placing the animal in the magnet. Diffusion-weighted images (100 mT/m) were acquired to identify the location of the orthotopic tumors. Vascular imaging was performed as previously described (3). pHe maps were obtained from a 4 mm thick slice using a 2D CSI sequence with VAPOR water suppression (TE = 23 ms). Optical images of GFP expression in freshly excised tumor sections, as well as in lymph nodes and in ascites fluid were obtained on a fluorescent microscope.

Results: Orthotopic tumors exhibited higher vascular volume and higher vascular permeability than heterotopic tumors. Figure 1 shows maps of vascular volume and vascular permeability in heterotopic and orthotopic tumors. pHe maps (Figure 3) acquired with the pH marker IEPA revealed no difference between orthotopic and subcutaneous tumors, with pHe values of 6.6 ± 0.4 (n=3) and 6.7 ± 0.3 (n=5) respectively. Hypoxic regions were visualized by GFP distribution within the tumor as shown in Figure 4. Hypoxia was observed in the orthotopic and heterotopic tumors. We also found numerous hypoxic cancer cells in the axillary lymph nodes (Figure 4c) and ascites fluid (Figure 4d) of orthotopically implanted mice, but not in the mice with heterotopic tumors.

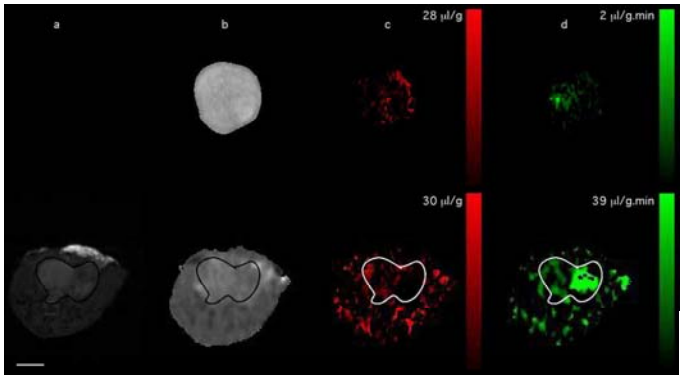

Figure 1 Vascular analysis: top row: subcutaneous tumor; bottom row: orthotopic tumor. (a) Diffusion-weighted image, (b) M_0 map, (c) Vascular volume map, (d) Permeability map (scale bar = 5mm).

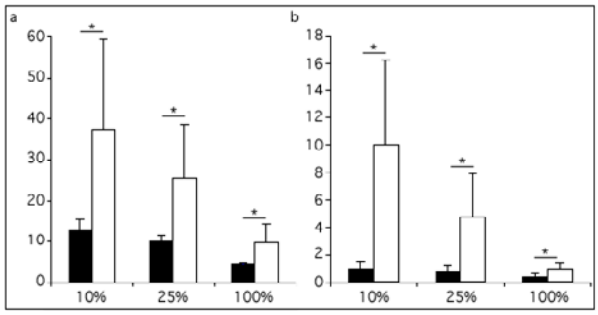

Figure 2 (a) Vascular volume quantification in subcutaneous tumor (black box) and orthotopic tumor (white box), (b) Permeability quantification in subcutaneous tumor (black box) and orthotopic tumor (white box) (*p<0.05) (highest 10%, 25% and all nonzero values of histogram are represented). 3 subcutaneous and 5 orthotopic tumors were studied.

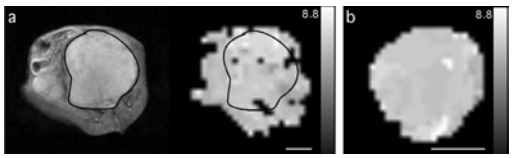

Figure 3 Diffusion weighted image and pHe map of an orthotopic tumor (a) and pHe map of a subcutaneous tumor (b) (scale bars=5 mm).

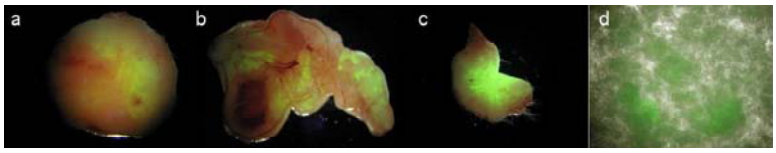

Figure 4 GFP maps overlaid with white light images. (a) GFP map from a subcutaneous excised tumor, (b) GFP map from an orthotopic excised tumor, (c) GFP expression in axillary lymph node from an orthotopically implanted mouse, (d) GFP expression in cancer cells in ascites fluid from an orthotopically implanted mouse.

Conclusion: These data demonstrate the feasibility of multi-modality imaging for understanding the role of the physiological tumor environment in cancer progression, invasion and metastasis. Although the vascular characteristics of the tumor were significantly different depending on the implantation site, there was no significant difference in pHe. Metastasis was frequently observed from orthotopic tumors, but rarely in subcutaneous tumors. These metastatic cells found in ascites fluid and axillary lymph nodes were frequently hypoxic, which may contribute to poor treatment outcome in metastatic disease. These results are consistent with the more aggressive growth and spread of orthotopic tumors. In 1889 Stephen Paget observed that the pattern of metastatic dissemination was not random but that the cancer cell or 'seed' had an affinity for certain sites or 'soil' a phenomenon he termed 'the seed and soil effect'. These results show that the 'soil' of the primary tumor also plays a critical role in the release of the 'seed'. Immunohistochemical analyses of tumor sections are currently under way to quantify GFP expression and vessel density in orthotopic and heterotopic tumors. The analysis of the correlation between hypoxia, pHe, vascularization and metabolism will provide a more detailed characterization of environments that are permissive or preventive for invasion and metastasis.

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References: (1) Waters et al, Prostate, 26:227-234,1995. (2) Raman et al, Cancer Res., 66:9929-9936,2006. (3) Bhujwalla et al., Clin. Cancer Res., 9:355-362,2003.