Characterizing the physiological environments of a prostate cancer xenograft in orthotopic and subcutaneous sites

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Introduction: The 'seed and soil' effect that the pattern of metastases is not random but that the cancer cell or 'seed' has an affinity for certain sites or 'soil' was first observed by Paget in 1889, and continues to be a seminal observation in cancer metastasis. Our ongoing focus is to understand and characterize the physiological environment that is part of the 'soil' in which the cancer cell grows, and its role in invasion and metastasis. Since human malignant lines metastasize more readily from relevant orthotopic sites than from anatomically irrelevant (heterotopic) sites, a comparison of the tumor physiological environments and metabolism in the orthotopic and heterotopic sites can provide insight into physiological environments permissive or preventive for the occurrence of metastasis. The availability of a stably transfected cell line with an HRE controlling GFP expression that fluoresces under hypoxia, and the use of an extracellular proton pH marker has provided an opportunity for characterizing hypoxia, extracellular pH (pHe), and metabolism for a human prostate cancer xenograft transplanted orthotopically and heterotopically in SCID mice.

Methods: We generated PC-3 tumors derived from cells stably transfected with the hypoxia response element (HRE) of human VEGF-A ligated to the enhanced green fluorescence protein (EGFP) gene. Intact tumor tissue < $1mm^3$ was implanted in the prostate by suturing it in the lobes of the gland under a surgical microscope to avoid spillage of cancer cells during inoculation. Sentinel mice were implanted subcutaneously with a similar piece of tumor tissue to provide a gauge of tumor growth in the orthotopic site, and for comparison of MR parameters with the orthotopic site. Studies were performed on seven orthotopic and three subcutaneous (heterotopic) tumors. Four of the seven orthotopic mice were characterized for pHe and three for metabolism. Metabolic and pHe maps of HRE-GFP PC-3 tumors were obtained on a Bruker Biospec 4.7T spectrometer using MRSI for metabolic or pHe imaging. Metabolic maps of total choline, lactate/lipid (TE=272ms) or pHe (TE=24ms) were obtained from a 4 mm thick slice, with an in-plane resolution of 1 mm x 1mm using a 2D CSI sequence with VAPOR water suppression. For the pHe maps, the extracellular pH marker 2-imidazole-1-yl-3-ethoxy-carbonyl propionic acid (IEPA) was administered intraperitoneally and analyzed as previously described [1]. Quantitative maps of total choline and lactate/lipid in arbitrary units were generated according to Bolan et al [2]. Diffusion weighted images were obtained to identify the tumor region for analysis in the orthotopic tumors. Optical imaging of GFP expression in freshly cut tumor sections was used to visualize hypoxia in relation to the noninvasively obtained MR metabolic and pHe maps.

Results: Examples of co-localized maps of a diffusion weighted image to identify the tumor, and maps of total choline, and choline/(lactate/lipid) ratio for an orthotopic tumor are shown in Figure 1a-c. The hypoxic regions visualized by the GFP distribution within this tumor are shown in Figure 1d. This animal had significantly enlarged axillary lymph nodes with strong GFP expression indicating that the metastatic deposits in the lymph nodes were hypoxic (Figure 1e). In addition, ascites fluid in this animal also contained fluorescing cancer cells suggesting that these cells were also hypoxic (Figure 1f). Of the seven animals studied with orthotopic tumors, two mice showed fluorescing cells in ascites fluid and one animal had fluorescing lymph nodes. In the orthotopic site, a heterogeneous distribution of total choline and choline/(lac/lip) was observed with elevated regions coarsely co-localizing with high GFP expression. A map of pHe obtained from a subcutaneous tumor together with an optical images showing GFP distribution superimposed over a white light image are show in Figure 2a and b. There is a coarse co-localization of GFP expression with lower pHe values. Hypoxia was observed in the orthotopic as well as subcutaneous tumors. Orthotopic tumors however, tended to have more acidic extracellular pH values than the subcutaneous tumors (5.9 $\pm$ 0.2; n= 4 versus 6.45 $\pm$ 0.45, n=2; values are mean $\pm$ S.E.M.).

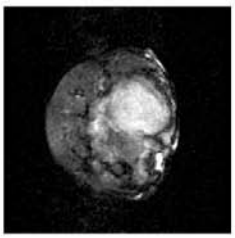

Figure 1a: Diffusion weighted image (DWI) from slice corresponding to metabolite maps. The tumor region is identified from the higher intensity.

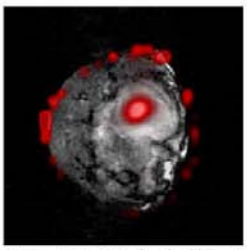

Figure 1b: Total choline map (Cho) in red overlaid on DWI demonstrating heterogenous distribution.

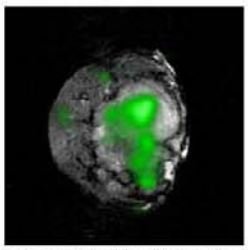

Figure 1c: Cho/(lac/lip) ratio map in green overlaid on DWI demonstrating elevated ratios in the tumor region.

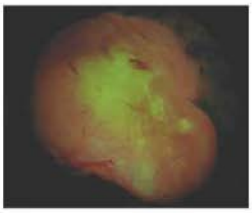

Figure 1d: GFP map from the excised tumor overlaid with white light image. A region of high GFP expression coarsely co-localized with high total choline.

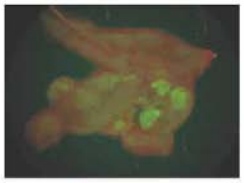

Figure 1e: GFP expression in axillary lymph node metastatic deposits from an orthotopic tumor overlaid with white light image.

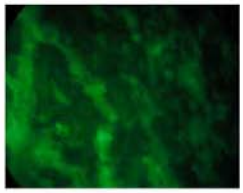

Figure 1f: GFP expression in cancer cells in ascites fluid.

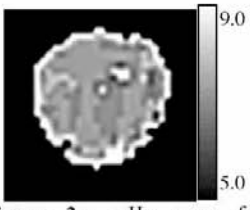

Figure 2a: pHe map from subcutaneous tumor (437 mm^3)

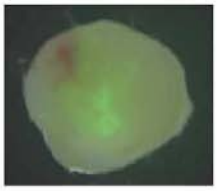

Figure 2b: GFP image from MR imaged slice in 2a overlaid with white light image of tumor slice.

Discussion: We are currently extending our studies to included co-localized maps of vascular volume and permeability as well as pHe and metabolism, for orthotopic and heterotopic tumors. Here we have observed that pHe in the PC-3 orthotopic prostate cancer xenograft model tended to be lower than in the corresponding subcutaneous tumors. Our data also suggest that lymph node metastases as well as ascites fluid may contain hypoxic cells that can contribute to the poor treatment outcome in late-stage disease.

References: 1. Bhujwalla, Z. M. et al., NMR in Biomedicine, 15: 114-119, 2002; 2. Bolan et al., Mag. Res. Med., 50: 1134-1143, 2003.

Acknowledgements: This work is supported by NIH 2 RO1 CA73850 and P50 CA103175. We thank Gary Cromwell for transplanting the tumors and maintaining cell lines, and Dr. Paul Winnard and Ms. Yelena Mironchik for characterizing the cell line. We thank Dr. Sebastian Cerdan and Dr. R. J. Gillies for useful discussions.