Hypoxia increases cellular phosphocholine and total choline levels in human prostate cancer cells

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Introduction

Cells adapt to hypoxia by stabilizing the hypoxia inducible factor (HIF-1α), which is ubiquitinated and degraded under normoxic conditions [1]. HIF-1α acts as a transcriptional activator for several genes, which have hypoxia response elements (HRE) with one or more HIF-1 binding sites. By placing green fluorescent protein (GFP) expression under the control of an HRE it is possible to indirectly detect HIF-1 activity and hypoxia in tissues. We have derived a human prostate cancer cell line stably transfected to express GFP under hypoxia (PC-3-HRE-GFP) [2]. Although increased total choline (tCho) and phosphocholine (PC) are hallmarks of cancer cells and occur due to distinct molecular alterations such as increased choline kinase expression and activity [3-5], a heterogeneous distribution of total choline is frequently detected in solid tumors. In our studies, combined MRSI and optical imaging of tumors (n=15) derived from stably transfected human PC-3 prostate cancer cells expressing green fluorescent protein (GFP) under hypoxic conditions (PC-3 HRE-GFP) routinely revealed a coarse co-localization between total choline maps obtained with MRSI, and hypoxic fluorescing regions detected with optical imaging (e.g. Figure 1a-d), for tumor volumes ranging from 30-600 mm^3 [2]. We therefore evaluated the effect of hypoxia on choline phospholipid metabolites as well as choline kinase levels of these PC-3 HRE-GFP cells.

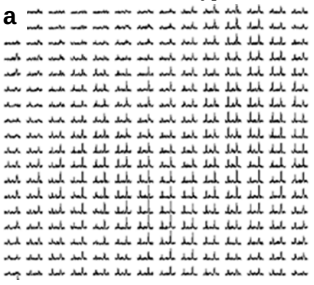

Figure 1a: Example of a CSI data set obtained from an PC-3 HRE-GFP tumor (550 mm3) with a spatial resolution of 0.5 x 0.5 x 4 mm

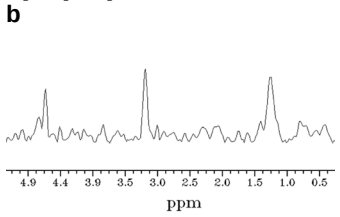

Figure 1b: Spectrum from a single 0.5 x 0.5 x 4 mm voxel showing total choline at 3.2 ppm, and lactate/lipid at 1.3 ppm (TE=272 ms).

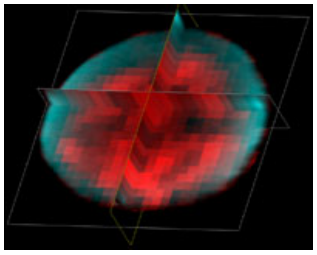

Figure 1c: Triplanar view of a total choline map (from Fig. 1a) displayed in red fused with a co-localized spin density map. A heterogeneous distribution of total choline is apparent, coarsely matching the GFP distribution in Fig. 1d.

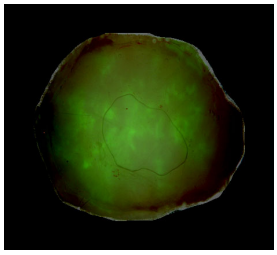

Figure 1d: GFP expression in hypoxic regions in a fresh tissue slice matching the MRSI slice, overlaid on a white light image. Comparison of Fig. 1c and d reveals a coarse co-localization between the GFP and total choline distributions.

Methods

Human PC-3 prostate cancer cells were stably transfected with the HRE of human VEGF-A ligated to the enhanced green fluorescent protein (EGFP). These PC-3 HRE-GFP cells were exposed to hypoxic conditions in a hypoxic chamber containing 0.3% O_2 and 5% CO_2 for 24 hours. Water-soluble cell extract fractions [6] and protein lysates were obtained from hypoxic as well as control cells. Fluorescence microscopy of live cells was performed before extraction. Fully relaxed ^{1}H MR spectroscopy of the water-soluble fractions was performed on Bruker Avance 500 spectrometer. The signal integrals were quantified relative to cell number and concentration of the internal standard 3-(trimethylsilyl)propionic-2,2,3,3,-d_4 acid. Choline kinase expression was determined by gel electrophoresis of cell lysates and Western blotting, and was probed for by a choline kinase antibody.

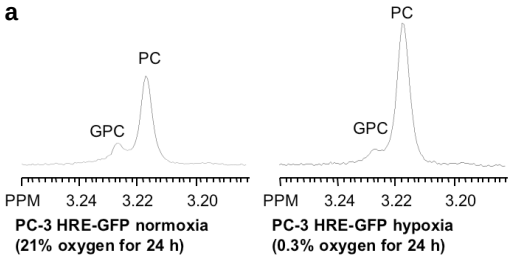

Figure 2a: Expanded regions of the ^{1}H MR spectra of normoxic (left) and hypoxic (right) PC-3 HRE-GPF prostate cancer cells. Assignments: Cho, free choline; GPC, glycerophosphocholine; PC, phosphocholine; tCho, total choline (Cho+GPC+PC).

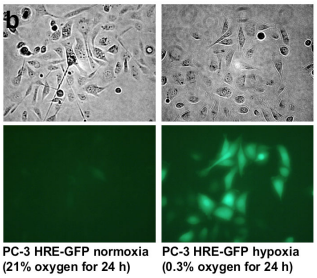

Figure 2b: Corresponding phase contrast (top) and fluorescence (bottom) microscopic images (40x lens).

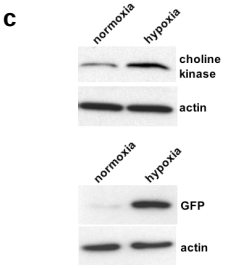

Figure 2c: Corresponding choline kinase (top) and GFP expression (bottom) probed with choline kinase or GFP antibodies, respectively. Actin was probed in loading controls.

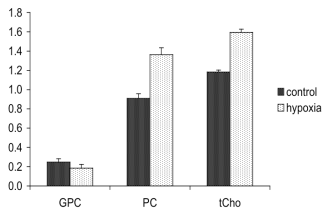

Figure 2d: Summary of changes in PC, GPC and total choline for two independent cell extract studies. Values are mean ± standard deviation.

Results

Human PC-3 HRE-GFP prostate cancer cells in culture exhibited significantly increased PC and tCho levels following 24h of hypoxia (Fig. 2a, d). GFP expression was significantly elevated in hypoxic PC-3 HRE-GFP cells (Fig. 2b, c), demonstrating HIF-1α elevation under hypoxia. Choline kinase was significantly overexpressed in hypoxic PC-3 HRE-GFP cells compared to normoxic controls (Fig. 2c).

Discussion

These cell studies as well as the solid tumor data indicate that choline kinase expression in prostate cancer cells can be driven by hypoxia, thereby leading to elevated PC and tCho levels in hypoxic tumor regions. Choline kinase most likely contains an HRE in its promoter region, which is activated by high cellular HIF-1α concentrations under hypoxia. The increase in choline kinase and PC in tumors may be an adaptive response to hypoxic stress.

Like most cancer cells, PC-3 cells exhibit high total choline as well as phosphocholine in culture, compared to normal prostatic epithelial cells. In vivo, however, a distinctly heterogeneous distribution of total choline is frequently observed in human tumor xenografts as well as clinical cancers. Our data suggest that hypoxia is associated with elevated total choline and may drive, in part, the heterogeneous distribution of total choline observed in vivo.

References & Acknowledgements [1] Semenza GL, *Crit Rev Biochem Mol Biol* 35, 71 (2000) [2] Bhujwalla ZM et al, *12th ISMRM Meeting, Kyoto, Japan*, Abstract #219 (2004) [3] Glunde K et al, *Cancer Res* 64, 4270 (2004) [4] Ackerstaff E et al, *Cancer Res* 61, 3599 (2001) [5] de Molina AR et al, *Biochem Biophys Res Commun* 296, 580 (2002) [6] Tyagi RK *et al*, *MRM* 35, 194 (1996) This work was supported by NIH 1R01 CA73850 and P50 CA103175 (JHU ICMIC Program). We thank Mr. Gary Cromwell for maintaining the cell lines.