Hypoxia regulates phosphocholine and total choline concentrations 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 functions as a transcriptional activator for several genes containing hypoxia response elements (HRE) with one or more HIF-1 binding sites. Placing green fluorescent protein (GFP) expression under the control of an HRE enabled us to indirectly detect HIF-1 activity and hypoxia in cells and tissues. A human prostate cancer cell line stably transfected to express GFP under hypoxia (PC-3-HRE-GFP) was generated in our laboratory [2]. We have frequently detected a heterogeneous distribution of total choline in solid tumors even though increased total choline (tCho) and phosphocholine (PC) were demonstrated to occur due to distinct molecular alterations such as increased choline kinase expression and activity in cancer cells [3-5]. Prostate tumors stably expressing 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 [2]. We therefore extended our study to assess the effects of hypoxia on choline phospholipid metabolites as well as choline kinase levels in PC-3 HRE-GFP cells as well as in wild-type human PC-3 prostate cancer cells.

Methods

Human PC-3 prostate cancer cells were stably transfected with the HRE of human VEGF-A ligated to the expression of enhanced green fluorescent protein. Wild-type human PC-3 prostate cancer cells and PC-3 HRE-GFP cells were exposed to hypoxic conditions in a hypoxic chamber containing 0.3% - 0.5% O_2 and 5% CO_2 for 24 hours. We obtained water-soluble cell extract fractions [6] and protein lysates from hypoxic as well as normoxic control cells. Fully relaxed ^{1}H MR spectroscopy of the water-soluble fractions was performed on a 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, probed for by a choline kinase antibody as previously described [7].

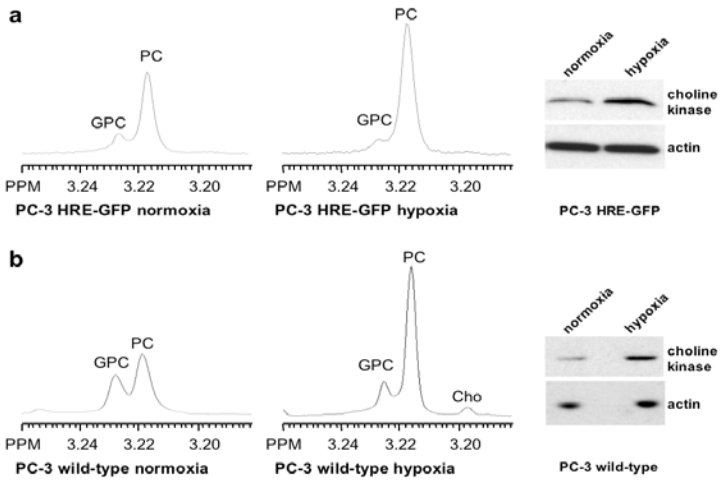

Figure: Expanded regions of the ^{1}H MR spectra of normoxic (left) and hypoxic (right) (a) PC-3 HRE-GPF prostate cancer cells and (b) wild-type PC-3 prostate cancer cells, and the corresponding Western blots probed with choline kinase antibody. Assignments: Cho, free choline; GPC, glycerophosphocholine; PC, phosphocholine.

Results

Human PC-3 HRE-GFP as well as wild-type PC-3 prostate cancer cells in culture exhibited significantly increased PC and tCho levels following 24h of hypoxia (Fig). Significantly increased GFP expression was detected in hypoxic PC-3 HRE-GFP cells by fluorescence microscopy as well as Western blots probed for by GFP antibody, demonstrating HIF-1α elevation under hypoxia. Choline kinase was significantly overexpressed in hypoxic PC-3 HRE-GFP cells as well as wild-type PC-3 prostate cancer cells compared to the corresponding normoxic control cells (Fig).

Discussion

These data demonstrate that choline kinase expression in prostate cancer cells is, at least in part, driven by hypoxia. This hypoxia-driven choline kinase expression may be the underlying cause for the elevated PC and tCho levels in hypoxic tumor regions. These findings indicate that choline kinase may contain an HRE in its promoter region, which is potentially activated by the high cellular HIF-1 concentrations under hypoxia. Increased choline kinase expression and subsequently increased PC levels in certain tumor regions may be an adaptive response to hypoxic stress in these regions. In cell culture, PC-3 cells contain elevated PC and tCho levels compared to normal prostatic epithelial cells [4]. However, human prostate tumor xenografts as well as clinical prostate tumors exhibit a distinctly heterogeneous distribution of total choline. The data presented here indicate that hypoxia is associated with elevated total choline and may partially drive the heterogeneous distribution of total choline in prostate cancer xenografts and clinical prostate cancers.

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) [7] Glunde K et al, *Cancer Res* 65, in press (2005)
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