Down regulation of HIF-1 alpha in MDA-MB-231 Human Breast Cancer Cells Alters Choline Phospholipid Metabolism

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Introduction: Hypoxia-inducible factor-1 (HIF-1) is a heterodimer made up of an oxygen-regulated HIF-1α subunit and a constitutively expressed HIF-1β subunit. Under hypoxic conditions, HIF-1α is stabilized and binds to hypoxia response elements that act as transcriptional controls of several genes. Among the 70 target genes of HIF-1 known so far, several are involved in angiogenesis, cell proliferation, cell viability, and glucose and iron metabolisms. HIF-1α over-expression has been associated with an increased patient mortality rate in many cancer types including breast cancer [1]. Suppression of HIF-1α gene expression has been shown to be sufficient in tumor growth repression [2]. Here we have studied the effect of HIF-1α silencing on the metabolism of MDA-MB-231 cells using an MR compatible cell perfusion assay. We found that HIF-1α silenced cells exhibited significantly reduced choline kinase (Chk) expression together with reduced total choline (tCho), and phosphocholine (PC) compared to parental cells.

Materials and Methods: The sequence for shRNA against HIF-1α was obtained from published reports [3] and cloned into a lentivirus vector with a green fluorescent protein (GFP) reporter construct (pRRL-pGK-GFP). Viral supernatant preparation and transduction of MDA-MB-231 breast cancer cells was performed as previously published [4]. Transduced cells were validated for HIF-1α knock-down by western blots and by quantitative real-time polymerase chain reaction (Q-RT-PCR). The cell perfusion studies were performed using an MR-compatible perfusion assay [5]. Intracellular levels of metabolites were derived from global, water suppressed diffusion-weighted (DW) 1D ^{1}H MR spectra acquired with 128 scans and 2K data points. DW 1D ^{1}H MR spectra obtained without water suppression were used to determine cell proliferation because the increase of slow-diffusing water, which represents intracellular water, was directly proportional to the number of cells. Energy metabolites, pH, and the choline phospholipid metabolites PC and PE were obtained from global 1D ^{31}P MR spectra with 4K scans and 2K data points. All MR spectra were processed and analyzed using XsOsNMR. Experiments were performed in triplicates and values are presented as a mean of three experiments. The Mann Whitney-U test was used to determine statistical significance ($p < 0.05$).

Results and Discussion: Western blots of HIF-1α and Chk expression, in response to treatment with the hypoxia mimetic $CoCl_2$, in parental MDA-MB-231 and HIF-1α silenced MDA-MB-231 cells are shown in Figure 1. HIF-1α protein expression increased with $CoCl_2$ treatment only in parental MDA-MB-231 cells while cells transduced with HIF-1α shRNA did not show HIF-1α stabilization following treatment with 200μM $CoCl_2$ (Figure 1)

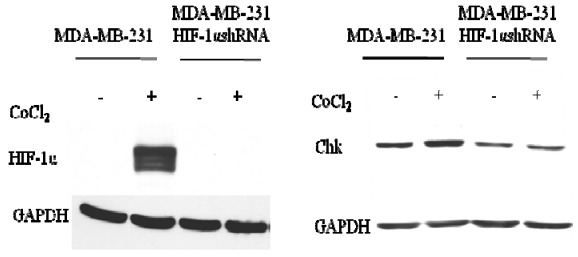

Figure 1. Western blot for HIF1α and Chk expression in parental and HIF-1α silenced MDA-MB-231 breast cancer cells after treatment with the hypoxia mimetic $CoCl_2$. Cells were exposed to 200 μM $CoCl_2$ for 4-6 h for HIF-1α levels or 48 h for Chk levels.

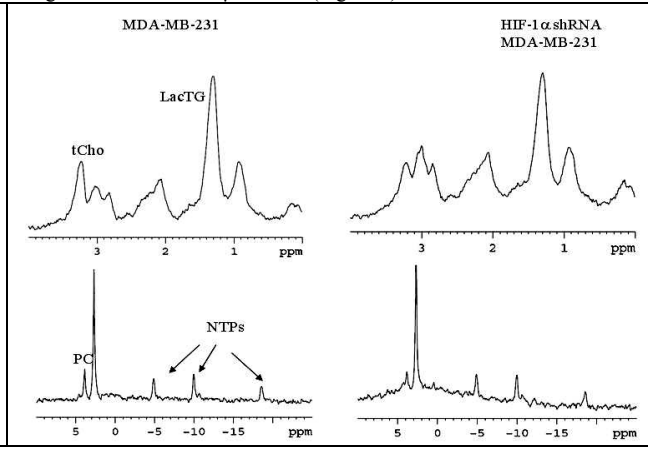

Figure 2. Representative ^{1}H and ^{31}P spectra from MDA-MB-231 and HIF1α shRNA MDA-MB-231 cells.

Reduced expression of Chk was observed in HIF-1α silenced cells while its expression was induced in parental cells with the hypoxia mimetic $CoCl_2$ treatment (Figure 1). A reduction of HIF-1α and Chk was also observed at the mRNA level for HIF-1α silenced cells (data not shown). Representative spectra from MDA-MB-231 and HIF-1α shRNA MDA-MB-231 cells show reduced levels of total choline and LacTG in ^{1}H spectra and reduced PC levels in ^{31}P spectra in HIF-1α silenced cells (Figure 2). Quantitative data from ^{1}H and ^{31}P studies showed significantly reduced levels of tCho ($p < 0.05$) and phosphocholine PC ($p < 0.01$) in HIF-1α silenced cells compared to parental MDA-MB-231 cells (Figure 3).

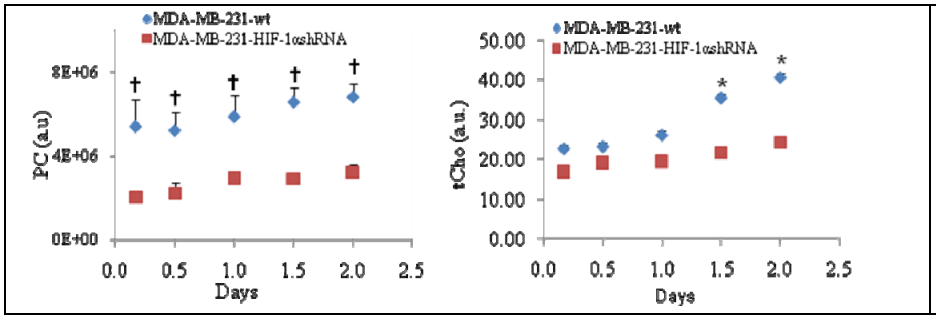

Figure 3. Quantification of ^{1}H spectra and ^{31}P spectra identified significantly reduced levels of tCho and PC respectively in HIF-1α silenced cells compared to parental MDA-MB-231 cells (n=3, † $p < 0.01$; *$p<0.05$).

Here we have shown that silencing of HIF-1α alters phospholipid metabolism by reducing levels of choline containing metabolites. We previously observed that Chk is upregulated under hypoxia and have established a HIF-1 binding site on the Chk promoter [6]. The reduced choline metabolites in HIF-1α silenced cells confirmed the role of HIF-1α in the regulation of Chk. The reduced total choline and PC levels in HIF-1α silenced cells are typical of a less aggressive metabolic phenotype.

References: [1]. van der Groep P *et al.* Breast Cancer Res Treat. 2008;111: 475-80. [2]. Yeo EJ *et al.* Biochem Pharmacol. 2004; 68:1061. [3]. Krishnamachary B *et al.* Cancer Res. 2006; 66: 2725-2731. [4] Krishnamachary B *et al*. Cancer Res. 2009; 69:3464-71. [5] Ackerstaff E *et al.* Neoplasia. 2007; 9:222-35. [6] Glunde K *et al.* Cancer Res 2008; 68:172-80. **Acknowledgement:** This work was supported by NIH RO1 CA82337 and P50CA103175. We thank Dr. Ackerstaff for useful discussions and Dr. Shungu for providing the XsOsNMR software.