Hypoxia Increases Degradation of the Extracellular Matrix by Human Breast Cancer Cells

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Introduction: Hypoxia commonly occurs in solid tumors and is often associated with poor prognosis [1]. Cancer cells display an adaptive response to hypoxia through the activation of several genes that is mediated through the binding of hypoxia inducible factor (HIF)-1 to hypoxia response elements (HRE) that regulate the transcription of these genes [2]. Under oxygenated conditions HIF-1α is rapidly degraded but under hypoxic conditions HIF-1α is stabilized. Amongst the genes activated are several proteolytic enzymes that play a role in invasion, metastasis and metabolism [3]. The MDA-MB-231 human breast cancer cell line is an aggressive and triple (estrogen receptor/progesterone receptor/Her-2 neu) negative cell line that is highly invasive and metastatic. Invasion and degradation are required in the metastatic cascade. In this study we have investigated the ability of MDA-MB-231 breast cancer cells perfused under hypoxic condition to degrade extracellular matrix (ECM) and have also determined the associated changes in metabolism using our MR compatible cell perfusion assay.

Material and Methods: Three days prior to the MR experiments, cells were seeded on Biosilon beads (Nunc, Denmark) at a cell density of 1.5 x 10^6 cells per 0.5 ml of beads in non–cell culture petri-dishes (Labtec, Nunc, Denmark) and grown to approximately 70% confluence. A detailed description of the MR cell perfusion system can be found in Ackerstaff *et al.* [4]. Experiments performed under normoxic conditions were carried out at oxygen tensions greater than 20%. Experiments performed under hypoxic conditions were carried out at 1% oxygen tension. MR data were acquired on a 9.4 T MR spectrometer (Bruker, Billerica, MA) every 12 h over a period of 2 days. Two layers of perfluorocarbon doped alginate beads were interspersed within the layers of cancer cells grown on Biosilon beads to monitor the oxygen tension in the sample using ^{19}F MR relaxometry. A chamber containing Matrigel® at a concentration of 8.8 mg/ml, which is part of the MR-compatible cell perfusion assay was used to determine the degradation of ECM by the cancer cells. Degradation of the ECM by cancer cells was determined at the 24 h time point relative to the initial time point from the proton images. Degradation of the ECM gel was estimated by drawing a region of interest (ROI) around the ECM gel region using NIH ImageJ software. The degradation index was defined as ROI_0/ROI_{24}. Intracellular metabolite levels including tCho, i.e., signals from PC + GPC + free choline, creatine/phosphocreatine (Cr/PCr), and lactate/triglycerides (LacTG) were derived from unlocalized, diffusion-weighted (DW) ^{1}H MR spectra. DW 1D ^{1}HMR spectra were acquired using lactate-editing to quantify the contribution of Lac and TG to the LacTG signal. Since the slow-diffusing water, which represents intracellular water, is proportional to the number of cells, DW 1D ^{1}H MR water spectra were obtained as an index of cell number to factor in cell proliferation. To quantify the contribution of lactate and triglycerides to the LacTG signal at 1.3 ppm in the unlocalized ^{1}H MR spectra, we acquired diffusion-weighted 1D ^{1}H MR spectra using a spin echo–based pulse sequence with an echo time of 136 ms, 2K data points and 256 scans and lactate editing. Energy metabolites, pH, and the choline phospholipid metabolites, PC, and glycerophosphocholine, were obtained from unlocalized 1D ^{31}P MR spectra.

Results and Discussion: Representative T_1-weighted ^{1}H MR images at various time points demonstrating degradation of ECM gel by parental MDA-MB-231 cells under normoxia and hypoxia are shown in Figure 1a. The ECM gel was completely degraded by MDA-MB-231 cells by 48 h under normoxic conditions. In contrast, the ECM was completely degraded by the MDA-MB-231 cells within 24 h under hypoxic conditions. As shown in Figure 1b there was a significant difference in the degradation index (ROI_0/ROI_{24}) of ECM gel by MDA-MB-231 breast cancer cells at the 24 h time point relative to the initial time point under hypoxic (3.26±0.55) compared to normoxic (1.97±0.45) ($p < 0.01$) conditions.

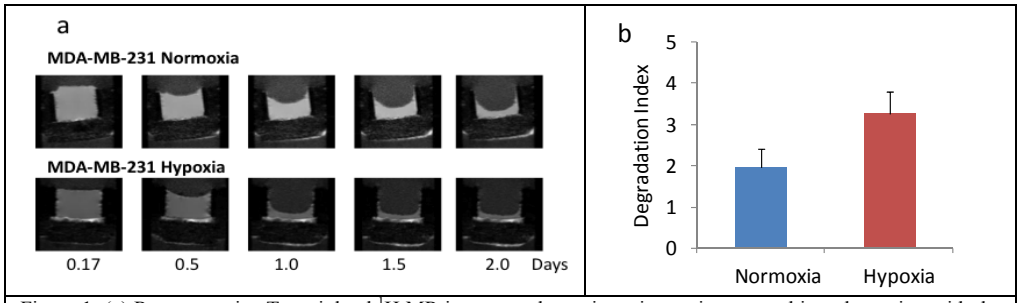

Figure 1: (a) Representative T_1-weighted ^{1}H MR images at the various time point zoomed into the region with the ECM chamber, showing degradation of ECM gel by parental MDA-MB-231 cells under normoxia and hypoxia. (b) Degradation index estimated on the basis of ECM gel degradation at 24h time point relative to start time point ($p < 0.01$, n=4).

This enhanced degradation of ECM gel under hypoxia may be driven by the upregulation and activation of a number of proteolytic enzymes involved in ECM degradation [3]. Representative proton spectra obtained under normoxic or hypoxic conditions acquired at the 24 h time point from MDA-MB-231 cells are shown in Figure 2a. Quantitative analyses of these metabolites, shown in Figure 2b, demonstrate significantly increased tCho ($p < 0.02$), lactate ($p < 0.02$) and triglycerides ($p < 0.02$) under hypoxic compared to normoxic conditions in MDA-MB-231 cells.

The increase of tCho is consistent with our previous observations that choline kinase expression increases under hypoxic conditions [5]. These results suggest that *in vivo* tumors with hypoxic regions will most likely exhibit significant alteration of the ECM that may influence drug delivery and metastasis. The combination of the metabolic changes of increased tCho, lactate and triglycerides represent metabolic changes that allow these cancer cells to adapt and survive under hypoxia.). These results demonstrate the profound effect on hypoxia on the ability of this triple negative breast cancer cell line to degrade the ECM.

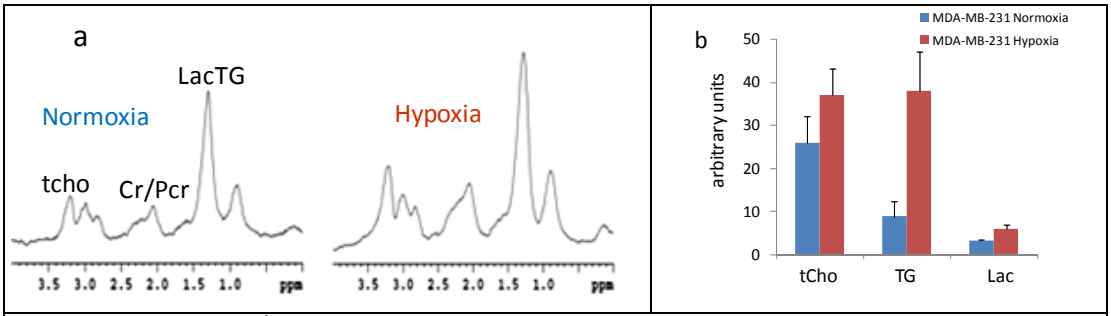

Figure 2: (a) Representative ^{1}H spectra acquired from MDA-MB-231 cells under hypoxic and normoxic condition at the 24 h time point. (b) Quantification of data from ^{1}H MR spectra in arbitrary units (a.u.) demonstrating differences in tCho, Lac and triglycerides level at the 24 h time point ($p < 0.02$).

References: (1) Ruan K, Song G, Ouyang G. J Cell Biochem 2009;107:1053-62. (2) Maxwell PH, Dachs GU, Gleadle JM *et al.* Proc Natl Acad Sci 1997; 94:8104-9. (3) E-pub. (4) Ackerstaff E, Gimi B, Artemov D, Bhujwalla ZM Neoplasia. 2007; 9: 222-35. (5) Glunde K, Shah T, Winnard PT Jr. *et al.* Cancer Res, 2008;68:172-80.

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