REVEALING CANCER PHENOTYPE-SPECIFIC BIOMARKERS IN A CELL PERFUSING SYSTEM BY 13C AND 1H MRS

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INTRODUCTION: Cancer development and progression is characterized by changes in the metabolic phenotype, most notably, enhanced aerobic glycolysis leading to acidification of the tumor microenvironment and increased choline metabolism. However, a majority of the increasing variety of cancer phenotypes identified on the bench are not yet identifiable in the clinic, and thus are not being utilized to help tailor treatment. MR-compatible cell perfusion systems have been reported (1,2) which allow studying cancer cells in vivo dynamically. We are currently using this approach to study the metabolic responses of several aggressive cancer cell lines to different stress conditions.

PURPOSE: To study cancer cells with different metastatic potential in a MR-compatible perfusion system and look for MRS-detectable biomarkers that distinguish them in defined environmental conditions.

METHODS: A cell perfusion system, based on a design described previously (2), has been built for a Bruker 7T horizontal magnet. For each study, cancer cells were cultured on microcarriers (Plastic Plus, SoloHill) for 3-5 days – seeding densities 1.5 to 3.0 x10⁶ cells. Cells were then loaded into the perfusion system: a custom-made 12 mm screw-cap tube where culture medium and gas were circulated, and the temperature controlled (37 °C) by a recirculating water bath and a heating water jacket. Data were acquired with a home-built dual RF coil, based on a solenoid (¹³C) inserted on a saddle coil (¹H). Our preliminary results were obtained with the highly metastatic mammary cancer cell line 4T1 (liver and lungs) and the brain metastasis-derived prostate cancer cell line DU-145. The cell lines were grown according to the following protocols: 4T1 (DME medium) – 25 mM glucose, 2 mM glutamine; DU-145 (MEM medium) – 5.5 mM glucose, 2 mM glutamine, and 1 mM pyruvate. The MR experiments consisted in acquiring ¹³C and ¹H data while challenging the perfused cells with different metabolic perturbations. These were carried out in six steps (I – VI, 4.5 hours each): I, regular medium (DME or MEM) and carbogen gas (95% O₂ / 5% CO₂); II, 100% ¹³C-glucose labeled medium, without glutamine (or pyruvate), and carbogen gas; III, same medium as in II but during hypoxia (95% N₂ / 5% CO₂); IV, still hypoxia but replacing the medium with 100% ¹³C-glucose with glutamine (and pyruvate, only for DU-145 cells); V, same medium as in IV but shifting the gas back to carbogen; VI, same as in I. 13C scans were acquired with a single block pulse (60º), 1200 ms TR, and 1800 excitations – 36 min scan time. ¹H data were acquired with PRESS volume selection and VAPOR water suppression; 2500 ms TR, 12/136 ms TE, 256 excitations – 11 min scan time. MRS data were analyzed by deconvolution of selected peak areas with software XsOsNMR v1.0.

RESULTS: Our preliminary results (Figure 1) suggest different metabolic responses of the two cancer cell lines subjected to identical stress conditions. In particular, 4T1 cells produced more choline than DU-145, which agrees with the higher doubling time of this cell line (~14h vs. ~30h); on the other hand, DU-145 cells store glucose as glycogen when glutamine (and pyruvate) is available in the medium, whereas 4T1 cells, growing in hyperglycemia, do not.

CONCLUSIONS: Our data indicates differences in cellular metabolism between two aggressive cancer cell lines. Further experiments are being carried out with other cancer cell lines. Results obtained with this setup may have a direct preclinical application which should facilitate translation to human studies.

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