Carbon Flux in Hypoxic Breast Cancer Cells


Introduction

Breast cancer is the second leading cause of cancer deaths in women, and despite advances in diagnosis and therapeutics, complete recovery is not always realized. Therefore, novel approaches towards predicting therapeutic success and/or outcome of breast cancer patients are being sought. Metabolism, particularly under hypoxic conditions, plays a role in the aggressiveness of cancer as well as the susceptibility to therapy. We hypothesize that understanding dominant metabolic pathways under varying conditions will lead to new targets for therapeutic intervention. This project explores the contribution of the pentose phosphate pathway (PPP) to the metabolic phenotype of cancer cells. Here, we approach a novel avenue of prediction through exploring nuclear magnetic resonance spectroscopy (NMR) techniques to evaluate carbon flux through glycolysis and PPP and correlate differences in flux to the known metastatic potential of human breast cancer cells (hBrC) by $^{13}$C spectroscopy and isotopomer analysis [1].

Experimental

MDA-MB-231 hBrC cells were grown in flasks, and exposed for 4 hours to media containing 15 mM uniformly-$^{13}$C-labeled glucose under either normoxic or hypoxic conditions, and then extracted [2]. The aqueous phase of the extract was examined by $^{31}$P & $^{13}$C NMR spectroscopy on a 500 MHz vertical bore Bruker magnet.

Results and Discussion

These preliminary extractions were expected to yield $^{13}$C isotopomeric information that would shed light on changes in metabolic pathways due to hypoxic conditions. There were differences observed between the two extracts (Fig.1), notably in the C2-alanine (51 ppm), C3/C5-fructose 1,6 diphosphate (84 ppm), C2/C4-citrate (46 ppm), and C1-fructose 1,6 diphosphate (65 ppm), all of which were higher in the hypoxic extract. The normoxic cells had a much more robust C4-glutamate (34.5 ppm) peak. Unfortunately, $^{13}$C spectra from these samples had insufficient signal for isotopomer analysis. This may be due to a number of factors: 1) the number of cells in the extractions was sub-optimal; 2) the hypoxic episode was prolonged and resulted in cell death; 3) the exposure time to the label was insufficient. These factors have been taken into consideration, and a recent experiment has been done to improve the signal-to-noise issue by improving the label exposure conditions (increased exposure time); increasing the number of cells; and optimizing the hypoxic episode. These samples are scheduled to be run in January on a 500 MHz magnet.

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References