DYNAMIC METABOLIC SIGNATURES OF METASTATIC AND NON-METASTATIC BREAST CANCER CELLS

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INTRODUCTION: Distinguishing metastatic from non-metastatic clinical breast cancer, non-invasively, would significantly improve evaluation of patient prognosis. Recent studies with two isogenic murine breast cancer lines, metastatic 4T1 and non-metastatic 67NR, revealed differences at the level of (a) LDH-A expression, in response to changing oxygen tensions in vitro, and (b) lactate accumulation, during tumor growth in vivo (1). Understanding how each cell line metabolically adapts to dynamic microenvironmental changes, such as oxygen availability vs. hypoxia, and major nutrient supply vs. deprivation (glucose and glutamine (2,3)), could provide unique non-invasive biomarkers of metastatic and non-metastatic breast cancer phenotypes, useful in developing new therapies.

PURPOSE: To study 4T1 and 67NR breast cancer lines in an NMR-compatible cell perfusion system and investigate differences in their dynamic metabolic responses to (a) oxygen availability vs. hypoxia, and (b) glutamine (Gln) supply vs. Gln deprivation.

METHODS: 4T1 and 67NR cells were initially studied in vitro to determine the response of cell growth to modified media composition (DME, supplemented with 1% FBS and 10% FBS: DMEcomp): 1) DMEcomp contains 25 mM glucose (Glc) and 6 mM Gln; 2) DME with 2 mM Gln; 3) DME without Gln; 4) DME without Glc. Cells were incubated for 48 h in each medium, harvested, and counted (ViaCount Assay, Guava Technologies). Three independent experiments, with duplicate samples per experiment, were performed for each cell line. Slight modifications were introduced in our cell perfusion system described previously (4) to accommodate its use in a 500 MHz vertical-bore Bruker magnet. For each study, 3.0 x 10^6 cells were seeded on microcarriers (Plastic Plus, SoloHill) and cultured for 3 days. Cells were then loaded into the perfusion system: a custom-made 10 mm screw-cap tube where culture medium and gas were circulated and the temperature controlled (37 °C) by a heating water jacket. The MR experiments consisted in repeatedly acquiring 13C, 1H, and 31P spectra (standard Bruker BBO probe) while challenging the perfused cells with different metabolic perturbations. These were carried out in six steps (I – VI, 5 h each): I) DMEcomp and carbogen gas (95% O2 / 5% CO2); II) 100% 13C(1)-Glc labeled medium, without Glc, 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(1)-Glc and 2 mM Glc; V, same medium as in IV but shifting the gas back to carbogen; VI, same as in I. Each experiment was repeated 3 times for each cell line. Spectral analysis of peak areas was carried out with AMARES (MRUI v4.0). Statistical analyses were carried out with the paired t-Test (SPSS 15.0) to compare 4T1 vs. 67NR data (significance level: <p>0.05).

RESULTS: As shown in Fig. 1, the in vitro cell growth is dependent on the availability of both Glc and Glc, in 4T1 and 67NR lines: cell growth drops by 85-85% in the absence of either Glc or Glc, and remains at 35-45% when Glc alone is decreased by 2/3; no significant differences were observed in growth response to each nutrient deprivation. As for dynamic cell perfusion studies, 31P-monitored ATP levels were kept within ±27% of their initial concentration during the experiments (I-VI), in both cell lines, although showing significant differences during stages IV-VI (data not shown). 1H-visible total choline compounds (3.21 ppm) and mobile lipids (1.29 ppm, mixed contribution of lactate) showed consistent stress-response changes at each stage, similar in both cell lines (data not shown). 13C-labeling studies revealed that 67NR cells are significantly more glycolytic than 4T1 cells in the experimental conditions tested: in 67NR cells, the rate of glucose-derived Lac (lactate) production is 2-fold higher in 4T1 cells. The glycolytic Lac synthesis during hypoxia is Glc-dependent in 4T1 cells (2-fold higher rate in presence of glutamine vs. deprivation), but not in 67NR cells. Supply of Gln vs. deprivation, generates significantly different dynamic responses between 4T1 and 67NR cells, as far as turnover of glycolytic intermediates, e.g. glyceraldehyde-3-phosphate, alanine synthesis, and TCA cycle activity. In aerobic conditions and presence of Gln, 4T1 cells incorporate more Glc into the TCA cycle (3-fold higher rate of glutamate synthesis) than 67NR cells, whereas the latter show higher glycolytic activity, i.e. 2.4-fold higher rate of Lac synthesis, and also 25-fold faster synthesis of alanine.

CONCLUSIONS: Although no significant differences in cell growth were observed in vitro between 4T1 and 67NR cells, when exposed to defined metabolite stress conditions each cell line adopts different metabolic strategies to cope with said changes, depending on the level of oxygen availability. Mostly, non-metastatic 67NR cells revealed higher glycolytic activity than metastatic 4T1 cells. Unlike in 67NR cells, glycolysis is Glc-dependent in the more aggressive cell line (4T1) during hypoxia, dropping when oxygen becomes available to favor TCA cycle activity. Ongoing 13C(3)-Gln studies should provide further insight into these metabolic differences.

ACKNOWLEDGMENTS: Work supported by NIH grants PO1 CA115675 and NCI P30 CA0874 (Cancer Center Support Grant).