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Metabolic Plasticity of Metastatic Breast Cancer Cells: Adaptation to Changes in the Microenvironment[1]

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Abstract

Cancer cells adapt their metabolism during tumorigenesis. We studied two isogenic breast cancer cells lines (highly metastatic 4T1; nonmetastatic 67NR) to identify differences in their glucose and glutamine metabolism in response to metabolic and environmental stress. Dynamic magnetic resonance spectroscopy of ^{13}C-isotopomers showed that 4T1 cells have higher glycolytic and tricarboxylic acid (TCA) cycle flux than 67NR cells and readily switch between glycolysis and oxidative phosphorylation (OXPHOS) in response to different extracellular environments. OXPHOS activity increased with metastatic potential in isogenic cell lines derived from the same primary breast cancer: 4T1 > 4T07 and 168FARN (local micrometastasis only) > 67NR. We observed a restricted TCA cycle flux at the succinate dehydrogenase step in 67NR cells (but not in 4T1 cells), leading to succinate accumulation and hindering OXPHOS. In the four isogenic cell lines, environmental stresses modulated succinate dehydrogenase subunit A expression according to metastatic potential. Moreover, glucose-derived lactate production was more glutamine dependent in cell lines with higher metastatic potential. These studies show clear differences in TCA cycle metabolism between 4T1 and 67NR breast cancer cells. They indicate that metastases-forming 4T1 cells are more adept at adjusting their metabolism in response to environmental stress than isogenic, nonmetastatic 67NR cells. We

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suggest that the metabolic plasticity and adaptability are more important to the metastatic breast cancer phenotype than rapid cell proliferation alone, which could 1) provide a new biomarker for early detection of this phenotype, possibly at the time of diagnosis, and 2) lead to new treatment strategies of metastatic breast cancer by targeting mitochondrial metabolism.

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Introduction

Breast cancer is the most prevalent type of cancer among women in the United States [1], and mortality is primarily caused by metastatic disease. The complex mechanisms of breast cancer invasion and metastasis [2] are intrinsically related to the malignant cell type [3], their interaction with stromal cells [4,5], and changes in the tumor microenvironment, related to poor perfusion, intermittent hypoxia, transient nutrient deprivation, and acidity [6,7]. Cancer cells adapt to dynamic stresses and proliferate by reprogramming their metabolism to support synthesis of an expanding biomass [8,9]. Due to oncogene-driven upregulation of key glycolytic enzymes [10], most cancer cells exhibit aerobic glycolysis known as the Warburg effect [11]. This metabolic phenotype has been studied by noninvasive techniques, such as ^{18}F-fluorodeoxyglucose positron emission tomography and magnetic resonance spectroscopy (MRS of ^{13}C-labeled substrates) [12,13]. Due to enhanced glycolysis, tumor cells synthesize high levels of lactate and export H^+, resulting in acidification of the microenvironment, which in turn promotes invasion and dissemination [14,15]. Recent studies with two isogenic murine breast cancer cell lines derived from the same spontaneous breast tumor, 4T1 and 67NR [16], have shown differences in lactate dehydrogenase (LDH) A expression during normoxia and hypoxia [17]. However, other studies have highlighted the importance of oxidative phosphorylation (OXPHOS) in tumorigenesis and progression [18–20].

Because it is well recognized that tumor cells are often hypoxic and nutritionally deprived *in vivo*[7], we have monitored in real time the metabolic changes in live 4T1 and 67NR cells under conditions that reflect these common, often transient, physiologic stresses. Our hypothesis is that the metabolism of metastases-forming breast cancer cells (4T1) is more adaptable to changes in the microenvironment than the metabolism of isogenic, nonmetastatic cells (67NR), thus providing 4T1 cells with a distinct advantage to grow, invade, and proliferate under different conditions. Previous metabolic studies using cell extracts have demonstrated marked differences in basal glucose consumption, lactate production, and oxygen consumption between these two cell lines grown in standard bidimensional tissue culture conditions [17,21]. However, these studies have not investigated the — potentially reversible — metabolic adaptations of these cancer cells to *in vivo* tumor conditions, which include changing microenvironmental stresses during tridimensional growth. We used a magnetic resonance (MR)–compatible cell perfusion system and time-course MRS of ^{13}C isotopomers to investigate how living cancer cells adapt their metabolism and growth to selective supply/deprivation of glucose and glutamine under both aerobic and hypoxic conditions. In contrast to standard 2D tissue culture studies, the MR-compatible cell perfusion system allows high-density 3D cancer cell growth and exposing cells dynamically and reversibly to various tissue growth environments in a single sample, more similar to the cellular microenvironment of small (<100 mm^3), well-perfused tumors. Moreover, compared with indirect metabolic measurements based on dynamic extracellular pH and O_2 changes (e.g. Seahorse XF analyzer studies), the cell perfusion system allows measuring real-time changes in intra- and extracellular metabolite levels and cellular bioenergetic profiles by sequential multinuclear (^{13}C, ^{31}P) MRS. Our assessment of the dynamic interplay between various environmental stresses and tumor cell metabolic response clearly demonstrates that 4T1 cells are more capable of adapting their metabolic responses to changes in the microenvironment than 67NR cells. This is largely accomplished in 4T1 cells by their greater plasticity and ability to more effectively metabolize glucose through either glycolysis or OXPHOS than 67NR cells, providing greater adaptability to a changing tumor and metastatic microenvironment.

Materials and Methods

Cell Lines

The 67NR, 168FARN, 4T07, and 4T1 cell lines were initially derived from a spontaneous breast tumor growing in a BALB/c mouse [16]. These cell lines were kindly provided by Dr. Fred Miller (Karmanos Cancer Institute, Detroit, MI) and grown in Dulbecco's modified Eagle's media

Table 1. MR Cell Perfusion Experiments

Study	Label (^{13}C)	Stage	Experiment Time (h)	Medium (mM)		Oxygenation
				Glucose	Glutamine	
A	–	A-1	0-1	25	6	Ox
	Glc C1	A-2	1-6	25	2	Ox
	Glc C1	A-3	6-32	25	6	Ox
B	–	B-1	0-1	25	6	Ox
	Gln C3	B-2	1-6	25	6	Ox
	Gln C3	B-3	6-11	25	6	H
	Gln C3	B-4	11-16	0	6	Ox
	Gln C3	B-5	16-21	0	6	H
	–	B-6	21-22	25	6	Ox
C	–	C-1	0-1	25	6	Ox
	Glc C1	C-2	1-6	25	0	Ox
	Glc C1	C-3	6-11	25	0	H
	Glc C1	C-4	11-16	25	2	H
	Glc C1	C-5	16-21	25	2	Ox
	–	C-6	21-22	25	6	Ox

Three different studies were carried out: A, B, and C. The first experimental hour of each experiment was reserved for loading the sample into the MR spectrometer, MR probe tuning, and matching, followed by sample shimming. Study A consisted of well-oxygenated cells perfused continuously for 31 hours with 99% 1-^{13}C-glucose–containing culture medium, initially with 2 mM glutamine (1-6 hours) and then with 6 mM glutamine (6-32 hours). In separate experiments, cells were studied during four sequential 5-hour stress conditions in the presence of either 99% 3-^{13}C-glutamine (study B) or 99% 1-^{13}C-glucose (study C) in the culture/perfusion medium. In study B, the experiment with 3-^{13}C-Gln–labeled medium consisted of the four conditions/stages, B2 to B5, evaluating the effects of the presence or absence of 25 mM glucose under well-oxygenated or hypoxic conditions on glutamine metabolism. In study C, the experiments with 99% 1-^{13}C-glucose–labeled culture medium were carried out in four stages/environmental conditions: C-2 to C-5. The perfusion media for stages C-4 and C-5 contained in addition to 2 mM Gln also a basal level of pyruvate (1 mM). Abbreviations: Glc C1: 1-^{13}C-glucose, Gln C3: 3-^{13}C-glutamine, Ox: well-oxygenated conditions, H: hypoxia.