MINI REVIEW

Adaptive landscapes and emergent phenotypes: why do cancers have high glycolysis?

Robert J. Gillies · Robert A. Gatenby

Abstract Investigating the causes of increased aerobic glycolysis in tumors (Warburg Effect) has gone in and out of fashion many times since it was first described almost a century ago. The field is currently in ascendance due to two factors. Over a million FDG-PET studies have unequivocally identified increased glucose uptake as a hallmark of metastatic cancer in humans. These observations, combined with new molecular insights with HIF-1α and c-myc, have rekindled an interest in this important phenotype. A preponderance of work has been focused on the molecular mechanisms underlying this effect, with the expectation that a mechanistic understanding may lead to novel therapeutic approaches. There is also an implicit assumption that a mechanistic understanding, although fundamentally reductionist, will nonetheless lead to a more profound teleological understanding of the need for altered metabolism in invasive cancers. In this communication, we describe an alternative approach that begins with teleology; i.e. adaptive landscapes and selection pressures that promote emergence of aerobic glycolysis during the somatic evolution of invasive cancer. Mathematical models and empirical observations are used to define the adaptive advantage of aerobic glycolysis that would explain its remarkable prevalence in human cancers. These studies have led to the hypothesis that increased consumption of glucose in metastatic lesions is not used for substantial energy production via Embden-Meyerhoff glycolysis, but rather for production of acid, which gives the cancer cells a competitive advantage for invasion. Alternative hypotheses, wherein the glucose is used for generation of reducing equivalents (NADPH) or anabolic precursors (ribose) are also discussed.

Keywords Aerobic glycolysis · Acid-base · Somatic evolution · Math models

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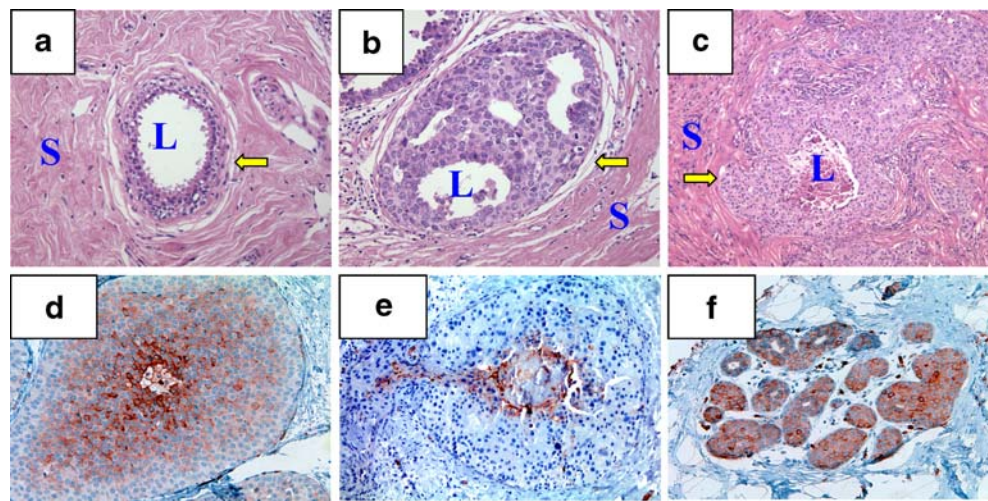Tucson, AZ 85724-5024, USA
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Selection for glycolysis in early carcinogenesis

Carcinogenesis involves a time-dependent but stochastic and disordered progression of cellular populations through a series of phenotypes culminating in emergence of an invasive cancer. Classical models of carcinogenesis, such as the Fearon-Vogelstein diagram (Fearon and Vogelstein 1990), are based on a sequence of mutations or epigenetic changes in expression or activity of oncogenes, protooncogenes, tumor suppressor genes or apoptotic pathways. Implicit in these models is the assumption that these genetic or epigenetic changes result in the augmentation of growth promoting signals and attenuation of growth inhibitors and hence, promote the emergence of increasingly aggressive phenotypes. Although this process is often described as "somatic evolution," models often ignore the fundamental role of selection forces within the microenvironment. It is a primary tenet of evolutionary theories that the phenotypic properties that emerge during evolution are the result of selection by specific growth constraints within the adaptive landscape of the environment. Hence, any phenotypic property commonly observed in an emergent population must *always* confer a competitive advantage. Thus, the high prevalence of aerobic glucose consumption

Fig. 1 Breast Carcinogenesis.
a is normal ductal epithelium;
b is ductal carcinoma in situ and
c is invasive ductal carcinoma.
In each figure Stroma is labeled
'*S*', Basement membrane is *thick
arrow*; and Lumen of duct is
labeled '*L*'. **d**–**f** show GLUT 1
distribution in DCIS and inva-
sive breast cancer. **d** Peri-
luminal upregulation of GLUT-1
with a distribution that parallels
that expected for hypoxia.
e GLUT-1 is periluminal as well
as in microinvasive process.
f In invasive cancer, nearly all
of the cells demonstrating
increased GLUT-expression

observed in human metastatic cancers (Czernin and Phepls 2002; Hawkins and Phepls 1988) is strong evidence that this metabolic behavior must confer a substantial adaptive advantage. We propose that emergence of this phenotype occurs in response to specific and identifiable environmental selection pressures during the process of carcinogenesis.

The proliferative advantage conferred by aerobic glycolysis is not immediately obvious. Glycolysis is substantially less efficient in energy production (2 moles of ATP per mole of glucose compared to about 36 moles of ATP per mole of glucose using aerobic pathways) and produces toxic by products, such as H^+. Indeed, as little as 1.0 μM free H^+ is invariably fatal to mammalian cells. If we assume that phenotypic traits are selected based on their contribution to fitness within a given adaptive landscape, there is no apparent reason for Darwinian forces to consistently select for use of the glycolytic pathways when aerobic metabolism is available. We have examined this progression puzzle by investigating microenvironmental parameters that would give rise to these diverse phenotypes and select for cells with high glycolysis in the presence of oxygen (Gatenby and Gillies 2004).

Carcinomas are derived from epithelial tissues that are physically separated from underlying stroma by a basement membrane (Fig. 1). Because of this anatomic property, blood vessels are confined to the stroma and separated from epithelial populations throughout early phases of carcinogenesis, which is hence, an *avascular* process. Carcinogenesis begins with hyperplasia, either through chronic inflammation or through genetic alterations. As this occurs, the cell layer becomes thicker and thicker and this physically pushes cells towards the lumen and further away from their blood supply. This requires substrate and metabolites to diffuse over increasing distances to reach the

cells adjacent to the lumen. Dating back to the work of Krogh (1919a,b) reaction-diffusion models have predicted that, among all substrates, oxygen is most limited due to its low solubility. In living tissue under physiologic conditions, the diffusion distance of oxygen (i.e. the distance at which oxygen concentrations go to zero) is predicted to be 150–200 microns (the Krogh cylinder). This has been verified with histology of tumor samples by Thomlinson and Gray (1955) and experimentally by Helmlinger et al. (1997). An example of reaction–diffusion modeling for developing epithelial tumors separated from their blood supply by a basement membrane is shown in Fig. 2. This shows that oxygen concentrations decline steeply with
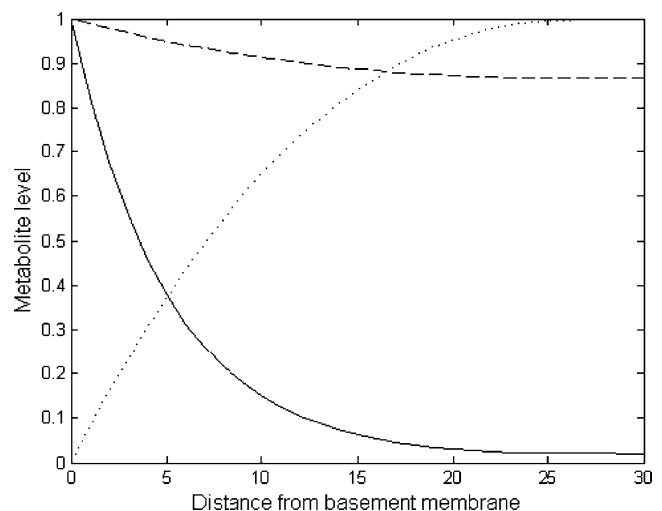

Fig. 2 Substrate and metabolite profiles found in premalignant intraductal tumors using reaction-diffusion modeling. Oxygen concentrations (*solid line*), glucose concentrations (*dashed line*) H^+ concentrations (*dotted line*) are shown

distance from the basement membrane resulting in severe hypoxia in regions only 5 to 10 cell layers from the membrane. Despite up-regulation of glycolysis with hypoxia by the Pasteur Effect, glucose concentrations decline only modestly with distance. However, there is a steep increase in H^+ concentrations in the region of hypoxia, suggesting that cell survival and proliferation in DCIS and other premalignant lesions is governed by hypoxia and acid-mediated toxicity. Thus, cells in a hyperplastic epithelium that are more than ca. 200 microns away from the basement membrane are oxygen-limited. Cells at further distances are acid limited. Biological hypoxia can be seen with immunohistochemical staining for hypoxia inducible proteins, such as GLUT-1 (Fig. 1d). GLUT-1 is up-regulated by the hypoxia inducible transcription factor, HIF-1 (see below), and consistently observed in periluminal cells of late-stage DCIS (Wykoff et al. 2001). Another consistent finding in such studies is the presence of necrotic cells adjacent to the GLUT-1 positivity, which is highly suggestive that these cells are under severe selection pressure.

Model simulations also demonstrate that the oxygen concentrations are cyclical because of metabolic switching. That is, hypoxia induces upregulated glycolysis, which leads to less oxygen consumption (Crabtree Effect), leading to increases in oxygen concentrations which then causes the cycle to repeat. Because of this and periodic variations in underlying oxygenated blood supply (Baudelet et al. 2004; Braun et al. 1999; Brurberg et al. 2006), it is likely that the border between oxygenated and de- oxygenated cells fluctuates somewhere between 150 and 200 microns. Thus, cells in these intervening volumes will be subjected to cycles of anoxia and re-oxygenation. Hypoxic re-oxygenation is associated with increased reactive oxygen species (ROS) stress. We have observed that subjecting MCF-10T breast epithelial cells in-vitro to hypoxia-reoxygenation with a periodicity of 1 cycle/week resulted in cells with 2X higher rates of aerobic glucose consumption and lactate production.

Thus, we hypothesize that the periodic hypoxia experienced by cells early in carcinogenesis results in selecting for cells with upregulated glucose consumption. It is not yet known whether this selection is due to the ability of cells to continue generating ATP during periods of hypoxia, or is due to the increased ability of cells to generate reducing equivalents (NADPH) to protect them from ROS stress during re-oxygenation.

Mathematically, this period of somatic evolution can be represented as a series of *adaptive landscapes*: using Evolutionary Game Theory (Gatenby and Vincent 2003). This approach defines a tissue landscape by the number and phenotypic properties of extant cellular populations, micro-environmental conditions and the evolutionary potential of each population.

Evolution by natural selection is, in this model, a "game" in that it has players, strategies, strategy sets, and payoffs. The players are the individual cells. Strategies are heritable phenotypes. A player's strategy set is the set of all strategies to which it can evolve during a biologically feasible time period.

This approach explicitly links cellular proliferation to its "fitness," which is defined by the interactions of the population's phenotypes with environmental selection forces.

In this game, winners proliferate and losers do not. The fitness of an individual eventually influences its progeny's frequency within the population as its strategy is passed from generation to generation. Hence, phenotypic properties are retained or lost depending on their contribution to individual fitness. Somatic evolution represents the gain or loss and persistence of a given strategy over time within a population of individuals that have many different potentially strategies.

The speed at which populations will evolve to higher fitness maxima is dependent on their phenotypic variance and the slope of the fitness curve. Thus, populations with a high level of phenotypic variation or in environments of strong clonal selection (e.g., inflammation or chronic trauma) will evolve most rapidly. Phenotypic heterogeneity can be achieved by a number of mechanisms, such as a "mutator phenotype" (e.g. chromosomal instability), a wide variety of epigenetic alterations (e.g. methylation or gene silencing), or through reduced activity of chaperone proteins (e.g. Hsp90), all of which have been associated with increased cancer risk.

Classical evolutionary game theory is focused primarily on temporal rather than spatial variations in adaptive landscapes. To better evaluate the regional variations in the microenvironmental selection forces and to track the evolutionary history of individual cell populations, we have used a modified cellular automaton approach to model intraductal carcinogens. Here, individual cells are imbued with the potential to evolve new properties based on their history and their environment (Anderson 2005; Patel et al. 2000; Smallbone et al. 2005).

We have used a "hybrid" cellular automaton approach in that it also incorporates reaction-diffusion kinetics so regional variations in oxygen, glucose and H^+ are generated. These interact with the cellular components of the model such that metabolism will alter local substrate and metabolite concentrations that, in turn, interact with the cell's phenotypic properties affecting survival and growth.

For modeling intraductal carcinogenesis, we used specific rule-sets that govern the probability for (Q) quiescence, (P) proliferation or (D) death. In normal cells, Q is the default decision. P is allowed only if the cell is adjacent to the basement membrane, there is an adjacent vacant

element, and the local substrate and metabolite concentrations are within physiologic range. D will ensue with a finite probability (senescence) that increases sharply if the crucial substrates (glucose and oxygen) fall below critical values. Simulations based on this model demonstrate that the initial events in carcinogenesis will be mutations that reduce cellular sensitivity to local growth control via cell-cell interactions. These events, which correspond to heritable changes in oncogenes in models such as the Fearon–Vogelstein diagram, serve to relax the prior requirement that a cell can proliferate only when it is in contact with the basement membrane and an adjacent vacant element. As a result, cells continue to proliferate even in the presence of inhibitory signals that would ordinarily prevent growth.

The models demonstrate that these changes yield only self-limited tumor growth because, as the cells extend further and further into the lumen, proliferation is halted by hypoxia and acidosis. The simulations demonstrate that the selection forces (i.e., hypoxia and acidosis) in the new adaptive landscape promote the emergence of a phenotype that is highly glycolytic and resistant to acid-induced toxicity. The models demonstrate that this population has a powerful growth advantage because it creates an acidic environment that is toxic to other populations but not to itself. This allows the population to invade even into normoxic regions. Notably, these automata predict patterns of GLUT-1 expression that are faithfully recapitulated in both patient samples and in spheroid culture models (Gatenby et al. in preparation).

While this model of carcinogenesis may explain why epithelial cancer cells assume a glycolytic phenotype early during carcinogenesis, there are a few inconsistencies that remain unexplained. First, it does not explain at all why malignant sarcomas and gliomas have high glycolysis. Because these cancers develop within mesenchymal tissues, they would not be subjected to the hypoxic selection that is experienced by epithelial-derived carcinomas, yet they consistently have high rates of glucose consumption, lactate production and FDG trapping (Beckner et al. 2005; Griguer et al. 2005; Kelloff et al. 2005; Rajendran et al. 2003). While this etiopathology may explain why sarcomas and gliomas are more prevalent in children, the elevated glucose metabolism in these cancers is an interesting conundrum. Second, this model does not explain why high glycolysis would persist in advanced invasive cancers and metastases. Figure 1e shows elevated expression of GLUT-1 is associated with invasive cells and Fig. 1f shows that metastatic foci invariably have elevated GLUT-1 expression. As discussed below, the answer to both of these problems may lie in the conversion of glucose to acid.

Glycolysis and the microenvironment during metastasis

As discussed above, both empirical studies and mathematical models demonstrate that the periodic hypoxia during early carcinogenesis will select for cells with elevated glycolysis (or pentose pathway activity). This occurs through the process of somatic evolution, the rate of which is directly dependent on the extent of phenotypic heterogeneity. As cells become metastatic, they retain the capacity to evolve, yet their adaptive landscape is altered.

This raises an important question: why do invasive and metastatic cancer cells continue to exhibit high rates of glucose consumption after the diffusion-reaction kinetic limitations imposed by intraductal carcinogenesis are no longer present? If glucose consumption is high for the purpose of energy production, persistence of such a phenotype lacks all logic. It is well-demonstrated that growth of micro-metastases beyond 1–2 mm diameter requires neo-angiogenesis, indicating that growth of metastatic lesions is oxygen-limited (Folkman and Folkman 2003; Naumov et al. 2006). Hence, even "hypoxic" cancers have well oxygenated regions that contain the proliferating cells (Brown and Giaccia 1998; De Jaeger et al. 1998; Fyles et al. 1998, 2002; Green and Giaccia 1998; Haugland et al. 2002; Kapp and Giaccia 1996; Kavanagh et al. 1996; Le et al. 2003; Semenza 2000). Since proliferating cells are responsible for evolution of phenotypes, metastatic cancers are evolving in an oxygenated microenvironment.

Notably, hypoxia (measured with ^{18}F misonidazole), and glucose consumption (measured with ^{18}F deoxyglucose) are not highly correlated in the same tumors (Bos et al. 2002; Cherk et al. 2006; Rajendran et al. 2003), indicating that maintenance of the glycolytic phenotype is not due to lack of oxygen. Aerobic glycolysis is inefficient and toxic, which would preclude its selection.

Glycolysis produces only 2 ATP/glucose, whereas complete oxidation produces 38 ATP/glucose. The metabolic products of glycolysis, such as lactic acid and hydrogen ions (H^+), cause a spatially heterogeneous but consistent acidification of the extracellular space which may result in cellular toxicity (Bhujwalla et al. 2002; Griffiths et al. 2001; Schornack and Gillies 2003). Despite these factors that would logically select *against* it, there is something about metastatic cancers that selects *for* a glycolytic phenotype.

What if the Warburg Effect has nothing to do with ATP? Most arguments to explain the Warburg Effect have assumed that aerobically consumed glucose was used for energy production (Pelicano et al. 2006), despite the fact that careful mass balance analyses demonstrate that a relatively minor fraction (<30%) of a cancer cells' aerobic ATP

production is derived from glycolysis (Guppy et al. 2002). What other by-product of glucose could provide evolutionary favoritism? There are two alternative possibilities:

1. Glucose is metabolized by the pentose phosphate pathway (PPP), which can provide a source of essential anabolic substrates, such as ribose for nucleic acid synthesis as well as reducing equivalents (NADPH) to combat reactive oxygen species (Langbein et al. 2006; Serkova et al. 2005).
2. Consumption of glucose through glycolysis produces hydrogen ions, and this hyperacidity provides a selective advantage to invading tumor cells.

Without discounting the importance of the PPP, we have propose that metastatic cancer cells consume glucose in order to produce H^+, thus providing a selective invasive advantage. It has been unequivocally demonstrated that solid tumors are significantly more acidic than normal tissues (Gillies et al. 2002). This low tumor pH favors cancer invasion. Although the mechanisms are ill-defined, modern data suggest that this involves release of cathepsins (B, D or L) through increased rate of endosomal turnover (Bhujwalla et al. 2001; Garcia et al. 1996; Hill et al. 2001; Jang and Hill 1997; Montcourrier et al. 1994, 1995; Rochefort et al. 1996; Rozhin et al. 1994; Schlappack et al. 1991; Turner 1979). Reversal of tumor acidosis via chronic bicarbonate-induced alkalinization reduces the rate of spontaneous metastases (Robey et al. in prep). Furthermore, pre-treatment of tumor cells with acidosis promotes experimental metastases (Rofstad et al. 2006; Schlappack et al. 1991). Finally, a penumbra of acidic pH surrounds growing tumors, and this correlates spatially with apoptosis and degradation in surrounding stroma (Gatenby et al. 2006a). This acid- mediated invasion hypothesis is also supported by mathematical models (Gatenby and Gawlinski 1996; Gatenby 1998; Gatenby and Vincent 2003; Patel et al. 2000).

Mechanisms of aerobic glycolysis

Although this review has focused on the "why?," rather than the "how?" of the Warburg Effect, it does lead to certain predictions that can be tested mechanistically. For example, this approach predicts that the phenotype is more important than the mechanism, i.e. the end justifies the means. Since we predict that the phenotype, not the genotype, is evolutionarily selected, then multiple mechanisms for upregulating glucose consumption would be observed. This appears to be the case, as elevated glucose consumption has been associated with, e.g. HIF-1α (Robey et al. 2005; Semenza 2002; Semenza et al. 2001), c-myc (Dang et al. 1997; Dang and Semenza 1999; Osthus et al. 2000), or Akt (Elstrom et al. 2004). Indeed, it may even be the case that these oncogenic factors are selected simply *because* they upregulate glucose consumption. We have investigated this question in a series of primary breast cancer cells, with the rationale that they will represent the genotypic/phenotypic heterogeneity observed in vivo (Table 1). Indeed, these cell lines exhibit a broad range of glucose consumption and lactate production rates, similar to those observed by FDG uptake in breast cancer patients. In these cell lines, phospho- and total c-myc and Akt levels were determined by Westerns and c-myc and HIF-1 activities were determined by ELISA. Of these factors, both c-myc and HIF-1 were highly correlated with glycolytic rates and inhibition of c-myc and HIF activities either pharmacologically or with siRNA resulted in decreased glycolysis (unpublished observations).

Table 1 Gene expression and metabolism in a series of low passage human breast cancer cell lines

Cell No.	2715	2925	732	2648	3133	1179	2087	2150	3199	3171	893	812
Path	id	id	A	id	l	A	A	id	id	met	id	id
Location	pl	as	pl	pl	pl	pl	pl	pl	ax	ax	br	br
Lac	1	3	3	4	4	6	7	7	8	8	12	14
Myc	-	-	-	-	-	+	+	++	-	-	++	+
pAkt	+	+	-	-	+	+	+	+	-	+	+	+
HIF	-	-	-	-	+	-	+	-	++	++	+	+
Her-2	-	-	+	-	+	+	-	+	-	-	+	+
EGF-R	-	-	-	-	-	-	+	-	+	-	-	-
ER/PR	+/+	-/-	-/+	+/+	+/-	+/+	-/-	+/+	-/-	-/-	-/-	+/-

blank not determined, *i/d/l/A* infiltrating/ductal/lobular/adenocarcinoma, *pl* pleural, *as* ascites, *br* primary, *ax* axillary node

Thus, in this system, glycolysis can be elevated by either c-myc and/or HIF-1 activities, consistent with the hypothesis that "the end justifies the means."

Summary

Development of cancers is a multi-stage multi-step process of somatic evolution. At each stage, cells are subjected to severe selective pressures and only those with phenotypes that confer increased fitness are able to survive. In early carcinogenesis, intermittent hypoxia selects for cells that have constitutively upregulated glycolysis. Mechanisms that can lead to this phenotype include stabilization of HIF-1α, activation of c-myc or activation of Akt. In turn, this glycolytic phenotype leads to severe acidosis that selects for cells that are invasive through a variety of mechanisms including increased turnover of endocytic vesicles and release of cathepsins. In metastases, cells that aerobically consume glucose are selected, and this may be due to their increased production of anabolic substrates, anti-oxidants or their production of H^+, which is toxic to the host tissue.

It is clear from FDG PET imaging that altered glucose metabolism is present in the vast majority of human cancers. We propose that it is essential to understand the evolutionary forces that both select for this phenotypic property during carcinogenesis and maintain it in invasive and metastatic cancers. By understanding the key parameters that govern these Darwinian dynamics, rational therapies can be designed to exploit these phenotypic properties for therapy or deeper understanding of carcinogenesis itself.

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