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Hypoxia and adaptive landscapes in the evolution of carcinogenesis

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Abstract Conceptual models of epithelial carcinogenesis typically depict a sequence of heritable changes that give rise to a population of cells possessing the hallmarks of invasive cancer. We propose the evolutionary dynamics that give rise to the phenotypic properties of malignant cells must be understood within the context of specific selection forces generated by the microenvironment. This can be accomplished by using an "inverse problem" approach in which we use observed typical phenotypic traits of primary and metastatic cancers to infer the evolutionary dynamics. This has led to the hypothesis that heritable changes in genes controlling cellular proliferation, apoptosis, and senescence, while necessary, are not usually sufficient to produce an invasive cancer. In addition to these evolutionary steps, we propose that the common observation of aerobic glycolysis in human cancers indicates, via the inverse problem analysis, that adaptation to hypoxia and acidosis must be a major component of the carcinogenic sequence. The details of the hypothesis are based on recognition that premalignant populations evolve within ducts and remain separated from their blood supply by a basement membrane. As tumor cells proliferate into the lumen, diffusion-reaction kinetics enforced by this separation result in hypoxia and acidosis in regions of the tumor the most distant from the basement membrane. This produces new evolutionary selection forces that promote constitutive upregulation of glycolysis and resistance to acid-induced toxicity. We hypothesize that these phenotypic adaptations are critical late steps in carcinogenesis conferring proliferative advantages even in normoxic conditions by allowing the population to produce an acidic environment (through aerobic glycolysis) which is toxic to other local cell populations and promotes extracellular matrix degradation, increasing invasiveness.

Keywords Hypoxia · Angiogenesis · Carcinogenesis · Acid-base balance · Glycolysis

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1 Introduction

Over the past decade, seminal papers by Fearon and Vogelstein [1] and Hanahan and Weinberg [2] have resulted in powerful iconography to describe the molecular etiopathology and essential characteristics of cancer. Figure 1 is drawn from the Fearon–Vogelstein model and Fig. 2 shows Hanahan and Weinberg's icons for six essential hallmarks of cancer, along with an additional new hallmark, i.e. elevated glucose consumption. Figure 2 also introduces icons for the environmental sequelae of pre-cancer growth that must be overcome for carcinogenesis to ensue. Both conceptual models focus on specific genotypic and phenotypic traits that must develop during the multistep process in which normal cells evolve to become malignant. This work both resulted from and contributed to a remarkable growth in the understanding of the molecular changes that occur during carcinogenesis to produce the observed phenotypic hallmarks of transformed cells.

This process of accumulating genetic and epigenetic changes during carcinogenesis is often described as "somatic evolution" because it is generally a prolonged, multistep process in which genotypes and phenotypes are sequentially generated and selected, ultimately leading to emergence of a malignant population [3, 4]. Much progress has occurred in understanding the alterations in the

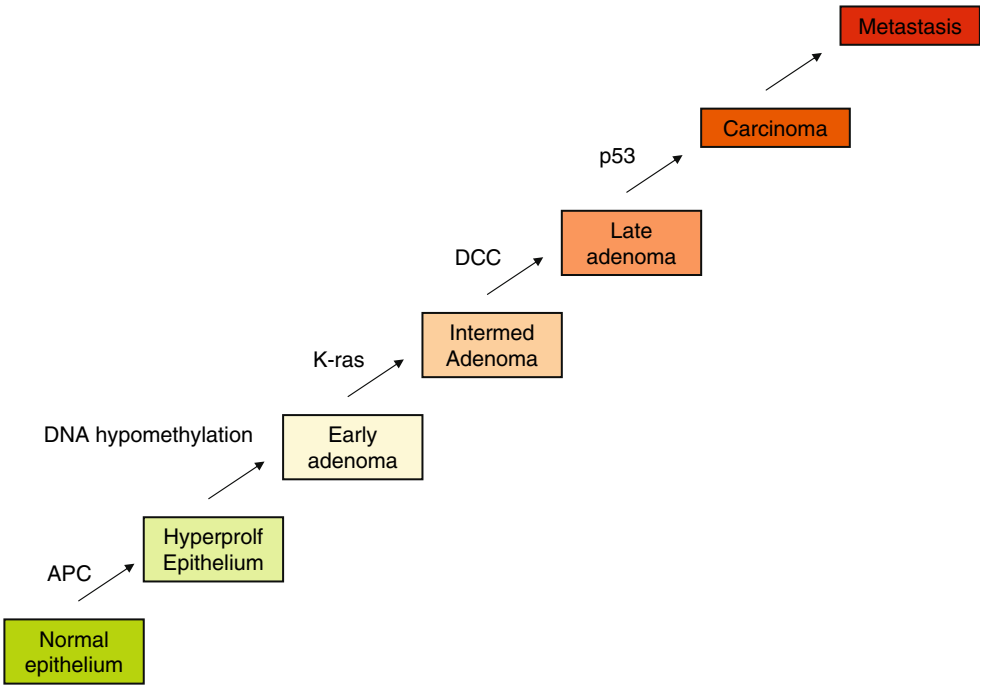

Fig. 1 Genetic events of colorectal carcinogenesis (adapted from Fearon and Vogelstein [1]). Multiple stages of carcinogenesis are shown along with the oncogene and tumor suppressor changes associated with progression from one stage to the next

molecular pathways that drive the intracellular evolutionary dynamics. However, these cellular events do not fully account for the biological forces governing somatic evolution because Darwinian dynamics also require understanding the environmental context of the observed phenotypic adaptations. That is, a basic tenet of evolutionary theory is that environmental selection forces interact with phenotypic properties and this interaction drives evolution by producing clonal expansion, death or stability of populations depending on the fitness of their phenotype. This aspect of Darwinian selection, viz. microenvironmental selection forces that actually drive observed phenotypic evolution, are not included in the original Fearon–Vogelstein and Hanahan–Weinberg iconographies.

Here, we focus on the physiological microenvironments of normal tissue and premalignant tumors to examine the selective pressures that such environments bring to bear on the etiopathology and the resulting phenotypes. Our goal is to understand the properties of the malignant phenotype not in terms of their molecular pathways but rather as adaptive responses to specific Darwinian selection forces that arise during the prolonged process of carcinogenesis. In other words we are interested in the "why" of phenotypic properties of tumor cells rather than the "how."

In many ways this can be considered an "inverse problem" approach. That is, we know the "answer" to the problem in the sense that common phenotypic properties of cancer cells are readily observed. These include, for example, the hallmarks of cancer enumerated by Hanahan and Weinberg. What we seek to know are the evolutionary dynamics that promote these properties during carcinogenesis, invasive growth and in the metastatic cascade. Specifically, we wish to understand the underlying dynamics that govern the interactions of observable properties of cancer cells with environmental selection forces and how specific phenotypic traits confer a selective growth advantage. This is based on the implicit assumption that, because

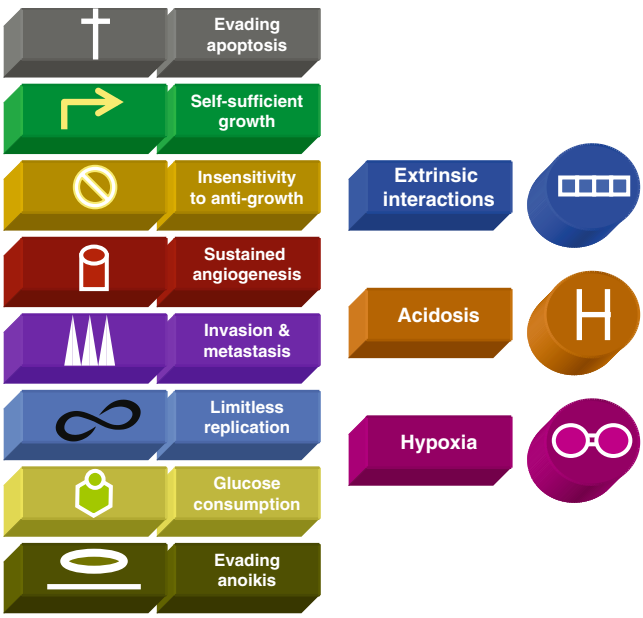

Fig. 2 Hallmarks of Cancer (adapted from Hanahan and Weinberg [2]). Icons and their descriptions on the *left* indicate hallmarks of cancer, the first six of which were adapted from Hanahan and Weinberg, and the last two (glucose consumption and evading anoikis) are introduced here. The *three icons on the right* indicate three important components of the physiological microenvironment that must be overcome for cancers to progress

cancer cells arise through a Darwinian process, only properties that confer a proliferative advantage will be commonly observed.

Over the past few years, we have developed a model of carcinogenesis and metastasis that invokes sequential evolutionary selection for phenotypes that culminate in populations of cells with characteristic phenotypic traits [5–7]. An essential component of our models is identification of dominant proliferative constraints in the physiological microenvironment [8–12]. Thus, in this communication, we will attempt to revisit the Fearon/Vogelstein and Hanahan/Weinberg models to include the altering physiological adaptive landscape throughout carcinogenesis and metastasis.

2 The anatomy of carcinogenesis

Epithelial cancers arise on mucosal surfaces that impose specific environmental constraints, as evidenced in Fig. 3. Typically, normal epithelium consists of a few layers of a single population of cells that are physically separated from the underlying stroma (including blood vessels and fibroblasts) by an intact basement membrane. This separation requires signaling molecules, substrates and metabolites to diffuse between the developing tumor on one side of the basement membrane and the stroma and blood vessels on the other side. These diffusion-reaction kinetics will persist throughout carcinogenesis until the basement membrane is breeched during the transition from carcinoma in-situ to invasive cancer.

3 The dynamics of carcinogenesis

The evolutionary events of carcinogenesis can be viewed as a simple population biology process in which new phenotypes continuously arise through random genetic and epigenetic changes in the epithelial or myo-epithelial cells, which then interact with selection forces in the local environment. This can be modeled using evolutionary game theory and here we present the results of our simulations based on these models, the mathematics of which are contained in prior publications [4, 6, 7, 13].

We can define the adaptive strategies of early carcinogenesis based on the environmental factors that control growth. In normal epithelium, proliferation of cells is constrained by three general sets of forces:

1. *Extrinsic interactions.* This encompasses the set of positive and negative growth signals that normal cells exchange with each other and the extracellular matrix. These factors ordinarily maintain a stable cell population within a normal mucosal surface.
2. *Senescence.* Normal cells, even if unconstrained by the local environment, can ordinarily proliferate only a fixed number of times before telomere shortening enforces cell death.
3. *Substrate availability.* Cells must have sufficient substrate (i.e. oxygen and carbon sources) to both maintain normal function and produce new individuals. Ordinarily, the blood supply in normal tissue is sufficient to maintain an abundance of substrate and metabolites. However, under pathologic conditions such as ischemia

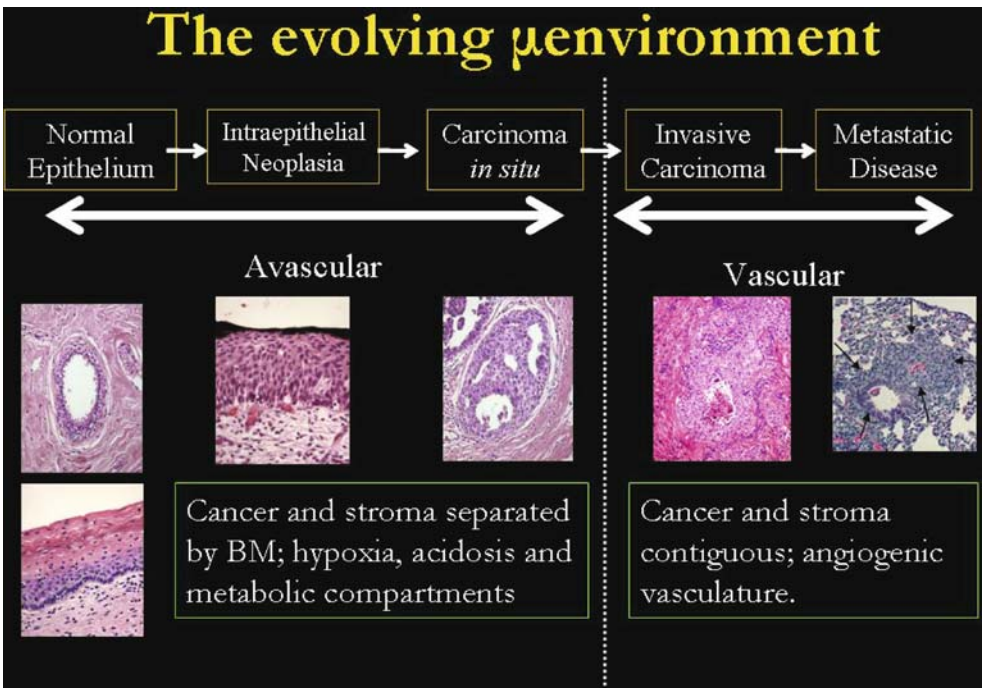

Fig. 3 The evolving microenvironment of breast cancer. The multiple stages of breast carcinogenesis are shown progressing from *left to right*, along with histological representations of these stages. As indicated the pre-invasive stages occur in an avascular environment, whereas cancer cells have direct access to vasculature following invasion

and hyperplasia, substrate concentrations may decline to levels that limit proliferation or even induce cell death.

4 From initiation to hyperplasia

In keeping with our inverse problem approach we can infer the genetic and epigenetic changes necessary for early carcinogenesis by turning to experimental observations. In colon carcinogenesis, for example, mutations in APC and k-ras are commonly found in small polyps indicating they are early events in the carcinogenesis cascade [1]. These gene products form critical components of pathways that either (a) suppress proliferation through contact inhibition, or (b) promote growth through translation of pro-proliferation growth factor signalling.

Viewed as an inverse problem, these observations lead to the conclusion that normal cell proliferation is entirely regulated by positive and negative growth signals from other cells and the extracellular matrix. That is, mutations in oncogenes and tumor suppressor genes during the early stages of carcinogenesis (initiation) must represent adaptations to overcome normal cell proliferation constraints. These changes can account for one of the hallmarks of cancer (Fig. 4), i.e. insensitivity to anti-growth signals. In addition, resistance to apoptosis may become apparent at this stage, if the initiation event was associated with genotoxic stress. The end result of these adaptive chances is a hyperplastic epithelium, culminating in interstitial neoplasias.

5 Hyperplasia to early carcinoma in situ

Even after normal tissue growth constraints are defeated, unrestricted tumor growth will not result because eventually additional growth constraints predominate in the adaptive landscape. The first is quite obvious—normal mammalian cells have limited proliferative potential due to onset of senescence regulated primarily by telomere shortening. This will require adaptations that overcome restrictions imposed by senescence pathways such as upregulation of telomerase. In fact the "telomere crisis" and subsequent changes in telomerase expression and activity are well-established and account for the limitless replicative ability that constitutes another of the hallmarks of cancer (Fig. 2) [14, 15]. Following this adaptation, the epithelial layer is unconstrained and will grow within the physical boundaries of the ductal lumen, i.e. a carcinoma in situ (Fig. 4).

6 Early to intermediate carcinoma in situ

Transition through the early stages of carcinoma in situ requires upregulation of glycolysis, which is characteristically observed in the malignant phenotype. First reported by Warburg nearly a century ago, increased fermentation of glucose (resulting in lactic acid production) is the result of both intratumoral hypoxia and a shift to glycolytic pathways even in the presence of oxygen (the Warburg effect) [16]. Increasing attention to this phenomenon has occurred during the past decade. In part this is due to clinical observations by FDG PET that increased

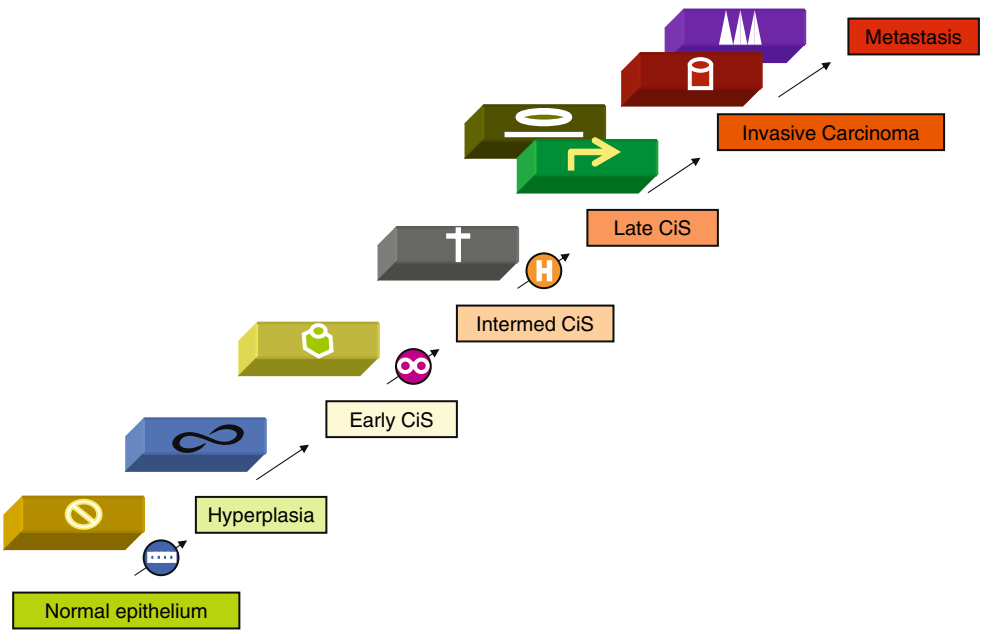

Fig. 4 Stages of cancer progression showing proposed environmental constraints which have to be overcome for carcinogenesis to ensue (extrinsic constraints, hypoxia and acidosis) as well as proposed timing for the accumulation of cancer's hallmarks

glucose flux is extraordinarily common in a wide range of clinical primary and metastatic cancers [17]. Increased interest also reflects remarkable progress in dissecting the molecular pathways (such as HIF-1) that control glucose metabolism in normal and transformed cells.

From an evolutionary point of view, aerobic glycolysis presents a dilemma because it (1) is substantially less energetically efficient than normal oxidative glucose metabolism (producing only two moles of ATP/mole of glucose compared to 36 moles of ATP/mole of glucose) and (2) produces copious amounts of acid which results in potentially toxic reductions of extracellular pH. We have approached this conundrum with the firm conviction that the Darwinian dynamics that govern somatic evolution unequivocally require that any common phenotypic property must confer a substantial proliferative advantage. The goal of the inverse problem, therefore, is to understand the adaptive advantage that is provided by increased aerobic glycolysis.

Analysis of the evolutionary dynamics of carcinogenesis using mathematical models, experiments in evolving tumor spheroids, and clinical observation have yielded a sequence of cellular and extracellular events which could plausibly lead to the common emergence of aerobic glycolysis in human cancers. Our proposal is fundamentally based on recognition that somatic evolution of epithelial cancers occurs entirely within a space contained by a basement membrane. This anatomic constraint results in separation of the evolving tumor cells from the underlying stroma including blood vessels so that carcinogenesis occurs in an avascular environment (Fig. 3). This enforces diffusion-reaction kinetics that limit substrate delivery to, and metabolite removal from, tumor cells. These substrate gradients become more severe as proliferation carries cells progressively further into the ductal lumen. As a result, even following multiple oncogene and tumor suppressor gene mutations and upregulation of telomerase, tumor cell proliferation is fundamentally limited by regional fluctuations in substrate and/or metabolites.

Based on well-established diffusion-reaction models we and others have demonstrated that oxygen concentrations will decline much more rapidly with distance from the basement membrane than will glucose concentrations. As a result, significant hypoxia will exist in populations even within five cell layers of the membrane. There is empirical and theoretical evidence that oxygenation of the periluminal layers will be periodic, with periods of anoxia followed by reoxygenation and this can have significance to upregulation of survival pathways [18–22]. To maintain ATP production, this environment will require transient and eventually fixed upregulation of glycolysis, likely mediated through constitutive increases in HIF-1 levels [23].

7 Intermediate to late carcinoma in situ

Upregulation of aerobic glycolysis alone is not sufficient for progression because it results in increased acid production and, therefore, a reduction of extracellular pH [24, 25] which can induce apoptosis through p53-dependent caspase activation [26]. Hence, this toxicity, in turn, requires adaptations that reduce sensitivity to extracellular acidosis such as upregulation of Na/H exchangers (NHE-1) or resistance to acid-mediated apoptosis through mutations in p53 or other pro-apoptotic pathways [22, 27]. Given the involvement of mitochondria in the regulation of both glycolysis and p53-mediated apoptosis, it is tempting to speculate a commonality of mechanisms. Our analysis indicates that these events may be connected but that they are not necessarily so.

8 Invasive cancer

We propose that the final outcome of this evolutionary sequence is a population with constitutive upregulation of glycolysis and the ability to survive and proliferate in acidic conditions. This constitutes a significant adaptive advantage because the phenotype creates an acidic environment (through upregulation of glycolysis) that is toxic to its competitors but less so to itself. We propose that this adaptive advantage promotes clonal expansion and invasive behavior and that these adaptations are necessary for evolution of invasive epithelial cancers. This conceptual model is supported by experimental observations of upregulation of cellular responses to hypoxia in regions of DCIS (ductal carcinoma in situ) most distant from the basement membrane. This includes upregulation of HIF (hypoxia-inducible factor) and related proteins such as carbonic anhydrase IX and GLUT-1 (glucose transporter 1) [11, 12]. Although acidic environments can promote invasive behavior [28–31], this alone is not sufficient for cancer cells to exit their luminal mileau and invade into the stroma. Cells must adopt a resistance to anoikis, which is a specialized paradigm of apoptosis induced by alterations in cell-ECM interactions [32, 33]. Additionally, at some point during this progression cascade, cells must acquire an ability to proliferate in the absence of positive growth signals (Fig. 4). The result of this phase is the invasion of cancer cells into the stroma, where they have increased access to a blood supply. The upregulation of HIF-1α and its transactivated genes, e.g. VEGF, leads to increased angiogenesis which promotes the growth of the invading cancer, as well as providing access for cells to escape and colonize distant sites.

9 Metastasis

A legacy of the above pre-invasive evolutionary events is aerobic glycolysis (the Warburg phenomenon [7]) in which cancer cells continue to use glycolytic metabolic pathways even in the presence of oxygen. Although aerobic glycolysis is common in primary tumors, it is a virtual hallmark of metastatic lesions. There are significant differences in the microenvironments between these primary and metastatic lesion sites. For example, pre-invasive cancers are avascular, whereas the proliferating and evolving components of invasive cancers and metastases have access to the vasculature. Given its inefficiency and toxicity, the persistence of aerobic glycolysis requires that it must continue to perform an essential function in metastatic lesions.

We propose that the competitive advantage for glycolysis is conferred by glucose-derived acid production. This can explain the increased glucose uptake observed by FDG PET in the overwhelming majority of metastatic human cancers [8, 9]. In-vivo studies with FDG PET have demonstrated the transition from DCIS to invasive breast cancer and colonic polyps to invasive colon cancers are invariably associated with a marked increase in tumor glucose uptake [13, 14].

The final stages of carcinogenesis are driven by cellular adaptations to hypoxia and acidosis—changes that are usually mediated by upregulation of HIF-1. These phenotypic changes result in the final two hallmarks of cancer: 1. the ability to invade and 2. persistent angiogenesis. We propose that the continued production of glucose-derived acid is an important component of the invasive-metastatic phenotype.

10 Conclusion

Based on an inverse problem analysis, we propose that upregulation of glycolysis in primary and metastatic cancers must serve an adaptive advantage critical for evolution of the invasive phenotype. We propose this trait arises as an adaptation to regional hypoxia and acidosis that develop in evolving tumors on mucosal surfaces as a result of their separation from underlying vascular supply by an intact basement membrane. This anatomic constraint enforces diffusion reaction kinetics that will inevitably lead to hypoxia as cellular proliferation carries individuals into the lumen and further from the source of substrate. Upregulated glycolysis is a straightforward adaptation to the hypoxic environment but proliferation is again constrained by the environmental acidosis that results from anaerobic metabolism of glucose. This new environmental selection force selects for phenotypes that are resistant to acid-mediated toxicity.

The final outcome of this evolutionary sequence is a phenotype that creates an acidic environment through aerobic glycolysis that is toxic to its competitors but not itself. This adaptive advantage is critical both for the invasive phenotype and persistent stimulation of angiogenesis—both hallmarks of the invasive phenotype. The advantages conferred by these phenotypic traits, in turn, explains the persistence of aerobic glycolysis in human cancers despite the negative consequences of this phenotype which includes inefficient energy production and increased acid production.

These results suggest that tumor prevention strategies aimed at interrupting the hypoxia-glycolysis-acidosis cycle and the resulting cellular adaptations could be explored to delay or prevent transition of in situ to invasive cancer. Furthermore, acknowledging the metabolic and physiologic hallmarks of cancer may lead to novel therapies once cancers are established [34, 35].

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