

Cancer may be a pathway to cell survival under persistent hypoxia and elevated ROS: A model for solid-cancer initiation and early development

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A number of proposals have been made in the past century regarding what may drive sporadic cancers to initiate and develop. Yet the problem remains largely unsolved as none of the proposals have been widely accepted as cancer-initiation drivers. We propose here a driver model for the initiation and early development of solid cancers associated with inflammation-induced chronic hypoxia and reactive oxygen species (ROS) accumulation. The model consists of five key elements: (i) human cells tend to have a substantial gap between ATP demand and supply during chronic hypoxia, which would inevitably lead to increased uptake of glucose and accumulation of its metabolites; (ii) the accumulation of these metabolites will cast mounting pressure on the cells and ultimately result in the production and export of hyaluronic acid; (iii) the exported hyaluronic acid will be degraded into fragments of various sizes, serving as tissue-repair signals, including signals for cell proliferation, cell survival and angiogenesis, which lead to the initial proliferation of the underlying cells; (iv) cell division provides an exit for the accumulated glucose metabolites using them towards macromolecular synthesis for the new cell, and hence alleviate the pressure from the metabolite accumulation; and (v) this process continues as long as the hypoxic condition persists. In tandem, genetic mutations may be selected to make cell divisions and hence survival more sustainable and efficient, also increasingly more uncontrollable. This model also applies to some hereditary cancers as their key mutations, such as BRCA for breast cancer, generally lead to increased ROS and ultimately to repression of mitochondrial activities and up-regulation of glycolysis, as well as hypoxia; hence the energy gap, glucose-metabolite accumulation, hyaluronic acid production and continuous cell division for survival.

The most popular theories about cancer and cancer drivers in the past century include: (i) Warburg's theory as summarized by him in 1966¹: "Cancer ... has countless secondary causes; But there is only one prime cause, (which) is the replacement of respiration of oxygen in normal body cells by a fermentation of sugar"; (ii) the genomic mutation theory of cancer, including mutations that lead to the formation of oncogenes and loss of function in tumor suppressor genes, which has been the most popular in the past four decades;

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and (iii) microbe-induced cancers such as cervical cancers induced by the infection of human papilloma virus² or liver cancer induced by hepatitis viruses.³

Among these major proposals, Warburg's proposal has been most intriguing and has received considerable renewed interests in the past few years.^{4,5} However, the proposal clearly lacks key information that connects the observed energy-metabolism reprogramming to cell proliferation, hence it remains as a proposal rather than a testable model. Various genetic mutation-centric driver models have been proposed since the first discoveries of oncogenes by Bishop and Varmus⁶ and tumor-suppressor genes by Knudson⁷ about 40 years ago. These models include APC gene mutation-based driver model for colon cancer⁸ and the Philadelphia chromosome-based model for chronic myelogenous leukemia.⁹ As of now, hundreds of "driver mutations" have been predicted for various cancers.¹⁰ One fundamental problem with these mutation-centric driver proposals is that activated oncogenes alone, even coupled with mutations in some tumor-suppressor genes, cannot lead to cell proliferation in a tissue environment as numerous conditions must be satisfied before the cells can divide, including: (i) the cells must be attached to their extracellular matrix¹¹; (ii) the associated

extracellular matrix (ECM) needs to have certain physical properties to support cell division^{12,13} (iii) the cells must overcome the contact-inhibition constraint designed to prevent cell over-growth¹⁴; (iv) cell survival signal(s) need to be present¹⁵; and (v) the cells need to be in specific morphology,¹⁶ among a few other conditions needed for tissue development, remodeling or repair.¹⁷ None of the suggested driver models have proposed mechanisms to overcome these tissue-level constraints, hence making them not applicable as candidates for cancer initiation drivers.

Virus-induced cancer models tend to lack molecular level details. For example, HPV-associated cervical cancers are among the most studied virus-induced cancers, but yet no models have been published that functionally link HPV infection to initiation of cervical cancer, other than a recent publication that observed integration of HPV DNA into the host genome.¹⁸ Overall, no experimentally testable models for cancer initiation have been published.

Here, we present a model for the initiation of solid cancers in general. The starting point of the model is the reprogrammed energy metabolism, which was proposed by Otto Warburg some 50 years ago to be the primary cause of all cancers. This reprogramming between the two energy metabolisms could be the result of chronic hypoxia and/or reactive oxygen species (ROS) accumulation,^{19,20} which may be induced by chronic inflammation or by genetic mutations in the case of hereditary cancers. It is worth-noting that chronic inflammation has long been linked to cancer development,^{21,22} and it can lead to hypoxia^{23–25} and ROS accumulation.²⁶ These conditions, if not inducing cell death, would lead to increased uptake of glucose from circulation and cellular accumulation of glucose metabolites.²⁷ However, the connection from here to cell proliferation has been elusive. Our model in this article suggests that the glucose metabolite accumulation will ultimately lead to the production and export of hyaluronic acid under persistent hypoxic conditions,²⁸ which is supported by the available transcriptomic data of both hypoxia-treated cell lines and cancer tissues.

It has been well established in the literature of tissue injury and repair that when a tissue is injured, its ECMs will be fragmented and the hyaluronic acid fragments of different sizes released from the ECM will serve as signals for tissue repair, including signals for cell proliferation, cell survival and angiogenesis.²⁹ Our analysis has shown that cancer-forming cells have used the hyaluronic acid synthesized from the accumulated glucose metabolites, which will be fragmented once exported into extracellular space, to mimic the ECM-released hyaluronic acid fragments as tissue repair signals, hence leading to cell proliferation, which will continue as long as the inflammation-induced hypoxic condition persists.

In addition, a proposal is made regarding how this model relates to genomic mutation-induced constitutive cell proliferation after the cell proliferation is already started. To the best of our knowledge, this represents the first such molecular

level model that connects chronic inflammation, hypoxia and/or ROS accumulation to cell proliferation.

Energy Gap During Hypoxia, Metabolite Accumulation and Evolutionary Pressure

Why do human, mouse and rat develop cancer but some other vertebrates such as naked mole rat, blind mole rat and turtle do not or rarely do? This question has puzzled medical practitioners and researchers for decades. A number of studies have been published, aiming to explain why some organisms such as amphibians, naked and blind mole rats do not develop cancers.^{30–34} The proposed mechanisms tend to be organism-specific, such as alterable immune systems being used by amphibians, special abilities to resist ROS in blind mole rats and a capability in producing long hyaluronic acid polymers by naked mole rats.^{30–34} We suspect that there is something more fundamental than these proposals, some common characteristics shared by the basic metabolisms of these organisms, which are distinct from those cancer-prone organisms.

We have examined the above question from the perspective of ATP demand and supply during hypoxia in human, mouse, rat, hypoxia-tolerant rat, naked mole rat, blind mole rat, frog and turtle. These organisms are selected because they are known to either develop cancer or rarely do and their ATP-consumption data are publicly available or can be reliably estimated based on transcriptomic data. The question we address here is: *What percentage of the ATP-consuming proteins is substantially repressed during hypoxia versus normoxia?*

According to the published data, proteins in the following six processes/enzyme families consume on average 84% of the ATPs in vertebrate cells: translation, Na⁺/K⁺ ATPase, Ca²⁺ ATPase, gluconeogenesis, urea synthesis and actin ATPase.³⁵ Among the eight organisms under consideration, naked mole rat and hypoxia-tolerant rat have both ATP consumption data by these proteins and their corresponding gene-expression data under hypoxia (1–5% oxygen) *versus* normoxia (21% oxygen)^{36,37} in the public domain. In addition, ATP consumption data under hypoxia *versus* normoxia are also publicly available for frog and turtle^{38,39} but without gene-expression data. For human, mouse, rat and blind mole rat, only gene-expression data under hypoxia *versus* normoxia are publicly available (see Supporting Information Section A for details).

We have derived a regression model between the reduced ATP consumption and the reduced gene-expression levels of the six groups of proteins for naked mole rat and hypoxia-tolerant rat (see the rationale and model in Supporting Information Sections B and C); and then applied this model to the reduced gene-expression levels of the same proteins in mouse, rat and blind mole rat, respectively, to estimate their reduced levels of ATP demand during hypoxia. The basic assumption here is that the relationship between gene-expression levels and the activity levels of the six groups of

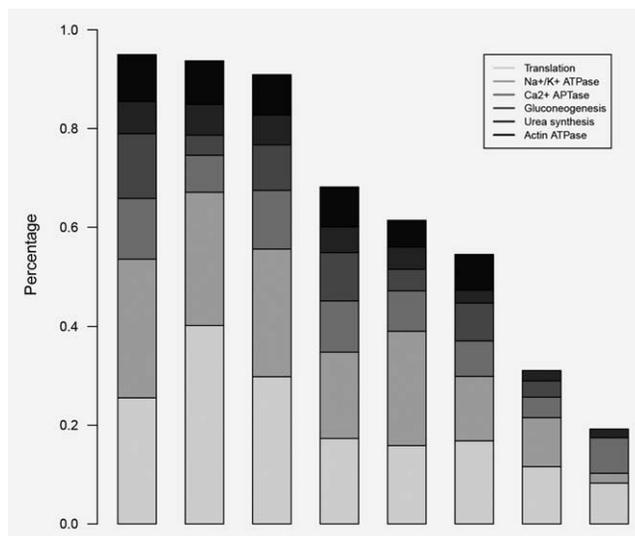


Figure 1. For human, rat, mouse, naked mole rat, hypoxia-tolerant rat, blind mole rat, frog and turtle (from left to right), 1.0 along the y-axis represents the total ATP demand by the six groups of proteins during normoxia. The height of each bar represents the percentage of ATP demand under the matching hypoxic condition, hence the difference between 1.0 and the percentage representing the percentage in reduction. Each bar is divided into six color-coded sections, each representing one group of relevant proteins under consideration. ATP consumption data for turtle, frog, naked mole rat and hypoxia-tolerant rat are collected from the literature and the data for human, rat, mouse and blind mole rat are predicted.

proteins in mouse and rat is highly similar to that in naked mole rat, hypoxia-tolerant rat and blind mole rat. This is a reasonable assumption as these five organisms are closely related except that the latter three have an additional regulatory mechanism that can turn down the gene expression levels of these proteins under hypoxia *versus* normoxia while the former two do not.^{40–44} It is worth noting that there is a strong correlation between gene-expression changes and protein abundance changes in metabolic proteins⁴⁵ (see details in Supporting Information Section D).

Using the regression model developed for naked mole rat and hypoxia tolerant rat as well as reduced expression data of mouse, rat and blind mole rat under hypoxia *versus* normoxia, we have predicted the reduction percentages in ATP consumption in mouse, rat and blind mole rat, respectively. Figure 1 shows the prediction result along with the known reduction percentages in the ATP consumption levels in naked mole rat, hypoxia-tolerant rat, turtle and frog. The predicted reductions in ATP consumption are highly consistent with the general knowledge about these organisms. For example, blind mole rats can substantially lower their metabolic rates, hence ATP consumption, when switching from normoxia to hypoxia⁴⁶; and they are known to be more tolerant to hypoxia than naked mole rats as they can stay viable at 3% oxygen³¹ while naked mole rats require a minimum of 10% oxygen, which can occasionally get down to 5%.⁴⁷ In

addition, naked mole rats are known to be able to live both above the ground and deep in the underground, while rats tend to live much closer to the ground when they are underground (more supporting data in Supporting Information Section D).

Based on this meaningful prediction, we further extrapolate this prediction to include human (see Fig. 1). The prediction indicates that under hypoxia, human cells have the least reduction in their ATP consumption.

It is worth noting that this figure is used to make a qualitative (rather than quantitative) statement that human, mouse and rat have substantially smaller reductions in their ATP consumption compared to the other five organisms when the condition switches from normoxia to hypoxia. To have more accurate estimates of ATP-consumption reductions by these organisms, we need substantially more data under multiple hypoxic conditions *versus* matching normoxia, which are currently not publicly available (the above are all the available data we can find in the public domain). Considering that these organisms use essentially the same glycolytic fermentation processes, we hypothesize that the percentages in the ATP-supply reduction are comparable across these organisms when the condition switches from normoxia to hypoxia. Hence, the estimated level of reduction percentage in ATP consumption reflects the gap between the ATP demand and ATP supply in each organism, that is, the smaller the reduction percentage, the larger the gap.

Knowing that frog and turtle can live well under very hypoxic conditions for extended periods of time, it is reasonable to assume that they have no gap between their energy supply and demand under hypoxia.⁴⁸ Similarly the energy gap in blind mole rats must be none or small as they can also live well under hypoxia for extended periods of time.^{42,44} Naked mole rat is an interesting case as it not only has reduced energy demand but also has an additional capability to avoid the glucose metabolite accumulation issue that humans have to deal with during hypoxia (see later sections). In contrast, human (and mouse, rat) all have substantial energy gaps during hypoxia, and they cannot live under hypoxia for long.^{48,49} This makes sense as the humans as a population have not lived under hypoxic conditions (*e.g.*, 10% oxygen or lower) for extended periods of time in the past millions of years of evolution, and hence human cells (possibly except for certain tissue types) have not been trained to adapt to such a condition by switching off some portions of the ATP-consuming metabolic reactions to keep the ATP demand within its supply under hypoxia.

Because of the large energy gap, human (and mouse, rat) cells would substantially increase their glucose uptake during hypoxia to make up for the reduced energy efficiency due to the switch from aerobic respiration to anaerobic fermentation, to meet the ATP needs of the hungry cells, which causes these cells to accumulate various glycolytic metabolites as widely observed.²⁷ The accumulation, we believe, is another result of human evolution. That is, there is a

mismatch between the influx rate of glucose, which is regulated by the ATP deficiency, and the maximum flux rate of the glycolytic fermentation pathway, which has been shaped by evolution. Knowing that human cells have not been under hypoxic conditions for long time, it is reasonable to infer that the glycolytic fermentation system has been serving only as a supplement to the aerobic respiration system for ATP production for very short periods of time, so its capacity has been evolutionarily determined correspondingly. Hence, we posit that the maximum flux rate of this system is intrinsically unable to meet the need for dealing with the substantially increased influx of glucose during hypoxia, resulting in the accumulation of glucose metabolites and other molecules (see next section).

Continuous accumulation of the metabolic derivatives, if not removed, will lead to cell death.^{50,51} Thus, we propose that the need for removing the accumulated metabolic derivatives forms the initial pressure for the affected cells to evolve; cell division may represent a most feasible way to remove the accumulated glycolytic metabolites using them towards DNA synthesis. In addition, we propose that it is this capability in switching off certain metabolic activities to keep the ATP demand within the ATP supply under hypoxia that decides if an organism has the potential to develop cancer or not.

In addition, as detailed in Supporting Information Section E and F, amino acid and fatty acid metabolisms can also contribute to the congestion of glycolysis pathway under hypoxia.

Hyaluronic Acid: A Key Facilitator of Cell Proliferation

Hyaluronic acid is a long chain of a repeating disaccharide, up to 2×10^5 disaccharides, each derived from one D-glucuronic acid (GlcUA) and one D-N-acetylglucosamine (GlcNAc).⁵² Hyaluronic acid is a key component of an ECM structure and mediates cell-ECM signaling. This large polymer has long been known to be associated with cancer development.^{53,54} The most relevant function of the molecule in this context is signaling roles played by its fragments related to tissue repair.

Briefly, when assaulted, an injured tissue releases ECM fragments, among which hyaluronic acid fragments serve as signals for repairing the injured tissue. Most interestingly, hyaluronic acid fragments of different sizes have been found to serve as signals for different purposes, including the induction of inflammation, anti-apoptosis, cell survival, cell-cycle activation, cell proliferation, activation of angiogenesis and cell motility, all related to injury response, maintenance of tissue integrity and tissue repair.^{29,55} It is worth emphasizing that all short hyaluronic acid fragments ($< \sim 5,000$ disaccharides) serve some signaling roles relevant to the above list. To see the connection between glucose metabolite accumulation and cell proliferation, we need to examine the synthesis pathway of hyaluronic acid (Fig. 2).

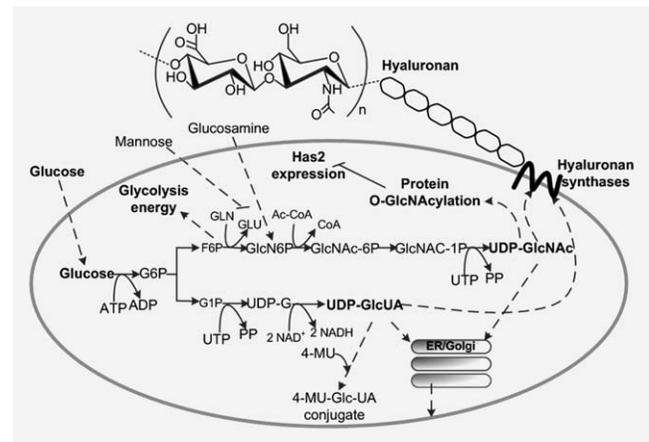


Figure 2. The synthesis pathway of hyaluronic acid from UDP-GlcUA and UDP-GlcNAc, both derived from the common precursor glucose (adapted from Ref. 56).

The upper part of the pathway goes from G6P (glucose 6-phosphate) to UDP-GlcNAc and the lower part goes from G6P to UDP-GlcUA. The upper part consists of five enzymes to catalyze the five reactions from left to right in the figure: phosphoglucose isomerase (*GPI*), glutamine-fructose-6-phosphate transaminase (*GFPT*), glucosamine phosphate *N*-acetyltransferase (*GNPNAT*), phosphoacetyl glucosamine mutase (*PGM3*) and acetylglucosamine pyrophosphorylase (*UAPI*). The lower part consists of three enzymes for the three reaction steps from left to right: phosphoglucomutase (*PGM*), UDP-glucose pyrophosphorylase (*UGP2*) and UDP-glucose dehydrogenase (*UGDH*). Three hyaluronic acid synthases (*HAS1–3*) are known to synthesize hyaluronic acid from one UDP-GlcNAc and one UDP-GlcUA.

Now we examine the conditions that can trigger the hyaluronic acid synthesis pathway. *GPI* is part of the glycolysis pathway and hence is activated whenever glycolysis is activated. The following three enzymes, *GNPNAT*, *PGM3* and *UAPI*, as part of the hexosamine pathway, can be activated when glucosamine is abundantly available and under hypoxia.^{57,58} *PGM* can be up-regulated by hypoxia,⁵⁹ so are *UGP2*⁶⁰ and *GFPT*.⁶¹ *UGDH* is positively regulated by *TGFβ* (transforming growth factor β).⁶² *HAS1–3* can be activated by various growth factors such as *TGFβ*, *PDGF* (platelet derived growth factor), *KGF* (keratinocyte growth factor), *FGF2* (fibroblast growth factor 2), *EGF* (epidermal growth factor), *IL1β* (interleukin-1 β) and *TNFα* (tumor necrosis factor α).⁵⁶ In addition, the level of UDP-GlcNAc has been found to control the expression of *HAS2*.⁵⁶

In sum, the upper part of the pathway will be activated when there is ample G6P under hypoxia; and the lower part will be activated under hypoxia and the availability of *TGFβ*. So the hyaluronic acid synthesis pathway can be activated by a condition with a plenty of G6P and availability of *TGFβ* under hypoxia. And hyaluronic acid synthases can be activated by *TGFβ* in conjunction with UDP-GlcNAc, the

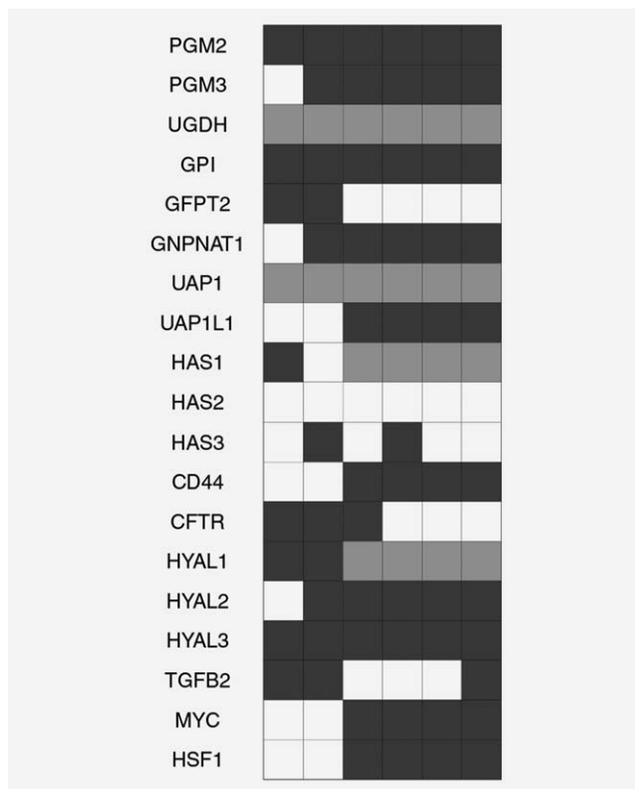


Figure 3. Differentially expressed hyaluronic acid metabolism related genes in inflammatory disease, colon adenoma, colon adenocarcinoma tissue samples of stages 1–4 (from left to right). Dark gray and light gray are for significantly up and down regulation in the disease samples comparing to normal colon samples (by a moderated *t*-test using a significance cutoff level at 0.05⁶⁹), respectively, while white represents no significant changes.

product of the upper part of the pathway. Clearly all these conditions are satisfied for an inflammatory tissue whose cells are accumulated with glucose metabolites under hypoxia, hence strongly suggesting the possibility that hyaluronic acid will be synthesized. It is worth noting that *TGFβ* is generally available in chronic inflammation.^{63,64}

Note that the synthesis of hyaluronic acid is done through repeated addition of one glucuronic acid and one *N*-acetylglucosamine to the nascent polydisaccharide as the molecule is extruded *via ABC* transporters or hyaluronic acid synthase into the extracellular space.^{65,66} The exported hyaluronic acid will be degraded by some of the hyaluronidases (*HYAL1–6*) or by ROS into fragments of different sizes if it is not incorporated into extracellular matrices.⁶⁷

We have examined gene-expression data collected on multiple normal human cells (see Supporting Information Sections G and H) treated with hypoxia between 3 and 48 hr, and found that hyaluronic acid synthases (*HAS1*) and hyaluronidases (*HYAL1, 3–4*) are up-regulated. These observations are consistent with past studies showing that hypoxia affects hyaluronic acid synthesis and turnover.⁶⁸ In addition, we have examined a set of gene-expression data collected on a

set of diseased colon tissue samples ranging from precancerous tissues to advanced adenocarcinoma (see Supporting Information Section H), and found that the hyaluronic acid synthesis pathway is indeed activated in the very early stage of the disease, starting from inflammatory colon disease or at latest colon adenoma. Figure 3 shows a heat-map of the expression level changes of the relevant genes in precancerous/cancer tissues *versus* the normal controls.

From Figure 3, we can see that all genes in the hyaluronic acid synthesis pathway are up-regulated in precancerous tissues except for *UAP1* and *UGDH*. Interestingly, one of *UAP1*'s homologs *UAP1L1* is up-regulated. Hence, we predict that *UAP1L1* is used for the same function. For *UGDH*, no homologous genes are found to be up-regulated but we suspect that another gene serving the same purpose is used here in its place as the rate-limiting factor gene *GFPT2* is up-regulated and hyaluronic acid is clearly being produced here as multiple hyaluronidase genes *HYAL 1–3* and one reported hyaluronic acid exporter gene *CFTR* (cystic fibrosis transmembrane conductance regulator)⁷⁰ are up-regulated. In addition, *TGFβ* is up-regulated in the precancerous stage; and its expression level goes back to the background level once the down-stream genes of hyaluronic acid synthesis such as *HSF1* (heat shock transcription factor) and *MYC* (*v-myc* avian myelocytomatosis viral oncogene homolog) are up-regulated, indicating that once the tissue becomes cancerous, cell proliferation will be driven by some factors other rather than hypoxia-induced hyaluronic acid production and fragmentation.^{71–73} Very interestingly, highly similar expression patterns of these genes are observed in precancerous, early stage and advanced stage melanoma (see Supporting Information Material Section I and Supporting Information Fig. S3). These are the only two gene-expression datasets covering both precancerous and cancer tissues at different stages we found on the Internet.

The above discussion and Figure 3 strongly suggest a pathway going from inflammation-induced hypoxia to cell proliferation: cells will accumulate glucose metabolites under hypoxia, which will ultimately leads to the synthesis and export of hyaluronic acid; the exported hyaluronic acid will be degraded into fragments. As all short hyaluronic acid fragments with size ranging from 4 to 5,000 disaccharides serve as signals for some aspects of tissue repair,²⁹ the “right” combinations of fragments will be generated in time by some cells, just like the combinations of hyaluronic acid fragments generated by a damaged ECM, hence starting the signaling process of tissue repair, including cell proliferation, cell survival and angiogenesis. Note that the fragmentation patterns of a damaged ECM should have a high degree of randomness as a tissue can be injured by different causes, hence possibly giving rise to different fragmentation patterns of hyaluronic acid, which all lead to the activation of the tissue repair system. Hence, we assume that the probability that the hypoxia-induced hyaluronic acid fragmentations will trigger the tissue repair system is high.

In addition, hyaluronic acid on the cell surface is known to facilitate anchorage-independent proliferation,^{74,75} hence bypassing the requirement for cells being connected to their ECM before they can proliferate; similarly it also facilitates the loss of the contact-inhibition constraint for cell proliferation.⁷⁶ Essentially hyaluronic acid and their fragments provide all the signals needed for cell proliferation *via* the utilization of “tissue repair” system. Interestingly several recent studies have shown that hypoxia can stimulate the synthesis of hyaluronic acid,^{68,77} hence providing a strong support to our model.

It was recently shown that inhibition of tumor growth caused by the abnormally large hyaluronic acid produced in naked mole rat tissues is reversed by treatment with *HYAL2*.³² Hence, we posit that hyaluronic acid turnover and the induced cell proliferation provide an exit for the accumulated glycolytic metabolites through stimulation of cell proliferation and subsequent utilization of the metabolites as building blocks for cell division. Our model is also consistent with recent studies showing that elevated cell sugar concentrations can increase the production of hyaluronic acid.⁵⁶

Hypoxia-Induced Signals Facilitate Additional Signals for Cell Division

In addition to the hypoxia-induced activation of tissue repair system discussed above, persistent hypoxia can lead to the generation of other signals to assist cell proliferation for their survival (*i.e.*, to provide an exit for the accumulated glucose metabolites). For example, hypoxia can lead to the accumulation of necrotic cells that release in-danger signals and trigger the production and release of growth signals such as *FGF2* (fibroblast growth factor 2) and *HDGF* (hepatoma-derived growth factor),^{78,79} hence providing additional signals to enhance the synthesis of hyaluronic acid (see the above section). In addition, a resistance signal to apoptosis can also be triggered by the necrotic cells to maintain tissue integrity.⁷⁹

A recent study suggests that hypoxia may play more direct roles in mediating cell division. Specifically, the authors demonstrated that hypoxia can change the actin cytoskeletal organization, leading to morphological changes of the cells⁸⁰ and hence preparing the cells for division. The study also showed that hypoxia leads to increase in cell volume, which we suspect is partially due to the metabolite accumulation discussed in the earlier sections. In addition, previous studies have established that the state of actin filament organization directly controls cell-cycle progression.^{81,82} Furthermore, the level of hypoxia has long been linked to the level of malignancy of a cancer.^{23,83} By integrating all this information, we can see that hypoxia may play a direct and essential role in the modulation of cell division. If this proves to be true, hypoxia probably plays a double role in early carcinogenesis: creating the pressure for cells to evolve to remove the accumulated glucose derivatives and facilitating the removal of the accumulated derivatives through cell division. Clearly this warrants further investigation.

Hypoxia is also known to mediate a few other activities that may facilitate sustained cell survival, such as up-regulation of telomerase,⁸⁴ genomic instability,⁸⁵ angiogenesis⁸⁶ and cell migration,⁸⁷ which will lead to more uncontrollable behaviors of the cells. Our recent transcriptomic analysis of precancerous and early stage cancer data strongly suggests that cellular hypoxia takes place no later than any of the cancer hallmark events (see Supporting Information Section J), hence providing independent evidence in support of the theory we are proposing.

In addition, lactate, generated due to the increased glycolytic fermentation activity due to the reprogrammed energy metabolism, is known to play a number of key roles in driving carcinogenesis such as (i) promotion of chronic inflammation,⁸⁸ (ii) wound over-healing,⁸⁹ (iii) secretion of *VEGF* and tumor angiogenesis⁸⁹ and (iv) immune escape,⁹⁰ hence providing additional signals in support of cell proliferation as well as cell survival.

Genomic Mutations and Cancer Development

Although genetic mutations are believed by many to be a primary reason for sporadic cancer development, some recent studies start to challenge this view.^{91,92} Within the driver model proposed here, genetic mutations, we believe, may dominantly play a facilitator's role rather than a primary driver's role in sporadic cancer. Specifically we suggest that the change-of-function genetic mutations in many cancer-related genes such as *P53* and *RAS* are selected as “permanent” replacements for on-going functions currently accomplished through regulation or other functional means, to facilitate cell division, hence survival, in a more sustained and more efficient manner. We use the following examples to illustrate the idea.

The functional form of *PKM2* (pyruvate kinase, muscle 2) is a homo-tetramer and it serves as a key enzyme in glycolysis, which catalyzes the conversion from phosphoenolpyruvate to pyruvate. It has been observed that the vast majority of advanced cancers have loss-of-function mutations in the *PKM2* gene.⁹³ This suggests that there is an evolutionary pressure for the affected cells to reduce their pyruvate production. It has been shown that oxidation of *PKM2* in specific locations by ROS can increase the possibility of disassociation of the tetramer to dimers or monomers,⁹⁴ hence reducing its normal function. This suggests the possibility that *PKM2* may gradually lose its functions due to oxidation, and natural selection may have gradually selected such cells as the loss of *PKM2* function may give such cells a survival advantage. Loss-of-function mutations in the *PKM2* gene may be just a permanent replacement for losing the function of the protein currently achieved through function-losing oxidation.

Another example is the loss of the contact-inhibition capability of cells that can terminate cell division when they are in proximity one to another.⁹⁵ The increased hyaluronic acid export can functionally diminish cellular contact-

inhibition⁷⁶ as discussed earlier, which allows sustained division by the underlying cells even when they get close to each other. At the end, mutations in genes responsible for activating the contact inhibition mechanism, such as *ING4* (inhibitor of growth family, member 4),⁹⁶ are selected, thus ensuring the permanent loss of this inhibition capability, as observed in advanced cancers in general.⁹⁷

Knowing that the mutation rates in cancer related genes go up as a cancer advances,⁹⁸ we suggest that genetic mutations are permanent replacements for on-going functions (or their repression) executed through functional regulation or other means, is generally applicable to cancer development.

Hereditary Cancer and the Hypoxia-Based Driver Model

Although the above inflammation/hypoxia-based driver model is for sporadic cancers, it is natural to ask if this or a similar model may apply to hereditary cancers. To address this issue, we have examined seven major types of hereditary cancers with known key mutations: breast cancer due to *BRCA* (breast cancer, early onset) mutations, kidney cancer due to mutations in *FH* (fumarate hydratase), colon cancer due to *APC* (adenomatous polyposis coli) mutations, retinoblastoma due to *RBI* (retinoblastoma 1) mutations, Li-Fraumeni syndrome due to *P53* mutations, Cowden syndrome due to *PTEN* (phosphatase and tensin homolog) mutations and Von Hippel-Lindau syndrome due to *VHL* (Von Hippel-Lindau tumor suppressor) mutations. A large number of studies have been performed, focused on linking these mutations to the development of the corresponding hereditary cancer.⁹⁹⁻¹⁰⁵ However, no general cancer initiation model has been proposed for these hereditary cancers, to the best of our knowledge. Our literature survey did reveal one commonality among all these loss-of-function mutations: they all result in increased production or accumulation of ROS. Potentially, this common role by loss-of-function mutations in the seven genes may prove to be the most essential role in the tumorigenesis of the relevant cancers. Details follow.

Recent studies have shown that *BRCA* mutations in normal breast cells can lead to the generation of hydrogen peroxide as well as increased glycolysis and decreased oxidative phosphorylation,¹⁰⁶ revealing the repression of the mitochondrial activities, which forces cells to increase their activity of glycolytic fermentation regardless of being cancer or non-cancer cells.

Regarding *FH*, recent publications have shown that loss-of-function *FH* mutations lead to pseudo-hypoxia and increased ROS constitutively, which further leads to increased glycolysis and decreased oxidative phosphorylation,¹⁰⁷ hence leading to induction of glycolytic fermentation pathway in time.

The loss-of-function mutations in the *APC* gene have been found to lead to constitutive activation of the *WNT* signaling pathway,¹⁰⁸ which activates a downstream gene called *RAC1*, a GTPase. The activation of *RAC1* has been shown to

lead to ROS production.¹⁰⁹ Hence, we posit that the same process leading to the reprogramming of the energy metabolism will take place in time like in the above cases.

The current understanding about the relation between *RBI* mutations and ROS production is that loss-of-function mutations in *RBI* leads to dysregulation of *E2F2*, a component of the transcription factor gene *E2F* involved in cell cycle regulation and DNA synthesis, which drives increased production of ROS.¹¹⁰

P53 gene mutations have long been linked to the production of ROS.¹¹¹ For example, mutations in *P53* can interfere with the normal response of human cells to oxidative stress through attenuating the activation and function of *NFE2* (nuclear factor, erythroid 2) related factor 2, a transcription factor that induces antioxidant response. This effect is manifested by decreased expression of phase 2 detoxifying enzymes *NQO1* (NAD(P)H dehydrogenase, quinone 1) and *HMOX1* (heme oxygenase [decycling] 1) and increased ROS levels; hence the energy-metabolism reprogramming will take in time as above.

The relationship between *PTEN* mutations and ROS production is an interesting one. A recent study has found that mutations in the ATP-binding motif of *PTEN* lead to disruption of the correct subcellular localization of the protein, which results in a significantly decreased nuclear *P53* protein level and transcriptional activity, and enhanced production of ROS.¹¹²

VHL-deficiency was recently found to constitutively activate *NOX* oxidases to maintain the *HIF2 α* (hypoxia inducible factor-2 α) protein expression while NADPH oxidases of the *NOX* family are the major sources of ROS.¹¹³ Hence, the same process of energy-metabolism reprogramming will take place in time.

The increased ROS accumulation associated with the above mutations, possibly in conjunction with some cell type-specific environment, will ultimately lead to repression of the mitochondrial activities, including oxidative phosphorylation, and further to energy-metabolism reprogramming as in the case of hypoxia-induced reprogramming of energy metabolism.¹¹⁴ In addition, it has been well established that mitochondrial ROS triggers hypoxia-induced transcription¹⁹ and inflammation.²⁶ Hence, we propose that the same cancer-driver model proposed earlier applies to these hereditary cancers except that the initial trigger is increased ROS instead of persistent hypoxia.

For the same reason, we suspect that aging-induced cancers may also follow this or a similar model as mitochondrial ROS accumulates and inflammatory cells increase, on top of cellular senescence¹¹⁵ as one ages; and at some point these cells may repress their mitochondrial activities when high enough ROS levels have accumulated, leading to the reprogrammed energy metabolism and associated phenomena discussed above. We believe that the various observed changes in these hereditary cancers as reported in the literature⁹⁹⁻¹⁰⁵ contribute to this general driver model proposed here.

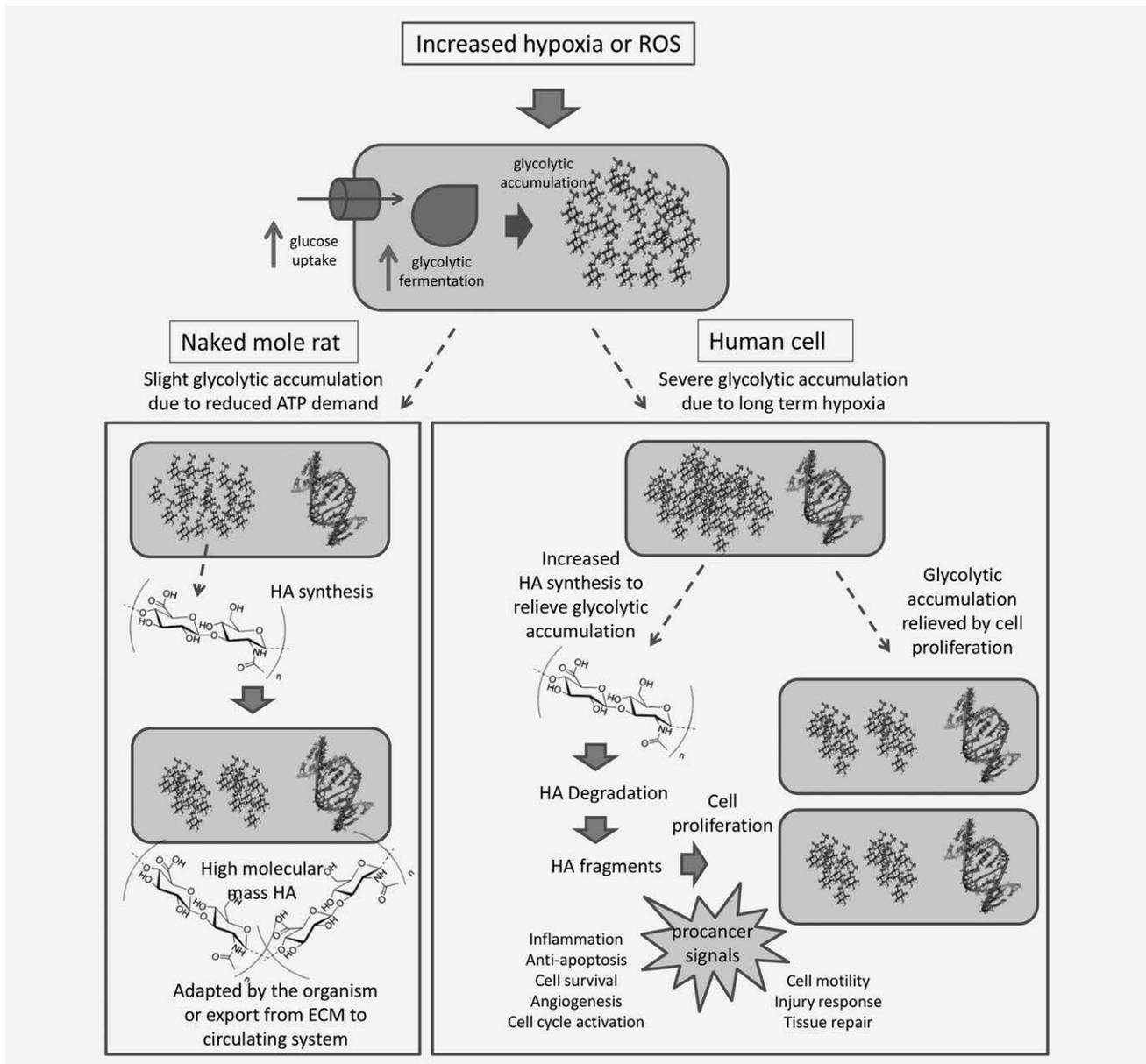


Figure 4. A driver model for the early phase of human carcinogenesis versus the exit pathway of the excess glucose derivatives in naked mole rats.

Interpretation of Naked Mole Rat Data Using Our Model

It is well known that naked mole rats do not or rarely develop cancer.^{14,32} We suspect that their reduced energy demand during hypoxia is one reason, as they will not accumulate as much glycolytic metabolites as in human, mouse and rat. A recent study³² suggests that naked mole rats may have additional abilities to resist cancer development. This study has found that naked mole rats synthesize and export an unusually long form of hyaluronic acid, multiple times longer than that in human. It was observed that knockdown of the species-specific mutant form of *HAS2* or fragmentation of hyaluronic acid by *HYAL2* leads to cancer development

from naked mole rat cells, which otherwise failed to form tumors under the same conditions.

Our model provides a natural and logical explanation of their observation. Specifically, we believe that naked mole rats have evolved systems to move the *HAS2* synthesized very long hyaluronic acid, hence with no overlap with the short hyaluronic acid as signaling molecules for tissue repair, into the circulation without being degraded, which may ultimately get deposited to their skins. The reason that blocking the activation of *HAS2* will lead to cancer development is, we speculate, that it will trigger alternative ways to synthesize hyaluronic acid, such as by *HAS3*, which tend to be much shorter, hence possibly directly serving as signaling molecules. The same can

be said about the activation of the degradation enzymes of hyaluronic acid, namely hyaluronidases, which will of course lead to the generation of tissue repair signals. Figure 4 shows a comparison of how human and naked mole rat cells handle the excessive production of glycolytic metabolites.

Concluding Remarks

It is the accumulation of glycolytic metabolites that puts cells on their way to becoming cancerous under chronic hypoxia and/or increased ROS conditions, coupled with chronic inflammatory condition. The accumulation is the direct result of the energy-metabolism reprogramming as Otto Warburg speculated 5 decades ago. The pressure for survival casted on the underlying cells with the glucose metabolite accumulation has clearly led the cells to create the conditions that can trigger the synthesis, export and degradation of hyaluronic acid chains and hence essentially make the whole tissue-repair system available to the affected cells for their survival through cell division as this provides an exit for the accumulated glucose metabolites. If the hypoxic and/or ROS condition persists, this process will continue, hence cell proliferation on a continuous basis. Along the way, some genetic mutations may be selected to provide permanent replacement for various on-going functions to make the proliferation and hence survival more sustainable and possibly more efficient, particularly knowing that hyaluronic acid can

directly activate a number of proto-oncogenes such as *HSF1* and *MYC*.^{71–73}

Although energy-metabolism reprogramming may be the key reason for a cancer to start, two “limitations” in our cellular systems shaped by the past evolution may be the fundamental reason of why human can develop cancer while organisms like blind mole rats or turtle do not: (i) the ATP demand could not drop to the level of ATP supply when energy-metabolism reprogramming takes place; and (ii) there is an intrinsic mismatch between the increased glucose influx triggered by the ATP-deficiency and the maximum flux of the pathway, both of which are due to the lack of “training” in the past. Overall, our model proposes a molecular level mechanism for how energy-metabolism reprogramming will lead to continuous cell proliferation for survival, that is, the initiation of a cancer, hence providing an explanation of Warburg’s speculation in 1960s: “Cancer . . . has countless secondary causes; but there is only one prime cause, (which) is the replacement of respiration of oxygen in normal body cells by a fermentation of sugar.”

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