Glucose metabolism in cancer cells

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Purpose of review

Cancer cells alter their metabolism in order to support their rapid proliferation and expansion across the body. In particular, tumor cells, rather than fueling glucose in the oxidative phosphorylation pathway, generally use glucose for aerobic glycolysis. In this review, we discuss some of the mechanisms thought to be responsible for the acquisition of a glycolytic phenotype in cancer cells and how the switch towards glycolysis represents a selective growth advantage.

Recent findings

Glucose deprivation can activate oncogenes and these can upregulate proteins involved in aerobic glycolysis. In turn, proteins implicated in increased glycolysis can render tumor cells more resistant to apoptosis. Aerobic glycolysis induces acidification of the tumor environment, favoring the development of a more aggressive and invasive phenotype. Altering the pH around tumors might represent a way to hamper tumor development as suggested by a recent work demonstrating that bicarbonate, which increases the pH of tumors, prevented spontaneous metastatization.

Summary

The acquisition of a glycolytic phenotype by transformed cells confers a selective growth advantage to these cells. Interfering with aerobic glycolysis, therefore, represents a potentially effective strategy to selectively target cancer cells.

Keywords

apoptosis, cancer, glucose, glycolysis, oncogene

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Introduction

Carcinogenesis is a complex, multistep process that requires the elimination of several cell-imposed barriers such as antiproliferative responses, programmed cell death-inducing mechanisms, and senescence. This occurs mostly through mutations in oncogenes and tumor suppressor genes [1,2]. The tumor microenvironment plays a crucial role in the transition from precancerous lesions to carcinogenesis by exerting an adaptive pressure that selects cells for their clonal expansion [3–5]. Cellular energy metabolism is one of the main processes that is affected during the transition from normal to cancer cells. In particular, glucose metabolism is very often altered in tumor cells. Glycolysis is a catabolic process that converts one molecule of glucose to two pyruvates with the production of two ATP and two reduced nicotinamide adenine dinucleotide (NADH) molecules. Pyruvate in the presence of oxygen undergoes oxidation to CO_2 and H_2O in the oxidative phosphorylation pathway, resulting in the production of approximately 36 molecules of ATP. Alternatively, in the absence of oxygen, pyruvate is transformed into lactic acid in the anaerobic glycolysis pathway. However, conversion of glucose to lactic acid can occur in the presence of oxygen and this is known as the Warburg effect or aerobic glycolysis [6]. Most cancer cells produce large amounts of lactate regardless of the availability of oxygen [7]. This increased aerobic glycolysis is considered by some to be the seventh hallmark of cancer [8] (the others, initially proposed by Hanahan and Weinberg [9], being limitless replicative potential, self-sufficiency in growth signals, resistance to apoptosis, insensitivity to antigrowth signals, sustained angiogenesis, and tissue invasion and metastasis).

In this review, we will discuss the different mechanisms responsible for the glycolysis switch in cancer and how they contribute to apoptosis resistance and survival of cancer cells. We will also present recent evidence indicating that interfering with glucose metabolism is a valuable anticancer approach.

Glucose utilization in tumors: the Warburg effect

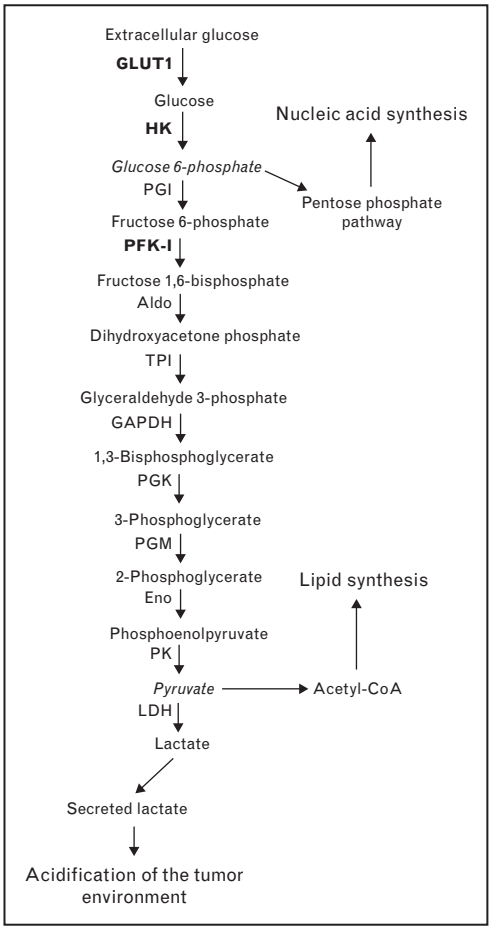
Normal cell proliferation in tissues is controlled by the availability of growth regulating factors and by the interaction with surrounding cells. The availability of nutrients and oxygen, necessary for cell proliferation and metabolism, largely depends on blood supply. Initial tumor growth occurs in the absence of formation of new blood vessels. In this phase, tumor cells ignore

environmental growth-controlling constraints. They can do so by acquiring the ability to proliferate independently of growth signals, through, for example, mutations in receptor-associated signaling molecules, and by becoming insensitive to antigrowth stimuli, such as those mediated by cell-to-cell contacts [9,10].

In the early carcinogenesis phase, uncontrolled cell proliferation moves tumor cells away from blood vessels and, therefore, from oxygen and nutrient supply. The only way oxygen and glucose can reach the inner cells of a nonvascularized tumor is by diffusion across the basement membrane and through the peripheral tumor-cell layers. However, partial oxygen pressure drops to very low values 100 μm away from blood vessels [11]. This implies that hypoxia and glucose shortage are rapidly generated in the inner mass of a growing tumor. Paradoxically, however, it is known since the 1920s [12] that tumor cells have a much higher rate of glucose consumption through a glycolysis pathway that does not send pyruvate to the Krebs cycle (i.e. the oxidative phosphorylation pathway) but that rather converts pyruvate to lactate: the so called Warburg effect [7] (Fig. 1). In fact, many tumors use this glucose to lactate pathway even in the presence of oxygen, explaining why the term aerobic glycolysis is often used as a substitute to the Warburg effect. It is important to note that the glycolytic switch occurring in cancer cells is not necessarily accompanied by a reduction in oxidative phosphorylation [13]. Nowadays, the augmented glycolytic activity of tumors is clinically exploited by positron emission tomography for the identification of metastatic lesions. This technique takes advantage of the increased ability of tumor cells to take up and metabolize glucose compared with normal tissues [5].

It would seem logical to assume that hypoxia is what drives tumor cells to fuel glucose in a nonoxidative 'glucose to lactate' pathway. However, it is currently believed that the glycolytic switch is acquired very early in carcinogenesis even before tumors experience hypoxia [7]. For example, lung cancers and leukemic cells, which are growing in the presence of oxygen, fuel glucose into the aerobic glycolysis pathway [14,15]. Consequently, the fact that, even in normoxic conditions, many tumors use aerobic glycolysis for their metabolic requirements indicates that the Warburg effect has functions that are not solely limited to hypoxia adaptation. We will come later to possible reasons why tumors turn on aerobic glycolysis but let us first discuss other responses induced by hypoxia in tumor cells.

Figure 1 Glycolysis in cancer cells



The metabolic switch towards aerobic glycolysis commonly observed in cancer cells – the Warburg effect – is set after upregulation of some enzymes (indicated in bold) that play an important role in glucose metabolism. The increased glucose utilization through the glycolytic pathway generates metabolic intermediates (indicated in italic) that cancer cells need to sustain their rapid proliferation. One of these intermediates, glucose 6-phosphate is used for the synthesis of nucleic acid, through the pentose phosphate pathway, to allow rapid DNA replication. The abundant production of pyruvate stimulates lipid synthesis that is necessary for the formation of membranes in dividing tumor cells. Finally, secretion of lactate by the tumor cells induces acidification of the tumor microenvironment that creates a particular niche that favors further tumor progression, as well as inhibiting the action of some anticancer drugs. Aldo, aldolase; Eno, enolase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GLUT1, glucose transporter 1; HK, hexokinase; LDH, lactate dehydrogenase; PFK-I, phosphofructokinase 1; PGI, phosphoglucose isomerase; PGK, phosphoglycerate kinase; PGM, phosphoglycerate mutase; PK, pyruvate kinase; TPI, triose phosphate isomerase.

Hypoxia adaptation and apoptosis resistance

A crucial molecule involved in the adaptation to hypoxia is the hypoxia-induced factor 1 (HIF-1). HIF-1 is a pleiotropic transcription factor that regulates genes involved in the hypoxia-induced metabolic switch, regulation of tumor pH, and angiogenesis [16]. The high glycolytic rate characteristic of hypoxic solid tumor is due in part to the greatly increased expression of hexokinase II (HK II) [17], a known transcriptional target of HIF-1. Hexokinase catalyses the first step in the glycolytic pathway where glucose is phosphorylated to

glucose-6-phosphate with conversion of one ATP to ADP (Fig. 1). There are four isoforms encoded by the mammalian genomes (I to IV) that are usually expressed at low levels in cells [18]. By increasing the expression level of HK II, hypoxia via HIF-1, can therefore modulate glucose metabolism.

HK II has additional features that are relevant in the context of cancer-cell apoptosis. HK II is normally associated with voltage-dependent anion channel (VDAC) [19], a 30 kDa pore protein inserted in the outer mitochondrial membrane that regulates the transport of metabolites in and out of the mitochondrial inter-membrane space [20]. Mitochondria are key components of the apoptotic cell-death process. Upon exposure to cell-death stimuli, mitochondria release cytochrome-*c* and other apoptogenic factors such as SMAC/DIABLO into the cytoplasm where they trigger caspase activation and apoptosis [21]. The release of cytochrome-*c* is orchestrated by members of the Bcl-2 family of proteins [22,23]. Among these proteins, only Bax and Bak are mandatory for the release of cytochrome-*c* [24] although the exact mechanism by which this happens is still debated. One model proposes that Bax, once activated by death stimuli, cooperates with VDAC to form a large cytochrome-*c* conducting channel through the mitochondrial membrane [25,26].

It can be envisioned that a protein interacting with VDAC, like HK II, has the potential to prevent the interaction of proapoptotic proteins with mitochondria and consequently interfere with apoptosis. Therefore in hypoxic tumors, the initial overexpression of HK II as a primary adaptation to hypoxia, may secondarily confer resistance to apoptosis. Supporting this notion is the observation that disruption of the binding of HK II to mitochondria, through activation of GSK3β and phosphorylation of VDAC, potentiates chemotherapy-induced cytotoxicity in transformed cells [27]. Moreover, methyl jasmonate, an anticancer agent that interacts directly with mitochondria, is able to induce apoptosis selectively in cancer cells apparently by detaching hexokinases from mitochondria [28]. Recently, it has also been shown that the release of HK II from mitochondria potentiates cisplatin-induced cytotoxicity [29•]. This was shown using a peptide that competed with HK II for VDAC binding and that enhanced cisplatin-induced apoptosis through Bak oligomerization and mitochondrial integrity loss.

The current evidence indicates, therefore, that hypoxia response in cancer cells, in additon to modulating the way they metabolize glucose, renders them more resistant to death stimuli. Hypoxic tumors are often more invasive and metastatic [30,31]. Whether this is linked to a higher resistance to apoptosis or to the altered glucose metabolism is currently not known.

Oncogenic stress and alteration of glucose metabolism

Hypoxia is not the only driving force that leads to abnormal glycolytic flux in cancer cells. It has been discovered in the last few years that oncogenes found in a wide variety of human cancers can directly activate HIF-1 and other components of glucose metabolism independently of hypoxia. One of such oncogenes is Akt. This serine/threonine kinase is involved in the modulation of several cellular processes such as proliferation, autophagy and cell metabolism [32,33]. Akt regulates factors involved in glucose metabolism, including HK II, whose association with mitochondria and its effect on apoptosis resistance was discussed in the previous section, phosphofructokinase-1 (PFK-1) [34], one of the rate-controlling enzymes of glycolysis, and GLUT1, the most widely expressed glucose transporter [35]. Recently, Akt was coined the 'Warburg kinase' able to promote the metabolic changes that tumor cells experience en route to a more malignant state [36•]. The fact that Akt promotes a glycolytic switch under normoxia conditions, without affecting the rate of oxidative phosphorylation, confirms that this occurs not only as an adaptation to low-oxygen flux but also when tumoral cells increase the production of metabolic intermediates required for rapid proliferation, such as pentose phosphates necessary for nucleic acid synthesis (Fig. 1).

These Akt-mediated metabolic changes render cancer cells dependent on aerobic glycolysis for their growth and survival. This can be seen for example in tumor cells bearing an activated form of Akt. These cells undergo rapid cell death when shifted to low-glucose conditions [37]. On the contrary, if tumor cells are given the chance to activate another metabolic pathway to sustain their high-energy demand, the addiction to aerobic glycolysis is overcome. This is indeed seen when cells are switched towards fatty acid metabolism by stimulating them with the AMPK activator 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) [38].

Another oncogene, K-Ras can alter glucose metabolism so as to provide tumor cells with a selective advantage. It has indeed recently been shown that expression of the GLUT1 glucose transporter is increased in cells with mutated K-Ras. Upregulation of this glucose receptor was associated with an increased glucose uptake, increased glycolysis and augmented lactate production, whereas mitochondrial functions and oxidative phosphorylation were not affected. When grown in low-glucose containing media, K-Ras mutated cells showed increased survival. Therefore, it was argued that agents able to inhibit glucose metabolism could selectively kill K-Ras mutant cells. Indeed the hexokinase inhibitor 3-bromopyruvate (3-BrPA) was found to be highly toxic to different cancer

cell lines bearing K-Ras mutation, but was much less toxic to cell lines lacking K-Ras mutation [39••]. Interestingly, the K-Ras mutations that lead to low-glucose adaptations can be induced by glucose deprivation [39••]. This suggests that a stress caused by nutrient shortage might be a favorable ground for the activation of oncogenes.

Aerobic glycolysis as a generator of a tumor friendly niche

There is evidence that an increased glycolysis rate contributes to the acquisition of resistance to chemical drugs by cancer cells, mainly through acidification of the tumor microenvironment. The large amounts of lactate secreted by tumor cells, as a direct consequence of the abnormal production of pyruvate, leads to acidification of the tumor surroundings [40]. Several anticancer drugs such as doxorubicin, mitoxantrone and vincristine are weak bases that are protonated in slightly acid tumor microenvironments. When protonated, these drugs cannot easily diffuse across the plasma membrane and consequently their cellular uptake is diminished. In this context it has recently been shown that addition of sodium bicarbonate in the drinking water raises the pH of the extracellular milieu in mice, which translated into a greater efficacy of doxorubicin to hamper the growth of xenotransplanted tumors [41]. The reverse situation was shown in another study where glucose administration to mice led to a decrease in the extracellular pH and a lower efficacy of doxorubicin on tumors [42]. In contrast to weak bases such as doxorubicin, weak acid anticancer drugs like chlorambucil are more efficacious when the pH of the extracellular milieu decreases [42]. These studies have important clinical implications because they suggest that appropriate modulation of the extracellular pH in patients with cancer based on the chemical properties of the used antitumor drugs could optimize the chemotherapy efficacy.

Additionally, pH lowering can influence more directly tumor progression and expansion. The extracellular conditions found within precancerous lesions (i.e. hypoxia, low-nutrient availability) typically results in necrosis or apoptosis of tumor cells through p53-dependent mechanisms and caspase-3-dependent mechanisms [43]. This initial beneficial response may, however, later favor the selection of cells that, in addition to upregulating glycolysis, acquire mutations allowing them to become immune to apoptotic-inducing pathways and potentially other antimalignant and anti-invasive checkpoints [5,9]. These cells are considered by some to be cancer stem-like cells [44]. The identification of these cancer stem-like cells and the mechanisms governing their anaerobic metabolic pathways may potentially open new perspectives in cancer treatment.

One peculiar feature of cancers is the ability to metastasize. Spontaneous metastasis consists in two main phases intravasation where transformed cells leave the primary tumor to the bloodstream and extravasation where tumor cells colonize new tissues. In-vitro studies have shown that tumor cell invasion can be stimulated by acidic conditions [45,46]. Moreover, acid pretreatment of tumor cells increases their ability to metastatize after injection in mice [47]. This effect could be attributed to an augmented release of cathepsin B that is involved in extracellular matrix remodeling. Recently, it has been shown that bicarbonate therapy significantly reduces the number and the size of metastases in a breast cancer mouse model by increasing tumor pH [48•]. Bicarbonate, by modulating the pH of the tumor environment, negatively affects the process of tumor cells extravasation without significantly influencing intravasation and circulation of tumor cells across the bloodstream [48•]. This finding is of high relevance if we consider that primary tumors rarely kill affected patients, but that it is rather formation of metastasis from primary tumors that is lethal. Therefore, strategies that aim at limiting spreading of primary tumors across the body could be highly beneficial for cancer patients.

Conclusion

Alteration of glucose metabolism can be the result of an adaptive response to the lack of oxygen or following activation of oncogenes. Aerobic glycolysis appears to represent a selective advantage for tumor cells as they become more resistant to apoptosis and acquire increased growth and invasive properties. At present it is still unclear if the molecular mechanisms controlling the switch towards aerobic glycolysis are directly involved in the acquisition of apoptosis resistance or whether this resistance is a secondary adaptation to hypoxia of a subpopulation of transformed cells in precancerous lesions. Regardless of the mechanisms, glycolysis upregulation represents a clear advantage for cancers cells and at the same time a target for new anticancer therapies.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

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- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 504–505).

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