Role of Oxygen in Cancer: Looking Beyond Hypoxia

Miguel López-Lázaro*

Department of Pharmacology, Faculty of Pharmacy, University of Seville, Spain

Abstract: Although cancer is considered to be a disease caused by DNA alterations, the high genetic variability of tumor cells makes it difficult to exploit these alterations for the treatment of cancer. The influence of non-genetic factors on cancer is increasingly being acknowledged and a growing line of research suggests that hypoxia (a decrease in normal oxygen levels) may play a fundamental role in the development of this disease. This line of research is supported by the fact that tumors often have hypoxic areas, that hypoxia activates the hypoxia-inducible factor 1 (HIF-1) and that HIF-1 activation plays a key role in cancer development. Evidence suggests, however, that the idea of hypoxia playing a central role in cancer development has some drawbacks. For instance, hypoxia has not been found in many tumors, HIF-1 activation has been observed in non-hypoxic tumor areas, and hypoxic tumor cells commonly have a reduced nutrient supply that restricts cell proliferation and tumor growth. This article reviews the literature that does not support the idea of hypoxia playing a central role in cancer development and discusses a broader view in which the role of oxygen in cancer is not limited to a reduction in its normal levels. According to this novel view, a deviation of the oxygen metabolism from the pathway that generates energy to the pathway that produces reactive oxygen species is crucial for cancer development. Interestingly, this switch in oxygen metabolism occurs under both hypoxic and normoxic conditions and may be exploited therapeutically.

Key Words: Glycolysis, HIF-1, reactive oxygen species, hydrogen peroxide, cell proliferation, tumor growth, metastasis, carcinogenesis.

1. INTRODUCTION

The most accepted view of cancer is that tumorigenesis is caused by DNA alterations in oncogenes, tumor-suppressor genes and stability genes [1]. According to this prevalent view, cancer may eventually be treatable by reversing these DNA alterations or by targeting them to eliminate the tumor cell. Much of cancer research over the past several decades has been devoted to finding out which genes are altered in tumor cells as compared with their normal counterparts. This research has revealed that cancer cells have a vast array of genetic alterations, which differ greatly between different cancer types and are heterogeneous even among cells within the same tumor. It is becoming clear that the cancer genome appears to be far more unstable than previously thought and that assembling a catalogue of these genomic changes in each cancer may not be sufficient to lead to major improvements in patient survival [2, 3].

The idea that cancer may not only be controlled by DNA mutations is increasingly being acknowledged. Evidence indicates that the most important cancer genes and pathways are directly or indirectly implicated in the activation of the hypoxia-inducible factor 1 (HIF-1) [1]. The most common cancer types have increased levels of HIF-1 and the activation of this transcription factor is involved in crucial aspects of cancer biology, including angiogenesis, cell survival, glucose metabolism, invasion and metastasis [4, 5]. Although HIF-1 can be activated by mutations in oncogenes and tumor-suppressor genes, it is recognized that the main activator of this transcription factor is hypoxia (a decrease in normal oxygen levels). This suggests that non-genetic factors, such as hypoxia, may play a key role in cancer development.

Clinical investigations carried out over the last two decades have shown that locally advanced tumors often have hypoxic and/or anoxic tissue areas. Such areas are heterogeneously distributed within the tumor mass and have been found in a wide range of malignancies. The extent of hypoxia is independent of clinical size, tumor stage, histopathologic type and grade of malignancy. Tumor hypoxia is known to reduce the effectiveness of radiotherapy, some chemotherapeutic agents and photodynamic therapy, and seems to be an adverse prognostic factor for patient outcome (see [6-8] and references therein).

Cells under hypoxic conditions activate HIF-1, a heterodimeric transcription factor that consists of a highly regulated alpha subunit (HIF-1α) and a constitutive beta subunit (HIF-1β). The activation of HIF-1 increases the transcription of a wide range of genes that are involved in pathways that either promote oxygen delivery to cells or allow cells to survive oxygen deprivation [9]. But many of the genes induced by HIF-1 are also involved in key aspects of cancer development [5]. Through the transcriptional regulation of hundreds of genes, HIF-1 plays important roles in every major aspect of cancer biology, including angiogenesis, invasion, metastasis, metabolic reprogramming, immortalization, genetic instability, maintenance of stem cell pools, cellular dedifferentiation, autocrine growth factor signaling, and treatment failure. In fact, inhibition of HIF-1 activity has marked effects on tumor growth in animal models. HIF-1α overexpression has been associated with increased patient mortality in several cancer types, and immunohistochemical detection of HIF-1α overexpression in biopsy sections is a prognostic factor in many cancers [5, 10, 11]. The key role of HIF-1 in cancer is widely acknowledged and evidence suggests that HIF-1 inhibition may be an important strategy for cancer chemoprevention [12] and therapy [5, 10, 11].

Knowing that hypoxia is a common feature of solid tumors, that hypoxia activates HIF-1, and that the activation of this transcription factor plays a fundamental role in cancer, it makes sense to think that hypoxia may have a central role in cancer development. The growing interest in this field of research is reflected in the increasing number of papers published on this topic in the last several years (Fig. 1). There are several lines of evidence, however, that do not support the idea of hypoxia playing a central role in cancer development. This article analyzes such evidence and discusses that it might be more appropriate to view cancer as a process in which oxygen metabolism is altered than as a process in which oxygen levels are reduced. This broader view can integrate the most critical changes occurring in cancer development under both hypoxic and normoxic conditions.

2. EVIDENCE NOT SUPPORTING THE IDEA OF HYPOXIA PLAYING A CENTRAL ROLE IN CANCER DEVELOPMENT

Accumulating experimental evidence has demonstrated that hypoxia participates in several key aspects of cancer development including, for instance, tumor invasion and metastasis [13, 14]. This evidence has fueled the idea that hypoxia may be necessary for
cancer development. Several lines of evidence that do not support this idea are discussed below.

**Fig. (1).** Increasing interest in the role of hypoxia in cancer. This figure represents the number of articles containing the words “hypoxia” and “cancer” in their title and/or abstract that has been added to PubMed between 1991/01/01 and 2008/12/31.

**Hypoxia has not been Found in Many Tumors**
A high number of clinical investigations have shown that up to 50-60% of locally advanced solid tumors have hypoxic areas (reviewed in reference [7]). This means that hypoxic areas have not been found in 40-50% of all solid tumors investigated and suggests that many tumors may develop despite the absence of hypoxia. These data suggest that hypoxia may not be necessary for cancer development and weaken the idea of hypoxia playing a central role in the development of this disease.

**Tumor Cells Activate HIF-1 Despite the Absence of Hypoxia**
Under standardized in vitro conditions, a clear association between expression of HIF-1α and oxygenation status has been observed. Analysis of cultured HeLa cells exposed to precisely defined oxygen concentrations revealed that reduction from 20 to 6% O2 resulted in a 2-fold increase in HIF-1α protein levels, whereas below 6%, HIF-1α levels increased exponentially, with a half-maximal response at 1.5-2% O2 and a maximal response at 0.5% O2 [15]. This clear association observed in vitro, however, is less obvious in tumors. Although immunohistochemical staining of HIF-1α in tumors has been found to increase as a function of distance from blood vessels, a diffuse pattern throughout the entire tumor has also been observed [16]. The poor correlation between HIF-1α expression and oxygenation status found in tumors [17] has been attributed to tumor heterogeneity. To reduce tumor heterogeneity, the expression of HIF-1α was examined in the same tumor microareas where oxygen levels had been measured; however, no correlation was observed [18]. Indeed, a wide range of HIF-1α expression was found in both severely hypoxic tumors and normoxic tumors; non-hypoxic tumors partially showed a pronounced expression of HIF-1α [18]. A lack of correlation was also observed between direct oxygen measurements and the expression of glucose transporter 1 (GLUT-1) [19] and carbonic anhydrase IX (CA-IX) [20], two key HIF-1 downstream genes (reviewed in [21]).

Despite insufficient recognition, it has been demonstrated that tumor cells can and do activate HIF-1 in the presence of normal oxygen levels [5, 18, 22-25]. This fact can explain the lack of correlation observed between direct oxygen measurements and HIF-1α expression in tumors [18]. Fig. (2) illustrates, in a simplified form, that HIF-1-mediated gene expression can be induced under both hypoxic and non-hypoxic conditions. This figure represents that hypoxia activates HIF-1 by inhibiting the activity of a family of oxygen-dependent enzymes, prolyl hydroxylases (PHDs), which hydroxylate HIF-1α and target it for proteasomal degradation. But, in addition to requiring oxygen, these enzymes require 2-oxoglutarate (2-OG) and Fe2+ for their activity; this means that a reduction in Fe2+ or 2-OG availability will lead to HIF-1 accumulation despite the presence of oxygen. It has been demonstrated that H2O2 activates HIF-1 under normoxic conditions; H2O2 may lead to the oxidation of Fe2+ to Fe3+ at the PHD catalytic center thus inducing HIF-1 gene expression even in the presence of oxygen [26]. It has also been observed that the accumulation of glucose metabolites and tricarboxylic acid (TCA) cycle intermediates (e.g., pyruvate, oxaloacetate, succinate, fumarate) induces HIF-1 activation under non-hypoxic conditions [24, 25, 27-29]. For example, pyruvate and oxaloacetate can bind to the 2-OG site of PHDs, can inactivate HIF-1α hydroxylation and can prevent its degradation [28]. Tumor cells are recognized to have increased HIF-1 levels because of hypoxia...
and/or mutations in oncogenes and tumor-suppressor genes [5]. The fact that non-malignant cells, which are not supposed to have mutations, can activate HIF-1 under non-hypoxic conditions [30] suggests that other mechanisms are possible. Interestingly, tumor cells produce high amounts of H₂O₂ [31, 32] and have increased glycolytic rates that can lead to the accumulation of glucose metabolites and TCA intermediates [33, 34]. This can contribute to explain why tumor cells activate HIF-1 under non-hypoxic conditions and why there is a poor correlation between HIF-1 activation and direct oxygen measurements in tumors. Although it is recognized that HIF-1 activation plays a crucial role in cancer development, the fact that tumor cells activate HIF-1 despite the absence of hypoxia weakens the idea of hypoxia playing a central role in the development of cancer.

**Tumor Cells with High Glycolytic Activity are not Hypoxic**

Our cells can obtain energy through the oxygen-dependent pathway of oxidative phosphorylation (oxphos) and through the oxygen-independent pathway of glycolysis. Because oxphos is more efficient in generating ATP than glycolysis, it is assumed that cells activate oxphos under aerobic conditions and glycolysis under hypoxic conditions (Pasteur effect). The widespread clinical use of the imaging technique positron-emission tomography using the glucose analogue tracer ¹⁸fluorodeoxyglucose (FdG PET) has shown that most primary and metastatic cancers have increased glucose consumption and up-regulation of glycolysis. This seems to indicate that tumor cells have increased glycolytic rates because they do not have enough oxygen [35]. The fact that the activation of glycolysis has been associated with key aspects of cancer development would support the idea of hypoxia playing a central role in tumor promotion.

The possible link between hypoxia and the activation of glycolysis was explored in several tumor types from 49 patients using PET-based molecular imaging [36]. This study showed that the presence of hypoxia and the activation of glycolysis were common but independent events in tumors. Indeed, although some hypoxic tumors had modest glucose metabolism, tumors having high glycolytic rates were not hypoxic [36]. This lack of association between hypoxia and glucose metabolism was also found in tumors from 21 patients with non-small cell lung cancer (NSCLC); this study also revealed that the hypoxic cell fraction of these tumors was consistently low [37]. The lack of correlation found between the presence of hypoxia and the activation of glycolysis in tumors is not surprising. *In vitro* experiments commonly evaluate the effects of hypoxia in tumor cells placed in cultured media rich in glucose. Under these conditions, when hypoxia is induced and energy generation through oxphos decreases, tumor cells take up glucose from the media and activate glycolysis to generate ATP and ensure their survival. In a tumor, however, the situation is rather different. Tumor hypoxia is predominantly caused by a reduced blood flow that limit both oxygen and nutrient availability. This suggests that a tumor cell that suffers from hypoxia also has a reduced glucose supply and, therefore, a limited glycolytic capacity (Fig. 3). Although the increased glycolytic metabolism of tumor cells plays an important role in cancer, the lack of correlation between tumor hypoxia and the increased glycolytic metabolism found in tumor cells [36, 37] weakens the idea of hypoxia playing a central role in cancer development.

**Hypoxia may Restrict Cell Proliferation and Tumor Growth**

Tumor growth requires cell proliferation. Cell proliferation requires that a cell duplicates all its cellular components to create two daughter cells. In order to do this, a tumor cell needs to take glucose and other nutrients from the blood. Hypoxic cells commonly have a reduced blood flow that limits their nutrient supply and, therefore, cell proliferation and tumor growth [34, 38-40]. Indeed, an inverse correlation between regions of hypoxia and regions of proliferation has been observed in human tumors [41]. Angiogenesis inhibitors reduce the formation of new blood vessels and decrease the delivery of oxygen and nutrients to tumor cells. These drugs can induce tumor hypoxia and, instead of promoting cancer, they are known to prevent tumor growth [2].

**Fig. (3). Lack of correlation between decreased oxygen levels (hypoxia) and activation of HIF-1, glycolysis and cell proliferation in tumors.** A lack of correlation between hypoxia and the activation of HIF-1 has been observed in tumor cells [18], probably because of the ability of tumor cells to activate HIF-1 in the presence of oxygen. The lack of correlation between hypoxia and the activation of glycolysis found in tumors [36, 37] may be due to the fact that oxygen-deprived tumor cells commonly have decreased levels of glucose and, therefore, a restricted glycolytic capacity. The inverse correlation between hypoxia and cell proliferation found in tumors [41] may be explained by the fact that cell proliferation requires the presence of nutrients (e.g. glucose, essential amino acids) and that these nutrients are commonly reduced in hypoxic cells (see text for further details).

**Populations Exposed to Lower Oxygen Levels have Lower Cancer Risk**

The prevalent view that hypoxia (a decrease in normal oxygen levels) promotes cancer development implies that oxygen plays a protective role in this disease. If this is the case, it makes sense to think that people exposed to reduced oxygen levels might have an increased cancer risk. Although the percentage of oxygen in inhaled air is constant at different altitudes, the fall in atmospheric pressure at higher altitudes decreases the partial pressure of inspired oxygen. The levels of inspired oxygen fall almost linearly with altitude, being 50% of the sea level value at an altitude of 5500 m. At high altitudes, the decreased oxygen in ambient air results in decreased oxygen levels in blood and tissues [42]. Therefore, populations living at high altitudes are exposed to lower oxygen levels than people living at sea level. Do populations living at high altitudes have an increased cancer risk?

In 1974 it was found that, above an altitude of 2000 feet (610 m), the mortality rate of leukemia in the United States decreased significantly with increasing altitude [43]. One year later, using data from the International Committee Against Cancer and from the World Health Organization, the relationship between altitude and global cancer incidence and mortality was analyzed [44]. This study showed a strong and statistically significant negative correlation between altitude and cancer incidence and mortality for the age groups above 60 years. For instance, in the upper age group, cancer
incidence in men living in cities or countries located at high altitudes was approximately 2.5 lower than the observed in men living in cities or countries located at low altitudes [44]. Several years later, the relationship between altitude and site-specific cancer mortality rates in the United States was studied and a deficit in cancer mortality at high altitudes was observed [45]. The largest differences between the low and high altitude groups were found for cancers of the tongue and mouth, esophagus, larynx, lung and melanoma [45]. This correlation between higher altitude and lower cancer risk does not seem to be biased by confounding factors such as inefficient collection of data, industrialization, urbanization or other cultural characteristics [44, 45]. These studies suggest that, instead of facilitating cancer, the reduced oxygen levels of inspired air at high altitudes may be protecting against cancer; this does not support the idea of hypoxia promoting cancer development. The increased oxygen levels at low altitudes may facilitate the formation of reactive oxygen species (ROS), which are known to have an important role in carcinogenesis.

3. KEY ROLE OF AN ALTERED OXYGEN METABOLISM (DYSOXIC METABOLISM) IN CANCER DEVELOPMENT

Cells predominantly use oxygen to generate ATP through oxidative phosphorylation (oxphos). In this process, ATP generation is coupled with a reaction in which oxygen (O\textsubscript{2}) is reduced to H\textsubscript{2}O in the mitochondria. In this reaction, four electrons and four protons are added to O\textsubscript{2} to form two molecules of H\textsubscript{2}O. But when a molecule of O\textsubscript{2} gains only one electron to form superoxide anion (O\textsuperscript{2}\textsuperscript{-}), this highly reactive oxygen species tends to gain three more electrons and four protons to form H\textsubscript{2}O\textsubscript{2}, this process involves several reactions and results in the production of other ROS such as hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}). Although the controlled production of ROS has an important physiological role [46], a high production of ROS that is not counterbalanced by the cellular antioxidant systems originates oxidative stress, which has been proposed to play an important role in the development of several diseases including cancer [47] (Fig. 4).

The fundamental framework for the oxidative stress theory of cancer (also called free radical theory of cancer) was laid down by several research groups in the 1950s (reviewed in [48]). Although this hypothesis was eclipsed by the somatic mutation theory of cancer, much evidence that supports this hypothesis has accumulated since then [47-56]. Evidence supports that tumor cells produce high levels of the ROS O\textsuperscript{2}\textsuperscript{-} and H\textsubscript{2}O\textsubscript{2}, that cell malignant transformation can be achieved by increasing the cellular levels of O\textsuperscript{2}\textsuperscript{-} and H\textsubscript{2}O\textsubscript{2}, and that the malignant phenotype of tumor cells can be reversed simply by decreasing the levels of these ROS (see [32, 54] and references therein). The most important carcinogenic agents and behaviors have been shown to induce oxidative stress, including most chemical carcinogens [48] (e.g. N-nitrosamines [57], asbestos [58], arsenic [59]), ultraviolet radiation [60, 61], cancer-associated viruses [62-65] or bacteria [66], inflammation [51], alcohol [67, 68], tobacco smoke [69, 70] and obesity [71, 72]. It is also recognized that age is the principal risk factor for most cancers [73] and that oxidative stress may be the most important causal factor in aging [74, 75].

In the first half of the 20\textsuperscript{th} century, the Nobel laureate Otto Warburg observed that tumor cells had increased rates of glycolysis despite the presence of oxygen. He proposed that the key alteration of cancer was a defect in respiration (oxphos) and that this defect was responsible for the increased glycolytic rates found in tumor cells even in the presence of oxygen [76]. His theory was rejected by most researchers mainly because the experimental evidence for a defective oxphos structure was lacking. But recent experiments have demonstrated that tumor cells from the most common cancer types have mutations and mitochondrial alterations that can produce oxphos repression [77-79]. At present, it is acknowledged that tumor cells commonly have a metabolic switch from oxidative phosphorylation to glycolysis despite the presence of oxygen (aerobic glycolysis or Warburg effect) and that this metabolic switch plays an important role in cancer development [34, 80].

![Fig. (4). Role of oxygen in oxidative stress and cancer. Accumulating data suggest that oxidative stress plays an important role in cancer. Oxidative stress is caused by an increase in the levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that is not counterbalanced by the cellular antioxidant defenses. The normal production of most ROS and RNS requires the formation of superoxide anion (O\textsuperscript{2}\textsuperscript{-}) from oxygen (O\textsubscript{2}). This suggests that oxygen plays an important role in cancer (see text for details).](image)

The fundamental ideas of the oxidative stress theory of cancer and of the Warburg hypothesis are merged in Fig. (5). A deviation from oxygen metabolism from the route that generates ATP (oxphos) to the route that produces ROS can occur under both hypoxic and non-hypoxic conditions and may explain key aspects of carcinogenesis [34, 38, 81, 82]. Several carcinogenic events that cannot be explained by a decrease in the normal oxygen levels (hypoxia) may be explained by this alteration in oxygen metabolism (dysoxic metabolism).

A Switch in Oxygen Metabolism may Explain that Tumor Cells Activate HIF-1 Despite the Absence of Hypoxia

The alteration in oxygen metabolism shown in Fig. (5) may lead to HIF-1 activation in tumor cells under both hypoxic and non-hypoxic conditions [81]. On the one hand, evidence suggests that hypoxic cells can activate HIF-1 via ROS production; indeed, hypoxia-induced HIF-1 activation is prevented in cells treated with antioxidants such as catalase [83-87]. In addition, it is known that hypoxic cells reduce oxphos activity and increase their glycolytic rates when glucose is available; the activation of glycolysis can increase HIF-1 via accumulation of glucose metabolites [24, 28]. On the other hand, experimental data have shown that cells with normal oxygen levels can activate glycolysis (aerobic glycolysis or Warburg effect) and increase HIF-1 activity via accumulation of glucose metabolites [24, 28]. Normoxic cells can also activate HIF-1 via ROS production [23, 81]. The switch in oxygen metabolism commonly observed in tumor cells may explain why these cells can activate HIF-1 under normoxic conditions [81] and why HIF-1
activation in solid tumors does not correlate well with direct oxygen measurements [18].

A Switch in Oxygen Metabolism may Explain that Tumor Cells Activate Glycolysis Under Non-Hypoxic Conditions (Aerobic Glycolysis or Warburg Effect)

A lack of correlation between hypoxia and the activation of glycolysis in tumor cells has been found in patients [36, 37]. Clinical data revealed that although some hypoxic tumors can have modest glucose metabolism, tumors having high glycolytic rates are not hypoxic [36]. Therefore, although it has been repeatedly observed that tumor cells have high glycolytic rates, evidence indicates that hypoxia cannot explain this observation. Most researchers acknowledge Warburg’s observation that tumor cells have aerobic metabolism, yet it is not clear why and how this phenomenon occurs. The possible reasons and mechanisms involved in the activation of glycolysis in the presence of oxygen have been discussed recently [34]. In brief, it has been proposed that tumor cells activate glycolysis in the presence of oxygen in order to proliferate and that the key mechanism that mediates this metabolic switch is a deviation of oxygen metabolism from the route that generates ATP to the route that produces ROS. This alteration in oxygen metabolism may be a key mechanism by which tumor cells activate glycolysis under both hypoxic and aerobic conditions [34].

A Switch in Oxygen Metabolism Allows Cell Proliferation and Tumor Growth

Uncontrolled cell proliferation is the most recognized feature of cancer. As discussed before, hypoxic tumor cells commonly have a reduced nutrient supply that restricts cell proliferation and tumor growth. A tumor cell with a sufficient blood flow and a dysoxic metabolism, however, would activate HIF-1 and glycolysis despite the presence of oxygen [34, 81], would have a sufficient nutrient supply required for cell proliferation and, therefore, would contribute to tumor growth [38].

A Switch in Oxygen Metabolism may Play an Important Role in Tumor Invasion and Metastasis

There is a consistent body of research supporting the idea of hypoxia playing an important function in tumor invasion and metastasis. Through the activation of HIF-1, hypoxic cells increase the transcription of many genes that play crucial roles in tumor invasion and metastasis [13, 14]. It has also been proposed that hypoxia activates glycolysis in tumor cells and that the activation of glycolysis in these cells may lead to tumor invasion through the acidification of its extracellular microenvironment [33]. It has been found, however, that hypoxia is not present in many tumors and that non-hypoxic tumor cells activate HIF-1 [18] and glycolysis [36, 37]. This suggests that other factors are probably involved in tumor invasion and metastasis in addition to hypoxia. For instance, it has been observed that an increased cellular production of H$_2$O$_2$ may be crucial for metastasis, as the H$_2$O$_2$-detoxifying enzyme catalase can prevent this process [88-92]. A dysoxic metabolism, which can occur under both hypoxic and non-hypoxic conditions, may play an important role in tumor invasion and metastasis by activating HIF-1, by increasing glycolysis-induced extracellular acidification and by rising the cellular production of H$_2$O$_2$ [82].

A Switch in Oxygen Metabolism may Participate in Cancer Initiation

It is considered that hypoxia mainly occurs in tumors as a result of the rapid proliferation in the tumor mass that distances the cells from the oxygen carrying blood vessels. In the initial stages of carcinogenesis, before the formation of the tumor mass, cells would be well oxygenated. Therefore, it is acknowledged that hypoxia plays an important role in cancer promotion but not in cancer initiation. If we considered that the role of oxygen in cancer is limited to hypoxia, we would be denying the key role that oxygen seems to play in cancer initiation. As mentioned before, most carcinogenic agents can increase the cellular levels of ROS and induce oxidative stress. An increase in the cellular levels of ROS can produce DNA alterations [93, 94] and induce cell malignant transformation [95-98]. This suggests that the production of ROS induced by most carcinogens may be important in cancer initiation. The fact that oxygen is required for the normal production of most ROS (Fig. (4) suggests that oxygen may play an important role in the initial stages of cancer). In non-hypoxic normal cells, an excessive deviation of oxygen metabolism from the route that produces ATP to the route that produces ROS (dysoxic metabolism) may participate in cancer initiation through the accumulation of ROS.

Tumor Cells Behave Differently Depending on their Location Within the Tumor

Some authors consider that tumor cells activate glycolysis in the presence of oxygen (aerobic glycolysis) and others that tumor cells activate glycolysis because they do not have oxygen (anaerobic glycolysis). This and other controversies found in the literature are probably due to the fact that tumor cells behave differently depending on their location within the tumor.

Cells located away from a blood vessel (Fig. (3), area 3) have a very limited supply of oxygen, glucose and other nutrients such as essential amino acids. Their lack of nutrients would restrict cell proliferation and, therefore, their capacity to increase the tumor mass. Although these cells may activate glycolysis (anaerobic glycolysis) temporarily, the limited supply of glucose, oxygen and other nutrients would probably make these cells engage autophagy, a process by which cells can obtain energy by metabolizing their own components [99-101]. A dysoxic metabolism may occur in cells that receive some oxygen, but is unlikely to happen in cells lacking it. The reduced oxygen supply would result in HIF-1 activation, which would increase the synthesis of proteins that favor cell motility and invasion. Their waste products (e.g. H$^+$ and CO$_2$) would not be removed because of their restricted blood flow and would accumulate in the extracellular milieu; this would lead to extracellular acidification and would also increase their invasive potential [102, 103]. These cells would eventually die unless they managed to invade adjacent regions and migrate to a less deprived area, or unless new blood vessels were generated next to them (angiogenesis). If these cells managed to reach the blood vessels or lymph channels, they could spread to different locations (metastasis).
Cells located closer to a blood vessel (Fig. 3, area 2) would receive oxygen, glucose and other nutrients, but in a limited supply. These hypoxic cells would probably have a dysoxic metabolism characterized by an increased production of ROS and the activation of glycolysis. Because of their limited supply of glucose and other nutrients, it is more likely that these cells used these nutrients to generate energy than to engage the energy-demanding process of proliferation. Like cells located in an oxygen and nutrient-deprived area (Fig. 3, area 3), these cells would probably have a reduced capacity to increase the tumor mass and an increased invasive and metastatic potential.

Cells located next to a blood vessel (Fig. 3, area 1) have an unlimited supply of oxygen, glucose and other nutrients. These cells can activate HIF-1 and glycolysis despite the presence of oxygen, possibly by deviating oxygen metabolism from the route that generates ATP to the route that produces ROS (dysoxic metabolism) [34, 81]. Because of their unlimited supply of nutrients, these non-hypoxic cells would not have a restricted proliferative capacity and would contribute to the growth of the tumor [38]. By increasing their \( \text{H}_2\text{O}_2 \) production and by activating HIF-1, these normoxic cells would also have the ability to invade adjacent tissues and metastasize to different locations [82].

**Therapeutic Implications**

It has been known for many years that hypoxia reduces the effectiveness of some forms of cancer therapy (e.g. radiotherapy) and is an adverse prognostic factor for patient outcome [7, 104]. Paradoxically, evidence suggests that tumor hypoxia may be exploited therapeutically and several strategies are being investigated in the laboratory and the clinic [105]. Despite the promise of these novel strategies, the benefits of exploiting tumor hypoxia have yet to be fully realized, and it is expected that this approach may only be useful in patients with hypoxic tumors [105].

Evidence suggests that the altered oxygen metabolism of cancer cells represented in Fig. 5 might be exploited to achieve therapeutic selectivity. In one aspect, experimental data have revealed that cancer cells generate higher levels of \( \text{H}_2\text{O}_2 \) than normal cells and that there is a threshold of \( \text{H}_2\text{O}_2 \) above which cells cannot survive [31, 32]. This may contribute to the growth of the tumor [38]. By increasing \( \text{H}_2\text{O}_2 \) production and activating HIF-1, these cells would have the ability to invade adjacent tissues and metastasize to different locations [82].

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