Tumorigenesis: cell defense against hypoxia?

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Abstract

Microenvironmental elements can directly contribute to the induction and the maintenance of tumor. Oxygen is the main element in the cell microenvironment and hypoxia can affect the process of tumorigenesis. In response to hypoxia, cells change their pattern and characteristics. These changes suggest that it is not just adaptation, but some sort of cell defense against hypoxia. If hypoxia is corrected, then cell defense mechanisms are interrupted. An examination of the process of tumorigenesis helps to design better therapeutic strategies.A systematic review of the English literature was conducted by searching PubMed, Google Scholar, and ISI Web databases for studies on changes that defend and help cells to live in a hypoxic microenvironment. Cells respond to hypoxia by de-differentiation and an increase in heat shock proteins. Angiogenesis and deviation of inflammatory response in favor of hypoxic cell survival also defend and save the oxygen-starved cells from death. Finally, anti-angiogenic therapies and more hypoxia enhance metastasis, as tumors with low oxygen concentration are more malignant than tumors with high oxygen concentration. All these enable cells to migrate away from low oxygen areas and seek a more conducive microenvironment. Therapies that make the microenvironment more hypoxic need to be revised. This has been done for anti-angiogenic therapies, previously considered to be anti-tumor approaches. Effective therapies may be *correcting therapies* which direct the tumor microenvironment towards natural physical/chemical condition. *Correcting therapies* either bring back tumor cells to a normal form (correct tumor cells) or help the immune system to eradicate tumor cells which can not be corrected.

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Introduction

Oxygen is a vital element required for cell survival and growth. Different levels of oxygen characterize healthy tissues, ranging from 150mmHg in the lung to 40-100 mmHg in the circulation and 4-20mmHg in the tissues.[1,2] Furthermore, the oxygen within a tissue is also variable and is proportional to the distance from the end of the nearest capillary;[3] this is known as physiological hypoxia. On the other hand, pathological hypoxia develops as an aberrant status when tissue is damaged or degenerated. Pathological hypoxia creates a unique microenvironment, conditioning the phenotype and function of every cell type and delivering important signals to promote angiogenesis for improving oxygen delivery, activate an alternative energy source, and recruit effector leukocytes to deal with the disease. Oxygen tension in chronically hypoxic regions is less than 5 mmHg.[4,5] Examples of pathological hypoxia include malignant solid tumors, inflammatory lesions, ischemic tissues, healing wounds, and obstructive pulmonary diseases.[6]

Adaptation to hypoxia is an essential homeostatic mechanism. Mammals are equipped with oxygen-sensing mechanisms that help them adapt quickly to hypoxia. If an inadequate supply of oxygen persists, additional mechanisms are activated to restore oxygenation or help the body adapt to hypoxia. These changes will be discussed with regard to tumor. Tumor cell adaptations to hypoxia mainly depend on the transcription factor hypoxia-inducible factor (HIF) which is activated in hypoxic conditions and is inactive when oxygen is abundant.[7,8] HIF regulates genes of intracellular proteins and receptors, and activation of HIF leads to changes in tumor cell function. These changes include de-differentiation, angiogenesis, invasion and metastasis formation,[9,10] and the glucose and energy metabolism.[11]

It is generally accepted that cancer is not merely an accumulation of genetic mutations that lead to unharnessed cell proliferation, but that it is mainly dependent up on the surrounding microenvironment. Since the cell microenvironment has a critical role in cancer formation and progression, this paper discusses the role of hypoxia as the main factor in tumourigenesis.

Hypoxia, inflammation and tumorigenesis

Inflammation is a defensive reaction of living tissue to injury. Clinical evidence indicates that hypoxia promotes inflammation. In a mutually exclusive fashion, hypoxia can promote inflammation and inflamed lesions are often severely hypoxic. Indeed, hypoxia can influence the tissue environment of the tissue, particularly by regulating oxygen-dependent gene expression.[12] Cancer is a clinical condition in which hypoxia and inflammation interact at several points.

As early as the 19th century, cancers were understood to arise from sites of chronic inflammation, infection and chronic irritation.[13,14] Characteristics of inflammation have been established even in tumors with no relationship to infection, such as breast tumors. Epidemiological studies are consistent with the notion that chronic inflammation predisposes to different types of cancer, suggesting a link between infections and inflammatory responses.[13] It is generally accepted that tumors and sites of inflammation, such as wounds, have many similarities in common. Tumors can be thought of as wounds that will not heal.[15] A variety of inflammatory mediators, such as interleukin (IL)-1, IL-6, tumor necrosis factor-α, vascular endothelia growth factor (VEGF), and eicosanoids affect tumor progression.[16]

At the site of tumor foci, inflammatory response recruits leukocytes through the production of various cytokines and chemokines, including VEGF and CXCR4, involved in angiogenesis and metstasis.[17] Hypoxia via HIF affects the performance of innate immunity. The tumor-recruited leukocyte population is heterogeneous and consists, in part, of tumor-associated neutrophils (TANs) and tumor-associated macrophages (TAMs) that acquire N2 and M2 phenotypes under the hypoxic environmental conditions of the tumor, respectively. TANs with N2 phenotype have a pro-tumorigenic effect secreting pro-tumorigenic factors, promoting angiogenesis and suppressing immune responses.[18-20] Recent studies have also suggested a pro-metastatic role for TANs with N2 phenotype. There is evidence to suggest that a hypoxic environment gives N2 phenotype to TANs at the tumor foci, whereas neutrophils seeding in the lungs with a normoxic environment acquire a N1 phenotype and have anti-metastatic effects.[21] In addition, hypoxia via HIF dramatically redirects differentiation of TAMs toward M2 in the tumor microenvironment. M2 macrophages produce cytokines and other mediators which favor tumor growth and progression.[22,23]

The hypoxic environment of the tumor influences adaptive immunity by stimulation of regulatory T-cell (Treg) activation and differentiation.[2,24,25] In addition, hypoxia affects denderitic cell differentiation in the same way that naïve T cells favor T-helper 2 (Th2) response.[12] Re-oxygenation of hypoxia-differentiated dendritic cells induces Th1 and Th17 cell differentiation, restricting tumor growth and progression.[26,27] In breast cancer, a high $CD4^+/CD8^+$ and Th2/Th1 ratio of tumor-infiltrating lymphocytes is indicative of poor prognosis.[28] On the other hand, TAMs are educated by Th2 $CD4^+$ T cells to produce pro-angiogenic and pro-metastatic factors stimulating mammary cancer progression and metastasis.[22] In addition, there is also evidence that many of the T-cell subsets found in solid tumors can aid tumor promotion, progression, or metastasis, including $CD8^+$ T cells,[29] interferon - producing Th1 cells,[30] Th2 cells[22,31] and Th17 cells.[32,33] Nevertheless, natural killer cells are the only cells that, so far, have not shown a pro-tumorigenic role. The determining factor that gives the same T-cell subset an anti-tumorigenic role in one cancer and a pro-tumorigenic role in another is for the most part not known.

Surprisingly, Treg cells, which are known to play a pro-tumorigenic role through suppression of anti-tumor immune responses,[34] may also play an anti-tumorigenic role under certain circumstances by virtue of their ability to suppress tumor-promoting inflammation.[35] All of the evidence mentioned above indicates that there is hypoxic microenvironment present in chronic inflammation and cancer, and that, therefore, they are closely linked. Angiogenesis is co-dependent with chronic inflammation and cancer;[36,37] this is a process dictated by a hypoxic microenvironment to overcome the starvation of oxygen and is observed in both conditions. Inflammatory cells, mainly M2 cells, and immunological mediators such as VEGF, chemokines, eicosanoids, and reactive oxygen, are involved in angiogenesis.[16]

Hypoxia and angiogenesis

The induction of new blood vessels by solid tumors was first recognized by Virchow nearly 150 years ago, but tumor angiogenesis is frequently linked to the name of Dr. Judah Folkman who proposed that the growth of all solid tumors is dependent on angiogenesis.[38] It is an important event in various biological processes, including embryonic vascular development and organ regeneration. Angiogenesis is also observed in many pathological conditions, such as inflammation, wound healing, and tumor growth. Nevertheless, tumor vasculature differs in many aspects from the vasculature of normal organs.[39]

Hypoxia is well known for its role in the induction of angiogenesis in hypoxic zones and for its essential involvement in further tumor development.[40] HIF regulates genes that are key players in cancer development. They include many angiogenic mediators, such as VEGF, genes involved in cellular matrix metabolism (urokinase-type plasminogen activator receptor, matrix metalloproteinases-2 MMP2) and glycolysis (phosphoglycerate kinase-1 and lactate dehydrogenase[41]). Haplode - letion of prolylhydroxylases, which hydroxylate and facilitate HIF destruction, in mice leads to *vascular normalization*, as defined by more sharply demarcated boundaries and branching points of tumor vessels[42] and tumor oxygenation. Vascular normalization is due to attenuation of tumor-vessel leakiness and vascular distortion and improvement of tumor-vessel architecture. This change reduces tumor invasiveness and the risk of metastasis.[43] However, other reports indicate that therapeutic strategies targeting the inhibition of neoangiogenesis lead to HIF activation and promotion of metastasis formation.[44,45] This suggests that oxygen-deprivation and prolonged enforced oxygen-deprivation of tumors by anti-angiogenic drugs can increase migratory activity and metastatic potential. Thus, therapeutic strategy in cancer treatment targeting inhibition of tumor vascularization leads to a sustained oxygen deprivation and may change the behavior of tumor cells so that they are either beneficial for the treatment, or can even worsen it. Therefore, the degree of hypoxia after treatment at the tumor foci can have a direct effect on the efficiency of the therapeutic strategy. Since the degree of hypoxia also affects cell differentiation status, some therapeutic strategies target this status in tumor cells.

Hypoxia, differentiation and tumorigenesis

Cell differentiation shows the degree of cell maturity. Fully differentiated (fully mature) cells have the same form and function as their parent cells and proliferate very slowly, if at all. In contrast, immature cells do not resemble their parents, are poorly differentiated, and are able to proliferate at a higher rate. Most cancer cells are less differentiated and less mature than normal cells and, therefore, proliferate readily.

The degree of cell differentiation is regulated by gene expression. Therefore, manipulation of gene expression can change the degree of differentiation. A number of natural compounds can induce differentiation in cancerous cells, decrease the proliferation rate of cancerous cells, and make them display fewer malignant characteristics.[46]

There is increasing evidence to suggest that hypoxia is the potent inhibitor of tumor cell differentiation and thus plays a key role in the maintenance of cancer stem cells, likely via the evolution of the tumor stromal microenvironment.[47]

The main hypoxia-activated pathway in cancer cell differentiation is mediated by HIF. HIF has been shown to inhibit or even reverse cell differentiation.[48] De-differentiation was induced by hypoxia and the cells exhibited downregulation of p53 gene expression.[49-53] Overexpression

of HIF has been shown to induce epithelial-mesenchymal transition (EMT), and metastatic phenotypic and migration activity change both *in vitro* and *in vivo*. Inversely, the process of EMT can be reversed by repression of HIF expression in hypoxic cells. HIF can regulate transcription factors, *e.g.* Snail, LOX or TWIST, involved in EMT.[54-56] Hypoxia can also act via activation of other key regulatory pathways in stem/progenitor cells, such as transforming-growth factor-β, CXCR4 and its ligand CXCL12 or stromal cell-derived factor-1, and erythropoietin-erythropoietin receptor pathway.[47]

The advantage of arresting tumorigenic cells in their undifferentiated state by hypoxia is that this makes immature stem cells more resistant to therapy compared to differentiated and partially differentiated stem cells, and also maintains their clonogenic/tumorigenic potential.[57] Presumably, de-differentiation occurs as a defensive mechanism to protect hypoxic cells against further hypoxia. Another important defense mechanism activated in response to hypoxia is increased expression of heat shock proteins (HSPs).

Hypoxia, cellular heat shock protein level and tumorigenesis

Heat shock proteins are highly conserved molecules, some of which are induced by cellular stresses, including hypoxia and oxidative damage.[58] HSP activation is critical to adaptation to hypoxia. There is a regulatory link between the cell oxygen sensors and heat shock pathways. Expression of inducible HSPs is regulated by heat shock factors (HSF), in particular HSF1.[59,60] HSF transcription is necessary for full HSP induction during hypoxia. Some reports have suggested that stresses associated with energy deprivation can activate HIF-1.[59-61] Expression of HSF is up-regulated during hypoxia due to direct binding by HIF-1 to HSF promoter.[62] Increased levels of HSPs have been detected in many solid tumors. The HIF-1-dependent increase in HSPs provide a transient protection from stress, as reduced production of HSPs decreases viability of multiple human cancer cell lines, but had no effect on normal cells, suggesting that HSF-1 relieves cancer cells from the cellular stresses.[63] HSPs provide a transient protection from stress acting as chaperones by regulation of protein folding to ensure correct conformation and translocation.[58] It may explain the ability of malignant cells to maintain protein homoeostasis even in the hostile hypoxic microenvironment of the tumor. Accordingly, HSPs may account for the ability of tumor cells to tolerate genetic alterations that would otherwise be fatal.[64] Presumably, HSPs support tumorigenesis by orchestrating a broad network of cellular pathways that counteract apoptosis and promote tumor cell survival, autonomous cell proliferation, and metastasis, even in hypoxic, acidotic, and growth factor-deprived conditions.[63,65,66] The cytoprotective function of HSPs is also explained by their anti-apoptotic function. The anti-apoptotic properties of HSPs can partly be explained by their ability to interact with different proteins of the apoptotic process at distinct key points, thereby directly blocking programmed cell death machinery/mechanisms. In addition, it has been reported that the increased HSP expression in tumor cells is due to loss of p53 function. This HSP action in tumor cells can account for inhibition of apoptosis.[62]

Apoptosis and differentiation share many common features. Many recent reports implicate HSPs in the differentiation process. Accordingly, HSPs are induced during oncogenesis, as well as at specific stages of development and differentiation.[67] This indicates that HSPs not only protect cancerous cells against a hostile hypoxic microenvironment but also act in orchestration with de-differentiation of cancerous cells in the hypoxic condition. Presumably, HSPs support tumorigenesis by orchestrating a broad network of cellular pathways that counteract apoptosis and promote tumor cell survival, autonomous cell proliferation, and metastasis, even in hypoxic, acidotic, and growth factor-deprived conditions.[63,65,66]

Hypoxia and metastasis

Metastasis is a process in which diverse interactions between cancer cells and their microenvironment allow the cells to populate and flourish in new tissue habitats. This follows organ dysfunction and death. Angiogenesis, and the proliferation of a network of blood vessels that penetrates into cancerous tissue supplying nutrients and oxygen, trigger metastasis.[68] Therapeutics strategies that impede angiogenesis have been proposed to combat malignancy.[69] However, some reports have suggested that angiogenic inhibitors may sometimes induce a more invasive type of tumor and increase risk of metastasis.[44,45] It is thought that hypoxia enhances metastases, as tumors with low oxygen concentration are more malignant than tumors with high oxygen concentration.[70] *In vitro* experiments have demonstrated that exposure to hypoxia increases migration, invasion and adhesion of cancerous cells. Inversely, RNA interference in *silencing* HIF-1 resulted in a marked decrease in cancerous cell migration, invasion and adhesion. Analysis of a number of genes which regulate cancer invasion and metabolism, such as CXCR4, angiopoietin-related protein, and pyruvate dehydrogenase kinase 1, revealed that they are also activated by hypoxia in cancerous cells.[71] Accordingly, *in vivo* experiments have also demonstrated that hypoxic conditions increase the expression of angiogenic factors, including VEGF, angiogenin, and IL-8, and molecules involved in motility and adhesion of cancerous cells, including autocrine motility factor, MMPs, and selectin-ligands, leading to the increase in angiogenesis, motility, and adhesion of cancerous cells through which they invade the blood circulation.[70] Accordingly, it has been revealed that CXCR4 is activated by the lack of oxygen and this activation makes tumor cells migrate and home in on a specific set of organs. Indeed, combined expression of HIF-1, CXCR4, and VEGF strongly correlates with lymph node metastasis and distant metastasis.[72,73]

Altogether, it seems that the proteins encoded by these HIF-responsive genes have a variety of functions and aid the hypoxic cells by either increasing tissue oxygenation, *e.g.* VEGF (which stimulates the outgrowth of new blood vessels) or by enhancing cellular glucose uptake and metabolism to allow energy generation when oxygen is scarce. All of these enable the cells to migrate away from low oxygen areas and home to specific, distant organs.[74]

Conclusions

In general, the term *adaptation* is used for the changes imposed by hypoxia. I propose *defense* instead of *adapt*, as I believe this describes the situation better, since events ending to tumorigenesis are not some sort of diplomatic negotiation but a battle. The term *defense* shows that a harsh disturbance, *i.e.* hypoxia, has been imposed on the microenvironment and cells have to respond by undergoing change. The changes made by defensive mechanisms are not optimal for cells but they are committed to making them. Our understanding of the process of tumorigenesis helps us design better therapeutic strategies.

Hypoxic cells defend against or respond to hypoxia by angiogenesis and by activating the expression of genes involved in better tissue oxygenation. This causes the cells to escape necrosis and apoptosis and survive. However, if the situation worsens, severe hypoxia, prolonged hypoxia, or anoxia may initiate a cascade of events that leads to apop-

totic cell death. Severe hypoxia, prolonged hypoxia, or anoxia may initiate apoptosis, whereas cells can often defend themselves against acute and mild hypoxia and survive. The severity of hypoxia determines whether cells can survive or enter apoptosis.[75] This is well illustrated by the experiments performed by Butterworth and co-workers who generated a human prostate cancer cell subline by extended oxygen-deprivation.[76] In other words, it seems that cells become cancerous in order to defend themselves against environmental insult, mainly hypoxia and hypoxia, increasing intrinsic tumorigenic potential.[47] This is shown more clearly by an increase in HSPs and de-differentiation under hypoxic conditions. The starving cells de-differentiate as an economically compensatory defense mechanism against hypoxia. Inversely, it has been shown that differentiation status can be improved and tumor arrest can be induced if exposed to higher oxygen concentrations.[51,77] In addition, experiments demonstrating vascular normalization and tumor oxygenation may be beneficial for treatment. It seems that the last line of cell immune response is performed by metastasis, as demonstrated by the notion that starving tumor cells of oxygen is one trigger for metastasis.[78,79] It seems that the starving cells travel and home into an environment that is more conducive to live in. Finally, under severe hypoxia or anoxia, the cells submit and commit suicide, as observed by apoptosis. Thus, effective therapeutic strategies may be those that correct the physical/chemical condition of the tumor microenvironment as this affects tumor cells, stromal cells of the tumor site, and the immune system. *Correcting therapies* which correct the physical/chemical condition of the tumor microenvironment may either help tumor cells to return to normal form (correct tumor cells) and/or help the immune system to eradicate tumor cells which do not correct or do not return to normal form (uncorrected cells). Based on the plasticity that protects cells from damage caused by hypoxia and enables cells to de-differentiate,[80] it may be possible to promote cancerous cells to its differentiated state by *correcting therapy*. The aim of this approach is to reprogram cancerous cells towards a normal state; this may depend on tissue type, as oxygen tension is different in different tissues.[77,80,81]

Notably, tumorigenesis does not happen suddenly, but is a process influenced by elements such as timing and target tissue. Accordingly, a simple oxygenation will not correct the microenvironment and so correct tumor cells. This is the same for any therapeutic agent. Any medication is administered according to a program, and *correcting therapy* also follows this rule. A model of malignancy is pregnancy and the fetus is regarded as a pseudomalignancy. The fetus is delivered/rejected after differentiation. As in embryogenesis, during which a gradient of hypoxia is involved in differentiation,[82-84] it has been suggested that *correcting therapy* in a programmed approach re-differentiates cancerous cells. Administration of *correcting therapy* should be set up in a programmed way, and may be administered with graded exposure or according to a planned gradient. It may even vary depending on the target tissue or may even complement it with current therapies.

In this review, *correcting therapies* using oxygen has been proposed because oxygen tension affects many aspects of the tumor microenvironment, such as acidity, which hampers current therapies.[85] The idea of *correcting therapy* is still in its infancy and its application needs to be developed, and, as with other therapeutics approaches, dose and planning of *correcting therapies* using oxygen need to be set up.

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