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REVIEW

Zhao et al: Detection and impact of tumor hypoxia

Tumor hypoxia: Classification, detection, and its critical role in cancer progression

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ABSTRACT

Hypoxia is a common feature of solid tumors and plays a critical role in cancer progression. A thorough understanding of tumor hypoxia is essential for gaining deeper insights into various aspects of cancer biology. This review examines the key factors contributing to tumor hypoxia, such as inadequate blood supply and oxygen delivery resulting from rapid tumor growth. We present a detailed classification of hypoxic regions and provide an overview of current methods used to identify these areas—from molecular techniques to imaging approaches—offering a comprehensive and multifaceted perspective. Additionally, we explore the mechanisms by which hypoxia drives tumor progression. Under low-oxygen conditions, tumor cells can alter their biological behavior, influencing processes such as cell proliferation, immune evasion, and the maintenance of tumor stem cells. By addressing these dimensions, we aim to enhance understanding of how hypoxia contributes to cancer development. Through this in-depth exploration, we hope this review will offer valuable insights to guide future research and clinical applications.

Keywords: Tumor; hypoxic; factors; classification; progression.

INTRODUCTION

Solid tumors, formed by the uncontrolled proliferation of abnormal cells, pose a serious threat to human health. The compression of surrounding tissues and the potential for metastasis to other sites can lead to organ dysfunction and life-threatening conditions [1, 2]. Tumor cells spread through the blood and lymphatic systems, giving rise to distant metastases and complicating treatment [3]. Some tumors exhibit high malignancy, characterized by rapid growth and poor treatment response, imposing significant burdens on patients. In the United States, cancer ranks as the second leading cause of mortality, following heart disease, with individuals aged 60 years and older facing cancer as the primary cause of death [4]. Research indicates that the tumor microenvironment, comprising cells, stroma, and molecular elements surrounding the tumor, plays a crucial role in the formation and progression of tumors, significantly influencing the development and successful implementation of cancer treatment strategies [5].

In the tumor microenvironment, cellular components primarily include tumor cells, immune cells, fibroblasts, and endothelial cells, among others [6]. The intricate interactions between these cells through complex signaling pathways significantly influence the biological behavior of tumors. Additionally, the extracellular matrix (ECM) is a vital component of the tumor microenvironment. The ECM, consisting of collagen, fibronectin, and various growth factors, forms the structural foundation supporting the growth and spread of tumor cells [7]. Changes in the vascular system are also notable features of the tumor microenvironment, with tumors inducing angiogenesis (new blood vessel formation) to meet their high metabolic demands and sustain growth [8]. Overall, the complexity and diversity of the tumor microenvironment contribute to the increased difficulty in treating tumors. Hypoxia, serving as a hallmark of the tumor microenvironment (TME), is prevalent in the majority of tumors and results from an imbalance between heightened oxygen

consumption and insufficient oxygen supply. It plays a crucial role in the progression of solid tumors [9].

In normal tissues, the oxygen pressure is generally greater than 5.3 kPa. By contrast, research indicates that the oxygen pressure in the surrounding environment of tumor tissues can be as low as 0.9 kPa or even lower [10]. The pervasive hypoxic conditions primarily arise from cancer cells undergoing unregulated growth, altered metabolism, and a lack of contact inhibition, resulting in an incomplete and inefficient tumor architecture [11]. Hypoxia triggers alterations in gene expression, leading to subsequent proteomic changes with profound impacts on diverse cellular and physiological functions. These alterations ultimately constrain patient prognosis [12]. For instance, cells that divide slowly in hypoxic regions may evade the effects of cytotoxic drugs designed to target rapidly dividing cells. Additionally, cancer stem cells could potentially reside in poorly oxygenated regions, ensuring the occurrence of epithelial-to-mesenchymal transition (EMT) [13].

Overall, gaining a deeper understanding of the hypoxic characteristics within the tumor microenvironment contributes to the exploration of novel treatment strategies. This, in turn, aims to enhance the efficacy of cancer treatments and alleviate the suffering of patients. Therefore, this study focuses on exploring the factors, classification, determination of hypoxic regions, and their role in promoting tumor progression in the context of tumor hypoxia.

Introduction to tumor hypoxia

Factors leading to tumor hypoxia

The internal hypoxia within tumors is a intricately complex phenomenon influenced by multiple factors. Firstly, the rapid proliferation of tumor cells far exceeds the rate of formation of the surrounding vascular network, resulting in the formation of avascular hypoxic regions within the solid tumor. This creates a challenging environment for the survival and proliferation of tumor cells [9]. Simultaneously, solid tumors are

characterized by a convoluted vascular system that is structurally and functionally flawed, with features such as tortuous vessels, erratic growth, and defective endothelial cells, which together result in an uneven blood supply that exacerbates hypoxic conditions and further complicates the already arduous task of sustaining tumor cell viability within this harsh microenvironment [14, 15].

Research has shown that newly formed solid tumors initially lack the ability to generate their own blood vessels, instead relying on the diffusion of oxygen from nearby host vasculature to sustain their growth and proliferation [16]. As these avascular tumor masses continue to expand, the increasing distance from the central regions to the nearest blood vessels inevitably leads to a condition of hypoxia [17, 18]. Furthermore, the challenges associated with the transport of essential substances between vessels exacerbate this issue [19]. Due to the high oxygen demand of rapidly proliferating tumor cells, intense competition between cells exacerbates, leading to a noticeable increase in oxygen consumption. This competition further widens the scope of hypoxic regions, forming a mutually constraining vicious cycle that makes it increasingly difficult for tumor cells to maintain their normal physiological activities in hypoxic environments [20].

Chemotherapy and radiation therapy are also factors that can lead to tumor hypoxia. The research conducted by Mizrachi et al. [21] explored the vascular damage of the chemotherapy drug doxorubicin (DOX) from both in vitro and in vivo perspectives. In vitro experiments demonstrated that DOX could significantly increase the activity of acid sphingomyelinase (ASMase), leading to the generation of reactive oxygen species (ROS) within cells and cell apoptosis. For in vivo observations, molecular imaging techniques were used to monitor blood flow in the femoral artery of mice, revealing that DOX could cause constriction of small vessels and destruction of the walls of large vessels. This suggests that the chemotherapy drug DOX primarily triggers ROS production through the ASMase pathway, resulting in the apoptosis of endothelial cells and acute vascular injury. Additionally, there is compelling evidence indicating that angiopathy is a common complication following radiation therapy (RT), a result of the

ensuing vascular damage. In both adult and pediatric patients, this condition can manifest in several ways: steno-occlusive changes in the blood vessels, intracranial hemorrhages, the development of aneurysms, and a range of other vascular abnormalities [22]. The vascular damage induced by radiotherapy and chemotherapy not only increases the likelihood of vascular malformations but also diminishes the stability of the vascular system, leading to a more chaotic distribution of blood flow. This not only makes it challenging to achieve the intended therapeutic effects but may also exacerbate the extent of hypoxic regions within the tumor, posing even greater challenges to treatment.

Anemia, typically caused by disease states or treatment processes, is a pathological condition characterized by a decreased number of red blood cells or hemoglobin levels, which can compromise the blood's oxygen-carrying capacity. This reduced oxygenation precipitates the emergence of hypoxic regions, adversely affecting the functionality and survival of tumor cells [23, 24]. Furthermore, research has elucidated that the oxygen-carrying capacity of blood deteriorates with advancing age and chronic smoking, which in turn may intensify the intratumoral hypoxic conditions [25, 26]. However, additional studies are warranted to elucidate the complex interactions between these systemic factors and their cumulative effects on the tumor microenvironment.

Lastly, physiological factors such as hormone levels may also influence tumor hypoxia. Research data suggests a connection between estrogen and the hypoxic pathway, where estrogen-mediated signals can directly drive hypoxia-inducible factor-1α (HIF-1α) expression and positively or negatively impact the hypoxic pathway in different cellular environments [27]. Additionally, changes in the activity of the immune system may lead to chronic inflammatory responses, increasing the infiltration of immune cells and thereby affecting the normal functioning of blood vessels. Immune cells intricately shape the phenotypes and functions of tumor vessels through the intricate interplay of various cytokines. Innate immune cells, such as mature dendritic cells (mDCs) and M1-like tumor-associated macrophages (TAMs), emit a repertoire of cytokines, such as IFN-α, IL-12, IL-18, or TNF, and chemokines like CXCL9, CXCL10, or CCL21, that

proficiently suppress tumor angiogenesis. Concurrently, adaptive immune cells, including CD8+ T cells and T helper 1 (TH1) cells, release IFN-γ, a potent cytokine that not only hampers angiogenesis but also instigates vascular normalization within the intricate milieu of the tumor microenvironment (TME) [28]. These changes in the tumor immune system lead to vascular malformations within the tumor, ultimately resulting in the occurrence of local hypoxia.

Therefore, we conclude that the explosive proliferation of tumor cells creates areas within the solid mass with insufficient blood supply. Known as hypoxic zones, these areas are caused by the increased distance from functional blood vessels, the abnormal structure and function of the tumor's vasculature, and the intense competition for oxygen among the rapidly dividing cells. External factors such as radiotherapy, chemotherapy, anemia, advanced age, smoking, immune dysregulation, and hormonal imbalances can all exacerbate the severity of tumor hypoxia (Figure 1). The detailed roles of various factors influencing hypoxia and its associated outcomes are outlined in Table 1. In this table, factors influencing tumor hypoxia are presented, such as cell proliferation, vascular issues, treatments, blood factors, and physiological factors. Alongside these factors are their effects, like outpacing vessel growth, causing vascular problems, and reducing oxygen - carrying capacity, as well as the consequences including tumor hypoxia. In comprehending the intricacies of this complex mechanism, we can observe that tumor hypoxia is not solely the result of a single factor but rather an outcome of the interaction of multiple factors. This profound understanding provides crucial clues for the development of more precise and effective therapeutic strategies.

Classification of tumor hypoxia

Research reports suggest that biological and therapeutic consequences seem to vary for different types of hypoxia. Thus, a distinction between and quantification of these subtypes may be necessary [29]. Factors contributing to the formation of tumor hypoxia mainly include abnormal vascular structure and function within solid tumors, increased distance for substance transport between vessels, intense competition for oxygen among rapidly proliferating tumor cells, and anemia induced by disease or treatment leading to a decrease in the blood's oxygen-carrying capacity [30-32]. Therefore, based on these hypoxia mechanisms and their duration, tumor hypoxia is primarily classified into three types: perfusion hypoxia, diffusion hypoxia, and anemic hypoxia [23, 33, 34]. Perfusion hypoxia, also referred to as acute hypoxia, typically occurs for periods ranging from minutes to hours during oxygen deprivation [29]. Acute hypoxia is believed to primarily stem from temporary interruptions in blood flow, largely attributed to physical obstructions within the blood vessels [33, 35]. These transient interruptions in blood flow can be prompted by various factors, including vascular thrombosis, vessel rupture, and alterations in hemodynamics. Vascular occlusion refers to the presence of blood clots or other substances within blood vessels, obstructing blood flow. This may result from thrombus formation or the invasion of blood vessels by tumor cells. It leads to a sudden interruption of blood flow, preventing the supply of blood to the occluded area, thereby causing hypoxia within the tumor [36]. Conversely, tumor-induced hypoxia can further contribute to the formation of blood clots, creating a vicious cycle [37, 38]. Vascular rupture may occur due to abnormal vascular structure or vascular fragility within tumor tissue, leading to rapid blood leakage into surrounding tissues and subsequent reduction in blood flow. Meanwhile, the hemodynamic changes in tumor tissue can result in the instability of blood supply, further contributing to the formation of a hypoxic environment within the tumor. Studies have shown that when cediranib is used in combination with radiotherapy and chemotherapy, approximately 50% of glioblastoma patients experience a certain degree of improvement in tumor perfusion, suggesting the potential of adjuvant treatment modalities to impact tumor hemodynamics [39]. Nevertheless, once a tumor establishes a hypoxic environment, it can, in turn, exert a detrimental effect on hemodynamic changes [40]. In summary, it is important to recognize that the combination of these aforementioned factors possesses the inherent capability to precipitate an acute onset of hypoxia.

Diffusion-related hypoxia, also known as chronic hypoxia, typically manifests during periods of oxygen deprivation lasting from hours to weeks [29]. Chronic hypoxia is detected in 65-86% of tumor tissues [33, 41]. The origins of chronic hypoxia encompass various factors, including compromised diffusion resulting from the considerable distance between the hypoxic region and blood vessels (tumor cells situated more than 70 μm from nutrient-carrying vessels are prone to oxygen deficiency); inadequate blood supply due to structural irregularities in the tumor's vascular network, such as perforations, blunt endings, tortuosity, sluggish flow, and poorly perfused vascular branches; intra-tumoral pressure induced by solid stress from non-fluid components or the interstitial pressure of fluid components; and alterations in diffusion 'geometry,' such as concurrent versus countercurrent blood flow within the tumor microvessel network. Each of these factors contributes to a sustained reduction in the delivery of oxygen, nutrients, growth factors, and impedes the transportation of anti-cancer and imaging agents [33, 35, 42].

Anemic hypoxia, also referred to as systemic hypoxia, denotes a state where tissues experience oxygen deficiency due to an inadequate oxygen-carrying capacity in the bloodstream. This deficiency primarily stems from a reduction in the oxygen-carrying elements of blood, specifically red blood cells and hemoglobin. Experimental investigations have revealed a substantial decrease in oxygen supply to tumors, coupled with heightened hypoxia, when hemoglobin levels dip below 10–12 g/dl. This phenomenon is particularly accentuated when there is a concurrent decrease in both oxygen transport capacity and perfusion rate [43, 44]. Consequently, effective management of anemia is imperative to enhance the outcomes of other therapeutic interventions.

There are three main therapeutic avenues for addressing anemia: iron supplementation, blood transfusion, and the use of erythropoiesis-stimulating agents (ESAs). Unfortunately, both transfusions and the administration of ESAs carry a significant risk of severe adverse effects, particularly thromboembolism. Given that iron-deficient anemia is a common cause of anemia in cancer patients, it becomes essential not only to assess hemoglobin levels but also to evaluate serum ferritin levels and saturation. In cases of absolute iron deficiency, iron supplementation is essential, while in instances of relative iron deficiency, iron supplementation needs to be complemented with the administration of ESAs [45, 46]. However, certain research [47] indicates that elevating

hemoglobin levels in patients with head and neck squamous cell carcinomas may have adverse effects, leading to decreased survival and compromised tumor control. These unanticipated outcomes may be attributed to a potential stimulation of tumor growth resulting from a sudden surge in oxygen availability or the activation of growth-promoting erythropoietin receptors within the tumor tissue.

In each of these hypoxia subtypes, while there is a critical reduction in oxygen supply to the tissues, various aspects such as the perfusion-dependent delivery of diagnostic and therapeutic agents, the provision of essential nutrients, the clearance of metabolic waste products, and the tissue's capacity for repair can vary or remain unaffected [29]. Martijn and his colleagues analyzed four clinical low-oxygen gene expression profiles and compared them with the corresponding acute and chronic low-oxygen gene expression profiles obtained from in vitro experiments. The results demonstrated that the acute hypoxia profile is a more robust prognostic indicator for patients with advanced-stage head and neck cancer than chronic hypoxia [48]. Nevertheless, most of the current research underscores the extensive analysis of both acute and chronic hypoxia within the confines of experimental and preclinical studies. Direct evidence regarding both acute and chronic hypoxia in the field of clinical oncology remains scarce, largely because of the lack of reliable detection and quantification methods. Consequently, the translation of experimental insights into clinical practice urgently requires the adoption of advanced technologies, including enhanced imaging techniques and the utilization of valid modeling, before any adjustments to current radiotherapy regimens can be effectively implemented [41]. In recent years, researchers have identified and classified the state of hypoxia in certain specific tumors, such as hepatocellular carcinoma, into two distinct subtypes through a meticulous analysis of hypoxic transcriptomes. This nuanced classification has unveiled the presence of unique clinical and pathological features characterizing each subtype. However, it is noteworthy that an observed heterogeneity in tumor-infiltrating immune cells further emphasizes the complex and varied microenvironment linked to the two distinct hypoxic tumor subtypes [49].

Determination of tumor hypoxic regions

Evidence suggests the presence of hypoxic regions within the internal milieu of many solid tumors [50]. The identification of hypoxic regions holds crucial significance for the treatment of tumors. Currently, the detection of tumor hypoxic regions primarily

involves two methods: invasive polarographic oxygen electrode detection and non-invasive medical imaging techniques.

Polarographic oxygen electrodes. While the concept of tumor tissue hypoxia was proposed 65 years ago based on histological findings, its confirmation awaited subsequent years when direct measurements of oxygen concentration in various tumor types were conducted using polarographic oxygen electrodes [51, 52]. The outcomes derived from polarographic oxygen electrodes offer a visual depiction of the oxygen levels within the tumor and are frequently acknowledged as the gold standard [53]. However, despite its effectiveness, the polarographic oxygen electrode method has some limitations. One major disadvantage is that it requires multiple measurements, which can be time-consuming and labor-intensive. Additionally, the electrode may experience pressure as it moves through the stroma and fibrous tissues, potentially introducing biases in the collected data. Furthermore, the invasive nature of the procedure, which involves inserting the electrode into the tumor, can cause discomfort to patients and even increase the risk of tumor metastasis [54]. Consequently, there is a pressing need for more contemporary and versatile techniques to overcome these limitations.

Medical imaging

Molecular imaging techniques, coupled with appropriate contrast agents, enable accurate detection of hypoxic tissues within tumors by overcoming the limitations of oxygen electrode measurements and providing comprehensive information on oxygen-deprived areas throughout the entire tumor tissue. Considering the close correlation between intratumoral hypoxia and malignancy, drug resistance, and other factors, non-invasive imaging of hypoxia using molecular imaging can offer critical information for the prognosis of tumor treatment. This approach aids in guiding clinical assessments more effectively, ultimately enhancing the success rate of treatments. Currently, multiple molecular imaging modalities can be employed for tumor hypoxia imaging, among which positron emission tomography (PET) imaging is included. Research shows that 18F-fluoromisonidazole (18F-FMISO) is a PET tracer derivative. It is mainly used to detect hypoxia in human studies and is the most widely used tracer for this purpose among such tracers [55]. The study conducted by Panek et al. enrolled 10 patients with locally advanced Head and Neck Squamous Cell Carcinoma (HNSCC). Prior to chemotherapy and radiotherapy, two 3T magnetic resonance imaging (MRI)

scans were performed at specific time intervals. The findings indicated that T2 measurement demonstrated a certain degree of reproducibility. Moreover, it was highly sensitive to variations in blood oxygen saturation, and significant changes could be detected under specific blood oxygen saturation conditions. This research outcome confirms that T2 measurement based on 3T MRI can effectively detect the oxygenation changes in tumor tissues, highlighting the crucial value of MRI in detecting tumor hypoxic regions [56]. In addition, optical imaging [57, 58] and photoacoustic imaging [59, 60] are also important tumor hypoxia imaging techniques.

Evidence suggests that methods such as tumor vascular analysis, determination of tumor metabolic activity, assessment of DNA damage in tumor cells, detection of radioresistant hypoxic markers, and endogenous biological hypoxia markers can also serve as means for detecting tumor hypoxia [61, 62]. Furthermore, significant recent technological advancements have been made in real-time, high-resolution oxygen mapping [63] and 3D microtumor “organoid” platforms [64]. These innovations indeed possess distinct advantages when it comes to understanding the hypoxic state of tumors. Overall, the detection of intratumoral hypoxia can provide a basis for clinical assessment of tumor prognosis and the adoption of intervention measures. Targeted therapy for hypoxic tumors can overcome resistance issues encountered in traditional tumor treatments, holding significant relevance for clinical strategies to prevent and treat hypoxia while enhancing the body's compensatory adaptive capabilities. Furthermore, there are documented indications that the deduction of tumor hypoxia can also be accomplished through gene expression profiling, with numerous gene expression profiles specifically designed for hypoxia having been put forth [48]. However, this type of detection can only determine whether a tumor is hypoxic and cannot precisely identify the hypoxic regions within the tumor.

Research into the hypoxia conditions at the micro - regional scale of tumors has always drawn significant attention. Studies have indicated that the structural adaptive deficiencies of the tumor microvascular network give rise to oxygen distribution heterogeneity. Its signal transduction capacity is diminished, and unlike normal microvessels, it cannot effectively regulate the blood vessel diameter in accordance with hemodynamic and metabolic requirements. Consequently, there is a discrepancy in blood vessel diameters, uneven blood flow distribution, inadequate blood perfusion in certain areas, restricted oxygen supply, the emergence of hypoxic regions, and

heterogeneous oxygen distribution. This heterogeneity influences tumor growth and development, rendering tumor cells in hypoxic areas more resilient to chemotherapy and radiotherapy and thereby reducing the treatment efficacy [65]. Moreover, some scholars have introduced a data - driven mechanistic modeling approach. This approach uses patient - derived tumor xenograft models of three distinct tumor types (breast cancer, ovarian cancer, and pancreatic cancer) as the research subjects. The marker density is extracted via image processing. Subsequently, these data are utilized to formulate reaction - diffusion equations for describing the oxygen distribution, and furthermore, a hypoxia distribution model is deduced. The results demonstrate that owing to the uneven distribution of blood vessels within the same tumor, there are variations in oxygen supply. Additionally, the hypoxia characteristics of different tumor types also exhibit notable disparities [66]. A comprehensive analysis and contrast of the variations in hypoxic regions across different clinical tumor types has been carried out. As shown in Table 2, for multiple tumor types including brain metastasis, breast cancer, cervical cancer etc., details such as stage, number of cases, oxygen level within the tumor, and the proportion of HF < 2.5mmHg are presented, revealing differences in hypoxic characteristics, intratumoral heterogeneity, and spatially variable hypoxia among tumor types.

Tumor hypoxia signaling pathway

HIF signaling pathway plays a central role when tumor cells respond to a hypoxic environment. It widely influences numerous physiological and pathological processes such as angiogenesis, metabolism, and tumor development [81, 82]. Many studies have focused on its related signaling mechanisms. Some research has pointed out that the small molecule inhibitor SU5416 can significantly downregulate the expression levels of VEGF and HIF-1α in ovarian cancer cells by inhibiting the PI3K/Akt signaling pathway. On the other hand, 4-hydroxyestradiol (4-OHE2) can promote the high expression of HIF-1α by regulating the PI3K/Akt signaling pathway. This fully highlights the crucial role of the PI3K/Akt signaling pathway in regulating the expression of HIF-1α [83, 84]. Apart from the PI3K/Akt signaling pathway, HIF is also interconnected with other signaling mechanisms. Some research has been conducted on liver injury after traumatic hemorrhage, elaborating on the close association between the MAPK pathway and HIF. Traumatic hemorrhage significantly reduces the activity of liver p38 MAPK and simultaneously induces a significant increase in the expression

of HIF-1α. When treated with the p38 MAPK inhibitor SB203580, the recovery of p38 MAPK activity is inhibited, and the decrease in the expression of HIF-1α is also prevented, confirming the role of the MAPK pathway in regulating the expression of HIF-1α [85]. In addition, HIF can also be regulated by multiple other signaling pathways, including the JAK-STAT3 signaling pathway [86], the NF-κB pathway [87], the Notch pathway [88] and the Wnt/β-catenin pathway [89].

Hypoxia and genetic damage repair

Hypoxia is a common feature of many solid tumors and is known to induce various genetic damages. When cancer cells are subjected to low oxygen levels, they may develop heightened resistance to treatments like radiotherapy and chemotherapy. This resistance is attributed to alterations in the frequency of DNA lesions, including single- and double-strand breaks (referred to as DNA-SSBs and DNA-DSBs), DNA-DNA cross-links, DNA base damage, and DNA-protein cross-links [90]. Studies have indicated that in both in vitro and in vivo models of hypoxic cancers, there is a notable increase in gene mutation rates, ranging from two to five times higher than normoxic conditions [91-93]. Furthermore, when comparing tumor cells grown in a controlled lab setting (in vitro) to those within a living organism, the latter exhibits more extensive genomic rearrangements and a higher prevalence of point mutations and small deletions in specific genes. In vitro studies have also observed that hypoxic stress can induce excessive DNA replication, resulting in gene amplification and the formation of genetically unstable regions. Most concerning is the finding that in mouse models, subjecting fibrosarcoma and melanoma cells to hypoxic conditions not only induces genomic instability but also enhances their capacity to generate metastases [94]. In summary, these aforementioned mutations and chromosomal breaks promote the activation of oncogenes, thereby facilitating the emergence of cancer cell variants with enhanced proliferative capacity [95]. However, there are also reports that exposing various cancer cell lines to physiological levels of chronic hypoxia (0.2% oxygen) can lead to the accumulation of γH2AX dependent on HIF, without causing detectable levels of DNA strand breaks [96].

Alfredo's research has demonstrated that severe hypoxia and acidosis in breast cancer cells can induce the expression of lncMat2B, which is observed in the hypoxic tumor-initiating cell (TIC) population within multicellular tumor spheroids (MCTS), and overexpression of lncMat2B enhances cancer cell resistance to cisplatin by reducing

cisplatin-induced DNA damage and promoting DNA repair [97]. Notably, not only severe hypoxia but also moderate hypoxia can induce replication stress and activate proteins involved in the DNA damage repair (DDR) pathway [98]. Besides, the type of hypoxia that occurs may also be a determining factor in the nature of DNA repair mechanisms involved in cancer development. Acute hypoxic stress rapidly alters DNA repair pathways through post-translational modifications; however, persistent hypoxia can lead to the transcriptional and/or translational downregulation of DNA repair proteins over an extended period. Additionally, prolonged moderate hypoxia can induce epigenetic regulation of DNA repair genes, contributing to the complex modulation of cellular responses under hypoxic conditions [94].

Ultimately, under conditions of hypoxia, both genetic damage and repair in cancer cells have the potential to stimulate tumor cell proliferation, presenting a multifaceted problem that warrants further extensive investigation.

Hypoxia-driven metabolic reprogramming

The metabolic reprogramming of tumor cells in response to hypoxia is a crucial area of research. This is because it not only significantly impacts tumor growth and survival but also holds great significance in formulating therapeutic strategies. Among the processes of hypoxia-induced metabolic reprogramming, HIF-1α plays a central role. HIF-1α gets activated under low oxygen conditions and coordinates a wide range of metabolic adaptations within cancer cells. For example, Faubert et al. [99] demonstrated that the loss of the tumor suppressor LKB1 promotes metabolic reprogramming through HIF-1α, thereby highlighting the vital role of this factor in cancer metabolism. Building on this understanding of HIF-1α's significance, similarly, Chen et al. [100] showed that miR-3662 suppresses hepatocellular carcinoma growth by inhibiting the HIF-1α-mediated Warburg effect—a characteristic feature of cancer metabolism that is characterized by enhanced glycolysis even in the presence of oxygen. This further exemplifies how different molecular mechanisms related to HIF-1α contribute to the complex landscape of cancer metabolic regulation. Moreover, the metabolic reprogramming of cancer-associated fibroblasts (CAFs) significantly impacts the tumor-stroma interaction. Fiaschi et al. [101] reported that CAFs contribute to tumor progression in multiple ways. They promote the ability of cancer cells to undergo EMT, enhance the traits of cancer stem cells, and facilitate metastatic dissemination. Simultaneously, CAFs can shift towards Warburg metabolism. This

metabolic shift not only alters their own metabolic profile but also affects the metabolic state of neighboring cancer cells. This reciprocal metabolic reprogramming underscores the importance of the tumor microenvironment in shaping cancer cell metabolism.

In addition to the above aspects, research has also explored the role of sirtuins, a family of NAD+-dependent deacetylases, in regulating metabolic reprogramming under hypoxic conditions. In the context of tumor metabolism, sirtuins, MYC, and HIF - 1α exhibit intricate interactions. SIRT6, a multifunctional deacetylase within the sirtuins family that relies on NAD^+, can inhibit the transcriptional activities of MYC and HIF - 1α, the key transcription factors. MYC plays a crucial role in promoting cell proliferation and biomass production, and HIF - 1α stimulates glycolysis under hypoxic conditions. SIRT6 inhibits these activities via deacetylation. Significantly, SIRT6 modulates the activities of both MYC and HIF - 1α. They form a complex network, and their dynamic balance determines the direction and intensity of tumor cell metabolism, profoundly influencing the biological behavior of tumors [102]. Furthermore, in recent years, a study has identified glycogen branching enzyme (GBE1) as a downstream target of HIF-1α in lung adenocarcinoma. It has established a connection between the expression of GBE1 and metabolic changes that drive tumor progression, highlighting the role of specific metabolic enzymes in mediating the effects of hypoxia on tumor progression [103].

In conclusion, the metabolic reprogramming of tumors in response to hypoxia is a multifaceted process. It involves various signaling pathways, metabolic enzymes, and interactions within the tumor microenvironment. HIF-1α serves as a central regulator of these adaptations, exerting a profound influence on cancer cell metabolism.

Hypoxia promotes tumor progression

The hypoxic microenvironment within tumor tissues, characterized by low oxygen levels, and the hypoxic tumor cells residing within these areas play a crucial role in propelling tumor progression [104]. This has been extensively validated through numerous studies that have elucidated how such conditions can facilitate tumor growth by activating various molecular pathways and signaling cascades, thereby contributing to the aggressive behavior and resistance of cancers.

Hypoxia promotes tumor angiogenesis

One of the crucial roles of the hypoxic microenvironment in tumor proliferation is to promote the construction of new blood vessels, a process that provides essential

nutrients for tumor growth. This process involves the participation of the hypoxia-inducible factor (HIF) family. Known HIFs include three subtypes, with HIF-1 having a significant association with angiogenesis [105, 106]. When exposed to low oxygen levels or hypoxic conditions, the HIF-1 plays a pivotal role in orchestrating the response of tumor cells. It exerts its influence by directly targeting these cells and inducing the production of Vascular endothelial growth factor (VEGF), a potent signaling protein. The action of VEGF is multifaceted; it not only promotes the division and multiplication of endothelial cells, which line blood vessels, but also increases the permeability of these vessels. This dual action facilitates a cascade that leads to the formation of new blood vessels in and around the tumor microenvironment. By fostering an increase in vascular permeability, VEGF enables a more efficient transport of essential nutrients to the tumor cells. Consequently, this improved nutrient supply supports the aggressive growth and survival of cancerous cells, as they require an abundance of resources to sustain their rapid proliferation. In addition, studies have shown that HIF can also activate carbonic anhydrase-9 (CA9), which also plays a crucial role in the process of hypoxia-induced angiogenesis [107]. In essence, HIF's activation under hypoxic conditions creates a proangiogenic state that bolsters the tumor's ability to thrive by ensuring a robust and continuous supply of nutrients via the enhanced vascular network [108, 109].

Extracellular vesicles, commonly found in the microenvironment, are small vesicles secreted by various cells involved in processes such as intercellular communication. In hypoxic conditions, tumor cells exhibit rapid proliferation and heightened metabolism, leading to a significant increase in the secretion of extracellular vesicles [110, 111]. These vesicles, brimming with an abundance of nucleic acid molecules, stand as vital repositories of pivotal data that orchestrate the relentless multiplication of tumor cells [112]. Of particular interest is the recent investigative spotlight cast on miR-23a, a microRNA residing within the exosomes shed by these malignant cells when subjected to hypoxic stress. Research evidence suggests that such a deficit in oxygen dramatically amplifies the concentration of miR-23a within these communicative vesicles. Delving deeper into this molecular dance, researchers have unveiled that miR-23a executes its nefarious influence by homing in on the gene for SIRT1, a protein crucial for normal cellular function, and dampening its production. By silencing SIRT1, miR-23a fuels the aggressive proliferation and recruitment of endothelial cells, which are the architects of

blood vessels. This hijacking of the endothelial cells' natural behavior is instrumental in the formation of new blood vessels, a hallmark of tumor growth and progression known as angiogenesis [113]. Additionally, the increased presence of extracellular vesicles in the hypoxic microenvironment, carrying abundant peroxidase, is also associated with the establishment of neovascularization in tumor tissues [114].

Hypoxia promotes tumor cell invasion

In a low-oxygen environment, the process begins with the activation of the small GTPase RhoA within breast cancer cells, an essential step for subsequent events. The activation of RhoA triggers a cascade of downstream signaling pathways, ultimately leading to an increase in the expression levels of the cell surface metalloproteinase MT1-MMP. MT1-MMP plays a crucial role in degrading proteins in the extracellular matrix (ECM), which in turn disrupts the ECM's integrity and provides avenues for tumor cell migration and invasion. As MT1-MMP expression increases, its activity is further modulated by hypoxic conditions. In this oxygen-deprived microenvironment, MT1-MMP activates another Matrix Metalloproteinase, MMP-2 [115]. Simultaneously, upon its activation, the small GTPase Ras plays a pivotal role in stabilizing HIF-1α, which can subsequently lead to the upregulation of the expression of MMP-9 [116] and fascin-a protein that bundles actin filaments and has been implicated in enhancing the migratory, dispersive, and invasive properties of cancer cells. Furthermore, fascin exerts regulatory influence on the expression of MMP-2 via the concerted action of protein kinase C (PKC) and extracellular signal-regulated kinase (ERK) [117]. Collectively, the enhanced expression and activation of MMP-2 and MMP-9 synergistically augment the invasive capacity of cancer cells, facilitating their ability to penetrate and spread within the body.

A pivotal event in cancer invasion is the EMT, during which cells undergo a transformation from the stationary epithelial phenotype (such as the expression of E-cadherin) characterized by a rigid cytoskeleton and extensive cell-cell interactions to an invasive mesenchymal phenotype (such as the expression of vimentin) [118]. In many cancer subtypes, the process of EMT is orchestrated by a consortium of transcriptional repressors, including TWIST, SLUG, SNAIL, ZEB1, and ZEB2. These repressors serve to diminish the expression of E-cadherin and other genes emblematic of epithelial cells. Of significance, at least one of these inhibitors is subject to the regulation of HIF - a pivotal player in the pathway governed by tumor hypoxia [119].

Nevertheless, accumulating evidence from diverse investigations indicates that the hypoxic tumor microenvironment can precipitate the erratic expression of the E-cadherin gene, culminating in a reduction of E-cadherin protein levels. This decline undermines E-cadherin's crucial role in preserving cellular cohesion and augments the tumor cells' propensity for invasion and metastasis [120-122]. Collectively, these findings suggest that hypoxia may facilitate the invasion of tumor cells by instigating the EMT.

In summary, hypoxia makes tumor cells generate RhoA, boosting the levels of cell surface enzyme MT1-MMP and HIF. MT1-MMP triggers MMP-2 activation. Active HIF enhances fascin expression, driving up MMP-2 and causing E-cadherin downregulation by inducing TWIST, SLUG, SNAIL, ZEB1, and ZEB2. These events culminate in EMT and facilitate tumor cell invasion (Figure 2).

Hypoxia promotes tumor immune escape

Hypoxia induces the release of immunosuppressive molecules from tumor cells. Under conditions of severe hypoxia, dying cells release ATP, which is then metabolized into adenosine by the enzymes CD73 and CD39 [123]. Extracellular adenosine can then bind to specific receptors on the surface of T cells, leading to an increase in intracellular cAMP levels. This biochemical change exerts an inhibitory effect on T cell function [124]. By harnessing this immunosuppressive mechanism, tumors are able to evade detection by the body's immune system, thereby facilitating their uncontrolled growth and metastasis. Under hypoxic conditions, tumor cells are capable of secreting both IL-10 and TGF-β, which play pivotal roles in modulating the tumor microenvironment. Specifically, IL-10 and TGF-β contribute to the polarization of TAMs into the M2 phenotype, characterized by immunosuppressive functions. These M2 macrophages can hinder the anti-tumor response by inhibiting T cell proliferation and effector activities [125]. Furthermore, TGF-β exerts a profound immunosuppressive effect by promoting the expansion of regulatory T cells (Tregs), which are instrumental in suppressing the immune response against cancer cells. TGF-β also impairs the cytolytic functions of natural killer (NK) cells by downregulating the expression of activating receptors, rendering them less effective at recognizing and destroying tumor cells [126]. In addition, TGF-β's influence extends to dendritic cells (DCs), where it diminishes the expression of the antigen-presenting surface molecule CD1d. This alteration impedes the ability of DCs to present tumor antigens to T cells, thereby hindering the initiation

of an effective anti-tumor T cell response [127].

Research has demonstrated that within hypoxic colon and head and neck squamous cell carcinomas, HIF-1 plays a pivotal role in orchestrating a complex immunosuppressive network. It activates the production of galectin-1 and galectin-3, which consequently induce the apoptosis of activated lymphocytes [128]. This cunning maneuver by the tumor cells is further compounded by their ability to upregulate COX-2, an enzyme that catalyzes the synthesis of prostaglandin E2 (PGE2). Elevated levels of PGE2 not only enhance the adenosine/cAMP signaling concentration within effector T cells [129], but also engage the EP-4 receptor on myeloid-derived suppressor cells (MDSCs), amplifying their immunosuppressive effects [130]. Collectively, these actions inhibit dendritic cell maturation and promote the differentiation of regulatory T cells, ultimately unleashing a potent wave of immune suppression that benefits the tumor's survival and progression.

Therefore, in the context of a hypoxic tumor microenvironment, the immune evasion tactics employed by cancer cells are primarily facilitated through a complex interplay of specific molecular signals and pathways. Central to this strategy is the generation of adenosine, a metabolite that dampens the anti-tumor immune response by engaging its receptor on immune cells. Additionally, tumors secrete immunosuppressive cytokines such as IL-10 and TGF-β, which further hinder the immune system's ability to recognize and eliminate cancer cells. Such mechanisms enable the tumor to establish a conducive microenvironment that nurtures its continued existence and unchecked growth (Fig. 3).

Hypoxia alters the expression of tumor cell checkpoint regulators. Hypoxia not only diminishes the cytolytic potential of immune effector cells but also profoundly modulates the tumor cells' immunological profile, rendering them more evasive to the body's immune attack. One mechanism by which this occurs is through the action of HIF-1α, which can stimulate the enzymatic activity of metalloproteinase ADAM10. Once activated, ADAM10 facilitates the cleavage of membrane-bound MHC class I chain–related molecule A (MICA), a process that results in the release of soluble MICA into the extracellular space [131]. This soluble form of MICA is less effective at engaging its NKG2D receptor on NK cells and T cells, thereby reducing their ability to recognize and destroy infected or malignant cells [132].

Furthermore, the hypoxic stress within tumor cells triggers the stabilization and accumulation of HIF-1α, which then upregulates the expression of the inhibitory co-

stimulatory molecule programmed death-ligand 1 (PD-L1). PD-L1, when bound to its receptor PD-1 on the surface of cytotoxic T lymphocytes (CTL), can deliver an inhibitory signal that dampens T cell responses and promotes T cell exhaustion or apoptosis [133]. Consequently, the engagement between PD-L1 and PD-1 on CTL reduces their cytolytic function, sparing the tumor cells from destruction by CTL-mediated cell lysis [134].

These dual actions of hypoxia-through the induction of ADAM10 activity leading to MICA shedding and through the upregulation of PD-L1 on the tumor cell surface-underscore the multifaceted mechanisms by which tumors can evade the immune system (Fig. 4). Studies have shown that 32-134D, a low-molecular-weight compound that inhibits HIF-1/2-mediated gene expression in hepatocellular carcinoma (HCC) cells, when combined with anti-PD-1, raises the HCC eradication rates in mice (from 25% to 67%) [135]. Findings like this point to potential treatment directions. Given the significance of such discoveries, comprehending how hypoxia impacts these immune checkpoints is essential for formulating targeted therapies capable of combating the immune evasion tactics of tumors.

The immune effects of hypoxia-induced angiogenesis. Increasing evidence suggests that hypoxia-induced angiogenesis is also associated with immune tolerance. Hypoxia triggers a plethora of physiological responses, notably the upregulation of VEGF, which is pivotal in angiogenesis [108, 109]. Reports have demonstrated that VEGF plays a pivotal role in obstructing the differentiation of DCs induced by tumors. In vitro experiments have shown that a VEGF-specific neutralizing antibody can counteract the negative impact of tumor cell-derived media on the differentiation of DCs from hematopoietic progenitor cells. Subsequent in vivo research has corroborated these findings, indicating that the administration of recombinant VEGF to tumor-free mice leads to the inhibition of DC development and an increase in the production of $GR1^{+}$iMCs. Notably, the administration of a VEGF-specific neutralizing antibody to tumor-bearing mice enhances DC differentiation and elevates the number of mature DCs. These observations suggest that VEGF can impede the maturation of DCs, thereby impairing their capacity to present tumor-associated antigens to helper T cells [136]. Furthermore, VEGF can promote the accumulation of MDSCs in tumor tissues and secondary lymphoid organs, suppressing anti-tumor T cell responses, while simultaneously mediating the release of factors that facilitate angiogenesis and

metastasis, thus promoting tumor progression [137]. Clinical studies have also explored the impact of VEGF on dendritic cells (DCs) and immature myeloid cells (iMCs) in the immune systems of cancer patients. The study by Osada et al. [138] included 41 cancer patients, comprising those with lung, breast, colorectal, and unknown primary cancers, as well as 30 healthy volunteers as controls. The research found that DCs in cancer patients exhibited lower maturity levels and a tendency towards an immunoregulatory DC2 phenotype, accompanied by an increased presence of iMCs. There was a positive correlation between VEGF levels and the numbers of DC2 cells and iMCs, suggesting that VEGF may influence immune function by inhibiting DC maturation. The researchers further observed that treatment with anti-VEGF antibodies, such as bevacizumab, in some cancer patients led to a reduction in iMC numbers and an increase in DC counts. Additionally, indicators of immune function, including IL-12 secretion and antigen presentation capacity, were enhanced. These findings indicate that VEGF may play a role in DC dysfunction and cancer immune suppression and suggest that anti-VEGF therapy may help improve the immune response in cancer patients.

The direct impact of hypoxia on immune effector factors. The direct impact of hypoxia on immune effector factors is mainly reflected in its influence on the function of T cells and NK cells. Firstly, hypoxia can suppress the functionality of T cells, which are essential components of the immune response, through various mechanisms. For instance, studies by Clambey [139] and others have revealed that T cells in a low-oxygen environment exhibit a significant increase in the expression of the FoxP3 gene, which leads to an elevation in the number of Treg cells while simultaneously inhibiting the proliferation of effector T cells. HIF-1α plays a critical role in this process by stabilizing the FoxP3 gene and directly influencing its function. Furthermore, it has been confirmed that hypoxia can suppress the expression of DC phenotypes associated with antigen presentation, such as MHC-II, CD80, and CD86, thereby impairing the functionality of effector T cells. These findings suggest that hypoxia exerts a regulatory effect on T cells by modulating immune effectors on their surface, potentially dampening anti-tumor immunity [140]. However, there are contrasting viewpoints. Research conducted by Palazon [141] and colleagues, for example, has illuminated a crucial interaction whereby HIF-1α enhances the transcription of CD137, a key receptor within the tumor necrosis factor (TNF) superfamily that is vital for T cell activation. Their groundbreaking work demonstrated that without HIF-1α, there is a substantial

decrease in CD137 expression, which ultimately undermines T cell activation. Taken together, these studies imply that hypoxia might have a dual regulatory impact on T cells, warranting further investigation to clarify its complex effects.

Secondly, studies have revealed that hypoxic conditions can significantly impair the functionality of NK cells, which are pivotal in the body's defense against cancerous cells such as those found in multiple myeloma. Specifically, the deficit in oxygen levels leads to a decrease in the number of NKG2D and CD16 receptors on the surface of NK cells. These receptors are essential for recognizing and targeting infected or malignant cells. Additionally, hypoxia results in reduced intracellular levels of perforin and granzyme B, which are proteins crucial for the cytotoxic activity of NK cells-their ability to directly kill compromised cells. Consequently, the diminished expression of these key molecules hampers the NK cells' capacity to exert their cytotoxic effects on multiple myeloma cells [142]. These findings underscore the profound implications that hypoxia has on the efficacy of immune effector cells like NK cells, highlighting the need for strategies to mitigate such immunosuppressive effects in the tumor microenvironment.

Hypoxia promotes proliferation of cancer stem cells

Research indicates that various solid cancers are indeed stem cell-driven, meaning they begin with a rare type of cell known as cancer stem cells (CSCs). Although only a tiny fraction of the total tumor, CSCs have the remarkable ability to regenerate and specialize into different cancer cells, giving them a big advantage in spreading and resisting treatments. This suggests that CSCs could be key players in both the formation and growth of tumors [143, 144].

Hypoxia has been identified as a pivotal element that propels the expansion of CSCs and amplifies their capacity to generate tumors throughout diverse cancer types. In the context of the particularly aggressive brain cancer known as glioblastoma, which is infamous for its vigorous angiogenesis, HIFs have been observed to modulate the tumorigenicity of glioma stem cells (GSCs). These GSCs, which are critically dependent on HIFs not just for their survival but also for their capacity to regenerate and contribute to the tumor's bulk, are strategically located within specific niches of the tumor, such as in the immediate vicinity of blood vessels and in necrotic zones, allowing them to exploit the low-oxygen conditions optimally [145, 146]. In HCC, hypoxia plays a role in accelerating the disease's advancement by rewiring the metabolism of

mesenchymal stem cells (MSCs) towards increased lipogenesis, thereby fueling their ability to foster tumor growth and contributing to the overall increase in malignancy [147].

In the context of the mechanisms by which hypoxia promotes the proliferation of CSCs, current research suggests that this phenomenon is mediated through several key pathways. Firstly, hypoxic conditions induce the upregulation of HIF-1α, which subsequently activates its target gene, Notch 1. The Notch 1 signaling pathway plays a pivotal role in maintaining the stemness of CSCs by regulating the balance between self-renewal and differentiation, primarily through the interaction of its ligands with specific receptors on the cell surface [148]. Furthermore, hypoxia can also result in the overexpression of HIF-2α, leading to the activation of the downstream gene Oct-4. The activation of Oct-4 may serve as a barrier to cellular differentiation and further contribute to the preservation of stem cell characteristics within the tumor [149, 150]. Collectively, these findings elucidate the complex interplay between hypoxia, CSCs, and the tumor microenvironment in driving tumor progression.

Hypoxia and therapy resistance

Tumor hypoxia exerts a profound influence on multiple treatment modalities, including chemotherapy, radiotherapy, and immunotherapy, through complex and intertwined mechanisms. Antiangiogenic therapies, designed to cut off the tumor's blood supply, present a double - edged sword. Reports have shown that while these therapies can initially suppress tumor growth, they frequently result in increased hypoxia within the tumor. This heightened hypoxia then drives the selection of more aggressive cancer phenotypes, ultimately correlating with poor patient prognosis. This duality underscores hypoxia's role as both a potential therapeutic target and a formidable resistance mechanism [151]. In the domain of chemotherapy and radiotherapy, hypoxia, often made worse by anemia, greatly weakens the effectiveness of these treatments. Hypoxia may lower the sensitivity of tumors to radiation therapy and chemotherapy through one or several indirect mechanisms, including proteomic and genomic changes. These effects, in turn, can give rise to increased invasiveness and metastatic potential, the loss of apoptosis, and disrupted angiogenesis, thus further increasing treatment resistance [152].

The role of HIF in mediating treatment resistance has been a focal point of extensive investigation. When there is hypoxia, research findings have shown that the expressions

of both DNA - dependent protein kinase (DNA – PK) and HIF - 1α increase. Moreover, DNA - PK can directly interact with HIF - 1. This regulatory relationship between DNA - PK and HIF - 1 leads to the therapeutic resistance of hypoxic tumor cells, providing a new basis for the development of strategies to improve the treatment efficacy of hypoxic tumor cells [153]. Furthermore, there are also complex relationships between HIF and various cell death pathways, such as autophagy. Hypoxia has been shown to trigger autophagy, a cellular survival mechanism that enables tumor cells to endure the stress imposed by therapeutic agents. This has been particularly evident in studies on glioblastoma, where hypoxia - induced autophagy promotes tumor cell survival, adding another layer of complexity to the treatment landscape [154].

The role of hypoxia in immunotherapy resistance has also come under scrutiny. Research has delved into the correlation between tumor hypoxia and resistance to PD - 1 blockade in head and neck squamous cell carcinoma. Under hypoxic conditions, tumor cells undergo metabolic alterations. In the murine head and neck cancer model, for instance, the anti - PD - 1 resistant cell lines exhibit heightened oxidative metabolism, thereby intensifying intratumoral hypoxia. Concurrently, this hypoxic state impacts immune cell infiltration. Specifically, elevated hypoxia levels lead to a reduction in the infiltration of crucial CD8+ T cells, thereby attenuating the immune system's capacity to eliminate tumor cells. Moreover, tumor hypoxia triggers the establishment of an immunosuppressive microenvironment. This involves inducing changes in the tumor microenvironment, modulating the expression of immunosuppressive molecules, or reshaping the functional distribution of immunosuppressive cells. These processes enable tumor cells to elude immune surveillance and cytotoxicity, ultimately culminating in resistance to immunotherapy [155]. Due to the complex interaction mechanisms between tumor hypoxia and resistance to immunotherapy described above, researchers have further explored and found that HIF - 1 has been identified as a key mediator of resistance to anti - PD - (L)1 therapies [156].

At present, the development of hypoxia - targeted therapies has encountered challenges, and the results of clinical trials have been inconsistent. Consequently, there is a growing consensus among researchers that a personalized approach is essential [157]. Nevertheless, there is reason for optimism as innovative strategies to overcome hypoxia - induced resistance are emerging. For example, a promising approach involves using

pH - responsive liposomes to enhance sonodynamic therapy by improving oxygen delivery to hypoxic tumors [158].

In summary, tumor hypoxia represents a multifaceted and complex challenge that significantly influences treatment resistance across a spectrum of cancer therapies. A comprehensive understanding of the underlying molecular, cellular, and microenvironmental mechanisms is essential for the development of innovative and personalized treatment strategies.

Hypoxia and cancer prognosis

Tumor hypoxia plays a pivotal role in cancer prognosis and therapeutic outcomes. Extensive research has delved into the correlation between hypoxia levels and disease progression across different cancer types. While select clinical investigations hint at no significant impact of tumor hypoxemia on patient survival [159], possibly influenced by dynamic changes within the tumor microenvironment, the prevailing body of evidence leans towards hypoxemia as a contributor to adverse prognostic outcomes.

As early as 2003, Koukourakis et al. [160] performed an analysis of 76 cases of non-small-cell lung cancer (NSCLC) tumor tissues, focusing on the correlation between Lactate dehydrogenase-5 (LDH-5) and HIFs expression and patient prognosis. By comparing patients with varying levels of LDH-5 and HIFs expression, they evaluated the impact of these biomarkers on survival. The research discovered that elevated LDH-5 expression, particularly when coupled with high levels of HIF1α and HIF2α, was associated with worse survival outcomes. Considering that LDH-5 and HIFs are critical molecules in the hypoxic state of tumors, this suggests that the hypoxic condition of tumor cells is a significant biological factor affecting prognosis in NSCLC. This is not an isolated occurrence, similar observations have been made regarding the upregulation of HIF-1α in gastric adenocarcinoma, which is also associated with an unfavorable prognosis [161, 162]. In the context of colorectal cancer, hypoxia exerts a substantial influence on the behavior of immune cells within the tumor microenvironment. This includes modulating the activity and polarization of immune cells such as T cells, macrophages, and tumor-infiltrating lymphocytes, particularly by upregulating the expression of chemokines like CCL2/4/5 and CSF1. This leads to an increased recruitment of immunosuppressive M2 macrophages, facilitating tumor escape and dampening anti-tumor immune responses. Consequently, the altered immune cell dynamics under hypoxic conditions play a pivotal role in determining the overall

prognosis of patients with colorectal cancer, as it affects disease progression and treatment response [163]. Additionally, research has revealed that hypoxia-related long non-coding RNAs (lncRNAs) can serve as potential biomarkers for predicting breast cancer prognosis and may inform future therapeutic strategies [164, 165].

However, there are also contradictory aspects regarding the impact of tumor hypoxia on patient prognosis. It's worth noting that Tribbles homolog 3 (TRIB3), a cytokine induced by hypoxia and involved in numerous cell survival pathways, shows elevated mRNA levels associated with poor prognosis in breast cancer patients. Conversely, its high protein expression is unexpectedly associated with better prognosis, highlighting the intricate relationship between TRIB3 and cancer progression, and also suggesting the complex interplay between hypoxia and tumor prognosis [166, 167]. When it comes to the impact on tumors, different HIF isoforms play distinct roles. In xenograft models, studies have shown that HIF2α, rather than HIF1α, drives tumor growth. Similarly, in animal models, there is evidence indicating that activating HIF2α within cancer cells or replacing HIF1α with HIF2α promotes aggressive tumor growth and invasion. On the contrary, overexpressing stable HIF1α can inhibit tumor growth. Despite these insights into their functions in cancer cells, the roles of HIF1α and HIF2α in the tumor stroma remain largely uninvestigated [168]. In addition, some studies have shown that hypoxia may induce adaptive responses that can be exploited for treatment. For example, Kucharzewska et al. demonstrated that exosomes secreted by glioblastoma cells under hypoxic conditions can mediate intercellular communication that promotes angiogenesis, suggesting that tumors may adapt to low oxygen levels through this potential mechanism. This adaptive response complicates the simple interpretation of hypoxia as a negative prognostic factor [169]. The findings of Li et al. further illustrate the paradoxical role of hypoxia in cancer. They explored the phenomenon of vasculogenic mimicry, which is related to hypoxia. Vasculogenic mimicry enables tumors to form a blood supply independent of endothelial vessels, enhancing the invasive and metastatic capabilities of tumors. Although this mechanism is associated with poor prognosis, it also highlights the plasticity of tumor cells in response to hypoxic stress, indicating that hypoxia does not always lead to adverse consequences [170]. Moreover, some researchers screened out 7 hypoxia - related genes to construct a prognostic model, which was verified by datasets to be effective in predicting survival rates. At the same time, significant differences were found in the immune

microenvironment indicators between the high - risk and low - risk groups under different hypoxia response patterns. That is, different hypoxia response patterns ultimately lead to significantly different prognostic situations in gastric cancer patients by affecting the immune microenvironment [171]. The above research results indicate that the impact of tumor hypoxia on patient prognosis is complex, and various factors exhibit complex and diverse relationships.

Overall, the relationship between tumor hypoxia and prognosis is multifaceted. The roles of LDH-5, HIF-1α, TRIB3, as well as the influence of hypoxia-related lncRNAs, highlight the intricate connections between cellular responses to low oxygen levels and cancer progression, underscoring the need for further investigation into these factors as potential therapeutic targets or prognostic indicators. Armed with knowledge of these intricate molecular mechanisms, clinical research to translate this understanding into effective therapies is crucial. Therefore, we've compiled an overview of existing hypoxia - targeted therapy clinical trials (https://www.clinicaltrials.gov) to offer a comprehensive view of the current research status. Table 3 details the clinical trials of hypoxia - targeting drugs for cancer treatment. These clinical trials are devised by leveraging hypoxia tracers and medications that target HIF and its downstream targets. Hypoxia tracers possess the ability to render tumor hypoxia visible and quantify it. This is conducive to enabling clinicians to conduct non - invasive detection and assessment of the tumor hypoxia level. By means of PET imaging, they can also be employed to monitor the dynamic therapeutic responses of patients, potentially facilitating individualized treatment strategies for patients. Medications targeting HIF and its downstream genes can effectively impede tumor growth, markedly enhance patient survival rates, and demonstrate favorable tolerability. However, these drugs have certain toxicities and side effects. Common adverse events include headache, fatigue, etc. Severe ones involve gastrointestinal bleeding, thrombosis, etc. Some clinical trials lack sufficient data to comprehensively analyze drug safety and efficacy [32].

CONCLUSION

Solid tumors typically experience hypoxia, characterized by significantly lower oxygen levels within the cancer cells when compared to normal tissues. Even in highly vascularized tumors like lung cancer, the oxygenation rate remains at a mere 2%. This

rate can drop even further in certain instances, particularly in pancreatic cancers, where it may reach as low as 0.3% [50]. The promotion of tumor development by hypoxia plays a crucial role in cancer biology research. This review delves into the multifaceted impacts of hypoxia on tumors, including its causes, classification, and the determination of hypoxic regions. Additionally, we extensively analyze how the hypoxic environment promotes tumor progression, involving tumor cell proliferation, immune evasion, and stem cell proliferation. This review summarizes the abundant research findings in this field, providing crucial insights for a deeper understanding of tumor biology and the formulation of future therapeutic strategies. Looking ahead, we encourage further exploration of the mechanisms underlying the interaction between hypoxia and tumors, particularly at the molecular and cellular levels. Utilizing newly discovered aspects related to hypoxia in cancer treatment, especially in developing innovative strategies targeting the hypoxic environment, holds immense potential. Continued promotion of interdisciplinary collaboration, integrating expertise from biology, medicine, and engineering, among other fields, will contribute to a comprehensive understanding and effective response to the impact of hypoxia on tumors.

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TABLES AND FIGURES WITH LEGENDS

Table 1. The detailed role of different factors affecting hypoxia and its consequences

Factor	Influence	Consequence
Rapid proliferation of tumor cells	Surrounding blood vessel growth outpaced	Tumor hypoxia
Vascular system abnormality	Structural distortion, irregular growth, and functional defects lead to uneven blood supply	
Newly formed solid tumors are located far from blood vessels	Blood vessels provide poor oxygen supply to tumor tissues	
Oxygen demand and competition	Increased oxygen consumption	
Chemotherapy	Generate ROS, leading to apoptosis of endothelial cells and damage to tumor blood vessels	
Radiation therapy	Tumor vascular abnormalities such as: vessel occlusion, rupture, and formation of aneurysms, etc.	
Anemia	A decrease in red blood cells or hemoglobin reduces the blood's ability to carry oxygen	
Advanced age and smoking	A decrease in the ability of the blood to carry oxygen	
Changes in hormone levels	Effects on hypoxia factor expression and impact on hypoxia levels in varied environments	
Immune system changes	Immunocytes release substances that inhibit tumor angiogenesis, leading to vascular malformations	

Table 2. Analyzing the disparities in hypoxic zones across different clinical categories of tumors

Tumor type	Stage	Number of cases	Oxygen level within tumor	HF < 2.5mmHg
Brain metastasis [67]	IV	5	Median: 3-23.7 mmHg	42.1%
Breast cancer [68]	Na	15	Median: 3-74 mmHg	5.8%
Cervical cancer [69]	Na	59	Mean: 10mmHg	29.0%
Cervical cancer [70]	I-IV	18	Mean: 21.1 mmHg	Na
Glioblastoma [67]	Na	10	Median: 0.1-24.3 mmHg	26.0%
Head and neck cancer [71]	III, IV	10	13.9 ± 8.0 mmHg	8.3%
Head and neck cancer [72]	Na	16	25.6 ± 20.2 mmHg	Na
Head and neck cancer [73]	Na	133	Primary: 12 (0–58 mmHg) Metastases: 13 (0-50 mmHg)	Na
Metastatic melanoma [74]	IV	18	Mean: 11.6 mmHg	15.0%
Non-small cell lung cancer [75]	I-III	20	16.6 (0.7–46 mmHg)	Na
Pancreatic tumor [76]	Na	7	Median: 0-5.3 mmHg	59.0%
Prostate cancer [77]	I-III	59	2.4 (0–65 mmHg)	Na
Rectal carcinoma [78]	Na	15	25.7 ±17.9 mmHg	Na
Renal cell carcinoma [79]	Na	3	13.3±17.4 mmHg	Na
Vulvar cancer [80]	Na	Primary 15	Primary: 16.4 ± 14.4 mmHg	Na

		Recurrent 19	Recurrent: 15.7 ± 13.4 mmHg	

Table 3. Clinical trials and hypoxia-targeting drugs in tumor therapy

Clinical trial ID	Tumor type	Primary outcome measures	Phase	Status	Target	Drug	Intervention
NCT04648033	Non-small cell lung cancer	MTD	I	Completed	Hypoxia regions	Atovaquone	Oral
NCT00495144	Unspecified	MTD, DLTs	I	Completed	Hypoxia regions	TH-302	Intravenous infusion
NCT01497444	Kidney cancer or liver cancer	DLTs, MTD, RR	I/II	Completed	Hypoxia regions	TH-302	Intravenous infusion
NCT01149915	Advanced leukemias	MTD, DLTs, adverse events	I	Completed	Hypoxia regions	TH-302	Intravenous infusion
NCT00743379	Pancreatic cancer, prostate cancer, lung cancer	MTD, DLTs	I/II	Completed	Hypoxia regions	TH-302	Intravenous infusion
NCT01522872	Myeloma	Adverse events, MTD, DLTs, recommended doses	I/II	Unknown	Hypoxia regions	TH-302	Intravenous infusion
NCT01746979	Pancreatic cancer	Overall survival	III	Completed	Hypoxia regions	TH-302	Intravenous infusion
NCT01440088	Soft tissue sarcoma	Overall survival	III	Completed	Hypoxia regions	TH-302	Intravenous infusion
NCT02528526	Hepato-pancreato-biliary neoplasm	Safety and tolerability	Ib/IIa	Unknown	Hypoxia regions	OXY111A	Intravenous infusion
NCT05281003	Esophageal squamous cell carcinoma	RR, major hypoxia signals	II	Recruiting	PD-1	Pembrolizumab	Intravenous infusion
NCT04114136	Unspecified	RR	II	Recruiting	PD-1	Nivolumab or	Intravenous

						pembroziluma b	infusion
NCT01763931	Breast cancer	Change in HIF-1α expression	II	Completed	HIF-1α	Digoxin	Oral
NCT04954599	Unspecified	Adverse events, biochemical test abnormalities	I/IIa	Recruiting	Hypoxia regions	CP-506	Intravenous infusion
NCT05119335	Kidney cancer	DLTs, recommended doses for expansion, RR, recommended phase 2 dose	I/II	Recruiting	HIF-2α	NKT2152	Oral
NCT03098160	Unspecified	Proper dose	I	Unknown	Hypoxia regions	Evofosfamide	Intravenous infusion
NCT02564614	Hepatocellular carcinoma	Change in HIF-1α expression	I	Completed	HIF-1α	RO7070179	Intravenous infusion
NCT00090727	Unspecified	Na	I	Unknown	Hypoxia regions	AQ4N	Intravenous infusion
NCT00466583	Lymphoma	MTD	I	Completed	HIF-1α	EZN-2968	Intravenous infusion
NCT00886405	Non-small cell lung cancer	Time to progression, over-all survival	II	Unknown	HIF-1α	Nitroglycerin	Skin adherence
NCT01950689	Head and neck squamous cell carcinoma	Locoregional control	III	Completed	Hypoxia regions	Nimorazole	Oral

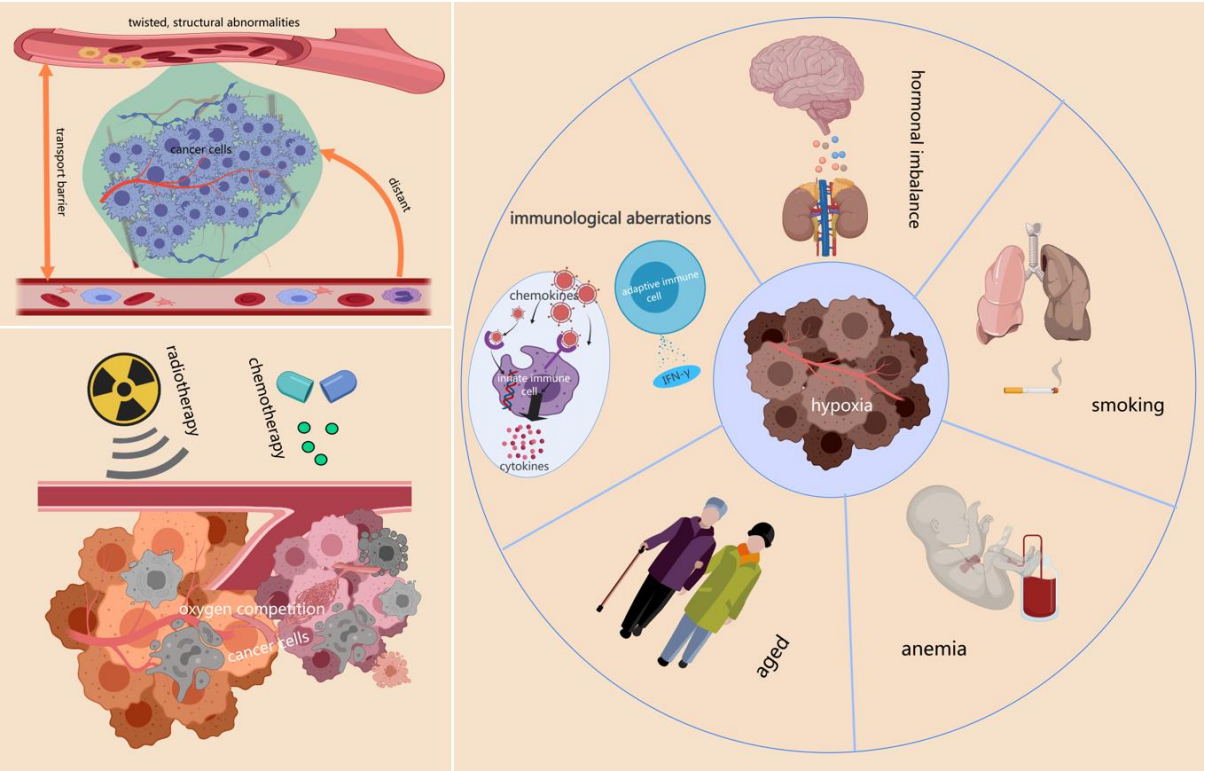

Figure 1. Factors leading to tumor hypoxia. The rapid proliferation of tumor cells far exceeds the formation speed of the surrounding vascular network, resulting in the formation of avascular hypoxic zones within solid tumors. The vascular system within solid tumors exhibits structural and functional abnormalities, including vascular distortion, abnormal proliferation, and dysfunction of endothelial cells. The distance between the tumor center and the nearest blood vessels increases, causing hindrance to the transport of substances between vessels. Intense competition among tumor cells leads to a significant increase in oxygen consumption. Factors such as radiation therapy, chemotherapy, anemia, advanced age, smoking, immunological abnormalities, and hormonal imbalances also contribute to tumor hypoxia.

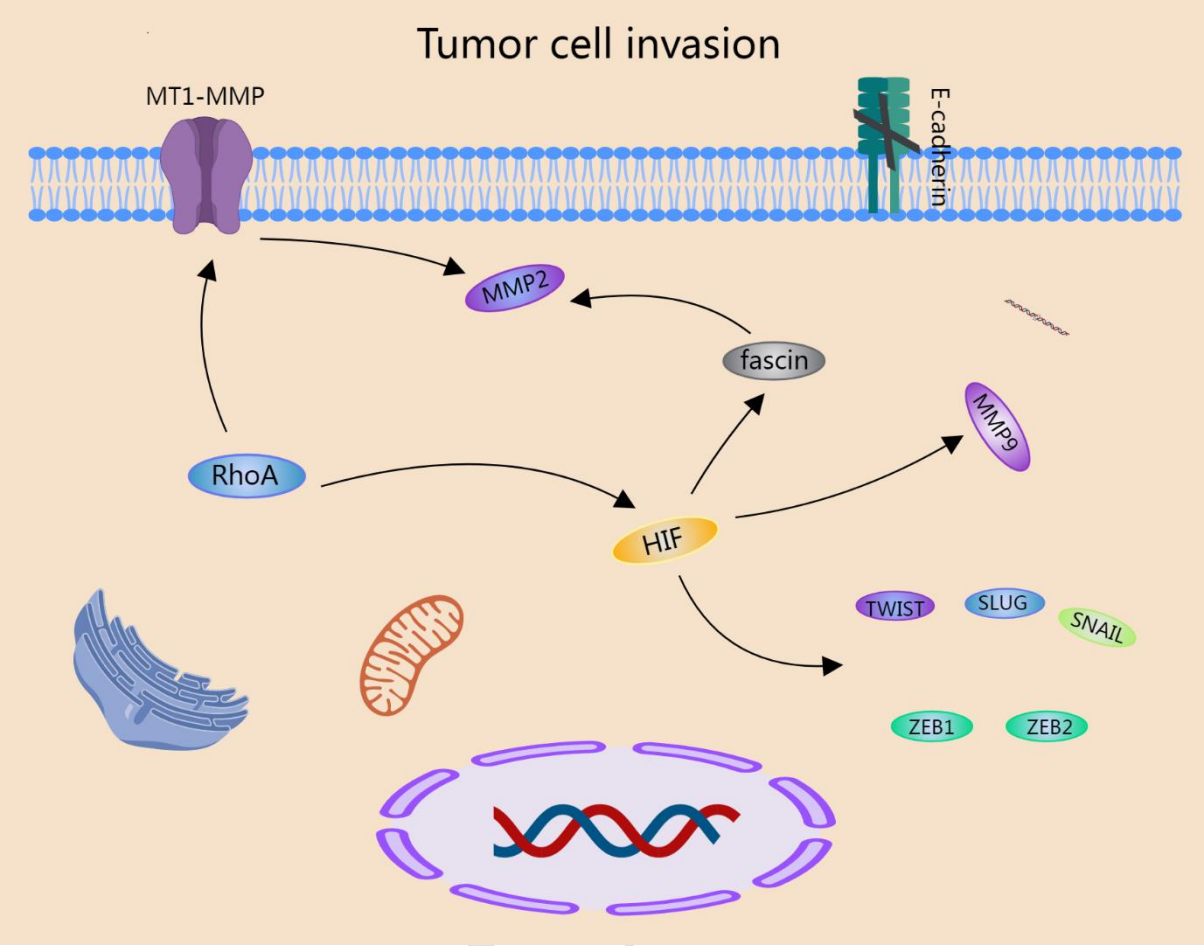

Figure 2. Hypoxia promotes tumor cell invasion. In the hypoxic environment, tumor cells produce RhoA, which leads to the upregulation of the cell surface matrix metalloproteinase MT1-MMP. MT1-MMP activates another matrix metalloproteinase, MMP-2, triggering EMT in tumor cells. Furthermore, RhoA regulates the expression level of HIF, subsequently increasing the expression of downstream factor fascin and thus enhancing the expression of MMP-2. High expression of HIF can promote the expression of molecules such as TWIST, SLUG, SNAIL, ZEB1, and ZEB2, which act to reduce the expression of E-cadherin on the cell surface, ultimately facilitating EMT and promoting tumor cell invasion.

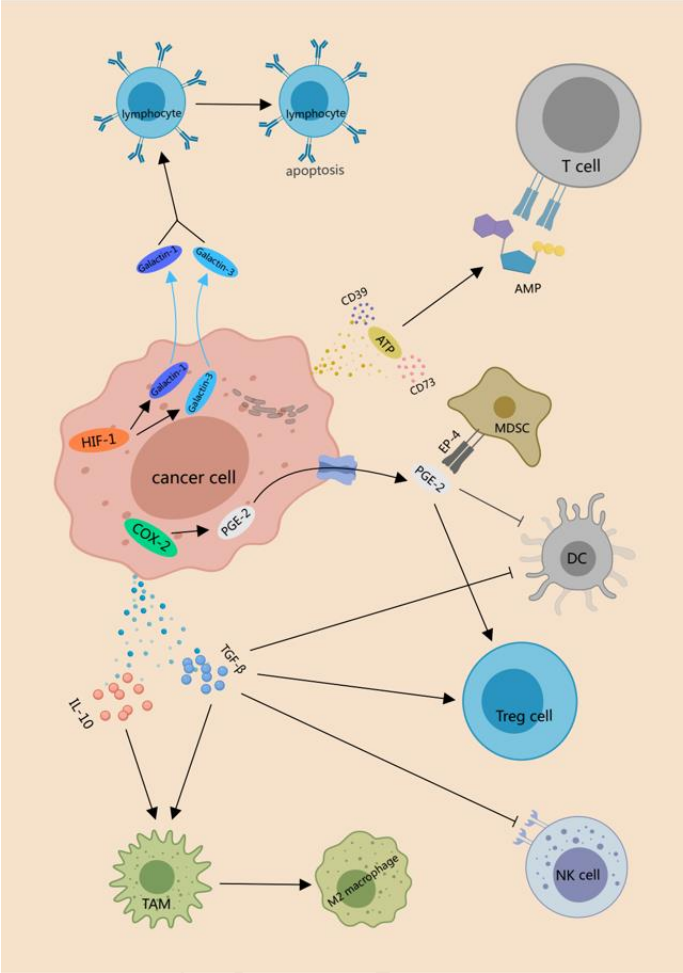

Figure 3. Hypoxia induces the release of immune suppressive molecules from tumor cells. Dying cancer cells release ATP, and CD73 and CD39 metabolize it into adenosine, which binds to specific receptors on T cells, elevating intracellular cAMP levels, thereby inhibiting T cell function. Under hypoxic conditions, tumor cells also release IL-10 and TGF-β, leading to the differentiation of TAMs into M2 macrophages with immune-suppressive activity. TGF-β inhibits T cell proliferation and effector functions, promotes the generation of Tregs, and simultaneously blocks the receptor expression necessary for NK cells to exert cytotoxic functions. TGF-β also downregulates the expression of CD1d, an antigen-presenting surface molecule on DCs, thereby inhibiting T cell differentiation and function. Hypoxia induces the activation of HIF-1, resulting in the production of galectin-1 and galectin-3, leading to apoptosis of activated lymphocytes. Hypoxic tumor cells, through upregulation of COX-2, increase the expression of PGE2, inhibiting DC maturation, promoting Treg differentiation, and triggering immune suppression. Additionally, PGE2 can bind to the EP-4 receptor on MDSCs, exerting immune-suppressive effects.

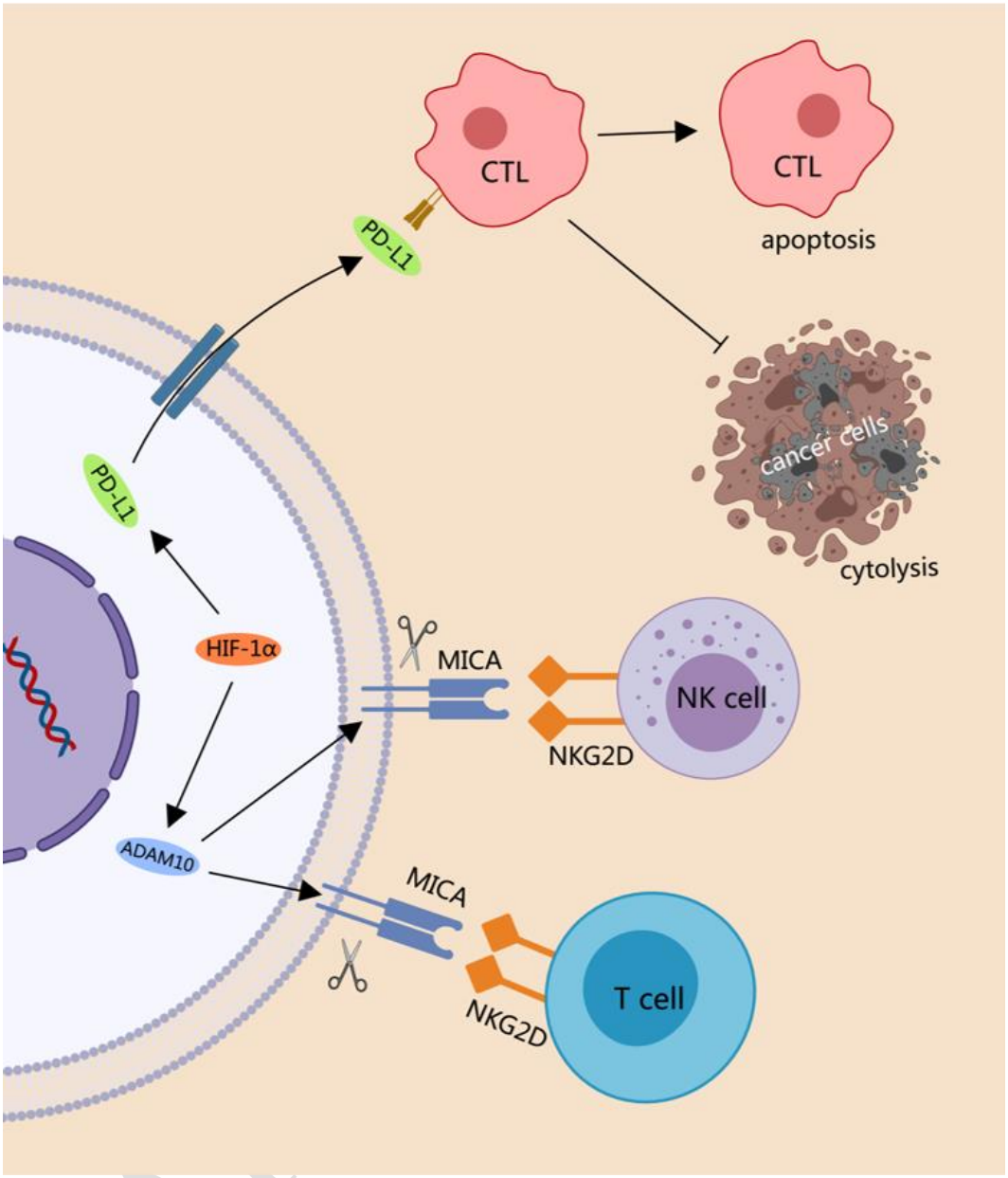

Figure 4. Hypoxia-induced changes in tumor cell checkpoint regulator expression. HIF-1α activates the expression of metalloproteinase ADAM10, leading to the shedding of cell surface pressure-induced MICA. This, in turn, reduces the binding with the receptor NKG2D on NK cells and T cells. Hypoxia-induced activation of HIF-1α in tumor cells also results in high expression of PD-L1. The binding of PD-L1 with the receptor PD-1 on CTL increases the apoptosis of CTL, simultaneously resisting CTL-mediated lysis of tumor cells.