Hypoxia studies in non-small cell lung cancer: Pathogenesis and clinical implications (Review)

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Abstract. Non-small cell lung cancer (NSCLC) is one of the most prevalent and lethal types of cancers worldwide and its high incidence and mortality rates pose a significant public health challenge. Despite significant advances in targeted therapy and immunotherapy, the overall prognosis of patients with NSCLC remains poor. Hypoxia is a critical driving factor in tumor progression, influencing the biological behavior of tumor cells through complex molecular mechanisms. The present review systematically examined the role of the hypoxic microenvironment in NSCLC, demonstrating its crucial role in promoting tumor cell growth, invasion and metastasis. Additionally, it has been previously reported that the hypoxic microenvironment enhances tumor cell resistance by activating hypoxia-inducible factor and regulating exosome secretion. The hypoxic microenvironment also enables tumor cells to adapt to low oxygen and nutrient-deficient conditions by enhancing metabolic reprogramming, such as through upregulating glycolysis. Further studies have shown that the hypoxic microenvironment facilitates immune escape by modulating tumor-associated immune cells and suppressing the antitumor response of the immune system. Moreover, the hypoxic microenvironment increases tumor resistance to radiotherapy, chemotherapy and other types of targeted therapy through various pathways, significantly reducing the therapeutic efficacy of these treatments. Therefore, it could be suggested that early detection of cellular hypoxia and targeted therapy based on hypoxia may offer new therapeutic approaches for patients with NSCLC. The present review not only deepened the current understanding of the mechanisms of action and role of the hypoxic microenvironment in NSCLC but also provided a solid theoretical basis for the future development of precision treatments for patients with NSCLC.

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1. Introduction

The International Agency for Research on Cancer (IARC) reported that in 2020, ~2.2 million new cases of lung cancer (LC) were diagnosed globally, leading to ~1.8 million related deaths. These figures represent 11.4% of all newly diagnosed cancer cases and 18.0% of all cancer-related mortalities (1). LC remains a significant global health concern, with ~28% of cases being diagnosed at an early stage and >50% of patients exhibiting distant metastasis at the time of the initial diagnosis (1,2). Furthermore, LC is characterized by high recurrence and metastasis rates, low cure rates and a poor overall 5-year survival rate of 22% (2,3). Histologically, LC is primarily classified into two primary types: Small cell lung cancer (SCLC) and non-SCLC (NSCLC), with NSCLC comprising ~80-85% of all LC cases. Therefore, the importance of early detection and prompt intervention for patient is important, as these factors are crucial for enhancing patient prognosis and improving survival rates.

Under normal physiological conditions, the body obtains energy through the respiratory process to maintain the normal functioning of its structure and functions, with oxygen serving an indispensable role in metabolic processes (4). The oxygen content in ambient air is ~21%, equivalent to an atmospheric pressure of 150 mmHg, whereas the oxygen level in most healthy mammalian tissues is maintained between 2-9%,

averaging ~40 mmHg. By contrast, the oxygen concentration within tumor tissue typically ranges from 1-2% (5,6). Hypoxia is generally defined as an oxygen concentration in tissues of ≤2%, while severe hypoxia refers to an oxygen content of ≤0.02% (6). Hypoxia is a characteristic of the solid tumor microenvironment (TME) (5). Solid tumors often exhibit acute, chronic or cyclic hypoxia, all of which stem from an imbalance between oxygen demand and supply (7). Acute hypoxia, or perfusion-limited hypoxia, is caused by irregular dilation of the tumor vasculature or excessive aggregation of tumor cells leading to vascular occlusion. Chronic hypoxia arises from the continuous proliferation of tumor cells, which consume a large amount of oxygen near the vasculature, resulting in inadequate oxygen supply to distal cells (8). Intermittent hypoxia is caused by transient occlusion of the immature and unevenly distributed tumor vasculature, lasting from several minutes to several days (5). The hypoxic TME is a critical factor driving tumor progression, affecting tumor metabolism and its surrounding microenvironment through various mechanisms, thereby influencing tumor biological characteristics and treatment efficacy.

Given the pivotal role of hypoxia in the progression of NSCLC, exploring its potential molecular mechanisms and possible therapeutic intervention strategies is important for improving patient prognosis and survival rates. While there is increasing awareness of the impact of hypoxia on NSCLC, the precise localization and interaction of hypoxic conditions with tumor biology are currently unknown. The present review integrated previously published research findings and aimed to elucidate how hypoxia affects the behavior of cancer cells through a series of mechanisms, offering a solid theoretical basis and practical operational guidance for early detection and precise targeted therapy of NSCLC.

2. Mechanisms of hypoxia in NSCLC

The TME is composed of multiple components, including tumor cells, blood vessels, immune cells, cancer-associated fibroblasts, signaling molecules and the extracellular matrix (ECM). All of these elements serve crucial roles in tumor progression (9,10). Tumor cells can actively alter the composition of the TME by secreting extracellular signals to adapt to or respond to changes in the host environment (11). In the hypoxic microenvironment, the host promotes the progression of NSCLC through various pathways.

Hypoxia-inducible factor (HIF). Cells adapt to hypoxic environments by modulating the expression of certain genes. HIF, a key transcription factor regulating gene expression, serves a central role in the hypoxic response (5). HIF is a heterodimer composed of an unstable, but critical, α subunit and a stable β subunit. The alpha subunit has three subtypes: HIF-1α, HIF-2α and HIF-3α. Although all subtypes are involved in regulating inflammatory responses, typically only HIF-1α is expressed *in vivo* (5). The structure of HIF-1α and HIF-1β are key to their interactions and activation during the hypoxic response (Fig. 1A). Under hypoxic conditions, HIF-α degradation is inhibited, which allows HIF-α to translocate to the nucleus and bind with HIF-β to form an active HIF transcription factor complex. This complex can interact with specific sequences in >70 target gene promoters, known as hypoxia response elements (HREs), to regulate the transcription of protein-coding and non-coding RNA genes (12) (Fig. 1B). These regulated genes serve critical roles in multiple key biological processes, including glucose uptake, tumor metabolism, angiogenesis, cell proliferation and apoptosis, helping cells effectively adapt to hypoxic stress (13).

HIF-1α can promote the expression of various cell proliferation factors, including insulin-like growth factor-2 and TGF-α. Upregulation of these factors stimulates the growth and proliferation of NSCLC cells, thereby promoting tumor development (14). HIF-1α can also activate the transcription of angiogenesis-related genes, including VEGF-A and angiopoietin-2. The increased expression of these factors enhances vasculogenesis, supplying essential oxygen and nutrients to support both local growth and metastasis of NSCLC cells. Under the influence of HIF-1α, the expression of proteases such as MMPs is significantly upregulated. These enzymes can degrade the ECM, facilitating tumor cell migration and invasion of surrounding tissues, thereby promoting NSCLC spread and metastasis (15). Additionally, hypoxia enhances the expansion and motility of NSCLC cells by inhibiting the expression of connexin (CX) proteins, thereby impairing intercellular communication. Under hypoxic conditions, the expression of certain connexin proteins, particularly CX26 and CX43, decreases, making these proteins more susceptible to degradation or internalization. The disruption of intercellular communication not only disrupts signal synchronization and metabolic exchange but also further promotes the proliferation and migration of tumor cells. Moreover, this communication barrier also activates the P53/MDM2 proto-oncogene signaling pathway, accelerating the malignant progression of NSCLC (16). Under hypoxic conditions, the activation of HIF-1α can induce EMT in NSCLC cells. During this process, epithelial cells lose their polarity and intercellular adhesion properties, transforming into cells with mesenchymal features. This transformation enhances their migration and invasion abilities, further promoting the malignant progression of tumors (17,18).

Release of exosomes. Exosomes are nanoscale extracellular vesicles enclosed by a lipid bilayer membrane and actively secreted by cells. Their biogenesis is a tightly regulated and complex process. The formation of exosomes is initiated by the invagination of the plasma membrane, leading to the generation of early endosomes. These early endosomes undergo intracellular maturation to form late endosomes, which subsequently give rise to multivesicular bodies (MVBs) through intraluminal vesicle budding (19). The fusion of MVBs with the plasma membrane facilitates the release of these vesicles into the extracellular milieu, a process defined as exosome secretion (Fig. 2) (19). Exosomes serve an essential role in intercellular communication by transporting a wide range of bioactive molecules, including mRNAs, circular RNAs (circRNAs), long non-coding RNAs (lncRNAs), microRNAs (miRNAs; miRs), proteins, lipids and other molecular entities (20). It has been previously reported that under hypoxic conditions, HIF-1 facilitates the release of tumor-derived exosomes by upregulating pyruvate kinase M2 (PKM2) mRNA expression levels. PKM2, upon upregulation

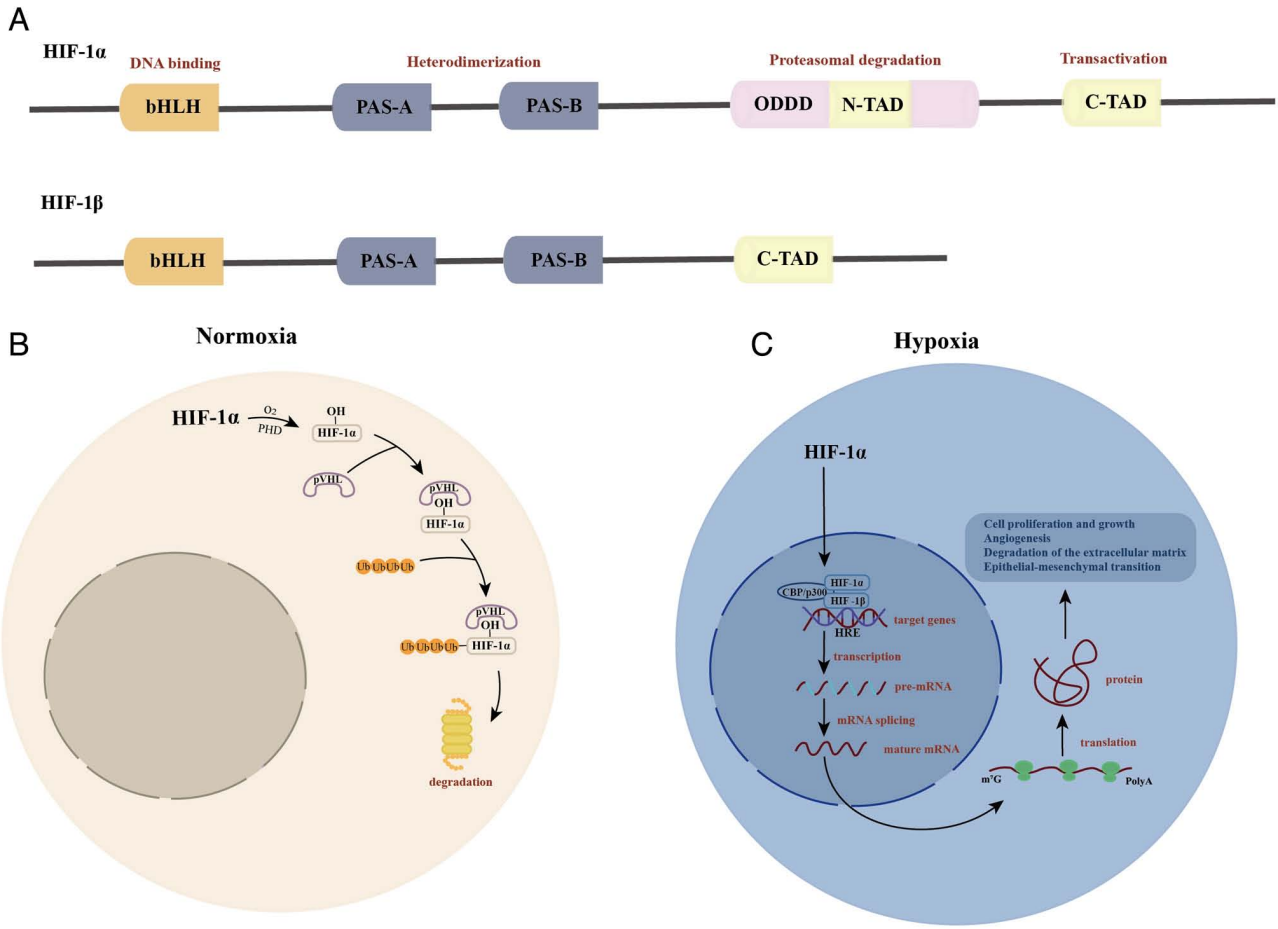

Figure 1. Structure of HIF-1α and HIF-1β and their role under normoxic and hypoxic conditions. (A) Structure of HIF-1α and HIF-1β. The bHLH structural domain binds to target DNA. The intermediate region is the PAS domain, which facilitates the formation of heterodimers. The ODDD senses the surrounding oxygen levels. The C-TAD enhances target gene expression by recruiting the transcriptional co-activators CBP/p300. (B) Role of HIF under normoxic conditions. In normoxia, PHDs hydroxylate the residues of the ODDD, enabling the VHL tumor suppressor protein to recognize and bind to HIF-1α, leading to its degradation via the ubiquitin-proteasome pathway. (C) Role of HIF under hypoxic conditions. Under hypoxia, HIF-1α degradation is inhibited, allowing it to translocate to the nucleus and dimerize with HIF-1β, thereby activating target gene expression. PAS, Per-ARNT-Sim; ODDD, oxygen-dependent degradation domain; C-TAD, C-terminal transcriptional activation domain; HIF, hypoxia-inducible factor; PHD, prolyl hydroxylase; VHL, von Hippel-Lindau; p, phosphorylated; ub, ubiquitin.

and phosphorylation in tumors, functions as a protein kinase, phosphorylating synaptosome-associated protein 23. This phosphorylation event promotes the exocytic release of exosomes (21,22). Exosomes, in turn, drive the growth and progression of NSCLC by transmitting key signals that influence processes such as glycolysis, angiogenesis, tumor cell migration, invasion and immune infiltration (23). Tumor cells in hypoxic environments exhibit abnormal proliferation and altered intercellular communication, often accompanied by increased exosome secretion. During this process, the hypoxic environment specifically regulates the expression of certain molecular components in exosomes, adapting to the needs of the TME by upregulating or downregulating key molecule regulators, such as miRNAs (23,24).

For example, miR-21 upregulates the expression and activity of HIF-1α, further activating glycolysis-related genes and increasing radiation resistance in NSCLC (25). HIF-1α also promotes endothelial cell (EC) angiogenesis by downregulating miR-186-5p and upregulating protein kinase C-α (26). Under chronic hypoxic conditions, miR-191 accelerates the proliferation and migration of NSCLC cells by reducing the levels of nuclear factor I A and its downstream oncogene CCAAT enhancer binding protein α (27). Under low oxygen conditions, lncRNA pvt1 is overexpressed in lung cancer cells and upregulates HIF-1α by binding to miR-199a-5p, thereby promoting cell proliferation and malignant transformation (28). However, intermittent hypoxia increases the expression level of miR-31-5p, activating WD repeat-containing protein 5, thereby enhancing EMT in tumor cells (29). Hypoxia can also stabilize HIF-1α by promoting its binding with mutant p53 and forming a complex that downregulates miR-129-1-3p. This, in turn, upregulates protein phosphatase, Mg^{2+}/Mn^{2+} dependent 1D, which dephosphorylates and inhibits p53, promoting the growth, survival and metastasis of NSCLC cells (30).

Macrophages are key immune cells that serve a pivotal role in early immune surveillance and response to tumors. They recognize and eliminate tumor cells and also activate other immune cells through antigen presentation, triggering specific immune responses. However, tumor cells interact with macrophages by secreting molecules such as exosomes that regulate macrophage polarization, thereby affecting their function. Tumor-associated macrophages (TAMs) can exhibit two

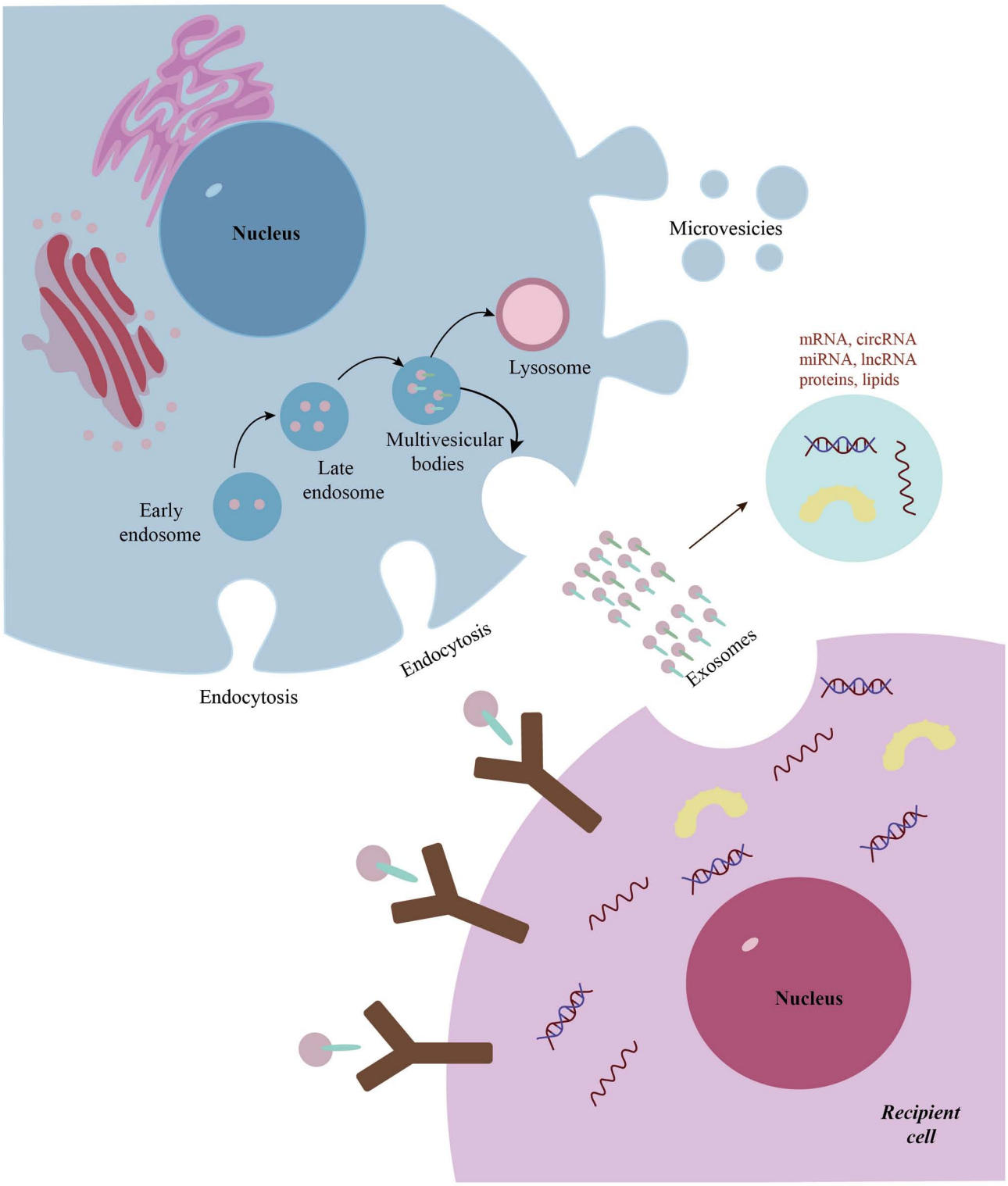

Figure 2. Cellular processes involved in exosome secretion. Adapted from doi: 10.2147/ijn.s479533 (20). CircRNA, circular RNA; miRNA, microRNA; lncRNA, long non-coding RNA.

distinct phenotypes: M1 and M2 (31). M1 phenotype macrophages promote inflammatory responses and inhibit tumor growth, while M2 phenotype macrophages have anti-inflammatory effects, suppress immune responses and promote tumor development (32). Under hypoxic conditions, LC cells further promote M2 polarization of TAMs by upregulating miR-21 and miR-1290. This polarization enhances angiogenesis and promotes tumor invasion and metastasis, thereby accelerating LC progression (33,34). Therefore, hypoxia is not only a result of tumorigenesis but also an important driving factor for tumor infiltration and growth (23,35). Depending on the duration and severity of hypoxia, its impact on tissues may be beneficial or harmful (36).

Metabolic reprogramming. Under hypoxic conditions, NSCLC cells undergo metabolic reprogramming to adapt to the

hypoxic environment and maintain their growth and survival. This process is related to the Warburg effect, where tumor cells convert glucose to lactate via glycolysis, even when oxygen is abundant and mitochondrial function is normal, rather than through the more efficient oxidative phosphorylation pathways (37). This metabolic transformation not only improves the efficiency of energy production but also provides necessary biosynthetic precursors for tumor cells to support their rapid proliferation needs (38). In NSCLC cells, the expression of key glycolytic enzymes, such as hexokinase, phosphoglycerate kinase 1, lactate dehydrogenase and glucose transporter 1 (GLUT-1) are increased, thereby promoting increased glucose uptake and lactate production (39). Lactate is not only a product of glycolysis but also serves multiple roles in the TME. It can create an acidic microenvironment that is conducive to the survival and proliferation of NSCLC cells and can also regulate certain signaling pathways to promote tumor progression (40). Specifically, lactate can activate the ERK/STAT3 signaling pathway, induce TAMs to polarize towards the M2 phenotype and stimulate angiogenesis, which together promote tumor growth and metastasis (41,42). Additionally, lactate also has immunosuppressive effects, which can regulate the function of immune cells, thereby limiting the effectiveness of antitumor immune responses (43,44). Although glycolysis is the primary energy source for NSCLC cells, fatty acid oxidation and mitochondrial oxidative phosphorylation also serve important roles in cellular metabolism, signal transduction and tumor progression (45). Mitochondria not only produce ATP through oxidative phosphorylation but also participate in fatty acid oxidation, redox regulation and the production of reactive oxygen species (ROS), which affect cell proliferation, survival and migration. Thus, NSCLC cells achieve adaptive growth in complex TMEs and promote tumor growth and metastasis by integrating multiple metabolic pathways such as glycolysis, lactate accumulation and mitochondrial metabolism (45).

Antitumor treatment. Hypoxia, a common characteristic of tumor growth, poses a significant challenge to cancer therapy. Hypoxic environments weaken the efficacy of antitumor therapy through various mechanisms. These mechanisms mainly include decreasing DNA damage repair and promoting the development of invasive phenotypes in tumor cells, thereby enhancing the survival rate of tumor cells (46). Therefore, the hypoxic microenvironment serves a critical role in determining the effectiveness of radiotherapy, chemotherapy, immunotherapy and targeted therapy.

Radiotherapy. Radiotherapy is a crucial approach for treating tumors and its mechanism of action involves utilizing high-energy electrons photons, such as X-rays or γ-rays, to interact with atoms in the body and release high-energy electrons. These electrons can trigger cellular damage, particularly DNA damage, thereby inhibiting the proliferation and survival of tumor cells (47). The mechanism of action of radiotherapy primarily encompasses two pathways: direct and indirect. In the direct mechanism, radiation photons directly interact with DNA molecules, causing single-strand or double-strand breaks, which can prevent cell division and may induce cell necrosis or apoptosis (48). The indirect mechanism involves the interaction between radiation and water molecules, generating free radicals such as hydrogen atoms, hydroxyl radicals and hydrogen peroxide. These free radicals further react with oxygen to generate ROS, which can damage macromolecules such as DNA, proteins and lipids, leading to cellular dysfunction, alterations in signal transduction and long-term cellular damage (49). The efficacy of radiotherapy is closely linked to the oxygen level of tissues. In hypoxic tumor cells, due to the insufficient oxygen available to support redox reactions, DNA repair mechanisms may not effectively repair radiation-induced damage (50). Therefore, to achieve similar damaging effects as in oxygen-rich cells, hypoxic tumor cells require an increase in radiation dose by 2-3 times, a phenomenon known as the oxygen enhancement ratio (OER) (51).

Chemotherapy. To support their rapid proliferation, tumors form an invasive and irregular vascular system. However, these neovessels often have abnormal structures and impaired functions, leading to low drug delivery efficiency and reduced permeability and distribution of chemotherapeutic agents. This significantly reduces drug exposure to tumor cells (52). Under hypoxic conditions, the HIF-1α and p53 genes play key roles in regulating the resistance of NSCLC to cisplatin. Specifically, HIF-1α promotes metabolic adaptation by enhancing glycolysis and limiting the accumulation of ROS, thereby increasing resistance to cisplatin. Simultaneously, p53 inhibits the progression of the cell cycle from the G1 phase to the S phase by activating the downstream target gene p21, further reducing the sensitivity of tumor cells to cisplatin (53). Additionally, cell cycle arrest under hypoxic conditions triggers metabolic reprogramming, including upregulation of glycolysis pathways and antioxidant responses, which further enhance cisplatin resistance (41).

Immunotherapy. Hypoxia can also impair the antitumor immune response. Under hypoxic conditions, HIF-1α is activated, leading to the upregulation of CD47 expression. CD47 is a transmembrane protein that interacts with the macrophage signal regulatory protein α, sending a signal to macrophages to prevent degradation. This signaling inhibits the recognition and phagocytosis of tumor cells by macrophages, promoting immune evasion and thus facilitating tumor growth and metastasis (54). Furthermore, dendritic cells (DCs) serve a crucial role in activating $CD4^+$ and $CD8^+$ T cells via antigen presentation, which is hindered by chronic hypoxia (55). Key factors such as IL-10 and VEGF induced by hypoxia can suppress the differentiation and maturation of DCs, thereby impairing T cell responses and reducing antitumor immunity (56,57). In the hypoxic microenvironment, activation of the mTOR-HIF-1α pathway also induces the upregulation of extracellular nucleotide enzymes CD39 and CD73 in myeloid-derived suppressor cells, converting extracellular ATP/ADP to adenosine. Conversely, adenosine suppresses immunotherapy, induces cell apoptosis and further impairs immune response by binding to adenosine A2A receptors on T cells and natural killer cells (58,59).

Targeted therapies. The hypoxic microenvironment can also affect the efficacy of targeted therapy. For example, gefitinib is a tyrosine kinase inhibitor commonly used to treat NSCLC. However, under hypoxic conditions, gefitinib upregulates IL-6 expression and activates inflammatory pathways such as the TNF, NF-κB and JAK-STAT pathways, leading to the enrichment of LC stem cells and initiation of the EMT,

Table I. Comprehensive summary of methods for detecting hypoxia in non-small cell lung cancer.

A, Direct detection

Method of detection	Analyte/ marker	Techniques or tools	Principles	Advantages	Disadvantages	(Refs.)
Physical	PO_2	Electrochemical probe	Measurement of current generated by electrode redox	Direct measurement of tissue PO_2 and is classed as the gold standard in clinical testing	Probes may damage tumor tissue and instantaneous detection cannot track changes over time	(64,65)
		Optical probe	Detecting the intensity or lifetime of probes	High sensitivity and resolution, non-invasive and enables real-time monitoring	Probes measure oxygen levels in blood rather than tissues and it necessitates particular environmental conditions	(66,67)

B, Indirect detection

Method of detection	Analyte/ marker	Techniques or tools	Principles	Advantages	Disadvantages	(Refs.)
Vascular system testing	Vascular function and morphology analysis	CT perfusion imaging, MRI and near-infrared spectroscopy	Evaluating tumor vascular abnormalities and blood flow	Non-invasive, analyses vascular function and allows early detection	Uneven blood flow and individual variations make blood flow parameters inadequate for assessing tissue oxygen supply	(68-71)
Hypoxia imaging	Oxygen metabolism tracers imaging	Positron emission tomography	Radiotracer probe-based oxygen metabolism evaluation	High sensitivity and specificity and can analyze the whole tumor tissue	Can cause radiation hazards and has a longer half-life of the tracer	(8,73,74)
		MRI	Blood oxygen level-dependent-MRI uses the magnetic property comparison of oxy-vs. deoxyhemoglobin whereas tissue oxygen level-dependent-MRI measures tissue oxygenation via longitudinal relaxation time changes	No radiation hazard and enhanced clarity of soft tissue visualization	Time-consuming and cannot be monitored in real-time	(75-78)
		Electron paramagnetic resonance imaging	Based on the spin distribution of unpaired electrons in free oxygen molecules	High sensitivity, experiences minimal interference from external factors and is effective in detecting low concentrations of free radicals	Low spatial resolution, rapid signal decay with tissue depth and high cost of imaging equipment	(79,80)

Table I. Continued.

B, Indirect detection

Method of detection	Analyte/ marker	Techniques or tools	Principles	Advantages	Disadvantages	(Refs.)
Measurement of gene expression levels	Hypoxia inducible factor-1α, solute carrier family 2 member 1, Egl-9 family hypoxia inducible factor 2, carbonic anhydrase 9 and VEGF	Quantitative PCR, RNA sequencing and Northern blotting	Hypoxia-induced gene expression for tumor hypoxia assessment	Can measure molecular changes in hypoxia and is useful for tissue sectioning	Tissue samples are required, is unable to assess dynamic changes *in vivo* and has a high cost	(81-84)
Measurement of protein expression levels	Solute carrier family 2 member 1, carbonic anhydrase 9, osteopontin and nitroreductase	Western blotting, immunohistochemistry, immunofluorescence and ELISA	Hypoxia-related protein expression for tumor hypoxia assessment	High sensitivity and is appropriate for clinical and laboratory analysis	Tissue samples are required, is unable to assess dynamic changes *in vivo* and has a high cost	(78,85-88)

PO_2, oxygen partial pressure.

ultimately enhancing resistance to gefitinib (60). In cases of NSCLC with EMAP like 4-ALK receptor tyrosine kinase (ALK) rearrangement, although ALK inhibitors usually show good efficacy, some patients develop resistance to agents such as crizotinib in hypoxic environments. The emergence of this resistance is closely related to the upregulation of HIF-1α and its downstream regulatory factor Slug under hypoxic conditions, which promote the EMT process and significantly enhance the migration and invasion ability of cancer cells, thereby conferring tumors with resistance to ALK inhibitors (61).

3. Hypoxia detection in NSCLC

Hypoxia serves a critical role in the progression of NSCLC. Early detection of the hypoxic state of tumor cells can facilitate timely intervention measures and effectively correct these conditions (62). Currently, hypoxia detection in laboratory cells and human tumors can be categorized based on the types of substances measured: physical substances, such as oxygen partial pressure and oxygen saturation, biological substances, such as enzymes and proteins, and chemical substances, such as signaling pathway products. Detection technology can be categorized as direct or indirect based on the measurement approach and can also be categorized as transient or dynamic based on the nature of the detection process (Table I) (63).

Direct detection of oxygen concentration. Physical sensors are among the most direct methods for detecting cellular hypoxia. Electrochemical probes and optical sensors can directly measure the oxygen partial pressure in tumor tissue, providing accurate tissue oxygenation values. The working principle of an electrochemical probe is to measure the oxygen partial pressure by detecting the current generated by the oxygen reduction reaction at the working electrode and the oxidation reaction at the anode (64). This technique remains the gold standard in the clinical field and with the assistance of CT-guided oxygen partial pressure measurement, it is now possible to detect hypoxia in deeper tumor regions (65). However, needle-based detection methods may cause damage to tumor tissue, making it difficult to distinguish between necrotic and hypoxic areas and these methods can only provide transient measurements, limiting the possibility of continuous monitoring (65). Alternatively, optical probes detect oxygen partial pressure by assessing the effect of oxygen partial pressure on the luminescent intensity of fluorescent substances, a process known as quenching (63). Metal-organic complexes, such as Pt(II), Pd(II), Ru(II) and Ir(III), are commonly used as phosphorescent probes (66). These probes interact with oxygen molecules, shortening their phosphorescence lifetime and leading to quenching phenomena. By measuring changes in phosphorescence intensity or lifetime, in combination with the Stern-Volmer equation, the oxygen partial pressure in tumor tissue can be quantified (67). These phosphorescent probes offer high sensitivity, high resolution and the ability to achieve real-time, non-invasive monitoring, thereby demonstrating significant advantages in effectively identifying tissue hypoxia.

Indirect detection of hypoxic microenvironment

Vascular system testing. The hypoxic state of tumors is closely linked to abnormalities in their vascular system. The structural

and functional defects of the tumor vasculature lead to insufficient oxygen supply, making it particularly important to evaluate these vascular parameters that are critical for detecting hypoxia. The generation of hypoxia is generally believed to be caused by three mechanisms: i) Diffusion, due to distance from perfusing blood vessels; ii) intermittent, fluctuations in vessel opening and closing; and iii) perfusion-related, low blood flow efficiency (68). Various imaging techniques, such as cryophotometry, near-infrared spectroscopy and MRI, have been used to evaluate tumor vascular features such as distance, density, distribution and oxygenation status (69). Additionally, CT perfusion imaging, a non-invasive functional imaging method, can evaluate tumor morphology and also assess microcirculation parameters such as blood volume, blood flow, mean transit time and permeability surface, which provide valuable insights for understanding hypoxia (70,71). However, due to uneven blood flow distribution, variations in oxygen consumption between individuals and increased hemoglobin levels caused by chronic hypoxic adaptation, relying solely on blood flow parameters cannot fully capture tissue-level oxygen supply and demand dynamics (72). Therefore, combining multiple parameters to evaluate the TME is essential for a comprehensive assessment of hypoxia.

Hypoxia imaging detection. Optical imaging provides a non-invasive and dynamic approach to assessing tissue oxygenation. Positron emission tomography (PET) is one of the most widely adopted methods of optical imaging. PET assesses oxygen metabolism in tissues and identifies preclinical or clinical hypoxia through the use of radioactive tracer probes (73). Unlike histological testing, PET can monitor the entire tumor comprehensively and boasts higher sensitivity and specificity compared with MRI, with fewer artifacts (8). Presently, two primary tracers are employed for hypoxia detection: ^{18}F-labeled nitroimidazole and Cu-labeled diacetylbis (N4-methylaminothiourea) analogs (74). The most widely used tracer is 18F-Fluoromisonidazole (^{18}F-FMISO), a highly lipophilic 2-nitroimidazole analog. However, its slow plasma clearance rate necessitates prolonged imaging times. To overcome this, new hydrophilic tracers such as fluazomycin cytarabine and fluorinated ethyl imidazole derivatives have been developed. These tracers have faster plasma clearance rates, which enable quicker imaging and improve the efficiency and accuracy of hypoxia detection (75).

MRI includes techniques like blood oxygen level-dependent (BOLD) and tissue oxygen level-dependent (TOLD) imaging. Compared with PET, MRI has the advantages of high soft tissue contrast and no radiation exposure, making it particularly suitable for individuals with conditions that contraindicate radiation. These include pregnant women, children, patients with thyroid disorders and individuals with suppressed immune systems (75). In BOLD-MRI, contrast arises from the differing magnetic properties of oxyhemoglobin (diamagnetic) and deoxyhemoglobin (paramagnetic), reflecting local concentrations of oxygenated and deoxygenated hemoglobin (76). TOLD-MRI detects tissue hypoxia by measuring free oxygen molecules within tissues. As mitochondria consume oxygen, the remaining oxygen dissolves in the plasma, leading to an increase in longitudinal relaxation time (T1), thereby evaluating tissue oxygenation (77). While MRI is highly effective in the 3D mapping of tumor hypoxia, it is time-consuming and does not allow for real-time monitoring (78).

Another method for detecting oxygen levels is electron paramagnetic resonance imaging (EPRI). Analogous to MRI which depicts proton distribution, EPRI measures the spin distribution of unpaired electrons in free oxygen molecules (76). Oxygen molecules are paramagnetic and possess two unpaired electrons in their ground state. When oxygen molecules interact with probes, which are typically paramagnetic substances, the collision between the unpaired electrons of oxygen and the probe alters the energy state of the probe (79). Specifically, this interaction alters the probe's spin state, which consequently shifts its resonance frequency. EPRI detects these frequency shifts, enabling precise the recording of oxygen levels (80). EPRI exhibits high sensitivity and is less influenced by external factors, making it a promising tool in clinical research.

Gene and protein markers. In the hypoxic microenvironment of NSCLC, HIF-1α serves as a key transcription factor that regulates the expression of various hypoxia-responsive genes. Due to the short half-life of HIF-1α and its difficulty for use in direct labeling, studies typically indirectly evaluate hypoxic status by measuring gene expression levels regulated by HIF-1α. For instance, solute carrier family 2 member 1 (SLC2A1) is a target gene of HIF-1α, which enhances glycolytic activity by upregulating the expression of SLC2A1, contributing to energy generation and biosynthesis in tumor cells. The upregulation of SLC2A1 expression is strongly associated with the prognosis of patients with lung squamous cell carcinoma and can also serve as a key hypoxia biomarker (81). Similarly, Egl-9 family hypoxia inducible factor 2 (EGLN2) is a HIF-1α hydroxylase that regulates hypoxia by promoting the hydroxylation and degradation of HIF-1α. During hypoxia, the decrease in EGLN2 activity leads to the stabilization of HIF-1α, thereby activating the hypoxic pathway. The expression of EGLN2 may offer diagnostic and prognostic value in lung adenocarcinoma (82). Additionally, genes such as carbonic anhydrase 9 (CA9) and VEGF, which are widely expressed in NSCLC, are closely correlated with the degree of cellular hypoxia, tumor invasiveness and the effectiveness of radiotherapy and immunotherapy (83,84).

To detect hypoxia in tumors, studies commonly employ immunohistochemistry and immunofluorescence techniques to assess protein expression associated with hypoxia, primarily involving biomarkers like GLUT-1 (SLC2A1), CA9 (CA-IX), osteopontin (OPN) and nitroreductase (NTR). SLC2A1 supports glycolysis by enhancing glucose uptake, providing tumor cells with the energy and raw materials necessary for metabolism (85). CA-IX catalyzes the formation of carbonic acid from carbon dioxide and water, promoting extracellular acidification and thereby enhancing tumor invasiveness (86). OPN, as a hypoxia biomarker, is closely related to increased invasion and metastasis potential in NSCLC (87,88). NTR is an enzyme expressed only under hypoxic conditions and can reflect the degree of tumor hypoxia based on its activity level (78). In hypoxic environments, nitroimidazole is reduced by intracellular NTRs to generate reactive intermediates that disrupt DNA repair enzymes and cell cycle regulators, thereby inhibiting tumor cell survival (76). Currently, derivatives of 2-nitroimidazole, such as pimonidazole and EF-5, have

Table II. Summary of targeted hypoxia therapies for non-small cell lung cancer.

Therapeutic approaches	Methods and drug treatments	Mechanism of action	Advantages	Disadvantages	(Refs.)
Enhancing tissue oxygenation	Hyperbaric oxygen therapy, normobaric hyperoxia and aerobic exercise	Increases oxygen levels in tumor tissues, inhibits cell proliferation and enhances therapeutic effects	Increases oxygen supply and enhances the effect of radiotherapy and chemotherapy	High-concentration oxygen induces oxygen toxicity, tissue damage and oxidative stresss and the efficacy of aerobic exercise remains unclear	(7,89-94)
Reduction of oxygen consumption	Metformin, Atovaquone, Papaverine, heme-segregating protein 2	Inhibition of oxidative phosphorylation reduces the oxygen demand of tumor cells	Improves the tumor hypoxic environment and enhances treatment sensitivity	Can cause metabolic toxicity and research on this therapy is currently underdeveloped	(98-102)
Restoration of blood flow	Nitroglycerin, and Cilengitide in combination with verapamil	Improved tumor blood flow and enhanced drug and oxygen delivery	Reduces glycolysis and improves distribution of chemotherapy and targeted drugs	Individual differences exist and excessive blood flow may promote tumor spread	(104-107)
Targeting pathways of HIF-1α	Acriflavine, Polyamides, Temsirolimus, Trametinib and Bevacizumab	Inhibits HIF-1α activity, targets HIF-1α signaling and inhibits angiogenesis	Inhibits tumor growth and improves the tumor hypoxic microenvironment	Monotherapy may induce resistance and the clinical efficacy and safety require further validation	(108-117)
Hypoxia-activated prodrugs	TPZ, TH-302, PR-104, and EO9	Targeting hypoxic tumor regions	Precisely kills hypoxic tumor cells while minimizing normal tissue damage	Limited reach to avascular areas and poor clinical performance of some drugs	(121-126)
Improvement of radiotherapy	Radiosensitizers, carbon ion radiotherapy and dose painting	Enhances radiotherapy sensitivity, higher linear energy transfer and precise dose distribution	More precise and higher-dose radiotherapy efficacy and minimizes impact on normal cells	Further clinical validation needed	(127-130)

HIF, hypoxia inducible factor.

become key tools for identifying and quantifying hypoxic regions within tumor tissues. By detecting specific antibodies like hypoxyprobe and ELK3-51, it is possible to efficiently map the distribution of hypoxia in tumors (76).

4. Targeted hypoxia in NSCLC

In NSCLC, the hypoxic microenvironment serves a crucial role in regulating tumor growth and invasion. Targeted therapy strategies aimed at this microenvironment have attracted widespread attention, as hypoxia not only affects the proliferation, invasion and metastasis of tumor cells but also activates a series of adaptive responses, such as angiogenesis, metabolic reprogramming and anti-apoptotic mechanisms. These responses enhance tumor invasiveness and lead to the development of therapeutic resistance. Therefore, targeted hypoxia therapy not only directly inhibits tumor progression but also has the potential to improve the efficacy of conventional treatment methods. Currently, several targeted therapies aimed at tumor hypoxia are being actively researched and developed (Table II).

Enhancing tissue oxygenation. The goal of oxygen therapy is to increase the levels of dissolved oxygen in plasma and enhance tissue oxygenation, thereby slowing down tumor progression and improving treatment efficacy. Currently, various key strategies including hyperbaric oxygen therapy (HBO), normobaric hyperoxia (NBO) and aerobic exercise are being explored to augment tissue oxygen levels. HBO involves administering 100% oxygen at a pressure of 1.5-2.5 atmospheres absolute. Previous studies have shown that HBO can significantly reduce the expression level of HIF-1α and its downstream

target genes, exert anti-angiogenic effects, reduce vascular density, inhibit tumor cell proliferation and thereby improve chemotherapy efficacy (7,89). NBO involves the delivery of high concentrations of oxygen via masks or shields, primarily by generating ROS to inhibit tumor growth (90). Although NBO is not as effective as HBO in enhancing the oxygenation of arteries and tumor tissues, it is considered more practically valuable due to its increased safety (91). However, the clinical use of HBO therapy faces certain challenges, mainly due to the possibility of causing some adverse reactions. These reactions include barotrauma to the middle ear, paranasal sinuses and lungs, ROS-induced damage to alveolar epithelium and pulmonary vascular endothelium and excessive production of nitric oxide (NO) due to aberrant activation of NO synthase. These mechanisms trigger pro-inflammatory pathways, such as the NF-κB pathway, amplifying local and systemic inflammation and worsening tissue damage (92).

Additionally, aerobic exercise has been shown to regulate the tumor vasculature and promote the relative normalization of dysfunctional tumor blood vessels (93). Jo *et al* (94) reported that sustained aerobic exercise improved lung function in NSCLC mice, increased oxygen saturation within tumor cells and elevated HIF-1α and ROS levels, thereby enhancing the efficacy of radiotherapy. In conclusion, oxygen-based therapies have potential in improving tumor oxygenation and enhancing treatment efficacy. However, a detailed assessment of the potential risks and benefits of this approach is particularly important for optimizing its clinical application.

Reduction of oxygen consumption rate. Tumor cells partially produce ATP through the mitochondrial oxidative phosphorylation (OXPHOS) pathway, in which hemoglobin serves a key role in the mitochondrial electron transport chain (ETC) complexes II-IV, thereby promoting ATP synthesis (95). In recent years, certain drugs approved by the US Food and Drug Administration (FDA), such as metformin, atovaquone and papaverine, have been shown to improve the hypoxic TME by inhibiting the OXPHOS pathway (96,97). Metformin binds to triphenylphosphine to generate MitoMet molecules, allowing the drug to accumulate in the mitochondria of tumor cells. MitoMet can inhibit mitochondrial complex I, thereby disrupting the function of ETC, increasing ROS production, inducing oxidative stress, leading to lipid peroxidation and ultimately causing tumor cell death (98). Atovaquone is an antimalarial drug that reduces hypoxia in NSCLC and improves cellular oxygenation by inhibiting complex III of OXPHOS (99). Papaverine is a smooth muscle relaxant and its derivative, papaverine pyrazole (PPV), temporarily reduces tumor hypoxia by reversibly inhibiting complex I, thereby enhancing the sensitivity of tumor cells to radiotherapy (100). Additionally, the inhibition mechanism of OXPHOS is closely associated with the action of heme-segregating proteins (HSPs), particularly HSP2. These proteins inhibit mitochondrial OXPHOS by limiting heme uptake in tumor cells, reducing oxygen consumption and ATP synthesis (101,102). Overall, targeting OXPHOS and related metabolic pathways offers a promising strategy for the treatment of NSCLC. Although these therapies have shown some potential, their toxicity and long-term efficacy still require further in-depth research to ensure their safety and effectiveness in clinical applications.

Restoration of blood flow. Acute hypoxia in tumors is often caused by vascular occlusion. Restoring blood flow can not only reduce the glycolytic metabolism of tumor cells but also improve the distribution of chemotherapy or targeted therapy, thereby significantly enhancing the therapeutic effect (103). Nitroglycerin, as a vasodilator, is primarily converted to NO through the mitochondrial aldehyde dehydrogenase and PI3K pathways, thereby inducing vasodilation, improving the oxygenation status of tumor tissue and enhancing the efficacy of anticancer drugs (104,105). Additionally, the Arg-Gly-Asp analogue cilengitide, can target αVβ3 and αVβ5 integrins on tumor ECs, regulate VEGFR2 expression and promote VEGF-dependent angiogenesis. Verapamil, a calcium channel blocker with vasodilatory effects, can enhance chemotherapy efficacy. When used in combination with cilengitide and gemcitabine, low-dose cilengitide significantly enhances the cytotoxic activity of gemcitabine against NSCLC cells (106). However, an excessive increase in blood flow may promote tumor growth, so the efficacy of this approach is highly dependent on tumor type and individual differences among patients. It is crucial to dynamically adjust treatment strategies within the time frame of vascular normalization to achieve optimal treatment outcomes (107).

Targeting upstream and downstream pathways of HIF-1α. HIF-1α serves a critical role in inflammation and hypoxia and strategies targeting HIF-1α have shown potential in reducing inhibiting the proliferation and invasion of NSCLC cells. Studies have demonstrated that Acriflavine binds to the Per-ARNT-Sim domain B subdomain of HIF-1α, thereby preventing the dimerization of the α and β subunits. In addition, polyamides and Echinomycin can inhibit the binding of the HIF-1 dimer to the HRE sequence of target genes. Compounds such as Chetomin, Bortezomib and Amphotericin B inhibit the transcriptional activity of HIF-1α through different mechanisms, while EZN-2968 suppresses the synthesis of the HIF-1α protein by blocking the translation process of HIF-1α mRNA (108). Collectively, these compounds effectively inhibit tumor growth and angiogenesis by modulating the activity of HIF-1α (Fig. 3B) (109).

Upstream pathways. Under hypoxic conditions, signaling pathways like PI3K-AKT-mTOR, RAS-RAF-MEK-ERK and JAK-STAT3 are activated, thereby promoting the synthesis of the HIF-1α protein (110). Specifically, the PI3K-AKT-mTOR pathway can enhance mRNA stability and protein synthesis efficiency, significantly increasing the transcription level of HIF-1α. Conversely, the RAS-RAF-MEK-ERK pathway phosphorylates the C-terminal activation domain of HIF-1α, facilitating its interaction with CBP/p300 and further enhancing HIF-1α-mediated gene transcription (111). Additionally, the STAT3 protein can be directly translocated to the nucleus to promote the transcription of HIF-1α and it can also work synergistically with the PI3K-AKT-mTOR pathway to trigger the transcription of HIF-1α through AKT-mediated mechanisms (110,112). Clinical trials have demonstrated that temsirolimus and rapamycin inhibit mTORC1 activity by binding to the FKBP prolyl isomerase 1A protein, while Voxtalisib exhibits broad antitumor activity by simultaneously inhibiting PI3K and mTOR targets (113). Additionally,

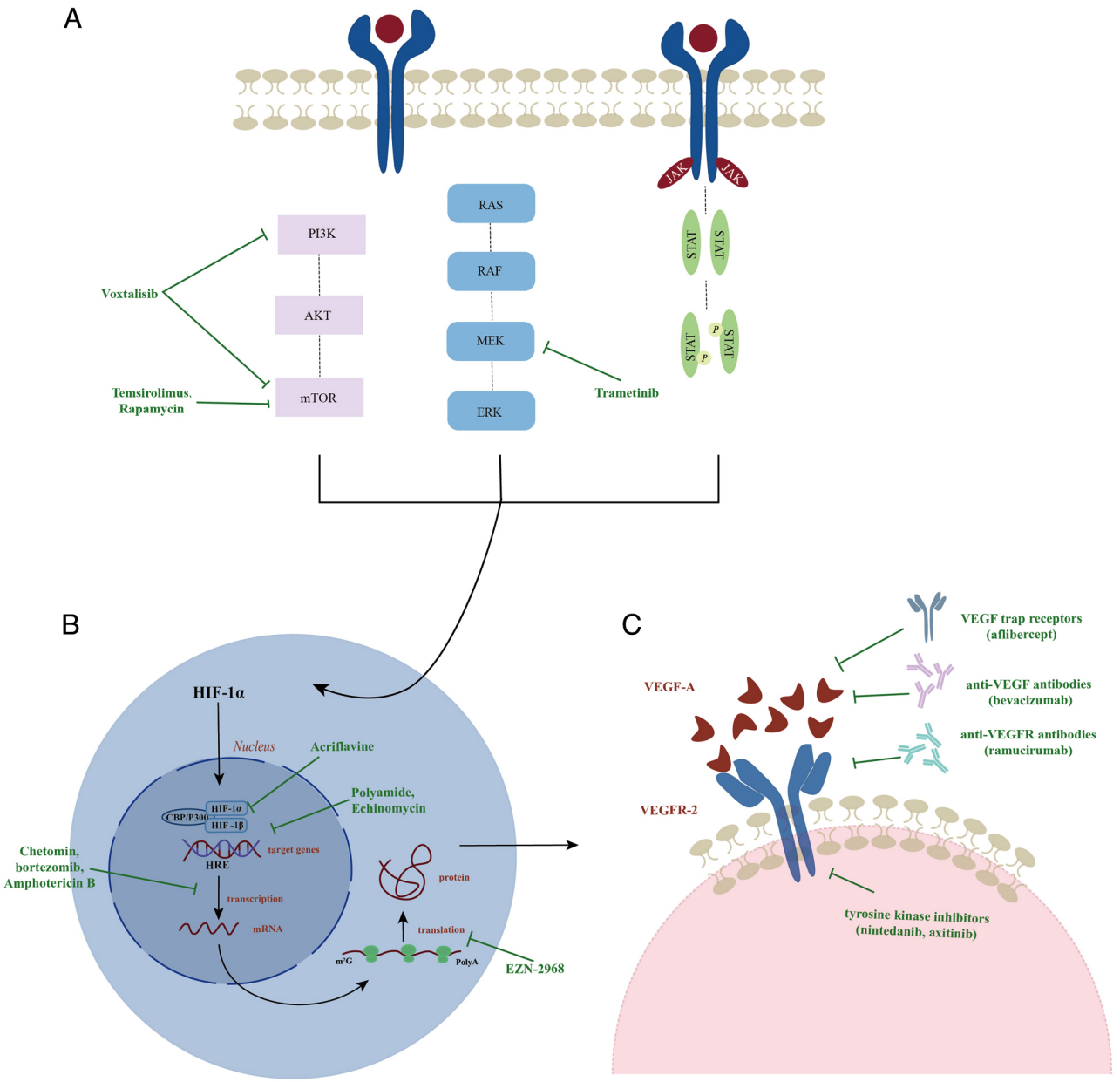

Figure 3. Targeted interventions in HIF-1α regulation and associated signaling pathways. (A) Intervention in the upstream signaling pathways of HIF-1α through interactions with the PI3K-AKT-mTOR, RAS-RAF-MEK-ERK and JAK-STAT3 pathways. (B) Intervention in HIF transcriptional and translational processes (C) Intervention in angiogenic pathways. Flat-headed arrows indicate inhibition, while pointed arrows represent activation. HIF, hypoxia inducible factor; HRE, hypoxia response element.

the use of MEK inhibitors alone did not significantly improve the survival rate of patients with NSCLC; however, when combined with molecular targets such as VEGF, EGFR, ALK and BRAF, it shows significant clinical efficacy. The combination therapy of trametinib and dabrafenib has been approved by the FDA for the treatment of these tumors (Fig. 3A) (114).

Downstream pathways. VEGF is the only angiogenic factor consistently expressed throughout the entire tumor cycle and serves as a key downstream target gene of HIF-1α. When VEGF-A binds to VEGFR-2, it initiates angiogenesis signals, fostering the survival and proliferation of ECs and enhancing vascular permeability, all aiding in improving tumor resistance (115). Consequently, anti-angiogenesis has become a crucial strategy in the treatment of NSCLC. The primary focus of this approach is to disrupt the interactions between VEGF and VEGFR or interfere with the transmission of angiogenesis signals to normalize the tumor vasculature. Currently, anti-angiogenic therapies encompass a variety of drugs, including anti-VEGF antibodies (such as bevacizumab), anti-VEGFR antibodies (such as ramucirumab), VEGF trap receptors (such as aflibercept) and small-molecule VEGFR tyrosine kinase inhibitors (such as nintedanib, axitinib, sorafenib, sunitinib, vandetanib and cediranib) (116). However, the sole use of anti-angiogenic drugs may prompt tumor cells to develop hypoxia-tolerant phenotypes, thereby promoting vascular remodeling and tumor invasion, without significantly improving patient prognosis (117). Therefore, in clinical practice, it is more common to combine anti-angiogenic therapy with other treatments such as radiotherapy, chemotherapy or anti-EGFR

therapy to enhance antitumor efficacy and prolong patient survival (Fig. 3C) (116).

Hypoxia-activated prodrugs (HAPs). HAPs, or bioreductive alkylating agents, typically consist of five chemical groups: Nitro, quinone, aromatic, aliphatic and transition metals (118). Due to the rarity of chronic or severe hypoxia in normal tissues, HAPs can selectively target tumor cells by disrupting DNA replication in the hypoxic tumor environment (119). *In vivo*, HAPs are activated by a single electron reductase system, generating free radical intermediates. Under normoxic conditions, these intermediates are rapidly oxidized, lose their activity or transform into non-toxic products. However, in hypoxic environments, the lifespan of these intermediates is prolonged, leading to DNA alkylation, inducing cell apoptosis or necrosis and selectively inducing tumor cell death (75,120).

Although HAPs were originally designed for hypoxic tumor regions, they cannot reach areas far from the vascular network, which limits their clinical efficacy (121). For instance, although the classic HAP tirapazamine demonstrated effective hypoxic cell-killing effects in early experiments, the overall survival rate and response rate of patients with advanced or metastatic NSCLC did not significantly improve in phase III trials (122). This limitation was mainly attributed to the insufficient efficacy of tirapazamine against normoxic tumor cells (123). Currently, researchers are developing several new HAPs, such as TH-302 (evofosfamide), PR-104 and Alpraziquinone (EO9), to overcome these limitations and improve clinical efficacy (124). Combining HAPs with conventional chemotherapy or radiotherapy can improve treatment efficacy. For example, the combination of ezetophosamide and erlotinib has been shown to reduce the resistance of patients with NSCLC to erlotinib (125), while the combination of HAPs and radiotherapy may increase the sensitivity of hypoxic tumor cells, thereby improving overall antitumor efficacy (126).

Improvement of radiotherapy in hypoxic NSCLC. Radiotherapy is a key component of comprehensive treatment for various stages of NSCLC. However, to overcome its limitations, particularly in hypoxic tumors, there is a need to develop effective radiosensitization strategies that enhance accuracy and improve outcomes. Radiosensitizers primarily augment the effectiveness of radiotherapy by inhibiting radiation-induced DNA repair, disrupting cell cycle progression, affecting organelle function or modulating gene expression (49). For example, compounds containing nitrogen oxides and NO act as oxygen mimetics, thereby increasing tumor sensitivity to radiation in low-oxygen environments. These reagents intensify the interaction between free radicals and DNA, exacerbating DNA damage (127,128). Nanomaterials serve a significant role in enhancing radiosensitization through multiple mechanisms, including amplifying ROS production, modulating the cell cycle and stimulating apoptosis (129). Furthermore, the natural compound deguelin has shown promising radiosensitizing effects in NSCLC. It achieves this by inhibiting FBXO22 expression, downregulating the FOXM1/Rad51 pathway and consequently impairing the DNA damage repair capability of tumor cells (130).

Although radiosensitizers have enhanced the efficacy of conventional radiotherapy, their effectiveness is still constrained by the inability of conventional photon radiation to effectively target radiation-resistant tumor areas. To address this challenge, carbon ion radiotherapy (^{12}C-ion) exhibits heightened tumor cell-killing effects due to its higher linear energy transfer (LET). The elevated LET of carbon ions leads to more localized deposition of radiation energy, resulting in complex and difficult-to-repair double-stranded DNA breaks, thereby increasing cell lethality (131). The advantages of carbon ion radiotherapy include: i) Increased relative biological effectiveness with radiation-killing effects 1.2-3.5 times greater than conventional photon or proton therapy at equivalent physical doses; ii) lower OER ensures effective therapeutic outcomes even in hypoxic TMEs; and iii) its weak dependence on the cell cycle renders it effective against S-phase tumor cells, which are typically resistant to photon radiation (132).

Despite the enhanced tumor-killing efficacy of carbon ion radiotherapy, its therapeutic potential needs to be optimized through precise dose distribution. Dose painting is an advanced technique that utilizes functional imaging techniques, such as PET and MRI, to personalize dose allocation based on specific tumor characteristics (133). The two primary strategies for dose painting include dose painting by contours (DPBC) and dose painting by numbers (DPBN). DPBC classifies tumors into high-risk, requiring higher doses, and low-risk areas, using conventional doses, based on imaging thresholds such as PET SUV values. Although clinically feasible, DPBC is constrained by variations in imaging thresholds, which affect treatment consistency. DPBN assigns continuous dose values to each voxel based on functional imaging data, more accurately reflecting tumor heterogeneity. However, this approach demands high image alignment precision and complex treatment planning (134).

In summary, the development of radiosensitization strategies and precise dose allocation techniques presents new opportunities for improving radiotherapy for NSCLC. The combination of efficient radiosensitizers and advanced radiotherapy approaches, such as carbon ion therapy and dose painting, may potentially optimize treatment strategies and enhance patient outcomes.

5. Discussion and conclusions

The present review summarized the role of the hypoxic microenvironment in the progression, metabolic reprogramming, immune escape and therapeutic resistance of NSCLC. Hypoxia is a key driving factor for tumor progression through multiple pathways. Specifically, HIF, as a central regulatory factor, serves a crucial role in the proliferation, angiogenesis and migration of tumor cells by regulating EMT and ECM degradation. Additionally, the hypoxic environment promotes intercellular communication through exosomes, thereby regulating processes such as glycolysis, angiogenesis, tumor invasion and immune evasion, which collectively promote the progression of NSCLC. At the metabolic level, hypoxia induces metabolic reprogramming, making NSCLC cells more inclined to rely on glycolytic pathways to generate lactate, thus meeting their energy demands and enhancing their adaptability to hypoxic conditions. The accumulation of lactate not only promotes tumor cell proliferation and immune escape but

also supports tumor growth and metastasis by modulating the TME. Moreover, under hypoxic conditions, the DNA damage repair capability of tumor cells is reduced, and invasive phenotypes are more likely to form, thereby increasing resistance to radiotherapy, chemotherapy, immunotherapy and targeted therapy.

To address the challenges posed by hypoxic microenvironments, further studies should focus on hypoxia detection and intervention strategies. Existing methods for hypoxia detection include electrochemical probes and optical sensors for directly measuring oxygen partial pressure, as well as imaging techniques such as PET, MRI and EPRI for evaluating hypoxic regions within tumors. Additionally, gene markers, such as SLC2A1, EGLN2, VEGF and CA9, and protein markers, such as GLUT-1, CA9 and OPN, are currently used to detect tissue hypoxia. However, there are currently still a number of challenges in overcoming the heterogeneity of oxygen distribution within tumors and achieving real-time and accurate hypoxia monitoring. Future research should focus on developing more precise, non-invasive and real-time hypoxia detection technologies, which may have profound implications for clinical treatment. Regarding hypoxia treatment, recent studies have proposed various strategies to improve TME and enhance treatment efficacy. These strategies include increasing tissue oxygenation, reducing tissue oxygen consumption, restoring blood perfusion, downregulating HIF-related signaling pathways, using HAPs and improving radiotherapy efficacy. Although these strategies have shown some promise in preclinical studies, they still face challenges such as inadequate targeting and potential adverse reactions in clinical applications.

The current hypoxia detection and therapeutic strategies have brought new perspectives to tumor treatment, but the development of more accurate, real-time and non-invasive monitoring techniques remains the key research area. For example, combining high-resolution imaging technology with the detection of hypoxia-related biomarkers can provide a dynamic and effective method for monitoring tumor hypoxia in clinical settings. Additionally, integrating artificial intelligence and machine learning into image processing and data analysis is expected to further advance real-time monitoring of the tumor hypoxic microenvironment. In terms of treatment strategies, a single intervention may not be sufficient to improve tumor hypoxia effectively. Future research should explore multi-target approaches, combined with various therapeutic approaches, to enhance therapeutic response, alleviate tumor hypoxia and thus improve overall treatment efficacy. However, the clinical application of these strategies still faces many challenges, requiring larger-scale interdisciplinary collaboration and more clinical trials of hypoxia-targeted therapy to achieve true therapeutic breakthroughs.

In a hypoxic microenvironment, NSCLC cells undergo a series of adaptive changes, including enhanced growth and metabolism, angiogenesis to maintain nutrient supply, increased cell energy through activation of glycolysis-related genes and enhanced invasion and migration capabilities. These adaptive mechanisms enable tumor cells to escape the primary site and spread to distant organs, thereby making treatment more complex and contributing to the emergence of treatment resistance. Thus, identifying hypoxic signals in tissues is crucial for early diagnosis and disease monitoring of NSCLC. The best detection method should have the characteristics of non-invasiveness, reproducibility, high sensitivity and specificity and be able to accurately assess hypoxia status to guide clinical decision-making. Future therapeutic strategies should be combined with conventional therapies and optimized for hypoxia. By improving tissue oxygenation, modulating hypoxic genes and signaling pathways and applying selective drug delivery methods, it is expected to enhance the clinical treatment efficacy for patients with NSCLC.

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Competing interests

The authors declare that they have no competing interests.

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