REVIEW

Second Clinical Medical College[1]; College of Medical Technology[2]; College of Life Sciences[3]; Basic Medical College[4], Zhejiang Chinese Medical University, Zhejiang, China

Status of hypoxia-inducible factor-1α expression in non-small cell lung cancer

Yangni Li[1], Yishu Zhang[2], Xuran Li[3], Yanfen Huang[3], Weiyan Chen[4], Lan He[1], Yuyan Zhang[3,*]

Received April 11, 2021, accepted May 3, 2021

**Corresponding author: Yuyan Zhang, Zhejiang Chinese Medical University, Zhejiang 310053, China yannoo7376@sina.com*

Pharmazie 76: 404-411 (2021) *doi: 10.1691/ph.2021.1524*

According to the latest statistics from WHO for all cancers, lung cancer tops the list with a 14.5% prevalence and a 22% death rate in men, similar to the prevalence in women, which is 13.8%. It is also the number one killer of cancer in China, with 40 in every 100,000 people suffering from lung cancer. HIF-1α is widely present in human cells in hypoxic environments. It regulates the body's response to hypoxia, cell oxygen balance, and hypoxia gene expression; participates in the proliferation and apoptosis of non-small cell lung cancer cells; participates in the invasion, metastasis, and neovascularization of tumor tissues; and affects the treatment and prognosis of non-small cell lung cancer. In view of the role of HIF-1α in the occurrence and development of non-small cell lung cancer, blocking HIF-1α by use of a single medication or combination chemotherapy has become a research hotspot. This review summarizes the role of HIF-1α in non-small cell lung cancer and provides new ideas for the treatment of this cancer type by synthesizing the research results of various authors.

Abbreviations
NSCLC: non-small cell lung cancer; HIF-1: hyoxia-inducible factor-1; HIF-1β: hyoxia-inducible factor-1β; HIF-1α: hyoxia-inducible factor-1α; bHLH-PAS: basic helix-loop-helix-PER-ARNT-SIM; TAD-N and TAD-C: transactivation domain; NLS: nuclear location signal; Pro/Ser/Thr: proline-serine-threonine; ODDD: oxygen-dependent degradation domain; ARNT: aryl hydrocarbon receptor nuclear translocator; HRE: hypoxia response element; VHL: von hippel-lindau; PHD: prolyl hydroxylase; IAP: inhibitor of apoptosis proteins; Bcl-2: B cell lymphoma-2; PCNA: proliferating cell nuclear antigen; VE: vascular endothelial; VEGF: vascular endothelial growth factor; TCT: thinprep cytology test; DLL4: Delta-like ligand 4; CD147: recombinant protein; MMP-2: matrix metalloproteinase-2; LPS: lipopolysaccharides; CXCR4: CXC motif chemokine receptor type 4; NF-κB: nuclear factor-κB; p-IκB: phosphorylated inhibitor of NF-κB; PSA: prostate-specific antigen; hCG: human chorionic gonadotropin; CA724: carbohydrate antigen 724; CA153: carbohydrate antigen 153; CHCHD2: coiled-coil-helix domain containing 2; RORyt: RAR-related orphan receptor gamma; EGLNs: HIF prolyl 4-hydroxylases; Pro-402 and Pro-564: proline residues; CpG: cytosine-guanine dinucleotide-containing; LUAD: lung adenocarcinoma; PPC: pulmonary pleomorphic carcinomas; PD-L1: programmed death 1; VE-Cad: vascular endothelial cadherin; TGF-β1: transforming growth factor-β1; VM: vasculogenic mimicry; ROS: reactive oxygen species; EMT: epithelial-mesenchymal transition; ROS1: ROS proto-oncogene 1; BRAF: v-raf murine sarcoma filtering toxin carcinogenic homolog B1; KRAS: Kirsten rat sarcoma 2 viral oncogene homolog; METex14: MET-exon-14-skipping; 2ME2: 2-methoxyestradiol; YC-1: 3-(5'-Hydroxymethyl-2'-furyl)-1-benzylindazole; HSP90: heat shock protein 90; CCI-779: Cell cycle inhibitor-779; Rad001: everolimus; CEA: carcinoembryonic antigen; NSE: neuron-specific enolase.

1. Introduction

Non-small-cell lung cancer (NSCLC) accounts for 80% of lung cancers, including squamous cell carcinoma, adenocarcinoma, large cell carcinoma, and other rare cancer types. It has the highest incidence rate worldwide, especially in China. The death rate of lung cancer worldwide is as high as 18.4% (Erratum 2020), and the five-year survival rate is 24% (Zhang et al. 2008).

Hypoxia inducible factor-1 (Chen et al. 2014) (HIF-1) is a heterodimeric transcription factor that consists of hypoxia inducible factor-1β (HIF-1β) and hypoxia inducible factor-1α (HIF-1α). Both are basic helical loop structural proteins (basic helix-loop-helix-PER-ARNT-SIM, bHLH-PAS) (Bersten et al. 2013). However, HIF-1α is the oxygen-sensitive regulatory and functional subunit of the HIF-1 heterodimer, consisting of 826 amino acids (Hirami et al. 2004). The human HIF-1α gene is located in the q21-24 region of chromosome 14 (14q21-24). Its N-terminal contains a fundamental bHLH-PAS configuration, which is necessary for binding to DNA and participating in dimerization (Semenza et al. 1997). The C-terminus of HIF-1α contains two transcription activation domains (transactivation domains) TAD-N and TAD-C, which function in transcription activation. There is also a nuclear localization signal (NLS) at the C-terminus to activate transcription. A downstream proline-serine-threonine (Pro/Ser/Thr) sequence is a specific structure that forms a heterodimer and binds to the target gene. There is also an oxygen-dependent degradation domain (ODDD) (Jiang et al. 1997), which is rich in Pro/Ser/Thr, and can assist the transport of the HIF-1α protein through the nuclear pore protein into the nucleus.

Due to the rapid renewal of tumor cells, the tumor blood vessel density increases early in tumor progression. This venous fluid pressure intensifies with the increase in tumor volume, compressing small blood vessels and causing ischemia or thrombosis. Therefore, although tumors have the ability to continuously induce angiogenesis and form a rich microvascular network, their oxygen consumption exceeds the amount of oxygen that microvessels can provide. This relative insufficiency in tumor microcirculation leads to tumor hypoxia and necrosis of the tumor center (Guo 2002).

HIF-1 plays an extremely important role in the information pathway of hypoxia-induced gene expression. It can regulate many genes to adapt to the hypoxic environment. At present, studies have found that there may be more than 1,000 target genes (Semenza 2014) transcribed by HIF. Therefore, HIF participates in a wide range of biological functions, including pathophysiological processes such as cell differentiation, migration, cell protection, apoptosis, cell cycle regulation, and mitochondrial function. Moreover, current studies have found that HIF-1α is closely related to multiple processes in the occurrence and development of NSCLC. This review summarizes the mechanism controlling HIF-1α expression and the role of this transcription factor in various aspects of the development of NSCLC, as well as provides new ideas for the treatment of NSCLC.

2. Oxygen regulation of HIF-1α transcriptional activity

The HIF-1α subunit is regulated by the intracellular oxygen concentration. HIF-1α is quickly degraded under normoxia, but not under low oxygen conditions. In addition, it forms a heterodimer with HIFβ (aryl hydrocarbon receptor nuclear translocator, ARNT) and binds to the target gene hypoxia response element (HRE) to initiate transcription (Hao and Li 2019). The HIF-1α pathway plays its role through the following mechanism (Wang and Hao 2013).

Oxygen-independent regulation: Hypoxia inhibits HIF-1α prolyl hydroxylation and prevents recognition of HIF-1α by the von Hippel-Lindau (VHL) protein. HIF-1α is protected from degradation and accumulates in the nucleus.

Oxygen-dependent regulation: At normal oxygen levels, HIF-1α is rapidly degraded by the proteasome. In the presence of O_2 and Fe^{2+}, two proline residues on the HIF-1α subunit bind to prolyl hydroxylase (PHD) and are hydroxylated (OH). The VHL-E3 (Ivan et al. 2001) ubiquitin ligase complex recognizes and forms a complex with the hydroxylated HIF-1α, resulting in the oxygen-dependent mode of degradation (Kamura et al. 1999).

3. Effect of HIF-1α on NSCLC cell proliferation and apoptosis

HIF-1α is mainly expressed in the nucleus and cytoplasm of NSCLC tissue and adjacent normal tissues. Gu (2010) found that 45% lung cancer tissue cells were positive for HIF-1α, while in adjacent normal tissues, the value was 6.67%. Yuan (2012) found that the positive expression of HIF-1α in NSCLC tissue is mainly localized in the nucleus of the cell. Wang et al. (2018) showed that HIF-1α expression is located in the cytoplasm. Of 107 specimens, 59 (55.14%) had HIF-1α expression in NSCLC tissues and four cases presented expression in adjacent tissues, with a positive rate of 3.73% (Table 1). In the meta-analysis by Yang et al. (2016), the authors concluded that the expression of HIF-1α in lung cancer tissues was significantly higher than that in normal lung tissues. They also found that HIF-1α expression in patients with squamous cell carcinoma was significantly higher than that in patients with adenocarcinoma.

Table 1: Expression of HIF-1α between NSCLC tissues and adjacent normal tissues (Wang 2018)

	Total	HIF-1α expression		χ^2	P
		+(%)	-(%)		
Cancer tissues	107	60(56.07)	47(43.93)	68.049	<0.0001
Adjacent normal tissues	107	4(3.73)	103(96.27)		

Abbreviations: HIF-1α, hypoxia-inducible actor 1α; NSCLC, non-small cell lung cancer. P<0.05.

It is known that HIF-1α participates in and regulates NSCLC cell proliferation and apoptosis under hypoxia. Survivin (Zhao 2019) is one of the inhibitors of apoptosis proteins (IAP), which has a dual role of inhibiting cell apoptosis and regulating cell division (Yang and Cao 2007). Chen et al. (2009) found that there is an HIF-1α binding site on the survivin promoter. The binding of HIF-1α to the survivin promoter upregulates the expression of survivin to promote tumor proliferation. Shen et al. (2011) showed that HIF-1α gene silencing leads to down-regulation of survivin expression, which coincided with Chen et al.'s findings.

Qian et al. (2016) knocked down HIF-1α from lung carcinoma NCI-H157 cells, which significantly increased the expression of caspases 3 and 9, but notably decreased B-cell lymphoma-2 (Bcl-2) expression. Caspases are considered the key enzymes involved in apoptosis, and B-cell lymphoma-2 (Bcl-2) is an anti-apoptotic protein (Reed 1997). The decrease in caspase protein is related to the occurrence of NSCLC (Li et al. 2020). The expression of Bcl-2 and HIF-1α in tumor tissue is closely related (Qun et al. 2015). Trisciuoglio's (Trisciuoglio et al. 2017) *in-vitro* experiments showed that Bcl-2 can stabilize and promote the expression of HIF-1α in pigmented tumor cells, and that it can inhibit (Andersen et al. 2011) the expression of HIF-1α in gastric cancer.

However, the effect of HIF-1α on NSCLC cells is still controversial. Proliferating cell nuclear antigen (PCNA) is a nuclear protein synthesized during the G/S phase of the cell cycle, which indicates cell proliferation. Fan et al. (2002) showed that HIF-1α is not associated with PCNA. Volm et al. (2000) studied the relationship between HIF-1α and CyclinA expression and the cell cycle in 91 cases of NSCLC and found that HIF-1α does not correlate with cell proliferation. However, Yuan (2019) used immunohistochemical staining to detect the cell markers Ki-67 and HIF-1α in 66 NSCLC tissue samples and found that HIF-1α and Ki-67 were positively correlated. These conflicting data may be related to differences in the cases selected and in methodology.

4. Effect of HIF-1α on the invasion and metastasis of NSCLC cells

Lymphatic tissues are widely distributed, and blood flow is abundant in lung cancer patients prone to lymph node metastasis (Li et al. 2017), which poses great challenges to clinical treatment (Guerrera et al. 2017) and prognosis. Qi et al. (2019) selected 92 patients with NSCLC who underwent lobectomy without lymph node metastasis and were followed up for 24 months. Among them, HIF-1α was highly expressed in the tumor in 27 and weakly expressed in 65 patients. The incidence of lymph node metastasis in patients was 92.6% and 26.9%, respectively. As a result, the lymph node metastasis rate of NSCLC patients with high HIF-1α expression was significantly higher than that of patients with low HIF-1α expression.

The steps of tumor metastasis can generally be divided into local invasion, intravasation, transfer to the blood circulatory system, extravasation, positioning, and proliferation in the target organ (Chambers et al. 2002). The mechanism (Liu et al. 2016) mainly involves the shedding of tumor cells into the extracellular matrix, which degrades the extracellular matrix via proteolytic enzymes, infiltrates adjacent tissues and the basement membrane, enters blood vessels or lymphatic vessels, and are then transported to other tissues and organs.

Before metastasis, the tumor in situ continues to proliferate and stimulates vascular endothelial cells to create a large number of capillaries in the tumor tissue, that is, angiogenesis. The cancer cells can escape the tumor *in situ* and enter the blood circulation system, that provides a convenient transportation system (Wyckoff et al. 2000). Hypoxia can increase the plasticity of tumor cells, which is conducive to the existence of the vascular endothelium (VE) (Xu 2017). Vascular endothelial growth factor (VEGF), a growth factor that specifically binds to blood vessels and promotes the growth of endothelial cells (Apte et al. 2019), is the major regulator of angiogenesis (Zhu et al. 2007). Chen et al. (2014) used a ThinPrep cytology test (TCT) and found that the relationship between HIF-1α and VEGF expression in NSCLC pleural effusion cells is positive. Roth et al. (1998) confirmed that hypoxia can regulate the expression of VEGF at both the transcriptional and post-transcriptional levels. HIF-1 initiates or increases the transcription of VEGF (Rho et al. 2009), thereby increasing its mRNA content.

Li et al. (2011) found that HPV-16 can promote the accumulation of HIF-1 in NSCLC A549 cells and enhance the expression of VEGF. When shRNA was used to silence HIF-1, the enhancement effect of HPV-16 on VEGF expression was eliminated. Therefore, it is believed that the upregulation of VEGF expression by HPV-16 in A549 cells is dependent on HIF-1, and the upregulation of VEGF by HIF-1 promotes *in vivo* angiogenesis of A549 cells. Delta-like ligand 4 (DLL4) is an important ligand in the Notch signal transduction pathway (Kang et al. 2013). It is upregulated in a variety of malignant tumors, including NSCLC (Soler et al. 2013), and plays a role in tumor angiogenesis (Zhang et al. 2013). DLL4 levels are elevated in hypoxic environments. Sun (2015) found that VEGF and DLL4/Notch signaling pathway molecules are overexpressed in lung cancer and are positively correlated with HIF-1α.

The extracellular matrix metalloproteinase inducer (recombinant protein, CD147), that is overexpressed in tumors, can induce the secretion of matrix metalloproteinases, degrade tumor extracellular matrix, enhance the penetration of tumor cells to the basement membrane and promote tumor invasion and metastasis (Brooks et al. 2010). Tan et al. (2016) used western blotting to detect the expression of CD147 and HIF-1α in NSCLC A549 and SPC-A-1 cells and normal lung epithelial BEAS-2B cells. The results showed that the positive expression rates of CD147 and HIF-1α in NSCLC tissues were 76% and 84%, respectively, which were higher than 10% and 30%, in normal lung tissues. There was a positive correlation between the expression of CD147 and HIF-1α in NSCLC ($P<0.05$). Matrix metalloproteinase-2 (MMP-2) can degrade type IV collagen, which is the main component of the extracellular matrix and basement membrane, and has important functions such as regulating cell migration, promoting tumor growth and angiogenesis, and benefiting tumor infiltration and metastasis (Yuki et al. 2008). Sun et al. (2015) found that the expression of HIF-1α and MMP-2 is positively correlated, indicating that the hypoxic environment caused by the rapid growth of tumor cells can induce the expression of HIF-1α, and that HIF-1α can regulate the expression of MMP-2 and enhance tumor invasion and transfer ability.

Some studies have shown that tumor invasion is related to lipopolysaccharides (LPS). For example, Liu et al. (2016) found that LPS can promote CXC motif chemokine receptor type 4 (CXCR4) expression, which leads to tumor invasion and migration. Western blotting was used to detect nuclear factor-κB (NF-κB) and confirmed that LPS can activate phosphorylated NF-κB (p-IκB) to increase CXCR4 expression. In addition, a study by Yang et al. (2017) revealed that HIF-1α is closely associated with LPS. They analyzed 187 human NSCLC specimens and concluded that, compared with normal lung tissues, the increase in HIF-1α is positively related with tumor invasion and staging. Moreover, to explore the relationship between HIF-1α and LPS, A549 cells were exposed to different concentrations of LPS, which increased the expression of HIF-1α (Table 2).

Table 2: Correlation of the expression of HIF-1α with clinicopathological features of the NSCLC cases (Yang et al. 2017)

		HIF-1α expression				
Characteristics	Total	-	+	++	+++	P-value
Total	187	21	73	67	26	0.5164
Gender						
Male	136	15	52	43	16	
Female	51	6	21	24	10	
Age (years)						0.6943
<57	92	13	34	34	11	
≥57	95	8	39	33	15	
Tumor size (cm)						0.0452[a]
≤3	86	9	46	24	7	
>3	101	12	27	43	19	
Histological type						0.3427
Squamous cell carcinoma	75	9	24	27	15	
Adenocarcinoma	112	12	49	40	11	
Lymph node metastasis						0.0049[b]
No	85	13	41	25	6	
Yes	102	8	32	42	20	
Differentiation status						0.0012
Well	43	12	23	6	2	
Moderate	81	6	37	29	9	
Poor	63	3	13	32	15	
TNM stage						0.0003[a]
I/II	146	19	66	50	11	
III/IV	41	2	7	17	15	

Abbreviations: HIF-1α, hypoxia-inducible actor 1α; NSCLC, non-small cell lung cancer; TNM, tumor-node-metastasis. [a]$P<0.05$.

5. Effect of HIF-1α on the stage of NSCLC cells

Accurate staging of lung cancer is critical to treatment selection, patient survival, and prognosis. Currently, the eighth edition of the TMN staging standard of the International Association for the Study of Lung Cancer is the most widely adopted staging system. Staging mainly relies on imaging examinations and does not consider patients with epidermal growth factor receptor (EGFR) gene mutations. Prognosis emphasizes the size of solid composition of the tumor rather than the changes in the ground glass area. However, with the continuous development of lung cancer staging, many genetic and molecular methods have emerged in recent years, which may make staging more accurate.

At present, a number of experimental studies have shown that HIF-1α is related to NSCLC staging. TMN staging is mainly based on the size of the lesion, lymph node metastasis, and local and distant metastases. Gu et al. (2010) found that tumor size is related to the expression of HIF-1α. The positive expression rate of HIF-1α in cases of primary tumors ≥5 cm in size was 69.23%, the positive expression rate of cases in primary tumors <5 cm in size was 26.47%, and the difference was statistically significant ($P<0.05$). Koshikawa et al. (2003) have found that the expression of HIF-1α in highly metastatic Lewis lung cancer cell lines is significantly increased, and that the expression of HIF-1α in poorly differentiated areas in tumor tissues was significantly increased compared to that in well-differentiated areas. This may be related to the expression of HIF-1α in tumor tissues that can activate cyclooxygenase, transferrin, VEGF, endothelin-1, and other genes related to vasodilation, angiogenesis, and tumor metastasis.

Fan et al. (2002) found that the expression level of HIF-1α in tumors is unrelated to the patient's age and sex, but is positively correlated with the clinical stage. The positive expression rate of HIF-1α in patients with metastasis was also higher than that in patients without metastasis. The mechanism may be that as the tumor continues to grow and increases in size, hypoxic necrosis occurs due to insufficient blood supply, which induces the expression of HIF-1α to promote the transcription of VEGF, one of its target genes. VEGF increases angiogenesis, tumor growth, invading surrounding tissues, and occurrence of distant metastasis; thus, the clinical stage increases and the survival rate decreases.

Gu's (2010) results showed that in 60 NSCLC tissue samples, the expression of HIF-1α was not affected by the patient's sex, age, or tumor location ($P>0.05$). It is related, however, to the TNM stage and pathological grade of NSCLC. The positive expression rate of HIF-1α in the stage III NSCLC group was 60.71%, which was significantly higher than the positive expression rate of 31.5% in the stage I + II group, and the difference was statistically significant ($P<0.05$) (Table 3).

Table 3: Relationship between the expression of HIF-1α in NSCLC and the characteristics of clinical cases (Gu 2010)

Group	Total	HIF-1α			
		Number of positive cases	%	c^2	P
Gender					
Male	41	18	43.90	0.063	0.802
Female	19	9	47.37		
Age (years)					
≥60	36	17	47.22	0.180	0.672
< 60	24	10	41.67		
TMN					
/	32	10	31.25	5.238	0.022
/	28	17	60.71		
Tumor size (cm)					
≥5	26	18	69.23	10.884	0.001
<5	34	9	26.47		
Lymphatic metastasis					
Yes	39	22	56.41	5.862	0.015
No	21	5	20.38		

Abbreviations: HIF-1α, hypoxia-inducible actor 1α; NSCLC, non-small cell lung cancer; TNM, tumor-node-metastasis. P<0.05.

Table 4: Relationship of VM with clinicopathological features (Lu et al. 2014)

Characters	Cases		VM		P
	n	%	(+)	(-)	
Gender					
Male	29	69.1	13	16	0.936
Female	13	30.9	6	7	
Age (years)					
≥60	23	54.8	12	11	0.320
<60	19	45.2	7	12	
Tumor history					
Squmous cell carcinoma	20	47.6	10	10	0.554
Adenocarcinoma	22	52.4	9	13	
T stage					
T1	3	7.1	1	2	0.589
T2	24	57.1	11	13	
T3	8	19.1	5	3	
T4	7	16.7	2	5	
N stage					
N0	21	50.0	13	8	0.030
N1-3	21	50.0	6	15	
M stage					
M0	26	61.9	15	11	0.039
M1	16	38.1	4	12	
Differentiation					
Well	1	2.4	1	0	0.233
Moderate	18	42.8	10	8	
Poor	23	54.8	8	15	

Abbreviations: VM, vasculogenic mimicry; T, tumor; N, node; M, metastasis; P<0.05.

Lu et al. (2014) separated 42 patients into two groups: 19 patients were in an early stage group (including stages I–II) and 23 patients were in a late stage group (including stages III–IV). The authors found that vasculogenic mimicry was closely related to tumor stage (Siddhesh et al. 2013). Vasculogenic mimicry was more frequent in stage I than stages III–IV. They also found that HIF-1α was positively expressed in 12 cases (63.2%) with vasculogenic mimicry, but in only seven cases (30.4%) without vasculogenic mimicry (Table 4).

Carbohydrate antigen 724 (CA724), carbohydrate antigen 153 (CA153), prostate-specific antigen (PSA), and human chorionic gonadotropin (hCG) play an important role in the early diagnosis and prognostic evaluation of tumors (Trisciuoglio et al. 2017; Peng et al. 2018; Wu et al. 2019). In terms of clinical staging, Liu et al. (2020) showed that the levels of CA724, CA153, PSA, and HCG in stage III and stage IV patients were significantly higher than those in stage I and stage II patients. The Spearman correlation analysis suggested that CA724 and PSA were significantly positively correlated with the clinical stage of lung cancer (P<0.05).

Coiled-coil-helix domain containing 2 (CHCHD2) is a newly discovered cancer-related protein that belongs to the CHCH domain protein family. Studies have shown that the overexpression of CHCHD2 in the fibroblast cell line NIH3T3 altered cell adhesion and rendered cells prone to migration (Seo et al. 2010), but the mechanism was not clarified. Zhang et al. (2018) used immunohistochemistry to detect the normal expression of CHCHD2 and HIF-1α proteins in NSCLC tissues. A total of 209 samples of NSCLC tissues and corresponding normal tissues were collected. Strong positive rates of CHCHD2 were found in stages I, II, and III NSCLC: 21.6% (11/51), 50.5% (46/91), and 88.1% (59/ 67). There were also positive rates of HIF-1α in stages I, II, and III NSCLC, respectively: 66.7% (34/51), 67.0% (61/91), and 85.1% (57/67). Immunofluorescence confirmed the co-localization of CHCHD2 and HIF-1 in NSCLC, indicating that these proteins may interact in NSCLC cells.

TH17 cells are a type of helper lymphocytes that can produce IL-17, which can regulate fibroblast VEGF, induce angiogenesis, and activate tumor cell activity. Zhao (2011) used flow cytometry and the enzyme-linked immunosorbent assay to detect the proportion of TH17 cells and CD4+CD25HiCD127LoTreg cells in the peripheral blood of 70 patients with primary lung cancer. The study evaluated patients with different TNM stages of lung cancer (seven cases of stage I–II, 30 cases of stage III, and 33 cases of stage IV) and the proportions of Th17 cells among peripheral blood CD4+T cells (2.90±c.35%, 3.27±1.21%, and 3.78±1.35%, respectively). However, the ratio of CD4+CD25HiCD127LoTreg cells to CD4+T cells in the peripheral blood of lung cancer patients with different TNM stages (6.24±2.16%, 7.58±1.94%, and 8.87±2.27%, respectively, according to staging) was statistically significantly different (P<0.05). This ratio in patients with stage IV lung cancer was significantly higher than that in patients with stages I–II and III (P<0.05). There was no statistically significant difference in peripheral blood Treg/Thl7 (2.73±1.65, 2.36±1.15, and 2.36±1.33, respectively) in lung cancer patients with different TNM stages (P>0.05). Liu et al. (2019) selected 40 patients with primary NSCLC and 40 healthy volunteers as controls. The study found that the higher the clinical stage, the higher the level of TH17 cells and IL-17 (P<0.05). In a research on rheumatoid arthritis, HIF-1 has been found to promote the proliferation of TH17 cells (Barbi et al.2013). The possible mechanism is that HIF-1α activates the transcription of RAR-related orphan receptor gamma (RORyt) and cooperates with RORyt and p300 to form a complex. The target of this complex is the gene promoter of IL17, which activates chemotaxis to regulate the expression of Th17 related genes (Du et al. 2020).

6. Effect of HIF-1α on the prognosis of NSCLC

Some researchers, according to multiple experimental data, have suggested that HIF-1α is positively correlated with NSCLC malignancy and prognosis. Andersen et al. (2011) used immunohistochemistry to stage the tumor and stromal tissue samples of 335

non-selected patients with stage I–IIIA NSCLC and found that the five-year survival rate of patients with high HIF-1 expression in tumor cells was significantly lower than that of patients with low HIF-1 expression. The five-year survival rate of patients with high expression of HIF-1 in the matrix was significantly higher than that of patients with low expression of HIF-1. At the same time, it was found that the high expression of HIF-1 in NSCLC squamous cell carcinoma cells and the low expression in the stroma were significantly related to poor prognosis. Yang et al. (2016) implemented a meta-analysis to further understand the prognostic role of HIF-1α in lung cancer. Four of the studies (Andersen et al. 2011; Swinson et al. 2004; Hung et al. 2009; Enatsu et al. 2006) showed that NSCLC and HIF-1α were significantly associated, while another three studies found no association (Yuji et al. 2004; Sanghui et al. 2011; Qian et al. 2011). Subgroup analysis showed that ethnicity may be the reason for the high heterogeneity found. Finally, it was concluded that the overall survival rate of patients with a positive expression of HIF-1α in NSCLC tissues is lower than that of patients with a negative expression of HIF-1α. This suggests that HIF-1α can be used as a prognostic biomarker and a potential therapeutic target in lung cancer.

The above-mentioned research directly derives the relationship between HIF-1 and NSCLC, but many researchers have found that HIF-1α can exert its effect indirectly through a series of genes and molecules in the body. Molecular characterizations such as DNA methylation (Wei et al. 2018) have been increasingly used to predict tumor prognosis (Sipeng et al. 2018). The expression of HIF prolyl 4-hydroxylases (EGLNs) is related to the prognosis of many cancers (Couvelard et al. 2008; Peurala et al. 2012; Xie et al. 2012). The proteins encoded by EGLN1, EGLN2, and EGLN3 can hydroxylate two proline residues (Pro-402 and Pro-564) of HIF-1α, and are HIF-1α degrading enzymes. Zhang et al. (2019) assessed the relationship between methylation and overall survival by analyzing EGLN DNA methylation data from tumor tissue samples of 1,230 patients with early stage NSCLC and gene expression data from the Cancer Genome Atlas. Studies have found that the expression of HIF-1α and EGLN3 in tumor tissues is negatively correlated. Among the 34 cytosine-guanine dinucleotide-containing (CpG) probes in EGLN, DNA methylation of cg25923056EGLN2 was determined to be significantly correlated with lung adenocarcinoma LUAD survival rate (HR=1.02, 95% CI: 1.01–1.03, P=9.90×10–5) and related to the expression of EGLN2 (r=–0.36, P=1.52×10-11). Chang et al. (2016) designed an experiment that included 112 pulmonary pleomorphic carcinomas (PPC) (Wang et al. 2013), a poorly differentiated NSCLC type, to study the relationship between overexpression of programmed death 1 (PD-L1) and HIF-1α and tumor necrosis. The results showed that PD-L1 membrane reactivity was found in 86 of the 122 patients (70.5%), and HIF-1α overexpression was detected in 92 of 122 PPCs (75.4%). These data have once again proven that the expression of PD-L1 is related to HIF-1α. However, they also revealed that the expression of PD-L1 may enhance immune evasion by the tumor and lead to poor clinical outcomes. Guo et al. (2019) found that a variety of human tumors can overexpress PD-L1, which is considered a poor prognostic indicator of overall survival and a predictive marker of good response to new immunotherapy drugs. HIF-1α, as a transcription factor, binds directly to the hypoxia response element in the PD-L1 promoter to increase the expression of PD-L1 in hypoxic cancer cells.

7. Effect of HIF-1α therapy on NSCLC

Oncology studies have shown that HIF-1α can regulate more than 100 downstream target genes (Schumacker 2006; Semenza 2003), such as survivin, VEGF, and insulin-like growth factor-2 (IGF-2), thereby affecting tumor proliferation, local invasion, and distant metastasis. Due to its strong influence on tumor development, including tumor angiogenesis mimicry, tumor proliferation, and tumor staging, HIF-1 is an important cancer drug target (N.Masoud and Li 2015). Treatment plans are adopted according to the patient's physical condition, cancer clinical stage, and pathological classification. Patients in stages I and II are mainly treated with radical surgical resection. However, since most patients are in the middle and advanced stages of lung cancer, chemotherapy, targeted therapy, and radiotherapy are particularly important.

Chemotherapy for lung cancer currently has problems such as poor targeting and tumor metastasis. Wang et al. (2019) found that co-delivery of liposomes for codelivery of daunorubicin and dioscin enhanced cellular uptake and had high cytotoxicity to tumor cells. After treatment, MMP-2, vascular endothelial cadherin (VE-Cad), transforming growth factor-β1 (TGF-β1), and HIF-1α decreased significantly. Since HIF-1α can induce vasculogenic mimicry under hypoxic conditions, regulate the expression of MMP-2 (Yuki et al. 2008), and enhance tumor invasion, it is likely that the targeted liposomes achieved therapeutic effects by inhibiting the expression of tumor-related proteins. HIF-1α plays a pivotal role in this process.

Some research shows that the radiation tolerance of HIF-1α-overexpressing cells is significantly increased, which produces resistance to radiotherapy. Wang (2017) observed changes in the radiosensitivity of NSCLC cells that highly express HIF-1α. The results showed that the radioresistance of H1299/M-HIF-1α cells was significantly enhanced, and, under hypoxia, the radioresistance of H1299/W-HIF-1α cells was also significantly increased. Studies have found that low- and medium-dose X-ray irradiation can induce the stabilization and accumulation of HIF-1α in normoxic NSCLC cells. The mechanism (Zhang et al. 2017; Bell et al. 2007) may be that radiation induces a large increase in reactive oxygen species (ROS). These, through the electron transfer pathway of the cytochrome respiratory chain, oxidize the iron in PHD, thereby inactivating PHD, blocking the proline hydroxylation of HIF-1α, and stabilizing it. Radiation resistance is the main reason for the failure of radiotherapy in NSCLC, and hypoxia is the main reason for radiation resistance (Moeller et al. 2007; Dewhirst et al. 2008). A number of studies have shown that the Notch pathway is abnormally activated in the occurrence of cancer, and that Notch3 is upregulated by radiation in NSCLC cell lines. Yasuyuki et al. (2017) used protein immunoassays to detect the expression of HIF-1 and Notch in the NSCLC cell line H460 and in the human lung cancer cell line HCC2429 after radiotherapy. The results showed that when the expression of HIF-1α was inhibited by small interfering RNA, the activation of Notch3 induced by radiation was also downregulated, suggesting that the Notch pathway was activated by HIF-1α after radiation. The mechanism may be that after HIF-1α is activated, the expression of the Notch pathway ligand, DLL4, increases, thereby generating effective Notch signals (Minhong and Greg 2007). In addition, there is another mechanism whereby VEGF or the epithelial-mesenchymal transition (EMT) can participate in the interaction between Notch and HIF, thereby regulating radiation-induced activation of the Notch pathway (Thurston et al. 2007). Therefore, a combination of radiation and HIF-1α inhibitors should be considered for NSCLC patients.

With the identification of lung cancer-related oncogenic driver genes, many studies and clinical practice have shown that targeted therapy greatly improves and prolongs the prognosis and survival of NSCLC patients with corresponding driver genes. In terms of targeted therapy for NSCLC, current drugs mainly target mutations in NSCLC. According to the genetic molecular typing found to date, the following mutation types mainly exist (Guiding Principles for the Clinical Application of New Anti-tumor Drugs 2020): EGFR gene mutation, anaplastic lymphoma kinase (ALK) gene rearrangement, ROS proto-oncogene 1 (ROS1) gene rearrangement, v-raf murine sarcoma filtering toxin carcinogenic homolog B1 (BRAF) point mutations, Kirsten rat sarcoma 2 viral oncogene homolog (KRAS) point mutations, and (MET-exon-14-skipping) METex14 mutations. Clinical drugs for these gene mutations include EGFR inhibitors such as gefitinib, dacomitinib, and osimertinib; ALK/ROS1/MET inhibitors such as crizotinib; and the BRAF mutation inhibitor dabrafenib. In addition, molecular monoclonal antibody drugs are being used, such as the humanized antibody bevacizumab, which inhibits tumor angiogenesis by binding to human VEGF and PD-1/PD-L1 inhibitors, which relieve

Table 5: Relationship between HIF-1α and various of molecules

NSCLC	Related molecules	Effect	The relationship with HIF-1α
Proliferation	PCNA	Cell proliferation	HIF-1α has nothing to do with PCNA
	CyclinA	Cell proliferation	HIF-1α has nothing to do with CyclinA
	Ki-67	Cell proliferation	HIF-1α and Ki-67 are positively correlated
Apoptosis	survivin	Inhibit cell apoptosis regulate cell division	HIF-1α binds to related binding sites on survivin to up-regulate survivin's expression
	Bcl-2	Inhibit cell apoptosis	Pigmented tumor cells:Bcl-2 can stabilize and promote the expression of HIF-1α. Gastric cancer: It can inhibit the expression of HIF-1α by down-regulating miRNA-27a.
	caspase	Key apoptosis enzymes	HIF-1α increases the expression of caspases 3 and caspases 9
Invasion and Metastasis	VEGF	Angiogenesis	HIF-1 initiates or strengthens the transcription of VEGF
	DLL4/Notch	Angiogenesis	DLL4/Notch signaling pathway molecules' overexpression is positively correlated with HIF-1α.
	CD147	Induce the secretion of matrix metalloproteinases, promote tumor invasion and metastasis	There is a positive correlation between the expression of CD147 and HIF-1α in NSCLC
	MMP-2	Degrade type IV collagen	HIF-1α can regulate the expression of MMP-2
	LPS	Tumor invasion and migration	LPS can increase the expression of HIF-1α.
Stage	CHCHD2	Promote migration	Co-localization of HIF-1α and CHCHD2 in NSCLC
	TH17	Produce IL-17 factor	Not confirmed
	IL-17	Adjust the vascular endothelial growth factor of fibroblasts	Not confirmed
Prognosis	EGLNs	HIF-1α degrading enzymes	The expression of HIF-1α and EGLN3 is negatively correlated
	PD-L1	Enhance immune evasion	The expression of PD-L1 is related with HIF-1α.

Abbreviations: HIF-1α, hyoxia-inducible factor-1α; NSCLC, non-small cell lung cancer; PCNA, proliferating cell nuclear antigen; Bcl-2, B cell lymphoma-2; VEGF, vascular endothelial growth factor; DLL4, Delta-like ligand 4; CD147, recombinant protein; MMP-2, matrix metalloproteinase-2; LPS, lipopolysaccharides; CHCHD2, coiled-coil-helix domain containing 2; EGLNs, HIF prolyl 4-hydroxylases; PD-L1, programmed death 1.

cancer cells from autoimmunity. The suppression of the system allows the patient's own immune system to kill the cancer cells.

Current targeted therapy requires patients to be tested after the target. Targeted drugs have limited coverage and are not suitable for all patients, and the existence of drug resistance may occur due to physiological compensation. As mentioned before, oncology studies have shown that HIF-1α can regulate more than 100 downstream target genes (Schumacker 2006; Semenza 2003). If HIF-1α is used as the target, all the downstream growth factors are inhibited as well; therefore, in theory, the application range of HIF-1α inhibitors is wider and the effect is greater. A large number of existing drugs are small molecule compounds that inhibit the function of HIF, and there is no specific HIF-1 inhibitor. At present, some anti-tumor drugs targeting HIF-1 are under research and development. The main small molecule inhibitors include topotecan, 103D5R, redox inhibitors, microtubule cytoskeleton inhibitors, heat shock protein 90 inhibitors, a PI3K-Akt-mTOR signal pathway inhibitor, HIF-1α transcriptional activity regulator, etc (Wei and You 2008). These small-molecule inhibitors can inhibit the expression of HIF-1 in tumor cells in vivo and in vitro, but clinical trial research is still in its infancy. Mabjeesh et al. (2003) found that the antitumor drug 2-methoxyestradiol (2ME2) not only inhibits the expression of VEGF induced by HIF-1 but also prevents the formation of tumor cytoskeleton microtubules. The drug 3-(5'-Hydroxymethyl-2'-furyl)-1-benzylindazole (YC-1) can also inhibit the activity of HIF-1, thereby inhibiting tumor growth (Isaacs et al. 2006). Geldanamycin, an inhibitor of heat shock protein 90 (HSP90), reduces the conformational stability of HIF-1α by competitively inhibiting the binding of HSP90 to HIF-1α (Majumder et al. 2004). The clinically used rapamycin and its derivatives, cell cycle inhibitor-779 (CCI-779) and Rad001 (everolimus), are specific inhibitors of mTOR and can reduce the expression of HIF-1α in cells (Hudson et al. 2002).

This review describes the mechanism of action of HIF-1α and its relationship with NSCLC tumor cell proliferation and apoptosis, infiltration and metastasis, staging, prognosis, and treatment. It is supported by clinical trials and deeply explored its specifics, mechanism, and Sorted out the relationship between various substances appearing in the article and HIF-1α. (**Tab V**)

8. Conclusion

This review describes the relationship between HIF-1α and NSCLC in terms of cell proliferation and apoptosis, infiltration and metastasis, staging, prognosis, and treatment. We showed that the expression of HIF-1α is closely related to that of many factors that play important roles in the development of NSCLC, confirming its clinical significance. In the treatment of NSCLC, current oral targeted drugs and immunotherapy are mainly applicable to NSCLC with distant metastasis, while the treatment of locally advanced NSCLC is still in a long-term stagnation state. Patients with EGFR mutations include up to 50% of the Asian population. ALK rearrangement patients account for 5% of NSCLC patients. At present, it is believed that local tissue hypoxia occurs in tumor progression. High expression of HIF-1α induced by hypoxia in tumor tissue has been confirmed in many types of tumors. Therefore, HIF-1α is an important treatment target for inhibiting tumor growth.

Currently, the diagnosis of lung cancer mainly relies on histological examination. Despite its high specificity, this is an invasive test and is prone to many complications. However, the detection of tumor markers is convenient and non-invasive, but it has low specificity. Many studies have found that the positive rate and accuracy are better for combined tests than for single tests. In the future, we should consider using common indicators for combined testing and diagnosis in clinical practice, such as detecting carcinoembryonic

antigen (CEA), neuron-specific enolase (NSE), and HIF-1α. It is hoped that more studies will be conducted in the future to improve the specificity and sensitivity of non-invasive testing and improve the gold standard for diagnosis.

The occurrence of NSCLC is usually caused by many mechanisms. This article describes the interaction between HIF-1α and a variety of transcription factors, genes, and molecules (summarized in Table 5). We hope that researchers will unify this complex system in the future. Small cell-targeted therapy is a new idea of approach to treatment. At present, targeted research is very popular, and many studies have proposed new therapeutic targets, but their conclusions have not been repeatedly verified, and the data analysis is not good. We also hope that research methods can be standardized to ease further understanding of HIF-1α and its role in NSCLC (Kaelin 2017).

Funding: This work was supported by the National Natural Science Foundation of China (No. 81774230), and Natural Science Foundation of Zhejiang Province (No. LY21H270012).

Availability of data and materials: All data analyzed during this study are included in this article.

Authors' contributions: YNL and YYZ designed the study. YNL and YSZ performed the literature search. YNL and XRL wrote the manuscript. LH, WYC, YFH and YYZ revised the manuscript. YYZ provided the funding. All authors read and approved the final manuscript.

Competing interests: There is no conflict of interest in this article, and all authors confirm its accuracy.

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