Role of hypoxia-inducible factor-2α in lung cancer (Review)

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Abstract. Hypoxia is a common phenomenon during tumorigenesis and tumour development. In recent years, studies have found that hypoxia-inducible factor (HIF)-2α, also referred to as endothelial PAS domain protein-1, plays an important role in tumours. HIF-2α is an important oncogene and a critical prognostic indicator in non-small cell lung cancer. However, no unified conclusion can be drawn concerning HIF-2α and small cell lung cancer, since few studies to date have focused on their association. An increasing number of studies have confirmed that HIF-2α is involved in tumorigenesis, cell proliferation, angiogenesis, metastasis, drug resistance and radiotherapy failure in lung cancer. Of note, HIF-2α plays a crucial role in lung cancer to maintain cancer cell stemness. Based on the importance of HIF-2α in lung cancer, HIF-2α-targeted therapy has been attracting increasing attention. Although this strategy currently appears to be promising *in vitro*, it has never been assessed as a therapy for lung cancer. The aim of the present review was to summarize the contribution of HIF-2α to various aspects of lung cancer, as well as its potential as targeted therapy.

Contents

1. Introduction

Hypoxia-inducible factor (HIF)-2α is a member of the hypoxia-inducible factor family. HIF-2α, also referred to as endothelial PAS domain protein-1 (EPAS1) (Fig. 1), was first identified in endothelial cells as a transcription factor stimulated under hypoxic conditions (1-3). HIF-2α shares 48% amino acid sequence identity with HIF-1α and enhances the expression of erythropoietin, vascular endothelial growth factor (VEGF) and various glycolytic enzymes *in vivo* (2). Despite this homology, HIF-α expression differs under different oxygen concentrations. For example, under normoxic and slightly hypoxic conditions, cells were shown to express HIF-2α more obviously than HIF-1α (3). An increasing number of studies have attempted to fully explore the role of HIF-α. HIF-2α expression has been identified in a number of non-vascular endothelial sites, such as the liver, kidney and sympathetic nervous system (4). Based on these findings, HIF-2α expression was indicated to be strongly associated with systems that were sensitive to hypoxia, including the vasculature and the nervous system. Moreover, tumours exhibiting extensive angiogenesis and high sensitivity to hypoxia have been demonstrated to be rich in HIF-2α. Nuclear expression of HIF-2α has been reported in various solid tumours of the bladder, brain, breast, colon, ovary, pancreas, prostate and kidney (5). HIF-2α was even found to be strongly expressed in subsets of tumour-associated macrophages, despite its lack of expression in the tumour (5). Although HIF-2α has been shown to serve as an oncogene in most tumours, some studies described opposite results, with inhibited tumour growth accompanied by overexpression of HIF-2α (6). In addition, HIF-2α was confirmed to be important in other types of diseases, such as preeclampsia (7), hypoglycaemia (8), non-alcoholic fatty liver (9) and osteoarthritis (10).

Given the significance of HIF-2α in cancer, targeted therapy recently emerged (11). Currently, the most mature applications involve HIF-2α/VEGF axis-targeted treatments in renal cell carcinoma. HIF-2α inhibitors, such as PT2385 and PT2399, were assessed in phase III clinical trials and were found to be effective for clear-cell renal cell carcinoma treatment (12,13). HIF-2α plays an important role not only in tumours, but also in other diseases. The downregulation of HIF-2α by small interfering RNA has been shown to inhibit the hypoxia-induced increase in soluble Fms-like tyrosine kinase-1 in trophoblast cells (14), which may be a new treatment strategy for preeclampsia. Furthermore, knockdown of HIF-2α in fatty liver was confirmed to improve triglyceride accumulation and steatosis (9).

The aim of the present review was to summarise our current knowledge of the role of HIF-2α in lung cancer and provide information on its initial discovery, structure, relationship with

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different pathophysiological processes and, finally, its potential as a targeted therapy strategy in lung cancer.

2. HIF-2α as an oncogene

HIF-2α has been explored and confirmed to act as an oncogene in various tumours from almost all human organ systems, including renal carcinoma (15), pancreatic cancer (16), hepatocellular carcinoma (17), colorectal adenocarcinoma (18), lung cancer (19), neuroblastoma (20), osteosarcoma (21), breast cancer (22), bladder cancer (23) and oral squamous cell carcinoma (24). However, some studies have suggested that HIF-2α is not associated with tumours. Its expression was strongly inhibited in small cell lung cancer cells (25), and no significant effects in functional tests *in vitro* were detected in hepatocellular carcinoma cells with efficient downregulation of HIF-2α (26). In some clinical studies, HIF-2α was identified as a tumour suppressor gene. For example, in large neuroblastoma patient datasets, high expression levels of HIF-2α were significantly associated with the expression of neuronal differentiation genes and a more favourable prognosis (27), whereas xenograft studies revealed that HIF-2α deficiency stimulated tumour growth and was correlated with advanced tumour stage in colon cancer (28). Therefore, the findings of different studies on the role of HIF-2α have been contradictory.

HIF-2α plays several roles in tumour-related processes, including tumorigenesis and proliferation, angiogenesis, metastasis, resistance to chemotherapy and radiotherapy, cancer cell stemness and targeted therapy (Fig. 2).

First, high expression levels of HIF-2α have been found to be closely associated with tumorigenesis and tumour cell proliferation. It was demonstrated that the clonogenic survival rate of head and neck squamous cancer cells decreased by ~30% following HIF-2α silencing (29). Furthermore, when PT2399 inhibited the expression of HIF-2α in clear-cell renal cell carcinoma, 10 of the 18 examined cell lines exhibited suppressed tumorigenic ability (11). Hypoxia-associated factor (HAF) overexpression enhanced the expression of HIF-2α to increase the proliferation and metastasis of clear-cell renal cell cancer cells, which also confirmed its positive association with tumorigenesis (30). The possible underlying mechanisms are variable and may include lactate dehydrogenase A (16), acetate-dependent acetyl-CoA synthetase 2 (31), nuclear enriched abundant transcript 1 (NEAT1) which is a nuclear long non-coding (lnc) RNA (32) CREB-binding protein (33) and sirtuin 1 (33).

Second, since it was first identified in blood vessel walls and vascular smooth muscle cells (4). HIF-2α has been shown to participate in cancer angiogenesis based on an increasing number of clinical studies and experiments. Two examples are provided below. Microvessel density (MVD) was found to be significantly higher in tumours exhibiting high HIF-2α expression compared with that in tumours with low HIF-2α expression among surgical specimens from 140 patients with non-small cell lung cancer (34). RAB11B-AS1, an lncRNA in breast cancer cells, was induced by HIF-2α and could promote tumour angiogenesis and distant metastasis without affecting primary tumour growth in mice (35). These effects of HIF-2α are most likely mediated via the HIF-2α/VEGF axis; for example, in mesothelioma cells, VEGF secretion was enhanced via upregulation of HIF-2α (36).

Third, metastasis is an important process in tumours, and a number of studies have reported that HIF-2α promotes cancer metastasis. In patients with clear-cell renal cell carcinoma who underwent nephrectomy, increased levels of HIF-2α were strongly associated with local invasion and distant metastasis (37). Similar findings were observed in pancreatic cancer cells (16), fibrosarcoma cells and animal models (31), non-small cell lung cancer cell lines (38), breast carcinomas (39), hepatocellular carcinoma (40) and bladder cancer (23), among others.

Fourth, resistance to chemotherapy and radiotherapy is responsible for tumour recurrence and treatment failure. In targeted therapy and chemotherapy, HIF-2α is positively correlated with treatment failure (41,42), but there remains controversy regarding the role of HIF-2α in radiotherapy resistance. In breast cancer, the protein and mRNA expression of HIF-2α was increased in a panel of antioestrogen-resistant breast cells (41). EGFR is known to contribute to antioestrogen resistance, and HIF-2α was shown to promote hypoxic induction of EGFR in breast cancer cells (41). Knockdown of HIF-2α was found to be positively associated with the increased treatment efficacy of doxorubicin in hepatocellular carcinoma (17). There have been relatively few studies on the association of radiotherapy and HIF-2α in cancer. Through investigating HIF-2α isoform-deficient non-small cell lung cancer cells, researchers demonstrated the strong radiosensitising effect of HIF-1α, but not of HIF-2α (43). In a continuous hyperfractionated accelerated radiotherapy randomized trial, HIF-2α was associated with radiotherapy failure in patients with head and neck cancer (44). However, further research on HIF-2α and tumour radiotherapy is needed.

Finally, the cancer stem cell (CSC) theory has been widely recognized, but there have been few studies on HIF-2α expression in CSCs. Research has shown that HIF-2α maintains self-renewal in leukaemia CSCs via Nanog and Sox2 (45). The overexpression of lncRNA HIF2PUT suppressed the sphere-forming ability of osteosarcoma stem cells by downregulating HIF-2α (21). According to these findings, HIF-2α appears to promote the proliferation of CSCs.

Given the important role of HIF-2α in tumours, HIF-2α-targeted therapies have emerged. The most representative HIF-2α-targeted therapies are PT2385 and PT2399 in renal cell carcinoma (12). PT2385 prevents the dimerization of HIF-2α and ARNT/HIF-1β, while PT2399 directly binds to the HIF-2α PAS B domain (11,13). PT2385 was utilized not only in renal cell carcinoma, but also in liver cancer. The administration of PT2385 restored the YTHDF2-programmed epigenetic machinery and inhibited the progression and metastasis of liver cancer (46). siRNA (42,47) and shRNA (48) are useful methods for downregulating HIF-2α in cell or animal studies. lncRNAs (32) and microRNAs (miRNAs/miRs) (49), as upstream sequences, could similarly regulate the level of HIF-2α. Finally, HIF-2α phosphorylation modification is also a potential method for regulating the expression of HIF-2α in basic research (50).

3. HIF-2α in lung cancer

In recent years, an increasing number of studies have shown that HIF-2α plays an important role in lung cancer (51-53). Current studies have confirmed the prognostic role of HIF-2α in patients with lung cancer; a high level of HIF-2α was

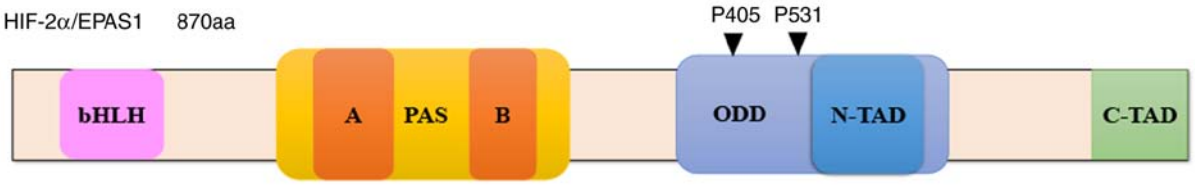

Figure 1. Components of HIF-2α. HIF-2α, also named EPAS1, contains 870 amino acids with bHLH, PAS, ODD domain, N-TAD and CADs. Prolyl hydroxylases drive hydroxylation of proline 405 and 531 of HIF-2α under normal oxygen supply conditions. HIF, hypoxia-inducible factor; EPAS1, endothelial per-Arnt-SIM (PAS) domain protein-1; bHLH, basic helix-loop-helix; ODD, oxygen-dependent degradation; N-TAD, N-terminal transactivation domain; CADs, C-terminal transactivation domains.

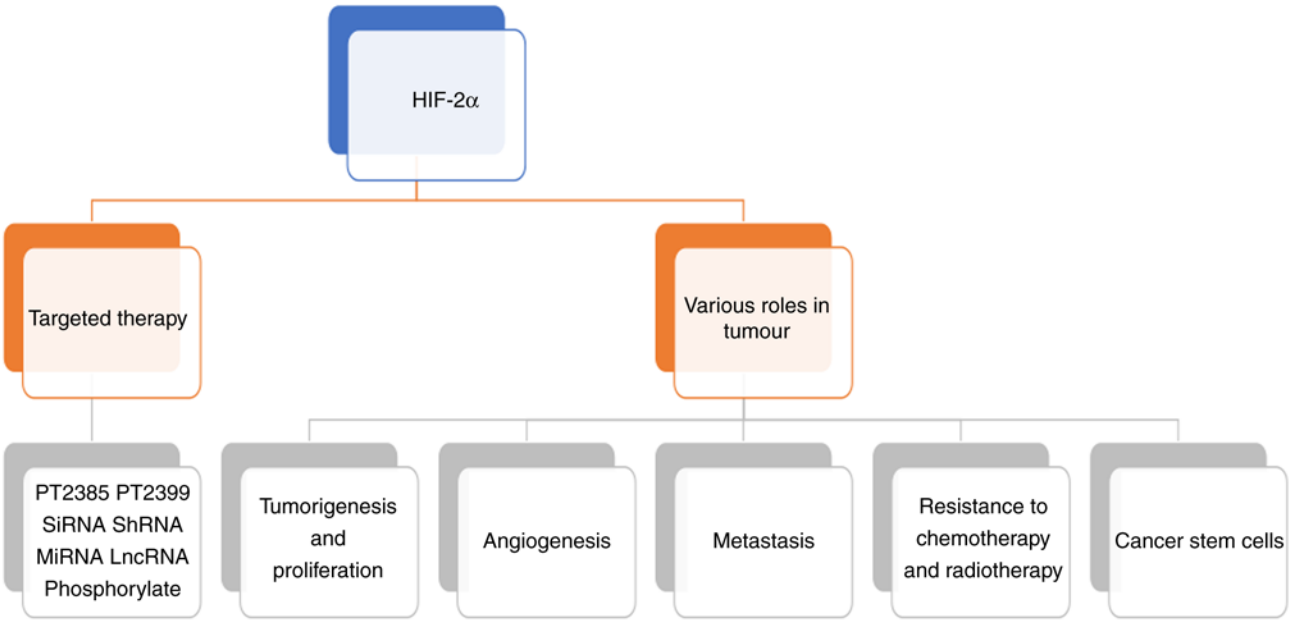

Figure 2. Various roles of HIF-2α in tumours. HIF-2α modulates key aspects of tumours, including tumorigenesis and proliferation, angiogenesis, metastasis, resistance to chemotherapy and radiotherapy, cancer cell stemness and targeted therapy. HIF, hypoxia-inducible factor.

strongly associated with poor prognosis (54). The Lung Cancer Consortium identified a SNP (rs12614710) in HIF-2α/EPAS1 that was associated with non-small cell lung cancer and achieved genome-wide significance (52). Of note, the findings of clinical studies on small cell lung cancer were contrary to those of basic studies. The high expression level of HIF-2α was found to be an independent poor prognostic index in patients with small cell lung cancer (53), but the expression of HIF-2α was inhibited in small cell lung cancer cells (25). HIF-2α regulated genes controlling important aspects of tumours, including tumorigenesis and proliferation, angiogenesis, metastasis, resistance to chemotherapy and radiotherapy and cancer cell stemness (Fig. 3).

Tumorigenesis and proliferation. The association between HIF-2α and tumorigenesis and proliferation in lung cancer has been investigated by several studies. The expression of HIF-2α was shown to promote the occurrence and proliferation of lung cancer, and the mechanisms were complex and variable. A clinical study on the association between HIF-2α gene polymorphisms and tumour susceptibility in lung adenocarcinoma was conducted in Japanese non-smoking females, and suggested that the frequency of lung cancer in female Japanese non-smokers with a minor G allele (A/G or G/G genotype) at rs4953354 was markedly higher compared with that of healthy controls (55). The genetic polymorphism of HIF-2α affected the susceptibility of non-smokers to lung adenocarcinoma, suggesting its importance in tumorigenesis (55). The mutation probability of EGFR in this type of population is significantly higher compared with that of the general population, which suggests its potential therapeutic significance (56). According to a tissue microarray study of 140 patients with stage I-III non-small cell lung cancer, immunohistological expression of HIF-2α was positively associated with histology, tumour size and stage, which further proved this hypothesis (57). It has been demonstrated that HIF-2α is involved in the malignant transformation of normal cells. Studies of arsenic-induced malignant transformation of bronchial epithelial cells indicated that HIF-2α is involved in the inflammatory response (58) and may block the ATM/CHK-2 pathway activated by arsenic (59). In addition, the suppression of HIF-2α prevented the effects of arsenic on benzopyrene-induced malignant transformation, cell migration and chromosomal aberrations (59). Downregulation or overexpression of HIF-2α in cell experiments or animal models provided more direct evidence. Knockdown of HIF-2α by shRNA enhanced the colonization of A549 cells (60), and tumour proliferation was inhibited in Lewis lung carcinoma mouse models through intravenous injection of HIF-2α

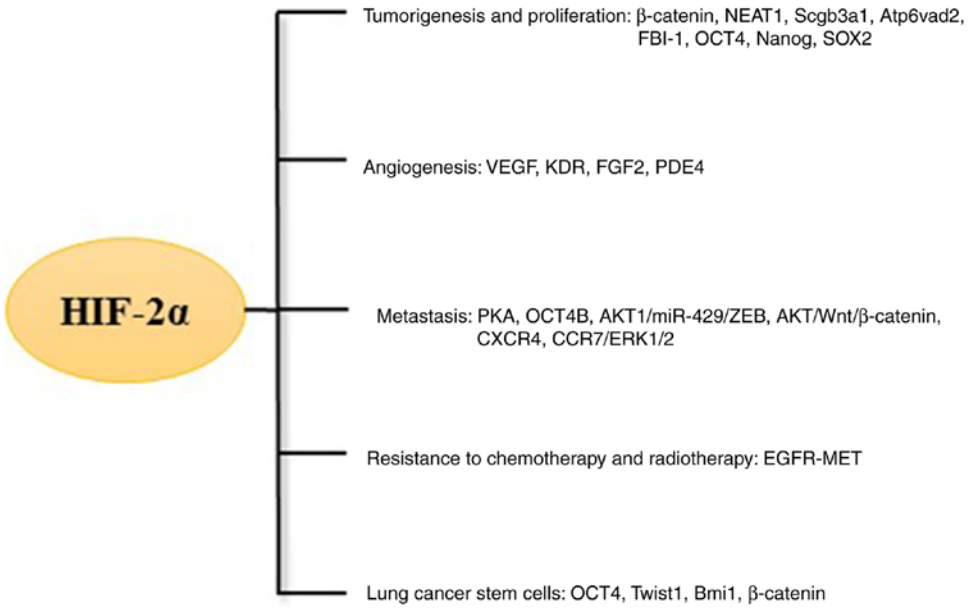

Figure 3. Mechanisms of action of HIF-2α in lung cancer. HIF-2α targets genes that regulate important aspects of tumours, including tumorigenesis and proliferation, angiogenesis, metastasis, resistance to chemotherapy and radiotherapy and cancer cell stemness. HIF, hypoxia-inducible factor.

siRNA (61). The lncRNA LINC01436 in non-small cell lung cancer cells, which acted as a miR-30a-3p sponge, upregulated the expression of HIF-2α/EPAS1 to promote cell proliferation *in vitro* (62). In addition, under hypoxic conditions, HIF-2α was required in anaplastic lymphoma kinase (ALK) regulation of the proliferation of ALK-rearranged non-small cell lung cancer cells (63).

According to previous studies, the mechanisms responsible for the HIF-2α involvement in the tumorigenesis and proliferation of lung cancer cells were summarized. The most important target protein was β-catenin (19,64). When pcDNA3.1-HIF-2α was transfected into A549 lung cancer cells under hypoxic conditions, the overexpression of HIF-2α enhanced the expression of NEAT1. A preliminary study revealed that NEAT1 was overexpressed in non-small cell lung cancer tissues and cell lines. Finally, tumour progression was detected after upregulation of NEAT1 via the miR-101-3p/SOX9/Wnt/β-catenin axis (19). Another study intuitively reflected the association between HIF-2α and β-catenin. Overexpression of HIF-2α was proven to increase the level of β-catenin to induce morphological changes in lung cancer cells, while knockdown of HIF-2α similarly inhibited β-catenin to reduce colony formation under conditions of prolonged hypoxia (64). Of note, β-catenin was not the only target gene of HIF-2α. When HIF-2α was abolished in established KRAS (G12D)-driven murine non-small cell lung cancer mouse models, the tumour burden was increased, which was linked to the depletion of Scgb3a1 (65). The consumption of Scgb3a1 appeared to be affected by HIF-2α, suggesting that it may be involved in regulating proliferation. HIF-2α and the macrophage-specific vacuolar ATPase subunit Atp6v0d2 were negatively and positively associated with survival, respectively, in a lung adenocarcinoma study; inhibiting the transcriptional activity of HIF-2α decreased the tumour progression susceptibility of Atp6v0d2$^{-/-}$ mice (51). Therefore, Atp6v0d2 may play the same role as Scgb3a1. Factor binding IST-1 (FBI-1) is an important oncogene in various tumours. Overexpression of HIF-2α/EPAS1 increased FBI-1 expression to enhance the survival and proliferation of human lung adenocarcinoma cells, which suggested that FBI-1 may also be a target gene of HIF-2α (66). Our team found that HIF-2α could regulate octamer-binding transcription factor 4 (OCT4), Nanog and Sox2 in human lung adenocarcinoma A549 stem cell spheres via the Wnt/β-catenin signalling pathway (unpublished data). This finding requires further in-depth studies.

Consistent conclusions have been drawn regarding the role of HIF-2α in tumorigenesis and cell proliferation in lung cancer. The specific mechanism is complex, and HIF-2α may be affected by a number of other factors. For example, patients with lung cancer who can be surgically treated are generally evaluated with positron emission tomography-computed tomography. An interesting study suggested that HIF-2α expression was significantly associated with cellular uptake of fluorodeoxyglucose, and both factors were positively linked to postoperative recurrence in patients with lung adenocarcinoma (67). This result indicated that HIF-2α may be associated not only with hypoxia-related diseases, but also with certain detection methods or treatments.

Angiogenesis. Angiogenesis plays an important role in lung cancer, while HIF-2α plays a key role in this process. HIF-2α may promote the secretion of VEGF in tumours to enhance angiogenesis, but in a hypoxic microenvironment, the association between MVD and the level of HIF-2α needs more research. HIF-2α expression was positively associated with MVD and poor outcome according to a tissue microarray study of 140 patients with non-small cell lung cancer (34). However, another study in lung cancer animal models demonstrated that long-term hypoxia suppressed MVD but increased the expression level of HIF-2α (68). In addition, HIF-2α could mobilize circulating endothelial progenitor cells in lung tumorigenesis in mice (69). VEGF is the best-known target gene of HIF-2α

in angiogenesis in lung cancer (70-72). Researchers found that HIF-2α was an independent prognostic indicator and linked to the intense vascularization activated by VEGF/KDR in a study of 108 tissue samples evaluating the immunohistochemical expression between non-small cell lung cancer and normal lung tissues (72). Long-term hypoxia plays a key role in the pathogenesis of chronic obstructive pulmonary disease (COPD), and lung cancer is a significant complication of COPD. It has been hypothesized that the hypoxic microenvironment promotes angiogenesis in lung cancer, and studies on this subject were undertaken. Tumour progression and high HIF-2α-regulated VEGF-A and FGF2 angiogenesis (71) were detected in mice with lung cancer exposed to hypoxic conditions, and these findings were partly contrary to those of a previous study on HIF-2α and MVD (68). Targeted regulation of the HIF-2α-VEGF axis provided more direct evidence. Downregulation of HIF-2α through siRNA downregulated PDE4 and reduced the secretion of VEGF to inhibit the growth of blood vessels in lung cancer animal models (73). These data suggested that the HIF-2α/VEGF axis is an important pathway in angiogenesis in lung cancer. However, more research is necessary to explore regulatory pathways other than VEGF that are involved in angiogenesis.

Metastasis. Clinicopathological and prognostic analyses indicate that metastasis is an independent prognostic indicator of lung cancer (53). When focusing on tumorigenesis and cell proliferation in lung cancer, studies have demonstrated that the expression of HIF-2α is closely associated with and promotes lung cancer metastasis. A clinical trial demonstrated that the expression of HIF-2α in lung cancer tissues was positively associated with lymph node metastasis (57). Epithelial-to-mesenchymal transition (EMT) was confirmed to participate in HIF-2α-associated lung cancer metastasis, and other pathways were also involved (74-76).

EMT is an important characteristic in lung cancer and is strongly associated with HIF-2α. A number of studies have focused on the relationship between these factors. Downregulation of HIF-1/2 inhibited hypoxia-mediated protein kinase A (PKA), and downregulation of PKA prevented hypoxia-mediated EMT, cell migration and invasion (76). In addition, an interesting study focused on the association between anaesthetics and lung cancer. Levobupivacaine induced the proliferation and metastasis of A549 lung cancer cells *in vitro* and *in vivo* through promoting EMT. According to gene expression chips, quantitative (q)PCR and western blotting, HIF-2α was upregulated in A549 cells after levobupivacaine treatment (75). OCT4, as a stem cell marker protein, was found to be associated with HIF-2α during metastasis of lung cancer. High levels of OCT4B inhibited epithelial barrier properties to reduce the migration and invasion of lung cancer cells. Downregulation of OCT4B attenuated HIF-2α-induced EMT to inhibit tumour metastasis in cell lines and animal models (77). Of note, mesothelial-to-mesenchymal transition (MMT) and mesenchymal-to-epithelial transition (MET) also actively participate in HIF-2α-related metastasis. Knockdown of EPAS1/HIF-2α by shRNA significantly suppressed the peritoneal metastasis of lung cancer *in vivo*, and the characteristics of MMT-inducing factors were closely associated with HIF-2α (74). Findings have shown that there is a special protein mediating metastasis through EMT and MET (78). Osteopontin (OPN) may be classified as secretory OPN (sOPN) and intracellular/nuclear OPN (iOPN), both of which promote metastasis through EMT and MET, respectively. Intracellular OPN interacting with HIF-2α regulates the AKT1/miR-429/ZEB pathway (78). Further *in vivo* experiments demonstrated that increased levels of OPN and MET in the nucleus were associated with tumour metastasis (78).

β-catenin is not only involved in proliferation, but also plays a role in the metastasis of lung cancer. Regulating HIF-2α could regulate β-catenin in the same manner, thereby affecting the migration of non-small cell lung cancer cells. The AKT/Wnt/β-catenin pathway may be a possible underlying mechanism, as evidence indicates that HIF-2α is necessary for Wnt activation and AKT1 is activated under hypoxic conditions (64). It was previously demonstrated that miRNAs may regulate HIF-2α. miRNA-184 inhibited metastasis, while miRNA-574-5p enhanced metastasis; they both participated in β-catenin signalling by suppressing EPAS1/HIF-2α according to microarray and qPCR analyses of small cell lung cancer (79).

Chemokine receptors, such as CCR7 and CXCR4, are induced and associated with metastasis in lung cancer cells under hypoxia (38,80). To investigate HIF-dependent cell invasion and migration, researchers knocked down HIF-1α or HIF-2α in two lung cancer cell lines by lentiviral vector-mediated RNA interference (RNAi). The results demonstrated that HIF-1α and HIF-2α were strongly associated with upregulation of CXCR4 and metastasis (38). CCR7 was positively correlated with HIF-1α and HIF-2α, and both factors were associated with lymph node metastasis as indicated in a study of 99 samples of tissues from patients with non-small cell lung cancer stained by immunohistochemistry (80). Further studies revealed that downregulation of HIF-1α or HIF-2α led to a decrease in CCR7 expression and inhibition of migration and invasion, although HIF-1α played a more significant role. According to this study, ERK1/2 was considered to be the downstream effector of CCR7 (80). The connection between HIF-2α and cytokines in lung cancer is a novel pathway implicated in metastasis, and further in-depth research is required.

Resistance to chemotherapy and radiotherapy. Ineffective treatments, including chemotherapy, radiotherapy and targeted therapy, markedly restrict the efficacy of lung cancer treatment, and it is crucial to explore the detailed mechanisms involved. Investigators found that sensitivity to cisplatin was enhanced after siRNA targeting HIF-2α was transfected into A549 cells (81). Another study focused on a wider variety of chemotherapy drugs. In the vinorelbine, vinflunine and methotrexate groups, the proliferation of lung cancer cells was effectively inhibited in both the 25 and 100 nmol/l siRNA HIF-1α and HIF-2α groups compared with the control group (82). From these experiments, it was inferred that HIF-2α expression indicated high resistance to chemotherapy drugs. Pyruvate dehydrogenase kinase (PDK)4 was detected early as a related gene involved in this process. According to clinical samples and analyses, the level of PDK4 was positively correlated with poor prognosis in lung adenocarcinoma, whereas upregulating PDK4 through lentivirus infection significantly increased the expression of EPSA1/HIF-2α and enhanced resistance to cisplatin (54). However, as regards sensitivity to radiotherapy,

the role of HIF-2α remains unclear. Isogenic H1299 non-small cell lung carcinoma cells lacking HIF-1α, HIF-2α, or both, were constructed by CRISPR gene editing. Double-HIF1/2 knockout cells exhibited the strongest radiosensitivity, followed by HIF-1α knockout cells, while HIF-2α had no radiosensitising effect (83). A different result was obtained in patients with non-small cell lung cancer, among whom patients with high HIF-2α expression levels had a shorter survival time and a faster recovery of carcinoembryonic antigen to the pre-radiation level (84). In recent years, targeted therapy accounts for at least half of the effective treatments of non-small lung cancer. Elucidating whether HIF-2α expression is related to targeted therapy resistance may provide some useful indicators. When non-small cell lung cancer cells expressing T790M EGFR were treated with a tyrosine kinase inhibitor (TKI), the overexpression of EPAS1/HIF-2α enhanced drug resistance, whereas the deregulation of EPAS1/HIF-2α obviously increased drug efficacy. Knocking down MET eliminated this EPAS1-dependent TKI resistance, which suggested that EPAS1/HIF-2α may act as a bridge in this process (85). Regardless of whether HIF-2α is a direct or indirect factor, it appears to be involved in treatment failure, and its detailed mechanisms require further clarification.

Lung CSCs. CSCs constitute a small part of the tumour mass and are characterized by self-renewal, differentiation and the ability to promote chemoresistance of tumours (86). CSCs have lower glucose and oxygen consumption and lower intracellular reactive oxygen species and ATP concentrations compared with other cells (87). Hypoxia is an important characteristic of the tumour microenvironment, and studies have indicated that HIF-2α is connected with lung CSCs (88). OCT4, as a homeobox transcription factor, is an important stem cell marker in lung CSCs (89-93). Human bronchial epithelial cells underwent EMT after long-term exposure to arsenite, and then acquired a malignant stem cell-like phenotype (94). Moreover, OCT4, Twist1 and Bmi1 participate in arsenic-induced EMT, which is directly regulated by HIF-2α (94). There have been few studies on HIF-2α in lung CSCs, but the target protein β-catenin, an important protein previously described in the tumour signalling pathway, was found to be possibly linked to these cells. Although there is no direct evidence of HIF-2α-mediated β-catenin in lung CSCs, the Wnt/β-catenin pathway was activated in liver CSCs (95) and lung CSCs (96). HIF-2α regulated the involvement of β-catenin in tumorigenesis, proliferation and metastasis, which has been described above. HIF-2α-mediated β-catenin regulation in lung CSCs may provide a novel perspective for exploring the pathway indicating that HIF-2α involved in maintaining the stemness of stem cell.

Studies have indicated that some specific characteristics of lung CSCs are related to HIF-2α. The expression of CD133 and HIF-2α and cell invasion ability were detected following CD133 siRNA transfection into lung cancer A549 stem cells. Compared with that on $CD133^-$ CSCs, the expression of HIF-2α was significantly enhanced on $CD133^+$ CSCs. The level of HIF-2α in CD133-si-A549 stem cells was decreased compared with that in control-si-A549 stem cells, and cell invasion was significantly reduced (88). Another study on HIF-2α and radiation sensitivity revealed that a high level of HIF-2α in lung CSCs was positively associated with its radioresistance effect (84).

The hypoxic microenvironment of lung cancer provides several perspectives for assessing HIFs. Several studies have focused on lung CSCs and HIF expression. HIF-2α is different from HIF-1α, but the upregulation of HIF-1 and HIF-2 in tumour sites correlates with the expansion of the CSC population (86). However, more studies are required to explore the function of HIF-2α in lung CSCs. The HIF-2α target proteins summarized were only based on existing research, and there may still be a number of mechanisms that remain unknown. Further investigations of lung CSCs based on the signalling pathways or target proteins previously summarized may be beneficial.

Possible HIF-2α-targeted therapy in lung cancer. At present, the most mature HIF-2α-targeted therapy is the application of PT2385 in renal clear-cell carcinoma, which was tested in a phase III clinical trial and exhibited significant efficacy (12). However, the application of HIF-2α-targeted therapy in lung cancer is not yet applicable in the clinical setting. Clinical studies, not only in small cell lung cancer, but also in non-small cell lung cancer, demonstrated that HIF-2α was negatively correlated with overall survival (53,97). Moreover, according to a meta-analysis, EPAS1/HIF-2α rs13419896 and rs4953344 gene polymorphisms were associated with overall survival (98) and progression-free survival (99), respectively. These findings indicate that HIF-2α-targeted therapy in lung cancer is meaningful and worth exploring.

Methods for downregulating or knocking out HIF-2α include siRNA (61,81,82), shRNA (60,74), lentiviral vectors (38) and gene editing (83). Gene editing is not in the consideration of clinical treatments due to ethical considerations, but other methods may be further explored in lung cancer. Certain methods may regulate HIF-2α through its upstream genes. For example, LINC01436 acted as a proto-oncogene to enhance lung cancer cell growth, migration and invasion *in vitro*, and promoted tumour growth and metastasis in nude mice *in vivo*. LINC01436 functions as a miR-30a-3p sponge to regulate the expression of its target gene EPAS1/HIF-2α (62). Given that miRNAs can be used in the laboratory, researchers can directly screen different miRNAs to regulate HIF-2α. miRNA-184 and miRNA-574-5p were revealed to have opposite effects in small cell lung cancer by regulating the EPAS1/β-catenin axis (79). In addition, miR-383 directly targeted EPAS1/HIF-2α by downregulating its expression to inhibit the migration and invasion of lung cancer cells *in vitro* and the tumorigenesis of lung cancer xenografts *in vivo* (100). This evidence provides a new suggestion for modifying HIF-2α expression, not only by regulating it per se, but also by regulating its driver genes. In addition, under hypoxic conditions, the mRNA and protein levels of EPAS1 in A549 lung cancer cells were increased, but the hypoxia-induced expression of EPAS1/HIF-2α may be abolished by a specific inhibitor of the Src family kinase PP1, confirming that Src family kinases mediate hypoxia-induced HIF-2α (101).

To date, few selectively targeted HIF-2α inhibitors have been discussed in lung cancer. Although HIF-2α inhibitors for lung cancer cells are currently being investigated in the laboratory, clinically applicable HIF-2α-specific inhibitors have yet to be developed. However, HIF-2α-targeted therapy may become a promising treatment for lung cancer in the future.

4. Conclusion and perspectives

Numerous studies have provided convincing evidence that HIF-2α plays an important role in critical aspects of lung cancer. HIF-2α is involved in tumorigenesis and proliferation, angiogenesis, metastasis and resistance of lung cancer to drugs or radiotherapy. Current studies on HIF-2α have indicated its role in lung cancer cell conversion to CSCs, which was confirmed as the main cause of treatment failure. Not only clinical evidence, but also cell experiments and animal models, have demonstrated that a high level of HIF-2α expression is correlated with the occurrence and poor prognosis of lung cancer. In conclusion, the HIF-2α signalling pathway may represent a useful biomarker for evaluating lung cancer, as well as a promising tool for lung cancer treatment.

However, a number of issues regarding HIF-2α remain to be addressed. First, studies have mostly focused on non-small cell lung cancer, whereas there are few studies on small cell lung cancer. Moreover, in small cell lung cancer, clinical evidence and laboratory studies may be contradictory, and it cannot be definitively concluded that this is an important oncogene. The reason for this phenomenon may be associated with the different biological characteristics of non-small cell lung cancer and small cell lung cancer. Second, although several HIF-2α-targeted genes were described in the development of lung cancer, the exact mechanisms remain elusive and the actual pathway may be unknown due to the lack of comprehensive research. Particularly in studies on lung CSCs, establishing the significance of HIF-2α requires more clinical data and laboratory results, and its signalling pathways and targeted genes should be thoroughly investigated. Third, the development of clinical-level HIF-2α-targeted therapy is still extremely challenging. HIF-2α does not exist alone, but is also associated with HIF-1α and HIF-3α under normoxic and hypoxic conditions. In particular, HIF-3α, as a regulatory factor, has been less extensively explored than the other two subunits in lung cancer. Finally, as previously discussed, HIF-2α plays a critical role in other diseases, such as preeclampsia and non-alcoholic fatty liver. Patients with lung cancer are prone to developing various diseases, such as pulmonary embolism and diffuse intracapillary coagulation, or this condition may be combined with other diseases, particularly hypoxic diseases, such as obstructive sleep apnoea/hypopnea syndrome. The role of HIF-2α may be complicated and variable, but may be worth exploring in the context of determining the cause for the high incidence of lung cancer. At present, however, the road to developing a HIF-2α-targeted therapy is long and further research on lung cancer is required.

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Authors' contributions

WJW and XQY developed and designed the idea and wrote the manuscript. CO, BY, CC, XFX and WJW performed retrieving articles and graphing. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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