Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2020, **18**(9): 696-703
doi: 10.1016/S1875-5364(20)60008-5
Chinese Journal of Natural Medicines

•Review•

Natural products as potent inhibitors of hypoxia-inducible factor-1α in cancer therapy

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Available online 20 Sep., 2020

[ABSTRACT] Hypoxia is a prominent feature of tumors. Hypoxia-inducible factor-1α (HIF-1α), a major subunit of HIF-1, is overexpressed in hypoxic tumor tissues and activates the transcription of many oncogenes. Accumulating evidence has demonstrated that HIF-1α promotes tumor angiogenesis, metastasis, metabolism, and immune evasion. Natural products are an important source of antitumor drugs and numerous studies have highlighted the crucial role of these agents in modulating HIF-1α. The present review describes the role of HIF-1α in tumor progression, summarizes natural products used as HIF-1α inhibitors, and discusses the potential of developing natural products as HIF-1α inhibitors for the treatment of cancer.

[KEY WORDS] Hypoxia-inducible factor-1α; Hypoxia; Cancer; Natural products; Anticancer treatment
[CLC Number] R965 **[Document code]** A **[Article ID]** 2095-6975(2020)09-0696-08

Introduction

The 2019 Nobel Prize for Physiology or Medicine was awarded to William G. Kaelin Jr, Sir Peter J. Ratcliffe, and Gregg L. Semenza, for their significant contribution to discovering how cells sense oxygen levels and regulate physiological hypoxia. Several Nobel Prize winners have laid the foundation for an understanding of how hypoxia affects cell metabolism and physiological functions. Their findings have also paved the way for new strategies to treat cancer, anemia, and many other diseases [1]. Hypoxia often occurs in a variety of physiological or pathological processes, especially cancer. Immature microvasculature and abnormal metabolism result in hypoxia of the tumor microenvironment [2]. Gregg L. Semenza identified a transcription factor that is regulated by hypoxia, and termed it hypoxia inducible factor-1α (HIF-1α). Later, Kaelin and Ratcliffe *et al*. reported that HIF-1α is involved in controlling the response of tumors to hypoxia [3]. Accordingly, the critical role of HIF-1α in tumors and the development of HIF-1α inhibitors have attracted widespread attention. Here, we provide an overview of the regulatory effects of HIF-1α on tumor development process and summarize potential HIF-1α inhibitors derived from natural products.

Structure and Function of HIF-1α

HIF-1 is a heterodimer that consists mainly of two subunits: HIF-1α and HIF-1β. Both subunits contain basic helix-loop-helix-PAS domains, which are necessary to form a heterodimer and combine with DNA. HIF-1β plays a constitutive role, while HIF-1α is regulated by anoxic signals and is regarded to be the active subunit of HIF-1 [4]. Under normoxic conditions, prolyl hydroxylase 2 (PHD2) uses O_2 as a substrate and adds hydroxyl functional groups to HIF-1α. The von Hippel-Lindau (VHL) protein then recognizes and combines with HIF-1α, leading to the ubiquitination and degradation of HIF-1α through ubiquitin ligases recruitments. Additionally, hydroxylation of HIF-1α precludes binding to p300/CBP, which blocks the transcriptional activation of HIF-1. Under hypoxic conditions, decreased hydroxylation leads to reduced formation of the VHL and HIF-1α complex, thereby promoting the stable expression of HIF-1α. Moreover, HIF-1α is able to combine with p300/CBP to exert its transcriptional activity [5-7].

[Received on] 16-Jun.-2020
[Research funding] This work was supported by the National Natural Science Foundation of China (Nos. 81973340, 81630095, and 81573455), the Local Innovative and Research Teams Project of Guangdong Pearl River Talents Program (No. 2017BT01Y036), the National Science and Technology Major Project (No. 2018ZX09711001-008-008), the National High-level Personnel of Special Support Program (2019, ZHANG Dong-Mei).
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These authors have no conflict of interest to declare.

HIF-1α and Cancer

Tumor cells metabolize significantly faster than normal cells, resulting in increased oxygen consumption and decreased oxygen content in tumor tissue, ultimately leading to hypoxia. Presently, approximately 60% of solid tumors contain < 1% oxygen, and therefore, HIF-1α overexpression can be detected in various tumor tissues [8]. HIF-1α affects development processes in tumors, such as angiogenesis, metastasis, metabolism, and immune evasion.

HIF-1α and tumor angiogenesis

Accumulating evidence indicates that angiogenesis is an adaptive response to hypoxia [9, 10]. HIF-1α expression in multiple tumors is positively correlated with the expression of pro-angiogenic factors, including vascular endothelial growth factor (VEGF), fibroblast growth factor-2, angiogenin, S100A8, matrix metallopeptidase 2 (MMP2), and platelet-derived growth factor-BB (PDGF-BB) [11-14]. In addition to directly regulating pro-angiogenic factors, HIF-1α facilitates angiogenesis through cellular microRNAs (miRNAs) and exosomes [15]. For example, when HIF-1α upregulates miR210 transcription, it causes a decrease of Ephrin-A3 levels in endothelial cells, which is followed by upregulation of VEGF expression and induction of angiogenesis [16]. Moreover, HIF-1α recruits other cells in the tumor environment to promote angiogenesis. For example, bone marrow-derived $CD45^+$ myeloid cells are recruited to trigger angiogenesis in glioblastoma by stromal cell-derived factor-1 α (SDF-1α), which is induced by HIF-1α [5]. HIF-1α also stimulates Jagged1-containing exosome secretion in mesenchymal stem cells; these exosomes are transported to endothelial cells (ECs) and enhance angiogenesis through the Jagged1/Notch pathway [17].

HIF-1α and tumor metastasis

An increasing number of studies have demonstrated that tumor cells in hypoxic environments are more likely to metastasize [18, 19]. Being a key regulator of tumor metastasis, HIF-1α plays a crucial role in epithelial-mesenchymal transition (EMT), tumor invasion, intravasation, extravasation, and premetastatic niche formation. HIF-1α drives EMT through multiple signaling pathways, including TGF-β/Smad, Wnt/β-catenin, and Jagged/Notch, which further activate downstream transcription factors, such as SNAIL, ZEB1, and TWIST, leading to tumor cell EMT and metastasis [11, 20-22]. HIF-1α also facilitates EMT by downregulating the RNase enzyme Dicer, which hinders the maturation of tumor suppressor miR-200b and leads to tumor cell metastasis [23]. HIF-1α also plays a crucial role in the initial stages of tumor intravasation through remodeling of the extracellular matrix (ECM) to overcome tissue barriers through MMPs such as MMP2, MMP9, and MMP13 [24]. In addition, HIF-1α also affects tumor intravasation by inducing tumor-endothelial cell adhesion and migration. HIF-1α induces CXCR4 expression in tumor cells and facilitates transendothelial migration of ECs expressing SDF-1 [25]. Moreover, HIF-1α is involved in tumor extravasation. HIF-1α induced tumor cells extravasate from the lung vasculature by downregulating angiopoietin-like 4 (ANGPTL4) and L1 cell adhesion molecule (LICAM), which attenuates EC-EC adhesion and facilitates tumor-EC interaction, thus promoting the extravasation of breast cancer cells [18]. The effects of HIF-1α on pre-metastatic niche formation are mainly mediated by the LOX family of proteins. Tumor cells produced LOX in a HIF-1α-dependent manner and subsequently promote ECM remodeling and metastatic growth [24].

HIF-1α and tumor metabolism

Compared to normal cells, tumor cells under the influence of HIF-1α differ in glucose, fatty acid, and amino acid metabolism. HIF-1α regulates tumor cell metabolism in several ways, including stimulating glucose production by upregulating glucose membrane transport (GLUT1 and GLUT3), mitochondrial dysfunction and upregulation of glycolytic enzymes, lactate production and lactate transporter transportation [26]. Various signaling molecules contribute to HIF-1α-induced glucose metabolism, such as mucin 1, extracellular matrix protein 1, and Jumonji C domain-containing dioxygenase 5 [27, 28]. Under hypoxic conditions, HIF-1α also participates in tumor fatty acid metabolism by inducing fatty acid synthesis and lipid accumulation and reducing fatty acid oxidation. For example, HIF-1α induces lipid droplet accumulation by increasing fatty acid binding protein (FABP) 3 and FABP7, leading to the growth and survival of tumor cells in both breast cancer and glioblastoma [29]. HIF-1α also reduces β-oxidation of fatty acids by repressing proliferator-activated receptor-γ coactivator-1α, long-chain acyl-CoA dehydrogenase and medium-chain acyl-CoA dehydrogenase, which is critical for tumor progression [30]. In addition to glucose and fatty acid metabolism, tumor cells need to produce increasing amounts of glutamine to support their proliferation and growth. HIF-1α directly converts glutamine into alpha-ketoglutarate to synthesize acetyl coenzyme A, which is available for *de novo* fatty acid synthesis [29]. Genetic deletion of liver kinase B1 (LKB1) triggers HIF-1α-mediated glutamine metabolism and faciliates lipogenesis. Further investigation has shown that the effect of LKB1 on HIF-1α is regulated by the mTOR and reactive oxygen species signaling pathways [31].

HIF-1α and immune evasion

HIF-1α serves as a major stimulator in orchestrating an immunosuppressive tumor microenvironment (TME) by regulating the function and differentiation of immunosuppressive cells. HIF-1α recruits myeloid-derived suppressor cells (MDSCs) to the TME and augments their immunosuppressive function [24]. HIF-1α increases the expression of ectonucleoside triphosphate diphosphohydrolase 2 in hepatocellular carcinoma cells, which facilitates the maintenance of MDSCs and promotes tumor growth [32]. In addition to triggering M2 macrophage polarization, HIF-1α is associated with macrophage-mediated T cell suppression; specific deletion of HIF-1α in macrophages can relieve the T cell-associated immune response [5].

Natural Products Targeting HIF-1α in Anticancer Treatment

Because HIF-1α is overexpressed in tumors and regulates the transcription of many genes, targeting the HIF-1α

pathway is believed to be a promising strategy in anticancer therapy. Many studies have reported that different types of natural products can inhibit the activity of HIF-1α to exert antitumor effects. Here, we classify and list these compounds according to their structures (Fig. 1), and summarize their mechanism of inhibiting HIF-1α (Fig. 2).

Alkaloids

Dictamnine is derived from *Dictamnus dasycarpus* and exerts its anticancer effects by downregulating HIF-1α protein expression. Mechanistic studies have shown that dictamnine inhibits the mammalian target of rapamycin (mTOR)/p70S6K/eIF4E and mitogen-activated protein kinase (MAPK) pathways to reduce HIF-1α synthesis, leading to suppression of the proliferation, migration, and invasion in HCT116 cells [33]. Another alkaloid, matrine, which is isolated from the roots of *Sophora flavescens*, significantly decreases messenger RNA (mRNA) and protein expression of HIF-1α to inhibit the growth of colon cancer cells [34]. Berber-

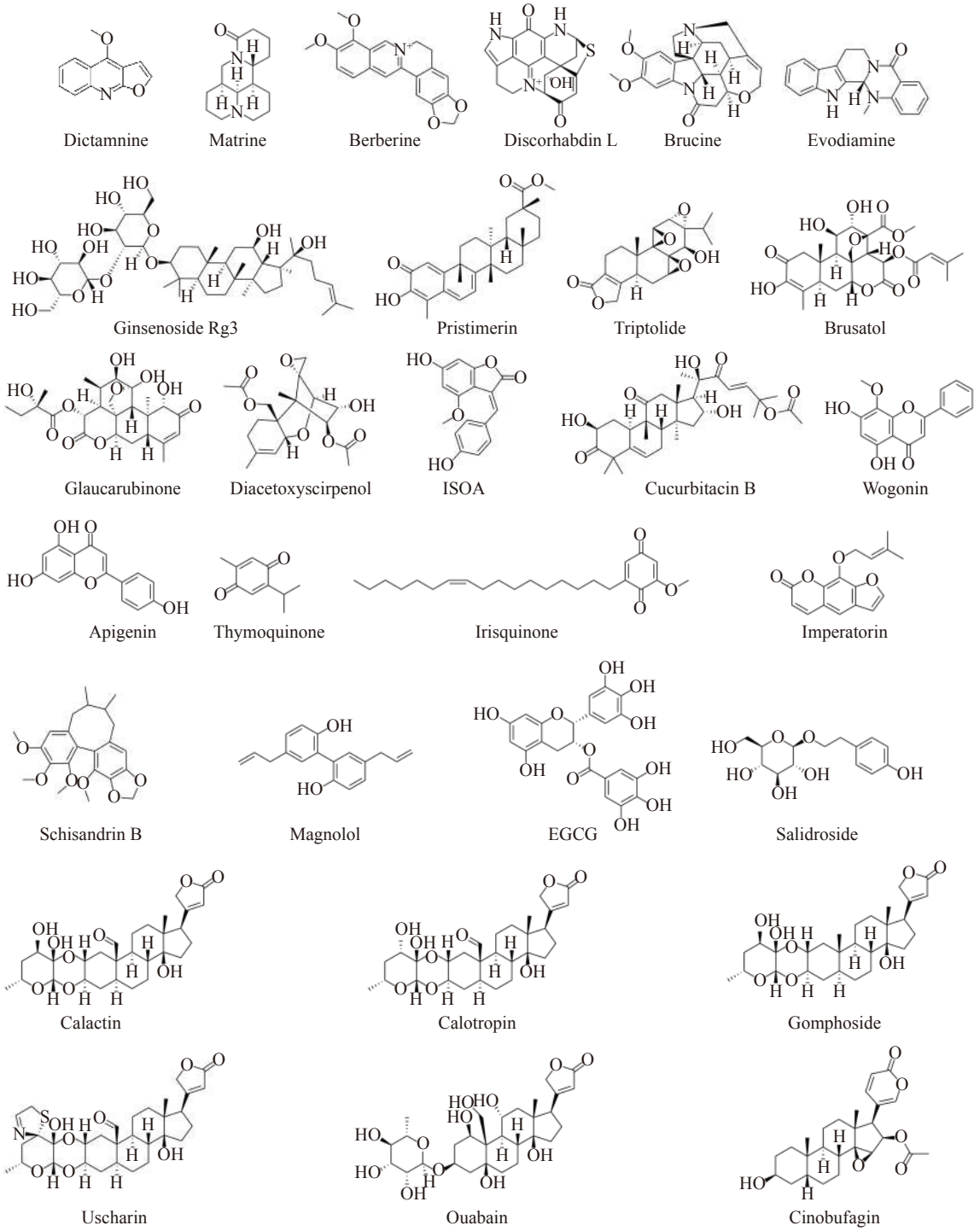


Fig. 1 Structures of the natural products targeting HIF-1α

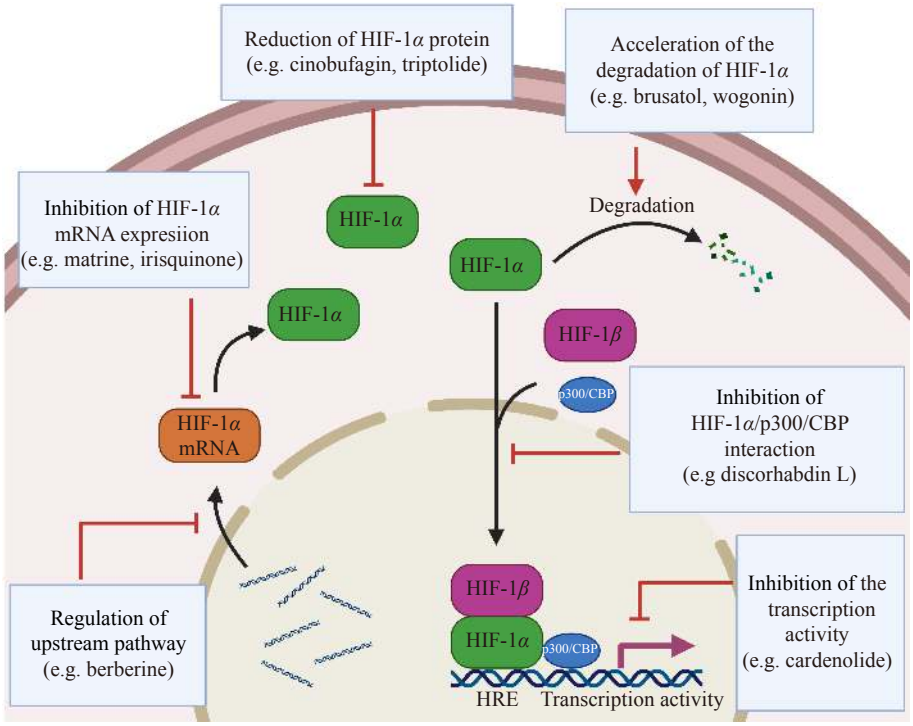


Fig. 2 Molecular mechanisms of natural products regulating HIF-1 pathway

ine has been also reported to reverse hypoxia-induced chemoresistance in breast cancer cells at the concentration of 2.5 μmol·L^{-1}. Berberine downregulates the AMPK-HIF-1α pathway, thus enhancing the sensitivity of MCF-7/hypoxia cells to doxycycline *in vivo* at a dose of 5 mg·kg^{-1} [35]. In a phase II/III trial, 429 patients with colorectal adenoma received berberine treatment (0.3 g twice daily) after complete polypectomy and 36% of them (155 patients) appeared to have recurrent adenoma, considerably lower than 47% in the placebo group (216 patients), and no serious adverse reactions have been reported (Table 1) [36]. A pyrroloiminoquinone alkaloid from the marine sponge *Latrunculia* species, discorhabdin L, exhibits the capability to inhibit the binding of HIF-1α/p300, thereby decreasing the HIF-1α transcription to exert an antitumor effect on HCT116 cells at a concentration of 0.1−10 μmol·L^{-1}; additionally, it also exerts an anti-tumor effect in an LNCaP xenograft model at the dose of 5 mg·kg^{-1} [37, 38]. Brucine, isolated from the seeds of *Strychnos nux-vomica*, has been shown to significantly inhibit the growth of H22 tumors at a dose of 15 mg·kg^{-1} and downregulate the transcription activity of HIF-1 [39]. Moreover, evodiamine, a quinolone alkaloid from *Evodia rutaecarpa*, reduces the expression of HIF-1α in LoVo cells at concentrations of 0.5, 1, and 2 μmol·L^{-1}; different doses of evodiamine (5, 10, and 20 mg·kg^{-1}) inhibited the growth of LoVo tumor xenografts and reduced HIF-1α expression *in vivo* [40].

Terpenoids

It has been reported that ginsenoside Rg3 reduces EGF-induced HIF-1α expression to inhibit Na^+/H^+ exchanger 1, thereby inducing apoptosis in liver cancer cells. Furthermore, 10 mg·kg^{-1} of ginsenoside Rg3 suppresses the tumor growth in HCCLM3 xenografts [41]. Also, there is a clinical trial on treating advanced gastric cancer with ginsenoside Rg3 plus chemotherapy which is still in process (Table 1). Pristimerin, a triterpenoid quinone methide isolated from *Maytenus ilicifolia*, concentration- and time-dependently inhibits HIF-1α protein levels to exert antitumor effects by reducing sphingosine kinase 1 expression, which is a modulator of HIF-1α under hypoxic conditions, at concentrations of 0.5 and 1 μmol·L^{-1} [42]. Moreover, the cytotoxic diterpenoid triptolide from *Tripterygium wilfordii* has been shown to decrease the HIF-1α level to inhibit the growth of pancreatic tumors at concentrations of 28, 56, and 139 nmol·L^{-1}, and reduces HIF-1α expression *in vivo* at a dose of 0.2 mg·kg^{-1} [43]. Furthermore, two clinical trials of triptolide on cancer therapy are still underway (Table 1). Brusatol, isolated from *Brucea* species, exhibites the ability to suppress the proliferation of HCT116 cells by downregulating HIF-1α. Further mechanistic studies showed that 60 nmol·L^{-1} brusatol accelerates the degradation of HIF-1α protein; approximately 55% of HIF-1α protein remained at 100 min, and impaired the transcriptional activity of HIF-1, resulting in decreased VEGF expression, glycolytic enzyme expression, and glucose consumption in HCT116 cells [44]. Glaucarubinone, isolated from the seeds of *Simarouba glauca*, inhibits the expression of HIF-1α in colorectal cancer (CRC) cells at the concentration of 0.5 μmol·L^{-1}, and 1 mg·kg^{-1} glaucarubinone significantly suppresses the growth of CRC xenografts and downregulates HIF-1α expression *in vivo* [45]. Diacetoxyscirpenol was shown to exert an antitumor effect in several types of cancer cells and lung carcinoma *in vivo*. Further mechanical study shows that diacetoxyscirpenol suppresses the synthesis of HIF-1α protein and inhibits the formation of HIF-1, resulting in reduced the transcriptional activity of HIF-1α at concentrations

Table 1 Natural products targeting HIF-1*α* in clinical trials

Natural product	Phase	Type of cancer	Combination	Status
Berberine	Phase 2	Lung adenocarcinoma		Completed
	Phase 2/3	Colorectal adenoma		Completed
	Phase 2/3	Colorectal cancer		Ongoing
	Phase 2/3	Colorectal adenoma	Gefitinib	Ongoing
Cinobufagin	Phase 1	Digestive system cancer	Transarterial	Ongoing
	Phase 2	Hepatoma	chemoembolization	Ongoing
	Phase 2/3	Diffuse large B cell lymphoma	vindesine, cyclophosphamide, epirubicin, prednisone,	Ongoing
	Phase 4	Liver cancer	rituximab	Ongoing
	Phase 4	Gastrointestinal neoplasms	chemotherapy	Ongoing
EGCG	Not applicable	Colorectal neoplasms		Completed
	Early phase 1	Colon cancer		Ongoing
	Phase 1	Stage I/II prostate cancer		Completed
	Phase 1	Uterine fibroids	Clomiphene citrate, letrozole	Ongoing
	Phase 2	Colorectal Adenomas		Completed
	Phase 2	Breast neoplasms		Ongoing
Ginsenoside Rg3	Not applicable	Stage I/II hepatocellular carcinoma		Completed
	Phase 2	Advanced gastric cancer	First-line chemotherapy	Ongoing
Thymoquinone	Phase 2	Premalignant lesion		Ongoing
Triptolide	Phase 1	Advanced cancer, gastric cancer, breast cancer, pancreatic cancer, prostate cancer metastatic, colorectal cancer, solid tumor, solid carcinoma, solid cancer of stomach		Ongoing
	Phase 2	Pancreatic cancer		Ongoing

ranged from 1.25 to 5 ng·mL^{-1} [46]. 4′, 6-Dihydroxy-4-methoxyisoaurone (ISOA), isolated from *Trichosanthes kirilowii* seeds, inhibits the expression of HIF-1*α* protein by suppressing the Akt/mTOR/p70S6K/4E-BP1 signaling pathway at concentrations of 1 and 3 μmol·L^{-1} in HeLa cells, resulting in disturbance of HIF-1 target gene expression [47]. Cucurbitacin B is a triterpenoid, isolated from *Trichosanthes kirilowii*. It has been reported that 0.3 μmol·L^{-1} cucurbitacin B significantly inhibits the expression of HIF-1*α* in HeLa cells, and cucurbitacin B (5 mg·kg^{-1}) suppresses HeLa tumors *in vivo* with a tumor inhibitory rate of 52.9% [48].

Flavones

A study demonstrated that wogonin, an anti-angiogenesis ingredient of *Scutellaria baicalensis*, inhibits the accumulation of HIF-1*α* protein by increasing the expression of PHD and VHL, followed by accelerated degradation of HIF-1*α*. It also inhibits binding between Hsp90 and HIF-1*α*, and reduces the nuclear translocation of HIF-1*α* [49]. Furthermore, apigenin inhibits mRNA and protein expression of HIF-1*α* in MDA-MB-453 breast cancer cells, and exerts an anti-tumor efficiency [50].

Quinones

Thymoquinone, an anti-tumor ingredient from *Nigella sativa*, has been confirmed to inhibit the binding of Hsp90 and HIF-1*α*, and trigger the degradation of HIF-1*α* through the ubiquitin-proteasome pathway, at a concentration of 5 μmol·L^{-1}; this subsequently reduces the transcription of HIF-1*α* downstream genes that initiate apoptosis in hypoxic renal cancer cells [51]. A clinical trial on using thymoquinone to treat oral potential premalignant lesions is still ongoing (Table 1). Moreover, irisquinone, an active compound from *Iris lacteal*, when used at the concentration of 4.2 nmol·L^{-1}, enhances the radiosensitivity of C6 rat glioma cells by decreasing mRNA and protein expression levels of HIF-1*α*. The results of immunohistochemical analysis demonstrate that irisquinone combined with irradiation treatment significantly reduces HIF-1α-positive cells in C6 tumors [52].

Phenylpropanoids

Imperatorin is a bioactive furocoumarin compound from *Angelica dahurica*, which has been shown to reduce the synthesis, but not the degradation, of HIF-1*α* protein by inhibiting the mTOR/p70S6K/4E-BP1 and MAPK signaling pathways. As a result, target genes of HIF-1*α* are suppressed, and the growth and angiogenesis of colon cancer HCT116 xenografts are also inhibited by imperatorin [53]. Schisandrin B, a dibenzo cyclooctadiene lignan from *Schisandra chinensis*, was shown to induce apoptosis in A549 cells. Furthermore, schisandrin B decreases the expression of HIF-1*α* to inhibit the invasion and migration of A549 cells [54]. Magnolol is a phenolic compound extracted from *Magnolia officinalis* that exhibits an anti-angiogenic effect *in vitro* at concentrations of 1 and 5 μmol·L^{-1} by reducing HIF-1*α* accumulation and VEGF secretion. Moreover, according to the immunohistochemistry data, magnolol (10 mg·kg^{-1}·d^{-1}) notably reduces the size and weight of T24 xenografts, and inhibits the HIF-1*α* expression of T24 tumors [55].

Phenols

Polyphenol epigallocatechin-3-gallate (EGCG) is a com-

mon component of green tea and has been proposed as a candidate antitumor agent. EGCG reduces HIF-1α protein levels to inhibit glycolysis in 4T1 cells, and demonstrates a significant anti-tumor effect on breast cancer xenograft at the doses of 5, 10 and 20 mg·kg^{-1} [56]. The results of a clinical trial on the prevention of colorectal adenomas by giving daily green tea extract (GTE) tablets (0.9 g·d^{-1} GTE, equal 0.6 g·d^{-1} EGCG) showed that the recurrence of metachronous adenomas in the GTE group (23.6%, 17 of 72) is lower than the control group (42.3%, 30 of 71), and the number of relapsed adenomas was also lower in GTE group (0.7 ± 1.1 in control group *vs* 0.3 ± 0.6 in GTE group, Table 1) [57]. Salidroside, isolated from *Rhodiola rosea*, improves the sensitivity of liver cancer cells to platinum and reverses drug resistance, which achieves a sensitization effect by decreasing protein expression levels of HIF-1α [58].

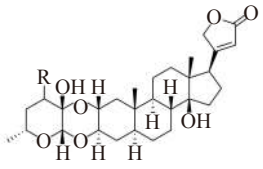
Steroids

Cardenolides are the bioactive components of *Calotropis gigantea*, and have demonstrated remarkable cytotoxic effects on multiple types of cancer cells. Studies have reported that cardenolides, such as calactin, calotropin, gomphoside, and uscharin, are potent inhibitors of HIF-1α and effectively suppress the transcriptional activity of HIF-1α at the concentration of 100 nmol·L^{-1}. Calactin, calotropin, gomphoside, and uscharin inhibit HIF-1 activity with half inhibitory concentrations of 22, 46, 28 and 28 nmol·L^{-1}, respectively [59, 60]. Moreover, ouabain, a cardiac glycoside, significantly inhibits the protein expression of HIF-1α, and reduces the growth and migration of glioma U-87MG cells at concentrations of 0.5 and 2.5 μmol·L^{-1} [61]. Cinobufagin, a bufanolide steroid, at the concentrations ranged from 0.1 to 1 μmol·L^{-1}, downregulates the expression of HIF-1α in HUVECs; it also suppresses HIF-1α expression and angiogenesis in hemangioendothelioma at the dose of 2 mg·kg^{-1} [62].

Conclusion and Perspectives

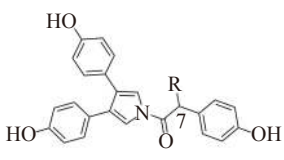
HIF-1α is overexpressed in various tumors under hypoxic conditions, which activates the transcription of genes related to tumor progression, including genes for angiogenesis, metastasis, metabolism, and immune evasion. Therefore, targeting HIF-1α in cancer therapy is a promising therapeutic strategy. Currently, most of the HIF-1α inhibitors are synthetic compounds, such as EZN-2968, EZN-2208, lificiguat, PX-478, and 2-methoxyestradiol. Some of these inhibitors have been studied in clinical trials; for example, EZN-2968 has been reported to inhibit the levels of HIF-1α protein in patients with refractory solid tumors in a pilot trial [63]. These HIF-1α inhibitors function by inhibiting HIF-1α mRNA transcription, translation, stabilization, dimerization, HIF-1α/DNA binding, and HIF-1α-related gene transcriptional activity. Although these synthesized HIF-1α inhibitors show more favorable antitumor activities in preclinical models than those of natural HIF-1α inhibitors, many of them do not specifically target HIF-1α, which undoubtedly increases the risk of adverse reactions. Therefore, exploiting specific and available HIF-1 inhibitors for cancer treatment remains a major challenge.

Natural products derived from plants, marine organisms, or microorganisms are important sources of anti-cancer drugs [64, 65]. The unique characteristics of natural products including novel structures, good biological activity and distinct mechanisms, make them promising candidates in the search for effective HIF-1α inhibitors. Multiple HIF-1α inhibitors have been discovered from natural products and have demonstrated favorable anti-tumor effects, as discussed in this review. Triptolide, berberine and EGCC exert potent inhibitory effects on HIF-1α and have been selected in clinical trials for cancer therapy, which suggests that these natural products are promising HIF-1α inhibitor as candidate drugs. However, concerns about these compounds persist, such as their low specificity and targeting properties, which undoubtedly increase the risk of adverse effects. In addition, the poor water solubility of natural products also hinders their development as HIF-1α inhibitors. Biotransformation and structural modification of these compounds to acquire highly active and less toxic derivatives are needed. 3′-*Epi*-gomphoside is the 3′α-epimer derivative of gomphoside. Compared to gomphoside, the change of configuration makes 3′-*epi*-gomphoside lose its ability to inhibit HIF-1, which suggests that the β-oriented substituent at C-3′ is crucial for cardenolides' HIF-1 inhibitory abilities [59]. The marine pyrrole alkaloid neolamellarin A has been reported to inhibit the HIF-1 transcriptional activity, and its 7-hydroxy substituted derivative, 7-hydroxyneolamellarin A, exerts more potent HIF-1 inhibitory activity with IC_{50} value of 1.9 μmol·L^{-1} than that of neolamellarin A [66] (Fig. 3). Generally, natural products can provide novel leading compounds for developing HIF-1α inhibitors; however, further structural modification is needed to obtain more potential drugs. To further improve the druggability of these



HIF-1 inhibitory activity (IC_{50}, nmol·L^{-1})

Gomphoside	R = β-OH	27.7
3′-*Epi*-gomphoside	R = α-OH	> 100



HIF-1 inhibitory activity (IC_{50}, μmol·L^{-1})

Neolamellarin A	R = H	> 10
7-Hydroxyneolamellarin A	R = OH	1.9

Fig. 3 Representative natural products and their derivatives as HIF-1α inhibitors

natural compounds, further investigations are warranted. The synthesis of antibody-drug conjugates is also a potential strategy for the development of HIF-1α targeting natural compounds. Moreover, to improve tumor-targeting properties and decrease the toxicity of natural products, designing pro-drugs or novel drug delivery systems, such as lipid nanoparticles may be an alternative approaches. In addition to modifying these molecules and agents, further studies are needed to understand the pharmacodynamic and pharmacokinetic characteristics of natural compounds targeting HIF-1α as well as their mechanism of action. Various molecular biology methods, including surface plasmon resonance, isothermal titration calorimetry and microscale thermophoresis, can be applied to validate the interaction of natural compounds with HIF-1α. Genetic point mutation, mass spectrometry, and protein eutectic methods can be used to determine the key binding sites of natural compounds with HIF-1α. In summary, natural products have great potential for further development as novel potent HIF-1α inhibitors and anti-cancer drugs, by enhancing their targetability and reducing their toxicity.

References

[1] Burki T. 2019 Nobel Prize awarded for work on oxygen regulation [J]. *Lancet*, 2019, **394**(10207): 1399-1400.

[2] Qiu GZ, Jin MZ, Dai JX, *et al*. Reprogramming of the tumor in the hypoxic niche: the emerging concept and associated therapeutic strategies [J]. *Trends Pharmacol Sci*, 2017, **38**(8): 669-686.

[3] Hurst JH. William Kaelin, Peter Ratcliffe, and Gregg Semenza receive the 2016 Albert Lasker Basic Medical Research Award [J]. *J Clin Invest*, 2016, **126**(10): 3628-3638.

[4] Semenza GL. HIF-1 and tumor progression: pathophysiology and therapeutics [J]. *Trends Mol Med*, 2002, **8**(4 Suppl): S62-S67.

[5] LaGory EL, Giaccia AJ. The ever-expanding role of HIF in tumour and stromal biology [J]. *Nat Cell Biol*, 2016, **18**(4): 356-365.

[6] Semenza GL, Agani F, Booth G, *et al*. Structural and functional analysis of hypoxia-inducible factor 1 [J]. *Kidney Int*, 1997, **51**(2): 553-555.

[7] Ivan M, Kondo K, Yang H, *et al*. HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing [J]. *Science*, 2001, **292**(5516): 464-468.

[8] Favaro E, Lord S, Harris AL, *et al*. Gene expression and hypoxia in breast cancer [J]. *Genome Med*, 2011, **3**(8): 55.

[9] Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: role of the HIF system [J]. *Nat Med*, 2003, **9**(6): 677-684.

[10] Acker T, Plate KH. Role of hypoxia in tumor angiogenesis-molecular and cellular angiogenic crosstalk [J]. *Cell Tissue Res*, 2003, **314**(1): 145-155.

[11] Tirpe AA, Gulei D, Ciortea SM, *et al*. Hypoxia: overview on hypoxia-mediated mechanisms with a focus on the role of HIF genes [J]. *Int J Mol Sci*, 2019, **20**(24): 6140.

[12] Kishimoto K, Yoshida S, Ibaragi S, *et al*. Hypoxia-induced up-regulation of angiogenin, besides VEGF, is related to progression of oral cancer [J]. *Oral Oncol*, 2012, **48**(11): 1120-1127.

[13] Ahn GO, Seita J, Hong BJ, *et al*. Transcriptional activation of hypoxia-inducible factor-1 (HIF-1) in myeloid cells promotes angiogenesis through VEGF and S100A8 [J]. *Proc Natl Acad Sci U S A*, 2014, **111**(7): 2698-2703.

[14] Xiao C, Pan Y, Zeng X, *et al*. Downregulation of hypoxia-inducible factor-1α inhibits growth, invasion, and angiogenesis of human salivary adenoid cystic carcinoma cells under hypoxia [J]. *Oncol Rep*, 2018, **40**(3): 1675-1683.

[15] Choudhry H, Harris AL. Advances in hypoxia-inducible factor biology [J]. *Cell Metab*, 2018, **27**(2): 281-298.

[16] Wang Z, Deng M, Liu Z, *et al*. Hypoxia-induced miR-210 promoter demethylation enhances proliferation, autophagy and angiogenesis of schwannoma cells [J]. *Oncol Rep*, 2017, **37**(5): 3010-3018.

[17] Gonzalez-King H, García NA, Ontoria-Oviedo I, *et al*. Hypoxia inducible factor-1α potentiates jagged 1-mediated angiogenesis by mesenchymal stem cell-derived exosomes [J]. *Stem cells*, 2017, **35**(7): 1747-1759.

[18] Rankin EB, Nam JM, Giaccia AJ. Hypoxia: signaling the metastatic cascade [J]. *Trends Cancer*, 2016, **2**(6): 295-304.

[19] Gilkes DM, Semenza GL, Wirtz D. Hypoxia and the extracellular matrix: drivers of tumour metastasis [J]. *Nat Rev Cancer*, 2014, **14**(6): 430-439.

[20] Yoshimoto S, Tanaka F, Morita H, *et al*. Hypoxia-induced HIF-1α and ZEB1 are critical for the malignant transformation of ameloblastoma *via* TGF-β-dependent EMT [J]. *Cancer Med*, 2019, **8**(18): 7822-7832.

[21] Yang MH, Wu MZ, Chiou SH, *et al*. Direct regulation of TWIST by HIF-1alpha promotes metastasis [J]. *Nat Cell Biol*, 2008, **10**(3): 295-305.

[22] Tian Q, Xue Y, Zheng W, *et al*. Overexpression of hypoxia-inducible factor 1α induces migration and invasion through Notch signaling [J]. *Int J Oncol*, 2015, **47**(2): 728-738.

[23] Lai HH, Li JN, Wang MY, *et al*. HIF-1α promotes autophagic proteolysis of Dicer and enhances tumor metastasis [J]. *J Clin Invest*, 2018, **128**(2): 625-643.

[24] Petrova V, Annicchiarico-Petruzzelli M, Melino G, *et al*. The hypoxic tumour microenvironment [J]. *Oncogenesis*, 2018, **7**(1): 10.

[25] Guan G, Zhang Y, Lu Y, *et al*. The HIF-1α/CXCR4 pathway supports hypoxia-induced metastasis of human osteosarcoma cells [J]. *Cancer Lett*, 2015, **357**(1): 254-264.

[26] Nagao A, Kobayashi M, Koyasu S, *et al*. HIF-1-dependent reprogramming of glucose metabolic pathway of cancer cells and its therapeutic significance [J]. *Int J Mol Sci*, 2019, **20**(2): 238.

[27] Shukla SK, Purohit V, Mehla K, *et al*. MUC1 and HIF-1alpha signaling crosstalk induces anabolic glucose metabolism to impart gemcitabine resistance to pancreatic cancer [J]. *Cancer Cell*, 2017, **32**(1): 71-87.

[28] Gan L, Meng J, Xu M, *et al*. Extracellular matrix protein 1 promotes cell metastasis and glucose metabolism by inducing integrin β4/FAK/SOX2/HIF-1α signaling pathway in gastric cancer [J]. *Oncogene*, 2018, **37**(6): 744-755.

[29] Xie H, Simon MC. Oxygen availability and metabolic reprogramming in cancer [J]. *J Biol Chem*, 2017, **292**(41): 16825-16832.

[30] Lee P, Chandel NS, Simon MC. Cellular adaptation to hypoxia through hypoxia inducible factors and beyond [J]. *Nat Rev Mol Cell Biol*, 2020, **21**(5): 268-283.

[31] Faubert B, Vincent EE, Griss T, *et al*. Loss of the tumor suppressor LKB1 promotes metabolic reprogramming of cancer cells *via* HIF-1α [J]. *Proc Natl Acad Sci U S A*, 2014, **111**(7): 2554-2559.

[32] Chiu DKC, Tse APW, Xu IMJ, *et al*. Hypoxia inducible factor HIF-1 promotes myeloid-derived suppressor cells accumulation through ENTPD2/CD39L1 in hepatocellular carcinoma [J]. *Nat Commun*, 2017, **8**(1): 517.

[33] Wang JY, Wang Z, Li MY, *et al*. Dictamnine promotes apoptosis and inhibits epithelial-mesenchymal transition, migration, invasion and proliferation by downregulating the HIF-1α and Slug signaling pathways [J]. *Chem Biol Interact*, 2018, **296**: 134-144.

[34] Hong X, Zhong L, Xie Y, *et al*. Matrine reverses the warburg effect and suppresses colon cancer cell growth negatively regulating HIF-1α [J]. *Front Pharmacol*, 2019, **10**: 1437.

[35] Pan Y, Shao D, Zhao Y, *et al*. Berberine reverses hypoxia-in-

duced chemoresistance in breast cancer through the inhibition of ampk-HIF-1α [J]. *Int J Biol Sci*, 2017, **13**(6): 794-803.
[36] Chen YX, Gao QY, Zou TH, *et al*. Berberine versus placebo for the prevention of recurrence of colorectal adenoma: a multicentre, double-blinded, randomised controlled study [J]. *Lancet Gastroenterol Hepatol*, 2020, **5**(3): 267-275.
[37] Harris EM, Strope JD, Beedie SL, *et al*. Preclinical evaluation of discorhabdins in antiangiogenic and antitumor models [J]. *Mar Drugs*, 2018, **16**(7): 241.
[38] Goey AKL, Chau CH, Sissung TM, *et al*. Screening and biological effects of marine pyrroloiminoquinone alkaloids: potential inhibitors of the HIF-1α/p300 interaction [J]. *J Nat Prod*, 2016, **79**(5): 1267-1275.
[39] Shu G, Mi X, Cai J, *et al*. Brucine, an alkaloid from seeds of *Strychnos nux-vomica* Linn., represses hepatocellular carcinoma cell migration and metastasis: the role of hypoxia inducible factor 1 pathway [J]. *Toxicol Lett*, 2013, **222**(2): 91-101.
[40] Huang J, Chen ZH, Ren CM, *et al*. Antiproliferation effect of evodiamine in human colon cancer cells is associated with IGF-1/HIF-1α downregulation [J]. *Oncol Rep*, 2015, **34**(6): 3203-3211.
[41] Li X, Tsauo J, Geng C, *et al*. Ginsenoside Rg3 decreases NHE1 expression *via* inhibiting EGF-EGFR-ERK1/2-HIF-1 pathway in hepatocellular carcinoma: A novel antitumor mechanism [J]. *Am J Chin Med*, 2018, **46**(8): 1915-1931.
[42] Lee SO, Kim JS, Lee MS, *et al*. Anti-cancer effect of pristimerin by inhibition of HIF-1α involves the SPHK-1 pathway in hypoxic prostate cancer cells [J]. *BMC Cancer*, 2016, **16**(1): 701.
[43] Ding X, Zhou X, Jiang B, *et al*. Triptolide suppresses proliferation, hypoxia-inducible factor-1α and c-Myc expression in pancreatic cancer cells [J]. *Mol Med Report*, 2015, **12**(3): 4508-4513.
[44] Lu Y, Wang B, Shi Q, *et al*. Brusatol inhibits HIF-1 signaling pathway and suppresses glucose uptake under hypoxic conditions in HCT116 cells [J]. *Sci Rep*, 2016, **6**: 39123.
[45] Huynh N, Beutler JA, Shulkes A, *et al*. Glaucarubinone inhibits colorectal cancer growth by suppression of hypoxia-inducible factor 1α and β-catenin *via* a p-21 activated kinase 1-dependent pathway [J]. *Biochim Biophys Acta*, 2015, **1853**(1): 157-165.
[46] Choi YJ, Shin HW, Chun YS, *et al*. Diacetoxyscirpenol as a new anticancer agent to target hypoxia-inducible factor 1 [J]. *Oncotarget*, 2016, **7**(38): 62107-62122.
[47] Mi C, Ma J, Shi H, *et al*. 4′, 6-Dihydroxy-4-methoxyisoaurone inhibits the HIF-1α pathway through inhibition of Akt/mTOR/p70S6K/4E-BP1 phosphorylation [J]. *J Pharmacol Sci*, 2014, **125**(2): 193-201.
[48] Ma J, Zi Jiang Y, Shi H, *et al*. Cucurbitacin B inhibits the translational expression of hypoxia-inducible factor-1α [J]. *Eur J Pharmacol*, 2014, **723**: 46-54.
[49] Song X, Yao J, Wang F, *et al*. Wogonin inhibits tumor angiogenesis *via* degradation of HIF-1α protein [J]. *Toxicol Appl Pharmacol*, 2013, **271**(2): 144-155.
[50] Seo HS, Ku JM, Choi HS, *et al*. Induction of caspase-dependent apoptosis by apigenin by inhibiting STAT3 signaling in HER2-overexpressing MDA-MB-453 breast cancer cells [J]. *Anticancer Res*, 2014, **34**(6): 2869-2882.
[51] Lee YM, Kim GH, Park EJ, *et al*. Thymoquinone selectively kills hypoxic renal cancer cells by suppressing HIF-1α-mediated glycolysis [J]. *Int J Mol Sci*, 2019, **20**(5): 1092.
[52] Wang H, Zhang Y, Yu W, *et al*. Radiosensitizing effect of irisquinone on glioma through the downregulation of HIF-1α evaluated by 18F-FDG and 18F-FMISO PET/CT [J]. *Nucl Med Commun*, 2016, **37**(7): 705-714.
[53] Mi C, Ma J, Wang KS, *et al*. Imperatorin suppresses proliferation and angiogenesis of human colon cancer cell by targeting HIF-1α *via* the mTOR/p70S6K/4E-BP1 and MAPK pathways [J]. *J Ethnopharmacol*, 2017, **203**: 27-38.
[54] Lv XJ, Zhao LJ, Hao YQ, *et al*. Schisandrin B inhibits the proliferation of human lung adenocarcinoma A549 cells by inducing cycle arrest and apoptosis [J]. *Int J Clin Exp Med*, 2015, **8**(5): 6926-6936.
[55] Chen MC, Lee CF, Huang WH, *et al*. Magnolol suppresses hypoxia-induced angiogenesis *via* inhibition of HIF-1α/VEGF signaling pathway in human bladder cancer cells [J]. *Biochem Pharmacol*, 2013, **85**(9): 1278-1287.
[56] Wei R, Mao L, Xu P, *et al*. Suppressing glucose metabolism with epigallocatechin-3-gallate (EGCG) reduces breast cancer cell growth in preclinical models [J]. *Food Funct*, 2018, **9**(11): 5682-5696.
[57] Shin CM, Lee DH, Seo AY, *et al*. Green tea extracts for the prevention of metachronous colorectal polyps among patients who underwent endoscopic removal of colorectal adenomas: A randomized clinical trial [J]. *Clin Nutr*, 2018, **37**(2): 452-458.
[58] Qin Y, Liu HJ, Li M, *et al*. Salidroside improves the hypoxic tumor microenvironment and reverses the drug resistance of platinum drugs *via* HIF-1α signaling pathway [J]. *EBioMedicine*, 2018, **38**: 25-36.
[59] Parhira S, Zhu GY, Chen M, *et al*. Cardenolides from *Calotropis gigantea* as potent inhibitors of hypoxia-inducible factor-1 transcriptional activity [J]. *J Ethnopharmacol*, 2016, **194**: 930-936.
[60] Parhira S, Zhu GY, Jiang RW, *et al*. 2'-*Epi*-uscharin from the latex of *Calotropis gigantea* with HIF-1 inhibitory activity [J]. *Sci Rep*, 2014, **4**: 4748.
[61] Yang XS, Xu ZW, Yi TL, *et al*. Ouabain suppresses the growth and migration abilities of glioma U-87MG cells through inhibiting the Akt/mTOR signaling pathway and downregulating the expression of HIF-1α [J]. *Mol Med Report*, 2018, **17**(4): 5595-5600.
[62] Li X, Chen C, Dai Y, *et al*. Cinobufagin suppresses colorectal cancer angiogenesis by disrupting the endothelial mammalian target of rapamycin/hypoxia-inducible factor 1α axis [J]. *Cancer Sci*, 2019, **110**(5): 1724-1734.
[63] Jeong W, Rapisarda A, Park SR, *et al*. Pilot trial of EZN-2968, an antisense oligonucleotide inhibitor of hypoxia-inducible factor-1 alpha (HIF-1α), in patients with refractory solid tumors [J]. *Cancer Chemother Pharmacol*, 2014, **73**(2): 343-348.
[64] Lv Y, So KF, Wong NK, *et al*. Anti-cancer activities of *S*-allylmercaptocysteine from aged garlic [J]. *Chin J Nat Med*, 2019, **17**(1): 43-49.
[65] Xiong L, Chen CF, Min TL, *et al*. Romipeptides A and B, two new romidepsin derivatives isolated from *Chromobacterium violaceum* No.968 and their antitumor activities *in vitro* [J]. *Chin J Nat Med*, 2019, **17**(2): 155-160.
[66] Liu R, Liu Y, Zhou YD, *et al*. Molecular-targeted antitumor agents. 15. Neolamellarins from the marine sponge Dendrilla nigra inhibit hypoxia-inducible factor-1 activation and secreted vascular endothelial growth factor production in breast tumor cells [J]. *J Nat Prod*, 2007, **70**(11): 1741-1745.

Cite this article as: ZHONG Jin-Cheng, LI Xiao-Bo, LYU Wen-Yu, YE Wen-Cai, ZHANG Dong-Mei. Natural products as potent inhibitors of hypoxia-inducible factor-1α in cancer therapy [J]. *Chin J Nat Med*, 2020, **18**(9): 696-703.