Role of Hypoxia-Inducible Factor (HIF) in the Initiation of Cancer and Its Therapeutic Inhibitors

10

Sasidhar Eda, Ramakrishna Vadde, and Rajeswari Jinka

Abstract

The inadequate oxygen (O2) supply to a large extent alters the cellular microenvironment and results in hypoxia or even anoxia. Hypoxia-inducible factor (HIF) facilitates the cellular response to hypoxia. HIF, a heterodimer composed of two subunits, the subunit α and subunit β, is involved in several signaling pathways which involves both survival and death pathways, their activation and regulation. HIF is believed to be the best molecular target in the treatment of cancer, and also numerous inhibitors for HIF-1α are available today. This chapter explains the HIF-1α role in cancer and its therapeutic applications that potentially target HIF pathway.

10.1 Introduction

Constant supply of O2 is required for all the cells to carry out oxidative phosphorylation in the mitochondria for the generation of ATP by oxidative phosphorylation. Under normal regularized conditions, with the normal supply of oxygen, the cells

---

The original version of this chapter was revised. The book was inadvertently published without Abstracts and Keywords, which are now included in all the chapters. An erratum to this chapter can be found at https://doi.org/10.1007/978-981-10-6728-0_39

S. Eda · R. Jinka (✉)
Department of Biochemistry, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India
e-mail: jinkarajeswari@gmail.com

R. Vadde
Department of Biotechnology & Bioinformatics, Yogi Vemana University,
Kadapa, Andhra Pradesh, India

divide in an orderly way and are replaced with new cells when they die, are worn out, or are damaged. In contrast, in an inadequate supply of oxygen, the lack of oxygen leads the cells to enter into abnormal and stressful conditions, where the regulated cell division becomes irregular and thereby activating several mechanisms in the process to sustain their viability. The inadequate supply of oxygen (O2) in large extent alters the cellular microenvironment and results in hypoxia or even anoxia leading to the cellular transformation [56]. Under the hypoxic conditions, the transformed cells divide rapidly and result in the formation of tumors by crowding out the normal cells. In such condition, the energy requirement and production are the most important aspects to understand the differences between the proliferating and nonproliferating cells [91]. The heterogenous cells in a complex structure of tumor are undergoing different stresses, e.g., low oxygen levels in the interior, so often the core of a tumor is necrotic [32, 60, 71, 94].

Under the hypoxic conditions, due to nonavailability of oxygen, tumor cells generate energy by non-oxidative breakdown of glucose, followed by fermentation of lactic acid in cytosol [25, 28, 32, 36, 47, 91]. In such conditions, hypoxia plays a major role at different stages of cancer (initiation, accumulation, angiogenesis, and metastasis) by initiating the changes in the microenvironment, altering the oncogenic genes and normal metabolism, and in the development of new blood vessels, thereby inducing the metastasis. The cellular response to hypoxia is mainly mediated by the HIF. HIF is found in mammalian cells grown under hypoxic condition. It is stimulated in response to intrahumoral hypoxia leading to genetic alterations by activating the oncogenes and inactivating the tumor suppressor genes. HIF plays an important role in adapting the cancer cells to low oxygen condition by triggering the transcription of over 100 target genes that regulate the tumor survival and progression [122–125].

10.2   HIF Structure

Hypoxia-inducible factor (HIF) is a heterodimer composed of two subunits, the subunit α and subunit β. The HIF-1α subunit is oxygen sensitive and is a cytoplasmic protein. It is degraded by the ubiquitin–proteasome system continuously in well-oxygenated cells. The HIF-1β subunit is also known as aryl hydrocarbon receptor nuclear translocator (ARNT), a nuclear protein, independent to oxygen tension and a heterodimeric partner of aryl hydrocarbon receptor (AhR). HIF-1β is constitutively expressed to levels within the nucleus that remain relatively constant and binds to AhR and facilitates its translocation. These two subunits (α and β) belong to the family of basic helix-loop-helix (bHLH) and PER-ARNT-single-minded protein (SIM) (PAS) transcription factors. The characteristic feature of these family proteins is that they have recognizable domains and can regulate their own transcription. Among all the family members, the PAS domain was the only domain that is conserved. The N-terminal region of this PAS domain is essential to mediate DNA binding and interaction with HIF-1β subunit [118].

The subunit α has three different isoforms, HIF-1α, HIF-2α, and HIF-3α. Analogs of α subunits of HIF-1α and HIF-2α are more comprehensively studied and were compared to HIF-3α. HIF-3α is less analyzed when compared with the other HIF-α homologs. The inhibitory PAS domain protein (IPAS), a spliced variant of HIF-3α discovery, led practical information about HIF-3α. It functions as dominant-negative regulator of hypoxia-inducible gene expression and does not show any intrinsic transactivation activity as compared to the COOH-terminal transactivation domain (C-TAD) of HIF-1α and HIF-2α [111, 148].

The analogs of HIF-1α and HIF-2α share high percentage sequence identity (48%) and can heterodimerize with HIF-1β subunit. These two analogs when heterodimerized with HIF-1β subunit have distinct tissue-specific expression. The ubiquitously expressed HIF-1α is constantly expressed and degraded in presence of induced hypoxic conditions. However, HIF-2α distribution is restricted to specific tissue origins like vascular endothelial cells, the kidney, catecholamine-producing cells, renal interstitial fibroblasts, and some glomerular cells [95].

HIF-1α in its C-terminal has two transactivation regions: the N-terminal transactivation region or N-TAD (AA 531–575) and the C-terminal transactivation region or C-TAD (AA 786–826) (Fig. 10.1). HIF-1α transcriptional activity is mostly dependent upon these two domains. Under hypoxia conditions the transcription of HIF-1α is modulated by C-TAD whereas stabilization by N-TAD. The requirement of C-TAD or N-TAD for different gene sets regulation is completely dependent on oxygen tension. N-TAD, also known as an oxygen-dependent degradation domain (ODDD), is responsible for stabilizing HIF-1α against degradation as hydroxylation of conserved prolyl residues resides in this region. This domain is also important in mediating oxygen regulation stability. Prolyl-4-hydroxylases (PHDs), 2-oxoglutarate-dependent oxygenase superfamily enzymes, mediate this hydroxylation and promote the subunit degradation [77].
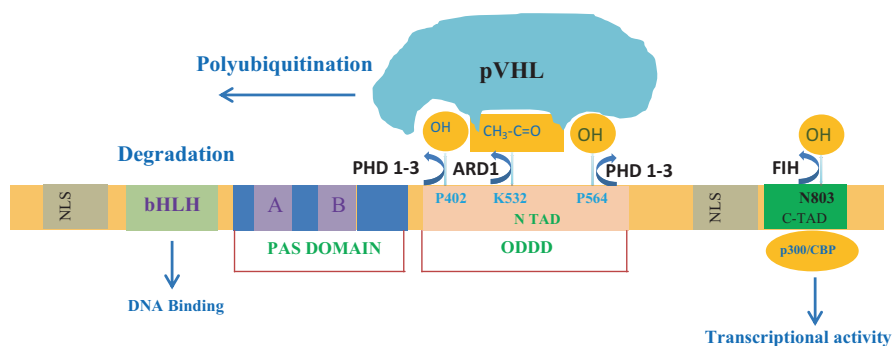

Fig. 10.1 Oxygen-dependent regulation of HIF-1α activity (This figure is adapted from [95, 121] with modifications)

HIF-1α hydroxylation does not occur in hypoxic conditions. In this condition α subunit along with other cofactors acts as transcription factor and thereby migrates to nucleus and dimerizes with β subunit and initiates its transcriptional program [18]. The resultant active protein that is HIF-1 is a messenger which is translocated to the nucleus to induce transcriptional responses to hypoxia [171].

The active HIF-1 protein activates transcription of target genes by adhering to specific hypoxic response elements (HRE) which comprises A/GCGTG consensus motif. Similarly HIF-2 and HIF-3 are resultant active heterodimers of HIF-2α or HIF-3α with ARNT [119]. The presence of two nuclear localization signals in bHLH domain (17–33 amino acids) and COOH-terminal regulatory domain (718–721 AA) results in translocation of HIF-1α into nucleus [110].

The interaction of C-TAD with coactivators CBP/p300 results in the change in transcription of HIF-1α under hypoxia. This interaction is governed by the CH1 region of p300/CBP and also improved by SRC-1, and synergistic effect was observed at limited concentrations. Phosphorylation of p300 by the MAPK pathway increases the HIF-1 α/p300 complex formation and thereby increases the transcriptional activity of HIF-1. Upon blocking of HIF-1α/p300 CH1 interaction, HIF-1 transactivation is inactivated as the p300-CH1 interacting protein and p35srj (for serine–glycine-rich junction) bind to p300/CBP. C-TAD interaction with p300/CBP does not occur in normal conditions. This is due to oxygen-dependent hydroxylation of N803 residue in the carboxyl-terminal transactivation domain (CAD) of HIF-1α by factor-inhibiting HIF (FIH-1), a 2-oxoglutarate-dependent dioxygenase enzyme [77]. It prevents the interaction of HIF-1α with transcriptional coactivators, p300 and CBP (cAMP response element-binding protein). Small redox protein thioredoxin-1(Trx-1) under both normoxic and hypoxic conditions has been reported to enhance the binding of CBP/p300 to the C-TAD of HIF-1α. This leads to the expression of HIF-1α and its downstream target VEGF and improved angiogenesis [39]. Transactivation of HIF-1 by Ref-1 leads decrease of a cysteine residue in the C-TAD of HIF-1α. But, the useful status of this cysteine residue and the consequence of CBP/p300 remains doubtful [59, 77].

The PHD enzymes (prolyl hydroxylase-domain protein) hydroxylate the proline 402 and 564 residues that are present in LXXLAP amino acid motif of ODDD of HIF-1α subunit under normal oxygen conditions. This allows modified HIF-1α at prolyl sites to bind to the von Hippel–Lindau (VHL) tumor suppressor protein. Only modified HIF-1α is able to bind to the VHL protein whose binding may also be promoted by acetylation of K532 residue by the arrest-defective-1(ARD1) acetyltransferase [42]. This VHL protein is a recognition component of an E3 ubiquitin-protein ligase. This ligase finally targets the HIF-1α for proteasomal degradation by 26S proteasome. OS-9 is another factor that impacts on the degradation of HIF-1α. OS-9 interacts HIF-1α directly, and the prolyl hydroxylases PHD2 and PHD3 and forms a ternary complex. This complex formation stabilizes the interaction between HIF-1α and PHDs, thus helping HIF-1α hydroxylation and pVHL-mediated ubiquitination, and finally leads to degradation of HIF-1α [34].

The HIF-1 activity depends on the regulation of its subunits (α and β) at several levels including transcription, translation, ubiquitin-mediated protein breakdown, and nuclear translocation. The loss of this activity decreases the vascularization, tumor growth, and energy metabolism. HIF-1, by employing transcriptional coactivators, controls the expression of many genes. The HIF-1 expression directly regulates the tumor growth. The overexpression of HIF-1 promotes the tumor growth by increasing HIF-1 transcription factor activity. The protein products play important roles in the severe and long-lasting adaptation to hypoxia, including angiogenesis, erythropoiesis, and pH regulation glycolysis. Pulse-chase studies of MCF-7 breast cancer cells stimulated with heregulin increase HIF-1α synthesis but do not activate transactivation-region function that was stopped by rapamycin in PC-3 prostate cancer cells. In another study when Rat-1 fibroblasts and breast cancer cells (MCF7) were overexpressed with BNIP3 (BCL2/adenovirus E1B 19 kDa interacting protein 3) and NIX (BNIP3 homolog) at the transcriptional level, it induced apoptosis. The cell death induced by BNIP3 is mediated by binding of BNIP3 to anti-apoptotic proteins Bcl-2 and Bcl-xL and inhibiting those proteins. This hypoxia-induced apoptosis may be HIF-1α dependent because BNIP3 promoter contains HRE [46].

10.2.1 Glucose Metabolism

The glycolytic rates in normal cells when compared to cancerous cells are very high even in the presence of oxygen, and energy required for cancerous cells is generated by glycolysis followed by fermentation of lactic acid in cytosol rather than oxidation of pyruvate in mitochondria, also defined as "aerobic glycolysis" [25, 28, 32, 36, 47, 91].

The aerobic glycolysis is an important pathway by which cells in the body could generate energy using glucose as main fuel source, whereas glutamine becomes the secondary fuel source for carcinogenic cells [91]. Glucose, the primary fuel source after entering the cell, is metabolized to pyruvate by a multistep set of reactions called glycolysis [32]. In typical normal cells, this pyruvate undergoes oxidative phosphorylation (OXPHOS) in mitochondria through Krebs cycle (TCA cycle) to generate energy (ATP) in order to meet the energy demands of the cell; however if oxygen levels are low, pyruvate is converted into lactate in cytoplasm through the action of lactate dehydrogenase (LDH) enzyme [28, 44]. In glycolysis one glucose molecule is broken down into two molecules of pyruvate thus generating two ATPs by consuming NAD+, whereas in OXPHOS one glucose molecule produces 30 ATPs by oxidation of NADH and FADH2, clearly stating that OXPHOS is more efficient than glycolysis [36, 139]. The main difference between cancer and normal cells dwells here. In cancer cells the pyruvate is converted into lactate even when an ample amount of oxygen is available [28]. For creation of new biomass such as nucleotides, lipids, amino acids, and nonessential amino acids, cancer cells require more nitrogen. The excess glucose that is generated is deviated to produce

nucleotides through pentose phosphate stunt (PPS) [32]. In multiple steps, PPS pathway by the action of malic enzyme generates NADPH reducing equivalents to produce more pyruvate. These NADPH reducing equivalents are required to produce acetyl CoA from citrate through the action of ATP-citrate lyase (ACL) in cytosol [25]. This production leads to synthesizes of fatty acids that are required for membrane production. Glutamine an essential metabolite acts as an intermediate in the bloodstream to transport reduced nitrogen and is also required for cell growth. This metabolite is utilized by tumor cells as secondary energy source because it plays a crucial role in uptake of essential amino acids and can replenish the TCA cycle by supplying carbon, and also through the action of malic enzyme, it can produce more pyruvate [24]. More NADPH in PPS pathway is produced by transactivation of TP-53-induced glycolysis and apoptosis regulator (TIGAR) by p53 oncogene. PI3K/Akt and Ras are activated through RTKs by stimulation of growth factor. RTK signaling to C-Myc activates many genes that are involved in lactate production and glycolysis [25, 28, 32, 47, 91].

The sequence initiation of angiogenesis and glycolysis in differentiating cells is arbitrated partly by triggering HIF-1. HIF-1 target genes are mainly the genes that are intricate in the glucose uptake and glycolysis. HIF-1 controls expressions of phosphoglycerate kinase 1, aldolase A, and pyruvate kinase M in the glycolytic pathway, as well as expression of the glucose transporters (GLUT1 and GLUT3), which facilitate uptake of glucose by the cells [62]. It also induces adaptive responses to ensure that the cells should have sufficient energy levels and thus allowing their survival in a hostile environment [77, 140].

10.3   HIF-Associated Pathways

Although HIF-1α transcription is constant, the mRNA translation and transactivation activity of HIF-1α are induced by associated pathways and cell surface receptors of tyrosine kinases and G protein-coupled receptors. In pseudohypoxia circumstances, HIF-1α subunits are stabilized by a variety of oxygen-independent signaling and cellular stress events. In hypoxia condition, in response to growth factor stimulation, the HIF-1α levels increase in a specific manner. If hypoxia is associated with decreased degradation of HIF-1α, growth factors, cytokines, and other signaling molecules stimulate synthesis of HIF-1α through stimulation of the phosphatidylinositol 3- kinase (PI3K) or mitogen-activated protein kinase (MAPK) pathways [98].

Activation of phosphatidylinositol-4, 5-bisphosphate-3-kinase (PI3K)/AKT pathway has been shown to upregulate the HIF-1α protein translation. Under non-hypoxic conditions, due to extremely short half-life, HIF-1α protein expression is particularly sensitive to changes in the rate of synthesis. In the phosphatidylinositol-3-kinase (PI3K) pathway, binding of a growth factor (e.g., insulin-like growth factor 1, IGF-1) to its cognate tyrosine kinase receptor activates PI3K by phosphorylation and stimulates the downstream serine/threonine kinase Akt (protein kinase B). This

stimulation subsequently phosphorylates mammalian target of rapamycin (mTOR), providing a link between the microenvironment and HIF signaling [118, 120]. mTOR increases protein translation and mediates its action by phosphorylation of the mRNA cap-binding protein eukaryotic initiation factor 4E (eIF4E)-binding protein (4E-BP1). mTOR provide a potential mechanism for increasing HIF-1a levels under normoxic conditions by disrupting the integrity of 4E-BP1, which is essential for inhibiting cap-dependent mRNA translation. In hypoxic conditions mTOR may increase HIF-1α levels by the mechanism in which it occurs independently without eIF4E. Alternatively, mTOR induces protein translation by phosphorylation of p70 S6 kinase (S6K) which promotes ribosomal protein S6 phosphorylation, a substrate. This pathway is upset by a tumor suppressor protein (PTEN) which backs the phosphorylation of PI3K products.

In MAPK pathway, certain growth factors are involved in activation of RAS; this activation in turn stimulates RAS/RAF/MEK/ERK kinase cascade and induces HIF-1α transactivation-domain function. Growth factors activate the mitogen-activated protein kinase (MAPK) to phosphorylate MAPK (extracellular signal-regulated kinase, ERK). Activated ERK is then capable of phosphorylating p70S6K1, 4E-BP1, S6K, and MAP kinase interacting kinase (MNK) [107, 161]. MNK can also phosphorylate eIF-4E directly that activates the translation initiator factor together with mTOR by inhibiting the 4E-binding protein (4E-BP). These signaling events result an increased rate of HIF-1α protein synthesis through its effects on eIF4E. ERK and p70S6K1 are essential factors that are required for HIF-1α mRNA translation. ERK regulates HIF-1α synthesis and also plays a pivotal role in its transcriptional activation. ERK phosphorylates the coactivator CBP/p300, hence increasing HIF-1α/p300 complex formation, and thus stimulates its transcriptional activation function (Fig. 10.2) [7, 26, 67, 70, 97].

The von Hippel–Lindau protein (pVHL) pathway along with p53, a tumor suppressor gene which induces apoptosis by regulating proteins such as Bax, regulates the levels of HIF-1α. In environmental stress or DNA damage, p21 mediates p53 to cause growth arrest (Fig. 10.2). The murine double minute 2 (Mdm2) ubiquitin-protein ligase mediates ubiquitination and proteasomal degradation of HIF-1α. Direct binding of the p53 tumor suppressor gene to the ODD domain of HIF-1α causes the ubiquitination and degradation [46]. It is evident that absence of p53 tumor suppressor gene in certain types of tumor cells enhances HIF-1α levels. In hypoxic tumors, mutations in tumor suppressor genes cancel the Mdm2-mediated degradation of HIF-1α. It was studied that Hsp90 inhibitors such as geldanamycin (GA) could nullify HIF-1α levels even in cell lines lacking von Hippel–Lindau protein (pVHL) regardless of the availability of oxygen. Mutation of prolyl residues (p^{402} and p^{564}) in HIF-1α does not protect HIF-1α from geldanamycin (GA)-induced degradation, suggesting that Hsp90 degradation involves a novel E3 ubiquitin ligase [46, 131, 140].

Redox (reduction-oxidation)-dependent processes displays a vital role in the control of HIF-1α. Some studies have shown that generation of ROS can start both MEK/ERK and PI3K/Akt signaling pathways. This activation leads to enhanced HIF-1α expression in human cancers such as ovarian, prostate, and breast cancer
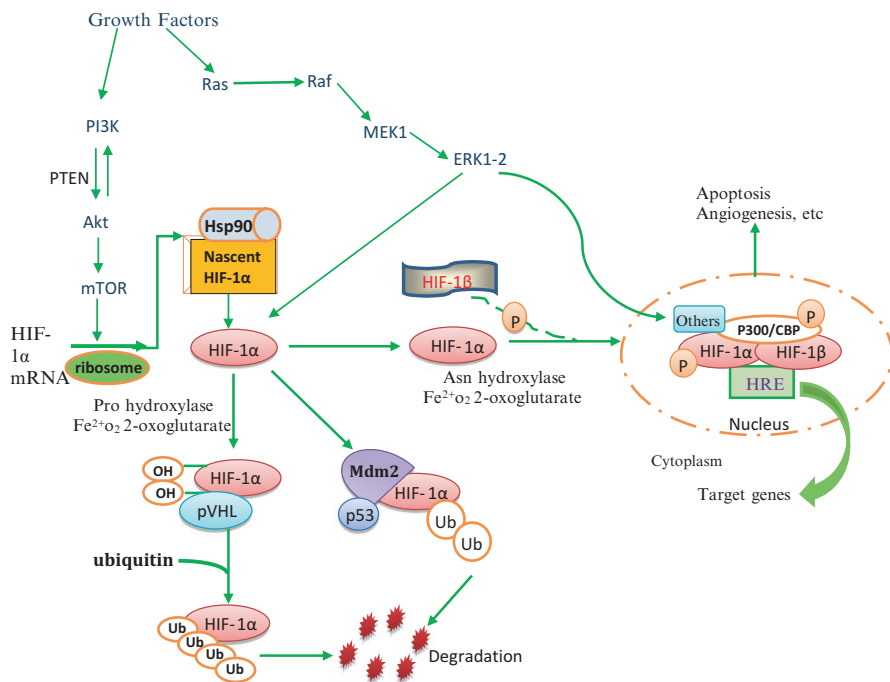
Fig. 10.2 Regulation of HIF-1α activity at different levels (This figure is adapted with some modifications from [95, 153])

[34, 171]. Breast carcinoma is characterized by persistent ROS generation. In human prostate cancer cells, carcinogens such as vanadium and arsenate were shown to elevate ROS and induce HIF-1α and VEGF expression through p70S6K1 activation. In human ovarian cancer cells, it is shown that p70S6K1 activation is stimulated by elevated epidermal growth factor (EGF) and its receptor (EGFR) which triggers H_2O_2 production.

Under hypoxia, mitochondrial ROS and intracellular secondary messengers such as CaM (calcium binding protein) levels increase and stimulate the accumulation of HIF-1α. CaM targets proteins (CaM kinase II, calcineurin, and actin) involved in the stimulation of transcriptional activity of HIF-1α expression. Thus, the inhibition of Ca2þ/CaM by a CaM-dominant mutant, Ca2þ/CaM antagonist such as HBC, or Ca2þ chelator downregulates the transcriptional activity of HIF-1, and subsequently angiogenesis is suppressed. The ROS levels in mitochondria increase through transfer of electrons from ubisemiquinone to molecular oxygen at the Q_0 site of complex III electron transport chain (ETC). HIF-1α activation is modulated by inhibiting its hydroxylation by the prolyl and asparaginyl hydroxylases. Mitochondrial ROS also induces signaling components of HIF-1α (ERK and p38 MAP kinase pathways) under hypoxic conditions. The activated ERK2 phosphorylates HIF-1α and increases its transcriptional activity [34, 42, 110].

10.3.1 HIF and Cell Cycle

Under hypoxia, there are different adaptive responses to lessen oxygen and nutrients for hypoxia-/hypoglycemia-regulated genes, which are involved in the cell cycle regulation. These genes are either HIF-1α dependent (p53, p21, Bcl-2) or HIF-1α independent (p27, GADD153). Hypoxia causes a HIF-1-dependent escalation in the expression of the cyclin-dependent kinase (CDK) inhibitors p21Cip1 and p27Kip-1and hypophosphorylation of retinoblastoma protein (Rb). Decreased activity of CDK complexes and hypophosphorylation of retinoblastoma protein regulate the cell cycle progression in response to hypoxia. HIF-1α activation may serve as a primary gatekeeper at the G1/S transition through at least two distinct mechanisms – the action of CKIs and another by cyclin E regulation. HIF-1α regulates cyclin E, not the cyclin A protein levels, but both may bind CDK2 and control its kinase activity dependent upon phase of cell cycle [15, 42, 43].

10.3.2 HIF and Cancer

HIFα is expressed in various types of cancers that include colorectal, liver, gastric, pancreatic, renal, gastrointestinal (IBD), esophagus, and many others. But mechanism and the factors that regulate the HIF1α expression remains poorly understood in cancer. Several studies demonstrated the associated mechanisms that activate the HIFα and their upstream or downstream factors. In this context, we explore recent updates on the impact of HIFα in different types of cancers.

HIF-1α and HIF-2α play a significant role and have overlapping and distant functions in inflammatory bowel disease (IBD) [154, 158]. IBD, a chronic inflammatory disease of the intestine, is characterized by repeated mucosa wounding and losing of intestinal epithelial barrier functions. It comprises two distinct pathological entities, ulcerative colitis (UC) and Crohn's disease (CD) [157, 158]. Immunohistochemical and immunostaining studies of surgical specimens from patients with IBD revealed higher vascular density in diseased tissue than in normal tissue [40].

Studies revealed that HIF was essential for restoration and intestinal barrier integrity [63]. Mouse models and cell studies demonstrated distinct functions for HIF-1α and HIF-2α and regulate diverse sets of genes to modulate the epithelial barrier [41, 92, 132, 154]. Regulation of HIF-1α and HIF-2α by different subset of genes also promotes disruption of intestinal tight junctions and increased barrier permeability. HIF-1α is a critical transcriptional factor in intestinal epithelial cells and is beneficial in regulating the epithelial barrier following inflammation. HIF-1α activation in intestinal epithelial cells decreases proinflammatory cytokines. Two mouse models of colon cancer, a sporadic and a colitis-associated colon cancer model, were assessed and proved that activation of HIF-1α in intestinal epithelial cells did not result in spontaneous tumor formation. HIF-2α activates several proinflammatory mediators and is important in wounding response, whereas its activation increases inflammation [157, 158, 168].

Pharmacologic inhibition of prolyl hydroxylases (PHDs) primes more vigorous activation of HIF-1α rather than HIF-2α. PHD inhibitors activate HIF-1α and HIF-2α in pulsatile manner and protect acute colitis in murine models. DMOG, a pan-hydroxylase inhibitor, activates the HIF pathway by mimicking hypoxia through the inhibition of hydroxylase activity, leading to stabilization and transactivation of HIF-1α [22]. AKB-4924, a HIF-1-specific prolyl hydroxylase inhibitor (PHDi), enhances innate immunity by robustly activating HIF-1α [61]. FG-4497, a novel PHD inhibitor, provides a protective adaptation in murine TNBS colitis [114].

HIF-1α is a critical protein in the development of colorectal cancer (CRC) [84]. Various studies have reported the role of HIF-1α in angiogenesis and tumor progression via regulation of VEGF in human colorectal carcinoma [75]. In colon cancer HIF isoforms have different cellular functions. In human colon cancer tissues, expression of HIF-1 α and, to a lesser extent, HIF-2 α was linked to upregulation of VEGF and tumor angiogenesis [52]. Overexpression of HIF-1α was found in the tissue of stage III and stage IV lymph nodes and liver metastases [13]. HIF-1α expression was strongly observed in the epithelium around the necrosis region of tumor compared to normal mucosa suggesting a significant correlation of HIF-1α expression along with CXCR4, VEGF, and microvessel density. Immunohistochemical studies of tumor cells in colon cancer cases by Wu et al. [152] also indicated that HIF-1α expression correlates with tumor TNM stage, lymph node status, tumor invasion, and distant metastases. JMJD2B upregulates hypoxia-inducible genes involved in cancer cell proliferation, apoptosis, cell cycle arrest, and invasion through specifically demethylating the H3K9me3 on their promoters. Study by Fu et al. [33] suggested a significant role of JMJD2B in CRC tumorigenesis and progression in HIF-1α-dependent manner under hypoxia. Activation of HIF-1α results in increasing transcription of STAT-3 and HSP90 in the CRC cell lines. This interaction between HIF-1α and STAT-3 in the CRC cell lines is dependent on the presence of an active HSP90 [35]. HSP90 in HCC cells regulated the levels of HIF-1α by inhibiting the ubiquitination and proteasomal degradation of HIF-1α. Further studies also analyzed a positive correlation between HSP90 and HIF-1α, with statistical significance, showing they may exert a synergistic effect on the occurrence, development, invasion, and metastasis of colorectal cancer [88, 155]. The results by Zhang et al. [167] and Zhang et al. [169] suggest that HIF-1α enhances EMT and cancer metastasis by binding to ZEB1 promoter in CRC and proposed a novel molecular mechanism for HIF-1α-inducing epithelial–mesenchymal transition (EMT) and cancer metastasis. LRG1 plays a crucial role in the progression of CRC by regulating HIF-1α expression thereby inducing VEGF-A expression and EMT markers of E-cadherin, VDR, N-cadherin, α-SMA, vimentin, and Twist1. In human CRC cells, HIF-1α under hypoxia induces B-cell CLL/lymphoma 9 protein (BCL-9) expression, an important underlying mechanism for increased BCL-9 expression [135].

In esophageal squamous cell carcinoma, HIF-1α expression levels significantly correlates with the expression of VEGF protein and with initial response to concurrent CRT. HIF-1α expression strongly apparent within nuclei and/or cytoplasm of

tumor cells and its expression are also found to be different in two separate tumor microenvironments: SCCs and ACs of the esophagus cancer proposing a different mechanism for HIF-1α expression in esophagus cancer [45, 96, 106].

Under hypoxic conditions, ERK1/2 phosphorylates and activates HIF-1α in pancreatic cancer cells. This activation contributes the ABCG₂ expression by inducing binding of HIF-1α to target promoter region for transcription [51]. Recent findings in pancreatic cancer patients indicated that HIF-2α induces cell migration, invasion in vitro, and regulated E-cadherin and MMPs protein expression; these are vital to epithelial–mesenchymal transition (EMT). It is regulated by binding of Twist2 protein to E-cadherin promoter; this indicates HIF-2α may act as an effective therapeutic target for prevention of pancreatic cancer [159].

HIF-1α is an important mediator and also acts as potential target for treatment of gastric cancer. The overexpression of HIF-1α in human gastric cancer proves the fact of it being a potential target. While regulating VEGF expression in cancer cells, it also plays a major role in the formation of complex proangiogenic microenvironment in tumors, and thereby affecting vessel morphology and vessel function. The in vitro studies in metastatic human gastric cancer cells evidenced that HIF-1α was not required for cellular proliferation. The inactivation of the HIF-1α activity by 2ME significantly reduced migratory, invasive, and adhesive features of gastric cancer cells. Inhibition of its function has proven the antitumor efficacy in rodent models and angiogenesis. In human gastric cancer cells, inhibition of HIF-1α activity by transfection with a construct expressing a dominant-negative mutant version of HIF-1α (pHIF-1αDN) that dimerizes with HIF-1β to form HIF-1 complexes that cannot activate transcription leads to impaired gastric tumor growth, angiogenesis, and vessel maturation [115, 131]. HIF-1α also regulates transcription factors (NF-κB1, BRCA1, STAT3, STAT1) and their corresponding network genes (MMP1, TIMP1, TLR2, FCGR3A, IRF1, FAS, and TFF3) that were associated with hypoxia, inflammation, and immune disorder in gastric cancer [145]. In the recent study, it is revealed a novel mechanism in three GC cell lines, 44As3, 58As9, and MKN45, and the integrity of mitochondrial autophagy (mitophagy) might determine the aggressiveness of cancer via the mitochondrial ROS (mtROS)/HIF-1α interplay under hypoxic conditions [127]. Relative mRNA expression of miR-421 (microRNAs), a crucial factor in carcinogenesis, was found to be upregulated by HIF-1α in gastric cancer tumor tissues [38]. Low expression of microRNA-186 (miR-186) facilitates aerobic glycolysis and suppresses cell proliferation induced by HIF-1α in gastric cancer cell lines. The in vivo xenograft tumor studies demonstrate that the miR-186/HIF-1α axis has an antioncogenic role in gastric cancer [86]. The in vitro and in vivo results revealed that dextran sulfate (DS) may reduce tumor metastasis through inhibition of HIF-1α and ITGβ1 expression in gastric cancer cells [156]. In hypoxic gastric cancer cells, angiopoietin-like protein 4 (ANGPTL4), a hypoxia-inducible gene expression, is independent of HIF-1α [73]. Expression of HSP60 or HIF2α serves as predictive marker for diagnosis of gastric cancer. In gastric cancer cells, HSP60 or HIF2α inhibition induce apoptosis and suppresses cell mobility by negative relation of MEK/ERK signaling [138].

10.3.3 HIF Pathway Inhibitors

Research is currently focused to target HIF involved pathways, and several drugs have been developed by considering the fundamental role of HIF and the analogs in the activation of various pathways involved in tumor progression in several cancers. Based on the mechanism of action, HIF inhibitors can be divided into the agents that modulate HIF1α *(1)* mRNA expression, *(2)* protein translation, *(3)* protein degradation, *(4)* DNA binding, and *(5)* transcriptional activity. The inhibitors representing each group are depicted in Fig. 10.3 and discussed below and listed in Table 10.1.

In diverse human cancer cell lines, the elevation of HIF-1α protein is by PI3K/Akt/mTOR signaling pathway. Various compounds for inhibiting PI3K/Akt/mTOR signaling pathway are under the exploitation stage, and few compounds are in clinical trials. Inhibitors *wortmannin*, *LY294002*, *GDC-0941*, and *PI-103* specifically inhibit PI3 kinase in dose-dependent manner [105]. FDA-approved drugs like *rapamycin* and its chemical derivatives (*temsirolimus* and *everolimus*) have more potency to target mTOR and inhibit the protein translation of HIF-1α at cellular levels [113].

Glyceollins, a set of phytoalexins present in soybean, potentially inhibit the HIF-1α synthesis and decrease stability by blocking the PI3K/AKT/mTOR pathway and interaction of Hsp90 with HIF-1α [81].

TSL-1, an agent in aqueous extracts of *Toona sinensis* (TS) leaves, which induces apoptosis via mitochondria-dependent pathway. TSL-1 stops cell division in G0/G1 phase via the decrease in cyclin D1, cyclin-dependent kinases (CDK2 and CDK4), and induced p53 expression. TSL-1 suppresses progression of cell cycle and motility through phosphorylation inhibition of JAK2/stat3, Akt, MEK/ERK, and mTOR. TSL-1 also inhibits p21, HIF-2α, c-*Myc*, VEGF, and MMP9 expressions and its anti-migration activity [19].

EZN-2968, an antisense oligodeoxynucleotide that precisely targets HIF-1α. A trial with administered EZN-2968 in patients with advanced solid tumors observed modulation of HIF-1α mRNA, protein, and its target genes [55]. In MCF-7 xenografts, *aminoflavone*, a potential therapeutic target for several human diseases, inhibited HIF-1α protein accumulation and expression of target genes [137].

GL 331, a topoisomerase II inhibitor, suppresses tumor-induced angiogenesis. In CL1-5 cells treated with GL331 downregulates HIF-1alpha expression through transcriptional repression. It also exerts cytotoxic effects on the glioma cells [16, 20].

Camptothecins (CPTs) analogs, topotecan and irinotecan, are active in different human tumors and shown significant anticancer activity against various tumors by inhibiting DNA topoisomerase I. *Topotecan* is the approved agent using in the treatment of lung cancer [37]. *Irinotecan* is a cytotoxic drug used for the patients suffering with colorectal cancer (CRC) in advanced stage. *SN-38* (10-hydroxy-7-ethyl-camptothecin) is the active metabolite of irinotecan prevents re-ligation of single-stranded DNA breaks induced during the DNA synthesis [37, 90]. These agents have shown the antitumor activity in xenograft model by inhibiting HIF-1α
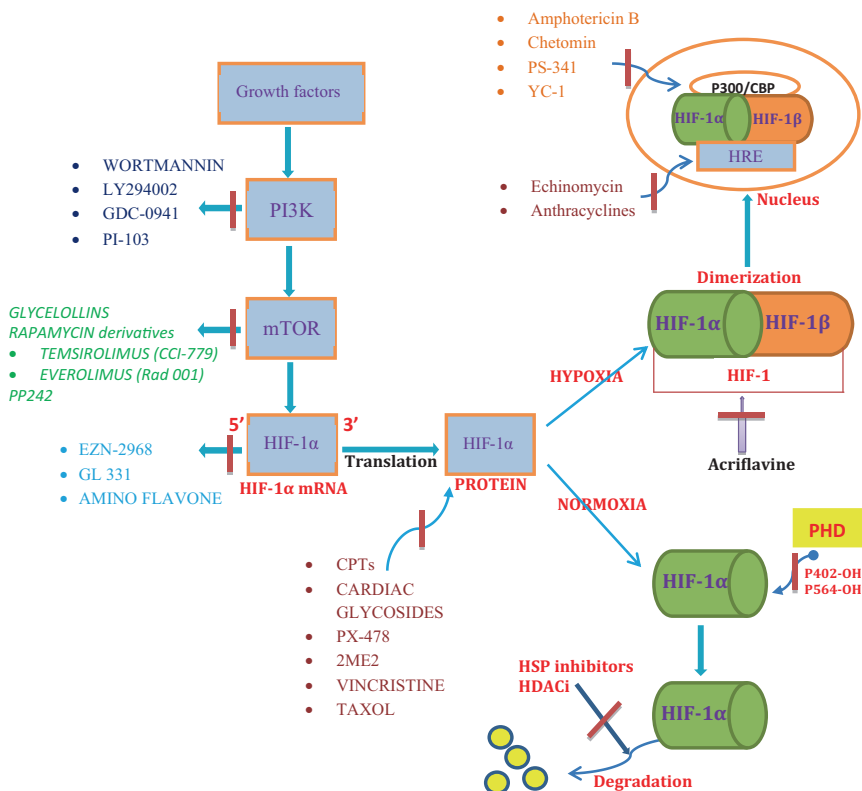
Fig. 10.3 Inhibitors that modulate different HIF-1α pathways

Table 10.1 Classification of HIF-1α pathway inhibitors and their molecular targets

Inhibitory mechanism	Target	Compound
PI3/AKT/mTOR inhibitors	PI3K	Wortmannin
		LY294002
		GDC-0941
		PI-103
	AKT/mTOR, Hsp90	Glyceollins
		Toona sinensis (TSL-1)
	mTOR	Rapamycin derivatives • Temsirolimus (CCI-779) Everolimus (Rad 001) PP242
mRNA expression	HIF-1α mRNA	EZN-2968
		GL 331
		Amino flavone
Protein translation	Topoisomerase I (top-1) inhibitor/ HIF-1α accumulation inhibitor	Camptothecins (CPTs) • Topotecan (NSC-609699) • (PEG-SN 38) • SN-38 Irinotecan

(continued)

Table 10.1 (continued)

Inhibitory mechanism	Target	Compound
	Topoisomerase II inhibitor	GL-331
	HIF-1α/mTOR-independent mechanism	Cardiac glycosides • Digoxin • Ouabain • Proscillaridin Strophanthidin glycoside
	HIF-1α/pVHL and p53-independent mechanism	PX-478
	Disrupts tumor interphase microtubules	2ME2 (2-methoxy-estradiaol) derivatives • ENMD-1198 • ENMD-1200 • ENMD-1237 Vincristine
		Taxol
HIF-1α degradation	HSP90 inhibitors	Geldanamycin derivatives • 17-AAG • 17-AG • 17-DMAG Radicicol derivatives • KF58333 • Apigenin IPI-504 (retaspimycin)
		Y-632
	FIH	YC-1
	Farnesyl transferase inhibitor	SCH66336
	Histone deacetylase inhibitors (HDACi)	Sirtuin1 (SIRT1)
		FK228 (romidepsin)
		Trichostatin A (TSA)
		LW6
		LAQ824
		LBH589
	TRX-1 signaling (thioredoxin-1)	PX-12
		Pleurotin
		AJM 290
		AW 464
HIF-α/HIF-1β dimerization inhibitors	HIF-1α/2α PAS B-domain	Acriflavine
	HIF-2α PAS B-domain	PT-2385
Transcriptional activity	p300 recruitment	Chetomin
		Bortezomib (PS-341)
	FIH interaction and p300 recruitment	Amphotericin B
	Hsp70	Triptolide
	Histone acetylation with repression of p300	FM19G11
DNA binding	HRE	Echinomycin

		Anthracyclines derivatives
		    Doxorubicin (DXR)
		    Daunorubicin (DNR)

accumulation. Clinical trials of these compounds are under progress to provide evidence as anticancer activity agents.

Cardiac glycosides, a group of natural products used in cardiac congestion and cardiac arrhythmias treatment. Recent studies suggested that cardiac glycosides have potential characteristic properties for the treatment of cancer [100]. Cardiac glycosides also inhibit cancer cell proliferation at nanomolar concentrations [117]. For example, *strophanthidin glycoside*, an organic solvent extract from *Crossosoma bigelovii*, showed the HIF-1α translation inhibitory effect [68].

Digoxin, a cardiac glycoside extracted from the foxglove plant, having antitumor activity against many cancers including lung, colon, prostate, and ovary. It shows activity through Erk and stress response pathways [30]. It exerts antitumor properties through antiproliferative and apoptosis mechanisms in HepG2 cell line cultured with different concentrations of digoxin [133]. Digoxin when treated also has shown to prolong tumor latency and hampers tumor xenograft growth in mice. It also inhibits HIF-1α expression and its target genes *VEGF*, *GLUT1*, *HK1*, and *HK2* [166]. Digitoxin in H1975 cells showed a significant cytotoxic effect by causing G2 phase arrest and suppressed microtubule polymerization through decreasing α-tubulin [170].

Ouabain is another cardiac glycoside used as novel anticancer HIF-1α antagonist. It can regulate HIF-1α translation and affects neither HIF-1α mRNA levels nor protein degradation. Studies revealed that inhibitory effect of ouabain on HIF-1α protein synthesis is by eIF4E rather than mTORC1, eIF2α signaling, or Na(+)/K(+)-ATPase inhibition. Mechanistically, ouabain straightly binds to eIF4E and disrupts association between IF4E/eIF4G complex rather than eIF4E/mRNA complex both in vitro and in vivo, finally suppressing the intracellular CAP-dependent translation [14].

Proscillaridin A exerts its cytotoxic activity by targeting both topoisomerase I and II enzymes simultaneously. In human fibroblasts it elevates intracellular Ca2+ concentration, activates caspase-3, and induces apoptosis relatively at high concentration. It exerts the antiproliferative and apoptotic activity at nanolevel drug concentrations (30 and 100 nM) [10, 151].

PX-478 (*S*-2-amino-3-[4′-*N*,*N*,-bis(chloroethyl)amino] phenyl propionic acid *N*-oxide dihydrochloride) decreases Hif-1α levels in both in vitro and in vivo by suppressing mRNA and blocking translation. PX-478 inhibitory mechanism is independent of pVHL or p53. This drug inhibits HIF-1α levels and transactivation in a variety of cancer cell lines including HT-29, PC-3, DU-145, MCF-7, Caki-1, and Panc-1. The effect of PX-478 is limited to hypoxia, as baseline levels of vascular endothelial growth factor is not altered under normoxic conditions [69, 149]. A recent study showed that PX-478 significantly decreased or inhibited extra skeletal bone formation by inhibition of Hif1α. This finding indicates that Hif-1α represents a promising target to prevent and treat pathologic extra skeletal bone or heterotopic ossification (HO) [2].

2-Methoxyestradiol (2ME2) is a natural estrogen metabolite having antiangiogenic, antiproliferative, and pro-apoptotic drug activities. It culminates induction of apoptosis by diverse cellular effects including microtubule disruption, commencement of signal transduction pathways, and generation of reactive oxygen species

[102]. $2ME_2$ targets apoptosis in rapidly proliferating cells of both the tumor cell and endothelial cell compartments and inhibiting blood vessel formation. The ability of $2ME_2$ to inhibit metastatic spread in several models adds to its therapeutic value for cancer treatment at various stages of the disease. Many genes regulating cell death and repression of growth/survival machinery were also induced transiently in multiple myeloma (MM) cells. Cells under normoxia and hypoxia conditions when exposed to 2-ME reduced mRNA expression of HIF-1α and HIF-2α were observed [4, 8]. 2ME2 significantly induced apoptosis in HIF-1α overexpressed AML cells by suppressing the expression HIF-1α. In vivo 2ME2 has been shown to downregulate HIF-1α target genes, such as for VEGF, phosphoglycerate kinase, glucose transporter-1, GLUT1, and HO-1 [8, 172]. In clinical trials the 2ME2 was noticed to target both tumor cells and neovasculature in preclinical models. The report of first Phase I trials of 2-methoxyestradiol, alone and in combination with docetaxel, was well tolerated in patients with metastatic breast cancer (MBC) [23, 53]. 2ME2 analogs (*ENMD-1198*, *ENMD-1200*, and *ENMD-1237*) with superior properties have been identified [76, 109, 128].

Few compounds like *Taxol* and *vincristine* also inhibit protein translation of HIF-1α by disrupting tumor interphase microtubules. Taxol induces static magnetic field (SMF) effect on microtubules to cause abnormal mitotic spindles that delay cell exit from mitosis [93]. Vincristine clinical trials in adults have demonstrated clinical activity without dose-limiting neurotoxicity. The safety, tolerability, and activity of vincristine might be reasons for FDA approval for adults with relapsed acute lymphoblastic leukemia [126].

Hsp90 antagonists induce degradation of HIF-1α proteins because binding of HSP90 to HIF-1α promotes HIF-1α activity [95]. Heat shock protein 90 is a 90-kDa ATPase-dependent molecular chaperone which is a ubiquitously expressed and highly conserved. The expression of Hsp90 in cancer cells is generally higher than that in normal cells. The Hsp90 proteins include a wide variety of signal-transducing proteins that regulate cell growth and differentiation; these are like protein kinases and steroid hormone receptors [101]. Hsp90 inhibitors may be organ-specific and should be carefully monitored, and they have some effects on cell adhesion-associated molecules. Hsp90 has long been regarded as an emerging drug target for a wide spectrum of cancers. Heat shock protein inhibitors are a diverse group of agents which have been verified to have pro-apoptotic effects on malignant cells [3, 129]. The high sensitivity of the inhibitor in cancer cells is proposed due to the formation of the Hsp90–cochaperone–client super complex that is highly unstable and possesses high ATPase activity [89]. Initial development of hsp90 inhibitors, *geldanamycin* and *17-AAG* (17-N-allylamino-17-demethoxygeldanamycin), showed nearly 100-fold higher binding affinity in cancer cells than in normal cells. The effect is restricted by hepatotoxicity and need for solvent carrying agents. On the other hand, *retaspimycin*, or *IPI-504*, a derivative of geldanamycin and 17-AAG, is highly soluble in water and has shown promising activity in gastrointestinal stromal tumor in Phase I/II trials [28]. Currently, Phase I/II trials are underway in the evaluation of dosing schedules and activity for IPI-504 in breast cancer [49, 146].

Y-632, a novel pyrimidine derivative, Hsp90 function suppressed through induced thiol oxidation and disruption of Hsp90–Hsp70/Hsp90 organizing protein complex. This further induces inhibition of cell adhesion, G_0/G_1 cell cycle arrest, and apoptosis [147].

17-DMAG (17-dimethylaminoethylamino-17-demethoxygeldanamycin), another geldanamycin derivative of the HSP90 inhibitor, stalled the viability of human lung cancer cell lines via reduced expression of client proteins, including the proto-oncogene RAF-1. 17-DMAG treatment in human SCLC cell line SBC-5 inhibited the formation of metastatic sites in both liver and bone [134].

KF58333, a novel oxime derivative of radicicol, binds to Hsp90 and destabilizes its associated signaling molecules. KF58333, without altering the HIF-lα mRNA expression, resulted in significant downregulation of HIF-1α under hypoxic conditions. KF58333 also inhibited tumor angiogenesis and vascular endothelial growth factor (VEGF) secretion in a dose dependently [74].

Apigenin a naturally occurring flavonoid exhibits antiproliferative and antiangiogenic activities. Apigenin inhibits VEGF expression via degradation of HIF-1α and interferes with the function of Hsp90 in endothelial cells of human umbilical artery. In pancreatic cancer cells, it inhibits HIF-1α, GLUT-1, and VEGF mRNA and protein expression in both normoxic and hypoxic conditions [99, 108]. It inhibits the growth of UV-induced skin cancer and thyroid cancer cells by activating AMP-activated protein kinase (AMPK), leading to suppression of basal mTOR activity. This suppression of mTOR activity inhibits cell proliferation and arrests the cell cycle at G2/M phase. Apigenin is shown to reduce CDK4 and cyclins D1 and A, but not the cyclin E, CDK2, and CDK6 protein expression. Its growth inhibitory effects are mediated by targeting signal transduction pathways and emerging as a promising anticancer agent [11, 163].

YC-1 [3-(5′-hydroxymethyl-2′-furyl)-1-benzyl indazole], a HIF-1 inhibitor, acts by reducing HIF-1α expression [50, 104]. YC-1 inhibits HIF-1α expression via the FIH-dependent CAD inactivation as well as protein downregulation [83]. YC-1 suppresses the hypoxic responses by posttranslationally inhibiting HIF-1α accumulation and exhibits novel antiangiogenic anticancer agent properties [21, 160].

SCH66336, a small molecule farnesyl protein transferase inhibitor that shares a common tricyclic nucleus and competes with peptide/protein substrates for binding to farnesyl protein transferase [87]. It also inhibits the interaction between HIF-1α and Hsp90 to inhibit VEGF production in NSCLC and HNSCC cells [48].

Under hypoxia, histone deacetylase (HDAC) inhibitor enhances p53 and von Hippel–Lindau expression and thereby stimulates angiogenesis. This stimulation leads to downregulation of HIF-1α and VEGF thus promoting HIF-1α degradation [65]. Stress-responsive genetic regulator, sirtuin 1 (Sirt1) gene expression, increases in a HIF-dependent manner, and loss of HIF signaling affects Sirt1 deacetylase activity during hypoxia [17]. SIRT1 downregulation was due to decreased NAD levels; this allowed the acetylation and HIF-1α activation. SIRT1 deacetylase and the HIF-1α transcription factor act as redox and oxygen sensors, respectively, whereas hypoxic HIF-1α stabilization requires SIRT1 activation [85]. Sirt1 regulates HIF-1α and HIF-2α by deacetylating Lys674 of HIF-1α and HIF-1α K674 and

HIF-2α K741 by PCAF and CBP, respectively. HIF-1α deacetylation blocks the recruitment of p300 to HIF-1α. This blockade consequently inactivates HIF-1α; represses HIF-1 target genes including VEGF, GLUT1, and MMP2; and finally promotes cancer cell invasion [58, 165].

Trichostatin A (TSA), an antifungal antibiotic showing histone deacetylase (HDAC) activity. In vitro and in vivo studies in human breast cancer and squamous cell carcinoma cell lines assessed the antitumor efficacy and toxicity of TSA [141]. It induced caspase-dependent or caspase-independent apoptosis according to cell types. In gastric cancer cells, TSA increased TRAIL-induced apoptosis [82]. In HSC-3 cells, TSA enhanced the Bim protein expression levels by dephosphorylating ERK1/2 pathway. In Ca9.22 cells TSA damaged MMP and increased cytosolic apoptosis-inducing factor (AIF) [54].

LW6, a small compound, inhibits the HIF-1α accumulation. LW6 degrade HIF-1α via VHL expression, with modifications of P402A and P564A, at hydroxylation sites in the oxygen-dependent degradation domain (ODDD), without affecting the activity of prolyl hydroxylase (PHD) [78]. A recent data revealed that angiogenesis suppression through LW6 inhibited HIF-1α stability via direct binding with calcineurin B homologous protein 1 (CHP1) [64].

LAQ824 and *LBH589*, the inhibitors of histone deacetylase (HDACi) and established cancer therapeutic agents. Both engage in the intrinsic apoptotic cascade which does not require p53. Mitochondrial damage is the key event for LAQ824 and LBH589 to mediate tumor cell death [31].

Thioredoxin-1 (Trx-1), a redox protein usually overexpressed in many human tumors. It increases aerobic and hypoxia-induced HIF-1α protein in the cells and leads to expression of HIF-regulated genes. Trx-1 controls multiple aspects of cell growth and survival [57].

PX-12 (1-methylpropyl 2-imidazolyl disulfide), an irreversible inhibitor of Trx-1. This is currently under clinical development [5, 112]. PX-12 decreases plasma VEGF levels and contributes to the antitumor activity [6]. PX-12 acts independently and increases nuclear Nrf2; this one interacts with PMF-1 to increase SSAT1 expression, and further SSAT1 binds to HIF-1α and RACK1, finally resulting in oxygen-independent HIF-1 ubiquitination and degradation [66].

Pleurotin, a growth inhibitory and antitumor agent shown to decrease HIF-1α protein levels, HIF-1-*trans*-activating activity, VEGF formation, inducible nitric oxide synthase, and the expression of downstream target genes [150].

AJM290 and *AW464* (quinols), two novel anticancer drugs that inhibit Trx-1 function and also inhibit HIF-1α CAD transcription activity and DNA binding. In contrast to other Trx inhibitors, these agents also inhibit HIF degradation [57].

Small molecules can inhibit HIF-1 dimerization and potentially inhibit the tumor growth and vascularization.

Acriflavine antagonizes HIF upon binding to the HIF-α PAS-B domain. It directly binds to HIF-1alpha and HIF-2alpha and suppresses dimerization of HIF-1 and transcriptional activity. It also induces cell death under hypoxic conditions and reduced the expression of the HIF-1 target genes *VEGF, PTGS2*, and *EDN1* [12, 80].

PT2385, HIF-2α inhibitor allosterically binds to PAS-B domain of HIF-2α, thereby preventing HIF-2α dimerization with ARNT (aryl hydrocarbon receptor nuclear translocator, HIF-1β). This results in decreased transcription and expression of HIF-2α downstream target genes, many of which regulate tumor cell growth and survival. Blocking HIF-2α reduces the proliferation of HIF-2α-expressing tumor cells. PT2385 is currently under evaluation in Phase I clinical trials for the treatment of clear cell renal carcinoma [144].

In hypoxic conditions, HIF-1α is translocated into nucleus, heterodimerizes with HIF-1β, and binds to hypoxia response element (HRE) DNA sequence. *Chetomin*, a metabolite complex, produced by several fungi of the genus *Chaetomium*, disrupts the ability of tumors to adapt to hypoxia by blocking the HIF pathway and reduces hypoxia-dependent transcription. Chetomin targets transcriptional coactivator p300 by disrupting its CH1 domain and impairs the interaction of between HIF-1α and p300 [130, 142].

Bortezomib, the first proteasomal inhibitor (PI) and also confirmed antitumor activity-containing agent in clinical setting. Bortezomib attenuates the transcriptional activity and impairs tumor growth only of HIF-1, and not HIF-2. Bortezomib inhibits HIF-1α protein expression at the translational level under both normoxic and hypoxic conditions and its nuclear targeting through inhibition of PI3K/Akt/mTOR and MAPK pathways, respectively, by dephosphorylation of phospho-Akt, phospho-p70S6 K, and phospho-S6RP [1, 9].

Amphotericin B (AmB), an agent that interferes the HIF-1α expression through CAD-FIH. AmB represses the C-terminal transactivation domain (CAD) of HIF-1α, a target site of the factor-inhibiting HIF-1 (FIH). CAD-FIH interaction inhibits the recruitment of p300 through CAD of HIF-1α [162].

Triptolide possesses anticancer, antiangiogenesis, and drug-resistance activities. Triptolide suppresses HIF-1α through c-Myc-dependent mechanism. Triptolide treatment in SKOV-3 cells resulted in loss of function of HIF-1α protein transcriptional activity and reduced mRNA levels of its target genes [29, 173].

FM19G11, an agent that inhibits HIF-alpha protein expression and suppresses target genes of two alpha subunits in several tumor cell lines. FM19G11 reduces overall histone acetylation with significant p300 repression and behaves as a target gene of HIF2alpha at nanomolar range of FM19G11 inhibiting transcriptional and translational expression of Oct4, Sox2, Nanog, etc. [103].

Echinomycin (NSC-13502), a small molecule that binds in a sequence-specific manner in the DNA and shows dual effect on HIF-1 activity under normoxic and hypoxic conditions. It inhibits binding of HIF-1α and HIF-1β proteins to a HRE sequence. It suppresses cell growth and induces apoptosis with decreased mRNA expression of HIF1 targets, glucose transporter-1 (GLUT1), and B-cell CLL/lymphoma-2 (BCL2). This agent has failed as anticancer agent due to its dual effect [72, 143, 164].

Anthracycline and its chemical derivatives (doxorubicin (DXR) and daunorubicin (DNR)) are the topoisomerase inhibitor family that suppresses hypoxia-inducible factor-1 (HIF-1) transcriptional activity by obstructing its binding to DNA. These

agents are using widely in the prevention of tumors [116]. Doxorubicin (DXR) weakens the transcriptional activity of the HIF by inhibiting the binding of the HIF heterodimer to the consensus – RCGTG – enhancer element and downregulated HIF target lysyl oxidase (LOX) family members [136]. Anthracyclines also inhibit the endogenous HIF-1 target gene expression. In hypoxic cells the VEGF and GLUT1 mRNA levels were significantly decreased by DNR, and DXR, in a dose-dependent manner [79].

10.3.4 Future Approaches

The thrust is continuously inundated in identifying the novel metastasis-associated oncogenes and tumor suppressor genes. Several therapeutic approaches that target HIF and its associated factors in tumor progression are emerging continuously. Further studies are needed for answering how the cells sense hypoxia and how HIF-1α activation occurred along with other signaling pathways. In recent studies, researchers have focused on the determination of the pathways (pro-survival and apoptosis) activated in response to hypoxia in cancer cells, and further it is needed to analyze the hypoxia-response gene expression patterns to the levels such as apoptosis, angiogenesis, and metastasis in human cancer cells through microarray analysis and other high-throughput technologies.

References

1. Abd-Aziz N, Stanbridge EJ, Shafee N (2015) Bortezomib attenuates HIF-1- but not HIF-2-mediated transcriptional activation. Oncol Lett 10:2192–2196
2. Agarwal S, Loder S, Brownley C, Cholok D, Mangiavini L, Li J, Breuler C, Sung HH, Li S, Ranganathan K et al (2016) Inhibition of Hif1α prevents both trauma-induced and genetic heterotopic ossification. Proc Natl Acad Sci 113:E338–E347
3. Aoyagi Y, Fujita N, Tsuruo T (2005) Stabilization of integrin-linked kinase by binding to Hsp90. Biochem Biophys Res Commun 331:1061–1068
4. Aquino-Gálvez A, González-Ávila G, Delgado-Tello J, Castillejos-López M, Mendoza-Milla C, Zúñiga J, Checa M, Maldonado-Martínez HA, Trinidad-López A, Cisneros J (2016) Effects of 2-methoxyestradiol on apoptosis and HIF-1α and HIF-2α expression in lung cancer cells under normoxia and hypoxia. Oncol Rep 35:577–583
5. Baker AF, Adab KN, Raghunand N, Chow HH, Stratton SP, Squire SW, Boice M, Pestano LA, Kirkpatrick DL, Dragovich T (2013) A phase IB trial of 24-hour intravenous PX-12, a thioredoxin-1 inhibitor, in patients with advanced gastrointestinal cancers. Investig New Drugs 31:631–641
6. Baker AF, Dragovich T, Tate WR, Ramanathan RK, Roe D, Hsu CH, Kirkpatrick DL, Powis G (2006) The antitumor thioredoxin-1 inhibitor PX-12 (1-methylpropyl 2-imidazolyl disulfide) decreases thioredoxin-1 and VEGF levels in cancer patient plasma. J Lab Clin Med 147:83–90
7. Balamurugan K (2016) HIF-1 at the crossroads of hypoxia, inflammation, and cancer. Int J Cancer 138:1058–1066
8. Becker CM, Rohwer N, Funakoshi T, Cramer T, Bernhardt W, Birsner A, Folkman J, D'Amato RJ (n.d.) 2-methoxyestradiol inhibits hypoxia-inducible factor-1α and suppresses growth of lesions in a mouse model of endometriosis. Am J Pathol 172:534–544

9. Befani CD, Vlachostergios PJ, Hatzidaki E, Patrikidou A, Bonanou S, Simos G, Papandreou CN, Liakos P (2012) Bortezomib represses HIF-1alpha protein expression and nuclear accumulation by inhibiting both PI3K/Akt/TOR and MAPK pathways in prostate cancer cells. J Mol Med (Berlin, Germany) 90:45–54

10. Bielawski K, Winnicka K, Bielawska A (2006) Inhibition of DNA topoisomerases I and II, and growth inhibition of breast cancer MCF-7 cells by Ouabain, digoxin and Proscillaridin a. Biol Pharm Bull 29:1493–1497

11. Bridgeman BB, Wang P, Ye B, Pelling JC, Volpert OV, Tong X (2016) Inhibition of mTOR by apigenin in UVB-irradiated keratinocytes: a new implication of skin cancer prevention. Cell Signal 28:460–468

12. Broekgaarden M, Weijer R, Krekorian M, van den IJssel B, Kos M, Alles LK, van Wijk AC, Bikadi Z, Hazai E, van Gulik TM et al (2016) Inhibition of hypoxia-inducible factor 1 with acriflavine sensitizes hypoxic tumor cells to photodynamic therapy with zinc phthalocyanine-encapsulating cationic liposomes. Nano Res 9:1639–1662

13. Cao D, Hou M, Guan YS, Jiang M, Yang Y, Gou HF (2009) Expression of HIF-1alpha and VEGF in colorectal cancer: association with clinical outcomes and prognostic implications. BMC Cancer 9:432

14. Cao J, He L, Lin G, Hu C, Dong R, Zhang J, Zhu H, Hu Y, Wagner CR, He Q et al (2014) Cap-dependent translation initiation factor, eIF4E, is the target for Ouabain-mediated inhibition of HIF-1alpha. Biochem Pharmacol 89:20–30

15. Carmeliet P, Dor Y, Herbert J-M, Fukumura D, Brusselmans K, Dewerchin M, Neeman M, Bono F, Abramovitch R, Maxwell P (1998) Role of HIF-1α in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. Nature 394:485–490

16. Chang H, Shyu KG, Lee CC, Tsai SC, Wang BW, Hsien Lee Y, Lin S (2003) GL331 inhibits HIF-1alpha expression in a lung cancer model. Biochem Biophys Res Commun 302:95–100

17. Chen R, Dioum EM, Hogg RT, Gerard RD, Garcia JA (2011) Hypoxia increases sirtuin 1 expression in a hypoxia-inducible factor-dependent manner. J Biol Chem 286:13869–13878

18. Chen S, Sang N (2016) Hypoxia-inducible factor-1: a critical player in the survival strategy of stressed cells. J Cell Biochem 117:267–278

19. Chen Y-C, Chien L-H, Huang B-M, Chia Y-C, Chiu H-F (2016) Aqueous extracts of Toona sinensis leaves inhibit renal carcinoma cell growth and migration through JAK2/stat3, Akt, MEK/ERK, and mTOR/HIF-2α pathways. Nutr Cancer 68:654–666

20. Chen Y, Lin TY, Chen JC, Yang HZ, Tseng SH (2006) GL331, a topoisomerase II inhibitor, induces radiosensitization of human glioma cells. Anticancer Res 26:2149–2156

21. Chun Y-S, Yeo E-J, Choi E, Teng C-M, Bae J-M, Kim M-S, Park J-W (2001) Inhibitory effect of YC-1 on the hypoxic induction of erythropoietin and vascular endothelial growth factor in Hep3B cells1. Biochem Pharmacol 61:947–954

22. Cummins EP, Seeballuck F, Keely SJ, Mangan NE, Callanan JJ, Fallon PG, Taylor CT (2008) The hydroxylase inhibitor dimethyloxalylglycine is protective in a murine model of colitis. Gastroenterology 134:156–165

23. Dahut WL, Lakhani NJ, Gulley JL, Arlen PM, Kohn EC, Kotz H, McNally D, Parr A, Parr A, Nguyen D et al (2006) Phase I clinical trial of oral 2-methoxyestradiol, an antiangiogenic and apoptotic agent, in patients with solid tumors. Cancer Biol Ther 5:22–27

24. Dang CV, Le A, Gao P (2009) MYC-induced cancer cell energy metabolism and therapeutic opportunities. Clin Cancer Res Off J Am Assoc Cancer Res 15:6479–6483

25. DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB (2008) The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. Cell Metab 7:11–20

26. Dehne N, Brune B (2009) HIF-1 in the inflammatory microenvironment. Exp Cell Res 315:1791–1797

27. Demetri G, Le Cesne A, Von Mehren M, Chmielowski B, Bauer S, Chow W, Rodenas E, McKee K, Grayzel D, Kang Y (2010) Final results from a phase III study of IPI-504 (retaspimycin hydrochloride) versus placebo in patients (pts) with gastrointestinal stromal tumors (GIST) following failure of kinase inhibitor therapies. Paper presented at: Gastrointestinal Cancers Symposium

28. Diaz-Ruiz R, Rigoulet M, Devin A (2011) The Warburg and Crabtree effects: on the origin of cancer cell energy metabolism and of yeast glucose repression. Biochim Biophys Acta 1807:568–576

29 Ding X, Zhou X, Jiang B, Zhao Q, Zhou G (2015) Triptolide suppresses proliferation, hypoxia-inducible factor-1alpha and c-myc expression in pancreatic cancer cells. Mol Med Rep 12:4508–4513

30. Einbond LS, Wu H-A, Sandu C, Ford M, Mighty J, Antonetti V, Redenti S, Ma H (2016) Digitoxin enhances the growth inhibitory effects of thapsigargin and simvastatin on ER negative human breast cancer cells. Fitoterapia 109:146–154

31. Ellis L, Bots M, Lindemann RK, Bolden JE, Newbold A, Cluse LA, Scott CL, Strasser A, Atadja P, Lowe SW et al (2009) The histone deacetylase inhibitors LAQ824 and LBH589 do not require death receptor signaling or a functional apoptosome to mediate tumor cell death or therapeutic efficacy. Blood 114:380–393

32. Ferreira LM (2010) Cancer metabolism: the Warburg effect today. Exp Mol Pathol 89:372–380

33. Fu L, Chen L, Yang J, Ye T, Chen Y, Fang J (2012) HIF-1α-induced histone demethylase JMJD2B contributes to the malignant phenotype of colorectal cancer cells via an epigenetic mechanism. Carcinogenesis 33:1664–1673

34. Galanis A, Pappa A, Giannakakis A, Lanitis E, Dangaj D, Sandaltzopoulos R (2008) Reactive oxygen species and HIF-1 signalling in cancer. Cancer Lett 266:12–20

35. Ganji PN, Park W, Wen J, Mahaseth H, Landry J, Farris AB, Willingham F, Sullivan PS, Proia DA, El-Hariry I et al (2013) Antiangiogenic effects of ganetespib in colorectal cancer mediated through inhibition of HIF-1α and STAT-3. Angiogenesis 16:903–917

36. Garber K (2004) Energy boost: the Warburg effect returns in a new theory of cancer. J Natl Cancer Inst 96:1805–1806

37. Garst J (2007) Topotecan: an evolving option in the treatment of relapsed small cell lung cancer. Ther Clin Risk Manag 3:1087–1095

38. Ge X, Liu X, Lin F, Li P, Liu K, Geng R, Dai C, Lin Y, Tang W, Wu Z (2016) MicroRNA-421 regulated by HIF-1α promotes metastasis, inhibits apoptosis, and induces cisplatin resistance by targeting E-cadherin and caspase-3 in gastric cancer. Oncotarget 7:24466

39. Giaccia A, Siim BG, Johnson RS (2003) HIF-1 as a target for drug development. Nat Rev Drug Discov 2:803–811

40. Giatromanolaki A, Sivridis E, Maltezos E, Papazoglou D, Simopoulos C, Gatter KC, Harris AL, Koukourakis MI (2003) Hypoxia inducible factor 1alpha and 2alpha overexpression in inflammatory bowel disease. J Clin Pathol 56:209–213

41. Glover LE, Bowers BE, Saeedi B, Ehrentraut SF, Campbell EL, Bayless AJ, Dobrinskikh E, Kendrick AA, Kelly CJ, Burgess A et al (2013) Control of creatine metabolism by HIF is an endogenous mechanism of barrier regulation in colitis. Proc Natl Acad Sci U S A 110:19820–19825

42. Goda N, Dozier SJ, Johnson RS (2003) HIF-1 in cell cycle regulation, apoptosis, and tumor progression. Antioxid Redox Signal 5:467–473

43. Goda N, Ryan HE, Khadivi B, McNulty W, Rickert RC, Johnson RS (2003) Hypoxia-inducible factor 1α is essential for cell cycle arrest during hypoxia. Mol Cell Biol 23:359–369

44. Gogvadze V, Zhivotovsky B, Orrenius S (2010) The Warburg effect and mitochondrial stability in cancer cells. Mol Asp Med 31:60–74

45. Goscinski MA, Nesland JM, Giercksky K-E, Dhakal HP (2013) Primary tumor vascularity in esophagus cancer. CD34 and HIFI-a expression correlate with tumor progression. Histol Histopathol 28:1361–1368

46. Greijer AE, van der Wall E (2004) The role of hypoxia inducible factor 1 (HIF-1) in hypoxia induced apoptosis. J Clin Pathol 57:1009–1014

47. Hamanaka RB, Chandel NS (2011) Cell biology. Warburg effect and redox balance. Science 334:1219–1220

48. Han J-Y, Oh SH, Morgillo F, Myers JN, Kim E, Hong WK, Lee H-Y (2005) Hypoxia-inducible factor 1α and antiangiogenic activity of farnesyltransferase inhibitor SCH66336 in human aerodigestive tract cancer. J Natl Cancer Inst 97:1272–1286

49. Hanson BE, Vesole DH (2009) Retaspimycin hydrochloride (IPI-504): a novel heat shock protein inhibitor as an anticancer agent. Expert Opin Investig Drugs 18:1375–1383

50. Harada H (2016) Hypoxia-inducible factor 1–mediated characteristic features of cancer cells for tumor radioresistance. J Radiat Res 57:i99

51. He X, Wang J, Wei W, Shi M, Xin B, Zhang T, Shen X (2016) Hypoxia regulates ABCG2 activity through the activation of ERK1/2/HIF-1α and contributes to chemoresistance in pancreatic cancer cells. Cancer Biol Ther 17:188–198

52. Imamura T, Kikuchi H, Herraiz MT, Park DY, Mizukami Y, Mino-Kenduson M, Lynch MP, Rueda BR, Benita Y, Xavier RJ (2009) HIF-1α and HIF-2α have divergent roles in colon cancer. Int J Cancer 124:763–771

53. James J, Murry DJ, Treston AM, Storniolo AM, Sledge GW, Sidor C, Miller KD (2007) Phase I safety, pharmacokinetic and pharmacodynamic studies of 2-methoxyestradiol alone or in combination with docetaxel in patients with locally recurrent or metastatic breast cancer. Investig New Drugs 25:41–48

54. Jang B, Kim L-H, Lee S-Y, Lee K-E, Shin J-A, Cho S-D (2016) Trichostatin A induces apoptosis in oral squamous cell carcinoma cell lines independent of hyperacetylation of histones

55. Jeong W, Rapisarda A, Park SR, Kinders RJ, Chen A, Melillo G, Turkbey B, Steinberg SM, Choyke P, Doroshow JH et al (2014) Pilot trial of EZN-2968, an antisense oligonucleotide inhibitor of hypoxia-inducible factor-1 alpha (HIF-1alpha), in patients with refractory solid tumors. Cancer Chemother Pharmacol 73:343–348

56. Jinka R, Kapoor R, Sistla PG, Raj TA, Pande G (2012) Alterations in cell-extracellular matrix interactions during progression of cancers. Int J Cell Biol 2012:219196

57. Jones DT, Pugh CW, Wigfield S, Stevens MF, Harris AL (2006) Novel thioredoxin inhibitors paradoxically increase hypoxia-inducible factor-alpha expression but decrease functional transcriptional activity, DNA binding, and degradation. Clin Cancer Res Off J Am Assoc Cancer Res 12:5384–5394

58. Joo HY, Yun M, Jeong J, Park ER, Shin HJ, Woo SR, Jung JK, Kim YM, Park JJ, Kim J et al (2015) SIRT1 deacetylates and stabilizes hypoxia-inducible factor-1alpha (HIF-1alpha) via direct interactions during hypoxia. Biochem Biophys Res Commun 462:294–300

59. Ju C, Colgan SP, Eltzschig HK (2016) Hypoxia-inducible factors as molecular targets for liver diseases. J Mol Med (Berl). 94(6):613–627

60. Kaelin WG Jr, Thompson CB (2010) Q&A: cancer: clues from cell metabolism. Nature 465:562–564

61. Keely S, Campbell EL, Baird AW, Hansbro PM, Shalwitz RA, Kotsakis A, McNamee EN, Eltzschig HK, Kominsky DJ, Colgan SP (2014) Contribution of epithelial innate immunity to systemic protection afforded by prolyl hydroxylase inhibition in murine colitis. Mucosal Immunol 7:114–123

62. Keith B, Simon MC (2007) Hypoxia-inducible factors, stem cells, and cancer. Cell 129:465–472

63. Kelly CJ, Glover LE, Campbell EL, Kominsky DJ, Ehrentraut SF, Bowers BE, Bayless AJ, Saeedi BJ, Colgan SP (2013) Fundamental role for HIF-1alpha in constitutive expression of human beta defensin-1. Mucosal Immunol 6:1110–1118

64. Kim BS, Lee K, Jung HJ, Bhattarai D, Kwon HJ (2015) HIF-1alpha suppressing small molecule, LW6, inhibits cancer cell growth by binding to calcineurin b homologous protein 1. Biochem Biophys Res Commun 458:14–20

65. Kim MS, Kwon HJ, Lee YM, Baek JH, Jang JE, Lee SW, Moon EJ, Kim HS, Lee SK, Chung HY et al (2001) Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. Nat Med 7:437–443

66. Kim YH, Coon A, Baker AF, Powis G (2011) Antitumor agent PX-12 inhibits HIF-1alpha protein levels through an Nrf2/PMF-1-mediated increase in spermidine/spermine acetyl transferase. Cancer Chemother Pharmacol 68:405–413

67. Kizaka-Kondoh S, Tanaka S, Harada H, Hiraoka M (2009) The HIF-1-active microenvironment: an environmental target for cancer therapy. Adv Drug Deliv Rev 61:623–632

68. Klausmeyer P, Zhou Q, Scudiero DA, Uranchimeg B, Melillo G, Cardellina JH, Shoemaker RH, Chang CJ, McCloud TG (2009) Cytotoxic and HIF-1alpha inhibitory compounds from Crossosoma bigelovii. J Nat Prod 72:805–812

69. Koh MY, Spivak-Kroizman T, Venturini S, Welsh S, Williams RR, Kirkpatrick DL, Powis G (2008) Molecular mechanisms for the activity of PX-478, an antitumor inhibitor of the hypoxia-inducible factor-1α. Mol Cancer Ther 7:90–100

70. Koh MY, Spivak-Kroizman TR, Powis G (2008) HIF-1 regulation: not so easy come, easy go. Trends Biochem Sci 33:526–534

71. Komarova NL, Wodarz D (2005) Drug resistance in cancer: principles of emergence and prevention. Proc Natl Acad Sci U S A 102:9714–9719

72. Kong D, Park EJ, Stephen AG, Calvani M, Cardellina JH, Monks A, Fisher RJ, Shoemaker RH, Melillo G (2005) Echinomycin, a small-molecule inhibitor of hypoxia-inducible factor-1 DNA-binding activity. Cancer Res 65:9047–9055

73. Kubo H, Kitajima Y, Kai K, Nakamura J, Miyake S, Yanagihara K, Morito K, Tanaka T, Shida M, Noshiro H (2016) Regulation and clinical significance of the hypoxia-induced expression of ANGPTL4 in gastric cancer. Oncol Lett 11:1026–1034

74. Kurebayashi J, Otsuki T, Kurosumi M, Soga S, Akinaga S, Sonoo H (2001) A radicicol derivative, KF58333, inhibits expression of hypoxia-inducible factor-1α and vascular endothelial growth factor, angiogenesis and growth of human breast cancer xenografts. Jpn J Cancer Res 92:1342–1351

75. Kuwai T, Kitadai Y, Tanaka S, Onogawa S, Matsutani N, Kaio E, Ito M, Chayama K (2003) Expression of hypoxia-inducible factor-1α is associated with tumor vascularization in human colorectal carcinoma. Int J Cancer 105:176–181

76. LaVallee TM, Burke PA, Swartz GM, Hamel E, Agoston GE, Shah J, Suwandi L, Hanson AD, Fogler WE, Sidor CF et al (2008) Significant antitumor activity in vivo following treatment with the microtubule agent ENMD-1198. Mol Cancer Ther 7:1472–1482

77. Lee JW, Bae SH, Jeong JW, Kim SH, Kim KW (2004) Hypoxia-inducible factor (HIF-1) alpha: its protein stability and biological functions. Exp Mol Med 36:1–12

78. Lee K, Kang JE, Park SK, Jin Y, Chung KS, Kim HM, Lee K, Kang MR, Lee MK, Song KB et al (2010) LW6, a novel HIF-1 inhibitor, promotes proteasomal degradation of HIF-1alpha via upregulation of VHL in a colon cancer cell line. Biochem Pharmacol 80:982–989

79. Lee K, Qian DZ, Rey S, Wei H, Liu JO, Semenza GL (2009) Anthracycline chemotherapy inhibits HIF-1 transcriptional activity and tumor-induced mobilization of circulating angiogenic cells. Proc Natl Acad Sci U S A 106:2353–2358

80. Lee K, Zhang H, Qian DZ, Rey S, Liu JO, Semenza GL (2009) Acriflavine inhibits HIF-1 dimerization, tumor growth, and vascularization. Proc Natl Acad Sci U S A 106:17910–17915

81. Lee SH, Jee JG, Bae JS, Liu KH, Lee YM (2015) A group of novel HIF-1alpha inhibitors, glyceollins, blocks HIF-1alpha synthesis and decreases its stability via inhibition of the PI3K/AKT/mTOR pathway and Hsp90 binding. J Cell Physiol 230:853–862

82. Li L, Fan B, Zhang L-H, Xing X-F, Cheng X-J, Wang X-H, Guo T, Du H, Wen X-Z, Ji J-F (2016) Trichostatin A potentiates TRAIL-induced antitumor effects via inhibition of ERK/FOXM1 pathway in gastric cancer. Tumour Biol. 37(8):10269–10278

83. Li SH, Shin DH, Chun Y-S, Lee MK, Kim M-S, Park J-W (2008) A novel mode of action of YC-1 in HIF inhibition: stimulation of FIH-dependent p300 dissociation from HIF-1α. Mol Cancer Ther 7:3729–3738

84. Li Z, Wang J, Zhou T, Ye X (2016b) Establishment of a colorectal cancer nude mouse visualization model of HIF-1α overexpression. Oncol Lett 11:2725–2732

85. Lim JH, Lee YM, Chun YS, Chen J, Kim JE, Park JW (2010) Sirtuin 1 modulates cellular responses to hypoxia by deacetylating hypoxia-inducible factor 1alpha. Mol Cell 38:864–878

86. Liu L, Wang Y, Bai R, Yang K, Tian Z (2016) MiR-186 inhibited aerobic glycolysis in gastric cancer via HIF-1[alpha] regulation. Oncogene 5:e224

87. Liu M, Bryant MS, Chen J, Lee S, Yaremko B, Lipari P, Malkowski M, Ferrari E, Nielsen L, Prioli N et al (1998) Antitumor activity of SCH 66336, an orally bioavailable tricyclic inhibitor of farnesyl protein transferase, in human tumor xenograft models and wap-ras transgenic mice. Cancer Res 58:4947–4956

88. Liu X, Chen S, Tu J, Cai W, Xu Q (2016) HSP90 inhibits apoptosis and promotes growth by regulating HIF-1α abundance in hepatocellular carcinoma. Int J Mol Med 37:825–835

89. Liu Y-F, Zhong J-J, Lin L, Liu J-J, Wang Y-G, He W-Q, Yang Z-Y (2016) New C-19-modified geldanamycin derivatives: synthesis, antitumor activities, and physical properties study. J Asian Nat Prod Res. 18(8):752–764

90. Lokich J (2001) Phase I clinical trial of weekly combined topotecan and irinotecan. Am J Clin Oncol 24:336–340

91. Lopez-Lazaro M (2008) The warburg effect: why and how do cancer cells activate glycolysis in the presence of oxygen? Anti Cancer Agents Med Chem 8:305–312

92. Louis NA, Hamilton KE, Canny G, Shekels LL, Ho SB, Colgan SP (2006) Selective induction of mucin-3 by hypoxia in intestinal epithelia. J Cell Biochem 99:1616–1627

93. Luo Y, Ji X, Liu J, Li Z, Wang W, Chen W, Wang J, Liu Q, Zhang X (2016) Moderate intensity static magnetic fields affect mitotic spindles and increase the antitumor efficacy of 5-FU and Taxol. Bioelectrochemistry 109:31–40

94. Luqmani YA (2005) Mechanisms of drug resistance in cancer chemotherapy. Med Princ Pract Int J Kuwait Univ Health Sci Cent 14(Suppl 1):35–48

95. Masoud GN, Li W (2015) HIF-1α pathway: role, regulation and intervention for cancer therapy. Acta Pharm Sin B 5:378–389

96. Matsuyama T, Nakanishi K, Hayashi T, Yoshizumi Y, Aiko S, Sugiura Y, Tanimoto T, Uenoyama M, Ozeki Y, Maehara T (2005) Expression of hypoxia-inducible factor-1α in esophageal squamous cell carcinoma. Cancer Sci 96:176–182

97. Maxwell PH (2005) The HIF pathway in cancer. Paper presented at: Seminars in cell & developmental biology (Elsevier)

98. Maxwell PH, Pugh CW, Ratcliffe PJ (2001) Activation of the HIF pathway in cancer. Curr Opin Genet Dev 11:293–299

99. Melstrom LG, Salabat MR, Ding XZ, Strouch MJ, Grippo PJ, Mirzoeva S, Pelling JC, Bentrem DJ (2011) Apigenin down-regulates the hypoxia response genes: HIF-1alpha, GLUT-1, and VEGF in human pancreatic cancer cells. J Surg Res 167:173–181

100. Milutinovic S, Heynen-Genel S, Chao E, Dewing A, Solano R, Milan L, Barron N, He M, Diaz PW, Matsuzawa S-I et al (2016) Cardiac glycosides activate the tumor suppressor and viral restriction factor Promyelocytic leukemia protein (PML). PLoS One 11:e0152692

101. Miyata Y (2005) Hsp90 inhibitor geldanamycin and its derivatives as novel cancer chemotherapeutic agents. Curr Pharm Des 11:1131–1138

102. Mooberry SL (2003) New insights into 2-methoxyestradiol, a promising antiangiogenic and antitumor agent. Curr Opin Oncol 15:425–430

103. Moreno-Manzano V, Rodriguez-Jimenez FJ, Acena-Bonilla JL, Fustero-Lardies S, Erceg S, Dopazo J, Montaner D, Stojkovic M, Sanchez-Puelles JM (2010) FM19G11, a new hypoxia-inducible factor (HIF) modulator, affects stem cell differentiation status. J Biol Chem 285:1333–1342

104. Nayak BK, Shanmugasundaram K, Friedrichs WE, Cavaglierii RC, Patel M, Barnes J, Block K (2016) HIF-1 mediates renal fibrosis in OVE26 type 1 diabetic mice. Diabetes 65:1387–1397

105. Nunoi K, Yasuda K, Tanaka H, Kubota A, Okamoto Y, Adachi T, Shihara N, Uno M, Xu LM, Kagimoto S et al (2000) Wortmannin, a PI3-kinase inhibitor: promoting effect on insulin secretion from pancreatic beta cells through a cAMP-dependent pathway. Biochem Biophys Res Commun 270:798–805

106. Ogawa K, Chiba I, Morioka T, Shimoji H, Tamaki W, Takamatsu R, Nishimaki T, Yoshimi N, Murayama S (2011) Clinical significance of HIF-1α expression in patients with esophageal cancer treated with concurrent chemoradiotherapy. Anticancer Res 31:2351–2359

107. Onnis B, Rapisarda A, Melillo G (2009) Development of HIF-1 inhibitors for cancer therapy. J Cell Mol Med 13:2780–2786

108. Osada M, Imaoka S, Funae Y (2004) Apigenin suppresses the expression of VEGF, an important factor for angiogenesis, in endothelial cells via degradation of HIF-1α protein. FEBS Lett 575:59–63

109. Pasquier E, Sinnappan S, Munoz MA, Kavallaris M (2010) ENMD-1198, a new analogue of 2-methoxyestradiol, displays both antiangiogenic and vascular-disrupting properties. Mol Cancer Ther 9:1408–1418

110. Powis G, Kirkpatrick L (2004) Hypoxia inducible factor-1α as a cancer drug target. Mol Cancer Ther 3:647–654

111. Quintero M, Mackenzie N, Brennan PA (2004) Hypoxia-inducible factor 1 (HIF-1) in cancer. Eur J Surg Oncol 30:465–468

112. Ramanathan RK, Stephenson JJ, Weiss GJ, Pestano LA, Lowe A, Hiscox A, Leos RA, Martin JC, Kirkpatrick L, Richards DA (2012) A phase I trial of PX-12, a small-molecule inhibitor of thioredoxin-1, administered as a 72-hour infusion every 21 days in patients with advanced cancers refractory to standard therapy. Investig New Drugs 30:1591–1596

113. Rini BI (2008) Temsirolimus, an inhibitor of mammalian target of rapamycin. Clin Cancer Res Off J Am Assoc Cancer Res 14:1286–1290

114. Robinson A, Keely S, Karhausen J, Gerich ME, Furuta GT, Colgan SP (2008) Mucosal protection by hypoxia-inducible factor prolyl hydroxylase inhibition. Gastroenterology 134:145–155

115. Rohwer N, Lobitz S, Daskalow K, Jöns T, Vieth M, Schlag P, Kemmner W, Wiedenmann B, Cramer T, Höcker M (2009) HIF-1α determines the metastatic potential of gastric cancer cells. Br J Cancer 100:772–781

116. Roncuzzi L, Pancotti F, Baldini N (2014) Involvement of HIF-1alpha activation in the doxo-rubicin resistance of human osteosarcoma cells. Oncol Rep 32:389–394

117. Schoner W, Scheiner-Bobis G (2007) Endogenous and exogenous cardiac glycosides: their roles in hypertension, salt metabolism, and cell growth. Am J Phys Cell Phys 293:C509–C536

118. Semenza GL (2000) HIF-1: mediator of physiological and pathophysiological responses to hypoxia. J Appl Physiol. (1985 88:1474–1480

119. Semenza GL (2001) Hypoxia-inducible factor 1: oxygen homeostasis and disease patho-physiology. Trends Mol Med 7:345–350

120. Semenza GL (2002) HIF-1 and tumor progression: pathophysiology and therapeutics. Trends Mol Med 8:S62–S67

121. Semenza GL (2003) Targeting HIF-1 for cancer therapy. Nat Rev Cancer 3:721–732

122. Semenza GL (2004) Hydroxylation of HIF-1: oxygen sensing at the molecular level. Physiology 19:176–182

123. Semenza GL (2010) Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. Oncogene 29:625–634

124. Semenza GL (2012) Hypoxia-inducible factors in physiology and medicine. Cell 148:399–408

125. Semenza GL (2012) Hypoxia-inducible factors: mediators of cancer progression and targets for cancer therapy. Trends Pharmacol Sci 33:207–214

126. Shah NN, Merchant MS, Cole DE, Jayaprakash N, Bernstein D, Delbrook C, Richards K, Widemann BC, Wayne AS (2016) Vincristine sulfate liposomes injection (VSLI, Marqibo®): results from a phase I study in children, adolescents, and young adults with refractory solid tumors or Leukemias. Pediatr Blood Cancer 63:997–1005

127. Shida M, Kitajima Y, Nakamura J, Yanagihara K, Baba K, Wakiyama K, Noshiro H (2016) Impaired mitophagy activates mtROS/HIF-1α interplay and increases cancer aggressiveness in gastric cancer cells under hypoxia. Int J Oncol 48:1379–1390

128. Snoeks TJ, Mol IM, Que I, Kaijzel EL, Lowik CW (2011) 2-methoxyestradiol analogue ENMD-1198 reduces breast cancer-induced osteolysis and tumor burden both in vitro and in vivo. Mol Cancer Ther 10:874–882

129. Song X, Zhao Z, Qi X, Tang S, Wang Q, Zhu T, Gu Q, Liu M, Li J (2015) Identification of epipolythiodioxopiperazines HDN-1 and chaetocin as novel inhibitor of heat shock protein 90

130. Staab A, Loeffler J, Said HM, Diehlmann D, Katzer A, Beyer M, Fleischer M, Schwab F, Baier K, Einsele H et al (2007) Effects of HIF-1 inhibition by chetomin on hypoxia-related transcription and radiosensitivity in HT 1080 human fibrosarcoma cells. BMC Cancer 7:213

131. Stoeltzing O, McCarty MF, Wey JS, Fan F, Liu W, Belcheva A, Bucana CD, Semenza GL, Ellis LM (2004) Role of hypoxia-inducible factor 1α in gastric cancer cell growth, angiogenesis, and vessel maturation. J Natl Cancer Inst 96:946–956

132. Synnestvedt K, Furuta GT, Comerford KM, Louis N, Karhausen J, Eltzschig HK, Hansen KR, Thompson LF, Colgan SP (2002) Ecto-5′-nucleotidase (CD73) regulation by hypoxia-inducible factor-1 mediates permeability changes in intestinal epithelia. J Clin Invest 110:993–1002

133. Tahervand A, Mahmoudi M, Roushandeh AM (2016) Digoxin effectively decreased proliferation of liver cancer cell line. Focus Sci 2

134. Takeuchi S, Fukuda K, Arai S, Nanjo S, Kita K, Yamada T, Hara E, Nishihara H, Uehara H, Yano S (2016) Organ-specific efficacy of HSP90 inhibitor in multiple-organ metastasis model of chemorefractory small cell lung cancer. Int J Cancer 138:1281–1289

135. Tan Z, Huang Q, Zang J, Teng S, Chen T, Wei H, Song D, Liu T, Yang X, Fu C (2016) HIF-1α activates hypoxia-induced BCL-9 expression in human colorectal cancer cells. Oncotarget 8(16):25885–25896

136. Tanaka T, Yamaguchi J, Shoji K, Nangaku M (2012) Anthracycline inhibits recruitment of hypoxia-inducible transcription factors and suppresses tumor cell migration and cardiac angiogenic response in the host. J Biol Chem 287:34866–34882

137. Terzuoli E, Puppo M, Rapisarda A, Uranchimeg B, Cao L, Burger AM, Ziche M, Melillo G (2010) Aminoflavone, a ligand of the aryl hydrocarbon receptor, inhibits HIF-1alpha expression in an AhR-independent fashion. Cancer Res 70:6837–6848

138. Tong W-W, Tong G-H, Kong H, Liu Y (2016) The tumor promoting roles of HSP60 and HIF2α in gastric cancer cells. Tumor Biol:1–6

139. Vander Heiden MG, Cantley LC, Thompson CB (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 324:1029–1033

140. Vaupel P (2004) The role of hypoxia-induced factors in tumor progression. Oncologist 9(Suppl 5):10–17

141. Vigushin DM, Ali S, Pace PE, Mirsaidi N, Ito K, Adcock I, Coombes RC (2001) Trichostatin A is a histone deacetylase inhibitor with potent antitumor activity against breast cancer in vivo. Clin Cancer Res Off J Am Assoc Cancer Res 7:971–976

142. Viziteu E, Grandmougin C, Goldschmidt H, Seckinger A, Hose D, Klein B, Moreaux J (2016) Chetomin, targeting HIF-1[alpha]/p300 complex, exhibits antitumour activity in multiple myeloma. Br J Cancer 114:519–523

143. Vlaminck B, Toffoli S, Ghislain B, Demazy C, Raes M, Michiels C (2007) Dual effect of echinomycin on hypoxia-inducible factor-1 activity under normoxic and hypoxic conditions. FEBS J 274:5533–5542

144. Wallace EM, Cao Z, Cheng T, Czerwinski R, Dixon DD, Du X, Goggin B, Grina J, Halfmann M, Han G (2015) Abstract DDT01-01: PT2385: first-in-class HIF-2α antagonist for the treatment of renal cell carcinoma. Cancer Res 75, DDT01-01-DDT01-01

145. Wang J, Ni Z, Duan Z, Wang G, Li F (2014) Altered expression of hypoxia-inducible factor-1α (HIF-1α) and its regulatory genes in gastric cancer tissues. PLoS One 9:e99835

146. Wang M, Shen A, Zhang C, Song Z, Ai J, Liu H, Sun L, Ding J, Geng M, Zhang A (2016) Development of Heat Shock Protein (Hsp90) Inhibitors To Combat Resistance to Tyrosine Kinase Inhibitors through Hsp90–Kinase Interactions. J Med Chem 59:5563

147. Wang W, Liu Y, Zhao Z, Xie C, Xu Y, Hu Y, Quan H, Lou L (2016) Y-632 inhibits heat shock protein 90 (Hsp90) function by disrupting the interaction between Hsp90 and Hsp70/Hsp90 organizing protein, and exerts antitumor activity in vitro and in vivo. Cancer Sci 107:782–790

148. Weidemann A, Johnson R (2008) Biology of HIF-1α. Cell Death Differ 15:621–627

149. Welsh S, Williams R, Kirkpatrick L, Paine-Murrieta G, Powis G (2004) Antitumor activity and pharmacodynamic properties of PX-478, an inhibitor of hypoxia-inducible factor-1α. Mol Cancer Ther 3:233–244

150. Welsh SJ, Williams RR, Birmingham A, Newman DJ, Kirkpatrick DL, Powis G (2003) The thioredoxin redox inhibitors 1-methylpropyl 2-imidazolyl disulfide and pleurotin inhibit hypoxia-induced factor 1α and vascular endothelial growth factor formation 1. Mol Cancer Ther 2:235–243

151. Winnicka K, Bielawski K, Bielawska A, Miltyk W (2010) Dual effects of ouabain, digoxin and proscillaridin A on the regulation of apoptosis in human fibroblasts. Nat Prod Res 24:274–285

152. Wu YG, Jin M, Xu HB, Zhang SM, He SB, Wang LA, Zhang YY (2010) Clinicopathologic significance of HIF-1 alpha, CXCR4, and VEGF expression in colon cancer. Clin Dev Immunol 2010

153. Xia Y, Choi HK, Lee K (2012) Recent advances in hypoxia-inducible factor (HIF)-1 inhibitors. Eur J Med Chem 49:24–40

154. Xie L, Xue X, Taylor M, Ramakrishnan SK, Nagaoka K, Hao C, Gonzalez FJ, Shah YM (2014) Hypoxia-inducible factor/MAZ-dependent induction of caveolin-1 regulates colon permeability through suppression of occludin, leading to hypoxia-induced inflammation. Mol Cell Biol 34:3013–3023

155. Xu Q-R, Liu X, Yao Y-M, Liu Q-G (2014) Expression of HSP90 and HIF-1α in human colorectal cancer tissue and its significance. Asian Pac J Trop Med 7:720–724

156. Xu Y, Jin X, Huang Y, Dong J, Wang H, Wang X, Cao X (2016) Inhibition of peritoneal metastasis of human gastric cancer cells by dextran sulphate through the reduction in HIF-1α and ITGβ1 expression. Oncol Rep 35:2624–2634

157. Xue X, Ramakrishnan S, Anderson E, Taylor M, Zimmermann EM, Spence JR, Huang S, Greenson JK, Shah YM (2013) Endothelial PAS domain protein 1 activates the inflammatory response in the intestinal epithelium to promote colitis in mice. Gastroenterology 145:831–841

158. Xue X, Ramakrishnan SK, Shah YM (2014) Activation of HIF-1alpha does not increase intestinal tumorigenesis. Am J Physiol Gastrointest Liver Physiol 307:G187–G195

159. Yang J, Zhang X, Zhang Y, Zhu D, Zhang L, Li Y, Zhu Y, Li D, Zhou J (2016) HIF-2α promotes epithelial-mesenchymal transition through regulating Twist2 binding to the promoter of E-cadherin in pancreatic cancer. J Exp Clin Cancer Res 35:1–10

160. Yeo E-J, Chun Y-S, Cho Y-S, Kim J, Lee J-C, Kim M-S, Park J-W (2003) YC-1: a potential anticancer drug targeting hypoxia-inducible factor 1. J Natl Cancer Inst 95:516–525

161. Yeo EJ, Chun YS, Park JW (2004) New anticancer strategies targeting HIF-1. Biochem Pharmacol 68:1061–1069

162. Yeo EJ, Ryu JH, Cho YS, Chun YS, Huang LE, Kim MS, Park JW (2006) Amphotericin B blunts erythropoietin response to hypoxia by reinforcing FIH-mediated repression of HIF-1. Blood 107:916–923

163. Yin F, Giuliano AE, Law RE, Van Herle AJ (2001) Apigenin inhibits growth and induces G2/M arrest by modulating cyclin-CDK regulators and ERK MAP kinase activation in breast carcinoma cells. Anticancer Res 21:413–420

164. Yonekura S, Itoh M, Okuhashi Y, Takahashi Y, Ono A, Nara N, Tohda S (2013) Effects of the HIF1 inhibitor, echinomycin, on growth and NOTCH signalling in leukaemia cells. Anticancer Res 33:3099–3103

165. Yoon H, Shin SH, Shin DH, Chun YS, Park JW (2014) Differential roles of Sirt1 in HIF-1alpha and HIF-2alpha mediated hypoxic responses. Biochem Biophys Res Commun 444:36–43

166. Zhang H, Qian DZ, Tan YS, Lee K, Gao P, Ren YR, Rey S, Hammers H, Chang D, Pili R et al (2008) Digoxin and other cardiac glycosides inhibit HIF-1α synthesis and block tumor growth. Proc Natl Acad Sci 105:19579–19586

167. Zhang J, Zhu L, Fang J, Ge Z, Li X (2016) LRG1 modulates epithelial-mesenchymal transition and angiogenesis in colorectal cancer via HIF-1α activation. J Exp Clin Cancer Res 35:1

168. Zhang W-J, Chen C, Zhou Z-H, Gao S-T, Tee TJ, Yang L-Q, Xu Y-Y, Pang T-H, Xu X-Y, Sun Q (2017) Hypoxia-inducible factor-1 alpha correlates with tumor-associated macrophages infiltration, influences survival of gastric cancer patients. J Cancer 8:1818–1825

169. Zhang W, Shi X, Peng Y, Wu M, Zhang P, Xie R, Wu Y, Yan Q, Liu S, Wang J (2015) HIF-1α promotes epithelial-mesenchymal transition and metastasis through direct regulation of ZEB1 in colorectal cancer. PLoS One 10:e0129603

170. Zhang YZ, Chen X, Fan XX, He JX, Huang J, Xiao DK, Zhou YL, Zheng SY, Xu JH, Yao XJ et al (2016) Compound library screening identified cardiac glycoside digitoxin as an effective growth inhibitor of gefitinib-resistant non-small cell lung cancer via downregulation of alpha-tubulin and inhibition of microtubule formation. Molecules (Basel, Switzerland) 21:374

171. Zhao T, Zhu Y, Morinibu A, Kobayashi M, Shinomiya K, Itasaka S, Yoshimura M, Guo G, Hiraoka M, Harada H (2014) HIF-1-mediated metabolic reprogramming reduces ROS levels and facilitates the metastatic colonization of cancers in lungs. Sci Rep 4:3793

172. Zhe N, Chen S, Zhou Z, Liu P, Lin X, Yu M, Cheng B, Zhang Y, Wang J (2016) HIF-1α inhibition by 2-methoxyestradiol induces cell death via activation of the mitochondrial apoptotic pathway in acute myeloid leukemia. Cancer Biol Ther:1–10

173. Zhou ZL, Luo ZG, Yu B, Jiang Y, Chen Y, Feng JM, Dai M, Tong LJ, Li Z, Li YC et al (2010) Increased accumulation of hypoxia-inducible factor-1alpha with reduced transcriptional activity mediates the antitumor effect of triptolide. Mol Cancer 9:268