Am J Cancer Res 2022;12(12):5351-5374
www.ajcr.us /ISSN:2156-6976/ajcr0141445

Review Article

Non-canonical approaches to targeting hypoxic tumors

Shuai Zhao[1,2,3,4,5], Wafik S El-Deiry[1,2,3,4,5,6]

[1]Laboratory of Translational Oncology and Experimental Cancer Therapeutics, Warren Alpert Medical School, Brown University, Providence, RI, USA; [2]Pathobiology Graduate Program, Brown University, Providence, RI, USA; [3]Department of Pathology and Laboratory Medicine, Brown University, Providence, RI, USA; [4]Joint Program in Cancer Biology, Brown University and Lifespan Cancer Institute, Providence, RI, USA; [5]Legorreta Cancer Center at Brown University, Warren Alpert Medical School, Brown University, Providence, RI, USA; [6]Hematology/Oncology Division, Lifespan Cancer Institute, Providence, RI, USA

Received December 31, 2021; Accepted August 22, 2022; Epub December 15, 2022; Published December 30, 2022

Abstract: Hypoxia is a common characteristic in solid cancers. Hypoxia-inducible factors (HIFs) are involved in various aspects of cancer, such as angiogenesis, metastasis and therapy resistance. Targeting the HIF pathway has been regarded as a challenging but promising strategy in cancer treatment with recent FDA approval of a HIF2α-inhibitor. During the past several decades, numerous efforts have been made to understand how HIFs participate in cancer development and progression along with how HIF signaling can be modulated to achieve anti-cancer effect. In this chapter, we will provide an overview of the role of hypoxia and HIFs in cancer, summarize the oxygen-dependent and independent mechanisms of HIF-1α regulation, and discuss emerging approaches targeting hypoxia and HIF signaling which possess therapeutic potential in cancer. We will emphasize on two signaling pathways, involving cyclin-dependent kinases (CDKs) and heat shock protein 90 (HSP90), which contribute to HIF-1α (and HIF-2α) stabilization in an oxygen-independent manner. Through reviewing their participation in malignant progression and the potential targeting strategies, we discuss the non-canonical approaches to target HIF signaling in cancer therapy.

Keywords: HIF1-alpha, CDK4/6, HSP90, hypoxia, angiogenesis

Hypoxia in cancer

Angiogenesis is among the key hallmarks of cancer. Despite the intensive vascularization, blood supply is often inadequate in tumors because of the abnormal blood vessel formation with the unbalanced level between pro-angiogenic and anti-angiogenic molecules. The blood vessels can be leaky, twisted, and lacking of the regular structures composed of arterioles, capillaries and venules. Meanwhile in tumors, the diffusion radius of oxygen is limited to 100 μm away from blood vessels [1]. The cancer cells located at a further distance become deprived of oxygen, due to a combination of excessive oxygen consumption during the rapid cell division and the disorganized tumor-associated vasculature, resulting in intratumoral hypoxia (**Figure 1**). More than half of the locally advanced solid tumors contain diversely distributed hypoxic regions [2], which contributes to the heterogeneity within the tumor mass.

In adaptation to hypoxia, a coordinated set of cellular responses is triggered. The main mediator in such response is the hypoxia-inducible factor (HIF). Hypoxia/HIF signaling affects a majority of the cancer hallmarks (**Figure 1**). It selects for the cell population with defective apoptotic capabilities (with p53-deficiency) [3]. It downregulates proapoptotic molecules (*e.g.* Bid & Bax) to promote survival [4]. It switches the energetics (*e.g.* increasing the expression of GLUT1) [5]. It stimulates angiogenesis [6] (*e.g.* induction of VEGF). It contributes to invasion [7] and metastasis [8]. It decreases DNA repair and increases genomic instability [9]. Notably, it also suppresses immune reactivity (*e.g.* driving the expression of PD-L1) [10, 11]. In clinic, it is linked with chemo- and radio-therapy resistance as well as aggressive disease and poor patient prognosis [12-14].

Given the involvement of hypoxia/HIF in many aspects of the cancer hallmarks and its association with more aggressive and resistant disease, hypoxic environment serves as an appeal-

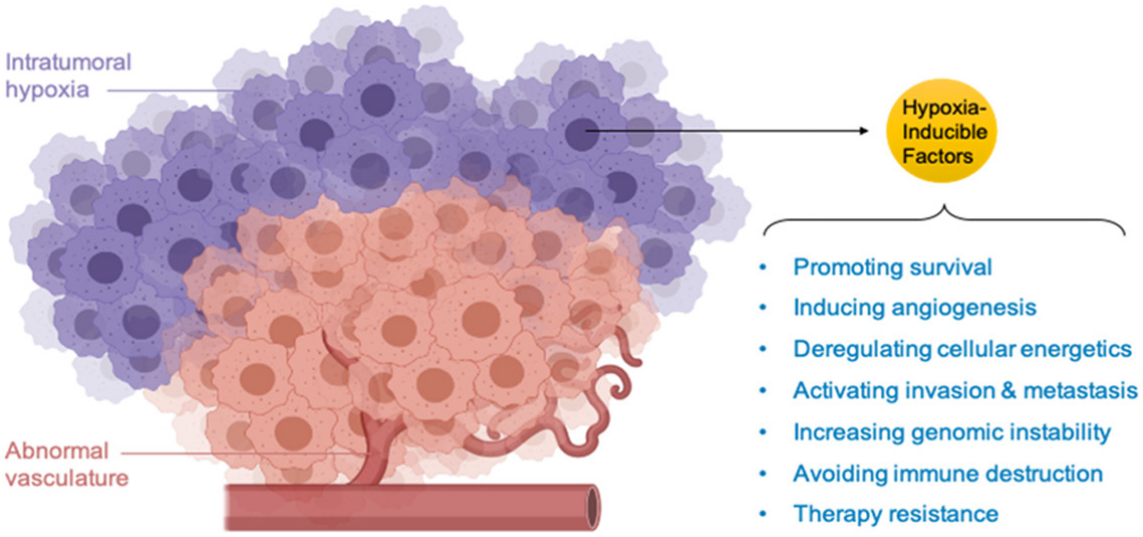

Figure 1. Intra-tumoral hypoxia and HIF signaling is implicated in most of the cancer hallmarks. Oxygen concentration is low at regions within a tumor far away from the blood supply, which is termed as intra-tumoral hypoxia. The tumor cells survive the hypoxic tensions by inducing adaptive responses mostly mediated by hypoxia-inducible factors. Hypoxia/HIF signaling plays a role in various hallmarks of cancer. (Picture created at Biorender.com).

ing target in cancer treatment. One potential approach to target hypoxia in cancer is through the bioreductive prodrugs based on certain chemical moieties (*e.g.* nitro groups) that can be metabolized in low-oxygen conditions [15]. These kinds of prodrugs undergo one-electron reduction by cellular reductases. The generated prodrug radical can be re-oxidized by oxygen in normoxic condition but is converted to the active drug form in hypoxia, by which way the treatment selects particularly for hypoxic cells. However, challenges exist with this concept, such as the need for determining biomarkers in patient selection and improving the pharmacokinetic properties (*e.g.* delivery into cells at the targeted site) of the prodrug. A main limitation of this approach is that conventionally the active drugs being released are DNA-damaging cytotoxins, which contributes to off-target toxicity as well as eliminates the possibility of combining with traditional chemotherapy. Replacing the active drug with protein components may provide new options [16].

Hypoxia-inducible factors

Hypoxia-inducible factors (HIFs) are the major mediators in hypoxia-induced cellular responses. The discovery of HIF dates back to the early 90s during the study of an enhancer upstream to the erythropoietin (*EPO*) gene, which identified a nuclear factor that activates the transcription of EPO in response to hypoxia, termed as hypoxia-inducible factor 1 (HIF-1) [17, 18]. Subsequent purification and characterization of the protein have shown that HIF-1 is composed of two separate subunits: HIF-1α and a smaller HIF-1β (**Figure 2A**) [19], both of which harbor a basic-helix-loop-helix-PAS (bHLH-PAS) structure [20]. HIF-1β is also named aryl hydrocarbon nuclear translocator (ARNT), since it was discovered as a heterodimer with aryl hydrocarbon receptor (AhR) before the identification of HIF-1α. As a transcription factor, HIF-1 acts by binding to the hypoxia-responsive element (HRE) in promoters and stimulates the transcription of its target genes. The base domain in the HIF subunits has DNA binding capabilities while the HLH domain is responsible for the protein dimerization. The core motif of HRE is a consensus sequence of 5'-(A/G)CGTG-3'. HIF-1α subunit contains two transactivation domains (TAD), referred to as N-TAD (N-terminal TAD) and C-TAD (C-terminal TAD) (**Figure 2B**). Hypoxia allows the interaction between C-TAD and co-activators p300/CBP, which recruits the transcriptional machinery and thus increases gene expression [21, 22]. N-TAD is also involved with transcriptional activity and is overlapping with the oxygen-dependent degradation domain (ODDD) which is essential for the protein stability regulation of HIF by oxygen availability. Interestingly, N-TAD is

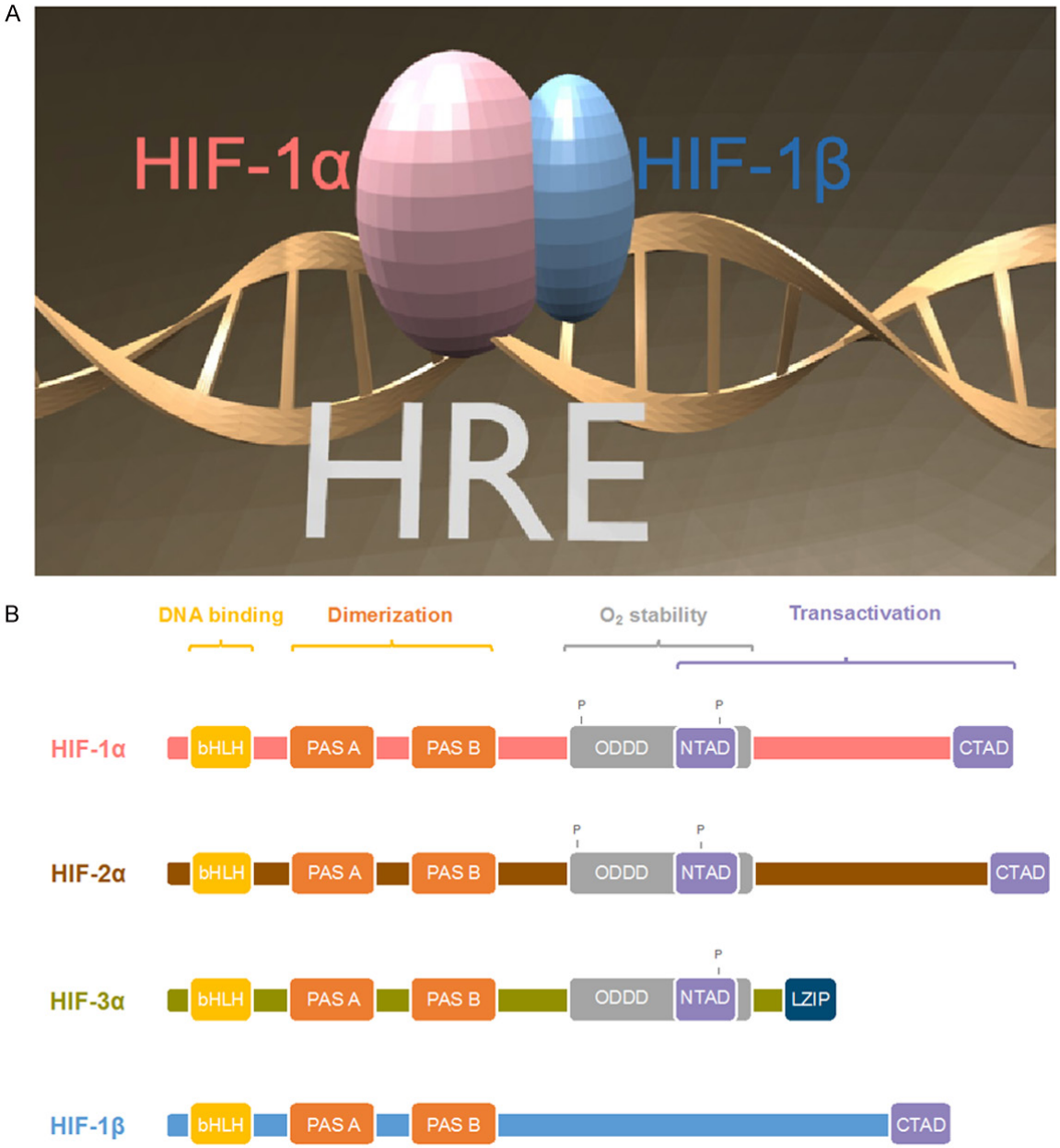

Figure 2. Hypoxia-inducible factor (HIF) subunits. (A) The heterodimer of HIF-1α and HIF-1β binds to the HRE region in target DNA to elicit HIF-1 signaling. (B) Comparison of domains in HIF subunits. (A is drawn in Bioblender. B is drawn with reference to Linda Ravenna, *et al*. 2015 with permission from *John Wiley and Sons*).

found to modulate the specificity of target gene selection between HIF-1α and HIF-2α [23].

The second main HIF, HIF-2α, was identified later in 1997 [24-27]. It is also known as endothelial PAS domain protein-1 (EPAS1). HIF-2α shares a similar structure composition as HIF-1α. HIF-2α also contains an ODDD domain. The sequence similarity in their DNA binding domains is 83% [23], while the similarity in the dimerization domains is 70% [23]. Their C-TAD is also comparable (67%) [23]. Regions surrounding their oxygen-dependent regulatory sites are alike as well (70%) [23]. This sequence homology is in line with the observation that HIF-2α heterodimerizes with HIF-1β and recognizes the same HRE. However, while HIF-1α serves as the "master regulator" [28] in maintenance of the oxygen homeostasis, HIF-2α, although can be induced under hypoxia [29],

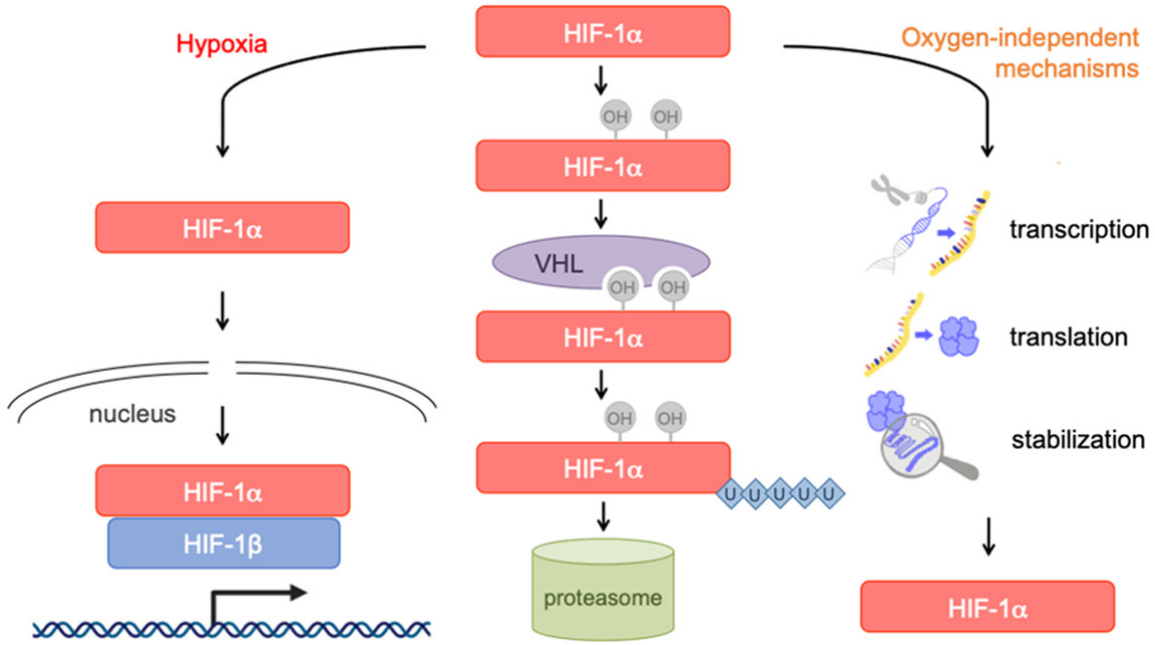

Figure 3. Regulation of the HIF-1α expression in an oxygen-dependent and independent manner. In normoxia, HIF-1α is hydroxylated and subsequently targeted by VHL for ubiquitination, resulting in proteasomal degradation (middle). In hypoxia, hydroxylation of HIF-1α is inhibited, which allows it to translocate into the nucleus and activate target gene transcription in a heterodimer with HIF-1β (left). Alternatively, HIF-1α is regulated through VHL-independent mechanisms at transcriptional, translational and post-translational levels. In this regard, HIF-1α stabilization can be achieved even in the presence of oxygen. (The three icons of transcription, translation and stabilization are adapted from https://innovativegenomics.org/glossary/).

seems to play distinct roles [30] and show specific tissue distributions (e.g. expressed in endothelial cells). While HIF-1α and HIF-2α have shared transcription activities, they each target unique genes as well [31-34].

Another HIF-α subunit, HIF-3α, unlike HIF-1α or 2α, carries a LZIP (leucine zipper) domain instead of the C-TAD domain at the C-terminal. It exists as several different variants according to the involvement of different promoters, transcription start sites, and splicing patterns. The full-length HIF-3α contains the ODDD domain and can exert oxygen-regulated transcription activities [35]. Some truncated variants lacking ODDD can act as a dominant negative inhibitor on HIF-1α/2α. Other variants are able to dampen the HIF-1α/2α signaling through competitive binding with HIF-1β. Further investigation is needed to unravel the complexity of HIF-3α.

HIF-1α regulation

Oxygen-dependent regulation of HIF-1α

Regulation of stability: All HIF-α subunits containing the ODDD domain go through post-translational regulation in an oxygen-dependent manner, while HIF-1β lacks the ODDD domain and is constitutively expressed. A group of prolyl hydroxylases, named as prolyl hydroxylase-domain protein (PHD) or HIF-1 prolyl hydroxylases (HPH), rely on molecular oxygen (as well as the activating metal iron, the co-substrate α-ketoglutarate, and the co-factor ascorbate) to modify the conserved proline residues at the LXXLAP motif in the ODDD domain [36-41]. In HIF-1α, for example, two proline residues (P402 & P564) are subject to such hydroxylation under normoxia, which allows the interaction with the von Hippel Lindau (VHL) protein. VHL is the substrate recognition component within an E3 ubiquitin ligase complex and is known as a tumor suppressor. The VHL complex (VHL together with the adaptor protein Elongin B/Elongin C, the scaffold protein Cullin-2, and the RING protein Rbx1) catalyzes polyubiquitination on HIF-1α, which results in protein degradation by the proteasome [42-44]. Under hypoxia, insufficient oxygen amount limits the PHD hydroxylation activity and hence prevents the VHL-dependent ubiquitination and degradation (**Figure 3**).

Additionally, a lysine residue, K532, located in the ODDD region of HIF-1α, is an acetylation target site of the arrest-defective-1 (ARD-1) acetyltransferase. The acetylation of K532 by ARD-1 enhances the binding of VHL to HIF-1α, and thus contributes to HIF-1α ubiquitination and degradation [45]. The finding that K532R mutation stabilizes HIF-1α in normoxia further supports the role of K532 acetylation in the regulation of HIF-1α [45]. ARD-1 does not require oxygen as a substrate for its function, however, hypoxic conditions downregulate the expression of ARD-1 at the transcriptional level [45].

Regulation of activity: Other than stability regulation, HIF-1α transactivation activity can be regulated by oxygen availability through its C-TAD domain. Asparagine residue N803 in the C-TAD domain of HIF-1α can be hydroxylated by an enzyme called factor inhibiting HIF (FIH) [46-48]. Modification of this residue is critical because it sits within the molecular interface in the binding between HIF-1α and CBP [49]. FIH also senses oxygen concentration in a similar way as PHD. However, it is noteworthy that the Km value of FIH for O_2 is three times lower than those of PHD hydroxylases, which indicates that such regulation of HIF-1α signaling is still able to occur even when HIF-1α is already stabilized by the PHD activity inhibition.

Oxygen-independent regulation of HIF-1α

Aside from the oxygen-sensing mechanisms, HIF-1α is as well-regulated at multiple levels in an oxygen-independent manner.

Regulation of HIF-1α transcription: At the transcriptional level, HIF1A (the gene encoding HIF-1α) is upregulated through the JAK-STAT3 pathway [50, 51]. Pro-inflammatory cytokine interleukin-6 (IL-6) induces the transcription of HIF-1α in lymphocytes [52] and MCF7 breast cancer cells [50], which requires the participation of STAT3. Moreover, HIF1A transcription is stimulated by the NF-κB pathway in myeloid cells [53]. The endotoxin lipopolysaccharide (LPS) increases the expression of HIF-1α mRNA [54] through toll-like receptor (TLR)-induced NF-κB activity [55]. Furthermore, the reactive oxygen species (ROS) increases the HIF1A transcription under both normoxic and hypoxic conditions through the extracellular signal-regulated kinase (ERK) and phosphatidyl inositol 3-kinase (PI3K) pathways [56, 57].

Regulation of HIF-1α translation: The PI3K pathway is also involved in the translation of HIF-1α. PI3K acts in a signaling cascade including its downstream target, protein kinase B (Akt), and a subsequent factor, mammalian target of rapamycin (mTOR) [58]. Activated PI3K-Akt-mTOR signaling is then transduced to mRNA translation activity. For example, mTOR releases the eukaryotic translation initiation factor 4E (eIF-4E) from its binding protein (4E-BP1) through 4E-BP1 phosphorylation, which allows the cap-dependent translation of mRNA. Alternatively, mTOR can activate the p70 S6 Kinase (S6K) which phosphorylates ribosomal protein S6, resulting in mRNA translation. The PI3K-Akt pathway is exploited by certain hormones (*e.g.* Insulin [59]) and growth factor signaling (*e.g.* human epidermal growth factor receptor 2 (HER2) [60]) to induce the translation of *HIF1A* and thus increases the expression of HIF-1α protein.

Regulation of HIF-1α stabilization: As in the canonical regulatory mechanism, targeting HIF-1α protein stability plays an essential part in the non-canonical HIF-1α regulation.

The HIF-1α-targeting PHD hydroxylases require oxygen as well as other co-factors such as ascorbate for their enzymatic activity. Exposure to Cobalt (II) has been found to deplete ascorbate and increase HIF-1α expression without the requirement for a hypoxic environment [61]. As a matter of fact, Cobalt chloride ($CoCl_2$) has been used in multiple circumstances as a chemical inducer to elevate the level of HIF-1α.

The VHL-dependent ubiquitination of HIF-1α is a downstream event to the oxygen-caused hydroxylation. Therefore, defective VHL function leads to the accumulation of HIF-1α regardless of oxygen concentration. For instance, the VHL protein is a substrate of WD repeat and SOCS box-containing protein 1 (WSB1) for ubiquitination and degradation, which consequently facilitates HIF-1α stabilization and promotes cancer cell migration and invasion [62]. Genetic deletion or mutations of the *VHL* gene leads to constantly stabilized HIF-1α (as well as HIF-2α). A clinical condition with *VHL* gene alterations is known as the VHL syndrome, which is related to human cancers such as hemangioblastomas (affecting brain, spinal cord, and retina) and clear cell renal cell carcinoma (ccRCC) [63].

In addition to VHL, several other E3 ubiquitin ligases have been revealed in HIF-1α regulation. The hypoxia-associated factor (HAF) ubiquitinates HIF-1α but not HIF-2α independently of oxygen [64]. The carboxy terminus of Hsp70-interacting protein (CHIP) ubiquitinates HIF-1α in high-glucose conditions with aid of Hsp40/Hsp70 [65]. The mouse double minute 2 homolog (MDM2) is recruited to ubiquitinate HIF-1α through interaction with the tumor suppressor p53 [66]. The receptor of activated protein kinase C (RACK1) acts both similarly as VHL to ubiquitinate HIF-1α by recruiting elongin-C and promote proteasomal degradation and distinctly from VHL in that it targets unhydroxylated HIF-1α [67]. The F-box and WD protein Fbw7 (FBW7) ubiquitinates the glycogen synthase kinase 3 (GSK-3)-phosphorylated HIF-1α [68-70]. The X-linked inhibitor of apoptosis (XIAP) also serves as an E3 ubiquitin ligase that targets HIF-1α for Lys63-linked polyubiquitination [71].

Other than proteasomal degradation, HIF-1α can be digested in the lysosome. The co-localization of HIF-1α and the lysosome-associated membrane protein type 2A (LAMP2A) was observed in RCC4 VHL-deficient clear cell renal cancer cells [72]. The heat shock cognate 70-kDa protein (HSC70) and LAMP2A bind to HIF-1α and escort it to lysosome via chaperone-mediated autophagy (CMA).

Another chaperone protein, the heat shock protein 90 (HSP90) promotes the stabilization of HIF-1α instead. HSP90 has been acknowledged to interact with HIF-1α since 1996 [73]. The binding is suggested to induce a conformational change that assists in the dimerization of HIF-1α and HIF-1β [74]. Inhibition of HSP90 by geldanamycin (GA) accelerates the destabilization rate of HIF-1α in UMRC2 ccRCC cells that lack a functional VHL [75], which indicates the stabilization of HIF-1α by HSP90 is VHL-independent. It is proposed that HSP90 stabilizes HIF-1α through competitively binding to it against RACK1, preventing the recruitment of elongin-C and subsequent ubiquitination [76].

Our lab unraveled a previously unrecognized mechanism by which CDK1 stabilizes HIF-1α through phosphorylation at its Serine 668 residue [77]. Knockdown or inhibition of CDK1 decreases the expression of HIF-1α in hypoxia. Overexpression of CDK1 or cyclin B1 raises the level of HIF-1α in normoxia, which is reversed by CDK1 inhibitor, Ro3306. The interaction of CDK1 and HIF-1α phosphorylates Ser668 which is located within a sequence (RTASPNR) that contains the CDK1 consensus motif pS/T-P-x-R. Point mutation S668E enhances the stabilization of HIF-1α while S668A inhibits HIF-1α expression, indicating the Ser668 phosphorylation plays an important role in HIF-1α stability. In consistence with the presence of CDK1 activity throughout cell cycle, HIF-1α expression is increased after 8 hours upon release from the synchronization at S phase, at which point most of the cells are in G2/M phase. In this study, CDK4 knockdown is also shown to inhibit HIF-1α expression.

Recently, we have shown a novel VHL-independent mechanism of HIF-1α regulation, which involves the SMAD specific E3 ubiquitin protein ligase 2 (Smurf2) [78]. Smurf2 is a E3 ubiquitin ligase that is known to have a classic substrate of Smad 2/3. We identified Smurf2 in the overlap of a HIF-1α-interacting protein mass spectrometry upon CDK4/6 inhibition and a bioinformatic prediction of HIF-1α-targeting E3 ubiquitin ligases. Smurf2 targets HIF-1α for ubiquitination and destabilization *in vitro*. The study provides additional components to HIF-1α-regulating profile and suggests potential strategies to therapeutically target HIF signaling.

HIF-1α in cancer

HIF-1α overexpression in cancer

HIF-1α is induced in a broad spectrum of solid tumors as a result of the hypoxic nature inside the malignant mass at both primary and metastatic sites. In breast cancer, for example, the median oxygen partial pressure is as low as 10 mm Hg compared to that being 65 mm Hg in normal breast tissue [79]. However, the intratumoral expression of HIF-1α is not limited to the deoxygenated core, but also appears under normoxia in the process of immune selection and can be located at tumor margins as well [80]. Thus HIF-1α signaling is upregulated both by hypoxia and through other mechanisms in normoxia in cancer.

In a manner similar to how HIF-1α is regulated independently of oxygen, HIF-1α overexpression in cancer can be attributed to non-hypoxic signaling pathways as well. Alterations in different oncogenes and tumor suppressors give rise to the upregulation of HIF-1α through multiple pathways. (1) As mentioned, VHL loss-of-func-

Table 1. HIF-1α target genes in cancer progression

Involvement in cancer hallmarks	HIF-1α target gene product
Angiogenesis	Angiopoietin 2, Inhibitor of differentiation 2, Placental growth factor, Stromal-derived factor 1, Vascular endothelial growth factor
Cell survival & proliferation	Insulin-like growth factor 2, Survivin, *WSB1,* Platelet-derived growth factor B, Transforming growth factor-α
Immortalization	Telomerase
Invasion & Metastasis	Angiopoietin-like 4, *C-ME, CXCR4,* Endothelin 1, Fibronectin 1, Lysyl oxidase, Matrix metalloproteinase 2 and 14, *TWIST1,* Urokinase plasminogen activator receptor, *ZEB1* (ZFHX1A), *ZEB2* (ZFHX1B)
Glucose uptake & metabolism	Glucose phosphate isomerase, Glucose transporter 1, Hexokinase 1 and 2, Lactate dehydrogenase A, Pyruvate dehydrogenase kinase 1, Pyruvate kinase M2
Genomic instability	*DEC1*
Immune evasion	NT5E (ecto-5'-nucleotidase/CD73)
Stemness	ABCG2, *JARID1B,* Kit ligand (stem cell factor), OCT4
Epigenetic reprogramming	*JMJD1A, JMJD2B, JMJD2C, PLU-1* [190]
Senescent cells	*IL8, CXCR2, GROα, IL6, PAI1* [191]

Adapted from G L Semenza, 2010 [189].

tion mutations result in constitutive HIF-1α stabilization [63]. (2) TP53 (encoding p53) is the most commonly mutated gene in human cancer [81]. And p53 deletion disrupts MDM2 binding with HIF-1α, which increases HIF-1α expression in colon cancer cells [66]. (3) Mutations in the isocitrate dehydrogenase-1 (IDH1) gene impair the catalytic activity of the enzyme a nd reduce the production of α-ketoglutarate (α-KG) [82]. α-KG is the indispensable co-substrate in PHD-mediated HIF-1α hydroxylation. IDH1 mutation enhances HIF-1α transcriptional activity and is associated with high HIF-1α level in human gliomas [82]. (4) Tuberous sclerosis complex 2 (TSC2) is a tumor suppressor that inhibits the activation of mTOR. Mutation in the TSC gene causes the tuberous sclerosis disease, with which patients develop benign tumors and have an increased risk for giant cell astrocytoma [83] and ccRCC kidney cancer [84]. It is known that mTOR is involved in the stimulation of HIF-1α transcription and translation. Genetic inactivation of TSC2 increases the expression of HIF-1α mRNA and protein as well as boosts its target gene transcription [85]. (5) Another tumor suppressor, Phosphatase and tensin homolog (PTEN), is a negative regulator of the PI3K/Akt pathway and is frequently mutated in glioblastoma, endometrial cancer, and prostate cancer. Nearly 70% of prostate cancer patients carry a single-allele PTEN loss [86]. Defects in PTEN activity contribute to the expression of HIF-1α which leads to the transcription of responsive genes in prostate cancer cells [87]. (6) Amplification of the c-myc oncogene occurs in more than 15% of breast cancer cases [88]. It encodes the protein c-Myc which post-transcriptionally increases HIF-1α expression regardless of hypoxia in breast cancer cells [89]. (7) The *RAS* genes are the most commonly mutated oncogenes in human cancer [90]. In colorectal cancer, KRAS mutation occurs at a frequency of more than 40% [91]. The KRAS G12V mutation activates both Akt and ERK pathways and induces HIF-1α transcription in colorectal cancer cells [92].

An elevated level of HIF-1α enables cell survival and promotes cancer progression by orchestrating an extensive set of target genes involved in energetic switch, angiogenic induction, apoptotic counteraction, immune evasion, proliferative capabilities, invasive and metastatic properties, therapy resistance, and stemness maintenance (**Table 1**). In patients, HIF-1α overexpression has been correlated with adverse prognosis among multiple cancer types, such as brain [93], head-and-neck [12], lung [94], breast [13, 95-97], gastric [98], pancreatic [99], colorectal [100-102], cervix [103-105], ovarian [106], bladder [107], and prostate cancers [108].

Targeting HIF in cancer treatment

Apart from the attempts to develop pro-drugs that are activated in the hypoxic microenvironment, efforts have been made to target HIF signaling (**Table 2**). Anti-angiogenic drugs that block VEGF and its receptor represent an example of indirect targeting of HIF signaling through the downstream components that mediate the HIF-induced cancer-promoting effects. Some other approaches target the upstream of HIF signaling to inhibit HIF-α mRNA and protein levels. For instance, it is not unexpected to see the mTOR inhibitor, rapamycin, decreases the

Table 2. Molecular targeting of HIF signaling

Inhibitory mechanism	Molecular targets	Targeting agents
mRNA/protein expression	PI3K	Wortmannin, LY94002, GDC-0941, PI-103
	mTOR	Rapamycin, PP242, Glyceollins
	Topoisomerase 1	Topotecan, PEG-SN38
	HIF-1α mRNA	EZN-2968
	Microtubules	2ME2, ENMD-1198
	Hsp90	Geldanamycin and analogs
	HDAC	Vorinostat
	Thioredoxin-1	PX-12, pleurotin
	IRP1/IRE interaction	HIF-2α translational inhibitors
HIF-α/HIF-1β dimerization	HIF-1α/2α PAS-B domain	Acriflavine
	HIF-2α PAS-B domain	PT2385
DNA binding	HRE	Echinomycin, Polyamides
Transcriptional activity	p300 recruitment	Chetomin, Bortezomib
	FIH-1 interaction and p300 recruitment	Amphotericin B
	Hsp70	Triptolide
	Thioredoxin-1	AJM290, AW464

Adapted from Caroline Wigerup, *et al*., 2016 [192].

expression of HIF-1α in normoxic and hypoxic conditions [109], given the role of PI3K/Akt/mTOR pathway in HIF-1α transcription and translation. Temsirolimus, a soluble ester of rapamycin, is the first mTOR inhibitor approved by the FDA to treat cancer (advanced renal cell carcinoma) [110]. Other compounds that target the same pathway may have similar effects. FDA-approved topotecan is a camptothecin analogue that suppresses topoisomerase I and inhibits HIF-1α translation [111]. ENZ-2968 is a RNA oligonucleotide that selectively binds to the HIF-1α mRNA, reducing the expression of HIF-1α mRNA and protein in various cancer cells [112]. 2-Methoxyestradiol is an estrogen metabolite but does not bind with estrogen receptors. It decreases the nuclear accumulation of HIF-1α and inhibits its transcriptional activity [113]. Histone deacetylase (HDAC) inhibitors have been clinically used in cancer treatment. They are shown to reduce the HIF-1α expression [114]. Moreover, HIF-1α transcriptional activity can be regulated through the interruption of its binding with HIF-1β (*e.g.* acriflavine [115]), DNA sequences (*e.g.* echinomycin [116]), or p300/CBP (*e.g.* Chetomin [117]). Until the present time, several targeted therapies have become standard of care, some of which induce changes that are involved in hypoxia/HIF signaling. Meanwhile developing direct HIF destabilizers remains a challenge. Although some attempts targeting HIF pathway did not eventually make it to clinical application because of the toxicity, lack of efficacy, undefined patient selection, or discontinuation of the development, targeting the HIF signaling represents a promising approach in cancer therapy as it provides a way to generally and simultaneously target multiple malignant properties. Notably, targeting HIF-2α is thought to be important in certain tumors (*e.g.* ccRCCs). And certain inhibitors are selected for a HIF-2α specificity (*e.g.* PT2385). An orally available selective HIF-2α inhibitor, Belzutifan (MK-6482, developed by Merck), has been approved by FDA for cancers associated with von Hippel-Lindau (VHL) disease in 2021. The inhibitor showed favorable tolerance and anti-tumor activity in patients with previously treated clear cell renal cell carcinoma (ccRCC) in phase I/II study [118] and had a 49% objective response rate in patients with renal cell carcinoma in a phase II trial [119]. On the other hand, a recent transgenic mouse model that monitors tumor evolution has revealed an essential role of HIF-1α at the tumor-initiating phase in clear cell renal cell carcinoma [120]. It has also reinforced the involvement of HIF-1α in glycolysis and revealed HIF-2α involvement in other mechanisms such as lipoprotein metabolism. Overall, HIF-1α and HIF-2α are both potential targets with context-dependent importance in cancer therapy.

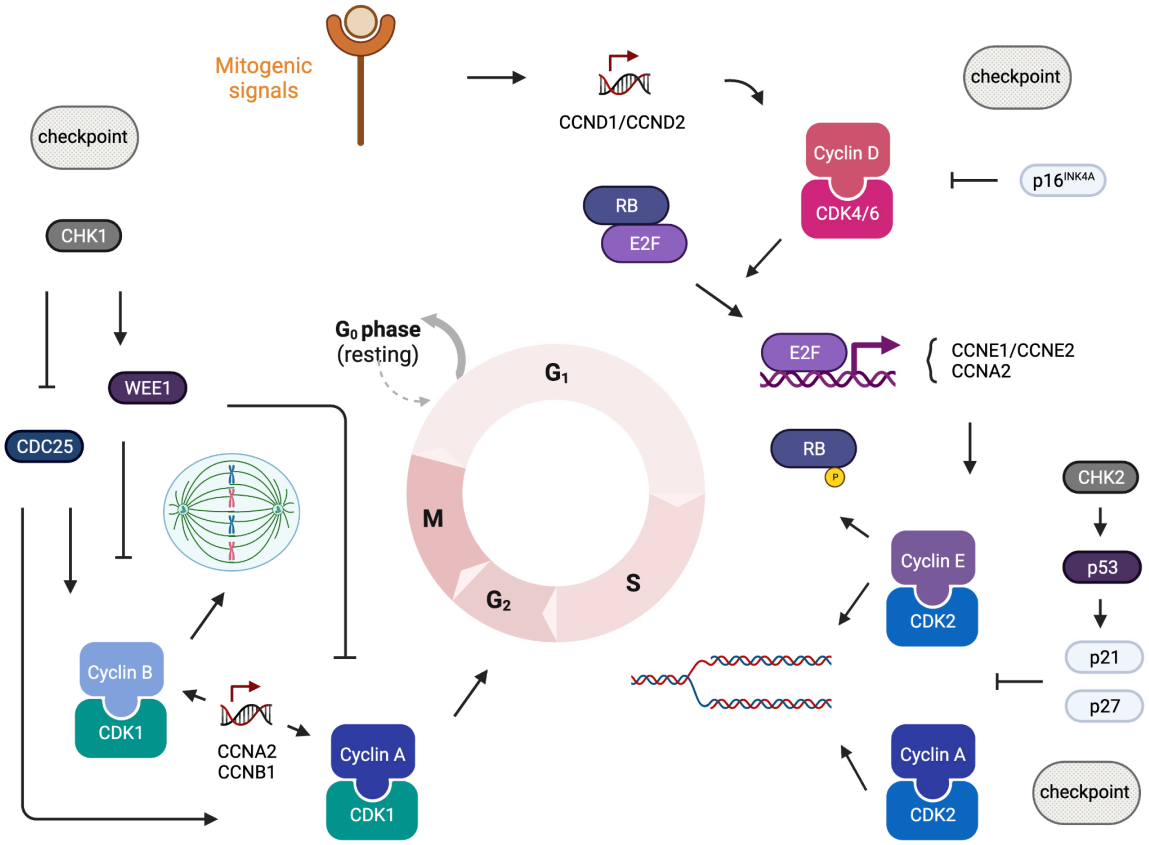

Figure 4. The role and regulation of CDKs through cell cycle progression. CDK4/6-cyclin D promotes G1-S transition, which is further mediated by CDK2 activity. CDK1 is essential in G2-M progression, which results in the accomplished mitosis. CDK1, CDK2 and CDK4/6 activities are regulated through stimulation upon synthesis of and interaction with cyclins, as well as inhibition by checkpoint kinase signaling. The suppression of CDK1 is through inhibitory phosphorylation, while CDK4/6 and CDK2 are inhibited by protein binding. INK4A protein targets CDK4/6, while p21 and p27 suppress CDK2. Phosphorylation of Rb relieves its inhibitory interaction with E2F and allows E2F transcriptional activity. (Picture created at Biorender.com).

Cyclin-dependent kinases

CDKs in cell cycle regulation

Eukaryotic cells proliferate by going through cell cycles where they duplicate the genetic materials as well as important organelles and divide equally into two. Each cell cycle is composed of G1, S, G2, and M phases. DNA replication takes place in the S phase, in between of the G1 and G2 gap phases during which cells grow in size, produce proteins and assemble organelles to prepare for DNA synthesis and mitosis, respectively. Mitosis in the M phase results in segregation of the chromosome replicas, followed by cytokinesis where cytoplasm is partitioned in cell division. Cells in the G1 phase can exit the cell cycle and enter a resting phase referred to as G0, where they become quiescent or senescent and not dividing.

Progression of the cell cycle requires participation of the cyclin-dependent kinases (CDKs) (**Figure 4**). The first member among the CDK family was discovered by screening for mutants in a yeast species, *Schizosaccharomyces pombe,* in 1986 [121], and was named as cdc2 with regard to its role in the cell division control. Next year, another protein serine kinase was identified as PSK-J3 (putative serine/threonine kinase, filter J colony 3) by homologous probing [122]. Soon after, it was demonstrated that the activation of cdc2 requires its association with a protein that belongs to a previously recognized group, termed as cyclins [123, 124]. In 1991, to unify the nomenclature, cdc2 was

designated as CDK1, and PSK-J3 was designated as CDK4, along with other members designated as CDK2 [125-128], CDK5 and CDK6 [129-131]. To date, CDK1 through CDK-20 have been identified, together with a series of cyclins. Cyclins bind to CDKs and allow them to catalyze the phosphorylation on their substrates. Cyclins are synthesized and degraded by ubiquitin-mediated proteolysis throughout the cell cycle, and thereby regulate the activity of different CDKs at corresponding stages. There are two categories of CDKs, with one being involved in cell cycle regulation (CDK1-CDK6, CDK14-CDK18) and the other one controlling gene transcription (CDK7-CDK13, CDK-19 and CDK20) [132].

The activity of CDK1 plays an essential part in the G2-M transition. At the entry to G2 phase, CDK1 interacts with A-type cyclins (*e.g.* cyclin A2). As cell cycle progresses from G2 to M phase, A-type cyclins are degraded and B-type cyclins are synthesized. Consequently, CDK1 binds to B-type cyclins (*e.g.* cyclin B1) in M phase. CDK1-cyclin B activity has been implicated in multiple events that are fundamental to mitosis such as centrosome separation, nuclear envelope disassembly, chromosome condensation and spindle formation [133]. To prevent premature mitosis, CDK1 activity is tightly regulated. In addition to the cyclin-mediated control, cell cycle checkpoints are key determinants to assess the cell status, ensure the proper pace and restrict the cell cycle progression in response to DNA damage. For instance, checkpoint kinase 1 (CHK1) phosphorylates and activates WEE1, which in turn confers the phosphorylation on CDK1 and inhibits its activity [134, 135]. CDK1 can be derepressed by CDC25 through its phosphatase activity [134]. Later in the M phase, elimination of B-type cyclins leads to the exit from mitosis.

CDK4 and CDK6 share high amino acid homology and the ability to bind D-type cyclins. The CDK4/6 activity is a key determinant in the G1-S phase transition. In the G1 phase, mitogenic signals upregulate the synthesis of D-type cyclins (D1-D3), which interact with CDK4/6. The complex phosphorylates the downstream substrates in the Rb (retinoblastoma) "pocket protein" family, including Rb, p107 and p130 proteins [136]. Rb functions by binding with the E2F transcription factors and thus inhibits the transcription of E2F-responsive targets [136]. The phosphorylation of Rb releases the sequestered E2F which activates the expression of genes that are required in G1-S progression. Among these stimulated genes are E-type cyclins, which subsequently upregulate CDK2 activity. CDK2-cyclin E hyper-phosphorylates Rb [137], resulting in irreversible cell cycle commitment. Activated CDK4/6 also derepresses CDK2 by interacting with $p21^{WAF1}$ and $p27^{KIP1}$ which inhibit CDK2 activity through interactions. As the cell cycle progresses, E-type cyclins are degraded while A-type cyclins are synthesized and bound by CDK2. The CDK2-cyclin A activity then assists the progression in S phase. The activity of CDK4/6 is regulated through binding by the INK4 family ($p16^{INK4A}$, $p15^{INK4B}$, $p18^{INK4C}$ and $p19^{INK4D}$) [138-140]. Interaction with $p18^{INK4C}$ disrupts the CDK4/6-cyclin D association and distorts the kinase catalytic region to suppress CDK4/6 activity [141-143].

Altered CDK4/6 signaling in cancer

Rapid and uncontrolled cell proliferation is a key trait of cancer. Examination of malignant tissues has revealed overexpression of promoting factors and abnormally activated progression signals in the cell cycle. After the activation of CDK4/6 in G1-S phase, the positive feedback loop between Rb phosphorylation and CDK2 activation forms a "restriction point", from where the extracellular signal becomes dispensable for cell division. This feature has made the CDK4/6-Rb axis a major hotspot of dysregulation exploited by cancer cells. The *CCND1* gene (encoding cyclin D1) is amplified in 10-20% in breast cancer (BC), and the overexpression of cyclin D1 has been reported in up to 81% of the BC cases [144-147]. *CDK4* amplification occurs in 15-20% glioblastomas [148]. Homozygous deletion of *CDKN2A* (encoding $p16^{INK4A}$) is found in up to 43% IDH-mutant gliomas [149]. *RB1* (encoding Rb protein) is the earliest identified tumor suppressor gene. Its mutation was detected in 75% in a cohort of small cell lung cancer (SCLC) [150].

Targeting CDKs in cancer therapy

The involvement of deregulated cell cycle and CDKs in tumorigenesis and cancer progression

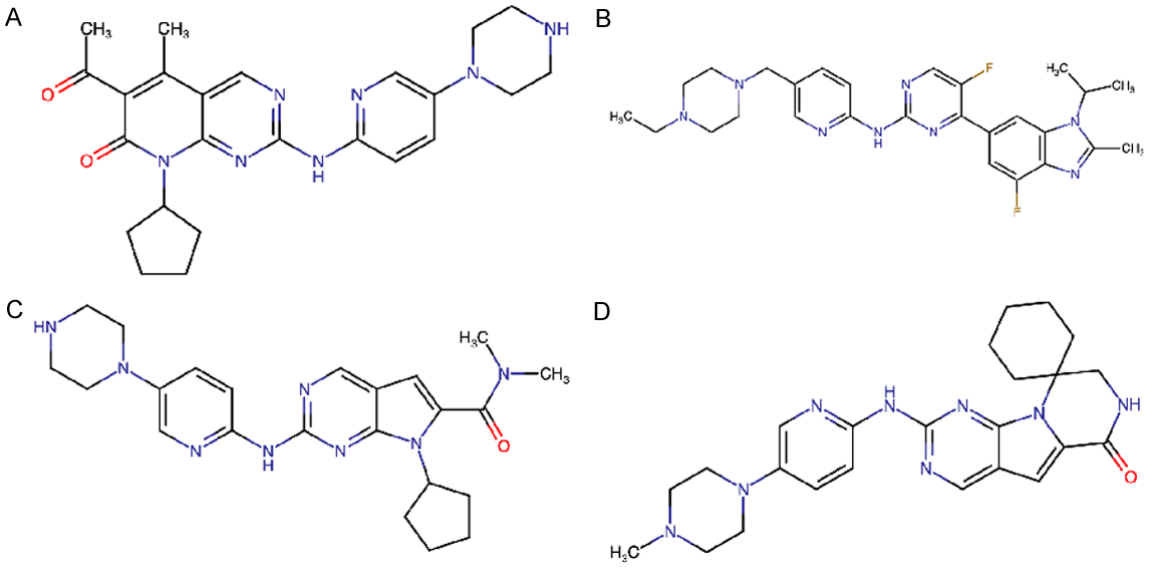

Figure 5. Approved CDK4/6 inhibitors. (A) Palbociclib, (B) abemaciclib, (C) ribociclib and (D) trilaciclib chemical structures are shown. Structures are drawn with *Chem Space*.

has prompted the development of CDK inhibitors in cancer treatment. Trials of Pan-CDK inhibitors (*e.g.* flavopiridol) suffer from the lack of specific biomarkers for patient selection and the undetermined therapeutic window. Flavopiridol also has significant toxicity. Whereas four of the selective CDK4/6 inhibitors have acquired FDA approval over the recent years, including palbociclib (Ibrance; Pfizer) (**Figure 5A**) [151], abemaciclib (Verzenio; Eli Lilly) (**Figure 5B**) [152], ribociclib (Kisqali; Novartis) (**Figure 5C**) [153] and trilaciclib (Cosela; G1 Therapeutics) (**Figure 5D**) [154]. Palbociclib and ribociclib have a higher selectivity to CDK4/6 than abemaciclib. However, abemaciclib has the advantage in crossing the blood-brain barrier which permits potential application on brain tumors and metastases [155]. These three CDK4/6 inhibitors are approved for use in hormone receptor-positive, HER2-negative advanced breast cancer and are taken orally. Trilaciclib is approved in February 2021 for the treatment of patients with small cell lung cancer (SCLC) in combination with chemotherapy. It is administered intravenously prior to chemotherapeutic agents to protect from chemo-induced myelosuppression [156, 157].

A remaining issue with targeted therapy inhibiting CDK4/6 is the development of resistance in patients. Some of the potential resistance mechanisms include amplification of CDK4/6, overexpression of cyclin D1/D2, upregulation of CDK2 and cyclin E1/E2, silencing of Rb, and activation of the growth factor signaling (**Figure 6**) [158]. To overcome the resistance, exploration of the combination treatment strategies is being investigated such as adding PI3K pathway inhibitors to the therapeutic plan of CDK4/6 inhibition plus endocrine therapy. Interestingly, inhibition of CDK4/6 has been shown to induce immune activity [159-161], which suggests a prospective strategy to combine CDK4/6 inhibitors with immunotherapy (*e.g.* Anti-PD1/PDL1). To date, there are still more CDK4/6 inhibitors and therapeutic plans with existing approved drugs under investigation in ongoing clinical trials [162] (**Figure 7**).

Heat-shock proteins

HSPs as chaperone proteins

The heat shock proteins (HSPs) were discovered unexpectedly in *Drosophila melanogaster* upon a temperature increase. In response to heat (and other cellular) stress(es), HSPs play a protective role on newly synthesized and misfolded proteins. HSPs are traditionally named as per their molecular weights. Accordingly, HSP90 proteins refer to a group of 90-kDa HSPs. Members within this family are highly

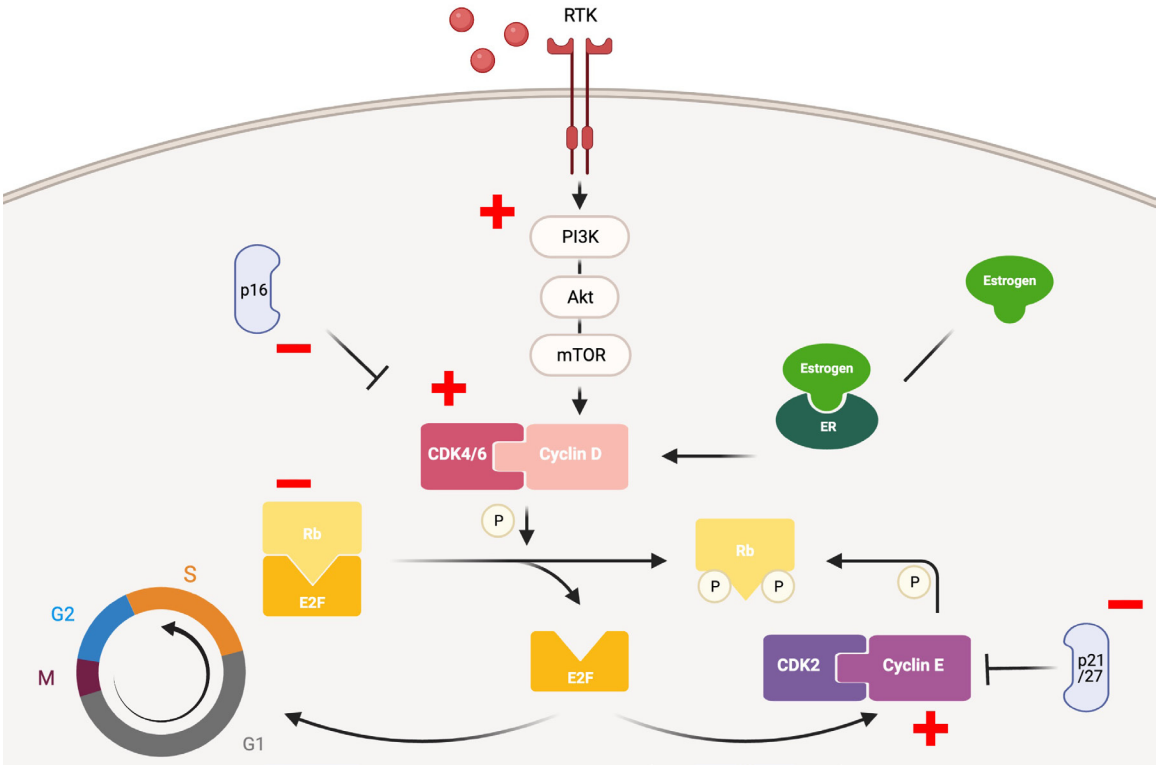

Figure 6. Potential mechanisms linked with the resistance to CDK4/6 inhibition. The red "+" and "-" indicate where alterations could occur. "+" implicates activation and overexpression, and "-" implicates loss of function. (Picture created at Biorender.com).

conserved and assist the folding of their clients. Mammalian HSP90 isoforms include cytosolic HSP90α (HSP90α_1 & HSP90α_2) and HSP90β, mitochondrial TRAP, and GRP94 that is located in endoplasmic reticulum [163]. The cytoplasmic HSP90s represent the primary HSP90 expression and interact with more proteins than the isoforms residing in the ER and mitochondria. HSP90 homologues contain an N-terminal domain (NTD), a middle domain (MD), a C-terminal domain (CTD), and a charged linker (CR) region (**Figure 8**). The CTDs mediate the homodimerization of HSP90, which in turn serves as an ATPase. The NTDs provide an ATP binding site and serve as motifs for co-chaperone associations. Client proteins and co-chaperones are recruited to the MD region. For example, the activator of HSP90 ATPase homologue 1 (AHA1) interacts with the NTD and the MD of HSP90 and promotes the ATPase activity of the HSP90 dimer through the induction of dynamic conformational changes [164].

HSP90 chaperoning in cancer

During the initiation and progression of cancer, increased malignancy is coupled with the overexpression and various mutations of numerous proteins. HSP90 chaperones a variety of oncoproteins, such as cancer-related kinases and transcription factors, maintains their activated forms and prevents them from misfolding and degradation [165, 166]. The functions of HSP90 clients span multiple hallmarks of cancer and alleviate external and internal stresses to facilitate cell survival (**Figure 9**). For example, the epidermal growth factor receptor (EGFR) is a tumor driver that typically induces PI3K/Akt signaling. Amplification and mutation of the *EGFR* gene often results in increased activation of the pathway and promotes cancer especially in lungs and breasts [167]. As an HSP90 client protein [168], EGFR is degraded upon HSP90 inhibition [169]. Another client of HSP90, DNA (cytosine-5)-methyltransferase 1 (DNMT1) [170], induces the hypermethylation at CpG is-

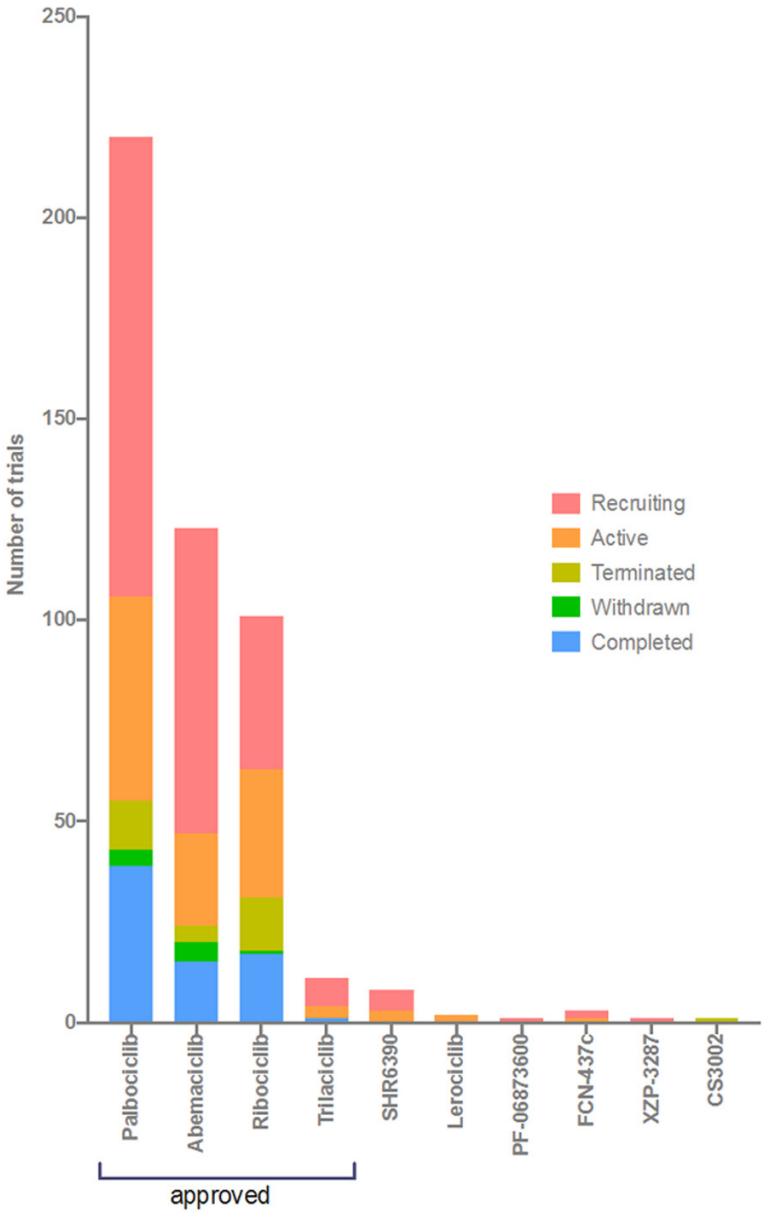

Figure 7. CDK4/6 inhibitors investigated in clinical trials. The status of CDK4/6 inhibitors in clinical trials for cancer treatment. Graph is generated based on information in the *ClinicalTrials.gov* database.

lands to silence tumor-suppressive genes [171] and promote cancer. Overexpression of DNMT1 has been reported in multiple types of cancer (*e.g.* colon [172], breast [173] and liver [174]). As mentioned, HIF-1α is stabilized by HSP90, through which the role of HSP90 is linked to angiogenesis and metabolism alterations in cancer. The overexpression of HSP90 itself has also been associated with low survival rate in breast cancer [175].

Targeting HSP90 in cancer therapy

HSP90 has been long perceived as a potential target in cancer treatment. A classic HSP90 inhibitor, geldanamycin (**Figure 10A**), was extracted as a natural product from *Streptomyces hygroscopicus* half a century ago [176]. It is structurally similar to ATP and able to dock at the ATP-binding pocket in the NTD of HSP90 [177-179]. Although the instability and hepatotoxicity have limited its applications in clinic, geldanamycin has been used *in vitro* for decades to elucidate the effects of HSP90 inhibition. The derivative of geldanamycin, 17-N-Allylamino-17-demethoxygeldanamycin (17-AAG) was the first HSP90 inhibitor to be tested in clinical trials two decades ago. A concern about 17-AAG is its poor aqueous solubility [180]. The development of 17-AAG was eventually discontinued. Over the past years, multiple HSP90 inhibitors have emerged and have been tested in more than 170 clinical trials (**Figure 11**) [181]. Some of the inhibitors developed during the recent decade include ganetespib (also known as STA-9090) [182] (**Figure 10B**), onalespib (also known as AT13387) [183] (**Figure 10C**), luminespib [184] (**Figure 10D**), XL888 [185] (**Figure 10E**), and TAS-116 [186] (**Figure 10F**). While most HSP90 inhibitors are similar in the way that they bind to the NTD region competitively against ATP, TAS-116 is unique in that it is the first-in-class to enter clinical trials with a selectivity for cytosolic HSP90 (HSP90α & HSP90β) [186]. An anti-cancer effect has been observed with TAS-116 in non-small cell lung cancer and gastrointestinal stromal tumor (GIST) in a Phase I study [187].

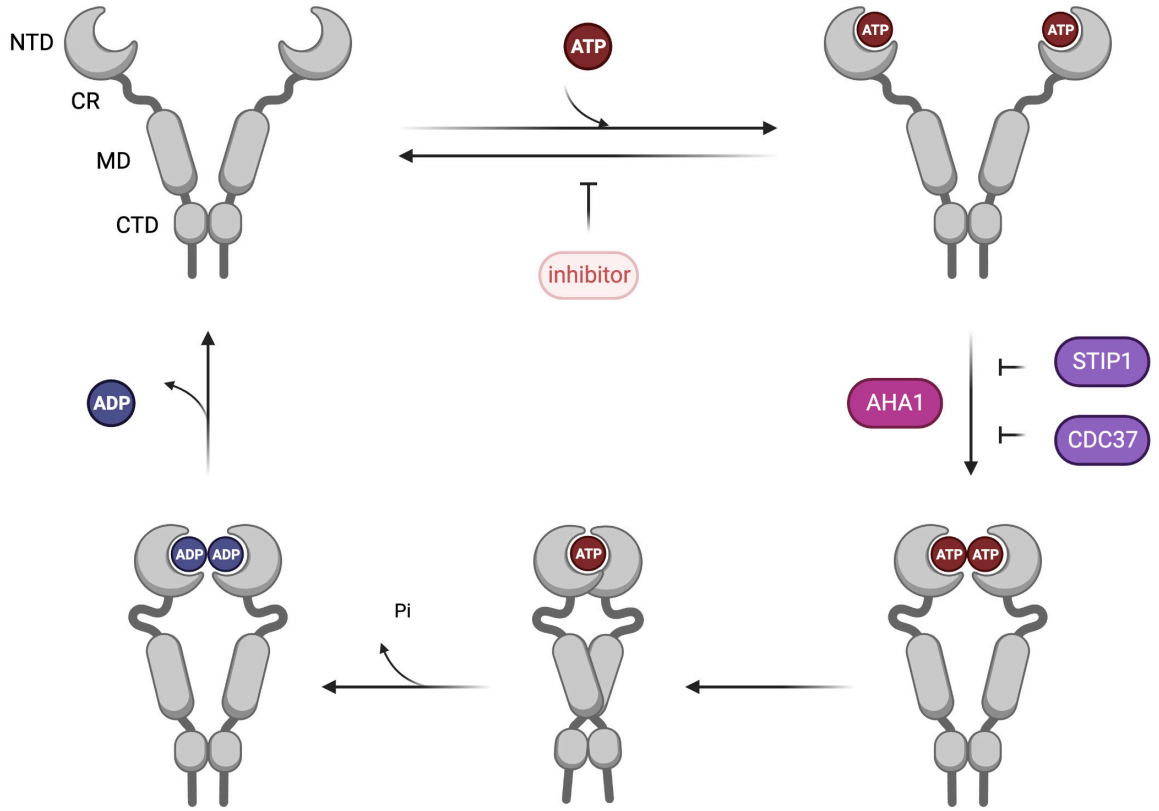

Figure 8. The model of HSP90 cycle with dynamic ATP and co-chaperone associations. AHA1 accelerates the conformational switch, which results in the ATPase activity of HSP90 dimer. (Picture created at Biorender.com).

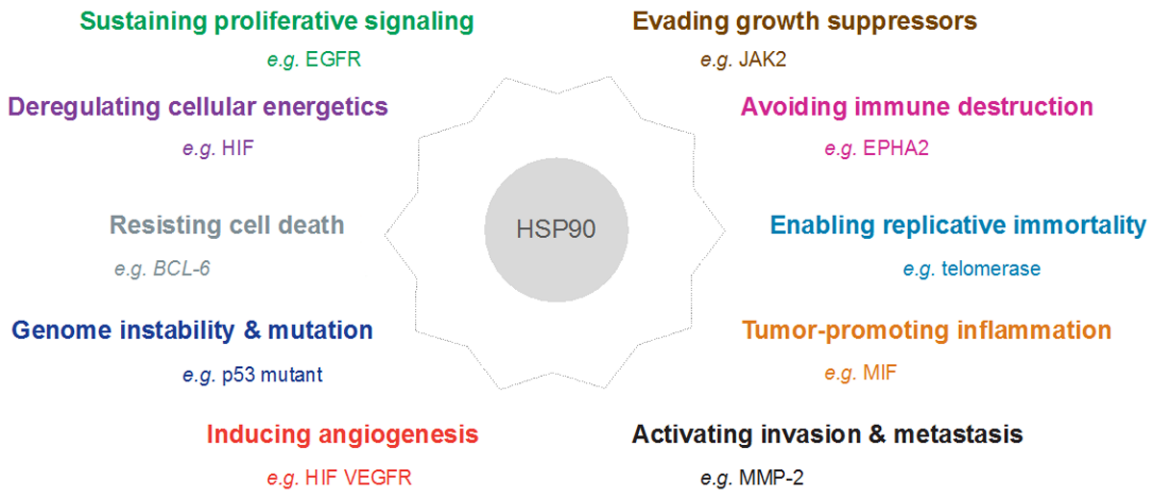

Figure 9. HSP90 regulates proteins involved in various cancer hallmarks. HSP90 clients are implicated in multiple pathways to help cancer cells survive the environmental stresses as well as to promote tumor growth and progression.

Summary

Accumulation of HIF-1α is a signature of hypoxia and potentiates tumor propagation and malignant progression. HIF-1α overexpression is associated with poor prognosis in various cancer types. Malignant induction of HIF-1α signaling is not restricted to intratumoral hy-

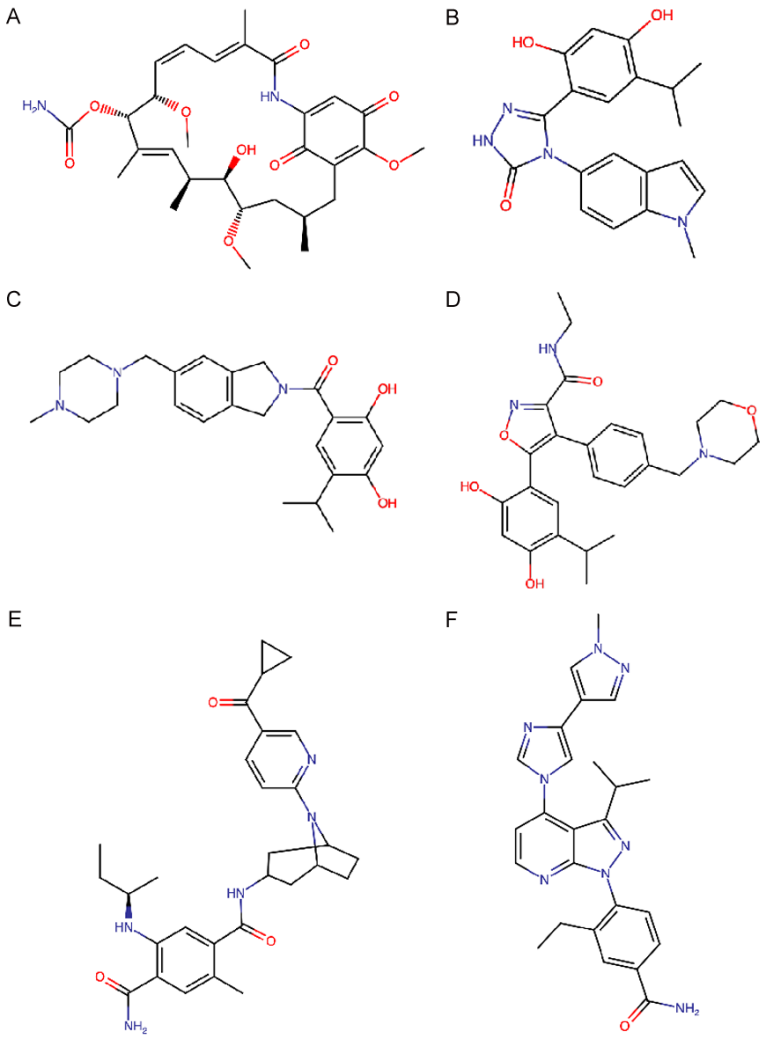

Figure 10. Structures of HSP90 inhibitors. (A) Geldanamycin, (B) ganetespib, (C) onalespib, (D) luminespib, (E) XL888 and (F) TAS-116 chemical structures are drawn with *Chem Space*.

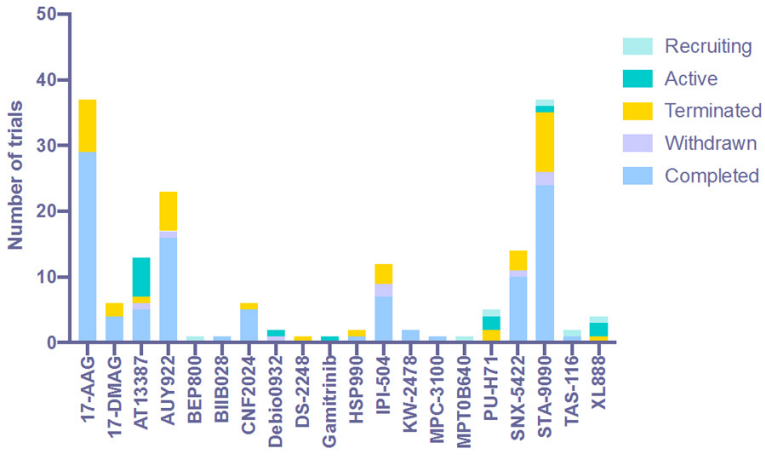

Figure 11. HSP90 inhibitors investigated in clinical trials. The status of HSP90 inhibitors in clinical trials for cancer treatment. Graph is generated based on information in the *ClinicalTrials.gov* database.

poxic regions. Understanding and targeting the mechanisms of cancer-related HIF-1α stabilization potentially help to improve current cancer treatments. Targeting HIF-1α in malignant cells under both normoxia and hypoxia represents a promising approach to generally target tumordriving molecules and pathways involved with cancer hallmarks.

We have previously shown that cyclin-dependent kinase 1 (CDK1) stabilizes HIF-1α through phosphorylation of its Ser668 residue in a VHL-independent manner. The stabilization occurs both under hypoxia and at G2/M under normoxia. Meanwhile we showed that CDK4/6 proteins also a role in HIF-1α stabilization. We have recently shown a convergence of CDK1 or CDK4/6 and HSP90 signaling on HIF-1α, provides the rationale and preclinical reference for targeting HIF-1α by strategies of combination therapy [188].

Understanding the different mechanisms of HIF-1α regulation will help to pave the way for therapeutic targeting of the related components in cancer as well as other physiological disorders which involve HIF-1α activity. The therapeutic targeting of HIF1-alpha and the non-canonical mechanism(s) of its destabilization during cancer therapeutic intervention is an area ripe for much research.

Disclosure of conflict of interest

None.

Address correspondence to: Wafik S El-Deiry, Laboratory of Trans-

lational Oncology and Experimental Cancer Therapeutics, Warren Alpert Medical School, Brown University, Providence, RI, USA. E-mail: wafik@brown.edu

References

[1] Carmeliet P and Jain RK. Angiogenesis in cancer and other diseases. Nature 2000; 407: 249-257.

[2] Vaupel P and Mayer A. Hypoxia and anemia: effects on tumor biology and treatment resistance. Transfus Clin Biol 2005; 12: 5-10.

[3] Graeber TG, Osmanian C, Jacks T, Housman DE, Koch CJ, Lowe SW and Giaccia AJ. Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. Nature 1996; 379: 88-91.

[4] Erler JT, Cawthorne CJ, Williams KJ, Koritzinsky M, Wouters BG, Wilson C, Miller C, Demonacos C, Stratford IJ and Dive C. Hypoxia-mediated down-regulation of Bid and Bax in tumors occurs via hypoxia-inducible factor 1-dependent and -independent mechanisms and contributes to drug resistance. Mol Cell Biol 2004; 24: 2875-2889.

[5] Ebert BL, Firth JD and Ratcliffe PJ. Hypoxia and mitochondrial inhibitors regulate expression of glucose transporter-1 via distinct Cis-acting sequences. J Biol Chem 1995; 270: 29083-29089.

[6] Carmeliet P, Dor Y, Herbert JM, Fukumura D, Brusselmans K, Dewerchin M, Neeman M, Bono F, Abramovitch R, Maxwell P, Koch CJ, Ratcliffe P, Moons L, Jain RK, Collen D and Keshert E. Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. Nature 1998; 394: 485-490.

[7] Pennacchietti S, Michieli P, Galluzzo M, Mazzone M, Giordano S and Comoglio PM. Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. Cancer Cell 2003; 3: 347-361.

[8] Chang Q, Jurisica I, Do T and Hedley DW. Hypoxia predicts aggressive growth and spontaneous metastasis formation from orthotopically grown primary xenografts of human pancreatic cancer. Cancer Res 2011; 71: 3110-3120.

[9] Bristow RG and Hill RP. Hypoxia and metabolism. Hypoxia, DNA repair and genetic instability. Nat Rev Cancer 2008; 8: 180-192.

[10] Barsoum IB, Smallwood CA, Siemens DR and Graham CH. A mechanism of hypoxia-mediated escape from adaptive immunity in cancer cells. Cancer Res 2014; 74: 665-674.

[11] Noman MZ, Desantis G, Janji B, Hasmim M, Karray S, Dessen P, Bronte V and Chouaib S. PD-L1 is a novel direct target of HIF-1α, and its blockade under hypoxia enhanced MDSC-mediated T cell activation. J Exp Med 2014; 211: 781-790.

[12] Koukourakis MI, Giatromanolaki A, Sivridis E, Simopoulos C, Turley H, Talks K, Gatter KC and Harris AL. Hypoxia-inducible factor (HIF1A and HIF2A), angiogenesis, and chemoradiotherapy outcome of squamous cell head-and-neck cancer. Int J Radiat Oncol Biol Phys 2002; 53: 1192-1202.

[13] Generali D, Berruti A, Brizzi MP, Campo L, Bonardi S, Wigfield S, Bersiga A, Allevi G, Milani M, Aguggini S, Gandolfi V, Dogliotti L, Bottini A, Harris AL and Fox SB. Hypoxia-inducible factor-1alpha expression predicts a poor response to primary chemoendocrine therapy and disease-free survival in primary human breast cancer. Clin Cancer Res 2006; 12: 4562-4568.

[14] Vergis R, Corbishley CM, Norman AR, Bartlett J, Jhavar S, Borre M, Heeboll S, Horwich A, Huddart R, Khoo V, Eeles R, Cooper C, Sydes M, Dearnaley D and Parker C. Intrinsic markers of tumour hypoxia and angiogenesis in localised prostate cancer and outcome of radical treatment: a retrospective analysis of two randomised radiotherapy trials and one surgical cohort study. Lancet Oncol 2008; 9: 342-351.

[15] Wilson WR and Hay MP. Targeting hypoxia in cancer therapy. Nat Rev Cancer 2011; 11: 393-410.

[16] Mistry IN, Thomas M, Calder EDD, Conway SJ and Hammond EM. Clinical advances of hypoxia-activated prodrugs in combination with radiation therapy. Int J Radiat Oncol Biol Phys 2017; 98: 1183-1196.

[17] Semenza GL, Nejfelt MK, Chi SM and Antonarakis SE. Hypoxia-inducible nuclear factors bind to an enhancer element located 3' to the human erythropoietin gene. Proc Natl Acad Sci U S A 1991; 88: 5680-5684.

[18] Semenza GL and Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol 1992; 12: 5447-5454.

[19] Wang GL and Semenza GL. Purification and characterization of hypoxia-inducible factor 1. J Biol Chem 1995; 270: 1230-1237.

[20] Wang GL, Jiang BH, Rue EA and Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A 1995; 92: 5510-5514.

[21] Ebert BL and Bunn HF. Regulation of transcription by hypoxia requires a multiprotein complex that includes hypoxia-inducible factor 1, an adjacent transcription factor, and p300/

CREB binding protein. Mol Cell Biol 1998; 18: 4089-4096.
[22] Ema M, Hirota K, Mimura J, Abe H, Yodoi J, Sogawa K, Poellinger L and Fujii-Kuriyama Y. Molecular mechanisms of transcription activation by HLF and HIF1α in response to hypoxia: their stabilization and redox signal-induced interaction with CBP/p300. EMBO J 1999; 18: 1905-1914.
[23] Hu CJ, Sataur A, Wang L, Chen H and Simon MC. The N-terminal transactivation domain confers target gene specificity of hypoxia-inducible factors HIF-1alpha and HIF-2alpha. Mol Biol Cell 2007; 18: 4528-4542.
[24] Tian H, McKnight SL and Russell DW. Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. Genes Dev 1997; 11: 72-82.
[25] Ema M, Taya S, Yokotani N, Sogawa K, Matsuda Y and Fujii-Kuriyama Y. A novel bHLH-PAS factor with close sequence similarity to hypoxia-inducible factor 1α regulates the VEGF expression and is potentially involved in lung and vascular development. Proc Natl Acad Sci U S A 1997; 94: 4273-4278.
[26] Flamme I, Fröhlich T, von Reutern M, Kappel A, Damert A and Risau W. HRF, a putative basic helix-loop-helix-PAS-domain transcription factor is closely related to hypoxia-inducible factor-1α and developmentally expressed in blood vessels. Mech Dev 1997; 63: 51-60.
[27] Hogenesch JB, Chan WK, Jackiw VH, Brown RC, Gu YZ, PrayGrant M, Perdew GH and Bradfield CA. Characterization of a subset of the basic-helix-loop-helix-PAS superfamily that interacts with components of the dioxin signaling pathway. J Biol Chem 1997; 272: 8581-8593.
[28] Semenza GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer 2003; 3: 721-732.
[29] Wiesener MS, Jurgensen JS, Rosenberger C, Scholze CK, Horstrup JH, Warnecke C, Mandriota S, Bechmann I, Frei UA, Pugh CW, Ratcliffe PJ, Bachmann S, Maxwell PH and Eckardt KU. Widespread hypoxia-inducible expression of HIF-2alpha in distinct cell populations of different organs. FASEB J 2003; 17: 271-273.
[30] Brusselmans K, Bono F, Maxwell P, Dor Y, Dewerchin M, Collen D, Herbert JM and Carmeliet P. Hypoxia-inducible factor-2alpha (HIF-2alpha) is involved in the apoptotic response to hypoglycemia but not to hypoxia. J Biol Chem 2001; 276: 39192-39196.
[31] Hu CJ, Wang LY, Chodosh LA, Keith B and Simon MC. Differential roles of hypoxia-inducible factor 1alpha (HIF-1alpha) and HIF-2alpha in hypoxic gene regulation. Mol Cell Biol 2003; 23: 9361-9374.
[32] Raval RR, Lau KW, Tran MG, Sowter HM, Mandriota SJ, Li JL, Pugh CW, Maxwell PH, Harris AL and Ratcliffe PJ. Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel-Lindau-associated renal cell carcinoma. Mol Cell Biol 2005; 25: 5675-5686.
[33] Grabmaier K, de Weijert MC, Verhaegh GW, Schalken JA and Oosterwijk E. Strict regulation of CAIX G250/MN by HIF-1 α in clear cell renal cell carcinoma. Oncogene 2004; 23: 5624-5631.
[34] Covello KL, Kehler J, Yu H, Gordan JD, Arsham AM, Hu CJ, Labosky PA, Simon MC and Keith B. HIF-2alpha regulates Oct-4: effects of hypoxia on stem cell function, embryonic development, and tumor growth. Genes Dev 2006; 20: 557-570.
[35] Duan C. Hypoxia-inducible factor 3 biology: complexities and emerging themes. Am J Physiol Cell Physiol 2016; 310: C260-269.
[36] Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara JM, Lane WS and Kaelin WG Jr. HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. Science 2001; 292: 464-468.
[37] Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, von Kriegsheim A, Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW and Ratcliffe PJ. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. Science 2001; 292: 468-472.
[38] Masson N, Willam C, Maxwell PH, Pugh CW and Ratcliffe PJ. Independent function of two destruction domains in hypoxia-inducible factor-alpha chains activated by prolyl hydroxylation. EMBO J 2001; 20: 5197-5206.
[39] Appelhoff RJ, Tian YM, Raval RR, Turley H, Harris AL, Pugh CW, Ratcliffe PJ and Gleadle JM. Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor. J Biol Chem 2004; 279: 38458-38465.
[40] Landazuri MO, Vara-Vega A, Viton M, Cuevas Y and del Peso L. Analysis of HIF-prolyl hydroxylases binding to substrates. Biochem Biophys Res Commun 2006; 351: 313-320.
[41] Koivunen P, Tiainen P, Hyvärinen J, Williams KE, Sormunen R, Klaus SJ, Kivirikko KI and Myllyharju J. An endoplasmic reticulum transmembrane prolyl 4-hydroxylase is induced by hypoxia and acts on hypoxia-inducible factor α. J Biol Chem 2007; 282: 30544-30552.
[42] Kershaw NJ and Babon JJ. VHL: Cullin-g the hypoxic response. Structure 2015; 23: 435-436.
[43] Tanimoto K, Makino Y, Pereira T and Poellinger L. Mechanism of regulation of the hypoxia-inducible factor-1α by the von Hippel-Lindau tu-

mor suppressor protein. EMBO J 2000; 19: 4298-4309.
[44] Kamura T, Maenaka K, Kotoshiba S, Matsumoto M, Kohda D, Conaway RC, Conaway JW and Nakayama KI. VHL-box and SOCS-box domains determine binding specificity for Cul2-Rbx1 and Cul5-Rbx2 modules of ubiquitin ligases. Genes Dev 2004; 18: 3055-3065.
[45] Jeong JW, Bae MK, Ahn MY, Kim SH, Sohn TK, Bae MH, Yoo MA, Song EJ, Lee KJ and Kim KW. Regulation and destabilization of HIF-1alpha by ARD1-mediated acetylation. Cell 2002; 111: 709-720.
[46] Mahon PC, Hirota K and Semenza GL. FIH-1: a novel protein that interacts with HIF-1alpha and VHL to mediate repression of HIF-1 transcriptional activity. Genes Dev 2001; 15: 2675-2686.
[47] Lando D, Peet DJ, Gorman JJ, Whelan DA, Whitelaw ML and Bruick RK. FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. Genes Dev 2002; 16: 1466-1471.
[48] Lando D, Peet DJ, Whelan DA, Gorman JJ and Whitelaw ML. Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. Science 2002; 295: 858-861.
[49] Dames SA, Martinez-Yamout M, De Guzman RN, Dyson HJ and Wright PE. Structural basis for Hif-1 alpha/CBP recognition in the cellular hypoxic response. Proc Natl Acad Sci U S A 2002; 99: 5271-5276.
[50] Xu Q, Briggs J, Park S, Niu GL, Kortylewski M, Zhang SM, Gritsko T, Turkson J, Kay H, Semenza GL, Cheng JQ, Jove R and Yu H. Targeting Stat3 blocks both HIF-1 and VEGF expression induced by multiple oncogenic growth signaling pathways. Oncogene 2005; 24: 5552-5560.
[51] Niu G, Briggs J, Deng J, Ma Y, Lee H, Kortylewski M, Kujawski M, Kay H, Cress WD and Jove R. Signal transducer and activator of transcription 3 is required for hypoxia-inducible factor-1α RNA expression in both tumor cells and tumor-associated myeloid cells. Mol Cancer Res 2008; 6: 1099-1105.
[52] Dang EV, Barbi J, Yang HY, Jinasena D, Yu H, Zheng Y, Bordman Z, Fu J, Kim Y, Yen HR, Luo W, Zeller K, Shimoda L, Topalian SL, Semenza GL, Dang CV, Pardoll DM and Pan F. Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. Cell 2011; 146: 772-784.
[53] Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel AS, Nizet V, Johnson RS, Haddad GG and Karin M. NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1alpha. Nature 2008; 453: 807-811.
[54] Blouin CC, Page EL, Soucy GM and Richard DE. Hypoxic gene activation by lipopolysaccharide in macrophages: implication of hypoxia-inducible factor 1alpha. Blood 2004; 103: 1124-1130.
[55] Jantsch J, Wiese M, Schödel J, Castiglione K, Gläsner J, Kolbe S, Mole D, Schleicher U, Eckardt KU and Hensel M. Toll-like receptor activation and hypoxia use distinct signaling pathways to stabilize hypoxia-inducible factor 1α (HIF1A) and result in differential HIF1A-dependent gene expression. J Leukoc Biol 2011; 90: 551-562.
[56] Sasabe E, Yang Z, Ohno S and Yamamoto T. Reactive oxygen species produced by the knockdown of manganese-superoxide dismutase up-regulate hypoxia-inducible factor-1alpha expression in oral squamous cell carcinoma cells. Free Radic Biol Med 2010; 48: 1321-1329.
[57] Movafagh S, Crook S and Vo K. Regulation of hypoxia-inducible factor-1a by reactive oxygen species: new developments in an old debate. J Cell Biochem 2015; 116: 696-703.
[58] Fruman DA, Chiu H, Hopkins BD, Bagrodia S, Cantley LC and Abraham RT. The PI3K pathway in human disease. Cell 2017; 170: 605-635.
[59] He Q, Gao Z, Yin J, Zhang J, Yun Z and Ye J. Regulation of HIF-1{alpha} activity in adipose tissue by obesity-associated factors: adipogenesis, insulin, and hypoxia. Am J Physiol Endocrinol Metab 2011; 300: E877-885.
[60] Laughner E, Taghavi P, Chiles K, Mahon PC and Semenza GL. HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1alpha (HIF-1alpha) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. Mol Cell Biol 2001; 21: 3995-4004.
[61] Salnikow K, Donald SP, Bruick RK, Zhitkovich A, Phang JM and Kasprzak KS. Depletion of intracellular ascorbate by the carcinogenic metals nickel and cobalt results in the induction of hypoxic stress. J Biol Chem 2004; 279: 40337-40344.
[62] Kim JJ, Lee SB, Jang J, Yi SY, Kim SH, Han SA, Lee JM, Tong SY, Vincelette ND, Gao B, Yin P, Evans D, Choi DW, Qin B, Liu T, Zhang H, Deng M, Jen J, Zhang J, Wang L and Lou Z. WSB1 promotes tumor metastasis by inducing pVHL degradation. Genes Dev 2015; 29: 2244-2257.
[63] Kim WY and Kaelin WG. Role of VHL gene mutation in human cancer. J Clin Oncol 2004; 22: 4991-5004.
[64] Koh MY, Darnay BG and Powis G. Hypoxia-associated factor, a novel E3-ubiquitin ligase, binds and ubiquitinates hypoxia-inducible factor 1alpha, leading to its oxygen-independent degradation. Mol Cell Biol 2008; 28: 7081-7095.

[65] Bento CF, Fernandes R, Ramalho J, Marques C, Shang F, Taylor A and Pereira P. The chaperone-dependent ubiquitin ligase CHIP targets HIF-1alpha for degradation in the presence of methylglyoxal. PLoS One 2010; 5: e15062.
[66] Ravi R, Mookerjee B, Bhujwalla ZM, Sutter CH, Artemov D, Zeng Q, Dillehay LE, Madan A, Semenza GL and Bedi A. Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1alpha. Genes Dev 2000; 14: 34-44.
[67] Liu YV, Baek JH, Zhang H, Diez R, Cole RN and Semenza GL. RACK1 competes with HSP90 for binding to HIF-1alpha and is required for O(2)-independent and HSP90 inhibitor-induced degradation of HIF-1alpha. Mol Cell 2007; 25: 207-217.
[68] Flugel D, Gorlach A, Michiels C and Kietzmann T. Glycogen synthase kinase 3 phosphorylates hypoxia-inducible factor 1alpha and mediates its destabilization in a VHL-independent manner. Mol Cell Biol 2007; 27: 3253-3265.
[69] Flugel D, Gorlach A and Kietzmann T. GSK-3beta regulates cell growth, migration, and angiogenesis via Fbw7 and USP28-dependent degradation of HIF-1alpha. Blood 2012; 119: 1292-1301.
[70] Cassavaugh JM, Hale SA, Wellman TL, Howe AK, Wong C and Lounsbury KM. Negative regulation of HIF-1alpha by an FBW7-mediated degradation pathway during hypoxia. J Cell Biochem 2011; 112: 3882-3890.
[71] Park CV, Ivanova IG and Kenneth NS. XIAP upregulates expression of HIF target genes by targeting HIF1alpha for Lys63-linked polyubiquitination. Nucleic Acids Res 2017; 45: 9336-9347.
[72] Olmos G, Arenas MI, Bienes R, Calzada MJ, Aragones J, Garcia-Bermejo ML, Landazuri MO and Lucio-Cazana J. 15-Deoxy-Delta(12,14)-prostaglandin-J(2) reveals a new pVHL-independent, lysosomal-dependent mechanism of HIF-1alpha degradation. Cell Mol Life Sci 2009; 66: 2167-2180.
[73] Gradin K, McGuire J, Wenger RH, Kvietikova I, Whitelaw ML, Toftgard R, Tora L, Gassmann M and Poellinger L. Functional interference between hypoxia and dioxin signal transduction pathways: competition for recruitment of the Arnt transcription factor. Mol Cell Biol 1996; 16: 5221-5231.
[74] Kallio PJ, Pongratz I, Gradin K, McGuire J and Poellinger L. Activation of hypoxia-inducible factor 1alpha: posttranscriptional regulation and conformational change by recruitment of the Arnt transcription factor. Proc Natl Acad Sci U S A 1997; 94: 5667-5672.
[75] Isaacs JS, Jung YJ, Mimnaugh EG, Martinez A, Cuttitta F and Neckers LM. Hsp90 regulates a von Hippel Lindau-independent hypoxia-inducible factor-1α-degradative pathway. J Biol Chem 2002; 277: 29936-29944.
[76] Liu YV and Semenza GL. RACK1 vs. HSP90: competition for HIF-1 alpha degradation vs. stabilization. Cell Cycle 2007; 6: 656-659.
[77] Warfel NA, Dolloff NG, Dicker DT, Malysz J and El-Deiry WS. CDK1 stabilizes HIF-1alpha via direct phosphorylation of Ser668 to promote tumor growth. Cell Cycle 2013; 12: 3689-3701.
[78] Zhao S and El-Deiry WS. Identification of Smurf2 as a HIF-1alpha degrading E3 ubiquitin ligase. Oncotarget 2021; 12: 1970-1979.
[79] Vaupel P, Mayer A and Höckel M. Tumor Hypoxia and Malignant Progression. In: editors. Methods Enzymol. Academic Press; 2004. p. 335-354.
[80] Lee YH, Bae HC, Noh KH, Song KH, Ye SK, Mao CP, Lee KM, Wu TC and Kim TW. Gain of HIF-1alpha under normoxia in cancer mediates immune adaptation through the AKT/ERK and VEGFA axes. Clin Cancer Res 2015; 21: 1438-1446.
[81] Kandoth C, McLellan MD, Vandin F, Ye K, Niu B, Lu C, Xie M, Zhang Q, McMichael JF, Wyczalkowski MA, Leiserson MDM, Miller CA, Welch JS, Walter MJ, Wendl MC, Ley TJ, Wilson RK, Raphael BJ and Ding L. Mutational landscape and significance across 12 major cancer types. Nature 2013; 502: 333-339.
[82] Zhao S, Lin Y, Xu W, Jiang W, Zha Z, Wang P, Yu W, Li Z, Gong L, Peng Y, Ding J, Lei Q, Guan KL and Xiong Y. Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1alpha. Science 2009; 324: 261-265.
[83] Goh S, Butler W and Thiele EA. Subependymal giant cell tumors in tuberous sclerosis complex. Neurology 2004; 63: 1457-1461.
[84] Iliopoulos O and Eng C. Genetic and clinical aspects of familial renal neoplasms. Semin Oncol 2000; 27: 138-149.
[85] Brugarolas JB, Vazquez F, Reddy A, Sellers WR and Kaelin WG. TSC2 regulates VEGF through mTOR-dependent and -independent pathways. Cancer Cell 2003; 4: 147-158.
[86] Chen Z, Trotman LC, Shaffer D, Lin HK, Dotan ZA, Niki M, Koutcher JA, Scher HI, Ludwig T, Gerald W, Cordon-Cardo C and Pandolfi PP. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. Nature 2005; 436: 725-730.
[87] Zhong H, Chiles K, Feldser D, Laughner E, Hanrahan C, Georgescu MM, Simons JW and Semenza GL. Modulation of hypoxia-inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogen-

esis and therapeutics. Cancer Res 2000; 60: 1541-1545.
[88] Deming SL, Nass SJ, Dickson RB and Trock BJ. C-myc amplification in breast cancer: a meta-analysis of its occurrence and prognostic relevance. Br J Cancer 2000; 83: 1688-1695.
[89] Doe MR, Ascano JM, Kaur M and Cole MD. Myc posttranscriptionally induces HIF1 protein and target gene expression in normal and cancer cells. Cancer Res 2012; 72: 949-957.
[90] Porru M, Pompili L, Caruso C, Biroccio A and Leonetti C. Targeting KRAS in metastatic colorectal cancer: current strategies and emerging opportunities. J Exp Clin Cancer Res 2018; 37: 57.
[91] Cox AD, Fesik SW, Kimmelman AC, Luo J and Der CJ. Drugging the undruggable RAS: mission possible? Nat Rev Drug Discov 2014; 13: 828-851.
[92] Wang Y, Lei F, Rong W, Zeng Q and Sun W. Positive feedback between oncogenic KRAS and HIF-1alpha confers drug resistance in colorectal cancer. Onco Targets Ther 2015; 8: 1229-1237.
[93] Liu Q and Cao P. Clinical and prognostic significance of HIF-1alpha in glioma patients: a meta-analysis. Int J Clin Exp Med 2015; 8: 22073-22083.
[94] Swinson DE, Jones JL, Cox G, Richardson D, Harris AL and O'Byrne KJ. Hypoxia-inducible factor-1α in non small cell lung cancer: relation to growth factor, protease and apoptosis pathways. Int J Cancer 2004; 111: 43-50.
[95] Dales JP, Garcia S, Meunier-Carpentier S, Andrac-Meyer L, Haddad O, Lavaut MN, Allasia C, Bonnier P and Charpin C. Overexpression of hypoxia-inducible factor HIF-1alpha predicts early relapse in breast cancer: retrospective study in a series of 745 patients. Int J Cancer 2005; 116: 734-739.
[96] Kronblad Å, Jirström K, Rydén L, Nordenskjöld B and Landberg G. Hypoxia inducible factor-1α is a prognostic marker in premenopausal patients with intermediate to highly differentiated breast cancer but not a predictive marker for tamoxifen response. Int J Cancer 2006; 118: 2609-2616.
[97] Yamamoto Y, Ibusuki M, Okumura Y, Kawasoe T, Kai K, Iyama K and Iwase H. Hypoxia-inducible factor 1α is closely linked to an aggressive phenotype in breast cancer. Breast Cancer Res Treat 2008; 110: 465-475.
[98] Griffiths EA, Pritchard S, Valentine HR, Whitchelo N, Bishop P, Ebert M, Price PM, Welch I and West CM. Hypoxia-inducible factor-1 α expression in the gastric carcinogenesis sequence and its prognostic role in gastric and gastro-oesophageal adenocarcinomas. Br J Cancer 2007; 96: 95-103.
[99] Sun HC, Qiu ZJ, Liu J, Sun J, Jiang T, Huang KJ, Yao M and Huang C. Expression of hypoxia-inducible factor-1 alpha and associated proteins in pancreatic ductal adenocarcinoma and their impact on prognosis. Int J Oncol 2007; 30: 1359-1367.
[100] Rajaganeshan R, Prasad R, Guillou PJ, Poston G, Scott N and Jayne DG. The role of hypoxia in recurrence following resection of Dukes' B colorectal cancer. Int J Colorectal Dis 2008; 23: 1049-1055.
[101] Schmitz KJ, Muller CI, Reis H, Alakus H, Winde G, Baba HA, Wohlschlaeger J, Jasani B, Fandrey J and Schmid KW. Combined analysis of hypoxia-inducible factor 1 alpha and metallothionein indicates an aggressive subtype of colorectal carcinoma. Int J Colorectal Dis 2009; 24: 1287-1296.
[102] Baba Y, Nosho K, Shima K, Irahara N, Chan AT, Meyerhardt JA, Chung DC, Giovannucci EL, Fuchs CS and Ogino S. HIF1A overexpression is associated with poor prognosis in a cohort of 731 colorectal cancers. Am J Pathol 2010; 176: 2292-2301.
[103] Birner P, Schindl M, Obermair A, Plank C, Breiteneckerr G and Oberhuber G. Overexpression of hypoxia-inducible factor 1α is a marker for an unfavorable prognosis in early-stage invasive cervical cancer. Cancer Res 2000; 60: 4693-4696.
[104] Bachtiary B, Schindl M, Potter R, Dreier B, Knocke TH, Hainfellner JA, Horvat R and Birner P. Overexpression of hypoxia-inducible factor 1alpha indicates diminished response to radiotherapy and unfavorable prognosis in patients receiving radical radiotherapy for cervical cancer. Clin Cancer Res 2003; 9: 2234-2240.
[105] Burri P, Djonov V, Aebersold DM, Lindel K, Studer U, Altermatt HJ, Mazzucchelli L, Greiner RH and Gruber G. Significant correlation of hypoxia-inducible factor-1alpha with treatment outcome in cervical cancer treated with radical radiotherapy. Int J Radiat Oncol Biol Phys 2003; 56: 494-501.
[106] Daponte A, Ioannou M, Mylonis I, Simos G, Minas M, Messinis IE and Koukoulis G. Prognostic significance of Hypoxia-Inducible Factor 1 alpha (HIF-1 alpha) expression in serous ovarian cancer: an immunohistochemical study. BMC Cancer 2008; 8: 335.
[107] Theodoropoulos VE, Lazaris A, Sofras F, Gerzelis I, Tsoukala V, Ghikonti I, Manikas K and Kastriotis I. Hypoxia-inducible factor 1 alpha expression correlates with angiogenesis and unfavorable prognosis in bladder cancer. Eur Urol 2004; 46: 200-208.
[108] Huang M, Du H, Zhang L, Che H and Liang C. The association of HIF-1alpha expression

with clinicopathological significance in prostate cancer: a meta-analysis. Cancer Manag Res 2018; 10: 2809-2816.
[109] Hudson CC, Liu M, Chiang GG, Otterness DM, Loomis DC, Kaper F, Giaccia AJ and Abraham RT. Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. Mol Cell Biol 2002; 22: 7004-7014.
[110] Rini BI. Temsirolimus, an inhibitor of mammalian target of rapamycin. Clin Cancer Res 2008; 14: 1286-1290.
[111] Rapisarda A, Zalek J, Hollingshead M, Braunschweig T, Uranchimeg B, Bonomi CA, Borgel SD, Carter JP, Hewitt SM, Shoemaker RH and Melillo G. Schedule-dependent inhibition of hypoxia-inducible factor-1alpha protein accumulation, angiogenesis, and tumor growth by topotecan in U251-HRE glioblastoma xenografts. Cancer Res 2004; 64: 6845-6848.
[112] Greenberger LM, Horak ID, Filpula D, Sapra P, Westergaard M, Frydenlund HF, Albaek C, Schroder H and Orum H. A RNA antagonist of hypoxia-inducible factor-1alpha, EZN-2968, inhibits tumor cell growth. Mol Cancer Ther 2008; 7: 3598-3608.
[113] Mabjeesh NJ, Escuin D, LaVallee TM, Pribluda VS, Swartz GM, Johnson MS, Willard MT, Zhong H, Simons JW and Giannakakou P. 2ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF. Cancer Cell 2003; 3: 363-375.
[114] Hutt DM, Roth DM, Vignaud H, Cullin C and Bouchecareilh M. The histone deacetylase inhibitor, vorinostat, represses hypoxia inducible factor 1 alpha expression through translational inhibition. PLoS One 2014; 9: e106224.
[115] Lee K, Zhang H, Qian DZ, Rey S, Liu JO and Semenza GL. Acriflavine inhibits HIF-1 dimerization, tumor growth, and vascularization. Proc Natl Acad Sci U S A 2009; 106: 17910-17915.
[116] Kong D, Park EJ, Stephen AG, Calvani M, Cardellina JH, Monks A, Fisher RJ, Shoemaker RH and Melillo G. Echinomycin, a small-molecule inhibitor of hypoxia-inducible factor-1 DNA-binding activity. Cancer Res 2005; 65: 9047-9055.
[117] Kung AL, Zabludoff SD, France DS, Freedman SJ, Tanner EA, Vieira A, Cornell-Kennon S, Lee J, Wang B, Wang J, Memmert K, Naegeli HU, Petersen F, Eck MJ, Bair KW, Wood AW and Livingston DM. Small molecule blockade of transcriptional coactivation of the hypoxia-inducible factor pathway. Cancer Cell 2004; 6: 33-43.
[118] Choueiri TK, Plimack ER, Bauer TM, Merchan JR, Papadopoulos KP, McDermott DF, Michaelson MD, Appleman LJ, Thamake S, Zojwalla NJ and Jonasch E. Phase I/II study of the oral HIF-2 α inhibitor MK-6482 in patients with advanced clear cell renal cell carcinoma (RCC). J Clin Oncol 2020; 38: 611-611.
[119] Jonasch E, Donskov F, Iliopoulos O, Rathmell WK, Narayan VK, Maughan BL, Oudard S, Else T, Maranchie JK, Welsh SJ, Thamake S, Park EK, Perini RF, Linehan WM, Srinivasan R and Investigators MK. Belzutifan for renal cell carcinoma in von Hippel-Lindau disease. N Engl J Med 2021; 385: 2036-2046.
[120] Hoefflin R, Harlander S, Schafer S, Metzger P, Kuo F, Schonenberger D, Adlesic M, Peighambari A, Seidel P, Chen CY, Consenza-Contreras M, Jud A, Lahrmann B, Grabe N, Heide D, Uhl FM, Chan TA, Duyster J, Zeiser R, Schell C, Heikenwalder M, Schilling O, Hakimi AA, Boerries M and Frew IJ. HIF-1alpha and HIF-2alpha differently regulate tumour development and inflammation of clear cell renal cell carcinoma in mice. Nat Commun 2020; 11: 4111.
[121] Russell P and Nurse P. Schizosaccharomyces pombe and Saccharomyces cerevisiae: a look at yeasts divided. Cell 1986; 45: 781-782.
[122] Hanks SK. Homology probing: identification of cDNA clones encoding members of the protein-serine kinase family. Proc Natl Acad Sci U S A 1987; 84: 388-392.
[123] Nurse P. Universal control mechanism regulating onset of M-phase. Nature 1990; 344: 503-508.
[124] Evans T, Rosenthal ET, Youngblom J, Distel D and Hunt T. Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. Cell 1983; 33: 389-396.
[125] Elledge SJ and Spottswood MR. A new human p34 protein kinase, CDK2, identified by complementation of a cdc28 mutation in Saccharomyces cerevisiae, is a homolog of Xenopus Eg1. EMBO J 1991; 10: 2653-2659.
[126] Paris J, Le Guellec R, Couturier A, Le Guellec K, Omilli F, Camonis J, MacNeill S and Philippe M. Cloning by differential screening of a Xenopus cDNA coding for a protein highly homologous to cdc2. Proc Natl Acad Sci U S A 1991; 88: 1039-1043.
[127] Tsai LH, Harlow E and Meyerson M. Isolation of the human cdk2 gene that encodes the cyclin A- and adenovirus E1A-associated p33 kinase. Nature 1991; 353: 174-177.
[128] Ninomiya-Tsuji J, Nomoto S, Yasuda H, Reed SI and Matsumoto K. Cloning of a human cDNA encoding a CDC2-related kinase by complementation of a budding yeast cdc28 mutation. Proc Natl Acad Sci U S A 1991; 88: 9006-9010.
[129] Meyerson M, Enders GH, Wu CL, Su LK, Gorka C, Nelson C, Harlow E and Tsai LH. A family of

human cdc2-related protein kinases. EMBO J 1992; 11: 2909-2917.
[130] Xiong Y, Zhang H and Beach D. D type cyclins associate with multiple protein kinases and the DNA replication and repair factor PCNA. Cell 1992; 71: 505-514.
[131] Hellmich MR, Pant HC, Wada E and Battey JF. Neuronal cdc2-like kinase: a cdc2-related protein kinase with predominantly neuronal expression. Proc Natl Acad Sci U S A 1992; 89: 10867-10871.
[132] Malumbres M. Cyclin-dependent kinases. Genome Biol 2014; 15: 122.
[133] Nigg EA. Mitotic kinases as regulators of cell division and its checkpoints. Nat Rev Mol Cell Biol 2001; 2: 21-32.
[134] Parker LL and Piwnica-Worms H. Inactivation of the p34cdc2-cyclin B complex by the human WEE1 tyrosine kinase. Science 1992; 257: 1955-1957.
[135] O'Connell MJ, Raleigh JM, Verkade HM and Nurse P. Chk1 is a wee1 kinase in the G2 DNA damage checkpoint inhibiting cdc2 by Y15 phosphorylation. EMBO J 1997; 16: 545-554.
[136] Cobrinik D. Pocket proteins and cell cycle control. Oncogene 2005; 24: 2796-2809.
[137] Lundberg AS and Weinberg RA. Functional inactivation of the retinoblastoma protein requires sequential modification by at least two distinct cyclin-cdk complexes. Mol Cell Biol 1998; 18: 753-761.
[138] Serrano M, Hannon GJ and Beach D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature 1993; 366: 704-707.
[139] Witkiewicz AK, Knudsen KE, Dicker AP and Knudsen ES. The meaning of p16(ink4a) expression in tumors: functional significance, clinical associations and future developments. Cell Cycle 2011; 10: 2497-2503.
[140] Lim S and Kaldis P. Cdks, cyclins and CKIs: roles beyond cell cycle regulation. Development 2013; 140: 3079-3093.
[141] Russo AA, Tong L, Lee JO, Jeffrey PD and Pavletich NP. Structural basis for inhibition of the cyclin-dependent kinase Cdk6 by the tumour suppressor p16INK4a. Nature 1998; 395: 237-243.
[142] Pavletich NP. Mechanisms of cyclin-dependent kinase regulation: structures of Cdks, their cyclin activators, and Cip and INK4 inhibitors. J Mol Biol 1999; 287: 821-828.
[143] Jeffrey PD, Tong L and Pavletich NP. Structural basis of inhibition of CDK-cyclin complexes by INK4 inhibitors. Genes Dev 2000; 14: 3115-3125.
[144] Gillett C, Fantl V, Smith R, Fisher C, Bartek J, Dickson C, Barnes D and Peters G. Amplification and overexpression of cyclin D1 in breast cancer detected by immunohistochemical staining. Cancer Res 1994; 54: 1812-1817.
[145] Zhang SY, Caamano J, Cooper F, Guo X and Klein-Szanto AJ. Immunohistochemistry of cyclin D1 in human breast cancer. Am J Clin Pathol 1994; 102: 695-698.
[146] Elsheikh S, Green AR, Aleskandarany MA, Grainge M, Paish CE, Lambros MB, Reis-Filho JS and Ellis IO. CCND1 amplification and cyclin D1 expression in breast cancer and their relation with proteomic subgroups and patient outcome. Breast Cancer Res Treat 2008; 109: 325-335.
[147] Al-Kuraya K, Schraml P, Torhorst J, Tapia C, Zaharieva B, Novotny H, Spichtin H, Maurer R, Mirlacher M, Kochli O, Zuber M, Dieterich H, Mross F, Wilber K, Simon R and Sauter G. Prognostic relevance of gene amplifications and coamplifications in breast cancer. Cancer Res 2004; 64: 8534-8540.
[148] Li M, Xiao A, Floyd D, Olmez I, Lee J, Godlewski J, Bronisz A, Bhat KPL, Sulman EP, Nakano I and Purow B. CDK4/6 inhibition is more active against the glioblastoma proneural subtype. Oncotarget 2017; 8: 55319-55331.
[149] Lu VM, O'Connor KP, Shah AH, Eichberg DG, Luther EM, Komotar RJ and Ivan ME. The prognostic significance of CDKN2A homozygous deletion in IDH-mutant lower-grade glioma and glioblastoma: a systematic review of the contemporary literature. J Neurooncol 2020; 148: 221-229.
[150] Bhateja P, Chiu M, Wildey G, Lipka MB, Fu P, Yang MCL, Ardeshir-Larijani F, Sharma N and Dowlati A. Retinoblastoma mutation predicts poor outcomes in advanced non small cell lung cancer. Cancer Med 2019; 8: 1459-1466.
[151] Fry DW, Harvey PJ, Keller PR, Elliott WL, Meade M, Trachet E, Albassam M, Zheng X, Leopold WR, Pryer NK and Toogood PL. Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts. Mol Cancer Ther 2004; 3: 1427-1438.
[152] Patnaik A, Rosen LS, Tolaney SM, Tolcher AW, Goldman JW, Gandhi L, Papadopoulos KP, Beeram M, Rasco DW, Hilton JF, Nasir A, Beckmann RP, Schade AE, Fulford AD, Nguyen TS, Martinez R, Kulanthaivel P, Li LQ, Frenzel M, Cronier DM, Chan EM, Flaherty KT, Wen PY and Shapiro GI. Efficacy and safety of abemaciclib, an inhibitor of CDK4 and CDK6, for patients with breast cancer, non-small cell lung cancer, and other solid tumors. Cancer Discov 2016; 6: 740-753.
[153] Infante JR, Shapiro G, Witteveen P, Gerecitano JF, Ribrag V, Chugh R, Issa I, Chakraborty A, Matano A, Zhao XM, Parasuraman S and Cassier P. A phase I study of the single-agent

CDK4/6 inhibitor LEE011 in pts with advanced solid tumors and lymphomas. J Clin Oncol 2014; 32: 2528-2528.
[154] Dhillon S. Trilaciclib: first approval. Drugs 2021; 81: 867-874.
[155] Raub TJ, Wishart GN, Kulanthaivel P, Staton BA, Ajamie RT, Sawada GA, Gelbert LM, Shannon HE, Sanchez-Martinez C and De Dios A. Brain exposure of two selective dual CDK4 and CDK6 inhibitors and the antitumor activity of CDK4 and CDK6 inhibition in combination with temozolomide in an intracranial glioblastoma xenograft. Drug Metab Dispos 2015; 43: 1360-1371.
[156] Weiss J, Goldschmidt J, Andric Z, Dragnev KH, Gwaltney C, Skaltsa K, Pritchett Y, Antal JM, Morris SR and Daniel D. Effects of trilaciclib on chemotherapy-induced myelosuppression and patient-reported outcomes in patients with extensive-stage small cell lung cancer: pooled results from three phase II randomized, double-blind, placebo-controlled studies. Clin Lung Cancer 2021; 22: 449-460.
[157] Domine Gomez M, Csoszi T, Jaal J, Kudaba I, Nikolov K, Radosavljevic D, Xiao J, Horton JK, Malik RK and Subramanian J. Exploratory composite endpoint demonstrates benefit of trilaciclib across multiple clinically meaningful components of myeloprotection in patients with small cell lung cancer. Int J Cancer 2021; 149: 1463-1472.
[158] Portman N, Alexandrou S, Carson E, Wang S, Lim E and Caldon CE. Overcoming CDK4/6 inhibitor resistance in ER-positive breast cancer. Endocr Relat Cancer 2019; 26: R15-R30.
[159] Goel S, DeCristo MJ, Watt AC, BrinJones H, Sceneay J, Li BB, Khan N, Ubellacker JM, Xie S, Metzger-Filho O, Hoog J, Ellis MJ, Ma CX, Ramm S, Krop IE, Winer EP, Roberts TM, Kim HJ, McAllister SS and Zhao JJ. CDK4/6 inhibition triggers anti-tumour immunity. Nature 2017; 548: 471-475.
[160] Deng J, Wang ES, Jenkins RW, Li S, Dries R, Yates K, Chhabra S, Huang W, Liu H, Aref AR, Ivanova E, Paweletz CP, Bowden M, Zhou CW, Herter-Sprie GS, Sorrentino JA, Bisi JE, Lizotte PH, Merlino AA, Quinn MM, Bufe LE, Yang A, Zhang Y, Zhang H, Gao P, Chen T, Cavanaugh ME, Rode AJ, Haines E, Roberts PJ, Strum JC, Richards WG, Lorch JH, Parangi S, Gunda V, Boland GM, Bueno R, Palakurthi S, Freeman GJ, Ritz J, Haining WN, Sharpless NE, Arthanari H, Shapiro GI, Barbie DA, Gray NS and Wong KK. CDK4/6 inhibition augments antitumor immunity by enhancing T-cell activation. Cancer Discov 2018; 8: 216-233.
[161] Schaer DA, Beckmann RP, Dempsey JA, Huber L, Forest A, Amaladas N, Li Y, Wang YC, Rasmussen ER, Chin D, Capen A, Carpenito C, Staschke KA, Chung LA, Litchfield LM, Merzoug FF, Gong X, Iversen PW, Buchanan S, de Dios A, Novosiadly RD and Kalos M. The CDK4/6 inhibitor abemaciclib induces a T cell inflamed tumor microenvironment and enhances the efficacy of PD-L1 checkpoint blockade. Cell Rep 2018; 22: 2978-2994.
[162] Álvarez-Fernández M and Malumbres M. Mechanisms of sensitivity and resistance to CDK4/6 inhibition. Cancer Cell 2020; 37: 514-529.
[163] Hoter A, El-Sabban ME and Naim HY. The HSP90 family: structure, regulation, function, and implications in health and disease. Int J Mol Sci 2018; 19: 2560.
[164] Trepel J, Mollapour M, Giaccone G and Neckers L. Targeting the dynamic HSP90 complex in cancer. Nat Rev Cancer 2010; 10: 537-549.
[165] Ciocca DR, Arrigo AP and Calderwood SK. Heat shock proteins and heat shock factor 1 in carcinogenesis and tumor development: an update. Arch Toxicol 2013; 87: 19-48.
[166] Vartholomaiou E, Echeverria PC and Picard D. Unusual suspects in the twilight zone between the Hsp90 interactome and carcinogenesis. Adv Cancer Res 2016; 129: 1-30.
[167] Sigismund S, Avanzato D and Lanzetti L. Emerging functions of the EGFR in cancer. Mol Oncol 2018; 12: 3-20.
[168] Ahsan A, Ramanand SG, Whitehead C, Hiniker SM, Rehemtulla A, Pratt WB, Jolly S, Gouveia C, Truong K, Van Waes C, Ray D, Lawrence TS and Nyati MK. Wild-type EGFR is stabilized by direct interaction with HSP90 in cancer cells and tumors. Neoplasia 2012; 14: 670-677.
[169] Sawai A, Chandarlapaty S, Greulich H, Gonen M, Ye Q, Arteaga CL, Sellers W, Rosen N and Solit DB. Inhibition of Hsp90 down-regulates mutant epidermal growth factor receptor (EGFR) expression and sensitizes EGFR mutant tumors to paclitaxel. Cancer Res 2008; 68: 589-596.
[170] Isaacs JS. Chapter Five - Hsp90 as a "Chaperone" of the Epigenome: Insights and Opportunities for Cancer Therapy. In: Isaacs J, Whitesell L, editors. Adv Cancer Res. Academic Press; 2016. p. 107-140.
[171] Rhee I, Bachman KE, Park BH, Jair KW, Yen RW, Schuebel KE, Cui H, Feinberg AP, Lengauer C, Kinzler KW, Baylin SB and Vogelstein B. DNMT1 and DNMT3b cooperate to silence genes in human cancer cells. Nature 2002; 416: 552-556.
[172] el-Deiry WS, Nelkin BD, Celano P, Yen RW, Falco JP, Hamilton SR and Baylin SB. High expression of the DNA methyltransferase gene characterizes human neoplastic cells and progression stages of colon cancer. Proc Natl Acad Sci U S A 1991; 88: 3470-3474.
[173] Girault I, Tozlu S, Lidereau R and Bieche I. Expression analysis of DNA methyltransferases

1, 3A, and 3B in sporadic breast carcinomas. Clin Cancer Res 2003; 9: 4415-4422.
[174] Oh BK, Kim H, Park HJ, Shim YH, Choi J, Park C and Park YN. DNA methyltransferase expression and DNA methylation in human hepatocellular carcinoma and their clinicopathological correlation. Int J Mol Med 2007; 20: 65-73.
[175] Pick E, Kluger Y, Giltnane JM, Moeder C, Camp RL, Rimm DL and Kluger HM. High HSP90 expression is associated with decreased survival in breast cancer. Cancer Res 2007; 67: 2932-2937.
[176] DeBoer C, Meulman PA, Wnuk RJ and Peterson DH. Geldanamycin, a new antibiotic. J Antibiot (Tokyo) 1970; 23: 442-447.
[177] Stebbins CE, Russo AA, Schneider C, Rosen N, Hartl FU and Pavletich NP. Crystal structure of an Hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor agent. Cell 1997; 89: 239-250.
[178] Grenert JP, Sullivan WP, Fadden P, Haystead TA, Clark J, Mimnaugh E, Krutzsch H, Ochel HJ, Schulte TW, Sausville E, Neckers LM and Toft DO. The amino-terminal domain of heat shock protein 90 (HSP90) that binds geldanamycin is an ATP/ADP switch domain that regulates HSP90 conformation. J Biol Chem 1997; 272: 23843-23850.
[179] Prodromou C, Roe SM, O'Brien R, Ladbury JE, Piper PW and Pearl LH. Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. Cell 1997; 90: 65-75.
[180] Sidera K and Patsavoudi E. HSP90 inhibitors: current development and potential in cancer therapy. Recent Pat Anticancer Drug Discov 2013; 9: 1-20.
[181] Sanchez J, Carter TR, Cohen MS and Blagg BSJ. Old and new approaches to target the HSP90 chaperone. Curr Cancer Drug Targets 2020; 20: 253-270.
[182] Wang Y, Trepel JB, Neckers LM and Giaccone G. STA-9090, a small-molecule HSP90 inhibitor for the potential treatment of cancer. Curr Opin Investig Drugs 2010; 11: 1466-1476.
[183] Woodhead AJ, Angove H, Carr MG, Chessari G, Congreve M, Coyle JE, Cosme J, Graham B, Day PJ, Downham R, Fazal L, Feltell R, Figueroa E, Frederickson M, Lewis J, McMenamin R, Murray CW, O'Brien MA, Parra L, Patel S, Phillips T, Rees DC, Rich S, Smith DM, Trewartha G, Vinkovic M, Williams B and Woolford AJ. Discovery of (2,4-dihydroxy-5-isopropylphenyl)-[5-(4-methylpiperazin-1-ylmethyl)-1,3-dihydrois oindol-2-yl]methanone (AT13387), a novel inhibitor of the molecular chaperone HSP90 by fragment based drug design. J Med Chem 2010; 53: 5956-5969.
[184] Wang CY, Guo ST, Wang JY, Liu F, Zhang YY, Yari H, Yan XG, Jin L, Zhang XD and Jiang CC. Inhibition of HSP90 by AUY922 preferentially kills mutant KRAS colon cancer cells by activating Bim through ER stress. Mol Cancer Ther 2016; 15: 448-459.
[185] Bussenius J, Blazey CM, Aay N, Anand NK, Arcalas A, Baik T, Bowles OJ, Buhr CA, Costanzo S, Curtis JK, DeFina SC, Dubenko L, Heuer TS, Huang P, Jaeger C, Joshi A, Kennedy AR, Kim AI, Lara K, Lee J, Li J, Lougheed JC, Ma S, Malek S, Manalo JC, Martini JF, McGrath G, Nicoll M, Nuss JM, Pack M, Peto CJ, Tsang TH, Wang L, Womble SW, Yakes M, Zhang W and Rice KD. Discovery of XL888: a novel tropane-derived small molecule inhibitor of HSP90. Bioorg Med Chem Lett 2012; 22: 5396-5404.
[186] Ohkubo S, Kodama Y, Muraoka H, Hitotsumachi H, Yoshimura C, Kitade M, Hashimoto A, Ito K, Gomori A, Takahashi K, Shibata Y, Kanoh A and Yonekura K. TAS-116, a highly selective inhibitor of heat shock protein 90alpha and beta, demonstrates potent antitumor activity and minimal ocular toxicity in preclinical models. Mol Cancer Ther 2015; 14: 14-22.
[187] Shimomura A, Yamamoto N, Kondo S, Fujiwara Y, Suzuki S, Yanagitani N, Horiike A, Kitazono S, Ohyanagi F, Doi T, Kuboki Y, Kawazoe A, Shitara K, Ohno I, Banerji U, Sundar R, Ohkubo S, Calleja EM and Nishio M. First-in-human phase I study of an oral HSP90 inhibitor, TAS-116, in patients with advanced solid tumors. Mol Cancer Ther 2019; 18: 531-540.
[188] Zhao S, Zhou L, Dicker DT, Lev A, Zhang S, Ross E and El-Deiry WS. Anti-cancer efficacy including Rb-deficient tumors and VHL-independent HIF1alpha proteasomal destabilization by dual targeting of CDK1 or CDK4/6 and HSP90. Sci Rep 2021; 11: 20871.
[189] Semenza GL. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. Oncogene 2010; 29: 625-634.
[190] Cimmino F, Avitabile M, Lasorsa VA, Montella A, Pezone L, Cantalupo S, Visconte F, Corrias MV, Iolascon A and Capasso M. HIF-1 transcription activity: HIF1A driven response in normoxia and in hypoxia. BMC Med Genet 2019; 20: 37.
[191] Otero-Albiol D and Carnero A. Cellular senescence or stemness: hypoxia flips the coin. J Exp Clin Cancer Res 2021; 40: 243.
[192] Wigerup C, Pahlman S and Bexell D. Therapeutic targeting of hypoxia and hypoxia-inducible factors in cancer. Pharmacol Ther 2016; 164: 152-169.