Minireview

Hypoxia-regulated gene network in drug resistance and cancer progression

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Abstract

Hypoxia is a common phenomenon of solid tumors and contributes to aggressive phenotype and treatment failure. Hypoxia-inducible factor (HIF), a versatile transcription factor that regulates more than 5% of total human genes, not only plays important roles in controlling physiological processes, but is also a crucial mediator in hypoxia-induced tumor progression and chemoresistance. Overexpression of HIF-1α is detected in a wide spectrum of cancers via different kinds of mechanisms, including reduced oxygen concentration, loss-of-function of tumor suppressor gene, activating mutation of oncogenes, and hyperactivation of protein kinase signaling pathways. HIF-regulated genes involve in many pathological processes such as metabolic switch, drug efflux, angiogenesis, cell proliferation, and anti-apoptosis, which ultimately leads to increased tumor growth and drug resistance. Due to the common failure of classic chemotherapeutic agents in treating hypoxic cancers, novel strategies have been developed to target tumors under hypoxic conditions including inhibition of HIF activity and administration of bioreductive drugs. These new strategies may provide more effective and specific methods in targeting hypoxic tumors.

Keywords: Drug resistance, hypoxia, metabolic switch, apoptosis, microRNA

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Introduction

Cancer is a primary factor leading to mortality and morbidity worldwide, mainly contributed by its invasion and metastasis. According to world health organization statistics, the incidence of 12.7 million new cancer cases in 2008 will rise to 21.4 million by 2030.[1] Although cancer development is a very complex, multi-step process with mechanisms largely unknown, hypoxia, no doubt, is one of the most important factors contributing to cancer progression and malignancy. When cancer cells start to grow, hypoxia occurs because of no adequate or normal microvasculature for the whole tumor develops concurrently.[2,3]

Hypoxia-inducible factor (HIF) plays a central role to regulate transcription of numerous genes for adapting to hypoxia, maintaining oxygen homeostasis, and keeping cells alive.[4] After HIF-1α was first discovered by the identification of a hypoxia response element (HRE) in 1990s,[5–7] scientists have proven it is a key regulator responsible for the induction of genes that facilitate adaptation and survival under low oxygen condition. Besides, HIF-1 activates the transcription of genes that are involved in pivotal aspects of cancer biology, including angiogenesis, survival, metabolism, and invasion. Intratumoral hypoxia and genetic alterations can lead to HIF-1α overexpression, which has been associated with tumor aggressiveness and treatment failure in many cancer types.[7–11] In preclinical studies and clinical trial, inhibition of HIF-1 activity has marked effects on tumor growth.[12]

Although the molecular mechanisms of hypoxia-induced tumor progression and drug resistance remain elusive, recent studies have provided many new and exciting findings that will advance our understanding of this complicated pathological process. In this article, we will review the current understandings of the roles of hypoxia and HIFs in tumor pathogenesis and drug resistance. In addition, current developments in targeting hypoxia as a cancer therapeutic approach will also be reviewed.

HIF and HIF-regulated genes

Regulation of HIF

Regulation of cell functions in response to hypoxia is mainly mediated by two transcription factors, namely HIF-1 and HIF-2. HIF is a heterodimer composed of one α and one β subunit. The HIF heterodimer binds to DNA at HRE located in the promoter region, 5′ untranslated region, or 3′ untranslated region of numerous genes to regulate their

transcription under hypoxic status.[13,14] In normoxia, HIF-α has very short half-life due to rapid degradation by ubiquitin-proteosome pathway[15,16] (Figure 1). In the presence of oxygen molecules, HIF-α is hydroxylated by prolyl hydroxylase domain-containing protein (PHD) at two proline residues within the oxygen-dependent degradation domain.[17,18] The proline-hydroxylated HIF-α is recognized by von Hippel-Lindau (VHL) protein, an E3 ubiquitin ligase. Binding of hydroxylated HIF-α by VHL initiates the process of polyubiquitination and protein degradation.[19–23] In addition, the α subunit of HIF is also hydroxylated by factor-inhibiting HIF (FIH) at the C-terminal transactivation domain (asparagine residue) under normal oxygen level, which prevents its interaction with transcription coactivator, P300.[24,25] Thus, the α subunit of HIF is constitutively transcribed, translated, and degraded under normoxic condition. Even with the presence of residual HIF protein, its transcription activity is markedly inhibited because of FIH-mediated hydroxylation of asparagine residue in the transactivation domain. In contrast, because both PHD and FIH require oxygen molecules to exert their enzymatic activity, the hydroxylation reaction cannot occur under hypoxia condition. Therefore, HIF-α protein level rapidly accumulates under hypoxia and binds to the β subunit of HIF (also known as aryl hydrocarbon receptor nuclear translocator, ARNT). The dimerized αβ heterodimer then translocates to the nucleus and binds to HRE to regulate the expression of target genes.

Besides the absolute requirement of oxygen molecules, the enzyme PHD also needs 2-oxoglutarate and ferrous (Fe^{+2}) as two cofactors to exert its enzymatic activity. Therefore, the reduction of either one of these two cofactors leads to the inhibition of PHD's activity and stabilization of HIF-α. Iron chelators such as desferrioxamine and transition metal ions such as cobalt ion that deplete and compete with Fe^{2+}, respectively, are good inhibitors of PHDs. Dimethyloxaloylglycine, the 2-oxoglutarate analog, also inhibits PHD's enzyme activity. Therefore, desferrioxamine, cobalt chloride, and dimethyloxaloylglycine are often used as chemical hypoxia mimetics in biomedical research to cause the increase of HIF-1α under normoxic condition.

Genes regulated by HIF

Considering hypoxia controls numerous physiological and pathological processes, it is of interesting to identify hypoxia-regulated genes that govern diverse cellular functions. Hypoxia controls gene expression mainly through two distinct mechanisms: HIF-dependent and HIF-independent. In the HIF-independent pathway, hypoxia controls gene expression via influencing the activity of oxygen- and 2-oxoglutarate-dependent enzymes, the dioxygenases. It has been shown that under hypoxic condition,

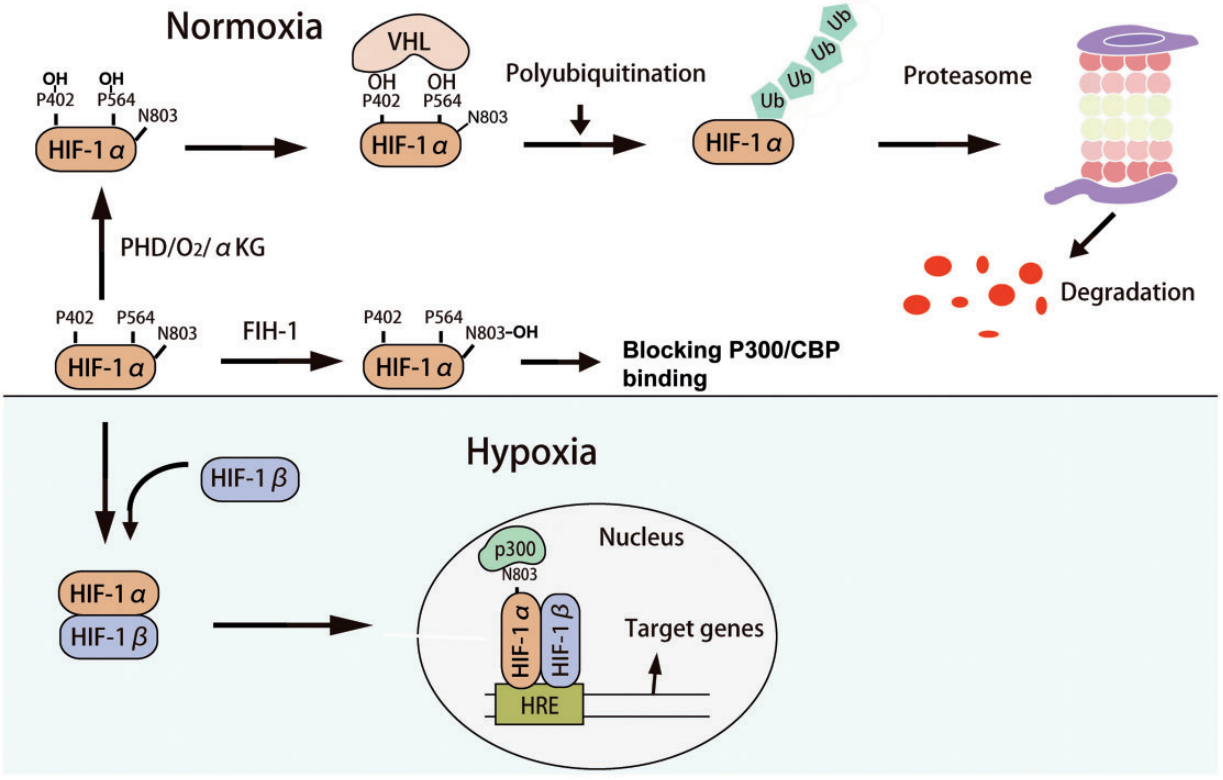

Figure 1 Regulation of hypoxia-inducible factors. In normoxia, HIF-1α is hydroxylated by prolyl hydroxylase domain (PHD) protein and factor-inhibiting HIF (FIH), respectively. The proline-hydroxylated HIF-1α is then recognized by von Hippel-Lindau E3 ubiquitin ligase (VHL) and subjected to be degraded via proteasome while the asparagine-hydroxylated HIF-1α fails to interact with transcriptional activator such as P300 or CREB-binding protein (CBP). In hypoxia, HIF-1α becomes stable and dimeries with HIF-1β. After translocation to the nucleus and recruitment of co-activators (e.g. CBP/p300), the HIF heterodimer binds the hypoxia response element (HRE) of target genes to regulate transcription. O_2: oxygen, α-KG: α-ketoglutarate. (A color version of this figure is available in the online journal.)

some transcription factors such as nuclear transcription factor (NF)-κB, CXXC finger protein 5, coiled-coil-helix-coiled-coil-helix domain 2, activation transcription factor (ATF)-3, and ATF-4 are upregulated due to loss of function of dioxygenase.[26–30] Alternatively, hypoxia may lead to phosphorylation of c-MYC transcription factor via PI3K pathway.[31] Upregulation or activation of these transcription factors results in induction of their downstream target genes independent of HIF transcription activity.

In contrast to the HIF-independent regulation, the majority of genes regulated by hypoxia are mediated directly by HIF-dependent transcriptional activation or repression. HIF-1α or HIF-2α dimerizes with HIF-1β, the constitutively expressed isoform, and recognizes the HRE motif. It is reported that the HRE motif consists of a core element, 5′-RCGTG-3′[32]; nevertheless, experimental data prove that this core HRE is necessary but not sufficient to determine the responsiveness of a gene to HIF transcription factors.[33] Therefore, it is reasonable to hypothesize that the sequences flanking the core element are important to determine the binding specificity. Indeed, recent studies by bioinformatic predictions[34–37] and genomewide screenings[38–41] reported that the flanking sequences, though not as conserved as the core element, are important for the binding of HIFs to their target genes.

Although both HIF-1α and HIF-2α recognize the same DNA motif, more and more experimental data demonstrate that HIF-1α and HIF-2α regulate a distinct subset of genes.[39,40,42,43] Therefore, it is believed that these two homologs exert evolutionally diverse physiological and pathological functions instead of just a pair of redundant genes. Further study is necessary to carefully dissect the regulation and function of these two transcription factors in order to paint better pictures of hypoxia-mediated biological processes.

Functional roles of HIF in cancer biology

Metabolism reprogramming

It has been known that cellular metabolism in the neoplasm is quite different from its surrounding normal tissue. The rapid growth and proliferating cancer cells demand large amount of different nutrients to provide ATP and building blocks to maintain cellular structure and morphology. In normal cells, the majority of ATP and building blocks are derived from oxidative phosphorylation of glucose. One molecule of glucose can generate 38 ATPs through cytoplasmic glycolysis followed by mitochondrial oxidative phosphorylation. Besides, the intermediate metabolites of tricarboxylic acid (TCA) cycle can serve as building blocks for the synthesis of cellular components. However, the oxidative phosphorylation requires large amount of oxygen molecules as fuel. Because the oxygen supply, which heavily depends on the availability of blood vessels, is limited, cancer cells have to develop a different approach to metabolize glucose. In cancer cells, glucose is mainly catalyzed via the glycolytic pathway without going through the TCA cycle, the so-called Warburg effect.[44] Although Warburg effect has been proposed by Otto Warburg in 1920s, detailed mechanism was not elucidated until 2006. Two studies reported that pyruvate dehydrogenase kinase 1 (PDK1) was markedly upregulated under hypoxia in a HIF-1α-dependent manner.[45,46] Upregulation of PDK1 phosphorylates pyruvate dehydrogenase (PDH) leading to the inhibition of its catalytic activity. As a result, pyruvate cannot be converted to acetyl-CoA, the initiated molecule to enter TCA cycle. Instead, accumulated pyruvate was converted by another hypoxia-inducible enzyme, lactate dehydrogenase A (LDHA), to become lactic acid (Figure 2). Later, it was reported that another member of PDK family, PDK3, is also a target of HIF-1α.[35] Induction of PDK3 also results in blockage of PDH enzyme activity. The same study carefully dissects molecular mechanism responsible for induction of PDK1 and PDK3. PDK1 is induced by both HIF-1α and HIF-2α while PDK3 only induced by HIF-1α.[35] Further study demonstrated that PDK3 but not PDK1 was elevated in colon cancer and elevation of PDK3 promotes metabolic switch, increases drug resistance, and is correlated with colorectal cancer malignancy and poor prognosis.[35,47]

Although cytoplasmic glycolysis is not limited by oxygen availability, it produces less than 6% of ATP per molecule of glucose compared to mitochondrial phosphorylation. Since cancer cells actually rely more on ATP production for the rapid proliferation, they need to uptake much more glucose than normal cells do. This is achieved by overexpression of glucose transporters and glycolytic enzymes including glucose transporters (GLUT1 and GLUT3), hexokinase (HK1 and HK2), aldolase A (ALDA), phosphoglycerate kinase-1 (PGK1), enolase (ENO1), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), pyruvate kinase M (PKM), and LDHA.[48–51] As expected, these glucose transporters and glycolytic enzymes are induced by HIF-1α.

How tumor cells sustain TCA cycle and building blocks for cellular components during hypoxia? Glutamine, one of the most abundant amino acid in plasma, plays an important role here. Glutamine, which is transformed to α-ketoglutarate, provides a carbon source for TCA cycle and nitrogen for phospholipids, amino acid, and nucleotides biosynthesis. Cancer cells overexpress a series of enzymes, including glutaminase, glutamate dehydrogenase, and transaminase that convert glutamine to α-ketoglutarate to maintain TCA cycle.[52,53] Interestingly, c-MYC, an oncogenic transcription factor that is activated by hypoxia, enhances mitochondrial glutaminase expression and causes glutamine addiction in tumor cells.[54,55]

An important issue accompanied with metabolic switch is the production of lactate and H^+. Excessive H^+ accumulation within the cytoplasm impairs cellular function and leads to cell death. Hypoxic cancer cells overcome this problem by upregulation of monocarboxylate transporter 4[56] and sodium-hydrogen exchanger 1[57] to pump lactate and H^+, respectively, out of the cell. In addition, hypoxia induces carbonic anhydrase 9, which also contributes to net efflux of H^+ from cancer cells.[58] As a result, the intracellular environment is more basic, which promotes proliferation, and the extracellular space is more acidic, which promotes invasion.

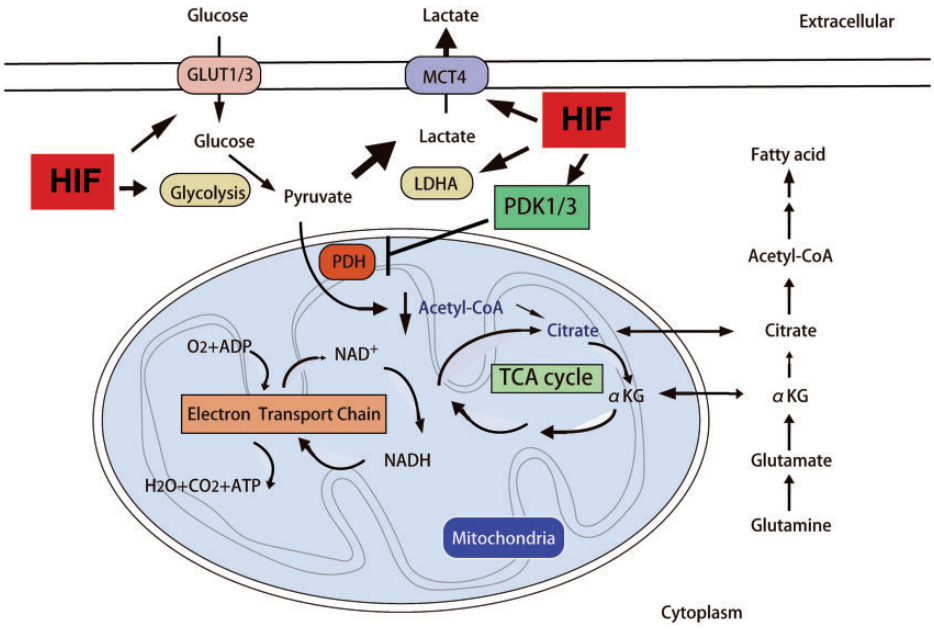

Figure 2 HIF-1 regulates the glucose metabolism. Under well oxygenated condition, glucose is metabolized to pyruvate. Then pyruvate dehydrogenase (PDH) converts pyruvate to acetyl-CoA for the entry into TCA cycle. Under hypoxia, HIF-1 targets and activates many glycolytic enzymes and lactate dehydrogenase A (LDHA) to increase lactate production. Besides, HIF1 promotes pyruvate dehydrogenase kinase 1/3 (PDK1/3) to inactivate PDH which prohibits the conversion of pyruvate to acetyl-CoA. In addition, glutamine is converted to α-ketoglutarate to provide intermediates for TCA cycle and fatty acid synthesis. (A color version of this figure is available in the online journal.)

Cell proliferation and survival

For cancer cell proliferation, increase expression of growth factors for autocrine signaling is needed (Figure 3). Indeed, it has been reported that various growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor-9 (FGF9), transforming growth factor-α (TGF-α), platelet-derived growth factor B (PDGFB), insulin-like growth factor-2 (IGF-2), erythropoietin (EPO), and endothelin 1 (ET-1) are regulated by HIF in different cancer cells.[59–63] It is of interest to know that hypoxia regulates these mitogenic factors not only at the transcriptional level but also at the translational level. Recent studies reveal that hypoxia induces translational switch from cap-dependent to cap-independent manner to maintain production of critical survival proteins.[63–65] In addition to peptide growth factors, HIF also induces cell proliferation via activation of extracellular signal-related kinase (ERK) signaling. Suppression of dual specificity phosphates-2 (DUSP2), an ERK-specific phosphatase, by HIF-1α prolongs ERK phosphorylation and thus results in promoting cancer cell proliferation.[66]

Keeping cell proliferating is the first priority besides trying to maintain survival when facing an unfavorable condition such as hypoxia. A group of anti-apoptotic genes such as B-cell lymphoma 2 (BCL2), B-cell lymphoma-extra-large (BCL-xL), myeloid cell leukemia sequence 1 (MCL-1), NK-κB, and survivin are upregulated by HIF-1α under hypoxic condition to enhance cell survival.[67,68] Autophagy, the process that occurs for compensating hypoxia, nutrient depletion, and metabolic stress, is a means for cells to avoid apoptosis or necrosis and counteracts the potentially deleterious effects caused by mitochondrial dysfunction and reactive oxygen species.[69,70] Under hypoxic condition, HIF activates BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) and BNIP 3-like (BNIP3L) proteins to induce mitochondrial-selective autophagy.[71,72] Upregulation of BNIP3 and BNIP3L has been shown to protect cancer cells from apoptosis.[72]

Angiogenesis

It is necessary for tumor cells to have adequate blood flow to gain nutrients and remove metabolic waste during rapid growth. Angiogenesis is a complex process that involves multiple gene products expressed by different cell types. Nonetheless, genes involved in different steps of angiogenesis are independently responsive to hypoxia. These include VEGF and its receptors, placental growth factor (PGF), PDGFB, angiopoietin 1 and 2 (Ang1 and Ang2), stromal-derived factor-1 (SDF-1), matrix metalloproteinases (MMPs), plasminogen activator receptors (uPAR) and inhibitors (PAI), and procollagen prolyl hydroxylase.[73–77] Since angiogenesis is the hallmark of cancer and VEGF is the signature gene regulating angiogenesis, anti-VEGF antibody has been used in combination with chemotherapy for treating various cancers.[78–80] For example, fluoropyrimidine-based chemotherapy plus the anti-VEGF antibody, bevacizumab, is standard first-line treatment for metastatic colorectal cancer, and treatment with bevacizumab has been shown to have significant impact on progression-free survival and overall survival for those patients.[79,81]

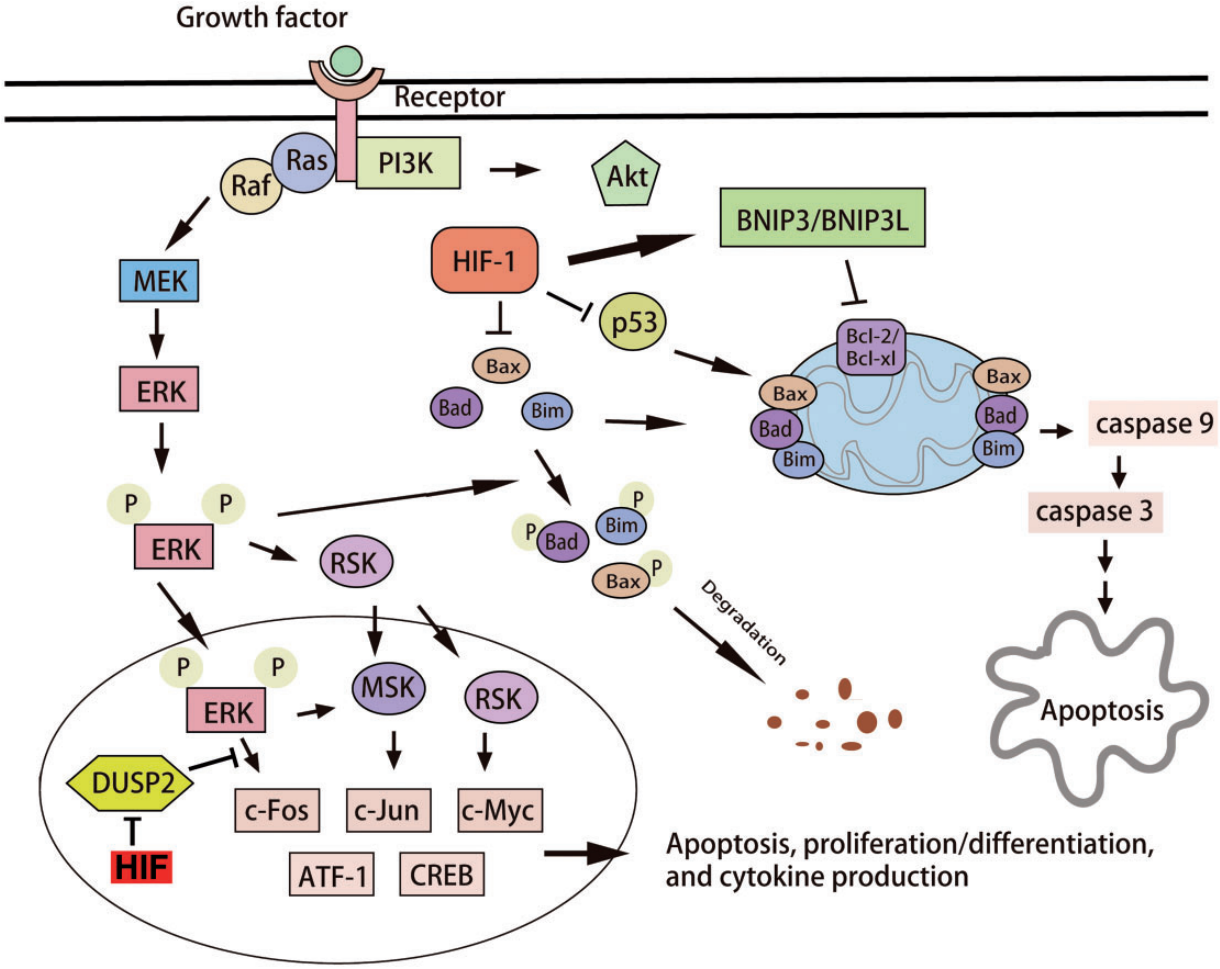

Figure 3 The pathways of HIF-1-modulated signaling in cell proliferation and anti-apoptosis under hypoxia. Hypoxia upregulates growth factors and their receptors to trigger signals through Ras-Raf-MEK-ERK cascades resulting in (1) induction of target genes to inhibit apoptosis and/or promote cell proliferation and survival, and (2) phosphorylation and inactivation of Bim, Bax, BAD, and caspase 3 to block apoptosis. HIF-1α further inhibits the expression of dual specificity phosphatase 2 (DUSP2) and thus enhances ERK phosphorylation and gene expression. CREB: c-AMP response element-binding protein, ATF1: activating transcription factor 1. (A color version of this figure is available in the online journal.)

Invasion and metastasis

Epithelial-mesenchymal transition (EMT) is thought to be the most important step of cancer invasion and metastasis initiation. HIF induces various transcriptional regulators to cause EMT, including Twist1, Snail 1 and 2, TGFA, zinc finger E-box-binding homeobox 1 and 2 (ZEB1 and 2), and transcription factor 3.[82–84] In addition, HIF inhibits E-cadherin and other proteins involved in maintaining epithelial cells characteristics while upregulates genes with functions in promoting mesenchymal phenotypes such as N-cadherin and vimentin.[85] After EMT, primary cancer cells need to go through multiple steps such as detachment from original site, extracellular matrix modulation, intravasation, circulation, extravasation, and homing to finally colonize on other distant organs. Among all these sequential processes, detachment from neighboring cells and extracellular matrix represent the first step of metastasis. Cell–cell and cell–matrix adhesion is strengthened by expression of members of tetraspanin superfamily including CD9 and CD151.[86] Expression of CD151 is downregulated under hypoxia but is restored after re-oxygenation, which perfectly facilitates cancer cells to detach from the hypoxic primary site and attach to the secondary site.[87] Upregulation of lysyl oxidase (LOX) and lysyl oxidase-like (LOXL) enzymes by HIF-1α is also a critical process to remodel extracellular matrix for cancer cell migration.[88] The induction of metalloproteases including MMP1, MMP3, and membrane type-1 MMP by HIF-1α and HIF-2α is associated with metastasis.[89,90] VEGF and Ang2 help cancer cells migrate into blood vessel while L1 cell adhesion molecule and other proteins may promote cancer cells invasion and metastases.[91] Furthermore, induction of monocarboxylate transporter 4 (MCT4) to efflux lactate and sodium hydrogen exchanger 1 (NHE-1) to pump H^+ outside cancer cells by hypoxia results in extracellular acidification, which is of beneficial for cancer cell invasion and metastasis.[56,57,92]

MicroRNA expression

MicroRNAs (miRNA) are small noncoding transcripts ranging from 19 to 24 nucleotides long and have been found to play key roles in physiological and pathological processes, including cancer development, proliferation, and

metastases.[93,94] The altered expression of miRNAs is associated with clinical prognosis of tumor, resistance to chemotherapy and radiation therapy, and tumor recurrence.[95] For most updated information regarding effects of hypoxia-regulated miRNAs in cancer development and drug resistance, please read the recent review by Liao et al.[96]

Role of HIF in drug resistance

HIF has been reported to participate in drug and radiotherapy resistance in cancer treatment. Radiotherapy (ionizing radiation) causes DNA damage directly and indirectly. Ionizing radiation and some chemotherapeutic drugs cause intracellular/tissue water ionizing to produce free radical (to impair DNA). Lack of oxygen reduces the cytotoxic effects of drugs and radiotherapy directly. Therefore, areas of hypoxic tumor tissue are more resistant to treatment and are associated with a poor clinical prognosis.[97] In addition to the direct effect of lack-of-oxygen molecules on chemo- and radio-reactivity, hypoxia also induces therapeutic resistance via gene upregulation. It has been shown that HIF induces drug resistance of cancer cells, which can be reversed after blocking HIF under normoxia or hypoxia.[98] As mentioned above, hypoxia/HIF can modulate at least hundreds of genes to control cell proliferation, survival, metabolism, and drug efflux, which ultimately leads to drug resistance (Figure 4). In the following sections, we will discuss drug resistance induced by hypoxia-controlled genes through different pathways.

Hypoxia-induced drug resistance through metabolic alteration

As described earlier, one of the hall marks of cancer cells is the elevated glucose uptake and the switch of cellular metabolism from oxidative phosphorylation to aerobic glycolysis, the so-called Warburg effect. The Warburg effect is not only a critical cellular metabolic adaptation to cyclic hypoxia, it is also an obviously beneficial trade-off for cancer cells to increase chemoresistance, mutation rate, and invasion/metastatic ability.[99] Recent data revealed that expression of PDK1 and PDK3 is upregulated by HIF-1α-dependent hypoxic stress and is linked to increased drug resistance in cell culture system.[35,45,46] However, upregulation of PDK3, but not PDK1, is associated with colon cancer malignancy and early recurrence.[47]

Despite their roles in energy biogenesis, mitochondria also play an important role in the control of cell death. Mitochondria regulate cell death pathways not only through control of intrinsic apoptotic pathways but also the generation of reactive oxygen species.[100] It has been shown that chemotherapy induces cell death and, at least in part, is mediated through the generation of reactive oxygen species. The generation of reactive oxygen species is suppressed in tumor cells under hypoxia by HIF-1α,[101–103] thus conferring the drug resistance of tumor cells.

Hypoxia-induced drug resistance through increased drug efflux

The ATP-binding cassette (ABC) transporter, a membrane-resident P-glycoprotein encoded by multidrug resistance 1

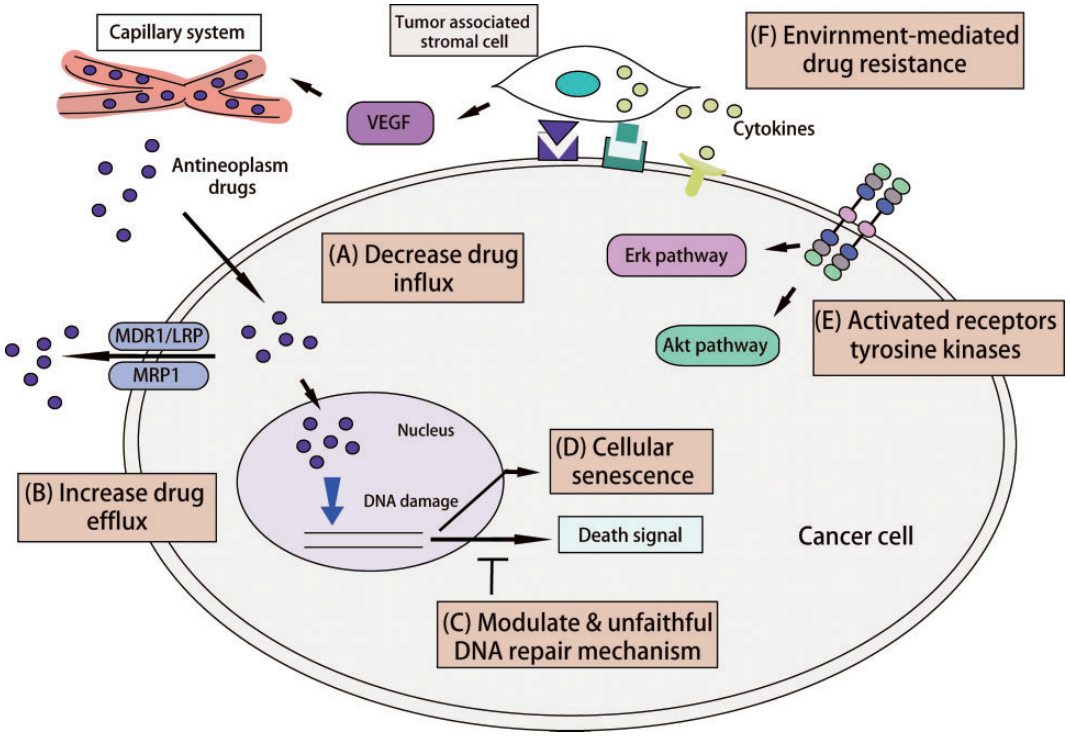

Figure 4 An overview of hypoxia-related chemoresistance. (A color version of this figure is available in the online journal.)

(MDR1) gene, is upregulated by hypoxia.[104] By acting as a drug efflux pump, MDR1 decreases the intracellular concentrations of chemotherapeutic drugs to cause drug resistance. An induction of MDR1 by HIF-1α has been reported in cancers of breast, colon, glia, and stomach.[105–109] Multidrug-resistance-associated protein, another ABC transporter, was also reported to be involved in HIF-1α-mediated drug efflux and chemoresistance.[106,109,110] EGR-1 is an important transcription factor that increases cell survival and is overexpressed in a wild spectrum of human cancers.[111–113] Recent data demonstrated that hypoxia-induced prolonged ERK activation induces the overexpression of EGR-1 and MDR1.[66] Taken together, these data demonstrate that HIF-1α can directly and indirectly upregulate MDR1 to increase the drug efflux ability of cancer cells.

Hypoxia-induced drug resistance through inhibition of apoptotic pathways

DNA damage is sensed by DNA damage response pathway,[114] which aims to prevent the transmission of damaged DNA and confer the genome stability. Typically, DNA damages are sensed by ATM/ATR kinases, which phosphorylate p53 to induce cell cycle arrest and apoptosis.[115,116] The classic chemotherapeutic agents that were designed to cause DNA damage of fast-growing cancer cells would kill most tumor cells but also give selective advantages to cells bearing mutations in genes involving in DNA repair pathway, leading to more aggressive phenotype and development of drug resistance. In addition to this colony selection phenomenon, it also has been shown that hypoxia could attenuate DNA damage-induced cell cycle arrest and apoptosis.

Deregulation of apoptosis and cell cycle under hypoxic condition are pivotal causes of tumor progression and drug resistance. Cancer cells display different response to hypoxia by expressing a subset of anti-apoptotic genes such as BCL2, BCL-xL, MCL-1, NF-κB, and survivin in a HIF-1-dependent manner.[67,68] Upregulation of these genes protects cancer cells from hypoxia-induced cell death and confers cancer cells the ability to resist chemotherapies. It has been shown that functional interference of HIF-1α results in enhanced apoptosis upon chemotherapeutic treatment in different cancer types. Through regulation of those anti-apoptotic genes, HIF-1α plays a crucial role in inhibition of apoptosis and promoting tumor cell survival upon chemotherapy.

Hypoxia-induced drug resistance through hyperactivation of ERK pathway

Sustained activation and nuclear translocation of ERK are prerequisites for controlling cell proliferation, differentiation, and survival.[117,118] Constitutive activation of ERK pathway is associated with malignancy in many cancers[119–121]; however, no activating mutation in ERK has been detected in human cancer.[122] Phosphorylation of ERK depends on activating mutation of upstream signaling kinases or loss-of-function of downstream phosphatases. It is evident that activating mutations of upstream molecules such as epidermal growth factor receptor, RAS, and RAF would result in constitutive ERK phosphorylation.[123–129] Nevertheless, aberrant activation of ERK has also been found in many cancers without detectable activating mutations of these genes,[119,120,130] suggesting that loss-of-function of the downstream phosphatases also plays important roles in causing ERK activation. The downstream phosphatases that specifically inactivate ERK signaling are members of DUSP family. Downregulation of DUSP2 and DUSP4 thus leading to prolonged ERK activation has been found in various cancer types.[66,131] Intriguingly, reduced expression of DUSP2 and DUSP4 is mediated by HIF-1α, which results in increasing chemoresistance and malignancy in human cancer cells.[66,132] In-depth investigation reveals that knockdown of DUSP2 upregulates several important genes downstream of ERK such as MDR1, EGR-1, CCN1, glucose response protein 78, and osteopontin,[66] which had been reported to confer drug resistance and aggressive tumor phenotypes.[133–135] Taken together, these findings suggest that hypoxia-dependent, DUSP-mediated ERK activation is an important pathway that increases drug resistance via several different pathways.

Targeting hypoxia for cancer treatment

HIF targeting drugs

Since HIF controls many processes of tumorigenesis, it is reasonable to consider inhibiting HIF expression and/or activity for cancer treatment. There were many new developed agents to inhibit HIF-1α protein expression by targeting different objectives (Table 1). Wortmannin, CCI-779, rapamycin, and LY294002 are inhibitors of PI3K-Akt-mTOR. Hsp90 inhibitors, histone deacetylase inhibitors, camptothecins analogs, topoisomerase I inhibitors, 2 ME2 and analogs, and RNA antagonist (e.g. EZN-2968) were proven to be effective to inhibit HIF-1 function preclinically or clinically. There are still many other molecules/drugs inhibiting HIF-1 function not only in reducing protein level but also inhibiting dimerization, DNA binding, and transcriptional activity. Acriflavine, a very old antibiotic, binds to PAS-B subdomain of HIF-1/2α and thus interrupts binding with HIF-1β to block HIF-dependent transcription. Doxorubicin and other anthracycline agents exert their ability to destroy HIF DNA binding activity and thus block transcription of genes required for tumor growth and vascularization. The FDA approved bortezomib, a proteasome inhibitor, binds to carboxyl-terminal transactivation domain of HIF-1α to change the structure and disrupt the function of HIF-1α-dependent transactivation. Inhibition of aldose reductase, an enzyme that catalyzes the reduction of lipid aldehydes and their glutathione conjugates, prevents increase of HIF-1α and VEGF by regulating 26S proteasome-mediated protein degradation in human colon cancer cells.

Bioreductive drugs

Although some exciting results have been shown by targeting HIF, it needs to bear in mind that HIF is indispensable for maintaining normal physiological functions as well. Ablation of HIF protein or inhibiting HIF activity will generate severe adverse effects. Therefore, the concept of

Table 1 Overview of drugs related to HIF-1 activity inhibition

Mechanism	Target	Drug Name	Drug Class	Status	Ref.
Blockage of HIF-1α protein synthesis	mTOR	Rapamycin; everolimus, temsirolimus	Allosteric binders to FKBP12- rapamycin binding domain	Approved	143–145
		WYE-125132	mTOR kinase inhibitor	Preclinical	146
	Topoisomerase I	Topotecan	Camptothecin analogues	Approved	147,148
	Topoisomerase II	Doxorubicin	Anthracycline	Approved	149,150
	Microtubule	2-methoxyestradiol (2ME); taxane; vinca alkaloid	Microtubule-targeting agent	Clinical trial (2ME); approved (Taxotere, Vincristine)	151
	Unknown	Digoxin	Cardiac glycoside	Approved	152
Disruption of HIF-1α protein stability	HSP90	17-AAG	Benzoquinone ansamycin antibiotics	Clinical trial	153, 154
	Histone deacetylase	LAQ824;	Histone deacetylase inhibitor	Suspended	155
		FK228(Romidepsin)		Approved	156,157
	FIH1	YC-1	Guanylate cyclase activator	Preclinical	158,159
Blockage of HIF heterodimerization	PAS-B subdomain	Acriflavine	Antimicrobial agent	Preclinical	160
Blockage of DNA bindings	DNA binding	Doxorubicin	Anthracycline	Approved	152
		Echinomycin	DNA intercalator	Suspended	161
Inhibition of HIF-1 transactivation	p300	Chetomin	Dithiodiketopiperazine	Preclinical	162
	Thioredoxin 1	PX12	Imidazole disulphide	Clinical trial	163
	26 proteasome	Bortezomib	Proteasome inhibitor	Approved	164,165
	FIH	Amphotericin B	Cyclooxygenase inhibitor	Approved	166
Inhibiting of HIF-1 target gene products	GLUT1	Glufosfamide	Glucose isophosphoramide mustard	Clinical trial	167
		Fasentin	Oxobutanilide	Preclinical	168
	MCT1	α-cyano-4-hydroxycinnamate	Lactate transport inhibitor	Preclinical	169
Blockage of HIF-1 signal transduction	VEGFR	Bevacizumab	Monoclonal antibody	Approved	170,171
	EGFR	Cetuximab	Monoclonal antibody	Approved	172,173
	BRAF	Sorafenib	ATP competitive kinase inhibitor	Approved	174
	COX2	Celecoxib	COX2 inhibitor	Approved	175

17-AAG: 17-allylaminogelanamycin, COX2: cyclooxygenase 2, EGFR: epidermal growth factor receptor, FIH1: factor inhibiting HIF-1, GLUT1: glucose transporter 1, HSP90: heat-shock protein 90, MCT1: monocarboxylate transporter 1, PX12: 1-methylpropyl 2-imidazolyl disulfide, VEGFR: vascular endothelial growth factor receptor.

pro-drugs has been developed. There are five chemical moieties including nitro groups, quinones, aromatic N-oxides, and transition metals that could be metabolized by enzymatic reduction under hypoxic conditions, thus providing the basis for targeting hypoxic tumor.[136–142] Most of those bioreductive drugs are reduced by cellular reductases and form the free radical intermediates. The re-oxidation of this intermediate is oxygen-dependent and thus the produced free radical cannot be eliminated in hypoxic cells. Such bioreductive pro-drugs can inhibit the growth of hypoxic cells with no or little effect on normoxic cells.

Conclusion

Malignancy, one of the leading causes of cancer-related death worldwide, is quite complicated and difficult in treatment. Despite receiving aggressive treatments, the mortality rate is still high. HIF, the product under hypoxia, is a predominant determinant in a wide range of cancer cell biology in mammal. Expression of HIF is correlated with cancer cell angiogenesis, proliferation, metastases, and treatment outcome. So, blocking any step of HIF-related pathway maybe helpful to win the battle against cancer. Clinically, some HIF-related inhibitors seem to have the potential to cure several types of cancers. However, the effectiveness is still not very high. And it should be very cautious in utility of HIF inhibitors due to its possibly wide range effects not only in cancer but also in normal cells. Therefore, detecting more underlying mechanisms of the HIF pathway and/or identifying more satisfying agents that targets molecules in the HIF pathway will be the first priority in the future.

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REFERENCES

1. Organization WH. Global status report on noncommunicable diseases 2010, http://www.who.int/nmh/publications/ncd_report2010/en/index.html (2011)
2. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science* 2005;**307**:58–62
3. Dewhirst MW, Cao Y, Moeller B. Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. *Nat Rev Cancer* 2008;**8**:425–37
4. Semenza GL. Regulation of oxygen homeostasis by hypoxia-inducible factor 1. Physiology *(Bethesda)* 2009;**24**:97–106
5. Wang GL, Jiang BH, Rue EA, 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**:5510–4
6. Semenza GL. Targeting HIF-1 for cancer therapy. *Nat Rev Cancer* 2003;**3**:721–32
7. Keith B, Johnson RS, Simon MC. HIF1alpha and HIF2alpha: sibling rivalry in hypoxic tumour growth and progression. *Nat Rev Cancer* 2012;**12**:9–22
8. Rohwer N, Cramer T. Hypoxia-mediated drug resistance: novel insights on the functional interaction of HIFs and cell death pathways. *Drug Resist Updat* 2011;**14**:191–201
9. Harris AL. Hypoxia – a key regulatory factor in tumour growth. *Nat Rev Cancer* 2002;**2**:38–47
10. Nurwidya F, Takahashi F, Minakata K, Murakami A, Takahashi K. From tumor hypoxia to cancer progression: the implications of hypoxia-inducible factor-1 expression in cancers. *Anat Cell Biol* 2012;**45**:73–8
11. Semenza GL. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. *Oncogene* 2010;**29**:625–34
12. Wilson WR, Hay MP. Targeting hypoxia in cancer therapy. *Nat Rev Cancer* 2011;**11**:393–410
13. Wang GL, Semenza GL. General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. *Proc Natl Acad Sci U S A* 1993;**90**:4304–8
14. Semenza GL, 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**:5447–54
15. Salceda S, Caro J. Hypoxia-inducible factor 1alpha (HIF-1alpha) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes. *J Biol Chem* 1997;**272**:22642–7
16. Huang LE, Gu J, Schau M, Bunn HF. Regulation of hypoxia-inducible factor 1alpha is mediated by an O2-dependent degradation domain via the ubiquitin-proteasome pathway. *Proc Natl Acad Sci U S A* 1998;**95**:7987–92
17. Bruick RK, McKnight SL. A conserved family of prolyl-4-hydroxylases that modify HIF. *Science* 2001;**294**:1337–40
18. Epstein AC, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, Mole DR, Mukherji M, Metzen E, Wilson MI, Dhanda A, Tian YM, Masson N, Hamilton DL, Jaakkola P, Barstead R, Hodgkin J, Maxwell PH, Pugh CW, Schofield CJ, Ratcliffe PJ. C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell* 2001;**107**:43–54
19. 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, Ratcliffe PJ. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. *Science* 2001;**292**:468–72
20. Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara JM, Lane WS, Kaelin WG Jr. HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. *Science* 2001;**292**:464–8
21. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER, Ratcliffe PJ. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* 1999;**399**:271–5
22. Luo W, Zhong J, Chang R, Hu H, Pandey A, Semenza GL. Hsp70 and CHIP selectively mediate ubiquitination and degradation of hypoxia-inducible factor (HIF)-1alpha but Not HIF-2alpha. *J Biol Chem* 2010;**285**:3651–63
23. Ohh M, Park CW, Ivan M, Hoffman MA, Kim TY, Huang LE, Pavletich N, Chau V, Kaelin WG. Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel-Lindau protein. *Nat Cell Biol* 2000;**2**:423–7
24. Mahon PC, Hirota K, 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**:2675–86
25. Coleman ML, Ratcliffe PJ. Signalling cross talk of the HIF system: involvement of the FIH protein. *Curr Pharm Des* 2009;**15**:3904–7

26. Ameri K, Hammond EM, Culmsee C, Raida M, Katschinski DM, Wenger RH, Wagner E, Davis RJ, Hai T, Denko N, Harris AL. Induction of activating transcription factor 3 by anoxia is independent of p53 and the hypoxic HIF signalling pathway. *Oncogene* 2007;**26**:284–9
27. Elvidge GP, Glenny L, Appelhoff RJ, Ratcliffe PJ, Ragoussis J, Gleadle JM. Concordant regulation of gene expression by hypoxia and 2-oxoglutarate-dependent dioxygenase inhibition: the role of HIF-1alpha, HIF-2alpha, and other pathways. *J Biol Chem* 2006;**281**:15215–26
28. Aras S, Pak O, Sommer N, Finley R Jr, Huttemann M, Weissmann N, Grossman LI. Oxygen-dependent expression of cytochrome c oxidase subunit 4-2 gene expression is mediated by transcription factors RBPJ, CXXC5 and CHCHD2. *Nucleic Acid Res* 2013;**41**:2255–66
29. Cummins EP, Comerford KM, Scholz C, Bruning U, Taylor CT. Hypoxic regulation of NF-kappaB signaling. *Meth Enzymol* 2007;**435**:479–92
30. Koditz J, Nesper J, Wottawa M, Stiehl DP, Camenisch G, Franke C, Myllyharju J, Wenger RH, Katschinski DM. Oxygen-dependent ATF-4 stability is mediated by the PHD3 oxygen sensor. *Blood* 2007;**110**:3610–7
31. Mizukami Y, Fujiki K, Duerr EM, Gala M, Jo WS, Zhang X, Chung DC. Hypoxic regulation of vascular endothelial growth factor through the induction of phosphatidylinositol 3-kinase/Rho/ROCK and c-Myc. *J Biol Chem* 2006;**281**:13957–63
32. Swanson HI, Yang JH. Specificity of DNA binding of the c-Myc/Max and ARNT/ARNT dimers at the CACGTG recognition site. *Nucleic Acid Res* 1999;**27**:3205–12
33. Ambrosini G, Nath AK, Sierra-Honigmann MR, Flores-Riveros J. Transcriptional activation of the human leptin gene in response to hypoxia. Involvement of hypoxia-inducible factor 1. *J Biol Chem* 2002;**277**:34601–9
34. Chen KF, Lai YY, Sun HS, Tsai SJ. Transcriptional repression of human cad gene by hypoxia inducible factor-1alpha. *Nucleic Acid Res* 2005;**33**:5190–8
35. Lu CW, Lin SC, Chen KF, Lai YY, Tsai SJ. Induction of pyruvate dehydrogenase kinase-3 by hypoxia-inducible factor-1 promotes metabolic switch and drug resistance. *J Biol Chem* 2008;**283**:28106–14
36. Ortiz-Barahona A, Villar D, Pescador N, Amigo J, del Peso L. Genome-wide identification of hypoxia-inducible factor binding sites and target genes by a probabilistic model integrating transcription-profiling data and in silico binding site prediction. *Nucleic Acids Res* 2010;**38**:2332–45
37. Wang HC, Chen YH, Kao HY, Tsai SJ. Inference of transcriptional regulatory network by bootstrapping patterns. *Bioinformatics* 2011;**27**:1422–8
38. Choi SM, Oh H, Park H. Microarray analyses of hypoxia-regulated genes in an aryl hydrocarbon receptor nuclear translocator (Arnt)-dependent manner. *FEBS J* 2008;**275**:5618–34
39. Wang V, Davis DA, Haque M, Huang LE, Yarchoan R. Differential gene up-regulation by hypoxia-inducible factor-1alpha and hypoxia-inducible factor-2alpha in HEK293T cells. *Cancer Res* 2005;**65**:3299–306
40. Schodel J, Oikonomopoulos S, Ragoussis J, Pugh CW, Ratcliffe PJ, Mole DR. High-resolution genome-wide mapping of HIF-binding sites by ChIP-seq. *Blood* 2011;**117**:e207–17
41. Mole DR, Blancher C, Copley RR, Pollard PJ, Gleadle JM, Ragoussis J, Ratcliffe PJ. Genome-wide association of hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha DNA binding with expression profiling of hypoxia-inducible transcripts. *J Biol Chem* 2009;**284**:16767–75
42. Aprelikova O, Wood M, Tackett S, Chandramouli GV, Barrett JC. Role of ETS transcription factors in the hypoxia-inducible factor-2 target gene selection. *Cancer Res* 2006;**66**:5641–7
43. Hu CJ, Wang LY, Chodosh LA, Keith B, Simon MC. Differential roles of hypoxia-inducible factor 1alpha (HIF-1alpha) and HIF-2alpha in hypoxic gene regulation. *Mol Cell Biol* 2003;**23**:9361–74
44. Warburg O. über den Stoffwechsel der Carcinomzelle. *J Mol Med* 1925;**4**:534
45. Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* 2006;**3**:177–85
46. Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metab* 2006;**3**:187–97
47. Lu CW, Lin SC, Chien CW, Lin SC, Lee CT, Lin BW, Lee JC, Tsai SJ. Overexpression of pyruvate dehydrogenase kinase 3 increases drug resistance and early recurrence in colon cancer. *Am J Pathol* 2011;**179**:1405–14
48. Sivitz WI, Lund DD, Yorek B, Grover-McKay M, Schmid PG. Pretranslational regulation of two cardiac glucose transporters in rats exposed to hypobaric hypoxia. *Am J Physiol* 1992;**263**:E562–9
49. Firth JD, Ebert BL, Pugh CW, Ratcliffe PJ. Oxygen-regulated control elements in the phosphoglycerate kinase 1 and lactate dehydrogenase A genes: similarities with the erythropoietin 3′ enhancer. *Proc Natl Acad Sci U S A* 1994;**91**:6496–500
50. Semenza GL, Roth PH, Fang HM, Wang GL. Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. *J Biol Chem* 1994;**269**:23757–63
51. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 2009;**324**:1029–33
52. Hensley CT, Wasti AT, DeBerardinis RJ. Glutamine and cancer: cell biology, physiology, and clinical opportunities. *J Clin Invest* 2013;**123**:3678–84
53. Topkan E, Parlak C, Topuk S, Pehlivan B. Influence of oral glutamine supplementation on survival outcomes of patients treated with concurrent chemoradiotherapy for locally advanced non-small cell lung cancer. *BMC Cancer* 2012;**12**:502
54. Gao P, Tchernyshyov I, Chang TC, Lee YS, Kita K, Ochi T, Zeller KI, De Marzo AM, Van Eyk JE, Mendell JT, Dang CV. c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. *Nature* 2009;**458**:762–5
55. Liu W, Le A, Hancock C, Lane AN, Dang CV, Fan TW, Phang JM. Reprogramming of proline and glutamine metabolism contributes to the proliferative and metabolic responses regulated by oncogenic transcription factor c-MYC. *Proc Natl Acad Sci U S A* 2012;**109**:8983–8
56. Pinheiro C, Longatto-Filho A, Azevedo-Silva J, Casal M, Schmitt FC, Baltazar F. Role of monocarboxylate transporters in human cancers: state of the art. *J Bioenerg Biomembr* 2012;**44**:127–39
57. Chiche J, Brahimi-Horn MC, Pouyssegur J. Tumour hypoxia induces a metabolic shift causing acidosis: a common feature in cancer. *J Cell Mol Med* 2010;**14**:771–94
58. Swietach P, Wigfield S, Cobden P, Supuran CT, Harris AL, Vaughan-Jones RD. Tumor-associated carbonic anhydrase 9 spatially coordinates intracellular pH in three-dimensional multicellular growths. *J Biol Chem* 2008;**283**:20473–83
59. Feldser D, Agani F, Iyer NV, Pak B, Ferreira G, Semenza GL. Reciprocal positive regulation of hypoxia-inducible factor 1alpha and insulin-like growth factor 2. *Cancer Res* 1999;**59**:3915–8
60. Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD, Semenza GL. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol Cell Biol* 1996;**16**:4604–13
61. Grimshaw MJ. Endothelins and hypoxia-inducible factor in cancer. *Endocr Relat Cancer* 2007;**14**:233–44
62. Szenajch J, Wcislo G, Jeong JY, Szczylik C, Feldman L. The role of erythropoietin and its receptor in growth, survival and therapeutic response of human tumor cells From clinic to bench – a critical review. *Biochim Biophys Acta* 2010;**1806**:82–95
63. Chen TM, Shih YH, Tseng JT, Lai MC, Wu CH, Li YH, Tsai SJ, Sun HS. Overexpression of FGF9 in colon cancer cells is mediated by hypoxia-induced translational activation. *Nucleic Acids Res* 2013;DOI: 10.1093/nar/gkt286
64. Braunstein S, Karpisheva K, Pola C, Goldberg J, Hochman T, Yee H, Cangiarella J, Arju R, Formenti SC, Schneider RJ. A hypoxia-controlled cap-dependent to cap-independent translation switch in breast cancer. *Molecular Cell* 2007;**28**:501–12
65. Uniacke J, Holterman CE, Lachance G, Franovic A, Jacob MD, Fabian MR, Payette J, Holcik M, Pause A, Lee S. An oxygen-regulated switch in the protein synthesis machinery. *Nature* 2012;**486**:126–9
66. Lin SC, Chien CW, Lee JC, Yeh YC, Hsu KF, Lai YY, Lin SC, Tsai SJ. Suppression of dual-specificity phosphatase-2 by hypoxia increases

chemoresistance and malignancy in human cancer cells. *J Clin Invest* 2011;**121**:1905–16
67. Chen N, Chen X, Huang R, Zeng H, Gong J, Meng W, Lu Y, Zhao F, Wang L, Zhou Q. BCL-xL is a target gene regulated by hypoxia-inducible factor-1{alpha}. *J Biol Chem* 2009;**284**:10004–12
68. Erler JT, Cawthorne CJ, Williams KJ, Koritzinsky M, Wouters BG, Wilson C, Miller C, Demonacos C, Stratford IJ, 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**:2875–89
69. Townsend KN, Hughson LR, Schlie K, Poon VI, Westerback A, Lum JJ. Autophagy inhibition in cancer therapy: metabolic considerations for antitumor immunity. *Immunol Rev* 2012;**249**:176–94
70. Eng CH, Abraham RT. The autophagy conundrum in cancer: influence of tumorigenic metabolic reprogramming. *Oncogene* 2011;**30**:4687–96
71. Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, Gonzalez FJ, Semenza GL. Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. *J Biol Chem* 2008;**283**:10892–903
72. Bellot G, Garcia-Medina R, Gounon P, Chiche J, Roux D, Pouyssegur J, Mazure NM. Hypoxia-induced autophagy is mediated through hypoxia-inducible factor induction of BNIP3 and BNIP3L via their BH3 domains. *Mol Cell Biol* 2009;**29**:2570–81
73. Shweiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature* 1992;**359**:843–5
74. Cao Y, Linden P, Shima D, Browne F, Folkman J. In vivo angiogenic activity and hypoxia induction of heterodimers of placenta growth factor/vascular endothelial growth factor. *J Clin Invest* 1996;**98**:2507–11
75. Semenza GL. Cancer-stromal cell interactions mediated by hypoxia-inducible factors promote angiogenesis, lymphangiogenesis, and metastasis. *Oncogene* 2013;**32**:4057–63
76. Khong TL, Thairu N, Larsen H, Dawson PM, Kiriakidis S, Paleolog EM. Identification of the angiogenic gene signature induced by EGF and hypoxia in colorectal cancer. *BMC Cancer* 2013;**13**:518.
77. Pan J, Mestas J, Burdick MD, Phillips RJ, Thomas GV, Reckamp K, Belperio JA, Strieter RM. Stromal derived factor-1 (SDF-1/CXCL12) and CXCR4 in renal cell carcinoma metastasis. *Mol Cancer* 2006;**5**:56
78. von Minckwitz G, Eidtmann H, Rezai M, Fasching PA, Tesch H, Eggemann H, Schrader I, Kittel K, Hanusch C, Kreienberg R, Solbach C, Gerber B, Jackisch C, Kunz G, Blohmer JU, Huober J, Hauschild M, Fehm T, Muller BM, Denkert C, Loibl S, Nekljudova V, Untch M. Neoadjuvant chemotherapy and bevacizumab for HER2-negative breast cancer. *N Engl J Med* 2012;**366**:299–309
79. Tol J, Koopman M, Cats A, Rodenburg CJ, Creemers GJ, Schrama JG, Erdkamp FL, Vos AH, van Groeningen CJ, Sinnige HA, Richel DJ, Voest EE, Dijkstra JR, Vink-Borger ME, Antonini NF, Mol L, van Krieken JH, Dalesio O, Punt CJ. Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. *N Engl J Med* 2009;**360**:563–72
80. Custodio A, Barriuso J, de Castro J, Martinez-Marin V, Moreno V, Rodriguez-Salas N, Feliu J. Molecular markers to predict outcome to antiangiogenic therapies in colorectal cancer: current evidence and future perspectives. *Cancer Treat Rev* 2013;**39**:908–24
81. Tol J, Punt CJ. Monoclonal antibodies in the treatment of metastatic colorectal cancer: a review. *Clin Ther* 2010;**32**:437–53
82. Tsai YP, Wu KJ. Hypoxia-regulated target genes implicated in tumor metastasis. *J Biomed Sci* 2012;**19**:102
83. Yang MH, Wu MZ, Chiou SH, Chen PM, Chang SY, Liu CJ, Teng SC, Wu KJ. Direct regulation of TWIST by HIF-1alpha promotes metastasis. *Nat Cell Biol* 2008;**10**:295–305
84. Peinado H, Olmeda D, Cano A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nat Rev Cancer* 2007;**7**:415–28
85. Imai T, Horiuchi A, Wang C, Oka K, Ohira S, Nikaido T, Konishi I. Hypoxia attenuates the expression of E-cadherin via up-regulation of SNAIL in ovarian carcinoma cells. *Am J Pathol* 2003;**163**:1437–47
86. Stipp CS, Kolesnikova TV, Hemler ME. Functional domains in tetraspanin proteins. *Trends Biochem Sci* 2003;**28**:106–12
87. Chien C-W, Lin S-C, Lai Y-Y, Lin B-W, Lin S-C, Lee J-C, Tsai S-J. Regulation of CD151 by hypoxia controls cell adhesion and metastasis in colorectal cancer. *Clin Cancer Res* 2008;**14**:8043–51
88. Erler JT, Bennewith KL, Nicolau M, Dornhofer N, Kong C, Le QT, Chi JT, Jeffrey SS, Giaccia AJ. Lysyl oxidase is essential for hypoxia-induced metastasis. *Nature* 2006;**440**:1222–6
89. Lin JL, Wang MJ, Lee D, Liang CC, Lin S. Hypoxia-inducible factor-1alpha regulates matrix metalloproteinase-1 activity in human bone marrow-derived mesenchymal stem cells. *FEBS Lett* 2008;**582**:2615–9
90. Petrella BL, Lohi J, Brinckerhoff CE. Identification of membrane type-1 matrix metalloproteinase as a target of hypoxia-inducible factor-2 alpha in von Hippel-Lindau renal cell carcinoma. *Oncogene* 2005;**24**:1043–52
91. Zhang H, Wong CC, Wei H, Gilkes DM, Korangath P, Chaturvedi P, Schito L, Chen J, Krishnamachary B, Winnard PT Jr., Raman V, Zhen L, Mitzner WA, Sukumar S, Semenza GL. HIF-1-dependent expression of angiopoietin-like 4 and L1CAM mediates vascular metastasis of hypoxic breast cancer cells to the lungs. *Oncogene* 2012;**31**:1757–70
92. Gatenby RA, Smallbone K, Maini PK, Rose F, Averill J, Nagle RB, Worrall L, Gillies RJ. Cellular adaptations to hypoxia and acidosis during somatic evolution of breast cancer. *Br J Cancer* 2007;**97**:646–53
93. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993;**75**:843–54
94. Iorio MV, Croce CM. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. *EMBO Mol Med* 2012;**4**:143–59
95. Bao B, Azmi AS, Ali S, Ahmad A, Li Y, Banerjee S, Kong D, Sarkar FH. The biological kinship of hypoxia with CSC and EMT and their relationship with deregulated expression of miRNAs and tumor aggressiveness. *Biochim Biophys Acta* 2012;**1826**:272–96
96. Liao WL, Lin SC, S. SH, Tsai SJ. Hypoxia-induced tumor malignancy and drug resistance: Role of microRNAs. *Biomarker Genomic Med* 2014;**6**:1–11
97. Bristow RG, Hill RP. Hypoxia and metabolism. Hypoxia, DNA repair and genetic instability. *Nat Rev Cancer* 2008;**8**:180–92
98. Nurwidya F, Takahashi F, Minakata K, Murakami A, Takahashi K. From tumor hypoxia to cancer progression: the implications of hypoxia-inducible factor-1 expression in cancers. *Anatomy Cell Biol* 2012;**45**:73
99. Kondoh H. Cellular life span and the Warburg effect. *Exp Cell Res* 2008;**314**:1923–8
100. Brizel DM, Sibley GS, Prosnitz LR, Scher RL, Dewhirst MW. Tumor hypoxia adversely affects the prognosis of carcinoma of the head and neck. *Int J Radiat Oncol Biol Phys* 1997;**38**:285–9
101. Zhang H, Gao P, Fukuda R, Kumar G, Krishnamachary B, Zeller KI, Dang CV, Semenza GL. HIF-1 inhibits mitochondrial biogenesis and cellular respiration in VHL-deficient renal cell carcinoma by repression of C-MYC activity. *Cancer Cell* 2007;**11**:407–20
102. Fruehauf JP, Meyskens FL Jr. Reactive oxygen species: a breath of life or death? *Clin Cancer Res* 2007;**13**:789–94
103. Rohwer N, Dame C, Haugstetter A, Wiedenmann B, Detjen K, Schmitt CA, Cramer T. Hypoxia-inducible factor 1alpha determines gastric cancer chemosensitivity via modulation of p53 and NF-kappaB. *PLoS One* 2010;**5**:e12038
104. Comerford KM, Wallace TJ, Karhausen J, Louis NA, Montalto MC, Colgan SP. Hypoxia-inducible factor-1-dependent regulation of the multidrug resistance (MDR1) gene. *Cancer Res* 2002;**62**:3387–94
105. Ding Z, Yang L, Xie X, Xie F, Pan F, Li J, He J, Liang H. Expression and significance of hypoxia-inducible factor-1 alpha and MDR1/P-glycoprotein in human colon carcinoma tissue and cells. *J Cancer Res Clin Oncol* 2010;**136**:1697–707
106. Liu L, Ning X, Sun L, Zhang H, Shi Y, Guo C, Han S, Liu J, Sun S, Han Z, Wu K, Fan D. Hypoxia-inducible factor-1 alpha contributes to hypoxia-induced chemoresistance in gastric cancer. *Cancer Sci* 2008;**99**:121–8
107. Li J, Shi M, Cao Y, Yuan W, Pang T, Li B, Sun Z, Chen L, Zhao RC. Knockdown of hypoxia-inducible factor-1alpha in breast carcinoma

MCF-7 cells results in reduced tumor growth and increased sensitivity to methotrexate. *Biochem Biophys Res Commun* 2006;**342**:1341–51
108. Nardinocchi L, Puca R, Sacchi A, D'Orazi G. Inhibition of HIF-1alpha activity by homeodomain-interacting protein kinase-2 correlates with sensitization of chemoresistant cells to undergo apoptosis. *Mol Cancer* 2009;**8**:1
109. Zhu H, Chen XP, Luo SF, Guan J, Zhang WG, Zhang BX. Involvement of hypoxia-inducible factor-1-alpha in multidrug resistance induced by hypoxia in HepG2 cells. *J Exp Clin Cancer Res* 2005;**24**:565–74
110. Chen L, Feng P, Li S, Long D, Cheng J, Lu Y, Zhou D. Effect of hypoxia-inducible factor-1alpha silencing on the sensitivity of human brain glioma cells to doxorubicin and etoposide. *Neurochem Res* 2009;**34**:984–90
111. Abdulkadir SA, Qu Z, Garabedian E, Song SK, Peters TJ, Svaren J, Carbone JM, Naughton CK, Catalona WJ, Ackerman JJ, Gordon JI, Humphrey PA, Milbrandt J. Impaired prostate tumorigenesis in Egr1-deficient mice. *Nat Med* 2001;**7**:101–7
112. Ma J, Ren Z, Ma Y, Xu L, Zhao Y, Zheng C, Fang Y, Xue T, Sun B, Xiao W. Targeted knockdown of EGR-1 inhibits IL-8 production and IL-8-mediated invasion of prostate cancer cells through suppressing EGR-1/NF-kappaB synergy. *J Biol Chem* 2009;**284**:34600–6
113. Mahalingam D, Natoni A, Keane M, Samali A, Szegezdi E. Early growth response-1 is a regulator of DR5-induced apoptosis in colon cancer cells. *Br J Cancer* 2010;**102**:754–64
114. Shiloh Y. The ATM-mediated DNA-damage response: taking shape. *Trends Biochem Sci* 2006;**31**:402–10
115. Hammond EM, Denko NC, Dorie MJ, Abraham RT, Giaccia AJ. Hypoxia links ATR and p53 through replication arrest. *Mol Cell Biol* 2002;**22**:1834–43
116. Green SL, Giaccia AJ. Tumor hypoxia and the cell cycle: implications for malignant progression and response to therapy. *Cancer J Sci Am* 1998;**4**:218–23
117. Yamamoto T, Ebisuya M, Ashida F, Okamoto K, Yonehara S, Nishida E. Continuous ERK activation downregulates antiproliferative genes throughout G1 phase to allow cell-cycle progression. *Curr Biol* 2006;**16**:1171–82
118. Brunet A, Roux D, Lenormand P, Dowd S, Keyse S, Pouyssegur J. Nuclear translocation of p42/p44 mitogen-activated protein kinase is required for growth factor-induced gene expression and cell cycle entry. *EMBO J* 1999;**18**:664–74
119. Gioeli D, Mandell JW, Petroni GR, Frierson HF Jr, Weber MJ. Activation of mitogen-activated protein kinase associated with prostate cancer progression. *Cancer Res* 1999;**59**:279–84
120. Hoshino R, Chatani Y, Yamori T, Tsuruo T, Oka H, Yoshida O, Shimada Y, Ari-i S, Wada H, Fujimoto J, Kohno M. Constitutive activation of the 41-/43-kDa mitogen-activated protein kinase signaling pathway in human tumors. *Oncogene* 1999;**18**:813–22
121. Sebolt-Leopold JS, Dudley DT, Herrera R, Van Becelaere K, Wiland A, Gowan RC, Tecle H, Barrett SD, Bridges A, Przybranowski S, Leopold WR, Saltiel AR. Blockade of the MAP kinase pathway suppresses growth of colon tumors in vivo. *Nat Med* 1999;**5**:810–6
122. Kohno M, Pouyssegur J. Targeting the ERK signaling pathway in cancer therapy. *Ann Med* 2006;**38**:200–11
123. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE, Meyerson M. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;**304**:1497–500
124. Downward J. Targeting RAS signalling pathways in cancer therapy. *Nat Rev* 2003;**3**:11–22
125. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA. Mutations of the BRAF gene in human cancer. *Nature* 2002;**417**:949–54
126. Wan PT, Garnett MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM, Jones CM, Marshall CJ, Springer CJ, Barford D, Marais R. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell* 2004;**116**:855–67
127. McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Wong EW, Chang F, Lehmann B, Terrian DM, Milella M, Tafuri A, Stivala F, Libra M, Basecke J, Evangelisti C, Martelli AM, Franklin RA. Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. *Biochimica et biophysica acta* 2007;**1773**:1263–84
128. Persons DL, Yazlovitskaya EM, Cui W, Pelling JC. Cisplatin-induced activation of mitogen-activated protein kinases in ovarian carcinoma cells: inhibition of extracellular signal-regulated kinase activity increases sensitivity to cisplatin. *Clin Cancer Res* 1999;**5**:1007–14
129. Fan M, Chambers TC. Role of mitogen-activated protein kinases in the response of tumor cells to chemotherapy. *Drug Resist Updat* 2001;**4**:253–67
130. Oka H, Chatani Y, Hoshino R, Ogawa O, Kakehi Y, Terachi T, Okada Y, Kawaichi M, Kohno M, Yoshida O. Constitutive activation of mitogen-activated protein (MAP) kinases in human renal cell carcinoma. *Cancer Res* 1995;**55**:4182–7
131. Balko JM, Cook RS, Vaught DB, Kuba MG, Miller TW, Bhola NE, Sanders ME, Granja-Ingram NM, Smith JJ, Meszoely IM, Salter J, Dowsett M, Stemke-Hale K, Gonzalez-Angulo AM, Mills GB, Pinto JA, Gomez HL, Arteaga CL. Profiling of residual breast cancers after neoadjuvant chemotherapy identifies DUSP4 deficiency as a mechanism of drug resistance. *Nat Med* 2012;**18**:1052–9
132. Balko JM, Schwarz LJ, Bhola NE, Kurupi R, Owens P, Miller TW, Gomez H, Cook RS, Arteaga CL. Activation of MAPK pathways due to DUSP4 loss promotes cancer stem cell-like phenotypes in basal-like breast cancer. *Cancer Res* 2013;**73**:6346–58
133. Dong D, Ko B, Baumeister P, Swenson S, Costa F, Markland F, Stiles C, Patterson JB, Bates SE, Lee AS. Vascular targeting and antiangiogenesis agents induce drug resistance effector GRP78 within the tumor microenvironment. *Cancer Res* 2005;**65**:5785–91
134. Lin MT, Chang CC, Chen ST, Chang HL, Su JL, Chau YP, Kuo ML. Cyr61 expression confers resistance to apoptosis in breast cancer MCF-7 cells by a mechanism of NF-kappaB-dependent XIAP up-regulation. *J Biol Chem* 2004;**279**:24015–23
135. Ohashi R, Tajima K, Takahashi F, Cui R, Gu T, Shimizu K, Nishio K, Fukuoka K, Nakano T, Takahashi K. Osteopontin modulates malignant pleural mesothelioma cell functions in vitro. *Anticancer Res* 2009;**29**:2205–14
136. Ahn GO, Botting KJ, Patterson AV, Ware DC, Tercel M, Wilson WR. Radiolytic and cellular reduction of a novel hypoxia-activated cobalt(III) prodrug of a chloromethylbenzindoline DNA minor groove alkylator. *Biochem Pharmacol* 2006;**71**:1683–94
137. Brown JM. SR 4233 (tirapazamine): a new anticancer drug exploiting hypoxia in solid tumours. *Br J Cancer* 1993;**67**:1163–70
138. Chowdhury G, Junnotula V, Daniels JS, Greenberg MM, Gates KS. DNA strand damage product analysis provides evidence that the tumor cell-specific cytotoxin tirapazamine produces hydroxyl radical and acts as a surrogate for O(2). *J Am Chem Soc* 2007;**129**:12870–7
139. Patterson LH. Rationale for the use of aliphatic N-oxides of cytotoxic anthraquinones as prodrug DNA binding agents: a new class of bioreductive agent. *Cancer Metastasis Rev* 1993;**12**:119–34
140. Shinde SS, Hay MP, Patterson AV, Denny WA, Anderson RF. Spin trapping of radicals other than the *OH radical upon reduction of the anticancer agent tirapazamine by cytochrome P450 reductase. *J Am Chem Soc* 2009;**131**:14220–1
141. Weiss GJ, Infante JR, Chiorean EG, Borad MJ, Bendell JC, Molina JR, Tibes R, Ramanathan RK, Lewandowski K, Jones SF, Lacouture ME, Langmuir VK, Lee H, Kroll S, Burris HA. Phase 1 study of the safety, tolerability, and pharmacokinetics of TH-302, a hypoxia-activated prodrug, in patients with advanced solid malignancies. *Clin Cancer Res* 2011;**17**, 3rd ed. 2997–3004

142. Wilson WR, Van Zijl P, Denny WA. Bis-bioreductive agents as hypoxia-selective cytotoxins: nitracrine N-oxide. *Int J Radiat Oncol Biol Phys* 1992;**22**:693–6
143. Hudson CC, Liu M, Chiang GG, Otterness DM, Loomis DC, Kaper F, Giaccia AJ, Abraham RT. Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. *Mol Cell Biol* 2002;**22**:7004–14
144. Ohtsu A, Ajani JA, Bai YX, Bang YJ, Chung HC, Pan HM, Sahmoud T, Shen L, Yeh KH, Chin K, Muro K, Kim YH, Ferry D, Tebbutt NC, Al-Batran SE, Smith H, Costantini C, Rizvi S, Lebwohl D, Van Cutsem E. Everolimus for previously treated advanced gastric cancer: results of the randomized, double-blind, phase III GRANITE-1 study. *J Clin Oncol* 2013;**31**:3935–43
145. Kruczek K, Ratterman M, Tolzien K, Sulo S, Lestingi TM, Nabhan C. A phase II study evaluating the toxicity and efficacy of single-agent temsirolimus in chemotherapy-naive castration-resistant prostate cancer. *Br J Cancer* 2013;**109**:1711–6
146. Yu K, Shi C, Toral-Barza L, Lucas J, Shor B, Kim JE, Zhang WG, Mahoney R, Gaydos C, Tardio L, Kim SK, Conant R, Curran K, Kaplan J, Verheijen J, Ayral-Kaloustian S, Mansour TS, Abraham RT, Zask A, Gibbons JJ. Beyond rapalog therapy: preclinical pharmacology and antitumor activity of WYE-125132, an ATP-competitive and specific inhibitor of mTORC1 and mTORC2. *Cancer Res* 2010;**70**:621–31
147. Rapisarda A, Shoemaker RH, Melillo G. Targeting topoisomerase I to inhibit hypoxia inducible factor 1. *Cell Cycle* 2004;**3**:172–5
148. Vergote IB, Garcia A, Micha J, Pippitt C, Bendell J, Spitz D, Reed N, Dark G, Fracasso PM, Ibrahim EN, Armenio VA, Duska L, Poole C, Gennigens C, Dirix LY, Leung AC, Zhao C, Soufi-Mahjoubi R, Rustin G. Randomized multicenter phase II trial comparing two schedules of etirinotecan pegol (NKTR-102) in women with recurrent platinum-resistant/refractory epithelial ovarian cancer. *J Clin Oncol* 2013;**31**:4060–6
149. Tanaka T, Yamaguchi J, Shoji K, Nangaku M. Anthracycline inhibits recruitment of hypoxia-inducible transcription factors and suppresses tumor cell migration and cardiac angiogenic response in the host. *J Biol Chem* 2012;**287**:34866–82
150. Moroney J, Fu S, Moulder S, Falchook G, Helgason T, Levenback C, Hong D, Naing A, Wheler J, Kurzrock R. Phase I study of the antiangiogenic antibody bevacizumab and the mTOR/hypoxia-inducible factor inhibitor temsirolimus combined with liposomal doxorubicin: tolerance and biological activity. *Clin Cancer Res* 2012;**18**:5796–805
151. Mabjeesh NJ, Escuin D, LaVallee TM, Pribluda VS, Swartz GM, Johnson MS, Willard MT, Zhong H, Simons JW, Giannakakou P. 2ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF. *Cancer Cell* 2003;**3**:363–75
152. Lopez-Lazaro M. Digoxin, HIF-1, and cancer. *Proc Natl Acad Sci U S A* 2009;**106**E26; (author reply E7)
153. Isaacs JS, Jung YJ, Mimnaugh EG, Martinez A, Cuttitta F, Neckers LM. Hsp90 regulates a von Hippel Lindau-independent hypoxia-inducible factor-1 alpha-degradative pathway. *J Biol Chem* 2002;**277**:29936–44
154. Modi S, Stopeck AT, Gordon MS, Mendelson D, Solit DB, Bagatell R, Ma W, Wheler J, Rosen N, Norton L, Cropp GF, Johnson RG, Hannah AL, Hudis CA. Combination of trastuzumab and tanespimycin (17–AAG, KOS-953) is safe and active in trastuzumab-refractory HER-2 overexpressing breast cancer: a phase I dose-escalation study. *J Clin Oncol* 2007;**25**:5410–7
155. de Bono JS, Kristeleit R, Tolcher A, Fong P, Pacey S, Karavasilis V, Mita M, Shaw H, Workman P, Kaye S, Rowinsky EK, Aherne W, Atadja P, Scott JW, Patnaik A. Phase I pharmacokinetic and pharmacodynamic study of LAQ824, a hydroxamate histone deacetylase inhibitor with a heat shock protein-90 inhibitory profile, in patients with advanced solid tumors. *Clin Cancer Res* 2008;**14**:6663–73
156. Harrison SJ, Quach H, Link E, Seymour JF, Ritchie DS, Ruell S, Dean J, Januszewicz H, Johnstone R, Neeson P, Dickinson M, Nichols J, Prince HM. A high rate of durable responses with romidepsin, bortezomib, and dexamethasone in relapsed or refractory multiple myeloma. *Blood* 2011;**118**:6274–83
157. Coiffier B, Pro B, Prince HM, Foss F, Sokol L, Greenwood M, Caballero D, Borchmann P, Morschhauser F, Wilhelm M, Pinter-Brown L, Padmanabhan S, Shustov A, Nichols J, Carroll S, Balser J, Balser B, Horwitz S. Results from a pivotal, open-label, phase II study of romidepsin in relapsed or refractory peripheral T-cell lymphoma after prior systemic therapy. *J Clin Oncol* 2012;**30**:631–6
158. Chun YS, Yeo EJ, Park JW. Versatile pharmacological actions of YC-1: anti-platelet to anticancer. *Cancer Lett* 2004;**207**:1–7
159. Li SH, Shin DH, Chun YS, Lee MK, Kim MS, Park JW. A novel mode of action of YC-1 in HIF inhibition: stimulation of FIH-dependent p300 dissociation from HIF-1{alpha}. *Mol Cancer Ther* 2008;**7**:3729–38
160. Lee K, Zhang H, Qian DZ, Rey S, Liu JO, Semenza GL. Acriflavine inhibits HIF-1 dimerization, tumor growth, and vascularization. *Proc Natl Acad Sci U S A* 2009;**106**:17910–5
161. Watanabe K, Oguri H, Oikawa H. Diversification of echinomycin molecular structure by way of chemoenzymatic synthesis and heterologous expression of the engineered echinomycin biosynthetic pathway. *Curr Opin Chem Biol* 2009;**13**:189–96
162. Cook KM, Hilton ST, Mecinovic J, Motherwell WB, Figg WD, Schofield CJ. Epidithiodiketopiperazines block the interaction between hypoxia-inducible factor-1alpha (HIF-1alpha) and p300 by a zinc ejection mechanism. *J Biol Chem* 2009;**284**:26831–8
163. Ramanathan RK, Kirkpatrick DL, Belani CP, Friedland D, Green SB, Chow HH, Cordova CA, Stratton SP, Sharlow ER, Baker A, Dragovich T. A Phase I pharmacokinetic and pharmacodynamic study of PX-12, a novel inhibitor of thioredoxin-1, in patients with advanced solid tumors. *Clin Cancer Res* 2007;**13**:2109–14
164. Kaluz S, Kaluzova M, Stanbridge EJ. Proteasomal inhibition attenuates transcriptional activity of hypoxia-inducible factor 1 (HIF-1) via specific effect on the HIF-1alpha C-terminal activation domain. *Mol Cell Biol* 2006;**26**:5895–907
165. Gay F, Magarotto V, Crippa C, Pescosta N, Guglielmelli T, Cavallo F, Pezzatti S, Ferrari S, Liberati AM, Oliva S, Patriarca F, Offidani M, Omede P, Montefusco V, Petrucci MT, Giuliani N, Passera R, Pietrantuono G, Boccadoro M, Corradini P, Palumbo A. Bortezomib induction, reduced-intensity transplantation, and lenalidomide consolidation-maintenance for myeloma: updated results. *Blood* 2013;**122**:1376–83
166. Yeo EJ, Ryu JH, Cho YS, Chun YS, Huang LE, Kim MS, Park JW. Amphotericin B blunts erythropoietin response to hypoxia by reinforcing FIH-mediated repression of HIF-1. *Blood* 2006;**107**:916–23
167. Ciuleanu TE, Pavlovsky AV, Bodoky G, Garin AM, Langmuir VK, Kroll S, Tidmarsh GT. A randomised Phase III trial of glufosfamide compared with best supportive care in metastatic pancreatic adenocarcinoma previously treated with gemcitabine. *Eur J Cancer* 2009;**45**:1589–96
168. Wood TE, Dalili S, Simpson CD, Hurren R, Mao X, Saiz FS, Gronda M, Eberhard Y, Minden MD, Bilan PJ, Klip A, Batey RA, Schimmer AD. A novel inhibitor of glucose uptake sensitizes cells to FAS-induced cell death. *Mol Cancer Ther* 2008;**7**:3546–55
169. Sonveaux P, Vegran F, Schroeder T, Wergin MC, Verrax J, Rabbani ZN, De Saedeleer CJ, Kennedy KM, Diepart C, Jordan BF, Kelley MJ, Gallez B, Wahl ML, Feron O, Dewhirst MW. Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. *J Clin Invest* 2008;**118**:3930–42
170. Goel HL, Mercurio AM. VEGF targets the tumour cell. *Nat Rev Cancer* 2013;**13**:871–82
171. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004;**350**:2335–42
172. Kolch W, Pitt A. Functional proteomics to dissect tyrosine kinase signalling pathways in cancer. *Nat Rev Cancer* 2010;**10**:618–29
173. Jonker DJ, O'Callaghan CJ, Karapetis CS, Zalcberg JR, Tu D, Au HJ, Berry SR, Krahn M, Price T, Simes RJ, Tebbutt NC, van Hazel G,

Wierzbicki R, Langer C, Moore MJ. Cetuximab for the treatment of colorectal cancer. *N Engl J Med* 2007;**357**:2040–8

174. Flaherty KT, Infante JR, Daud A, Gonzalez R, Kefford RF, Sosman J, Hamid O, Schuchter L, Cebon J, Ibrahim N, Kudchadkar R, Burris HA, 3rd, Falchook G, Algazi A, Lewis K, Long GV, Puzanov I, Lebowitz P, Singh A, Little S, Sun P, Allred A, Ouellet D, Kim KB, Patel K, Weber J. Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. *N Engl J Med* 2012;**367**:1694–703
175. Arber N, Eagle CJ, Spicak J, Racz I, Dite P, Hajer J, Zavoral M, Lechuga MJ, Gerletti P, Tang J, Rosenstein RB, Macdonald K, Bhadra P, Fowler R, Wittes J, Zauber AG, Solomon SD, Levin B. Celecoxib for the prevention of colorectal adenomatous polyps. *N Engl J Med* 2006;**355**:885–95