Pediatrics & Therapeutics
ISSN: 2161-0665

Adamaki et al., Pediatr Therapeut 2012, S1
DOI: 10.4172/2161-0665.S1-002

Review Article Open Access

Cancer and the Cellular Response to Hypoxia

Maria Adamaki*, Anastasia Georgountzou and Maria Moschovi

First Department of Pediatrics Hematology-Oncology Unit, National University of Athens, Aghia Sofia Children's Hospital, Athens, Greece

Abstract

Hypoxia is defined as the reduction of oxygen levels below normal (normoxia), i.e. below 5% and may occur naturally in certain physiological processes such as normal embryo development, stem cell function and angiogenesis [1-6]. However, hypoxia also plays a major role in many human pathological conditions, including cancer, inflammation, vascular disease and chronic kidney disease [7-10]. When an organism or tissue is exposed to hypoxia, a series of events takes place within that organism or tissue so as to reinstate oxygen homeostasis. Even though the physiological responses to hypoxia are well-documented, the molecular changes taking place at the cellular level are still being investigated to this very day. Detection of hypoxia by the cell is achieved through oxygen sensor relays residing inside the cell, a class of deoxygenases called PHDs (prolylhydroxylases), which activate special transcription regulators that lead to changes in the gene expression profile of the cell [11]. The changes in gene expression are mainly commanded by a family of hypoxia-responsive transcription factors called HIFs (hypoxia-inducible factors) which, since their discovery in the early 1990s [12] have greatly facilitated molecular research in the field; research on HIFs has led to the discovery of other hypoxia-responsive transcription factors, as well as additional molecular processes that take place following hypoxia, which play a very distinctive role in the transcriptional outcome of the cell. Overall, hypoxia causes a cell cycle arrest at the G1 phase [13] and ultimately, a hypoxia-responsive mechanism for the remodelling of chromatin leads to the activation or repression of specific downstream target genes, to changes in the translational profile of the cell and even to epigenetic post-translational modifications in the cell [14-17].

Introduction

Hypoxia is defined as the reduction of oxygen levels below normal (normoxia), i.e. below 5% and may occur naturally in certain physiological processes such as normal embryo development, stem cell function and angiogenesis [1-6]. However, hypoxia also plays a major role in many human pathological conditions, including cancer, inflammation, vascular disease and chronic kidney disease [7-10]. When an organism or tissue is exposed to hypoxia, a series of events takes place within that organism or tissue so as to reinstate oxygen homeostasis. Even though the physiological responses to hypoxia are well-documented, the molecular changes taking place at the cellular level are still being investigated to this very day. Detection of hypoxia by the cell is achieved through oxygen sensor relays residing inside the cell, a class of deoxygenases called PHDs (prolylhydroxylases), which activate special transcription regulators that lead to changes in the gene expression profile of the cell [11]. The changes in gene expression are mainly commanded by a family of hypoxia-responsive transcription factors called HIFs (hypoxia-inducible factors) which, since their discovery in the early 1990s [12] have greatly facilitated molecular research in the field; research on HIFs has led to the discovery of other hypoxia-responsive transcription factors, as well as additional molecular processes that take place following hypoxia, which play a very distinctive role in the transcriptional outcome of the cell. Overall, hypoxia causes a cell cycle arrest at the G1 phase [13] and ultimately, a hypoxia-responsive mechanism for the remodelling of chromatin leads to the activation or repression of specific downstream target genes, to changes in the translational profile of the cell and even to epigenetic post-translational modifications in the cell [14-17]. Hypoxia has long been recognised for its multifaceted role in cancer development and is regarded one of the most important features of solid tumours [18]. The hypoxic tumour microenvironment diverts the glucose flux into the pentose phosphate pathway (ie. there is no oxidative respiration) and cancer cells gain a proliferative advantage due to the limitation of oxidative damage by ROS [19]; as a result they evade apoptosis and immune surveillance, obtain genomic instability and unlimited proliferation potential, express various growth factors to promote angiogenesis and ultimately invade and metastasize [10]. In addition, this microenvironment presents an obstacle to conventional anti-cancer therapies [20], thus increasing the likelihood for malignant progression and metastasis. Therefore, tumour hypoxia is seriously considered in the prognosis and treatment of cancer patients, whereas therapeutic strategies for targeting the hypoxic cancer microenvironment are well underway.

In this article we give an overview of the current knowledge on the cellular response to hypoxia, including a summary of the transcription factors regulating it and the molecular processes resulting from it. We will give particular emphasis on the role of hypoxia in cancer development and treatment, with respect to changes in the transcriptional and translational profile of the cancer cell. Finally, we will discuss the present therapeutic modalities in overcoming hypoxia-mediated drug resistance and the progress in the pharmacological design of hypoxia inhibitors as new cancer chemotherapeutics. Overall, since our field of expertise is pediatric oncology, we will at the same time attempt to present this information in relation to pediatric cancers.

The Cellular Response to Hypoxia

The HIF transcription factors

The HIF family of transcription factors are highly conserved heterodimeric proteins composed of α and β subunits. HIF- α consists of three isoforms: HIF- 1α, HIF-2α and HIF-3α, whereas HIF-β, also known as ARNT (aryl hydrocarbon receptor nuclear translocator), has

***Corresponding author:** Maria Adamaki, First Department of Pediatrics, University of Athens, Oncology Research Laboratory, "Aghia Sofia" Children's Hospital, Thivon & Levadias Street, 11527 Goudi, Athens, Greece, Tel: 0030-210-7452172; Fax: 0030-7795538, E-mail: madamaki@med.uoa.gr

Received December 29, 2011; **Accepted** February 04, 2012; **Published** February 05, 2012

Citation: Adamaki M, Georgountzou A, Moschovi M (2012) Cancer and the Cellular Response to Hypoxia. Pediatr Therapeut S1:002. doi:10.4172/2161-0665.S1-002

only one isoform, HIF-1 β [21]. The *α* subunits are similar in structure and contain basic helix-loop-helix (bHLH) and PAS (PRE-ARNT-SIM) domains, in addition to an ODD (oxygen-dependent degradation) domain that renders them labile in the presence of oxygen [11]. The HIF-3*α* isoform lacks the C-terminal transactivation domain that the other two possess, thus suggesting an inhibitory role on HIF-1 α and HIF-2 α [22]. Interestingly, the PAS domain, due to its primitive origin and involvement in circadian rhythms, is believed to suggest a link between the circadian light-dark cycle and oxygen availability [23,24]. Overall, ARNT is essentially a constitutively expressed subunit not regulated by oxygen levels, whereas HIF- *α* subunits are actively involved in conferring oxygen homeostasis [25]. Under normoxic conditions (≥ 5% O_2), the special sensors called PHDs catalyse the hydroxylation (hence prolyl hydroxylation) of the ODD domain in the HIF- *α* and act as a signal for HIF- *α* recognition by the VHL (von Hippel-Lindau) tumor suppressor protein [26]. The VHL, in turn, acts as an E3 ligase substrate recognition component and promotes ubiquitination of HIF-*α*, thus marking it for rapid degradation by the proteasome [27]. In addition, a second type of hydroxylation of HIF-*α* may take place, the so-called asparaginyl hydroxylation, catalysed by a class of deoxygenases called FIHs (factors inhibiting HIF). FIHs negatively regulate the transactivation domain of HIF- *α* by preventing it from binding to its co-activators p300 and CBP, thus repressing its transcriptional activity [28]. In other words, normoxia results in HIF- *α* being either transcriptionally repressed or degraded by hydroxylation. On the other hand, in an acutely hypoxic environment, where oxygen-dependent hydroxylation is inhibited, HIF-*α* translocates to the nucleus where it dimerizes with ARNT, thus avoiding degradation and increasing in stability [29]. Consequently, the HIF-*α*/ARNT complex recruits the necessary co-activators p300/CBP (and/or p160/SRC in the case of ARNT) in order to modify chromatin structure via histone acetylation and to be able to bind to target genes via its recognition sequence 5'-(A/G)CGTG-3', thus increasing transcription of the target sequences [30,31]. Figure 2 diagrammatically represents the regulation of gene expression by HIF-1. Several hundreds of genes have been recognised as direct targets of HIF binding and transactivation, the most important ones regulating erythropoiesis, angiogenesis, glycolysis, vascular development, mitochondrial function, metabolism, cellular proliferation, cell migration and cancer. Examples include erythropoietin (*Epo*), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), glucose transporter-1 (Glut-1), GAPDH, lactate dehydrogenase A (LDHA), p53 and MYC [24,32,33]. Recent evidence shows that HIF-1α preferentially binds to loci that have been transcriptionally active prior to the onset of hypoxia, further implying that the pre-existing differences in the basal gene expression of the cell may be responsible, at least in part, for the cell-type specificity in the response to hypoxia, as well as the promiscuity of certain genes to transactivation by the HIFs [34].

HIF-independent response to hypoxia

In addition to the HIFs, there are several other transcription factors and pathways that show altered activity as a result of hypoxia. These are outlined below.

Mammalian target of rapamycin (mTOR): Being a main regulator of cellular energy, mTOR's normal function is to phosphorylate the ribosomal protein S6 kinase (S6K) and the eIF4E-binding protein 1 (4E-BP1) and to promote the translation of mRNAs that are essential for cell growth and survival [35]. Under hypoxic conditions, however, mTOR phosphorylation of S6K and 4E-BP1 is markedly suppressed, thus inhibiting ribosomal biogenesis and cap-dependent protein translation, respectively, in order to save cellular energy (ATP consumption) in the oxygen-limiting environment [36]. More specifically, hypoxia inhibits mTOR activity via the hypoxia-inducible gene REDD1, through the TSC1/TSC2 tumour suppressor complex and the constitutive activation of S6K promotes cell death [37]. On the other hand, it has been suggested that in a hypoxic tumour microenvironment inhibition of the mTOR pathway might induce new energy conservation strategies in cancer cells and thus be critical for maintaining their malignant phenotype via growth retardation and accumulation in the G1 phase [36,38]. This could have major implications in the way we regard cancer therapy, as attempts towards a forced activation of mTOR signalling are already proving more effective than mTOR suppression in inhibiting cancer growth in studies with mice [39].

Endoplasmic reticulum (ER): Under conditions of hypoxic stress, in order to maintain protein quality or to induce cell death, the ER activates the unfolded protein response (UPR), a coordinated cell-survival program mediated by three resident regulator kinases: PERK, IRE1 and ATF6. More specifically, upon hypoxic exposure, the ER induces phosphorylation of the eukaryotic initiator factor 2 alpha (eIF2 α) on Ser51 via activation of PERK, resulting in the rapid down-regulation of protein synthesis [40]. Inactivation of PERK or inhibition of eIF2 α phosphorylation has a negative effect on cell survival and tumour cells possessing these properties show a higher apoptotic rate than tumours with a normal functioning UPR [41]. In addition, activation of the IRE1 kinase has been found to promote the splicing of the X-box binding protein (XBP1) pre-mRNA, resulting in hypoxic tolerance and tumour growth in vitro [42].

Nuclear factor-kappa B (NF-κB): This is essentially a family of seven transcription factors, encoded by the following five genes: RelA(p65), RelB, c-Rel, NF-*κ*B1(p50/p105) and NF-*κ*B2(p52/p100), all of which share an N-terminal DNA-binding and dimerisation domain, the Rel homology domain (RHD) [43]. Apart from being one of the most important regulators in the immune system and in inflammatory responses, the NF-*κ*B pathway is also known for its implication in cell cycle progression and cancer [44,45]. Under hypoxic conditions, down-regulation of PHD2 directs an increase in NF-*κ*B levels and up-regulates the expression of IL-8 and angiogenin genes, thus causing angiogenesis [46]. The exact mechanism for the hypoxia-mediated induction of NF-*κ*B has not been fully elucidated but a dual and opposing mode of action has been well established. For example, even though NF-*κ*B acts as a survival signal on most cell systems, it has a pro-death effect on neuronal cells [47-49], a property currently subject to intense scrutiny in the research field. Recent studies have shown NF-*κ*B to directly modulate HIF-1 α transcriptionally as a response to hypoxic stress [50,51]. Subsequent studies will reveal which subunits are involved in HIF activation and whether a reciprocal relationship exists.

Tumor suppressor p53: Encoded by the gene TP53, the p53 protein is so well-known for its tumour suppressor properties that it has been tagged "the guardian of the genome". The importance of p53 in preventing cancer is highlighted in the fact that the gene is mutated in over 50% of all the human cancers, whereas p53 null mice develop cancer very early in life [52,53]. Normally, p53 has a half-life of only a few minutes, as the Mdm2 ubiquitin ligase directly binds to it and mediates its proteolytic degradation; upon activation, however, p53 becomes subject to various post-translational modifications that disrupt the

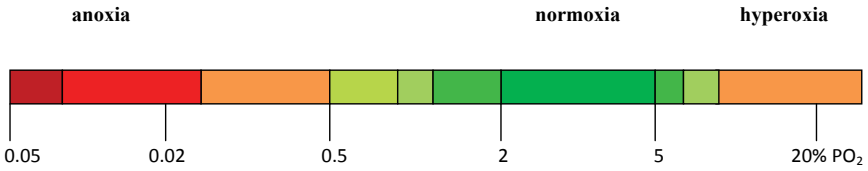

Figure 1: Diagrammatic representation of oxygen levels in adult brain cells. PO_2 (oxygen tension) varies with the cell type and the microenvironment of the respective organ. In the brain, physiological oxygen levels range between 2-5% whereas in the bone marrow physiological oxygen levels have been calculated to be highest (~5%) near the sinuses and lowest (1%) inside the cortical bone. Adapted from David M. Panchision (2009).

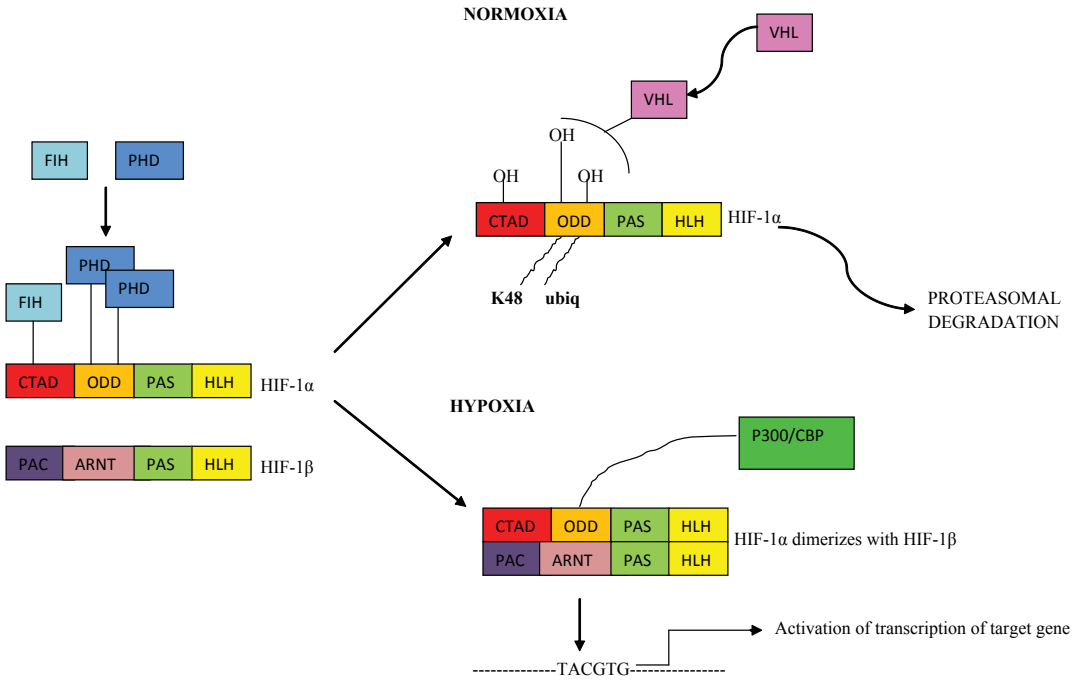

Figure 2: Regulation of gene expression by HIF-1α. In normoxic conditions, FIH and PHDs hydroxylate residues in the CTAD and ODD domains of HIF-1α, which in turn activates VHL binding and ubiquitination of HIF-1α, ultimately leading to its proteasomal degradation. Under hypoxic conditions, however, the lack of oxygen inhibits the action of FIH and PHDs, so HIF-1α is able to stabilise and dimerize with HIF-1β, ultimately activating target gene transcription through recruitment of co-activators. Adapted from Kenneth and Rocha (2008).

binding of Mdm2 and render p53 stable and transcriptionally active for a good few hours [54,55]. Activation is usually conferred as a response to DNA damage and results in either cell cycle arrest or apoptosis [53]; in particular, p53 interacts with the co-activator p300, as well as other transcription factors (eg. TFIID), so it is able to bind to damaged DNA in a sequence-dependent manner and to induce the transcription of downstream genes that are responsible for cell cycle inhibition or apoptosis [56,57]. Many studies have documented that p53 activation by hypoxia can be achieved by both HIF-1-dependent and HIF-1-independent mechanisms but it appears to be a very atypical response, in the sense that it does not induce the transactivation of the same set of genes as other stress signals, as for example in the case of Bnip3L [58-61]. Even though not all the p53 transcription targets have been defined so far, the role of p53 in hypoxia-induced apoptosis is profound. Under hypoxic conditions, p53 levels are believed to increase through an HIF-1 α-mediated decrease in Mdm2 [62] but others have speculated that p53 protein levels may also be influenced by the severity (i.e. 5.0-0.1% O_2) and duration of hypoxia (in hours versus days), as well as the cell type affected [63-65]. Like NF-κB, p53 also has a dual and opposing mode of activity in that it can serve either as a pro-survival signal or as a pro-death signal. Under moderate hypoxia (± 1.0% O_2), p53 levels may be reduced so as to protect the cells from apoptosis, whereas under severe hypoxic conditions (≤ 0.1% O_2, reaching anoxia) the situation may reverse, in that HIF-1 may induce p53 stabilisation and lead to apoptosis [63,66].

MYC: This refers to a family of four transcription factors containing a bHLH/LZ (bHLH/Leucine Zipper) domain: c-Myc, N-Myc, L-Myc and S-Myc [67]. The Myc protein can bind DNA via its bHLH domain and form heterodimers with its partner transcription factor, Max, through the LZ domain. The Myc-Max heterodimers are able to bind to specific DNA sequences, the Enhancer Box Sequences (E-boxes), in the promoters of their target genes, recruit histone acetyltransferases (HATs) and activate transcription [68,69]. On the other hand, Myc can also act as a transcriptional repressor through displacing the p300 co-activator and binding to the Miz-1 transcription factor instead, hence inhibiting the expression of Miz-1 genes [70]. Overall, Myc proteins have essential functions in many biological processes, such as cell growth, proliferation, angiogenesis and genomic instability

[67,71]. The gene is frequently mutated in human cancers, resulting in its constitutive expression and leading to the unregulated expression of many other genes; a common example is the t(8;14) translocation, characteristic to the pathogenesis of Burkitt's Lymphoma [72]. Under normoxic conditions, Myc promotes cell growth and proliferation via the repression of cyclin-dependent kinase inhibitors; under hypoxic conditions however, Myc activity is compromised by the antagonistic relationship between Myc and HIF for the binding sites of target genes [13,73,74]. More specifically, in low oxygen levels, c-Myc is replaced by HIF-1 and so induces expression of the cyclin-dependent kinase inhibitor p21 and causes cell-cycle arrest [75]. Many reports have also shown that in hypoxic cells HIF-1 α can reduce the expression of Myc target genes and inhibit transformation via direct binding to Myc or to its partners [74-76]. Interestingly, HIF-2 α has the opposite effect and promotes hypoxic cell proliferation by inducing c-Myc activity; it does so via stabilization of the Myc-Max heterodimer, which allows it to activate the expression of its target genes [77]. Overall, both Myc and HIF are directly involved in angiogenesis and cancer development, while recent studies have also shown that HIF-1 α and c-Myc cooperation is essential in c-Myc-induced tumorigenesis [78,79]. Finally, HIF and c-Myc have been found to synergise for the induction of shared target genes VEGF, HK2 (hexokinase) and PDK1 (pyruvate dehydrogenase kinase 1), in a cell-type-specific manner [80], so speculations are also being made on additional functional relationships between other important transcription factors, such as p53 and NF-κB.

Activator protein 1 (AP-1): This refers to any heterodimeric protein combination resulting from dimers between the Jun, Fos and ATF (activating transcription factor) families, so, depending on the cell type and microenvironment, the role of AP-1 is multifaceted and highly complex. Overall, AP-1 is able to respond to a variety of stimuli, including cytokines, growth factors, stress signals, infections and has been associated with many important biological processes, such as embryonic development, differentiation, proliferation, apoptosis and even tumorigenesis [81,82]. Activation of certain AP-1 dimer combinations (such as the c-Jun homodimer or the c-Jun/c-Fos heterodimer) induces the transcription of genes containing the TPA DNA recognition element (TRE; 5'-TGAG/CTCA-3') via site-specific binding [83]. It has been well documented that hypoxia induces AP-1 activity and mediates alterations in the gene expression of tyrosine hydroxylase, VEGF and endothelial nitric oxide synthase (eNOS) [84-86]. In addition, a functional cooperation seems to exist with other transcription factors in order to increase gene transcription; interestingly, AP-1 and HIF-1 cooperate to induce VEGF transcription, thus promoting vasculogenesis and angiogenesis [85,87,88]. AP-1 has also been shown to be strongly activated by hypoxia in a series of different tumour types, such as colon cancer, glioblastoma and malignant melanoma [89]. Last but not least, a synergistic relationship has also been reported between AP-1 and NF-κB in the activation of common target genes, such as IL-8, contributing to the malignant progression of pancreatic cancer [90].

Other transcription factors: ATF-4 (activating transcription factor 4) is activated and stabilised independently of HIF by anoxia rather than hypoxia [91]; Egr-1 (early growth response 1), a transcription factor known for modulating the expression of genes involved in synaptic plasticity, cell growth and cell survival, is also up-regulated by hypoxia independently of HIF and is actively involved in the pathogenesis of pulmonary thrombosis and vascular remodelling [92-94]; Ets-1 is induced by hypoxia in an HIF-regulated manner and plays a very important role in angiogenesis and cancer invasion [95]. Other transcription factors responding to hypoxia via transcriptome regulation include RTEF-1 (related transcriptional enhancer factor-1), GATA-2, the STAT family, Mash-2 (mammalian achaete-scute homologous protein-2) and GADD153 (growth arrest and DNA damage-153) [94].

Chromatin modifications in hypoxia

The cellular response to hypoxia recruits special transcription factors to the promoters or enhancers of their target genes, where they bind to specific DNA sequences and ultimately alter the gene expression profile of the cell. Considering that DNA is packaged into dense structures of chromatin called nucleosomes, accessibility of the transcription factors to their target areas may not always be an easy task. Adjustment of the chromatin structure to accommodate for the accessibility of transcriptional activators or repressors seems to be an essential mechanism for the appropriate gene expression. Chromatin is distributed into euchromatic and heterochromatic regions corresponding to transcriptionally active or repressed regions, respectively, depending on how tightly the structure is packed. Heterochromatin is the higher-order packed chromatin, generally inaccessible to transcription factors. The nucleosome is the basic repeat unit of chromatin and consists of an octamer that has two molecules of each of the four histones H2A, H2B, H3, H4 and 146bp of DNA wrapped twice around it; the globular domains of the histones are enclosed in the nucleosome, whereas their N-terminal tails protrude from it and facilitate histone modifications [96]. Linker histones (such as H1 and H5) are used to connect the nucleosomes together and to be able to fold the DNA into more compact structures, such as the heterochromatin regions [97]. Overall, chromatin structure can be altered so as to allow for alterations in the regulation of transcription mainly via three basic mechanisms:

Post-translational modifications of the histones via their flexible tails: These refer to the covalent modifications of histones, such as phosphorylation, methylation, acetylation, ubiquitination, SUMOylation and poly(ADP-ribosyl)ation, that either alter the charge and structure of chromatin, or provide accessible DNA binging sites that are recognised by specific structural domains (eg. bromo- and chloro-domains) [98].

ATP-dependent nucleosome remodelling: This is essentially chromatin remodelling at the nucleosome level, via the utilisation of ATP as a source of energy by complexes such as the SWI/SNF, the ISWI and the MI-2/CHD [99]. These remodelling complexes create a shift of the DNA segments in the histone-DNA interactions and facilitate the disruption of the nucleosome structure [100].

Histone replacement modifications: This refers to the incorporation into nucleosomes of chromosome variants (mainly H2A and H3 variants) that are assembled and synthesized independently of DNA replication and which have profound epigenetic consequences in the transcriptional profile of the cell [101].

Under hypoxic conditions, the histone acetyltransferase (HAT) complex p300/CBP interacts with HIF and the acetylate histones in target genes, via inhibition of FIH-1, leading to an increase in localized histone acetylation and transcriptional activation of the target genes [17,30]. Other observations, however, have shown that the HIF-p300/CBP interaction is responsible for the altered expression of only 30-50% of the target genes, further demonstrating that not all of the hypoxia-responsive genes are transactivated by histone acetylation [102]. Histone deacetylases (HDACs) on the other hand, whilst

generally known for facilitating repression of transcription, in hypoxia can cooperate with HIF and regulate transcription either positively or negatively [14]. Accordingly, histone deacetylase inhibitors, which usually promote transcriptional activation, in the hypoxic environment turn into transcriptional repressors of HIF targets and promote HIF-regulated angiogenesis [103-105]. Changes in histone methylation are not an exception to the chromatin modification repertoire in the hypoxic environment. In a recent study it was observed that following exposure to hypoxia, the responsive promoters of the VEGF and Egr-1 genes displayed an increase of histone H3K4 trimethylation (usually associated with transcriptional activation) and a decrease in histone H3K27 trimethylation (generally associated with transcriptional repression), demonstrating a bivalent chromatin behaviour under hypoxic influence [15]. This may further suggest important epigenetic changes caused by modifications in the neighbouring *cis*- and *trans*-acting elements of the histones or even by the recruitment of different co-activators and co-repressors that are yet to be determined. In the context of ATP-dependent chromatin remodelling, the SWI/SNF complex also seems to contribute to the activation of HIF target genes in the hypoxic response. More specifically, the catalytic subunits of SWI/SNF enhance HIF-mediated activation of two highly homologous ATPases, an erythropoietin (Epo)-driven promoter and a synthetic 6XHRE-driven reporter; with the recruitment of HIF-1, these ATPases form two distinct remodelling complexes and target the promoters of two different genes, Epo and VEGF [106,107]. Even though both enzymes are recruited, along with HIF-1, to the promoters of these genes in a hypoxia-responsive manner and they induce transcriptional activation of Epo, they do not seem to be essential for the transactivation of VEGF [107]. This further demonstrates that chromatin remodelling due to the hypoxic response may be sufficient for the transcriptional activation of one gene but not for the successful transactivation of another. Further analysis of the cellular pathways involved in chromatin remodelling due to hypoxia will help to define the mechanisms that recruit specific transcription factors and their co-factors to specific hypoxia-responsive target genes.

The Role of Hypoxia in Cancer Development

Cancer development is a multistep process that requires the acquisition of a certain number of genetic or epigenetic mutations, resulting from genetic instability in the dividing cell. This instability is usually caused by defects in the mechanisms that control the cell cycle and normal cell differentiation and usually include: cell cycle arrest, resistance to DNA repair and to growth inhibition, evasion of immune surveillance and apoptosis, unlimited replication potential, angiogenesis, invasion and metastasis [108]. In addition, the carcinogenic microenvironment employs unique strategies so as to be able to overcome the suppressive effects of the normal surroundings and to facilitate disease progression, whilst also becoming resistant to conventional cancer therapies [109]. Hypoxia plays a very important role in both triggering the malignant transformation process and promoting adaptive cell responses within the tumor microenvironment. Most solid tumors contain regions with extremely low oxygen concentrations, a necessary prerequisite for cancer progression. Hypoxia in the tumor microenvironment usually occurs as a result of rapid cell proliferation, which distances cells from blood vessels, often occurring at a distance of 100-200μm from them. The newly formed vessels are usually aberrant and cannot meet the high nutritional demands of the proliferating cancer cells, or may become compressed or obstructed by tumor growth [110]. This forces the tumor cells to develop adoptive responses that will allow them to survive and proliferate under hypoxic conditions. A central player in these adoptive responses is the HIF-pathway.

The HIF-pathway in cancer development

As mentioned in the previous section of this review, HIF-1 mediates the cellular adaptive response to hypoxia by increasing dramatically in transcriptional activity and inducing the transactivation of at least 100 hypoxia-responsive target genes. Many studies have demonstrated that most of the genetic alterations in tumor cells are synergistically interconnected with HIF-1 transcriptional activity, further highlighting the critical role of HIF-1 in cancer development. Indeed, HIF-1 is prevalent in many types of solid tumours and high expression usually correlates with poor clinical outcomes [111,112]. HIF-1α expression is usually an aggressive marker for prostate, oropharyngeal, oesophageal, head and neck, lung, ovarian and breast cancer, whereas HIF-2α is more frequently up-regulated in hepatic cancer, gliomas and neuroblastomas [113-116]. Hypoxia may additionally induce the expression of various growth factors that synergise with HIF-1 and promote cellular proliferation. Examples include EGF (epidermal growth factor), insulin, IGF-1 (insulin-like growth factor-1), IGF-2 and PDGF (platelet-derived growth factor) [117]. At the same time, in order to promote cancer cell proliferation and survival, certain growth-inhibitory events may also be mediated by the hypoxia-responsive genes; for example, mutations in the PTEN gene, a marked tumor suppressor, have been shown to promote tumor growth in glioblastoma cell lines in an HIF-1-coordinated manner [118]. On the other hand, certain animal model studies have shown that inhibition of HIF-1 decreases tumor growth, thus further supporting HIF-1-mediated cancer progression [119,120], while others have linked HIF-1 expression to higher apoptotic rates and increased patient mortality [121]. The latter, as mentioned earlier, is attributed to mechanisms such as the functional cooperation between HIF-1 and p53, which causes the activation of pro-apoptotic genes such as Bnip3L and hence promote hypoxia-induced apoptosis [60,122]. Overall, the finding that genetic and epigenetic alterations leading to oncogene activation and loss of tumor suppressor genes are correlated with increased HIF-1 activity, suggests that HIF over-expression represents a final common pathway in tumor pathogenesis, even if HIF activation is caused by conditions mimicking the effect of hypoxia [123]. To summarise, many studies have demonstrated the role of HIF-regulated gene expression in cancer development, including proliferation (MYC), angiogenesis (VEGF, PDGF), apoptosis (BNIP3), metabolism (PDK1, LDHA), DNA damage response (GADD45A), microRNAs (MIR210), extracellular matrix remodelling (LOX, MMP1), cell migration and invasion (CXCR4, SDF1) [124-127].

Hypoxia on cancer stem cells

Hypoxia seems to play an important role in maintaining the tumor stem cell (TSC) niche in the development of invasive cancer phenotypes, as shown from studies with cell cultures derived from pediatric patients. More specifically, it has been demonstrated through studies on pediatric neuroblastoma and rhabdomyosarcoma cell lines that the tumor stem cells, similar to normal stem cells, may share the unique property of migrating to the area of hypoxia and necrosis, where their highly tumorigenic fraction may be maintained and expanded [128]. In other studies with cancer stem cells, it was observed that hypoxia promotes the self-renewal capability of both the stem and the non-stem cell population; interestingly, the stem-like phenotype is induced more profoundly in the non-stem cell population and is accompanied by the upregulation of important stem cell factors, such as Oct4, c-Myc

and Nanog. This effect of hypoxia on cancer stem cells seems to be primarily mediated by HIF-2α, since its loss seems to cause a decrease in the stem cell proliferation capacity and self-renewal [113,129]. All of these findings suggest that, in a restricted oxygen environment, the TSC fraction is enhanced via the acquisition of the stem cell state but at the same time it is critically dependent on the HIFs for survival, self-renewal and proliferation.

Metabolism in hypoxic cells

Under physiological normoxic conditions (~ 5% O_2), cells convert glucose into energy (in the form of ATP) via the consecutive processes of glycolysis and oxidative respiration. The glycolytic enzyme pyruvate kinase (PK) catalyses the final step of glycolysis, i.e. the production of pyruvate from phosphoenol pyruvate (PKP), which is then shuffled from the cytoplasm into the mitochondria for oxidative respiration to take place (Figure 3). In highly proliferating cells, such as cancer cells and in anaerobic conditions, pyruvate is converted to lactate and is actively excreted from the cells [130]. As a matter of fact, many cancer cells seem to prefer the much less efficient glucose fermentation and lactate production, instead of oxidative respiration and pyruvate production, as a means of meeting their energy demands, even in the presence of oxygen, a condition described as the Warburg effect [131,132]. Therefore, in the absence of oxygen, additional and highly inter-connected to the HIF-1 pathway in mediating tumorigenesis is the altered intrinsic glucose metabolism of the cell, i.e. the adaptive shift from oxidative to glycolytic metabolism [133,134]. In this procedure, carbonic anydrases and in particular CA9, seem to relieve hypoxic tumor cells from intracellular acidosis that has been caused by the increased glycolysis and lactate production, hence contributing to their survival [135]. In particular, HIF-1 seems to mediate this metabolic switch via inhibition of pyruvate dehydrogenase, which in turn down-regulates cell-cycle activity and mitochondrial oxygen consumption [136,137]. More recently, evidence has come forward demonstrating that inhibition of PK, apart from down-regulating pyruvate production, also mediates redox balance in cells by activating the pentose phosphate pathway (Figure 3); this activation consequently

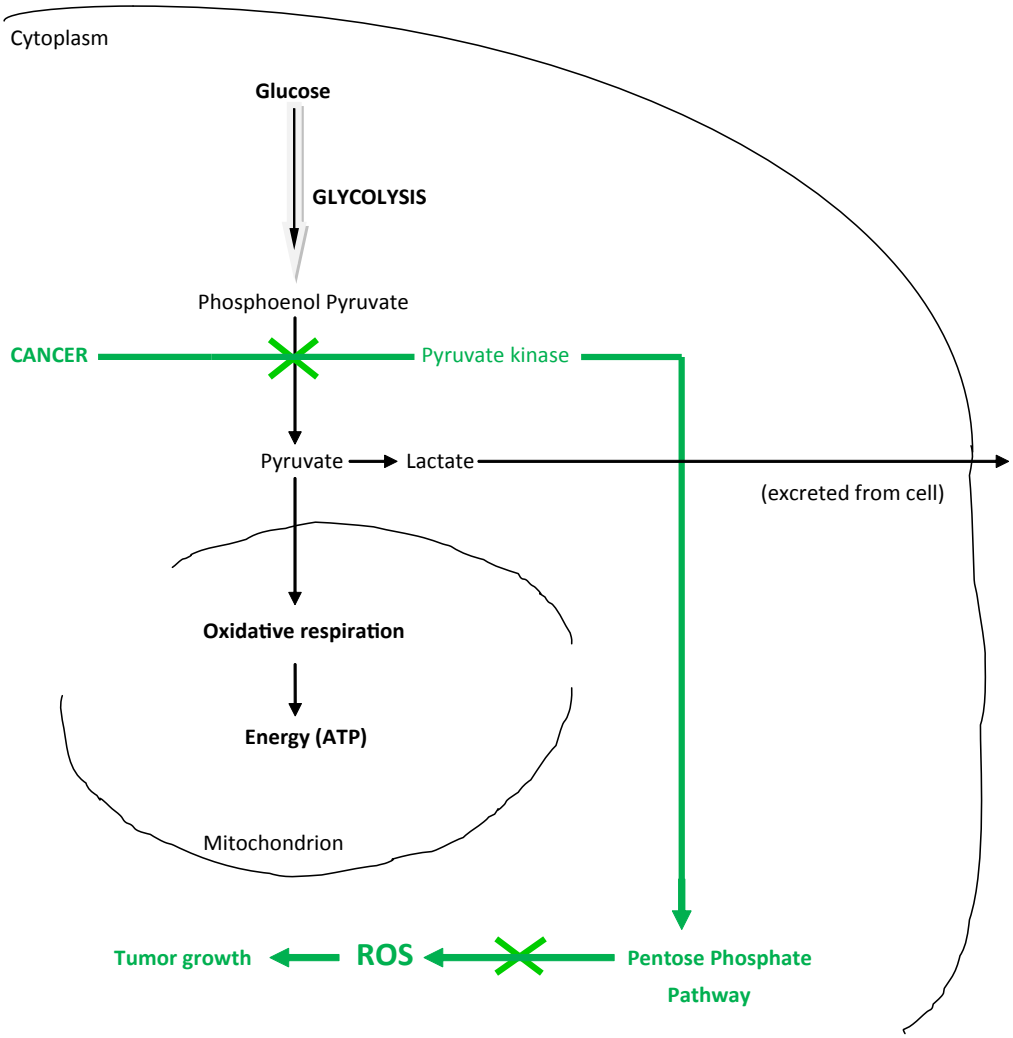

Figure 3: The cellular metabolic response to hypoxia. Under normal oxygen levels, glucose is converted into pyruvate through the process of glycolysis and then enters the mitochondrion and generates energy (in the form of ATP) through the process of oxidative respiration. Under hypoxic conditions, and in highly proliferating cells, however, pyruvate is converted into lactate which is actively secreted from the cell. In cancer, the reduced activity of the catalytic enzyme pyruvate kinase induces the pentose phosphate pathway which in turn limits ROS accumulation, diminishes oxidative damage and so promotes tumour growth. Adapted by Grüning and Ralser (2011).

limits the accumulation of mitochondrial reactive oxygen species (ROS) and oxidative stress, thus saving cancer cells from death due to oxidative damage and facilitating tumor growth [138]. In addition, it was also recently revealed that in human lung cancer cells, the activation of PK splice variant M2 (or PKM2) is inhibited via the oxidation of PKM2 residue Cys^{358} by acute increases in the production of intracellular ROS and diverts the glucose flux into the pentose phosphate pathway [19]. As a consequence, sufficient reducing potential is generated for the detoxification of ROS. However, when endogenous PKM2 was replaced by a PKM2 oxidation-resistant mutant that had Cys^{358} replaced by Ser^{358}, the cells exhibited increased sensitivity to oxidative stress and impaired tumor formation in a xenograft model, further highlighting the therapeutic potential of this metabolic reconfiguration [19]. More importantly, this latest finding adds new understanding to the Warburg effect (i.e. that cancer cells prefer the inefficient glycolysis even in the presence of oxygen), as it appears that the maintenance of the redox balance can be more limiting to tumor growth than insufficient energy levels. Interestingly, another recent report has demonstrated that PKM2 acts as a co-activator of HIF-1, interacting directly with the HIF-1α subunit and greatly enhancing its transcriptional activity [139]. In particular, it was shown that the PHD3-mediated phosphorylation of PKM2 promotes the transactivation of HIF-1 target genes by enhancing HIF-1α DNA binding and p300 recruitment to hypoxia-responsive elements. Even more specifically, it was shown that the PKM2-HIF-1α interaction is mediated by exon 10 of PKM2 (a region not present in the PKM1 variant), which contains the specific sequence motif responsible for the hydroxylation of HIF-1α by PHD2 and another two hydroxylated proline residues that appear mutated when the PKM2-HIF-1α interaction is lost [139,140]. In addition, a link was established between PHD3, PKM2 and HIF-1-mediated glycolysis from the observation that depletion of either PHD3 or PKM2 downregulates the transcription of HIF-1 metabolic target genes and reverses the Warburg effect [139,141]. Therefore, PKM2 expression in tumors appears to participate in a positive feedback loop that promotes alteration of gene expression via HIF-1 transactivation and reprograms glucose metabolism in cancer cells. This metabolic response to hypoxia is also accompanied by an increased expression of the genes coding for glycolytic enzymes and glucose transporters, which permits tumor cells to maintain a sufficient level of ATP energy for survival and proliferation [142,143]. Furthermore, HIF-1α induces over-expression of many glycolytic protein isoforms, such as glucose transporters GLUT-1 and GLUT-2 which, under hypoxic conditions, suppress apoptosis via inhibition of the stress-activated protein kinase pathway and promote cell migration [144,145]. In particular, it has been reported that, via the induction of GLUT-1, hypoxia protects rhabdomyosarcoma and Ewing sarcoma cells from apoptosis due to glucose deprivation in an HIF-1a-dependent manner [146]. Glycolysis may also be increased via repression of c-Myc, as shown by studies with VHL-deficient renal cell carcinoma [147], with the functional collaborations of HIF-1α with both c-Myc and mTOR having well-established roles in cancer [78,148]. In relation to pediatric cancer, experimental data from Wilms' tumors have shown over-expression of CA9 and HIF-1α, with concomitant high expression of VEGF and GLUT-1, further highlighting the importance of the functional relationship between the four hypoxia markers in cancer development [149].

Evasion of immune surveillance

Tumor cells commonly escape elimination by innate and adaptive immune responses using strategies such as the active suppression of effector immune cells. Under hypoxic conditions, through the activation of HIF-1 and HIF-2, tumor cells produce chemoattractants and soluble factors (eg. CSFI, VEGF and TGF-β) that stimulate and recruit monocytes and macrophages to tumor sites [150]. Following recruitment, macrophages mature into tumor-associated macrophages (TAMs) and hypoxia induces the secretion of potent immunosuppressive factors, such as prostaglandin E_2 and IL-10; these inhibit the TAMs immunosuppressive effect by repressing their ability to present antigens to T-cells and to phagocytose dead cells [114,151,152]. HIF-1α in particular, inhibits T-cells from undergoing activation-induced cell death and thus protects tumor cells from immune attack in the hypoxic environment [153,154]. A recent study has linked the hypoxia-induced transactivation of HIF-1α with an increase in the expression of metalloproteinase ADAM10 and a decrease in the surface MHC class I chain-related (MIC) levels, further highlighting the resistance of tumor cells to innate immune-mediated lysis [155]. Expression of HIF-2α, on the other hand, is associated with an unfavourable prognosis when found in the TAMs of breast and cervical cancers, whereas HIF-2α deletion from the myeloid cells in animal models of hepatocellular carcinoma and colitis-associated colon carcinoma correlates with a decreased recruitment of TAMs to tumor sites and a reduced tumor grade [156-158].

Evasion of apoptosis

To date, the exact mechanisms of apoptosis regulation under hypoxic conditions are not fully elucidated. Hypoxia is however known to induce apoptosis in both normal and cancer cells, with the latter developing mechanisms that allow them to increase their resistance and escape HIF-1-mediated apoptosis. More specifically, hypoxia has been found to increase the transcriptional activity of anti-apoptotic genes IAP-2, Bcl-2 and Bcl-XL, to activate the PI-3k/Akt survival pathway, a major regulator of cell survival and proliferation and to increase cell resistance to apoptosis via over-expression of the p53 negative regulator MDM2 [10,18,159]. Experimental data have also shown that hypoxia-induced apoptosis can be mediated by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a potent apoptosis inducer that specifically limits tumor growth without damaging normal cells and tissues *in vivo* [159]. In particular, hypoxia dramatically inhibits TRAIL-induced apoptosis by blocking Bax translocation from the cytosol to the mitochondria, hence blocking a pivotal signaling molecule for the effective induction of apoptosis [159]. Last but not least, human cancer cells may acquire the property of immortalization through the maintenance of telomere lengths, which is dependent on expression of the hTERT and hTR telomerase genes [160]. Indeed, several studies have demonstrated that cells with increased telomerase activity can divide beyond the Hayflick limit (the number of times a normal cell population can divide before it stops) without entering senescence or apoptosis and this leads to unlimited proliferative capacity, i.e. cellular immortalization [161-163]. It has also been shown that telomerase can synergize with certain oncogenes and convert normal human epithelial cells and fibroblasts into cancer cells [164] and hypoxia may contribute to the immortality of cancer cells by increasing telomerase activity via transcription in the promoters of both gene variants, with active involvement of HIF-1α [165,166].

Genomic instability and hypoxia

Several reports have linked hypoxia to increased genomic instability, which may also contribute into cancer formation. More specifically, it has been reported that hypoxia is responsible for increasing mutagenesis via down-regulation of the DNA mismatch

repair (MMR) system, which normally maintains genomic integrity by correcting replication errors [167,168]. In other words, the genomic destabilization seen in tumor cells is responsible for the cellular changes that confer progressive transformation on cancer cells and is further promoted by the hypoxic stress in the tumor microenvironment [169]. According to recent experimental evidence, hypoxia down regulates the expression of key genes within the MMR and homologous recombination (HR) pathways, such as MLH1 and MSH2, leading to increased mutagenesis, while suppressing the transcription of many critical HR-mediators, such as BRCA1, BRCA2 and RAD1, leading to down-regulation of recombinatorial repair and hence genomic instability [170,171]. In addition to this, a link has been made between genetic instability and HIF-1α, with HIF-1α inhibiting the expression of genes responsible for recognizing and repairing DNA base mismatches (such as MSH2, MSH6 and NBS1), despite notions from previous reports that repression of MMR and HR is HIF-independent [172,173]. On the other hand, several reports support the notion that "physiological" normoxia (O_2 levels about 5%, similar to natural niches), is protecting cells from falling into "genomic instability", as compared to the atmospheric oxygen level (about 20%), also enhancing stem cell clonal recovery and reducing chromosomal abnormalities [174,175]. Others have also shown that neural stem cells (NSCs) exist within a "physiological" hypoxia (1-5% O_2) in both embryonic and adult brains and that hypoxia can promote the growth and survival of NSCs *in vitro* (Figure 1) [176]. In addition, *in vivo* studies have shown that hypoxia can positively influence the production and differentiation of NSCs, as well as that of other types of stem cell [176-179]. Furthermore, there is enough evidence to suggest that hypoxia can initiate and promote the process of malignant transformation when a low percentage of cells overcome and escape cellular senescence [180]. As hypoxia causes the progressive elevation in mitochondrial ROS production (chronic ROS), this leads to oxidative DNA damage due to the continuously accumulating ROS; HIF-2α expression represses the DNA repair mechanisms in the hypoxic cells, enabling them to survive with sustained levels of elevated ROS along with the mutations that drive the malignant transformation [181]. In addition, it has recently been speculated that optimal "physiological" ROS levels confer minimal DNA damage due to adequate DNA repair, whereas both reduced and excessive ROS levels lead to genomic instability due to deficient DNA repair and oxidative DNA damage, respectively [182].

Angiogenesis

Hypoxia can promote angiogenesis via the activation of a number of angiogenic factors, such as VEGF, VEGF receptor-1, IL-8, platelet-derived growth factor (PDGF), adrenomedullin, angiopoietin-2, cyclooxygenase-2, endothelin-1 and -2, fibroblast growth factor-3, hepatocyte growth factor, histone deacetylase, monocyte chemotactic protein-1, nitric oxide synthase, osteopontin, placental growth factor, Tie-2 (an angiopoietin receptor) and transforming growth factors [110]. Since VEGF is a major component of the blood vessel formation procedure in hypoxic tissues, it is only logical to accept that it also plays a major role in the pathological angiogenesis of tumor development. It has long been reported that the hypoxia-mediated HIF-1α activation leads to VEGF up-regulation, which in turn triggers angiogenesis while at the same time suppressing angiogenic inhibitors, such as thrombospondin 1 [183,184]. Others support the notion that hypoxia is not responsible for the initiation of angiogenesis, that the initiation takes place via non-hypoxia-mediated mechanisms such as the activation of certain oncogenes and that hypoxia only contributes in accelerating the process [10]. Experimental data from glioblastoma mouse models show that HIF-1*α*, the direct effector of hypoxia, promotes neovascularization in glioblastomas via activation of VEGF; notably, when VEGF activity is impaired by ablation of either HIF-1α or matrix metalloproteinase-9 (MMP-9) and angiogenesis is disabled, tumor cells invade deeper into the brain in the perivascular compartment, thus being characterized by a more invasive phenotype [185]. Up to date, no studies have been conducted exclusively on pediatric cancers so only a few referrals exist on the role of hypoxia-promoted angiogenesis in childhood tumors or tumors found in both adults and children. Nonetheless, significantly elevated levels of VEGF secretion have been found in hypoxic tumor stem cells from malignant gliomas, including a pediatric glioblastoma xenograft [186]. Recently it was reported that in the highly vascularized human rhabdomyosarcoma tumors, in addition to VEGF, hypoxia induces the up-regulation of IL-8 both at the mRNA and protein level, thus highlighting its implication in promoting angiogenesis [187]. Last but not least, another group demonstrated that HIF-1α activity is a necessary prerequisite for hypoxia microRNA-16 (mir-16) down-regulation, which in turn induces VEGF expression in anaplastic lymphoma kinase (ALK)-positive anaplastic large-cell lymphomas, thus strongly suggesting the importance of mir-16 in regulating VEGF expression and angiogenesis [188].

Invasion and metastasis

As already mentioned in previous sections of this review, hypoxia induces the activation of a number of genes responsible for increased aggressiveness, invasion and metastasis of tumors, which subsequently leads to poor patient prognosis. Semenza [123,189] has already summarized the HIF-1 target genes, whose products actively contribute to cancer invasion and metastasis and regrouped the immunohistochemical studies in which increased levels of HIF-1α (or HIF-2α) protein in diagnostic tumor biopsies were associated with a decrease in patient survival. In search of data linking HIF expression to childhood cancers, we have found that HIF-1α protein accumulation has been associated with poor patient survival in oligodendroglioma, whereas HIF-2α over-expression is linked to increased patient mortality in childhood neuroblastoma and astrocytoma [124,190]. In a study conducted on both children and adult patients with osteosarcoma, HIF-1α expression significantly correlated with surgical stage, percentage of dead cells and microvessel density (MVD), as well as with shorter overall survival (OS) and disease-free survival (DFS) [191]. More recently, still in the context of pediatric tumors, the effects of hypoxia on primary Ewing's sarcoma family tumor (ESFT) cells were studied *in vitro* and were found to enhance the cells' malignant properties by stimulating the invasiveness and soft-agar colony formation; as expected, the Ewing's sarcoma oncoprotein EWS-FLI1 was up-regulated by hypoxia in a HIF-1α dependent manner [192]. Generally speaking, the recent literature supports the notion that the hypoxia-induced tumor aggressiveness is associated with the expansion of the cancer stem cell marker CD133+ in pancreatic cancer cells in a predominantly HIF-1α-dependent manner and that this might also play a key role during the transition from *in situ* to invasive breast cancer [193,194]. In explaining how hypoxia favors metastasis, it has been described that, through HIF activation, hypoxia facilitates the disruption of tissue integrity through the repression of the transmembrane molecule E-cadherin, therefore promoting tumor invasion and metastasis [195]. In the same study, it was also concluded that hypoxia enhances proteolytic activity at the invasive front, through upregulation of urokinase-type plasminogen activator receptor (uPAR) and alters the interactions between integrins and components of the extracellular matrix, thereby enabling cellular invasion through the basement membrane and the underlying stroma

[195]. It is believed that epithelial-mesenchymal transition (EMT), referring to the conversion of well-polarized, adhesive epithelial cells to non polarized mesenchymal cells, may be one of the initial steps involved in metastasis; both hypoxia and HIF-1α overexpression have been shown to promote EMT [10]. In addition, it has been observed that neuroblastoma cells can adjust to a hypoxic environment by losing their differential gene expression patterns and by developing stem cell-like phenotypes [196]. Seeing that there is a correlation in neuroblastoma between low stage of differentiation and high (aggressive) clinical stage, it can easily be assumed that hypoxia-induced dedifferentiation of neuroblastoma cells in hypoxic tumor regions contribute to the tumor heterogenicity and increased malignancy [196]. Finally, hypoxia-induced hepatocyte growth factor (HGF)-MET is known to increase cell motility, promoting cell migration towards the blood or lymphatic microcirculation, while hypoxia-induced VEGF promotes angiogenesis and lymphangiogenesis in the primary tumor and induces changes in vascular integrity and permeability, providing the necessary routes for dissemination [195,197].

Drug resistance

As mentioned at the beginning of this section, tumor hypoxia, acting through direct and indirect mechanisms, has long been recognized as a major factor involved in the resistance to radiotherapy and many chemotherapeutic agents and is thus linked to a poorer clinical outcome [198]. Radiosensitivity rapidly declines when tumor pO_2 is <25–30 mmHg and it has been found that radiation therapy is about two to three times less effective in destroying hypoxic cells than normoxic cells [199,200]. Oxygen increases DNA damage either through the formation of oxygen-derived free hydroxyl radicals, after the interaction of radiation with intracellular water, or by enhancing the stabilization ("fixation") of the highly reactive hydroxyl radicals that cause DNA damage. Since oxygen contributes in reducing the ability of the tumor cells to repair their damaged DNA after radiation therapy, it is believed that hypoxia can protect some malignant cells from radiation damage, subsequently causing local disease recurrence. Interestingly, this may occur after even a single fraction of radiation treatment but it may also explain to a certain extent the radioresistance following fractionated therapy. Taking into consideration that between the radiotherapy sessions the patterns of re-oxygenation of tumor cells are variable, it is possible that some cells remain hypoxic and are thus still protected. In addition, hypoxia may promote radioresistance indirectly, by inducing proteomic and genomic changes. Hypoxic stress can lead to the selection of a number of tumor cells with diminished apoptotic potential and influence the cell cycle, slowing proliferation and increasing the number of cells in the Go phase, thus reducing tumor radiosensitivity. It also leads to the increased transcriptional production of repair enzymes or resistance-related proteins, such as heat shock proteins, allowing cells to survive otherwise lethal conditions [200]. Radiotherapy is an important component of the treatment of many pediatric tumors, but very few studies refer to the role of hypoxia in radioresistance in children. Experimental data, however, have shown that both non-interrupted and cycling hypoxia pre-treatment significantly increases cell resistance to ionizing radiation compared with normoxic controls in U87 glioma xenografts and that cycling hypoxia treatment, through increased HIF-1 synthesis and stabilization, has a greater effect in increasing radiation resistance compared with non-interrupted hypoxia treatment [201]. Other groups have documented that the antitumor activity of ionizing radiation in U87 glioma xenografts is enhanced by improving intra-tumoral oxygenation [202]. On the other hand, acute hypoxia, resulting from poor and fluctuating blood flow in irregular newly formed tumor blood vessels, as well as chronic hypoxia, which is due to increased diffusion distances, can result in the diminished and uneven distribution of chemotherapeutic agents, subsequently affecting their therapeutic efficacy [198]. Hypoxia can also directly limit the chemotherapy induced DNA damage by reducing the generation of free radicals and this has been proposed as the mechanism of chemoresistance for agents such as bleomycine and anthracyclines. In particular, hypoxia induces the elevation of glutathione levels and DNA-repair enzymes seem to favor resistance to alkylating agents, bleomycin and platinum compounds [200]. Others have shown that hypoxia is able to induce 4-HPR (the chemopreventive retinoid *N*-(4 hydroxyphenyl) retinamide) resistance in Molt-4 cells (ALL cell line) and the potential mechanism may be the inhibition of 4HPR-induced regulation of mitochondrial pathway-related proteins associated in signaling apoptosis [203]. In addition, hypoxia mediates cycle cell modification and especially G_1/S-phase arrest can be incriminated for the resistance to vinca alkaloids and methotrexate [204]. Increased glycolysis with extracellular acidosis, a common feature in hypoxic tumor regions, may also favor chemoresistance by affecting the transport of drugs across the cell membrane, the intracellular drug accumulation (e.g.anthracyclines, bleomycin) and drug activity (e.g. vinblastine, doxorubicin, bleomycin). Some chemotherapeutic agents, such as cyclophosphamide, carboplatin and doxorubicin have been shown to be oxygen dependent under both *in vivo* and *in vitro* conditions [200]. In recent years, the contribution of HIF-1 to drug resistance has been observed in a wide spectrum of neoplastic cells [20]. One of the first reported molecular mechanisms explaining this contribution was that HIF-1α is able to activate the multidrug resistance 1 (MDR1) gene in response to hypoxia, coding for a membrane glycoprotein and finally leading to the decrease of intracellular concentration in a range of chemotherapeutic drugs, such as vinca alkaloids, anthracyclines and paclitaxel [205]. HIF-1- mediated changes in drug efflux have been shown to promote chemoresistance in many tumor cell lines, including glioblastoma cell resistance to adriamycin [205]. As mentioned earlier, HIF-1α is also linked to defective apoptosis and/or changes in cell cycle regulation, a phenomenon initially attributed to the HIF-1α anti-apoptotic target genes, but recent data have proposed additional mechanisms such as the suppression of p53 apoptosis by HIFs [206]. Overall, apoptosis inhibition is highly related to drug resistance in many adult tumor studies, but again little is known about the importance of hypoxia in pediatric tumors. However, a group have reported that hypoxia, in an HIF-1α-dependent manner, promotes resistance to apoptosis by etoposide and vincristine in neuroblastoma cells derived from pediatric patients [207]. As for the changes in cell cycle control promoting chemoresistance, more *in vivo* data are needed, since the functional importance of HIF appears to be variable, depending on the cell-type and the context. HIF activation due to hypoxia may, however, lead to chemoresistance through gene mutations that lead to the inhibition of DNA damage, as well as through the suppression of mitochondrial activity, which is strongly connected to the activation of cellular death pathways [20].

Implication of hypoxia in childhood cancer

In contrast to the well-investigated impact of hypoxia and HIFs in adult malignancies, their role in pediatric tumors has remained largely unaddressed. A great portion of our knowledge comes from tumors affecting both adults and children but the studies referring to cancer types that occur mostly among pediatric patients are scarce. We sought to collect the available data regarding the implication of hypoxia and

HIF activation in childhood cancer and, from our point of view, its clinical significance is becoming increasingly apparent. (Available data summarized in Table 1). First of all, hypoxia is a significant regulator at the level of the TSC niche. Researchers have found that a highly tumorigenic fraction of neuroblastoma and rhabdomyosarcoma cell lines is localized in the hypoxic zones *in vivo* and that this fraction is further increased by hypoxia [128]. Others report elevated levels of VEGF secretion, further induced by hypoxia, by TSC from malignant gliomas, including a pediatric GBM xenograft, while conditioned medium from the TSC increases endothelial cell migration *in vitro* [186]. Moreover, several studies highlight the importance of hypoxia as an adverse feature in almost every step of the cancerous procedure in pediatric tumours, mainly suggested by the presence of hypoxia-related markers/surrogate markers, such as HIF-1α, VEGF, the facilitative glucose transporter Glut-1 and carbonic anhydrase IX (CA IX). In relation to cellular adaptations, hypoxia has been shown to induce glycolytic activity in hepatomas. Gwak et al report an HIF-1α-dependent induction of hexokinase II expression and others an almost 3-fold increase in hexokinase II promoter activation [208,209]. Experimental data from Wilms' tumours has shown over-expression of CA9 and HIF-1α, with concomitant high expression of VEGF and GLUT-1 [149], whereas GLUT-1 and aldolase induction are also reported to take place in an HIF-1α-dependent manner, in rhabdomyosarcoma and Ewing sarcoma cells [146]. Finally, the expression of lactate dehydrogenase 5 (LDH5), the major LDH isoenzyme sustaining the anaerobic transformation of glycolysis, is highly upregulated in B-cell non-Hodgkin lymphomas, in direct relation to the expression of HIFs [210]. With angiogenesis being regarded a very important step in tumor development, VEGF is the most studied hypoxia-related factor in childhood malignancies. Patients with osteosarcoma, Ewing's sarcoma, neuroblastoma, rhabdomyosarcoma and Wilms' tumor were found to have increased serum levels of VEGF and the highest levels were associated with metastatic disease [210]. Evidence of elevated VEGF levels is linked to hypoxia and/or HIF activation in Wilms' tumor [149,211], rhabdomyosarcoma, [187], anaplastic large-cell lymphoma [188], neuroblastoma [212,213], as well as in cell lines and in hypoxic tumor stem cells from malignant gliomas and a pediatric glioblastoma xenograft [186]. Evasion of apoptosis as directly related to hypoxia has been shown in rhabdomyosarcoma and Ewing sarcoma cell lines, where it was speculated that the HIF-1α-mediated increase in glucose uptake plays an important role in conferring apoptosis resistance and indirectly in hepatomas, where inhibition of hexokinase II (found to be upregulated by hypoxia) led to apoptotic cell death [146,208]. More extensive appear to be the pediatric data linking hypoxia to tumor aggressiveness, invasion, metastasis and, consequently, prognosis. Degradation of the extracellular matrix implicated in tumor invasion is achieved partly through the proteolytic activity of matrix melloproteinases (MMP), found to be up-regulated in an HIF-1α-

Tumorigenic implication	Cancer type	HIF mediation	Effect	Reference
TSC niche	Neuroblastoma		↑Tumorigenic fraction	Das et al. [128]
	Rhabdomyosarcoma			
	GBM xenograft		↑ Endothelial cell migration	Bao et al. [186]
Cellular adaptations	Hepatoma	HIF-1a	↑Hexokinase II	Gwak et al. [208] Mathupala et al. [209]
	Wilms tumors	HIF-1α	↑GLUT-1, CA9	Dungwa et al. [149]
	Rhabdomyosarcoma	HIF-1α	↑ GLUT-1, aldolase	Kilic et al. [146]
	Ewings' sarcoma	HIF-1α		
	B-cell non-Hodgkin lymphomas	HIF1α, HIF2α	↑ LDH5	Giatromanolaki et al. [210]
Angiogenesis	Wilms' tumor	HIF-1α	↑ VEGF	Karth et al. [211] Dungwa et al. [149]
	Rhabdomyosarcoma	no	↑ IL-8	Wysoczynski et al. [187].
	Anaplastic large-cell lymphoma	HIF-1a	↑ VEGF	Dejean et al. [188]
	Neuroblastoma		↑ VEGF	Rössler et al. [212] Jögi et al. [213]
	GBM xenograft TSC		↑ VEGF secretion	Bao et al. [186]
Apoptosis evasion	Rhabdomyosarcoma	HIF-1α	↑ Glucose uptake	Kilic et al. [146]
	Ewings' sarcoma	HIF-1α		
	Hepatoma	HIF-1a	↑ Glucose uptake	Gwak et al. [208]
Invasion and metastasis	Glioma	HIF-1a	↑Proteolytic activity of matrix melloproteinases degradation of the extracellular matrix	Fujiwara et al. [214]
	Hepatoma			Miyoshi et al. [215]
	Glioma	HIF-1a	↑ Cell migration	Zagzag et al. [216]
	Ewings' sarcoma	HIF-1a	↑ Invasiveness and soft-agar colony formation	Aryee et al. [192]
	Neuroblastoma		Cell dedifferentiation, tumor heterogenicity , increased malignancy	Jogi et al. [196]
	Medulloblastoma	HIF-1 α	Activation of the Notch signaling pathway,↑ stem cell viability and expansion	Pistollato et al. [217]

Table 1: Summary of pediatric data linking hypoxia to childhood malignancies.

dependent manner in gliomas and hepatomas [214,215]. In addition to this, in glioma cells, inhibition of HIF-1α by geldanamycin has been found to reduce cell migration *in vitro*, thus hinting at a potential role for HIF-1α in glioma cell invasion [216]. In osteosarcoma, HIF-1α expression significantly correlates with surgical stage, percentage of dead cells and microvessel density (MVD), as well as with shorter overall survival (OS) and disease-free survival (DFS) [191]. In primary Ewing's sarcoma family tumor (ESFT) cells hypoxia has been found to enhance the cells' malignant properties by stimulating the invasiveness and soft-agar colony formation through an HIF-1α-mediated manner [192]. In neuroblastoma cells, the hypoxia-induced de-differentiation of hypoxic tumor regions has been found to contribute to the tumor heterogenicity and increased malignancy [196]. In medulloblastoma cells, hypoxia, through HIF-1α and by activation of the Notch signalling pathway (maintaining Notch1 in its active form) has been found to promote stem cell viability and expansion [217]. It has also been reported that HIF-1α protein accumulation is associated with poor patient survival in oligodendroglioma, whereas HIF-2α over-expression is linked to increased patient mortality in childhood neuroblastoma and astrocytoma [124,190,218]. Finally, hypoxia has been reported to contribute to chemoresistance and radioresistance in some pediatric malignancies. For example, hypoxia has been shown to protect rhabdomyosarcoma (A204 RMS) and Ewing's sarcoma (A673ES) cells against doxorubicin-, vincristin-, actinomycin D-induced apoptosis in a time- and dose-dependent manner [146]. In neuroblastoma cell lines (SH-EP1 and SH-SY5Y) short periods of hypoxia (1% O2) of up to 16 hours appears to have no effect on drug-induced apoptosis to the clinically relevant drugs vincristine, etoposide and cisplatin, whereas prolonged hypoxia of 1 to 7 days results in the reduction of vincristine- and etoposide-induced apoptosis [207]. HIF-1-mediated chemoresistance to adriamycin has also been found in many tumor cell lines, including glioblastoma cells [205]. Certain experimental data have shown that both non-interrupted and intermittent hypoxia contribute to cell resistance to ionizing radiation in U87 glioma xenografts, while others have documented that the antitumor activity of ionizing radiation in U87 glioma xenografts is enhanced by improving intra-tumoral oxygenation [201,202].

Hypoxia Targets as New Cancer Chemotherapeutics

Hypoxic cells are genetically unstable, resistant to apoptosis, invasive and metastatic. These properties make them more resistant to ionizing radiation and chemotherapy, so the way we regard cancer diagnosis and treatment today is highly associated with approaches that target tissue hypoxia [219]. At the same time advances in hypoxia research are beginning to unravel the molecular mechanisms responsible for the hypoxic tumor microenvironment, so the signalling molecules of the hypoxic cascade are becoming potential targets for cancer therapy.

General hypoxia-based therapeutic strategies

The most logical strategy to employ in enhancing radio- and chemo-sensitivity is the administration of high pressure oxygen. At the same time, the development of hypoxia-based radio-sensitizers is proving very promising in targeting tumor cells in their hypoxic microenvironment.

Hyperbaric Oxygen Therapy (HBO): This is the administration of pure oxygen at a pressure higher than 1 atmosphere to the tumor sites; studies have shown that intermittent HBO therapy increases the radio-curability and life expectancy in many cancers, especially head and neck cancer, but it also sensitizes chemotherapy by increasing tumor perfusion and cellular sensitivity, as seen in *in vitro* studies with doxorubicin and taxol [220-222].

Radio-sensitizers: These are agents that simulate the action of oxygen, thus compensating for the low oxygen concentration and the increase in radiation-induced damage. Despite the initial disappointment in the use of nitroimidazoles as active compounds, it was later shown that, in association with radiotherapy, nimorazole induces a higher cancer-related survival in head and neck carcinomas, whereas misonidazole increases 1-year survival by 8% in astrocytomas [223,224]. More recently, molecular research has focused on the development of bi-functional hypoxic cell radio-sensitizers, thus allowing for the simultaneous inhibition of certain tumor hypoxia responses, such as angiogenesis and metastasis. Many new agents, including p53-inhibiting agents, are currently being tested in clinical trials in the hope that they can be effectively used in the development of bi-functional radio-sensitizers for cancer therapy [225-229].

ARCON (Accelerated Radiotherapy with Carbogen and Nicotinamide): This is a method additional to radio-sensitizing, in which radiotherapy is administered in association with inhaling hyperoxic gas so as to decrease diffusion-limited hypoxia and nicotinamine so as to decrease perfusion-limited hypoxia [222]. Despite the lack of response in clinical studies with non-small cell lung cancer, ARCON has shown some promising results in xenograft models of breast cancer and preliminary studies of bladder cancer [230-232].

Hypoxic cytotoxins: With tirapazamine being the most widely studied compound of this group, hypoxic cytotoxins are bio-reductively activated in tumor cells and give rise to cytotoxic DNA breaks, thus potentiating the anti-tumor effects of radiation and chemotherapy [233,234]. Despite having no effect when administered together with paclitaxel and carboplatin, the combination of tirapazamine and cisplatin has been shown to increase response rate and overall survival in clinical trials of non-small cell lung cancer; in addition, the combination with cisplatin and radiation in locally advanced squamous cell carcinoma of the head and neck has proved far more effective in terms of survival rates than fluorouracil, cisplatin and radiation together [235-237].

Recombinant anaerobic bacteria: This is essentially the injection of non-pathogenic strains of bacteria, such as *Clostridium*, in the form of spores, in tumor areas. These spores only become activated and grow in the hypoxic environment, exerting either direct anti-tumor activity or carrying enzymes that can be manipulated for anti-tumor activity. The strain *C.oncolyticum*, in particular, has been genetically modified to express cytosine deaminase, an E.coli enzyme able to metabolise the non-toxic 5-fluorocytosine to the toxic anti-tumor 5-fluorouracile and shows potent activity in many animal studies [238,239].

Erythropoietin (Epo): Recombinant human Epo (rHuEpo) has shown great potential as a therapeutic tool in cancer patients, as many studies have shown that it may improve the radio- and chemo- tumor sensitivity by increasing oxygenation, as well as oxygen-sensitization of other chemotherapeutic drugs [240,241].

HIF-based therapeutic targets

Since HIF-1 is such an important regulator of the cellular response to hypoxia, two strategies really stand out when it comes to targeting hypoxia for therapeutic purposes:

Inhibition of the signalling pathways that regulate HIF-1 function

mTOR inhibitors: Rapamycin is well-known for its anti-proliferative effect in many human and animal cell lines, as well as for its strong inhibitory effects on tumor growth and angiogenesis [242-247]. Temsirolimus, or CCI-779 (Wyeth), an ester analogue of rapamycin, has been shown to inhibit HIF-1α-mediated VEGF endothelial proliferation in a breast cancer line, as well as mTOR-dependent angiogenesis and tumor growth in rhabdomyosarcoma xenograft models under hypoxic conditions [248,249]. Everolimous, or RAD001 (Novartis), an orally available rapamycin analog, was found to inhibit cell proliferation in lymphoid and smooth muscle cells, whilst at the same time exhibiting immunosuppressive effects in human T-cell clones; hence it is now clinically used both as an immunosuppressant in autoimmune disorders and as a tumor suppressor drug in cancer treatment [250-252]. Deforolimus, or AP23573 (Ariad Pharmaceuticals), a phosphorous rapamycin analog, has shown strong anti-proliferative effects in tumor cell lines *in vitro* and mouse xenografts *in vivo* [253]. Current efforts are focusing on the development of selective mTOR inhibitors, i.e. ones that compete with ATP in the catalytic site of mTOR, in the hope that these will be more effective in blocking cell proliferation than rapamycin; these include: PP242, PP30, Torin1, Ku-0063794, WAY-600, WYE-687 and WYE-354 [254]. Finally, dual specificity inhibitors of both mTOR and PI3K signaling pathways are also being investigated for their potentiality as cancer therapeutics and include: GNE477, NVP-BEZ235, PI-103, XL765 and WJD008 [254].

EGFR inhibitors: Gefitinib (Iressa) and Erlotinib (Tarceva) are both small molecule inhibitors already used in the treatment of non-small cell lung cancer but they have also been found to inhibit VEGF expression in squamous cell carcinoma *in vitro* [255]. Cetuximab, or C225 (Erbitux), a monoclonal antibody that was shown to inhibit HIF-1α protein levels, has been approved for the treatment of metastatic colorectal carcinoma and squamous cell carcinoma of the head and neck [256]. Trastuzumab (herceptin), on the other hand, is a humanized monoclonal antibody that targets the human EGF receptor 2 (Her2) and prevents it from inducing activation of HIF-1α and VEGF in breast cancer cells, hence promoting anti-angiogenesis [257-259].

VEGF inhibitors: Bevacizumab, an anti-VEGF neutralizing antibody currently in clinical use for cancer therapy, has shown strong anti-angiogenic activity *in vivo* and the ability to suppress the growth of xenografts derived from stem cell-like glioma cells [186].

Tyrosine kinase inhibitors: Imatinib mesylate, or Gleevec (Novartis), the small molecule inhibitor of the oncogenic fusion BCR-ABL used in the treatment of leukemias, has also been found to inhibit induction of HIF-1α and VEGF expression in small cell lung cancer cell lines [260].

Microtubule disrupting agents: Being a natural estrogen metabolite, 2ME2 (2-methoxyestradiol) promotes microtubule disruption via inhibition of tubulin polymerisation and causes mitotic arrest [261]. In pre-clinical models 2ME2 has shown increased anti-tumor activity in association with decreased HIF-1α protein levels and a newly formulated version, Panzem™, already approved in the treatment of rheumatoid arthritis, is currently in phase II clinical trial for cancer patients [262,263].

Targeting the HIF-1α-responsive genes and transcription factors

Topoisomerase-I inhibitors: Topotecan, the best characterized molecule of this group, is a potent inhibitor of HIF-1α that causes DNA damage and cytotoxicity by reversibly binding and stabilising the Topoisomerase-1 enzyme on the DNA [264]. Whilst already approved for second line therapy of small cell lung cancer and ovarian cancer, topotecan has also been show to inhibit tumor growth with a concomitant HIF-1α protein level reduction in glioma xenograft models [222,265]. Furthermore, a recent study has demonstrated that topotecan inhibits VEGF-mediated angiogenic activity induced by hypoxia in human neuroblastoma cells [266].

PX-478: being one of the most potent HIF-1α inhibitors, PX-478 has shown anti-tumor activity which positively correlates with HIF-1α levels in both cell lines and large xenograft models [267]. PX-478 has completed Phase I clinical trial for advanced solid tumours and lymphomas, where it was associated with stable disease and dose-dependent inhibition of HIF-1α [268].

YC-1 (3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole): This is a pharmacological agent initially formulated for circulatory disorders, acting as an inhibitor of platelet aggregation and vasodilation via activation of soluble guanylate cyclise (sGC) [269,270]. YC-1 has shown significant anti-tumor activity with concomitant reduction of HIF-1α protein levels in hepatoma cells and five xenograft models [271,272].

Heat-shock protein 90 (Hsp90) inhibitors: Hsp90 is a chaperone protein involved in the conformation, stability, maturation and function of many other proteins, mainly transcription factors and signalling kinases, including regulation of HIF-1α activation and cell cycle control [273]. Galdanamycin (GA) is a naturally occurring Hsp90 inhibitor that works by competing with Hsp90 for its ATP binding site, causing ubiquitination and proteasome-mediated degradation of the associated proteins; interestingly, GA has been found to cause the degradation of HIF-1α in renal cell carcinoma and prostate cancer cell lines, so GA analogs are currently being tested in clinical trials for their efficacy in treating renal tumors, metastatic breast cancer, malignant melanoma, thyroid carcinoma and lymphoma [222,274,275]. Similarly, other Hsp90 inhibitors have shown promising results in xenograft models and clinical trials of hypoxia in that they inhibit HIF-1α activity and VEGF expression; examples include radicicol, KF58333, SCH66336 and apigenin [275-279].

Histone deacetylase inhibitors (HDACI): Studies have shown that HDACI (especially HDAC-6 and HDAC-4) are actively implicated in the proteasome-dependent degradation of HIF-1 α, either by a VHL- and ubiquitin-independent pathway mediated by an HDAC-6-dependent hyperacetylation of Hsp90, or by an increased acetylation and poly-ubiquitination pathway mediated by the direct interaction between the HDACI and HIF-1α [280,281]. Vorinostat (ZOLINZA™, Merck) is an FDA-approved HDACI for the treatment of cutaneous T-cell lymphoma (CTCL), while several other HDACI are currently being tested in phase I/II studies for their efficacy as cancer therapeutics; examples include: valproic acid (also used as an anticonvulsant and mood stabilizing drug in epilepsy and bipolar disorder), MGCD0103, FK228, LBH589, Trichostatin A, AR-42 and CUDC101 [282].

Thioredoxin inhibitors: Since redox protein thioredoxin (Trx-1) over-expression has been found to correlate with increased HIF-1α protein levels and VEGF production in many human cancers, the next logical step is to design inhibitors that will prevent Trx-1 signalling

[283]. Examples include PX-12 and pleurotin, already showing encouraging results in decreasing HIF-1α levels and expression of HIF-1α-responsive genes *in vitro* [284].

Chetomin: Being a metabolite of the fungus *Chaetomium* with anti-microbial properties, chetomin has also shown strong potency in disrupting the interaction of HIF-1α with p300, hence inhibiting hypoxia-mediated transcription and tumor growth in xenograft models [285].

Echinomycin: Despite disappointing results when extensively tested in phase I/II clinical trials in the 1990s, echinomycin, essentially an antibiotic, has shown strong potency in inhibiting HIF-1α activity *in vitro*, paradoxically hinting at a possible therapeutic efficacy if exploited accordingly [286-288].

Miscellaneous: Ascorbate, Fara-A (nucleotide analog) and certain anti-inflammatory drugs such as ibuprofen and indomethacin have been found to inhibit HIF-1α activity and angiogenesis in both normal and cancer cells *in vitro* [289-292]. On the other hand, flavopiridol, a protein kinase inhibitor, has demonstrated strong potency in inhibiting VEGF and HIF-1α expression in human monocytes and glioma cells [293,294]. Recently, the development of a synthetic polyamide especially designed to inhibit the binding of the HIF-1 α/ARNT heterodimer to its cognate DNA sequence and its successful delivery in mammalian cells has shed new light in the mechanisms regarding HIF-1α inhibition [295,296]. Considerations are also being made for the development of proteasome inhibitors as HIF-1α transcriptional inhibition-mediated tumor therapeutics [297], whereas natural products such as curcumin (component of spice tumeric), berberin (Chinese herb component), resveratrol (found in grapes) and certain flavonoids such as genistein are also being screened for their ability to inhibit HIF-1α activity in certain human cell lines and xenograft models [298-302]. Finally, recent studies have demonstrated that zinc supplementation downregulates HIF-1α activity and protein levels in highly invasive and angiogenic prostate cancer and glioblastoma cells, resulting in the inhibition of VEGF expression and in the prevention of angiogenesis and tumor invasiveness, thus indicating that zinc could become a useful HIF-1α inhibitor in anti-cancer therapies [303,304].

PHD-based therapeutic targets

Since PHDs are actively implicated in the proteasome-mediated degradation of HIF-1α in the normoxic environment, as discussed at the beginning of this review, strategies employing their activation are well under consideration. Examples include:

Cyclosporin A: Essentially an immunosuppressive agent used in organ transplantation, cyclosporine A has also been found to inhibit the HIF-1α-mediated cellular response to hypoxia via induction of PHD activation in glioma cells *in vitro* [222,305].

R59949: This agent has shown potent activity in inhibiting HIF-1α protein accumulation via activation of PHD *in vitro* and therefore is a promising candidate for further *in vivo* testing [306].

Antioxidants: Such as ascorbic acid, N-acetylcysteine and vitamin C have been shown to decrease HIF-1α protein levels, possibly by maintaining the reduced active state of the catalytic ferrous ion of PHD and by inducing VHL-mediated HIF-1α degradation [307].

Clinical setbacks in the field of anti-angiogenic cancer therapy

Despite the FDA approval of several VEGF blockers for cancer therapy and the reported prolonged survival of the responsive cancer patients, recent findings show that progression-free survival (PFS) is very short, usually in the order of a few months and not always followed by overall survival (OS) [308,309]. Similarly, approvals of oral small molecule anti-angiogenic receptor tyrosine kinase inhibitors (TKIs) have also been associated with a number of failures in randomized phase III trials, whether administered alone or with chemotherapy [310,311]. The anti-VEGF neutralizing antibody bevacizumab, in particular, has produced extremely disappointing results in preclinical testing and in colorectal cancer phase III trials, with worse clinical outcomes appearing in patients who received bevacizumab plus chemotherapy compared to just chemotherapy [312,313]. Even though such therapeutic strategies may initially elicit a beneficial response by reducing tumor size, they can also result in hypoxia and hence eventually enhance tumor aggressiveness by reducing drug efficacy due to HIF-1α expression. As a result, hypoxia might select for the malignant metastatic cells that expand to more invasive metastatic disease, ultimately leading to shorter life expectancy [312,314,315]. Several mechanisms have been proposed to explain the aforementioned setbacks, mostly regarding changes in the tumor cells, as is for example the tumor microenvironment, where VEGF blockade induces hypoxia as a result of reducing tumor microvascularity, tumor vessel blood flow and blood perfusion [315,316]. Should other angiogenic factors be upregulated at a more advanced tumor stage, as for example PIGF, FGFs, chemokines and ephrins, not only will VEGF-blockade as cancer therapy no longer be effective, but tumor vascularisation will be rescued, leading to tumor invasiveness and metastasis [317]. Notably, depending on the cell type affected, certain tumor types may be less sensitive to VEGF blockade, as is for example pancreatic cell carcinoma, due to its hypovascular stroma structure [315]. As a consequence, there is an urgent need for devising strategies that will allow the anti-angiogenic drugs to be effectively combined with chemotherapy in targeting tumor hypoxia and HIF-1α expression. One such strategy could be metronomic chemotherapy, i.e. repetitive, low doses of chemotherapeutic drugs designed to minimise toxicity and target the tumor stroma, as opposed to targeting the tumor itself. It has already been shown that combining anti-angiogenic drugs with metronomic chemotherapy produces more potent anti-tumor effects *in vitro*, whereas a randomized phase II clinical trial of metronomic cyclophosphamide/capecitabine in combination with becavizumab for the treatment of metastatic breast cancer has shown a significant enough overall clinical benefit so as to move the treatment forward to phase III clinical trial testing [318-321].

Conclusions/Future Perspectives

To summarize, hypoxia induces the activation of many pathways within the cell, some being HIF-mediated and some HIF-independent, which interconnect and cooperate with each other in response to the hypoxic stress. Chromatin remodelling, changes in gene expression and altered translational processes are all aspects of the cellular response to hypoxia and are actively involved in cancer formation. The research and development of novel chemotherapeutic targets based on these features of the hypoxic response has been the subject of intense scrutiny in the past 20 years. Despite the fact that many hypoxia-based therapeutic agents show promising results *in vitro* and *in vivo*, with some of them having successfully passed onto Phase I/II clinical trials, we still haven't fully deciphered the molecular mechanisms interconnecting the various signalling pathways involved in the hypoxic response or gained enough insight into how gene expression affects the malignant phenotype. In addition, very little data exists on the effect of hypoxia on childhood tumorigenesis, so current therapeutic modalities are almost

exclusively based on data acquired from studies in adults. Future research in this area will lead to a better understanding of how the hypoxic cascade affects cancer progression in this particularly fragile patient population and hopefully lead to better and more effective therapeutic and prognostic outcomes.

References

1. Dunwoodie SL (2009) The role of hypoxia in development of the Mammalian embryo. Dev Cell 17: 755-773.
2. Simon MC, Keith B (2008) The role of oxygen availability in embryonic development and stem cell function. Nat Rev Mol Cell Biol 9: 285-296.
3. Forristal CE, Wright KL, Hanley NA, Oreffo RO, Houghton FD (2010) Hypoxia inducible factors regulate pluripotency and proliferation in human embryonic stem cells cultured at reduced oxygen tensions. Reproduction 139: 85-97.
4. Szablowska-Gadomska I, Zayat V, Buzanska L (2011) Influence of low oxygen tensions on expression of pluripotency genes in stem cells. Acta Neurobiol Exp (Wars) 71: 86-93.
5. Hickey MM, Simon MC (2006) Regulation of angiogenesis by hypoxia and hypoxia-inducible factors. Curr Top Dev Biol 76: 217-257.
6. Fraisl P, Mazzone M, Schmidt T, Carmeliet P (2009) Regulation of angiogenesis by oxygen and metabolism. Dev Cell 16: 167-179.
7. Semenza GL (2001) Hypoxia-inducible factor 1: oxygen homeostasis and disease pathophysiology. Trends Mol Med 7: 345-350.
8. Nangaku, M (2006) Chronic hypoxia and tubulointerstitial injury: a final common pathway to end-stage renal failure. J Am Soc Nephrol 17: 17-25.
9. Shi H (2009) Hypoxia inducible factor 1 as a therapeutic target in ischemic stroke. Curr Med Chem 16: 4593-4600.
10. Ruan K, Song G, Ouyang G (2009) Role of hypoxia in the hallmarks of human cancer. J Cell Biochem 107: 1053-1062.
11. Kenneth NS, Rocha S (2008) Regulation of gene expression by hypoxia. Biochem J 414: 19-29.
12. Semenza GL, Wang GL (1992) 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 12: 5447-5454.
13. Gardner LB, Li Q, Park MS, Flanagan WM, Semenza GL, et al. (2001) Hypoxia inhibits G1/S transition through regulation of p27 expression. J Biol Chem 276: 7919-7926.
14. Kato H, Tamamizu-Kato S, Shibasaki F (2004) Histone deacetylase 7 associates with hypoxia-inducible factor 1alpha and increases transcriptional activity. J Biol Chem 279: 41966-41974.
15. Johnson AB, Denko N, Barton MC (2008) Hypoxia induces a novel signature of chromatin modifications and global repression of transcription. Mutat Res 640: 174-179.
16. Koritzinsky M, Seigneuric R, Magagnin MG, van den Beucken T, Lambin P, et al. (2005) The hypoxic proteome is influenced by gene-specific changes in mRNA translation. Radiother Oncol 76: 177-186.
17. Perez-Perri JI, Acevedo JM, Wappner P (2011) Epigenetics: new questions on the response to hypoxia. Int J Mol Sci 12: 4705-4721.
18. Zhou J, Schmid T, Schnitzer S, Brüne B (2006) Tumor hypoxia and cancer progression. Cancer Lett 237: 10-21.
19. Anastasiou D, Poulogiannis G, Asara JM, Boxer MB, Jiang JK, et al. (2011) Inhibition of pyruvate kinase M2 by reactive oxygen species contributes to cellular antioxidant responses. Science 334: 1278-1283.
20. Rohwer N, Cramer T (2011) Hypoxia-mediated drug resistance: novel insights on the functional interaction of HIFs and cell death pathways. Drug Resist Updat 14: 191-201.
21. Reisz-Porszasz S, Probst MR, Fukunaga BN, Hankinson O (1994) Identification of functional domains of the aryl hydrocarbon receptor nuclear translocator protein (ARNT). Mol Cell Biol 14: 6075-6086.
22. Bárdos JI, Ashcroft M (2005) Negative and positive regulation of HIF-1: a complex network. Biochim Biophys Acta 1755: 107-120.
23. Taylor BL, Zhulin IB (1999) PAS domains: internal sensors of oxygen, redox potential, and light. Microbiol Mol Biol Rev 63: 479-506.
24. Webb JD, Coleman ML, Pugh CW (2009) Hypoxia, hypoxia-inducible factors (HIF), HIF hydroxylases and oxygen sensing. Cell Mol Life Sci 66: 3539-3554.
25. Wang GL, Jiang BH, Rue EA, Semenza GL (1995) Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci USA 92: 5510-5514.
26. Bruegge K, Jelkmann W, Metzen E (2007) Hydroxylation of hypoxia-inducible transcription factors and chemical compounds targeting the HIF-alpha hydroxylases. Curr Med Chem 14: 1853-1862.
27. Salceda S, Caro J (1997) 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 272: 22642-22647.
28. Mahon PC, Hirota K, Semenza GL (2001) FIH-1: a novel protein that interacts with HIF-1alpha and VHL to mediate repression of HIF-1 transcriptional activity. Genes Dev 15: 2675-2686.
29. Jiang BH, Rue E, Wang GL, Roe R, Semenza GL (1996) Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor 1. J Biol Chem 271: 17771-17778.
30. Arany Z, Huang LE, Eckner R, Bhattacharya S, Jiang C, et al. (1996) An essential role for p300/CBP in the cellular response to hypoxia. Proc Natl Acad Sci USA 93: 12969-12973.
31. Ruas JL, Poellinger L, Pereira T (2005) Role of CBP in regulating HIF-1-mediated activation of transcription. J Cell Sci 118: 301-311.
32. Semenza GL (2001) HIF-1 and mechanisms of hypoxia sensing. Curr Opin Cell Biol 13: 167-171.
33. Wenger RH (2002) Cellular adaptation to hypoxia: O2-sensing protein hydroxylases, hypoxia-inducible transcription factors, and O2-regulated gene expression. FASEB J 16: 1151-1162.
34. Xia X, Kung AL (2009) Preferential binding of HIF-1 to transcriptionally active loci determines cell-type specific response to hypoxia. Genome Biol 10: R113.
35. Bjornsti MA, Houghton PJ (2004) The TOR pathway: a target for cancer therapy. Nat Rev Cancer 4: 335-348.
36. Arsham AM, Howell JJ, Simon MC (2003) A novel hypoxia-inducible factor-independent hypoxic response regulating mammalian target of rapamycin and its targets. J Biol Chem 278: 29655-29660.
37. Brugarolas J, Lei K, Hurley RL, Manning BD, Reiling JH, et al. (2004) Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. Genes Dev 18: 2893-2904.
38. Hamanaka Y, Mukai M, Shimamura M, Kitagawa T, Nishida T, et al. (2005) Suppression of PI3K/mTOR pathway rescues LLC cells from cell death induced by hypoxia. Biochem Biophys Res Commun 330: 318-326.
39. Esumi H, Izuishi K, Kato K, Hashimoto K, Kurashima Y, et al. (2002) Hypoxia and nitric oxide treatment confer tolerance to glucose starvation in a 5'-AMP-activated protein kinase-dependent manner. J Biol Chem 277: 32791-32798.
40. Koumenis C, Naczki C, Koritzinsky M, Rastani S, Diehl A , et al. (2002) Regulation of protein synthesis by hypoxia via activation of the endoplasmic reticulum kinase PERK and phosphorylation of the translation initiation factor eIF2alpha. Mol Cell Biol 22: 7405-7416.
41. Koumenis C (2006) ER stress, hypoxia tolerance and tumor progression. Curr Mol Med 6: 55-69.
42. Romero-Ramirez L, Cao H, Nelson D, Hammond E, Lee AH, et al. (2004) XBP1 is essential for survival under hypoxic conditions and is required for tumor growth. Cancer Res 64: 5943-5947.
43. Hayden MS, Ghosh S (2004) Signaling to NF-kappaB. Genes Dev 18: 2195-2224.
44. Schmidt D, Textor B, Pein OT, Licht AH, Andrecht S, et al. (2007) Critical role for NF-kappaB-induced JunB in VEGF regulation and tumor angiogenesis. EMBO J 26: 710-719.
45. Barré B, Perkins ND (2007) A cell cycle regulatory network controlling NF-kappaB subunit activity and function. EMBO J 26: 4841-4855.
46. Chan DA, Kawahara TL, Sutphin PD, Chang HY, Chi JT, et al. (2009) Tumor

vasculature is regulated by PHD2-mediated angiogenesis and bone marrow-derived cell recruitment. Cancer Cell 15: 527-538.

47. Baetz D, Regula KM, Ens K, Shaw J, Kothari S, et al. (2005) Nuclear factor-kappaB-mediated cell survival involves transcriptional silencing of the mitochondrial death gene BNIP3 in ventricular myocytes. Circulation 112: 3777-3785.

48. Nijboer CH, Heijnen CJ, Groenendaal F, May MJ, van Bel F, et al. (2008) A dual role of the NF-kappaB pathway in neonatal hypoxic-ischemic brain damage. Stroke 39: 2578-2586.

49. Nijboer CH, Heijnen CJ, Groenendaal F, May MJ, van BF, et al. (2008) Strong neuroprotection by inhibition of NF-kappaB after neonatal hypoxia-ischemia involves apoptotic mechanisms but is independent of cytokines. Stroke 39: 2129-2137.

50. Bonello S, Zähringer C, BelAiba RS, Djordjevic T, Hess J, et al. (2007) Reactive oxygen species activate the HIF-1alpha promoter via a functional NFkappaB site. Arterioscler Thromb Vasc Biol 27: 755-761.

51. van Uden P, Kenneth NS, Rocha S (2008) Regulation of hypoxia-inducible factor-1alpha by NF-kappaB. Biochem J 412: 477-484.

52. Greenblatt MS, Bennett WP, Hollstein M, Harris CC (1994) Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res 54: 4855-4878.

53. Lozano G (2007) The oncogenic roles of p53 mutants in mouse models. Curr Opin Genet Dev 17: 66-70.

54. Haupt Y, Maya R, Kazaz A, Oren M (1997) Mdm2 promotes the rapid degradation of p53. Nature 387: 296-299.

55. Vousden KH (2002) Activation of the p53 tumor suppressor protein. Biochim Biophys Acta 1602: 47-59.

56. Levine AJ (1997) p53, the cellular gatekeeper for growth and division. Cell 88: 323-331.

57. Dai C, Gu W (2010) p53 post-translational modification: deregulated in tumorigenesis. Trends Mol Med 16: 528-536.

58. Koumenis C, Alarcon R, Hammond E, Sutphin P, Hoffman W, et al. (2001) Regulation of p53 by hypoxia: dissociation of transcriptional repression and apoptosis from p53-dependent transactivation. Mol Cell Biol 21: 1297-1310.

59. Hammond EM, Giaccia AJ (2006) Hypoxia-inducible factor-1 and p53: friends, acquaintances, or strangers? Clin Cancer Res 12: 5007-5009.

60. Fei P, Wang W, Kim SH, Wang S, Burns TF, et al. (2004) Bnip3L is induced by p53 under hypoxia, and its knockdown promotes tumor growth. Cancer Cell 6: 597-609.

61. Hammond EM, Mandell DJ, Salim A, Krieg AJ, Johnson TM, et al. (2006) Genome-wide analysis of p53 under hypoxic conditions. Mol Cell Biol 26: 3492-3504.

62. Chen D, Li M, Luo J, Gu W (2003) Direct interactions between HIF-1 alpha and Mdm2 modulate p53 function. J Biol Chem 278: 13595-13598.

63. Wouters A, Pauwels B, Lambrechts HA, Pattyn GG, Ides J, et al. (2009) Chemoradiation interactions under reduced oxygen conditions: Cellular characteristics of an in vitro model. Cancer Lett 286: 180-188.

64. Sermeus A, Michiels C (2011) Reciprocal influence of the p53 and the hypoxic pathways. Cell Death Dis 2: e164.

65. Schmid T, Zhou J, Köhl R, Brüne B (2004) p300 relieves p53-evoked transcriptional repression of hypoxia-inducible factor-1 (HIF-1). Biochem J 380: 289-295.

66. Halterman MW, Federoff HJ (1999) HIF-1alpha and p53 promote hypoxia-induced delayed neuronal death in models of CNS ischemia. Exp Neurol 159: 65-72.

67. Grandori C, Cowley SM, James LP, Eisenman RN (2000) The Myc/Max/Mad network and the transcriptional control of cell behavior. Annu Rev Cell Dev Biol 16: 653-699.

68. Blackwell TK, Kretzner L, Blackwood EM, Eisenman RN, Weintraub H (1990) Sequence-specific DNA binding by the c-Myc protein. Science 250: 1149-1151.

69. Blackwood EM, Lüscher B, Eisenman RN (1992) Myc and Max associate in vivo. Genes Dev 6: 71-80.

70. Dominguez-Sola D, Ying CY, Grandori C, Ruggiero L, Chen B, et al. (2007) Non-transcriptional control of DNA replication by c-Myc. Nature 448: 445-451.

71. Adhikary S, Eilers M (2005) Transcriptional regulation and transformation by Myc proteins. Nat Rev Mol Cell Biol 6: 635-645.

72. Haluska FG, Finver S, Tsujimoto Y, Croce CM (1986) The t(8; 14) chromosomal translocation occurring in B-cell malignancies results from mistakes in V-D-J joining. Nature 324: 158-161.

73. Goda N, Ryan HE, Khadivi B, McNulty W, Rickert RC, et al. (2003) Hypoxia-inducible factor 1alpha is essential for cell cycle arrest during hypoxia. Mol Cell Biol 23: 359-369.

74. Koshiji M, Huang LE (2004) Dynamic balancing of the dual nature of HIF-1alpha for cell survival. Cell Cycle 3: 853-854.

75. Koshiji M, Kageyama Y, Pete EA, Horikawa I, Barrett JC, et al. (2004) HIF-1alpha induces cell cycle arrest by functionally counteracting Myc. EMBO J 23: 1949-1956.

76. Hurlin PJ, Huang J (2006) The MAX-interacting transcription factor network. Semin Cancer Biol 16: 265-274.

77. Gordan JD, Bertout JA, Hu CJ, Diehl JA, Simon MC (2007) HIF-2alpha promotes hypoxic cell proliferation by enhancing c-myc transcriptional activity. Cancer Cell 11: 335-347.

78. Dang CV, Kim JW, Gao P, Yustein J (2008) The interplay between MYC and HIF in cancer. Nat Rev Cancer 8: 51-56.

79. Huang LE (2008) Carrot and stick: HIF-alpha engages c-Myc in hypoxic adaptation. Cell Death Differ 15: 672-677.

80. Kim JW, Gao P, Liu YC, Semenza GL, Dang CV (2007) Hypoxia-inducible factor 1 and dysregulated c-Myc cooperatively induce vascular endothelial growth factor and metabolic switches hexokinase 2 and pyruvate dehydrogenase kinase 1. Mol Cell Biol 27: 7381-7393.

81. Wagner EF (2002) Functions of AP1 (Fos/Jun) in bone development. Ann Rheum Dis 2: ii40-ii42.

82. Hess J, Angel P, Schorpp-Kistner M (2004) AP-1 subunits: quarrel and harmony among siblings. J Cell Sci 117: 5965-5973.

83. Conrad PW, Rust RT, Han J, Millhorn DE, Beitner-Johnson D (1999) Selective activation of p38alpha and p38gamma by hypoxia. Role in regulation of cyclin D1 by hypoxia in PC12 cells. J Biol Chem 274: 23570-23576.

84. Millhorn DE, Raymond R, Conforti L, Zhu W, Beitner-Johnson D, et al. (1997) Regulation of gene expression for tyrosine hydroxylase in oxygen sensitive cells by hypoxia. Kidney Int 51: 527-535.

85. Damert A, Ikeda E, Risau W (1997) Activator-protein-1 binding potentiates the hypoxia-inducible factor-1-mediated hypoxia-induced transcriptional activation of vascular-endothelial growth factor expression in C6 glioma cells. Biochem J 327 : 419-423.

86. Hoffmann A, Gloe T, Pohl U (2001) Hypoxia-induced upregulation of eNOS gene expression is redox-sensitive: a comparison between hypoxia and inhibitors of cell metabolism. J Cell Physiol 188: 33-44.

87. Michiels C, Minet E, Michel G, Mottet D, Piret JP, et al. (2001) HIF-1 and AP-1 cooperate to increase gene expression in hypoxia: role of MAP kinases. IUBMB Life 52: 49-53.

88. Salnikow K, Kluz T, Costa M, Piquemal D, Demidenko ZN, et al. (2002) The regulation of hypoxic genes by calcium involves c-Jun/AP-1, which cooperates with hypoxia-inducible factor 1 in response to hypoxia. Mol Cell Biol 22: 1734-1741.

89. Kunz M, Ibrahim SM (2003) Molecular responses to hypoxia in tumor cells. Mol Cancer 2: 23.

90. Shi Q, Le X, Abbruzzese JL, Wang B, Mujaida N, et al. (1999) Cooperation between transcription factor AP-1 and NF-kappaB in the induction of interleukin-8 in human pancreatic adenocarcinoma cells by hypoxia. J Interferon Cytokine Res 19: 1363-1371.

91. Ameri K, Lewis CE, Raida M, Sowter H, Hai T, et al. (2004) Anoxic induction of ATF-4 through HIF-1-independent pathways of protein stabilization in human cancer cells. Blood 103: 1876-1882.

92. Yan SF, Mackman N, Kisiel W, Stern DM, Pinsky DJ (1999) Hypoxia/Hypoxemia-Induced activation of the procoagulant pathways and the

pathogenesis of ischemia-associated thrombosis. Arterioscler Thromb Vasc Biol 19: 2029-2035.

93. Thiel G, Cibelli G (2002) Regulation of life and death by the zinc finger transcription factor Egr-1. J Cell Physiol 193: 287-292.

94. Cummins EP, Taylor CT (2005) Hypoxia-responsive transcription factors. Pflugers Arch 450: 363-371.

95. Oikawa M, Abe M, Kurosawa H, Hida W, Shirato K, et al. (2001) Hypoxia induces transcription factor ETS-1 via the activity of hypoxia-inducible factor-1. Biochem Biophys Res Commun 289: 39-43.

96. Luger K, Richmond TJ (1998) The histone tails of the nucleosome. Curr Opin Genet Dev 8: 140-146.

97. Hayes JJ, Hansen JC (2001) Nucleosomes and the chromatin fiber. Curr Opin Genet Dev 11: 124-129.

98. Shilatifard A (2006) Chromatin modifications by methylation and ubiquitination: implications in the regulation of gene expression. Annu Rev Biochem 75: 243-269.

99. Vignali M, Hassan AH, Neely KE, Workman JL (2000) ATP-dependent chromatin-remodeling complexes. Mol Cell Biol 20: 1899-1910.

100. Varga-Weisz PD, Becker PB (2006) Regulation of higher-order chromatin structures by nucleosome-remodelling factors. Curr Opin Genet Dev 16: 151-156.

101. Jin J, Cai Y, Li B, Conaway RC, Workman JL, et al. (2005) In and out: histone variant exchange in chromatin. Trends Biochem Sci 30: 680-687.

102. Kasper LH, Boussouar F, Boyd K, Xu W, Biesen M, et al. (2005) Two transactivation mechanisms cooperate for the bulk of HIF-1-responsive gene expression. EMBO J 24: 3846-3858.

103. Drummond DC, Noble CO, Kirpotin DB, Guo Z, Scott GK, et al. (2005) Clinical development of histone deacetylase inhibitors as anticancer agents. Annu Rev Pharmacol Toxicol 45: 495-528.

104. Fath DM, Kong X, Liang D, Lin Z, Chou A, et al. (2006) Histone deacetylase inhibitors repress the transactivation potential of hypoxia-inducible factors independently of direct acetylation of HIF-alpha. J Biol Chem 281: 13612-13619.

105. Chen S, Sang N (2011) Histone deacetylase inhibitors: the epigenetic therapeutics that repress hypoxia-inducible factors. J Biomed Biotechnol 2011: 197946.

106. Kadam S, Emerson BM (2003) Transcriptional specificity of human SWI/SNF BRG1 and BRM chromatin remodeling complexes. Mol Cell 11: 377-389.

107. Wang F, Zhang R, Beischlag TV, Muchardt C, Yaniv M, et al. (2004) Roles of Brahma and Brahma/SWI2-related gene 1 in hypoxic induction of the erythropoietin gene. J Biol Chem 279: 46733-46741.

108. Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100: 57-70.

109. Roskelley CD, Bissell MJ (2002) The dominance of the microenvironment in breast and ovarian cancer. Semin Cancer Biol 12: 97-104.

110. Harris AL (2002) Hypoxia--a key regulatory factor in tumour growth. Nat Rev Cancer 2: 38-47.

111. Semenza GL (2000) Hypoxia, clonal selection, and the role of HIF-1 in tumor progression. Crit Rev Biochem Mol Biol 35: 71-103.

112. Gordan JD, Simon MC (2007) Hypoxia-inducible factors: central regulators of the tumor phenotype. Curr Opin Genet Dev 17: 71-77.

113. Li Z, Rich JN (2010) Hypoxia and hypoxia inducible factors in cancer stem cell maintenance. Curr Top Microbiol Immunol 345: 21-30.

114. Talks KL, Turley H, Gatter KC, Maxwell PH, Pugh CW, et al. (2000) The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. Am J Pathol 157: 411-421.

115. Zhong H, Semenza GL, Simons JW, De Marzo AM (2004) Up-regulation of hypoxia-inducible factor 1alpha is an early event in prostate carcinogenesis. Cancer Detect Prev 28: 88-93.

116. Trastour C, Benizri E, Ettore F, Ramaioli A, Chamorey E, et al. (2007) HIF-1alpha and CA IX staining in invasive breast carcinomas: prognosis and treatment outcome. Int J Cancer 120: 1451-1458.

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

118. Zundel W, Schindler C, Haas-Kogan D, Koong A, Kaper F, et al. (2000) Loss of PTEN facilitates HIF-1-mediated gene expression. Genes Dev 14: 391-396.

119. Carmeliet P, Dor Y, Herbert JM, Fukumura D, Brusselmans K, et al. (1998) Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. Nature 394: 485-490.

120. Ryan HE, Lo J, Johnson RS (1998) HIF-1 alpha is required for solid tumor formation and embryonic vascularization. EMBO J 17: 3005-3015.

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

122. Hammond EM, Giaccia AJ (2005) The role of p53 in hypoxia-induced apoptosis. Biochem Biophys Res Commun 331: 718-725.

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

124. Bertout JA, Patel SA, Simon MC (2008) The impact of O2 availability on human cancer. Nat Rev Cancer 8: 967-975.

125. Kaelin WG Jr (2008) The von Hippel-Lindau tumour suppressor protein: O2 sensing and cancer. Nat Rev Cancer 8: 865-873.

126. Majmundar AJ, Wong WJ, Simon MC (2010) Hypoxia-inducible factors and the response to hypoxic stress. Mol Cell 40: 294-309.

127. Huang X, Ding L, Bennewith KL, Tong RT, Welford SM, et al. (2009) Hypoxia-inducible mir-210 regulates normoxic gene expression involved in tumor initiation. Mol Cell 35: 856-867.

128. Das B, Tsuchida R, Malkin D, Koren G, Baruchel S, et al. (2008) Hypoxia enhances tumor stemness by increasing the invasive and tumorigenic side population fraction. Stem Cells 26: 1818-1830.

129. Heddleston JM, Li Z, McLendon RE, Hjelmeland AB, Rich JN (2009) The hypoxic microenvironment maintains glioblastoma stem cells and promotes reprogramming towards a cancer stem cell phenotype. Cell Cycle 8: 3274-3284.

130. Cairns RA, Harris IS, Mak TW (2011) Regulation of cancer cell metabolism. Nat Rev Cancer 11: 85-95.

131. WARBURG O (1956) On the origin of cancer cells. Science 123: 309-314.

132. Gatenby RA, Gillies RJ (2004) Why do cancers have high aerobic glycolysis? Nat Rev Cancer 4: 891-899.

133. Gatenby RA, Gillies RJ (2008) A microenvironmental model of carcinogenesis. Nat Rev Cancer 8: 56-61.

134. Wheaton WW, Chandel NS (2011) Hypoxia. 2. Hypoxia regulates cellular metabolism. Am J Physiol Cell Physiol 300: C385-C393.

135. Kim JW, Gao P, Dang CV (2007) Effects of hypoxia on tumor metabolism. Cancer Metastasis Rev 26: 291-298.

136. Kim JW, Tchernyshyov I, Semenza GL, Dang CV (2006) HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab 3: 177-185.

137. Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC (2006) HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metab 3: 187-197.

138. Grüning NM, Rinnerthaler M, Bluemlein K, Mülleder M, Wamelink MM, et al. (2011) Pyruvate kinase triggers a metabolic feedback loop that controls redox metabolism in respiring cells. Cell Metab 14: 415-427.

139. Luo W, Hu H, Chang R, Zhong J, Knabel M, et al. (2011) Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. Cell 145: 732-744.

140. Tennant DA (2011) PK-M2 Makes Cells Sweeter on HIF1. Cell 145: 647-649.

141. Chen N, Rinner O, Czernik D, Nytko KJ, Zheng D, et al. (2011) The oxygen sensor PHD3 limits glycolysis under hypoxia via direct binding to pyruvate kinase. Cell Res 21: 983-986.

142. Semenza GL (2002) Involvement of hypoxia-inducible factor 1 in human cancer. Intern Med 41: 79-83.

143. Brahimi-Horn MC, Pouysségur J (2007) Hypoxia in cancer cell metabolism and pH regulation. Essays Biochem 43: 165-178.

144. Lin Z, Weinberg JM, Malhotra R, Merritt SE, Holzman LB, et al. (2000) GLUT-1 reduces hypoxia-induced apoptosis and JNK pathway activation. Am J Physiol Endocrinol Metab 278: E958-966.

145. Marín-Hernández A, Gallardo-Pérez JC, Ralph SJ, Rodríguez-Enríquez S, Moreno-Sánchez R (2009) HIF-1alpha modulates energy metabolism in cancer cells by inducing over-expression of specific glycolytic isoforms. Mini Rev Med Chem 9: 1084-1101.

146. Kilic M, Kasperczyk H, Fulda S, Debatin KM (2007) Role of hypoxia inducible factor-1 alpha in modulation of apoptosis resistance. Oncogene 26: 2027-2038.

147. Zhang H, Gao P, Fukuda R, Kumar G, Krishnamachary B, et al. (2007) HIF-1 inhibits mitochondrial biogenesis and cellular respiration in VHL-deficient renal cell carcinoma by repression of C-MYC activity. Cancer Cell 11: 407-420.

148. Wouters BG, Koritzinsky M (2008) Hypoxia signalling through mTOR and the unfolded protein response in cancer. Nat Rev Cancer 8: 851-864.

149. Dungwa JV, Hunt LP, Ramani P (2011) Overexpression of carbonic anhydrase and HIF-1α in Wilms tumours. BMC Cancer 11: 390.

150. Herr B, Zhou J, Werno C, Menrad H, Namgaladze D, et al. (2009) The supernatant of apoptotic cells causes transcriptional activation of hypoxia-inducible factor-1alpha in macrophages via sphingosine-1-phosphate and transforming growth factor-beta. Blood 114: 2140-2148.

151. Murata Y, Ohteki T, Koyasu S, Hamuro J (2002) IFN-gamma and pro-inflammatory cytokine production by antigen-presenting cells is dictated by intracellular thiol redox status regulated by oxygen tension. Eur J Immunol 32: 2866-2873.

152. Lewis C, Murdoch C (2005) Macrophage responses to hypoxia: implications for tumor progression and anti-cancer therapies. Am J Pathol 167: 627-635.

153. Makino Y, Nakamura H, Ikeda E, Ohnuma K, Yamauchi K, et al. (2003) Hypoxia-inducible factor regulates survival of antigen receptor-driven T cells. J Immunol 171: 6534-6540.

154. Sitkovsky M, Lukashev D (2005) Regulation of immune cells by local-tissue oxygen tension: HIF1 alpha and adenosine receptors. Nat Rev Immunol 5: 712-721.

155. Barsoum IB, Hamilton TK, Li X, Cotechini T, Miles EA, et al. (2011) Hypoxia Induces Escape from Innate Immunity in Cancer Cells via Increased Expression of ADAM10: Role of Nitric Oxide. Cancer Res 71: 7433-7441

156. Leek RD, Talks KL, Pezzella F, Turley H, Campo L, et al. (2002) Relation of hypoxia-inducible factor-2 alpha (HIF-2 alpha) expression in tumor-infiltrative macrophages to tumor angiogenesis and the oxidative thymidine phosphorylase pathway in Human breast cancer. Cancer Res 62: 1326-1329.

157. Kawanaka T, Kubo A, Ikushima H, Sano T, Takegawa Y, et al. (2008) Prognostic significance of HIF-2alpha expression on tumor infiltrating macrophages in patients with uterine cervical cancer undergoing radiotherapy. J Med Invest 55: 78-86.

158. Imtiyaz HZ, Williams EP, Hickey MM, Patel SA, Durham AC, et al. (2010) Hypoxia-inducible factor 2alpha regulates macrophage function in mouse models of acute and tumor inflammation. J Clin Invest 120: 2699-2714.

159. Kim M, Park SY, Pai HS, Kim TH, Billiar TR, et al. (2004) Hypoxia inhibits tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis by blocking Bax translocation. Cancer Res 64: 4078-4081.

160. Feng J, Funk WD, Wang SS, Weinrich SL, Avilion AA, et al. (1995) The RNA component of human telomerase. Science 269: 1236-1241.

161. Yang J, Chang E, Cherry AM, Bangs CD, Oei Y, et al. (1999) Human endothelial cell life extension by telomerase expression. J Biol Chem 274: 26141-26148.

162. Ouellette MM, Liao M, Herbert BS, Johnson M, Holt SE, et al. (2000) Subsenescent telomere lengths in fibroblasts immortalized by limiting amounts of telomerase. J Biol Chem 275: 10072-10076.

163. Ramirez RD, Morales CP, Herbert BS, Rohde JM, Passons C, et al. (2001) Putative telomere-independent mechanisms of replicative aging reflect inadequate growth conditions. Genes Dev 15: 398-403.

164. Reichman TW, Albanell J, Wang X, Moore MA, Studzinski GP (1997) Downregulation of telomerase activity in HL60 cells by differentiating agents is accompanied by increased expression of telomerase-associated protein. J Cell Biochem 67: 13-23.

165. Seimiya H, Tanji M, Oh-hara T, Tomida A, Naasani I, et al. (1999) Hypoxia up-regulates telomerase activity via mitogen-activated protein kinase signaling in human solid tumor cells. Biochem Biophys Res Commun 260: 365-370.

166. Minamino T, Mitsialis SA, Kourembanas S (2001) Hypoxia extends the life span of vascular smooth muscle cells through telomerase activation. Mol Cell Biol 21: 3336-3342.

167. Yuan J, Glazer PM (1998) Mutagenesis induced by the tumor microenvironment. Mutat Res 400: 439-446.

168. Yuan J, Narayanan L, Rockwell S, Glazer PM (2000) Diminished DNA repair and elevated mutagenesis in mammalian cells exposed to hypoxia and low pH. Cancer Res 60: 4372-4376.

169. Huang LE, Bindra RS, Glazer PM, Harris AL (2007) Hypoxia-induced genetic instability--a calculated mechanism underlying tumor progression. J Mol Med (Berl) 85: 139-148.

170. Bindra RS, Crosby ME, Glazer PM (2007) Regulation of DNA repair in hypoxic cancer cells. Cancer Metastasis Rev 26: 249-260.

171. Bristow RG, Hill RP (2008) Hypoxia and metabolism. Hypoxia, DNA repair and genetic instability. Nat Rev Cancer 8: 180-192.

172. Koshiji M, To KK, Hammer S, Kumamoto K, Harris AL, et al. (2005) HIF-1alpha induces genetic instability by transcriptionally downregulating MutSalpha expression. Mol Cell 17: 793-803.

173. To KK, Sedelnikova OA, Samons M, Bonner WM, Huang LE (2006) The phosphorylation status of PAS-B distinguishes HIF-1alpha from HIF-2alpha in NBS1 repression. EMBO J 25: 4784-4794.

174. Busuttil RA, Rubio M, Dollé ME, Campisi J, Vijg J (2003) Oxygen accelerates the accumulation of mutations during the senescence and immortalization of murine cells in culture. Aging Cell 2: 287-294.

175. Forsyth NR, Musio A, Vezzoni P, Simpson AH, Noble BS, et al. (2006) Physiologic oxygen enhances human embryonic stem cell clonal recovery and reduces chromosomal abnormalities. Cloning Stem Cells 8: 16-23.

176. Zhu LL, Wu LY, Yew DT, Fan M (2005) Effects of hypoxia on the proliferation and differentiation of NSCs. Mol Neurobiol 31: 231-242.

177. Cipolleschi MG, Dello Sbarba P, Olivotto M (1993) The role of hypoxia in the maintenance of hematopoietic stem cells. Blood 82: 2031-2037.

178. IvanoviÄ Z, Dello Sbarba P, Trimoreau F, Faucher JL, Praloran V (2000) Primitive human HPCs are better maintained and expanded in vitro at 1 percent oxygen than at 20 percent. Transfusion 40: 1482-1488.

179. Ramírez-Bergeron DL, Simon MC (2001) Hypoxia-inducible factor and the development of stem cells of the cardiovascular system. Stem Cells 19: 279-286.

180. Passos JF, Simillion C, Hallinan J, Wipat A, von Zglinicki T (2009) Cellular senescence: unravelling complexity. Age (Dordr) 31: 353-363.

181. Ralph SJ, Rodríguez-Enríquez S, Neuzil J, Saavedra E, Moreno-Sánchez R (2010) The causes of cancer revisited: «mitochondrial malignancy» and ROS-induced oncogenic transformation - why mitochondria are targets for cancer therapy. Mol Aspects Med 31: 145-170.

182. Li TS, Marbán E (2010) Physiological levels of reactive oxygen species are required to maintain genomic stability in stem cells. Stem Cells 28: 1178-1185.

183. Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, et al. (1996) Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol Cell Biol 16: 4604-4613.

184. Laderoute KR, Alarcon RM, Brody MD, Calaoagan JM, Chen EY, et al. (2000) Opposing effects of hypoxia on expression of the angiogenic inhibitor thrombospondin 1 and the angiogenic inducer vascular endothelial growth factor. Clin Cancer Res 6: 2941-2950.

185. Du R, Lu KV, Petritsch C, Liu P, Ganss R, et al. (2008) HIF1alpha induces the recruitment of bone marrow-derived vascular modulatory cells to regulate tumor angiogenesis and invasion. Cancer Cell 13: 206-220.

186. Bao S, Wu Q, Sathornsumetee S, Hao Y, Li Z, et al. (2006) Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. Cancer Res 66: 7843-7848.

187. Wysoczynski M, Shin DM, Kucia M, Ratajczak MZ (2010) Selective upregulation of interleukin-8 by human rhabdomyosarcomas in response to hypoxia: therapeutic implications. Int J Cancer 126: 371-381.

188. Dejean E, Renalier MH, Foisseau M, Agirre X, Joseph N, et al. (2011) Hypoxia-microRNA-16 downregulation induces VEGF expression in anaplastic lymphoma kinase (ALK)-positive anaplastic large-cell lymphomas. Leukemia 25: 1882-1890.

189. Semenza GL (2009) Regulation of cancer cell metabolism by hypoxia-inducible factor 1. Semin Cancer Biol 19: 12-16.

190. Khatua S, Peterson KM, Brown KM, Lawlor C, Santi MR, et al. (2003) Overexpression of the EGFR/FKBP12/HIF-2alpha pathway identified in childhood astrocytomas by angiogenesis gene profiling. Cancer Res 63: 1865-1870.

191. Yang QC, Zeng BF, Dong Y, Shi ZM, Jiang ZM, et al. (2007) Overexpression of hypoxia-inducible factor-1alpha in human osteosarcoma: correlation with clinicopathological parameters and survival outcome. Jpn J Clin Oncol 37: 127-134.

192. Aryee DN, Niedan S, Kauer M, Schwentner R, Bennani-Baiti IM, et al. (2010) Hypoxia modulates EWS-FLI1 transcriptional signature and enhances the malignant properties of Ewing's sarcoma cells in vitro. Cancer Res 70: 4015-4023.

193. Hashimoto O, Shimizu K, Semba S, Chiba S, Ku Y, et al. (2011) Hypoxia induces tumor aggressiveness and the expansion of CD133-positive cells in a hypoxia-inducible factor-1Î±-dependent manner in pancreatic cancer cells. Pathobiology 78: 181-192.

194. Gatenby RA, Smallbone K, Maini PK, Rose F, Averill J, et al. (2007) Cellular adaptations to hypoxia and acidosis during somatic evolution of breast cancer. Br J Cancer 97: 646-653.

195. Sullivan R, Graham CH (2007) Hypoxia-driven selection of the metastatic phenotype. Cancer Metastasis Rev 26: 319-331.

196. Jögi A, Øra I, Nilsson H, Poellinger L, Axelson H, et al. (2003) Hypoxia-induced dedifferentiation in neuroblastoma cells. Cancer Lett 197: 145-150.

197. Chan DA, Giaccia AJ (2007) Hypoxia, gene expression, and metastasis. Cancer Metastasis Rev 26: 333-339.

198. Teicher BA (1995) Physiologic mechanisms of therapeutic resistance. Blood flow and hypoxia. Hematol Oncol Clin North Am 9: 475-506.

199. Kumar P (2000) Impact of Anemia in Patients with Head and Neck Cancer. Oncologist 2: 13-18.

200. Harrison L, Blackwell K (2004) Hypoxia and anemia: factors in decreased sensitivity to radiation therapy and chemotherapy? Oncologist 5: 31-40.

201. Hsieh CH, Lee CH, Liang JA, Yu CY, Shyu WC (2010) Cycling hypoxia increases U87 glioma cell radioresistance via ROS induced higher and long-term HIF-1 signal transduction activity. Oncol Rep 24: 1629-1636.

202. McGee MC, Hamner JB, Williams RF, Rosati SF, Sims TL, et al. (2010) Improved intratumoral oxygenation through vascular normalization increases glioma sensitivity to ionizing radiation. Int J Radiat Oncol Biol Phys 76: 1537-1545.

203. Yang B, Fan L, Fang L, He Q (2006) Hypoxia-mediated fenretinide (4-HPR) resistance in childhood acute lymphoblastic leukemia cells. Cancer Chemother Pharmacol 58: 540-546.

204. Ogiso Y, Tomida A, Lei S, Omura S, Tsuruo T (2000) Proteasome inhibition circumvents solid tumor resistance to topoisomerase II-directed drugs. Cancer Res 60: 2429-2434.

205. Nardinocchi L, Puca R, Sacchi A, D'Orazi G (2009) Inhibition of HIF-1alpha activity by homeodomain-interacting protein kinase-2 correlates with sensitization of chemoresistant cells to undergo apoptosis. Mol Cancer 8: 1.

206. Maddika S, Ande SR, Panigrahi S, Paranjothy T, Weglarczyk K, et al. (2007) Cell survival, cell death and cell cycle pathways are interconnected: implications for cancer therapy. Drug Resist Updat 10: 13-29.

207. Hussein D, Estlin EJ, Dive C, Makin GW (2006) Chronic hypoxia promotes hypoxia-inducible factor-1alpha-dependent resistance to etoposide and vincristine in neuroblastoma cells. Mol Cancer Ther 5: 2241-2250.

208. Gwak GY, Yoon JH, Kim KM, Lee HS, Chung JW, et al. (2005) Hypoxia stimulates proliferation of human hepatoma cells through the induction of hexokinase II expression. J Hepatol 42: 358-364.

209. Mathupala SP, Rempel A, Pedersen PL (2001) Glucose catabolism in cancer cells: identification and characterization of a marked activation response of the type II hexokinase gene to hypoxic conditions. J Biol Chem 276: 43407-43412.

210. Giatromanolaki A, Koukourakis MI, Pezzella F, Sivridis E, Turley H, et al. (2008) Lactate dehydrogenase 5 expression in non-Hodgkin B-cell lymphomas is associated with hypoxia regulated proteins. Leuk Lymphoma 49: 2181-2186.

211. Karth J, Ferrer FA, Perlman E, Hanrahan C, Simons JW, et al. (2000) Coexpression of hypoxia-inducible factor 1-alpha and vascular endothelial growth factor in Wilms' tumor. J Pediatr Surg 35: 1749-1753.

212. Rössler J, Breit S, Havers W, Schweigerer L (1999) Vascular endothelial growth factor expression in human neuroblastoma: up-regulation by hypoxia. Int J Cancer 81: 113-117.

213. Jögi A, Vallon-Christersson J, Holmquist L, Axelson H, Borg A, et al. (2004) Human neuroblastoma cells exposed to hypoxia: induction of genes associated with growth, survival, and aggressive behavior. Exp Cell Res 295: 469-487.

214. Fujiwara S, Nakagawa K, Harada H, Nagato S, Furukawa K, et al. (2007) Silencing hypoxia-inducible factor-1alpha inhibits cell migration and invasion under hypoxic environment in malignant gliomas. Int J Oncol 30: 793-802.

215. Miyoshi A, Kitajima Y, Ide T, Ohtaka K, Nagasawa H, et al. (2006) Hypoxia accelerates cancer invasion of hepatoma cells by upregulating MMP expression in an HIF-1alpha-independent manner. Int J Oncol 29: 1533-1539.

216. Zagzag D, Nomura M, Friedlander DR, Blanco CY, Gagner JP, et al. (2003) Geldanamycin inhibits migration of glioma cells in vitro: a potential role for hypoxia-inducible factor (HIF-1alpha) in glioma cell invasion. J Cell Physiol 196: 394-402.

217. Pistollato F, Rampazzo E, Persano L, Abbadi S, Frasson C, et al. (2010) Interaction of hypoxia-inducible factor-1Î± and Notch signaling regulates medulloblastoma precursor proliferation and fate. Stem Cells 28: 1918-1929.

218. Holmquist-Mengelbier L, Fredlund E, Löfstedt T, Noguera R, Navarro S, et al. (2006) Recruitment of HIF-1alpha and HIF-2alpha to common target genes is differentially regulated in neuroblastoma: HIF-2alpha promotes an aggressive phenotype. Cancer Cell 10: 413-423.

219. Brown JM, Wilson WR (2004) Exploiting tumour hypoxia in cancer treatment. Nat Rev Cancer 4: 437-447.

220. Kalns J, Krock L, Piepmeier E Jr (1998) The effect of hyperbaric oxygen on growth and chemosensitivity of metastatic prostate cancer. Anticancer Res 18: 363-367.

221. Bennett M, Feldmeier J, Smee R, Milross C (2005) Hyperbaric oxygenation for tumour sensitisation to radiotherapy. Cochrane Database Syst Rev.

222. Benizri E, Ginouvès A, Berra E (2008) The magic of the hypoxia-signaling cascade. Cell Mol Life Sci 65: 1133-1149.

223. Overgaard J, Hansen HS, Overgaard M, Bastholt L, Berthelsen A, et al. (1998) A randomized double-blind phase III study of nimorazole as a hypoxic radiosensitizer of primary radiotherapy in supraglottic larynx and pharynx carcinoma. Results of the Danish Head and Neck Cancer Study (DAHANCA) Protocol 5-85. Radiother Oncol 46: 135-146.

224. Huncharek M (1998) Meta-analytic re-evaluation of misonidazole in the treatment of high grade astrocytoma. Anticancer Res 18: 1935-1939.

225. Kerbel RS (2006) Antiangiogenic therapy: a universal chemosensitization strategy for cancer? Science 312: 1171-1175.

226. Nagasawa H, Uto Y, Kirk KL, Hori H (2006) Design of hypoxia-targeting drugs as new cancer chemotherapeutics. Biol Pharm Bull 29: 2335-2342.

227. Albertella MR, Loadman PM, Jones PH, Phillips RM, Rampling R, et al. (2008) Hypoxia-selective targeting by the bioreductive prodrug AQ4N in patients with solid tumors: results of a phase I study. Clin Cancer Res 14: 1096-1104.

228. Barth RF, Coderre JA, Vicente MG, Blue TE (2005) Boron neutron capture therapy of cancer: current status and future prospects. Clin Cancer Res 11: 3987-4002.

229. Pietrancosta N, Maina F, Dono R, Moumen A, Garino C, et al. (2005) Novel cyclized Pifithrin-alpha p53 inactivators: synthesis and biological studies. Bioorg Med Chem Lett 15: 1561-1564.

230. Rojas A (1992) ARCON: accelerated radiotherapy with carbogen and nicotinamide. BJR 24: 174-178.

231. Bernier J, Denekamp J, Rojas A, Trovò M, Horiot JC, et al. (1999) ARCON: accelerated radiotherapy with carbogen and nicotinamide in non small cell lung cancer: a phase I/II study by the EORTC. Radiother Oncol 52: 149-156.

232. Hoskin PJ, Rojas AM, Phillips H, Saunders MI (2005) Acute and late morbidity in the treatment of advanced bladder carcinoma with accelerated radiotherapy, carbogen, and nicotinamide. Cancer 103: 2287-2297.

233. Wouters BG, Wang LH, Brown JM (1999) Tirapazamine: a new drug producing tumor specific enhancement of platinum-based chemotherapy in non-small-cell lung cancer. Ann Oncol 5: S29-S33.

234. Weitman S, Mangold G, Marty J, Dexter D, Hilsenbeck S, et al. (1999) Evidence of enhanced in vivo activity using tirapazamine with paclitaxel and paraplatin regimens against the MV-522 human lung cancer xenograft. Cancer Chemother Pharmacol 43: 402-408.

235. Williamson SK, Crowley JJ, Lara PN Jr, McCoy J, Lau DH, et al. (2005) Phase III trial of paclitaxel plus carboplatin with or without tirapazamine in advanced non-small-cell lung cancer: Southwest Oncology Group Trial S0003. J Clin Oncol 23: 9097-9104.

236. von Pawel J, von Roemeling R, Gatzemeier U, Boyer M, Elisson LO, et al. (2000) Tirapazamine plus cisplatin versus cisplatin in advanced non-small-cell lung cancer: A report of the international CATAPULT I study group. Cisplatin and Tirapazamine in Subjects with Advanced Previously Untreated Non-Small-Cell Lung Tumors. J Clin Oncol 18: 1351-1359.

237. Rischin D, Peters L, Fisher R, Macann A, Denham J, et al. (2005) Tirapazamine, Cisplatin, and Radiation versus Fluorouracil, Cisplatin, and Radiation in patients with locally advanced head and neck cancer: a randomized phase II trial of the Trans-Tasman Radiation Oncology Group (TROG 98.02). J Clin Oncol 23: 79-87.

238. Theys J, Landuyt W, Nuyts S, Van Mellaert L, van Oosterom A, et al. (2001) Specific targeting of cytosine deaminase to solid tumors by engineered Clostridium acetobutylicum. Cancer Gene Ther 8: 294-297.

239. Liu SC, Minton NP, Giaccia AJ, Brown JM (2002) Anticancer efficacy of systemically delivered anaerobic bacteria as gene therapy vectors targeting tumor hypoxia/necrosis. Gene Ther 9: 291-296.

240. Silver DF, Piver MS (1999) Effects of recombinant human erythropoietin on the antitumor effect of cisplatin in SCID mice bearing human ovarian cancer: A possible oxygen effect. Gynecol Oncol 73: 280-284.

241. Stüben G, Thews O, Pöttgen C, Knühmann K, Vaupel P, et al. (2001) Recombinant human erythropoietin increases the radiosensitivity of xenografted human tumours in anaemic nude mice. J Cancer Res Clin Oncol 127: 346-350.

242. Seufferlein T, Rozengurt E (1996) Rapamycin inhibits constitutive p70s6k phosphorylation, cell proliferation, and colony formation in small cell lung cancer cells. Cancer Res 56: 3895-3897.

243. Hosoi H, Dilling MB, Shikata T, Liu LN, Shu L, et al. (1999) Rapamycin causes poorly reversible inhibition of mTOR and induces p53-independent apoptosis in human rhabdomyosarcoma cells. Cancer Res 59: 886-894.

244. Grewe M, Gansauge F, Schmid RM, Adler G, Seufferlein T (1999) Regulation of cell growth and cyclin D1 expression by the constitutively active FRAP-p70s6K pathway in human pancreatic cancer cells. Cancer Res 59: 3581-3587.

245. Pang H, Faber LE (2001) Estrogen and rapamycin effects on cell cycle progression in T47D breast cancer cells. Breast Cancer Res Treat 70: 21-26.

246. van der Poel HG, Hanrahan C, Zhong H, Simons JW (2003) Rapamycin induces Smad activity in prostate cancer cell lines. Urol Res 30: 380-386.

247. Guba M, von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, et al. (2002) Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. Nat Med 8: 128-135.

248. Del Bufalo D, Ciuffreda L, Trisciuoglio D, Desideri M, Cognetti F, et al. (2006) Antiangiogenic potential of the Mammalian target of rapamycin inhibitor temsirolimus. Cancer Res 66: 5549-5554.

249. Wan X, Shen N, Mendoza A, Khanna C, Helman LJ (2006) CCI-779 inhibits rhabdomyosarcoma xenograft growth by an antiangiogenic mechanism linked to the targeting of mTOR/Hif-1alpha/VEGF signaling. Neoplasia 8: 394-401.

250. Gabardi S, Cerio J (2004) Future immunosuppressive agents in solid-organ transplantation. Prog Transplant 14: 148-156.

251. Tanaka C, O'Reilly T, Kovarik JM, Shand N, Hazell K, et al. (2008) Identifying optimal biologic doses of everolimus (RAD001) in patients with cancer based on the modeling of preclinical and clinical pharmacokinetic and pharmacodynamic data. J Clin Oncol 26: 1596-1602.

252. O'Donnell A, Faivre S, Burris HA 3rd, Rea D, Papadimitrakopoulou V, et al. (2008) Phase I pharmacokinetic and pharmacodynamic study of the oral mammalian target of rapamycin inhibitor everolimus in patients with advanced solid tumors. J Clin Oncol 26: 1588-1595.

253. Mita M, Sankhala K, Abdel-Karim I, Mita A, Giles F (2008) Deforolimus (AP23573) a novel mTOR inhibitor in clinical development. Expert Opin Investig Drugs 17: 1947-1954.

254. Zhou H, Luo Y, Huang S (2010) Updates of mTOR inhibitors. Anticancer Agents Med Chem 10: 571-581.

255. Pore N, Jiang Z, Gupta A, Cerniglia G, Kao GD, et al. (2006) EGFR tyrosine kinase inhibitors decrease VEGF expression by both hypoxia-inducible factor (HIF)-1-independent and HIF-1-dependent mechanisms. Cancer Res 66: 3197-3204.

256. Luwor RB, Lu Y, Li X, Mendelsohn J, Fan Z (2005) The antiepidermal growth factor receptor monoclonal antibody cetuximab/C225 reduces hypoxia-inducible factor-1 alpha, leading to transcriptional inhibition of vascular endothelial growth factor expression. Oncogene 24: 4433-4441.

257. Hudis CA (2007) Trastuzumab--mechanism of action and use in clinical practice. N Engl J Med 357: 39-51.

258. Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL, et al. (2001) 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 21: 3995-4004.

259. Koukourakis MI, Simopoulos C, Polychronidis A, Perente S, Botaitis S, et al. (2003) The effect of trastuzumab/docatexel combination on breast cancer angiogenesis: dichotomus effect predictable by the HIFI alpha/VEGF pre-treatment status? Anticancer Res 23: 1673-1680.

260. Litz J, Krystal GW (2006) Imatinib inhibits c-Kit-induced hypoxia-inducible factor-1alpha activity and vascular endothelial growth factor expression in small cell lung cancer cells. Mol Cancer Ther 5: 1415-1422.

261. Melillo G (2007) Targeting hypoxia cell signaling for cancer therapy. Cancer Metastasis Rev 26: 341-352.

262. Kang SH, Cho HT, Devi S, Zhang Z, Escuin D, et al. (2006) Antitumor effect of 2-methoxyestradiol in a rat orthotopic brain tumor model. Cancer Res 66: 11991-11997.

263. Ricker JL, Chen Z, Yang XP, Pribluda VS, Swartz GM, et al. (2004) 2-methoxyestradiol inhibits hypoxia-inducible factor 1alpha, tumor growth, and angiogenesis and augments paclitaxel efficacy in head and neck squamous cell carcinoma. Clin Cancer Res 10: 8665-8673.

264. Pommier Y (2006) Topoisomerase I inhibitors: camptothecins and beyond. Nat Rev Cancer 6: 789-802.

265. Rapisarda A, Uranchimeg B, Sordet O, Pommier Y, Shoemaker RH, et al. (2004) Topoisomerase I-mediated inhibition of hypoxia-inducible factor 1: mechanism and therapeutic implications. Cancer Res 64: 1475-1482.

266. Puppo M, Battaglia F, Ottaviano C, Delfino S, Ribatti D, et al. (2008) Topotecan inhibits vascular endothelial growth factor production and angiogenic activity induced by hypoxia in human neuroblastoma by targeting hypoxia-inducible factor-1alpha and -2alpha. Mol Cancer Ther 7: 1974-1984.

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

268. Lee K, Kim HM (2011) A novel approach to cancer therapy using PX-478 as a HIF-1Î± inhibitor. Arch Pharm Res 34: 1583-1585.

269. Chun YS, Yeo EJ, Park JW (2004) Versatile pharmacological actions of YC-1: anti-platelet to anticancer. Cancer Lett 207: 1-7.

270. Tulis DA (2004) Salutary properties of YC-1 in the cardiovascular and hematological systems. Curr Med Chem Cardiovasc Hematol Agents 2: 343-359.

271. Chun YS, Yeo EJ, Choi E, Teng CM, Bae JM, et al. (2001) Inhibitory effect of YC-1 on the hypoxic induction of erythropoietin and vascular endothelial growth factor in Hep3B cells. Biochem Pharmacol 61: 947-954.

272. Yeo EJ, Chun YS, Cho YS, Kim J, Lee JC, et al. (2003) YC-1: a potential anticancer drug targeting hypoxia-inducible factor 1. J Natl Cancer Inst 95: 516-525.

273. Minet E, Mottet D, Michel G, Roland I, Raes M, et al. (1999) Hypoxia-induced activation of HIF-1: role of HIF-1alpha-Hsp90 interaction. FEBS Lett 460: 251-256.

274. Isaacs JS, Jung YJ, Mimnaugh EG, Martinez A, Cuttitta F, et al. (2002) Hsp90 regulates a von Hippel Lindau-independent hypoxia-inducible factor-1 alpha-degradative pathway. J Biol Chem 277: 29936-29944.

275. Mabjeesh NJ, Post DE, Willard MT, Kaur B, Van Meir EG, et al. (2002) Geldanamycin induces degradation of hypoxia-inducible factor 1alpha protein via the proteosome pathway in prostate cancer cells. Cancer Res 62: 2478-2482.

276. Hur E, Kim HH, Choi SM, Kim JH, Yim S, et al. (2002) Reduction of hypoxia-induced transcription through the repression of hypoxia-inducible factor-1alpha/aryl hydrocarbon receptor nuclear translocator DNA binding by the 90-kDa heat-shock protein inhibitor radicicol. Mol Pharmacol 62: 975-982.

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

278. Han JY, Oh SH, Morgillo F, Myers JN, Kim E, et al. (2005) Hypoxia-inducible factor 1alpha and antiangiogenic activity of farnesyltransferase inhibitor SCH66336 in human aerodigestive tract cancer. J Natl Cancer Inst 97: 1272-1286.

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

280. Kong X, Lin Z, Liang D, Fath D, Sang N, et al. (2006) Histone deacetylase inhibitors induce VHL and ubiquitin-independent proteasomal degradation of hypoxia-inducible factor 1alpha. Mol Cell Biol 26: 2019-2028.

281. Qian DZ, Kachhap SK, Collis SJ, Verheul HM, Carducci MA, et al. (2006) Class II histone deacetylases are associated with VHL-independent regulation of hypoxia-inducible factor 1 alpha. Cancer Res 66: 8814-8821.

282. Chen S, Sang N (2011) Histone deacetylase inhibitors: the epigenetic therapeutics that repress hypoxia-inducible factors. J Biomed Biotechnol 2011: 197946.

283. Welsh SJ, Bellamy WT, Briehl MM, Powis G (2002) The redox protein thioredoxin-1 (Trx-1) increases hypoxia-inducible factor 1alpha protein expression: Trx-1 overexpression results in increased vascular endothelial growth factor production and enhanced tumor angiogenesis. Cancer Res 62: 5089-5095.

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

285. Kung AL, Zabludoff SD, France DS, Freedman SJ, Tanner EA, et al. (2004) Small molecule blockade of transcriptional coactivation of the hypoxia-inducible factor pathway. Cancer Cell 6: 33-43.

286. Muss HB, Blessing JA, DuBeshter B (1993) Echinomycin in recurrent and metastatic endometrial carcinoma. A phase II trial of the Gynecologic Oncology Group. Am J Clin Oncol 16: 492-493.

287. Chang AY, Kim K, Boucher H, Bonomi P, Stewart JA, et al. (1998) A randomized phase II trial of echinomycin, trimetrexate, and cisplatin plus etoposide in patients with metastatic nonsmall cell lung carcinoma: an Eastern Cooperative Oncology Group Study (E1587). Cancer 82: 292-300.

288. Kong D, Park EJ, Stephen AG, Calvani M, Cardellina JH, et al. (2005) Echinomycin, a small-molecule inhibitor of hypoxia-inducible factor-1 DNA-binding activity. Cancer Res 65: 9047-9055.

289. Knowles HJ, Raval RR, Harris AL, Ratcliffe PJ (2003) Effect of ascorbate on the activity of hypoxia-inducible factor in cancer cells. Cancer Res 63: 1764-1768.

290. Fang J, Cao Z, Chen YC, Reed E, Jiang BH (2004) 9-beta-D-arabinofuranosyl-2-fluoroadenine inhibits expression of vascular endothelial growth factor through hypoxia-inducible factor-1 in human ovarian cancer cells. Mol Pharmacol 66: 178-186.

291. Jones MK, Szabó IL, Kawanaka H, Husain SS, Tarnawski AS (2002) von Hippel Lindau tumor suppressor and HIF-1alpha: new targets of NSAIDs inhibition of hypoxia-induced angiogenesis. FASEB J 16: 264-266.

292. Palayoor ST, Tofilon PJ, Coleman CN (2003) Ibuprofen-mediated reduction of hypoxia-inducible factors HIF-1alpha and HIF-2alpha in prostate cancer cells. Clin Cancer Res 9: 3150-3157.

293. Melillo G, Sausville EA, Cloud K, Lahusen T, Varesio L, et al. (1999) Flavopiridol, a protein kinase inhibitor, down-regulates hypoxic induction of vascular endothelial growth factor expression in human monocytes. Cancer Res 59: 5433-5437.

294. Newcomb EW, Ali MA, Schnee T, Lan L, Lukyanov Y, et al. (2005) Flavopiridol downregulates hypoxia-mediated hypoxia-inducible factor-1alpha expression in human glioma cells by a proteasome-independent pathway: implications for in vivo therapy. Neuro Oncol 7: 225-235.

295. Olenyuk BZ, Zhang GJ, Klco JM, Nickols NG, Kaelin WG Jr, et al. (2004) Inhibition of vascular endothelial growth factor with a sequence-specific hypoxia response element antagonist. Proc Natl Acad Sci U S A 101: 16768-16773.

296. Nickols NG, Jacobs CS, Farkas ME, Dervan PB (2007) Improved nuclear localization of DNA-binding polyamides. Nucleic Acids Res 35: 363-370.

297. Kaluz S, Kaluzová M, Stanbridge EJ (2006) 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 26: 5895-5907.

298. Choi H, Chun YS, Kim SW, Kim MS, Park JW (2006) Curcumin inhibits hypoxia-inducible factor-1 by degrading aryl hydrocarbon receptor nuclear translocator: a mechanism of tumor growth inhibition. Mol Pharmacol 70: 1664-1671.

299. Lin S, Tsai SC, Lee CC, Wang BW, Liou JY, et al. (2004) Berberine inhibits HIF-1alpha expression via enhanced proteolysis. Mol Pharmacol 66: 612-619.

300. Zhang Q, Tang X, Lu QY, Zhang ZF, Brown J, et al. (2005) Resveratrol inhibits hypoxia-induced accumulation of hypoxia-inducible factor-1alpha and VEGF expression in human tongue squamous cell carcinoma and hepatoma cells. Mol Cancer Ther 4: 1465-1474.

301. Hasebe Y, Egawa K, Yamazaki Y, Kunimoto S, Hirai Y, et al. (2003) Specific inhibition of hypoxia-inducible factor (HIF)-1 alpha activation and of vascular endothelial growth factor (VEGF) production by flavonoids. Biol Pharm Bull 26: 1379-1383.

302. Büchler P, Reber HA, Büchler MW, Friess H, Lavey RS, et al. (2004) Antiangiogenic activity of genistein in pancreatic carcinoma cells is mediated by the inhibition of hypoxia-inducible factor-1 and the down-regulation of VEGF gene expression. Cancer 100: 201-210.

303. Nardinocchi L, Pantisano V, Puca R, Porru M, Aiello A, et al. (2010) Zinc downregulates HIF-1α and inhibits its activity in tumor cells in vitro and in vivo. PLoS One 5: e15048.

304. Sheffer M, Simon AJ, Jacob-Hirsch J, Rechavi G, Domany E, et al. (2011) Genome-wide analysis discloses reversal of the hypoxia-induced changes of gene expression in colon cancer cells by zinc supplementation. Oncotarget 2: 1191-1202.

305. D'Angelo G, Duplan E, Vigne P, Frelin C (2003) Cyclosporin A prevents the hypoxic adaptation by activating hypoxia-inducible factor-1alpha Pro-564 hydroxylation. J Biol Chem 278: 15406-15411.

306. Temes E, Martín-Puig S, Acosta-Iborra B, Castellanos MC, Feijoo-Cuaresma M, et al. (2005) Activation of HIF-prolyl hydroxylases by R59949, an inhibitor of the diacylglycerol kinase. J Biol Chem 280: 24238-24244.

307. Gao P, Zhang H, Dinavahi R, Li F, Xiang Y, et al. (2007) HIF-dependent antitumorigenic effect of antioxidants in vivo. Cancer Cell 12: 230-238.

308. Burger RA, Brady MF, Bookman MA, Walker JL, Homesley HD, et al. (2010) Phase III trial of bevacizumab (BEV) in the primary treatment of advanced epithelial ovarian cancer (EOC), primary peritoneal cancer (PPC), or fallopian tube cancer (FTC): A Gynecologic Oncology Group study. J Clin Oncol 28.

309. Ebos JM, Kerbel RS (2011) Antiangiogenic therapy: impact on invasion, disease progression, and metastasis. Nat Rev Clin Oncol 8: 210-221.

310. Barrios CH, Liu MC, Lee SC, Vanlemmens L, Ferrero JM, et al. (2010) Phase III randomized trial of sunitinib versus capecitabine in patients with previously treated HER2-negative advanced breast cancer. Breast Cancer Res Treat 121: 121-131.

311. Crown J, Dieras V, Staroslawska E, Yardley DA, Davidson N, et al. (2011) Phase III trial of sunitinib (SU) in combination with capecitabine (C) versus C in previously treated advanced breast cancer (ABC). J Clin Oncol 28.

312. Ebos JM, Lee CR, Cruz-Munoz W, Bjarnason GA, Christensen JG, et al. (2009) Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. Cancer Cell 15: 232-239.

313. Van Cutsem E, Lambrechts D, Prenen H, Jain RK, Carmeliet P (2011) Lessons from the adjuvant bevacizumab trial on colon cancer: what next? J Clin Oncol 29: 1-4.

314. Pàez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, et al. (2009) Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer Cell 15: 220-231.

315. Carmeliet P, Jain RK (2011) Molecular mechanisms and clinical applications of angiogenesis. Nature 473: 298-307.

316. Franco M, Man S, Chen L, Emmenegger U, Shaked Y, et al. (2006) Targeted anti-vascular endothelial growth factor receptor-2 therapy leads to short-term and long-term impairment of vascular function and increase in tumor hypoxia. Cancer Res 66: 3639-3648.

317. Bergers G, Hanahan D (2008) Modes of resistance to anti-angiogenic therapy. Nat Rev Cancer 8: 592-603.

318. Pietras K, Hanahan D (2005) A multitargeted, metronomic, and maximum-tolerated dose «chemo-switch» regimen is antiangiogenic, producing objective responses and survival benefit in a mouse model of cancer. J Clin Oncol 23: 939-952.

319. Hashimoto K, Man S, Xu P, Cruz-Munoz W, Tang T, et al. (2010) Potent preclinical impact of metronomic low-dose oral topotecan combined with the antiangiogenic drug pazopanib for the treatment of ovarian cancer. Mol Cancer Ther 9: 996-1006.

320. Dellapasqua S, Bertolini F, Bagnardi V, Campagnoli E, Scarano E, et al. (2008) Metronomic cyclophosphamide and capecitabine combined with bevacizumab in advanced breast cancer. J Clin Oncol 26: 4899-4905.

321. Kerbel RS (2011) Reappraising antiangiogenic therapy for breast cancer. Breast 3: S56-S60.

This article was originally published in a special issue, **Pediatric Oncology: Diagnosis and Therapy** handled by Editor(s). Dr. Anna Petryk, University of Minnesota, USA