

Recent developments in the regulation of the angiogenic switch by cellular stress factors in tumors

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Abstract

Angiogenesis in tumors is controlled by the so-called "angiogenic switch" which allows the passage from low invasive and poorly vascularized tumors to highly invasive and angiogenic tumors. A number of cellular stress factors such as hypoxia, nutrient deprivation or inducers of reactive oxygen species (ROS) are important stimuli of angiogenic signalling. The HIF system plays a significant role in several of these effects and the molecular mechanisms of its regulation have recently been characterized. In addition, HIF-independent mechanisms have been described which involved number of other molecules and transcription factors such as nuclear factor- κ B (NF- κ B) and p53.

p53 is an important intracellular mediator of the stress response and is now also recognized as a modifier of the angiogenic response. p53 may interact with the HIF system but may also have direct effects on angiogenesis regulators or interfere with translation mechanisms of angiogenesis factors.

1. Introduction

Tumor development is critically regulated by the ingrowth of the vasculature into the tumor, a process designated as tumor angiogenesis [33]. Below 1-2 mm³, tumors are, in general, avascular and grow slowly. To further increase in size, tumor cells express a set of molecules that initiate tumor vascularization. The passage to a vascularized phenotype has been called the angiogenic switch. First insight into the molecular mechanisms of the angiogenic switch was gained through the analysis of tumor progression in the RIP-Tag mouse model [22]. The overexpression of the T antigen under the control of the insulin promoter induces an insulinoma that progresses to highly malignant and vascularized lesions. Tumor nodules from the early stage do not induce angiogenesis when co-cultured with endothelial cells *in vitro*. At the later stage, however, angiogenesis is potently induced. This model has greatly contributed

to clarify the role of some of the angiogenesis regulators in the control of the angiogenic switch.

In the past few years, significant advances have been made in the understanding of how angiogenesis factors are expressed in tumor cells and how they act on the surrounding vasculature. Especially noteworthy are studies related to the role of hypoxia in the regulation of these processes which lead to the identification of an entirely new regulatory system involving the hypoxia-inducible transcription factors and the intracellular enzymes called proline hydroxylases.

The aim of this article is to summarize some recent developments in the understanding of the angiogenic switch and to discuss its role in tumor angiogenesis. Emphasis is given in this review to the role of hypoxia, nutrient deprivation, reactive oxygen species (ROS) and p53 in the regulation of the angiogenic response.

2. The angiogenic switchbox

The system that regulates the angiogenic response in tumors can be conceptualized as follows. A main device within the tumor cell named “switchbox”, is the critical entity able to generate angiogenic signals. The angiogenic switchbox is made of two parts: a “switch” that contains all the molecular devices able to respond to external constraints for the initiation/modulation of the angiogenic response and switchbox effectors that are soluble molecules able to interact with target molecules on the vasculature. Therefore, the switchbox can be modulated at both the level of the switcher (by acting on the external constraints or on the molecular devices) and at the level of the effectors (by acting on their expression or activity).

Switchbox effectors may positively or negatively affect the vasculature and may also contribute to a “quality-modification” of vessels such as vessel maturation and stabilization. Major prototypes include the Vascular Endothelial Growth factor family (VEGFs), fibroblast growth factors (FGFs), platelet derived growth factors (PDGFs), angiopoietins, or thrombospondins (for review see: [11,19,31,52]).

The roles of some of the major stimuli and molecular systems in the regulation of switchbox effectors are described below.

3. Hypoxia and the Hypoxia-Inducible Factor (HIF) system

First identified as a DNA binding factor that mediates hypoxia-inducible activity of the erythropoietin 3'enhancer, HIF-1 α has rapidly become the major actor of cellular response to hypoxic stress. It regulates the expression of many genes whose products are involved in glucose transport, glycolysis, erythropoiesis, iron transport and angiogenesis [25,94]. This allows both an immediate switch of the cellular metabolism and an adaptative response of the hypoxic tissues and organism by stimulating blood and oxygen supply. Despite certain recent results which suggest that the effects of HIF on angiogenesis and tumor growth may depend upon the location of the xenograft in mice systems, the VEGF-A gene is still one of the best known HIF target genes involved in angiogenesis [13,94].

The HIF transcription factor is a heterodimer of a constitutive nuclear subunit (HIF-1 β) and an inducible α subunit. Each of these subunits exists as a series of isoforms encoded by distinct genetic loci. Among the three HIF- α protein isoforms, HIF-1 α and HIF-2 α (also termed EPAS-1, HLF, HRF or MOP2) have extensive homologies and the same general organization in functional domains. Both proteins are basic helix-loop-helix mammalian transcription factors and are able to bind the Hypoxia Responsive Element (HRE) found in the promoter region of known hypoxia-induced genes. However, their activation pathways seem

to be slightly different. In contrast, HIF-3 α seems to have inhibitory function in the cellular response to hypoxia [94].

HIF- α subunit regulation is a multi-step process involving mRNA splicing, nuclear accumulation, phosphorylation, acetylation and hydroxylation.

HIF-1 α is the best studied HIF- α subunit (Figure 1). Under normoxia, HIF-1 α is hydroxylated by oxygen-dependent Prolyl Hydroxylases (PHD) at two distinct proline residues (402 and 564). These hydroxylations ensure its binding to the VHL (Von Hippel Lindau) subunit of the Ubiquitine E3 ligase that mediates its extremely rapid proteolysis by the 26S proteasome [108]. Binding to VHL is also favoured by the acetylation of the Lys532 by the ARD1 acetyltransferase [54]. Moreover, in the presence of oxygen, β -hydroxylation of the C-terminal Asn 803 by the hydroxylase FIH-1 Factor, located in the transactivation domain of HIF-1 α , inhibits its transcriptional activity by preventing the binding of the co-activator p300 [24,59,67]. Since the activity of these hydroxylases is completely dependent on molecular oxygen, lowering of oxygen concentration allows both the escape from degradation and the activation by p300 co-activator binding.

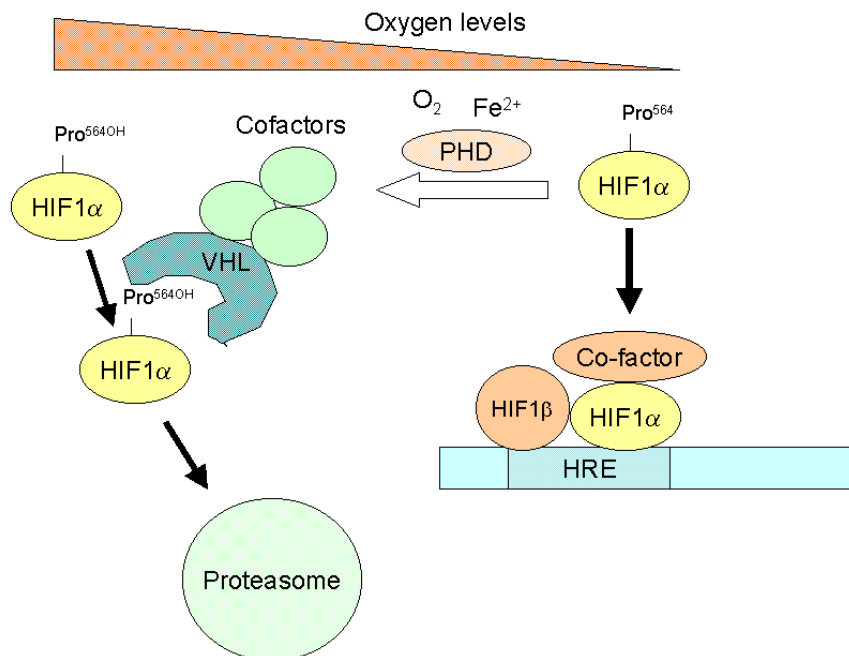


Figure 1: The HIF system

The HIF transcription factor is composed of two subunits, a ubiquitous HIF1 β subunit and a hypoxic responsive subunit HIF1 α . Constitutively expressed, HIF1 α is hydroxylated on proline residue 564 by proline hydroxylases (PHD1, 2 and 3). This post translational modification is necessary to its binding to the VHL (Von Hippen Lindau protein) which ensures its degradation by the proteasomal pathway. Activity of these PHD is dependent on the presence of regular concentration of oxygen (O₂) and Fe²⁺. In response to hypoxia, the inactivation of the PHDs allows HIF1 α protein stabilization, dimerization with HIF1 β subunit, binding of the dimmer to the Hypoxia Responsive Element (HRE) of HIF target genes and activation of the transcription of these genes.

Activation of HIF-1 α also involves phosphorylation by the p42/44 Mitogen Activated Protein kinase [83] that enhances its transcriptional activity. These phosphorylations seem to favour the binding of HIF-1 α to HIF-1 β , rather than to other proteins such as the tumor suppressor p53 protein [105]. It has been suggested that the level of hypoxia (from low to severe hypoxia and anoxia) could regulate the extent of HIF-1 α phosphorylation and be responsible for the binding of HIF-1 α to HIF-1 β or p53 [105]. This would lead either to an adaptative response to low hypoxia or to a p53-mediated cell death in the case of severe hypoxia [79].

In addition to the oxygen-dependent stabilization of the HIF protein, HIF-1 α is also regulated at the expression level by certain growth factors to ensure the maintenance of oxygen homeostasis in normal growing tissues. In this case, HIF activation results from the tissue specific activation of signalling pathways involving the phosphoinositide 3-kinase (PI3K) or the mitogen-activated protein kinase (MAPK). This activation by growth factors implicates signalling pathways involving PI3K/AKT/mTOR, leading to the activation of the translation factor eIF-4E which in turn enhances HIF-1 α mRNA translation [94]. In addition, activation of the RAF-MEK-ERK pathway stimulates HIF-1 α transactivation function [83,98] by phosphorylation of its co-activator p300 by ERK [86].

HIF-2 α is thought to play a critical but distinct role in tumor development. Gene inactivation studies of HIF-1 α and HIF-2 α in mice reveal distinct phenotypes, suggesting different functional and developmental stage-specific activities of the two related transcription factors. *HIF-1 α ^{-/-}* embryos die between embryonic day (E)10.0 and E11.0 and displayed major morphological alterations including deficiencies and abnormalities in the vascular network [51,84]. In comparison, *HIF-2 α ^{-/-}* embryos usually die between E9.5 and E13.5, a strain-dependent postnatal survival being also observed [78,92,109]. HIF-2 α seems to be essential for vascular remodeling after vasculogenesis has occurred. HIF-2 α could be involved in mitochondrial homeostasis by decreasing the reactive oxygen species (ROS)-dependent effects. *HIF-2 α ^{-/-}* mice that survive postnatally develop multiple-organ pathology whose general phenotype is consistent with mitochondrial disease. This phenotype is associated with metabolic abnormalities, an enhanced generation of ROS and a concomitant decrease in the expression of genes encoding primary antioxidant enzymes [92].

In vivo, the expression patterns of HIF-1 α and HIF-2 α mRNAs are quite different: in mammalian cells, HIF-1 α is widely expressed at low levels whereas HIF-2 α is more abundant and is restricted to some tissues and cells including endothelial cells [28,32]. In addition, protein expression, subcellular distribution and regulation of DNA binding of the two transcription factors may differ in cells subjected to hypoxia, to glucose deprivation as well as to various redox conditions [17,28,60,77]. In endothelial cells, HIF-2 α participates to the regulation of the expression of the Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) gene (Flk-1). Indeed, the carboxy terminus of HIF-2 α , but not HIF-1 α , binds to Ets-1 and therefore cooperates with Ets-1 in activating transcription of VEGFR-2 *via* a HIF-2 α /Ets binding site found in the VEGFR-2 promoter region (Elvert 2003).

4. Nutrient deprivation

Nutrient deprivation modulates gene expression and may also contribute to the activation of the angiogenic process. One of the key nutrients to cellular life is glucose. Its consumption meets several distinct metabolic requirements and is based on both catabolic and anabolic pathways. Glucose degradation through glycolysis and mitochondrial respiration lead to the production of ATP, the energy currency of the cell. It is also an essential precursor of various amino acids and sugars needed for the production of proteins and nucleic acids, for cellular proliferation and tissue expansion. Finally, glucose metabolism associated to the hexose monophosphate pathway (HMP) produces NADPH as the reducing power for several biosynthetic processes and for the protection of cells against oxydative stresses.

Glucose deprivation occurs in many solid tumors as the consequence of the local decrease in blood supply and is an intrinsic parameter of ischemia [29,113,114]. Abnormally low concentrations of glucose were measured in tumor interstitial fluids *vs* normal tissues [30,43,47,111,114,124]. The severity of glucose deprivation may even be aggravated by a higher nutrient consumption in the neoplastic tissue [37,121]. For example, under hypoxia,

cells increase glucose uptake and glycolysis in order to compensate for the decrease in respiration and ATP production (the Pasteur effect) [102]. The Pasteur effect is dependent upon HIF-mediated transactivation of genes encoding glycolytic enzymes [93].

Besides, many tumor cells consume glucose at a high rate even in the presence of O₂, to meet the high metabolite requirement of rapidly dividing cells. This phenomenon, known as the Warburg effect [115] can be induced by oncogenic transformation [56] or by the constitutive activation of HIF-dependent pathways, even under normoxic conditions [70]. The Warburg effect results in the activation of key regulatory enzymes of the glycolytic pathway. For example, the expression of the 6-Phosphofructo-2-Kinase/Fructose-2,6-Bisphosphatase (isoform PFKFB3) is increased in many human tumors as compared to normal tissues [8]. PFKFB3 catalyzes the production of a potent allosteric activator of glycolysis, the fructose-2,6-bisphosphate (F2,6BP), whose concentration is also increased in *ras* or *fps* oncogene-transformed cells [14,56]. PFKFB3 gene expression depends on HIF-1 α activation, and is increased in VHL-deficient cells [70], providing a possible genetic basis for the Warburg effect in cancer cells [101].

There are numerous metabolic consequences of glucose deprivation. Decrease in glucose uptake leads to a transient “hypoxia-like” type of stress by decreasing the total pool of cellular ATP, but also mobilizes alternative energetic pathways including degradation of cellular proteins and amino-acids [69]. It also induces oxidative stress and the modification of the intracellular reduced/oxidized ratios of NAD(P)H/NAD(P)⁺ and glutathione (GSH/GSSG). Glucose deprivation-induced oxidative stress is of special concern in angiogenesis as it activates protein kinases that are part of signal transduction pathways (ERK1/2, JNK and PKC), proto-oncogenes (Raf, Ras, c-Fos, c-Myc and c-Jun), and the expression or release of angiogenic growth factors [99]. Glucose deprivation, as well as hypoxic or reductive stresses, also causes accumulation of unfolded and misfolded proteins in the endoplasmic reticulum (ER) and induces the coordinate increase of the expression of a set of glucose/hypoxia-regulated proteins (GRP/ORPs) [55]. This cellular response, defined as the unfolded-protein response (UPR), has cytoprotective and anti-apoptotic functions and also confers drug resistance to cells, which is of great interest in the context of cancer therapy [82]. Transcripts of the two pro-angiogenic factors VEGF and interleukin-8 are up-regulated in tumor cell lines *in vitro* concomitantly with several GRP/ORPs in response to glucose and glutamine deprivation, suggesting a direct relationship between the ER stress and this effect [68]. The inducible molecular chaperone ORP150 was also reported to control tumor angiogenesis by regulating the extracellular release of VEGF [74].

Acute deprivation of glucose induces VEGF gene expression in normal and tumor cells [76,84,87,97,100]. The molecular mechanisms that stimulate VEGF production are, in part, different between hypoxic and hypoglycemic cells. Hypoxia increases VEGF gene transcription through HIF-dependent mechanisms, and also stabilizes its mRNA *via* the 3'-UTR sequence of the transcript [50,63,64,100]. In comparison, the increase in VEGF transcripts in response to glucose deprivation is not mediated by HIF-1 α [51,57] but depends on the 3'UTR sequence [49].

A better understanding of the interrelationships between nutrient metabolism and tumor development will help to define new approaches for cancer therapy. For example, using a strategy based on cancer resistance to nutrient starvation, Lu et al. isolated a natural compound, kigamicin D, that decreases tumor cell viability preferentially under nutrient-deprived conditions [66].

5. Reactive Oxygen Species/ Oxidative stress

Oxidative stress represents another stimulus that may contribute to tumor angiogenesis [20]. Such stress can induce and modify tumor cell phenotype by damaging DNA as well as modulating gene expression and activity of various metabolic pathways [2,4]. Oxidative stress may have several origins including the incomplete reduction of oxygen during respiration, exposure to hypoxia/reoxygenation or to a variety of chemicals or biological compounds, poisoning and irradiation. All these conditions produce derivatives of molecular oxygen (reactive oxygen species; ROS) which are mediators of the various oxidative stress effects. Cancer cells produce high amounts of ROS, including H₂O₂ [106]. Although H₂O₂ is toxic at high concentrations, it may contribute to neoplastic transformation and angiogenesis at lower concentrations, inducing tumor cell proliferation and endothelial cell differentiation [18,119,120].

The NOX family of NAD(P)H oxidases is a cellular source of ROS. NAD(P)H oxidase-derived ROS are implicated in the angiogenic switch in non-tumor tissue [7,40], with the likelihood of similar mechanisms acting in tumors. NAD(P)H oxidases influence tumor cell proliferation *via* the redox-regulated transcription factor NF- κ B, which in turn regulates numerous genes involved in apoptosis, cell proliferation, metastasis and angiogenesis [16]. NF- κ B is also constitutively expressed in numerous malignancies [16].

The effects of ROS on angiogenesis may be in part mediated by the up-regulation of VEGF expression at both the protein and mRNA levels [58,88]. The transcriptional activation is mediated by the transcription factors Sp1 and Sp3 and involves two GC-boxes located on the promoter region of VEGF. MAPK and JNK-dependent signaling pathways are involved in the redox regulation [88]. H₂O₂ also induces IL-8 expression in endothelial cells, which contributes to the angiogenic phenotype [96].

ROS-sensitive mechanisms may also lead to the stabilization of HIF-1 α protein under normoxia, suggesting hypoxia-independent pathways, potentially through the Shc-Ras signaling pathway [1,40,83].

The redox state may also critically impact on HIF-1 α and HIF-2 α activities. Cysteinyll residues on HIF-1 α , HIF-2 α and p53 also act as sensors of oxidative stress and differently modulate the angiogenesis process in response to the redox environment in tumor cells. HIF-1 α and HIF-2 α share 83% identity between their bHLH domains. Both proteins recognize the same DNA sequence in the VEGF gene promoter [28,110]. However, due to the presence of a cysteine (C₂₅) in the bHLH region of HIF-2 α , that is replaced by a serine (S₂₈) in the corresponding sequence of HIF-1 α , both proteins are subjected to distinct redox control mechanisms [60]. Another conserved cysteinyl residue in the C-terminal activation domains (CAD) of both HIF-1 α and HIF-2 α proteins is involved in transactivation by CAD and in its binding with the CREB/p300 transcriptional activator, and is also redox-sensitive [27]. The redox state of these cysteines is in part under the control of the redox factor 1 (Ref-1), a nuclear protein whose reducing activity also increases DNA binding activity of other transcription factors such as Fos, Jun, NF- κ B, Myb [117] and p53 [53]. Ref-1 is identical to the DNA repair enzyme APE/HAP-1 and possesses two distinct activities, a N-terminal reducing function acting on cysteines and a C-terminal DNA repair domain [118]. Another redox protein, thioredoxin, activates HIF-dependent pathways by similar mechanisms [27]. Overexpression of thioredoxin is observed in several human tumors, which may contribute to the HIF-induced transcriptional up-regulation of VEGF and tumor angiogenesis [27,116].

6. The p53 tumor-suppressor protein

Considered as one of the most potent onco-suppressive protein, p53 is a stress inducible transcription factor which is mutated in more than 50% of human tumors [44] (see also the p53 mutation database: <http://www.iarc.fr/>). This factor is known to be involved in the cellular response to genotoxic stress and, to a lesser extent, to various other forms of stress including hypoxia [73,80]. In response to stress, the protein accumulates in the nucleus and adopts a conformation that allows binding to the p53 responsive elements of different genes. Some of them are repressed (p53 repressed genes also called Prgs), some are activated (p53 inducible genes or Pigs, [73]). p53 protein also interacts directly with other proteins such as helicases or other transcription factors such as HIF-1 α . Depending on the kind of stress, signalling pathway or intensity of activation, the p53 response may differ, mediating either cell cycle arrest in G2 and/or G1 phase of the cell cycle (activation of genes such as p21, 14-3-3sigma, GADD45), DNA repair (GADD45, ERCC1), or apoptosis (Bax and PUMA activation, Bcl2 repression, [73]).

Figure 2 A and B : A role for p53 in regulating the angiogenic switch ?

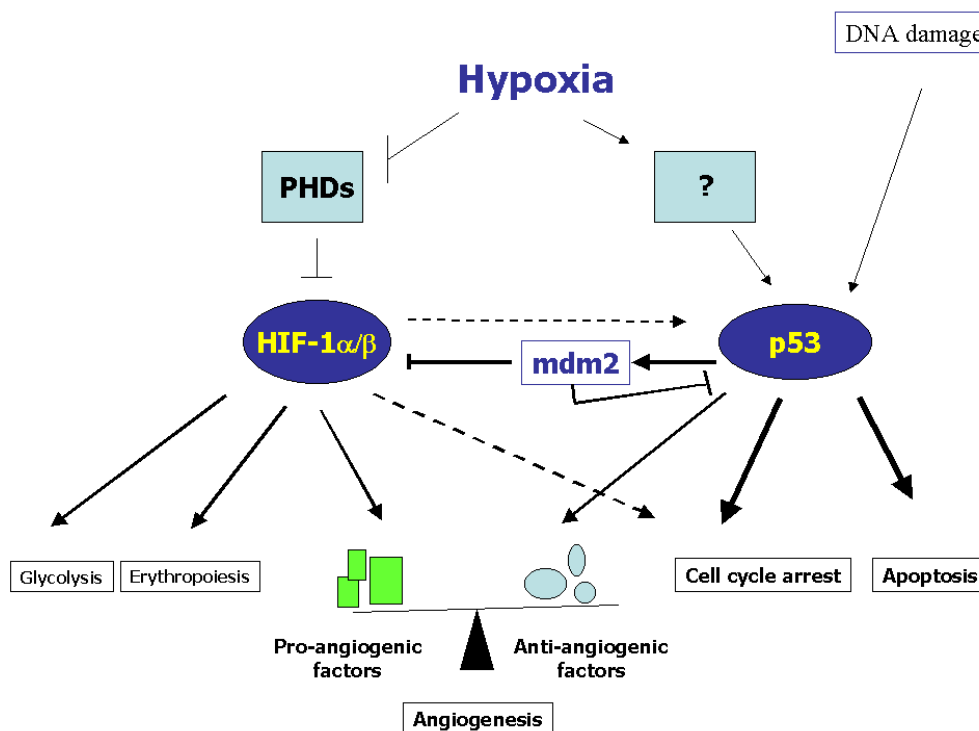


Figure 2 A : In normal cells exposed to hypoxia, HIF1 α and p53 are able to be activated by hypoxia alone (HIF) or hypoxia and DNA damage (p53). Activation of HIF1 α in response to hypoxia involves inactivation of Proline hydroxylases (PHDs) and mediates activation of genes that are involved in glucose metabolism, Erythropoiesis, and angiogenesis (pro-angiogenic factors). Activation of HIF could also contribute to cell cycle arrest either directly or *via* p53 activation (dotted arrows). In response to DNA damage and/or hypoxia p53 activation leads to the expression of genes involved in cell cycle arrest or apoptosis but also to the activation of anti-angiogenic genes and repression of pro-angiogenic factors. Therefore, p53 activation in response to stress can modulate the effects of HIF on angiogenesis switch. P53 is also capable of destabilizing HIF by activating mDM2 gene expression. Mdm2 protein is involved in the degradation of p53 in response to DNA damage and of the degradation of HIF1 α in response to hypoxia. The combination of these effects mainly results in the cell growth arrest or cell death observed in normal cells exposed to hypoxia.

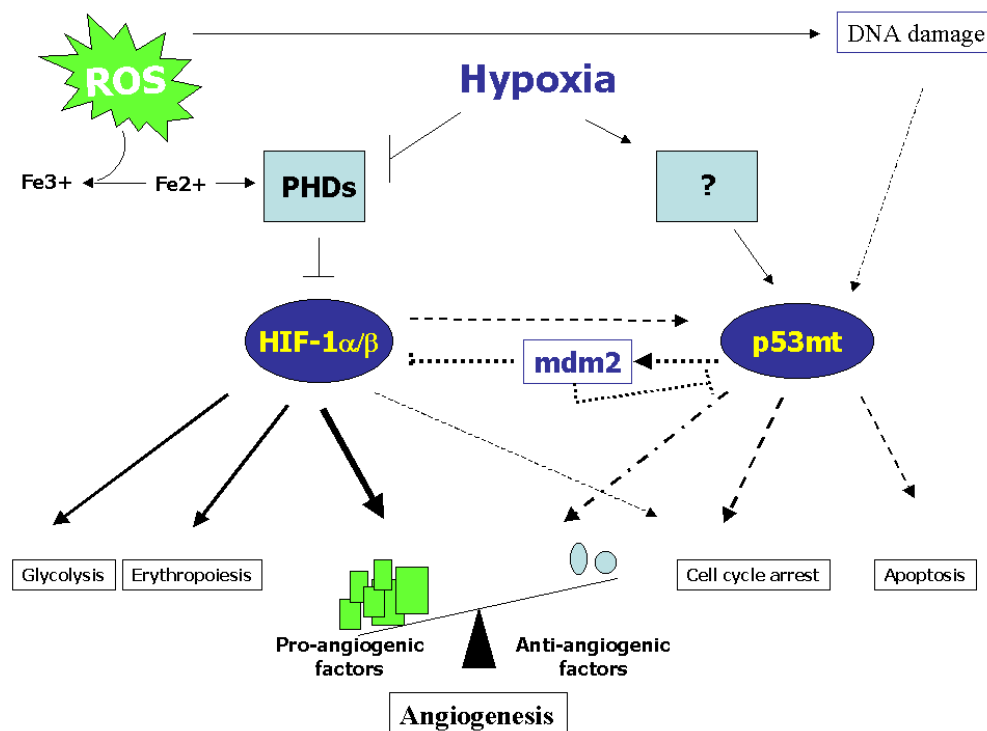


Figure 2 B : In cancer cells, p53 pathway is often altered by mutations on p53 (p53 mt) which limits the capability of the cells to respond to DNA damage that can be made by Reactive Oxygen Species (ROS) accumulation. Increased production of ROS and hypoxia maintain high levels of HIF1 α by inhibiting the activity of Proline Hydroxylases (PHDs). This activation of HIF is no more counter-balanced by p53 activation and this favours the accumulation of pro-angiogenic factors by cancer cells. Hypoxic cancer cells go on proliferating and mediate the angiogenic switch.

The best described p53 activation pathway is the DNA damage pathway that leads either to cell cycle arrest or apoptosis depending on both the extend of the lesion and the genetic background of the cells. This pathway has been extensively studied and involves strand breaks-induced kinases that phosphorylate both p53 and p53 regulating proteins, acetylation of the C-terminus regulating loop of p53, binding to p300 co-activator and escape from degradation by the proteasome [73]. p53, much like HIF, is constitutively expressed in normal cells and degraded in non stressed cells. p53 is principally degraded through ubiquitination by the Ubiquitine ligase mdm2. Interestingly, this Ub-ligase is encoded by a p53 target gene and thus ensures the rapid and short term burst of p53 activation in response to genotoxic stress [112].

The activation pathway of p53 by hypoxia is still unclear and at least partly different from the one elicited by DNA damage [6,75]. Activation of p53 by hypoxia induces cell death in normal neuronal cells [62] and could be implicated in the regulation of angiogenesis. Indeed, within a tumor, cells are exposed to ischemia that leads to acidosis, hypoxia and hypoglycaemia. Under these conditions, p53 activation may play a role in controlling angiogenic signalling, either by acting directly on the balance of pro and anti-angiogenic effectors or signalling molecules, or on modulating the HIF response to hypoxia (Figure 2).

p53 has been reported to inhibit the expression of pro-angiogenic factors such as VEGF-A [71,123], FGF [35,36,95], MMP1 [104] and Cox2 [103] and more recently the human Focal Adhesion Kinase FAK [39] which is highly expressed in endothelial cells in high grade malignant glioma [46]. These inhibitions may occur at the transcriptional (as for FAK) or translational (as for FGF) level [35,36].

p53 is also involved in the activation of anti-angiogenic factors such as TSP1 [23,61], BAI1 [34,72], MMP2 [10] or Eph2A [15,26]. It has become clear that expression of wt p53 in tumor cells promotes both dormancy and inhibition of metastasis by inhibiting angiogenesis *via* a change in the TSP1/VEGFA ratio [38,48]. Moreover, loss of p53 apparently confers a selective advantage for cancer cells to survive in hypoxic conditions. Therefore, hypoxia could exert an important pressure for the selection of p53 mutant cells during carcinogenesis [41,122]. Mice bearing tumors derived from p53^{-/-} HCT116 human colorectal cancer cells are also less responsive to anti-angiogenic therapy than mice with p53^{+/+} tumors [122]. These latter properties are probably directly linked to the ability of p53 to regulate HIF activity in different manners.

Despite evidence of a link between p53/HIF-1 α in tumors, the precise molecular mechanisms are only starting to be understood [65,81,94]. First evidences came in 1998 when An et al. immunoprecipitated a complex between HIF-1 α and p53 in MCF7 cells exposed to hypoxia. In these cells, activation of p53 by hypoxia is dependent upon the presence of the functional HIF-1 complex [6]. Moreover, over-expression of HIF-1 α in normoxia resulted in an increase in p53 activity measured on reporter plasmid. Two distinct regions of HIF-1 α interact with p53 DNA binding domain, these two regions encompassing the two proline residues that are involved in HIF hydroxylation and binding to pVHL [45]. The phosphorylation status of HIF seems to be critical for interaction with p53 since only the dephosphorylated form of HIF-1 α does bind to p53 and would be responsible for its proapoptotic properties [105].

How does the binding of HIF-1 α to p53 influence p53 activity? p53 bound to HIF-1 α may be protected from mdm2-dependent degradation [21] or its core domain held in an active conformation. Different results have been reported in ES cells where p53 activation by hypoxia is, at least in part, independent of HIF-1 α and HIF-2 α activities [75]. These differences may be explained by the fact that regulation of p53 in ES cells is completely different than in normal cells due to particularly high level of inactive p53 [3,85,91].

Other studies have shown that p53 down-regulates HIF-1 α transcriptional activity by competition with the co-activator p300 that may have a better affinity for p53 rather than for HIF-1 α [12,89]. It has also been reported that high levels of p53 activation induce a mdm2-dependent, but VHL-independent destabilization of HIF-1 α [81,89]. p53 may be considered, in this respect, as a molecular chaperone that facilitates the recruitment of mdm2 ubiquitinase to HIF-1 α and thereby contributes to terminate the HIF-1 response. This would explain the lower rate of vascularization of p53^{+/+} xenografts as compared to p53^{-/-} HCT116 xenografts [122]. One could imagine that p53 escapes the mdm2-dependent degradation by directing its ubiquitinase-like activity towards HIF-1 α . However, a recent publication showed that, at least in normoxic cells, mdm2 positively regulates HIF-1 α [9], which suggests that regulatory feed back loops involving HIF and p53 are rather complexes and stress or context-dependent.

Taken together, these results suggest that p53 and mdm2 play an important role in regulating HIF-1 activity in normoxic and in hypoxic cells. Apparent discrepancies between the data provided by different groups may be related to the cell type considered. Nevertheless, these different studies indicate that there is interplay between HIF and p53 pathways that forms a big regulatory loop involving p53, mdm2, pVHL and HIF-1 α . Noteworthy, the expression or function of all these proteins are found altered in most of human tumors.

An additional role for p53 in angiogenesis has been reported that involves the metastasis associated Mts1/S1004A protein. Expression of Mts1/S1004A increases with progression of tumor stage [107]. It acts as a pro-angiogenic factor, enhancing both endothelial cell mobility and corneal neovascularization *in vivo*. The angiogenic effects were

attributed to the capacity of Mts1 to activate the MMP13 collagenase when overexpressed in endothelial cells [5,90]. Another target of Mts1 is the p53 protein. Mts1 binds to the C-terminus regulatory loop of p53, thus inhibiting its transcriptional activity on Pigs and apparently increasing its pro-apoptotic functions [42]. The inhibition of p53 transcriptional activity could therefore participate to both, the pro-angiogenic function of Mts1 and the loss of p53 wild type cells during cancer progression.

7. Concluding remarks

Cellular stresses are able to critically regulate the angiogenic switch in many ways in tumors. Hypoxia, nutrient deprivation, or ROS generating stimuli act *via* HIF-dependent and independent regulatory pathways. Hypoxia targets mainly the HIF system. The molecular mechanisms of HIF activity have been recently elucidated. A number of key players participate in the regulation of HIF activity at the transcriptional or post-transcriptional level. Major molecular players that have been recently identified are the proline hydroxylases that control HIF protein stability.

Nutrient deprivation may use HIF-dependent and independent pathways for the regulation of angiogenesis signals. In particular, endoplasmic reticulum signalling following ER-stress may actively participate in the regulation of angiogenic factors such as VEGF by increasing its synthesis and export from cells.

Reactive oxygen species (ROS) also induces angiogenesis programming and may stimulate expression of angiogenesis factors. Multiple targets for this effect have been identified including HIF, NF- κ B, or growth factors signalling pathways.

Several recent studies have also pointed to a link between the HIF system and p53. p53 is able to associate with HIF-1 α and is possibly involved in HIF-1 α destabilization. p53 is also involved in other intracellular processes important for angiogenesis such as translational regulation of FGF or binding to Mts1.

These intracellular regulatory systems may differentially participate in the stress response. For example, the HIF system may be more important for the regulation of angiogenesis in certain tumors located in poorly vascularized regions whereas the other stress response pathways activated by ROS or nutrient deprivation may be predominant in highly vascularized tissues such as brain. Indeed, in rodent experimental tumor models with subcutaneous implantation, overexpression of HIF-1 α in response to ischemia is associated with a faster tumor growth whereas blocking HIF-1 α activity leads to slower tumor growth and reduced angiogenesis. However, VEGF expression could also be observed independently of the HIF system in gliomas implanted in the brain which is highly vascularized [13].

Nevertheless, the key role of the HIF system in regulating tumor growth and angiogenesis is further emphasized by the fact that it is overexpressed in many different human cancers such as cervical cancer, non small cell lung carcinomas or Head and neck cancers. This overexpression is attributed either to the chronic hypoxic state of the tumor cells or to a number of mutations in oncogenes (MEK-ERK or PI3K-AKT-mTOR or IGF1R signalling) and tumor-suppressor genes such as VHL or p53 which are implicated in its stability [94]. This interplay between the overexpression of HIF-1 and oncogene or anti-oncogene mutations found in cancer cells may be a crucial link between cell cycle, apoptosis and angiogenesis that are the main targets of anti-cancer therapies.

REFERENCES

- [1] J; Abe, B.C. Berk, Hypoxia and HIF-1alpha stability: another stress-sensing mechanism for Shc, *Circ. Res.* 91 (2002) 4-6.
- [2] V. Adler, Z. Yin, K.D. Tew, Z. Ronai, Role of redox potential and reactive oxygen species in stress signaling, *Oncogene* 18 (1999) 6104-6111.
- [3] M.I. Aladjem, B.T. Spike, L.W. Rodewald, T.J. Hope, M. Klemm, R. Jaenisch, G.M. Wahl, ES cells do not activate p53-dependent stress responses and undergo p53-independent apoptosis in response to DNA damage, *Curr. Biol.* 8 (1998) 145-155.
- [4] R.G. Allen, M. Tresini, Oxidative stress and gene regulation, *Free Radic. Biol. Med.* 28 (2000) 463-499.
- [5] N. Ambartsumian, J. Klingelhofer, M. Grigorian, C. Christensen, M. Kriajevska, E. Tulchinsky, G. Georgiev, V. Berezin, E. Bock, J. Rygaard, R. Cao, Y. Cao, E. Lukanidin, The metastasis-associated Mts1(S100A4) protein could act as an angiogenic factor, *Oncogene* 20 (2001) 4685-4695.
- [6] W.G. An, M. Kanekal, M.C. Simon, E. Maltepe, M.V. Blagosklonny, L.M. Neckers, Stabilization of wild-type p53 by hypoxia-inducible factor 1alpha, *Nature* 392 (1998) 405-408.
- [7] J.L. Arbiser, J. Petros, R. Klatfer, B. Govindajaran, E.R. McLaughlin, L.F. Brown, C. Cohen, M. Moses, S. Kilroy, R.S. Arnold, J.D. Lambeth, Reactive oxygen generated by Nox1 triggers the angiogenic switch, *Proc. Natl. Acad. Sci. U S A* 99 (2002) 715-720.
- [8] T. Atsumi, J. Chesney, C. Metz, L. Leng, S. Donnelly, Z. Makita, R. Mitchell, R. Bucala, High expression of inducible 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (iPFK-2; PFKFB3) in human cancers, *Cancer Res.* 62 (2002) 5881-5887.
- [9] J.I. Bardos, N.M. Chau, M. Ashcroft, Growth factor-mediated induction of HDM2 positively regulates hypoxia-inducible factor 1alpha expression, *Mol. Cell. Biol.* 24 (2004) 2905-2914.
- [10] J. Bian, Y. Sun, Transcriptional activation by p53 of the human type IV collagenase (gelatinase A or matrix metalloproteinase 2) promoter, *Mol. Cell. Biol.* 17 (1997) 6330-6338.
- [11] A. Bikfalvi, R. Bicknell, Recent advances in angiogenesis, anti-angiogenesis and vascular targeting., *Trends Pharmacol. Sci.* 23 (2002) 576-582.
- [12] M.V. Blagosklonny, W.G. An, L.Y. Romanova, j. Trepel, T. Fojo, L. Neckers, p53 inhibits hypoxia-inducible factor-stimulated transcription, *J. Biol. Chem.* 273 (1998) 11995-11998.
- [13] B. Blouw, H. Song, T. Tihan, j. Bosze, N. Ferrara, H.P. Gerber, R.S. Johnson, G. Bergers, The hypoxic response of tumors is dependent on their microenvironment, *Cancer Cell* 4 (2003) 133-146.
- [14] L. Bosca, M. Mojena, J. Ghysdael, G.G. Rousseau, L. Hue, Expression of the v-src or v-fps oncogene increases fructose 2,6-bisphosphate in chick-embryo fibroblasts. Novel mechanism for the stimulation of glycolysis by retroviruses, *Biochem J.* 236 (1986) 595-599.
- [15] D.M. Brantley, N. Cheng, E.J. Thompson, Q. Lin, R.A. Brekken, P.E. Thorpe, R.S. Muraoka, D.P. Cerretti, A. Pozzi, D. Jackson, C. Lin, J. Chen, Soluble Eph A receptors inhibit tumor angiogenesis and progression in vivo, *Oncogene* 21 (2002) 7011-7026.
- [16] S.S. Brar, T.P. Kennedy, M. Quinn, J.R. Hoidal, Redox signaling of NF-kappaB by membrane NAD(P)H oxidases in normal and malignant cells, *Protoplasma* 221 (2003) 117-127.

- [17] K. Brusselmans, F. Bono, P. Maxwell, Y. Dor, M. Dewerchin, D. Collen, J.M. Herbert, P. Carmeliet, Hypoxia-inducible factor-2alpha (HIF-2alpha) is involved in the apoptotic response to hypoglycemia but not to hypoxia, *J Biol Chem* 276 (2001) 39192-39196.
- [18] R.H. Burdon, V. Gill, C. Rice-Evans, Oxidative stress and tumour cell proliferation, *Free Radic. Res. Commun.* 11 (1990) 65-76.
- [19] P. Carmeliet, Angiogenesis in health and disease, *Nat. Med.* 9 (2003) 653-60.
- [20] P. Carmeliet, R.K. Jain, Angiogenesis in cancer and other diseases, *Nature*, 407 (2000) 249-257.
- [21] D. Chen, M. Li, J. Luo, W. Gu, Direct interactions between HIF-1 alpha and Mdm2 modulate p53 function, *J. Biol. Chem.* 278 (2003) 13595-13598.
- [22] G. Christofori, D. Hanahan, Molecular dissection of multi-stage tumorigenesis in transgenic mice, *Semin. Cancer Biol.* 5 (1994) 3-12.
- [23] K.M. Dameron, O.V. Volpert, M.A. Tainsky, N. Bouck, Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1, *Science* 265 (1994) 1582-1584.
- [24] S.A. Dames, M. Martinez-Yamout, R.N. De Guzman, H.J. Dyson, P.E. Wright, Structural basis for Hif-1 alpha /CBP recognition in the cellular hypoxic response, *Proc. Natl. Acad. Sci. USA* 99 (2002) 5271-5276.
- [25] N.C. Denko, L.A. Fontana, K.M. Hudson, P.D. Sutphin, S. Raychaudhuri, R. Altman, A.J. Giaccia, Investigating hypoxic tumor physiology through gene expression patterns, *Oncogene* 22 (2003) 5907-5914.
- [26] M. Dohn, J. Jiang, X. Chen, Receptor tyrosine kinase EphA2 is regulated by p53-family proteins and induces apoptosis, *Oncogene* 20 (2001) 6503-6515.
- [27] M. Ema, K. Hirota, J. Mimura, H. Abe, J. Yodoi, K. Sogawa, L. Poellinger, Y. Fujii-Kuriyama, Molecular mechanisms of transcription activation by HLF and HIF1alpha in response to hypoxia: their stabilization and redox signal-induced interaction with CBP/p300, *Embo J.* 18 (1999) 1905-1914.
- [28] M. Ema, S. Taya, N. Yokotani, K. Sogawa, Y. Matsuda, Y. Fujii-Kuriyama, A novel bHLH-PAS factor with close sequence similarity to hypoxia-inducible factor 1alpha regulates the VEGF expression and is potentially involved in lung and vascular development, *Proc. Natl. Acad. Sci. USA* 94 (1997) 4273-4278.
- [29] C.J. Eskey, A.P. Koretsky, M.M. Domach, R.K. Jain, Role of oxygen vs. glucose in energy metabolism in a mammary carcinoma perfused ex vivo: direct measurement by ³¹P NMR, *Proc. Natl. Acad. Sci. USA*, 90 (1993) 2646-2650.
- [30] S.N. Ettinger, C.C. Poellmann, N.A. Wisniewski, A.A. Gaskin, J.S. Shoemaker, J.M. Poulson, M.W. Dewhirst, B. Klitzman, Urea as a recovery marker for quantitative assessment of tumor interstitial solutes with microdialysis, *Cancer Res.* 61 (2001) 7964-7970.
- [31] N. Ferrara, H.P. Gerber, J. LeCouter, The biology of VEGF and its receptors, *Nat. Med.* 9 (2003) 669-676.
- [32] I. Flamme, T. Frohlich, M. von Reutern, A. Kappel, A. Damert, W. Risau, HRF, a putative basic helix-loop-helix-PAS-domain transcription factor is closely related to hypoxia-inducible factor-1 alpha and developmentally expressed in blood vessels, *Mech. Dev.* 63 (1997) 51-60.
- [33] J. Folkman, Tumor angiogenesis: therapeutic implications, *N. Engl. J. Med.* 285 (1971) 1182-1186.
- [34] Y. Fukushima, Y. Oshika, T. Tsuchida, T. Tokunaga, H. Hatanaka, H. Kijima, H. Yamazaki, Y. Ueyama, N. Tamaoki, M. Nakamura, Brain-specific angiogenesis inhibitor 1 expression is inversely correlated with vascularity and distant metastasis of colorectal cancer, *Int. J. Oncol.* 13 (1998) 967-970.

- [35] B. Galy, L. Creancier, L. Prado-Lourenco, A.C. Prats, H. Prats, p53 directs conformational change and translation initiation blockade of human fibroblast growth factor 2 mRNA, *Oncogene* 20 (2001) 4613-4620.
- [36] B. Galy, L. Creancier, C. Zanibellato, A.C. Prats, H. Prats, Tumour suppressor p53 inhibits human fibroblast growth factor 2 expression by a post-transcriptional mechanism, *Oncogene* 20 (2001) 1669-1677.
- [37] R.A. Gatenby, The potential role of transformation-induced metabolic changes in tumor- host interaction, *Cancer Res.* 55 (1995) 4151-4156.
- [38] A. Gautam, C.L. Densmore., S. Melton, E. Golunski, J.C. Waldrep, Aerosol delivery of PEI-p53 complexes inhibits B16-F10 lung metastases through regulation of angiogenesis, *Cancer Gene Ther.* 9 (2002) 28-36.
- [39] V. Golubovskaya, A. Kaur, W. Cance, Cloning and characterization of the promoter region of human focal adhesion kinase gene: nuclear factor kappa B and p53 binding sites, *Biochim. Biophys. Acta* 1678 (2004) 111-125.
- [40] A. Gorlach, I. Diebold, V.B. Schini-Kerth, U. Berchner-Pfannschmidt, U. Roth, R.P. Brandes, T. Kietzmann, R. Busse, Thrombin activates the hypoxia-inducible factor-1 signaling pathway in vascular smooth muscle cells: Role of the p22(phox)-containing NADPH oxidase, *Circ. Res.* 89 (2001) 47-54.
- [41] T.G. Graeber, C. Osmanian, T. Jacks, D.E. Housman, C.J. Koch, S.W. Lowe, A.J. Giaccia, Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours, *Nature* 379 (1996) 88-91.
- [42] M. Grigorian, S. Andresen, E. Tulchinsky, M. Kriaievska, C. Carlberg, C. Kruse, M. Cohn, N. Ambartsumian, A. Christensen, G. Selivanova, E. Lukanidin, Tumor suppressor p53 protein is a new target for the metastasis-associated Mts1/S100A4 protein: functional consequences of their interaction, *J.Biol.Chem.* 276 (2001) 22699-22708.
- [43] P.M. Gullino, Extracellular compartments of solid tumors. In B. FF (Ed.), *Cancer*, Vol. 3, Plenum Press, New York, 1975, pp. 327-354.
- [44] P. Hainaut, M. Hollstein, p53 and human cancer: the first ten thousand mutations, *Adv Cancer Res.* 77 (2000) 81-137.
- [45] L.O. Hansson, A. Friedler, S. Freund, S. Rudiger, A.R. Fersht, Two sequence motifs from HIF-1alpha bind to the DNA-binding site of p53, *Proc.Natl.Acad.Sci. USA* 99 (2002) 10305-10309.
- [46] H. Haskell, M. Natarajan, T.P. Hecker, Q. Ding, J. Stewart, Jr., J.R. Grammer, C.L. Gladson, Focal adhesion kinase is expressed in the angiogenic blood vessels of malignant astrocytic tumors in vivo and promotes capillary tube formation of brain microvascular endothelial cells, *Clin. Cancer Res.* 9 (2003) 2157-2165.
- [47] G. Helmlinger, A. Sckell, M. Dellian, N.S. Forbes, R.K. Jain, Acid production in glycolysis-impaired tumors provides new insights into tumor metabolism, *Clin. Cancer Res.* 8 (2002) 1284-1291.
- [48] L. Holmgren, G. Jackson, J. Arbiser, p53 induces angiogenesis-restricted dormancy in a mouse fibrosarcoma, *Oncogene* 17 (1998) 819-824.
- [49] K. Iida, Y. Kawakami., H. Sone, H. Suzuki, S. Yatoh, K. Isobe, K. Takekoshi, N. Yamada, Vascular endothelial growth factor gene expression in a retinal pigmented cell is up-regulated by glucose deprivation through 3' UTR, *Life Sci.* 71 (2002) 1607-1614.
- [50] E. Ikeda, M.G. Achen, G. Breier, W. Risau, Hypoxia-induced transcriptional activation and increased mRNA stability of vascular endothelial growth factor in C6 glioma cells, *J. Biol. Chem.* 270 (1995) 19761-19766.

- [51] N.V. Iyer, L.E. Kotch, F. Agani, S.W. Leung, E. Laughner, R.H. Wenger, M. Gassmann, J.D. Gearhart, A.M. Lawler, A.Y. Yu, G.L Semenza, Cellular and developmental control of O₂ homeostasis by hypoxia- inducible factor 1 alpha, *Genes Dev.* 12 (1998) 149-162.
- [52] S. Javerzat, P. Auguste, A. Bikfalvi, The role of fibroblast growth factors in vascular development, *Trends Mol. Med.* 8 (2002) 483-.
- [53] L. Jayaraman, K.G. Murthy, C. Zhu, T. Curran, S. Xanthoudakis, C. Prives, Identification of redox/repair protein Ref-1 as a potent activator of p53, *Genes Dev.* 11 (1997) 558-570.
- [54] J.W. Jeong, M.K. Bae, M.Y. Ahn, S.H. Kim, T.K. Sohn, M.H. Bae, M.A. Yoo, E.J. Song, K.J. Lee, K.W. Kim, Regulation and destabilization of HIF-1alpha by ARD1-mediated acetylation, *Cell* 111 (2002) 709-720.
- [55] R.J. Kaufman, D. Scheuner, m. Schroder, X. Shen, K. Lee, C.Y. Liu, S.M. Arnold, The unfolded protein response in nutrient sensing and differentiation, *Nat. Rev. Mol. Cell Biol.* 3 (2002) 411-21.
- [56] H.K. Kole, R.J. Resnick, M. Van Doren, E. Racker, Regulation of 6-phosphofructo-1-kinase activity in ras-transformed rat-1 fibroblasts, *Arch. Biochem. Biophys.* 286 (1991) 586-590.
- [57] L.E. Kotch, N.V. Iyer, E. Laughner, G.L. Semenza, Defective vascularization of HIF-1alpha-null embryos is not associated with VEGF deficiency but with mesenchymal cell death, *Dev. Biol.* 209 (1999) 254-267.
- [58] M. Kuroki, E.E. Voest, S. Amano, L.V. Beerepoot, S. Takashima, M. Tolentino, R.Y. Kim, R.M. Rohan, K.A. Colby, K.-T Yeo, A.P. Adamis, Reactive oxygen intermediates increase vascular endothelial growth factor expression in vitro and in vivo, *J. Clin. Invest.* 98 (1996) 1667-1675.
- [59] D. Lando, D.J. Peet, J.J. Gorman, D.A. Whelan, M.L. Whitelaw, R.K. Bruick, FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor, *Genes Dev.* 16 (2002) 1466-1471.
- [60] D. Lando, I. Pongratz, L. Poellinger, M.L. Whitelaw, A redox mechanism controls differential DNA binding activities of hypoxia-inducible factor (HIF) 1alpha and the HIF-like factor, *J. Biol. Chem.* 275 (2000) 4618-4627.
- [61] J. Lawler, M.W. Miao, M. Duquette, N. Bouck, R.T. Bronson, R.O. Hynes, Thrombospondin-1 gene expression affects survival and tumor spectrum of p53-deficient mice, *Am. J. Pathol.* 159 (2001) 1949-1956.
- [62] R.R. Leker, M. Aharonowiz, N.H. Greig, H. Ovadia, The role of p53-induced apoptosis in cerebral ischemia: effects of the p53 inhibitor pifithrin alpha, *Exp. Neurol.* 187 (2004) 478-486.
- [63] A.P. Levy, N.S. Levy, S. Wegner, M.A. Goldberg, Transcriptional regulation of the rat vascular endothelial growth factor gene by hypoxia., *J. Biol. Chem.* 270 (1995) 13333-13340.
- [64] N.S. Levy, S. Chung, H. Furneaux, A.P. Levy, Hypoxic stabilization of vascular endothelial growth factor mRNA by the RNA-binding protein HuR, *J. Biol. Chem.* 273 (1998) 6417-6423.
- [65] B. Linderholm, B. Lindh, B. Tavelin, K. Grankvist, R. Henriksson, p53 and vascular-endothelial-growth-factor (VEGF) expression predicts outcome in 833 patients with primary breast carcinoma, *Int. J. Cancer* 89 (2000) 51-62.
- [66] J. Lu, S. Kunimoto, Y. Yamazaki, M. Kaminishi, H. Esumi, Kigamicin D, a novel anticancer agent based on a new anti-austerity strategy targeting cancer cells' tolerance to nutrient starvation, *Cancer Sci.* 95 (2004) 547-552.

- [67] P.C. Mahon, K. Hirota, G.L. Semenza, FIH-1: a novel protein that interacts with HIF-1 α and VHL to mediate repression of HIF-1 transcriptional activity, *Genes Dev.* 15 (2001) 2675-2686.
- [68] P.L. Marjon, E.V. Bobrovnikova-Marjon, S.F. Abcouwer, Expression of the pro-angiogenic factors vascular endothelial growth factor and interleukin-8/CXCL8 by human breast carcinomas is responsive to nutrient deprivation and endoplasmic reticulum stress, *Mol Cancer* 3 (2004) 4-6.
- [69] S. Mazurek, E. Eigenbrodt, The tumor metabolome, *Anticancer Res.* 23 (2003) 1149-1154.
- [70] A. Minchenko, I. Leshchinsky, I. Opentanova, N. Sang, V. Srinivas, V. Armstead, J. Caro, J., Hypoxia-inducible factor-1-mediated expression of the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3) gene. Its possible role in the Warburg effect, *J. Biol. Chem.* 277 (2002) 6183-6187.
- [71] D. Mukhopadhyay, I. Tsiokas, V.P. Sukhatme, Wild-type p53 and v-Src exert opposing influences on human vascular endothelial growth factor gene expression, *Cancer Res.* 55 (1995) 6161-6165.
- [72] H. Nishimori, T. Shiratsuchi, T. Urano, Y. Kimura, K. Kiyono, K. Tatsumi, S. Yoshida, M. Ono, M. Kuwano, Y. Nakamura, T. Tokino, A novel brain-specific p53-target gene, BA11, containing thrombospondin type 1 repeats inhibits experimental angiogenesis, *Oncogene* 15 (1997) 2145-2150.
- [73] M. Oren, Decision making by p53: life, death and cancer, *Cell. Death Differ.* 10 (2003) 431-42.
- [74] K. Ozawa, Y. Tsukamoto, O. Hori, Y. Kitao, H. Yanagi, D.M., Stern, S. Ogawa, Regulation of tumor angiogenesis by oxygen-regulated protein 150, an inducible endoplasmic reticulum chaperone, *Cancer Res.* 61 (2001) 4206-4213.
- [75] Y. Pan, P.R. Oprysko, A.M. Asham, C.J. Koch, M.C. Simon, p53 cannot be induced by hypoxia alone but responds to the hypoxic microenvironment, *Oncogene* 23 (2004) 4975-4983.
- [76] S.H. Park, K.W. Kim, Y.S. Lee, J.H. Baek, M.S. Kim, Y.M. Lee, M.S. Lee, Y.J. Kim, Hypoglycemia-induced VEGF expression is mediated by intracellular Ca²⁺ and protein kinase C signaling pathway in HepG2 human hepatoblastoma cells, *Int. J. Mol. Med.* 7 (2001) 91-6.
- [77] S.K. Park, A.M. Dadak, V.H. Haase, L. Fontana, A.J. Giaccia, R.S. Johnson, Hypoxia-induced gene expression occurs solely through the action of hypoxia-inducible factor 1 α (HIF-1 α): role of cytoplasmic trapping of HIF-2 α , *Mol. Cell. Biol.* 23 (2003) 4959-4971.
- [78] J. Peng, L. Zhang, L. Drysdale, G.H. Fong, The transcription factor EPAS-1/hypoxia-inducible factor 2 α plays an important role in vascular remodeling, *Proc. Natl. Acad. Sci. USA* 97 (2000) 8386-8391.
- [79] J.P. Piret, D. Mottet, M. Raes, C. Michiels, Is HIF-1 α a pro- or an anti-apoptotic protein? *Biochem. Pharmacol.* 64 (2002) 889-892.
- [80] O. Pluquet, P. Hainaut, Genotoxic and non-genotoxic pathways of p53 induction, *Cancer Lett.* 174 (2001) 1-15.
- [81] R. Ravi, B. Mookerjee, Z.M. Bhujwalla, C.H. Sutter, D. Artemov, Q. Zeng, L.E. Dillehay, A. Madan, G.L. Semenza, A. Bedi, Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1 α , *Genes Dev.* 14 (2000) 34-44.
- [82] R.K. Reddy, C. Mao, C., Baumeister, P., Austin, R.C., Kaufman, R.J. and Lee, A.S., Endoplasmic reticulum chaperone protein GRP78 protects cells from apoptosis

- induced by topoisomerase inhibitors: role of ATP binding site in suppression of caspase-7 activation, *J. Biol. Chem.* 278 (2003) 20915-20924.
- [83] D.E. Richard, E. Berra, E. Gothie, D. Roux, J. Pouyssegur, p42/p44 mitogen-activated protein kinases phosphorylate hypoxia-inducible factor 1alpha (HIF-1alpha) and enhance the transcriptional activity of HIF-1, *J. Biol. Chem.* 274 (1999) 32631-32637.
- [84] H.E. Ryan, J. Lo, R.S. Johnson, HIF-1 alpha is required for solid tumor formation and embryonic vascularization, *Embo J.* 17 (1998) 3005-3015.
- [85] K. Sabapathy, M. Klemm, R. Jaenisch, E.F. Wagner, Regulation of ES cell differentiation by functional and conformational modulation of p53, *Embo J.* 16 (1997) 6217-6229.
- [86] N. Sang, D.P. Stiehl, J. Bohensky, I. Leshchinsky, V. Srinivas, J. Caro, MAPK signaling up-regulates the activity of hypoxia-inducible factors by its effects on p300, *J. Biol. Chem.* 278 (2003) 14013-14019.
- [87] S. Satake, M. Kuzuya, H. Miura, T. Asai, M.A. Ramos, M. Muraguchi, Y. Ohmoto, A. Iguchi, Up-regulation of vascular endothelial growth factor in response to glucose deprivation, *Biol. Cell.* 90 (1998) 161-168.
- [88] G. Schafer, T. Cramer, G. Suske, W. Kemmner, B. Wiedenmann, M. Hocker, Oxidative stress regulates vascular endothelial growth factor-A gene transcription through Sp1- and Sp3-dependent activation of two proximal GC-rich promoter elements, *J. Biol. Chem.* 278 (2003) 8190-8198.
- [89] T. Schmid, J. Zhou, R. Kohl, B. Brune, p300 relieves p53-evoked transcriptional repression of hypoxia-inducible factor-1 (HIF-1), *Biochem. J.* 380 (2004) 289-295.
- [90] B. Schmidt-Hansen, D. Ornas, M. Grigorian, J. Klingelhofer, E. Tulchinsky, E. Lukanidin, N. Ambartsumian, Extracellular S100A4(mts1) stimulates invasive growth of mouse endothelial cells and modulates MMP-13 matrix metalloproteinase activity, *Oncogene* (2004) 1-9.
- [91] P.K. Schmidt-Kastner, K. Jardine, M. Cormier, M.W. McBurney, Absence of p53-dependent cell cycle regulation in pluripotent mouse cell lines, *Oncogene* 16 (1998) 3003-3011.
- [92] M. Scortegagna, K. Ding, Y. Oktay, A. Gaur, F. Thurmond, L.J. Yan, B.T. Marck, A.M. Matsumoto, J.M. Shelton, J.A. Richardson, M.J. Bennett, J.A. Garcia, Multiple organ pathology, metabolic abnormalities and impaired homeostasis of reactive oxygen species in *Epas1*^{-/-} mice, *Nat. Genet.* 35 (2003) 331-340.
- [93] T.N. Seagroves, H.E. Ryan, H. Lu, B.G. Wouters, M. Knapp, P. Thibault, K. Laderoute, R.S. Johnson, Transcription factor HIF-1 is a necessary mediator of the pasteur effect in mammalian cells, *Mol. Cell. Biol.* 21 (2001) 3436-3444.
- [94] G.L. Semenza, Targeting HIF-1 for cancer therapy, *Nat. Rev. Cancer* 3 (2003) 721-732.
- [95] Z.A. Sherif, S. Nakai, K.F. Pirollo, A. Rait, E.H. Chang, Downmodulation of bFGF-binding protein expression following restoration of p53 function, *Cancer Gene Ther.* 8 (2001) 771-782.
- [96] T. Shono, M. Ono, H. Izumi, S.I. Jimi, K. Matsushima, T. Okamoto, K. Kohno, M. Kuwano, Involvement of the transcription factor NF-kappaB in tubular morphogenesis of human microvascular endothelial cells by oxidative stress, *Mol. Cell. Biol.* 16 (1996) 4231-4239.
- [97] D. Shweiki, M. Neeman, A. Itin, E. Keshet, Induction of vascular endothelial growth factor expression by hypoxia and by glucose deficiency in multicell spheroids: implications for tumor angiogenesis, *Proc. Natl. Acad. Sci. USA* 92 (1995) 768-772.
- [98] A. Sodhi, S. Montaner, V. Patel, M. Zohar, C. Bais, E.A. Mesri, J.S. Gutkind, The Kaposi's sarcoma-associated herpes virus G protein-coupled receptor up-regulates

- vascular endothelial growth factor expression and secretion through mitogen-activated protein kinase and p38 pathways acting on hypoxia-inducible factor 1alpha, *Cancer Res.* 60 (2000) 4873-4880.
- [99] D.R. Spitz, D.R., Glucose deprivation-induced oxidative stress in human tumor cells, *Annals NY Acad. Sci.* 899 (2000) 349-362.
- [100] I. Stein, M. Neeman, D. Shweiki, A. Itin, E. Keshet, Stabilization of vascular endothelial growth factor mRNA by hypoxia and hypoglycemia and coregulation with other ischemia-induced genes, *Mol. Cell. Biol.* 15 (1995) 5363-5368.
- [101] C. Stolle, G. Glenn, B. Zbar, J.S. Humphrey, P. Choyke, M. Walther, S. Pack, K. Hurley, C. Andrey, R. Klausner, W.M. Linehan, Improved detection of germline mutations in the von Hippel-Lindau disease tumor suppressor gene, *Hum. Mutat.* 12 (1998) 417-423.
- [102] M. Stubbs, C.L. Bashford, J.R. Griffiths, Understanding the tumor metabolic phenotype in the genomic era, *Curr. Mol. Med.* 3 (2003) 49-59.
- [103] K. Subbaramaiah, N. Altorki, W.J. Chung, J.R. Mestre, A. Sampat, A.J. Dannenberg, Inhibition of cyclooxygenase-2 gene expression by p53, *J. Biol. Chem.* 274 (1999) 10911-10915.
- [104] Y. Sun, J.M. Cheung, J. Martel-Pelletier, J.P. Pelletier, L. Wenger, R.D. Altman, D.S. Howell, H.S. Cheung, Wild type and mutant p53 differentially regulate the gene expression of human collagenase-3 (hMMP-13), *J.Biol.Chem.* 275 (2000) 11327-11332.
- [105] H. Suzuki, A. Tomida, T. Tsuruo, Dephosphorylated hypoxia-inducible factor 1alpha as a mediator of p53- dependent apoptosis during hypoxia, *Oncogene* 20 (2001) 5779-5788.
- [106] T.P. Szatrowski, C.F. Nathan, Production of large amounts of hydrogen peroxide by human tumor cells, *Cancer Res.* 51 (1991) 794-798.
- [107] K. Takenaga, H. Nakanishi, K. Wada, M. Suzuki, O. Matsuzaki, A. Matsuura, Endo, H., Increased expression of S100A4, a metastasis-associated gene, in human colorectal adenocarcinomas, *Clin. Cancer Res.* 3 (1997) 2309-2316.
- [108] K. Tanimoto, Y. Makino, T. Pereira, L. Poellinger, Mechanism of regulation of the hypoxia-inducible factor-1 alpha by the von Hippel-Lindau tumor suppressor protein, *Embo J.* 19 (2000) 4298-4309.
- [109] H. Tian, R.E. Hammer, A.M. Matsumoto, D.W. Russell, S.L. McKnight, The hypoxia-responsive transcription factor EPAS1 is essential for catecholamine homeostasis and protection against heart failure during embryonic development, *Genes Dev.* 12 (1998) 3320-3324.
- [110] H. Tian, S.L. McKnight, D.W. Russell, Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells, *Genes Dev.* 11 (1997) 72-82.
- [111] Vander, Sherman and Luciano, *Human Physiology: The Mechanisms of Body Function*, 9th edn., Mc Graw Hill, 2004.
- [112] D.A. Vargas, S. Takahashi, Z. Ronai, Mdm2: A regulator of cell growth and death, *Adv. Cancer Res.* 89 (2003) 1-34.
- [113] P. Vaupel, F. Kallinowski, P. Okunieff, Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review, *Cancer Res.* 49 (1989) 6449-6465.
- [114] S. Walenta, T. Schroeder, W. Mueller-Klieser, Metabolic mapping with bioluminescence: basic and clinical relevance, *Biomol. Eng.* 18 (2002) 249-262.
- [115] O. Warburg, On the origin of cancer cells, *Science* 123 (1956) 309-314.

- [116] S.J. Welsh, W.T. Bellamy, M.M. Briehl, G. Powis, 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 (2002) 5089-5095.
- [117] S. Xanthoudakis, T. Curran, Identification and characterization of Ref-1, a nuclear protein that facilitates AP-1 DNA-binding activity, *Embo J.* 11 (1992) 653-665.
- [118] S. Xanthoudakis, G.G. Miao, T. Curran, The redox and DNA-repair activities of Ref-1 are encoded by nonoverlapping domains, *Proc. Natl. Acad. Sci. USA* 91 (1994) 23-27.
- [119] M. Yasuda, Y. Ohzeki, S. Shimizu, S. Naito, A. Ohtsuru, T. Yamamoto, Y. Kuroiwa, Stimulation of in vitro angiogenesis by hydrogen peroxide and the relation with ETS-1 in endothelial cells, *Life Sci.* 64 (1999) 249-258.
- [120] A.V. Yeldandi, M.S. Rao, J.K. Reddy, Hydrogen peroxide generation in peroxisome proliferator-induced oncogenesis, *Mutat. Res.* 448 (2000) 159-177.
- [121] M. Younes, L.V. Lechago, J.R. Somoano, m. Mosharaf, J. Lechago, Wide expression of the human erythrocyte glucose transporter Glut1 in human cancers, *Cancer Res.* 56 (1996) 1164-1167.
- [122] J.L. Yu, J.W. Rak, B.L. Coomber, D.J. Hicklin, R.S. Kerbel, Effect of p53 status on tumor response to antiangiogenic therapy, *Science* 295 (2002) 1526-1528.
- [123] L. Zhang, D. Yu, M. Hu, S. Xiong, A. Lang, L.M. Ellis, R.E. Pollock, Wild-type p53 suppresses angiogenesis in human leiomyosarcoma and synovial sarcoma by transcriptional suppression of vascular endothelial growth factor expression, *Cancer Res.* 60 (2000) 3655-3661.
- [124] A. Ziegler, M. von Kienlin, M. Decorps, C. Remy, High glycolytic activity in rat glioma demonstrated in vivo by correlation peak 1H magnetic resonance imaging, *Cancer Res.* 61 (2001) 5595-5600.

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