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Review

Targeting cyclic hypoxia to prevent malignant progression and therapeutic resistance of cancers

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Summary. Emerging evidence shows that cyclic hypoxia exists in most solid cancers. It is believed that under cyclic hypoxic conditions cancer cells exhibit more malignant biological behaviors than under chronic hypoxic conditions. In this review, we provide a collection of evidence showing the molecular mechanisms by which cyclic hypoxia induces aggressiveness, malignant progression, and therapeutic resistance in cancers. Moreover, we propose that cyclic hypoxia is responsible for the regulation of cancer stem cells, which possess typical biological characteristics of therapeutic resistance. Based on the present findings, some key factors regulated by cyclic hypoxia may serve as potential targets for the prevention of malignant progression and the treatment of solid cancers. Much research is necessary to gain further insights into the biological aspects of cyclic hypoxia in the development and progression of cancers.

Key words: Cyclic hypoxia, Cancer cell biology, Cancer stem cell, Therapeutic resistance, Targeted therapy

Introduction

Hypoxia is an abnormal status defined as insufficient oxygenation in tissues. It is acknowledged that there exist poorly oxygenated regions with variable oxygen partial pressure (PO_2) in most solid tumors. Generally, there are two types of hypoxia in a tumor, chronic hypoxia and cyclic hypoxia. Chronic hypoxia, a status resulting from the relative distance from vessels or low oxyhemoglobin saturation, causes limited O_2 diffusion within tumor tissues. However, cyclic hypoxia is a separate condition characterized by alternating changes of O_2 levels between hypoxia and reoxygenation (hypoxia/reoxygenation, H/R), which is also known as acute hypoxia, fluctuating hypoxia, or intermittent hypoxia. In fact, cyclic hypoxia in tumors is a consequence of transient exposure to hypoxia caused by dynamic changes in blood perfusion, not least as a result of the abnormal vasculature and the mechanical instability of microvessel walls caused by proliferating tumor cells and/or circulating blood cells. To date, chronic hypoxia has been extensively studied while investigations on cyclic hypoxia have been scarce. Nevertheless, emerging evidence has shown that cyclic hypoxia exists in many solid tumors. It has been demonstrated that tumor cells exposed to cyclic hypoxia exhibit more malignant biological behaviors in comparison to those under chronic hypoxia. Therefore, further insight into the mechanisms by which cyclic hypoxia induces malignant behaviors of cancers could offer hope for reversing hypoxia-induced malignant progression and therapeutic resistance.

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Pervasive existence of cyclic hypoxia in solid tumors

As early as 1979, Brown (1979) suggested that due to the temporary 'closure' of blood vessels, cyclic hypoxia could appear simultaneously with chronic hypoxia in tumor tissues. Since then, many methods have been introduced to further confirm the existence

and define the features of cyclic hypoxia, leading to the recognition that cyclic hypoxia is pervasive in solid tumors (Toffoli and Michiels, 2008; Matsumoto et al., 2010). For example, Minchinton et al. (1990) detected the existence of fluctuations of intratumor blood flow in KHT sarcoma implanted in mice by a microelectrode and double hypoxia marker technique; Durand and Aquino-Parsons. (2001) found distinguished features of fluctuation in blood flow in cervical carcinoma, head and neck squamous carcinoma, and mouse xenograft colon carcinoma by carrying out a dual staining mismatch measurement; and using phosphorescence lifetime imaging, Cardenas-Navia et al. (2008) detected fluctuating changes of vascular PO_2 in rat fibrosarcomas, 9L gliomas, and R3230 mammary adenocarcinomas transplanted in dorsal skin-fold window chambers, and they found that O_2 delivery to tumors was constantly unstable and that the spatial distribution of oxygen depended on the tumor types. These findings suggest that cyclic hypoxia is not only pervasive but also heterogeneously varied among different tumors.

Although hypoxia is a general concept, chronic hypoxia and cyclic hypoxia have to be further classified and treated separately. According to Bayer et al. (2011), chronic hypoxia can be separated into three subtypes, diffusion-limited hypoxia, hypoxemic hypoxia, and hypoxia due to the loss of a pressure difference between the arterial and venous ends of microvessels. Diffusion-limited hypoxia is caused by an adequate distance of oxygen diffusion from vessels to tumor cells (Bayer et al., 2011). Therefore, the severity of hypoxia resulting from the diffusion-limiting effect may vary from mild to severe, depending on the distance of the tumor cells from microvessels. Hypoxemic hypoxia is caused by malignancy- and/or therapy-induced anemia, such as dyscrasia and some chemicals capable of reducing the production of red blood cells. What is worse, carbon monoxide (CO) absorbed in the blood of cancer patients addicted to smoking competes with oxygen to bind hemoglobin and exacerbates the status of hypoxemic hypoxia. Hypothetically, the third type of chronic hypoxia mentioned above results from a stagnant flow caused by the loss of a perfusion pressure difference between arteries and veins. This is supported by the fact that tumor microvessels are tortuous and leaky, often accompanied with aberrant lymphatics and even increased interstitial fluid pressure. First, a tortuous vascular structure leads to a prolonged duration of blood flow through capillaries, thereby causing hypoxia. Second, leaky vessels reduce the intra-lumen blood tension and perfusion pressure difference between arterial and venous ends of microvessels, attenuate intra-microvascular blood flow, and thereby cause local circulating hypoxia. Finally, aberrant and nonfunctional lymphatic structures, along with transmural coupling of microvessels caused by a high interstitial fluid pressure, results in stasis of blood flow.

Similarly, cyclic hypoxia can be divided into two entities, ischemic hypoxia and hypoxemic hypoxia. Ischemic hypoxia is caused by aberrant physical vascular kinetics and/or physical obstruction, such as aggregates of tumor cells, blood cells, or fibrin clots in the vessel lumen. In 1996, Kimura and colleagues (Kimura et al., 1996) measured microvessel red cell flux (RCF) and perivascular PO_2 by using Fischer-344 rats with R3230Ac mammary carcinomas implanted in dorsal flap window chambers. It was observed that the baseline RCF and PO_2 underwent a highly dynamic process and maintained a linear correlation, demonstrating that cyclic hypoxia is common within a tumor. More recently, Yasui et al. (2010) succeeded in visualizing cyclic hypoxia noninvasively in a SCCVII murine carcinoma and a HT29 human colon carcinoma, using a combined system of electron paramagnetic resonance imaging (EPRI) and magnetic resonance imaging (MRI). They found that cyclic hypoxia existed in both tumors, and each region of interest in the tumor mass exhibited a distinct type of PO_2 fluctuation, characteristic of tumor type-dependent heterogeneity of cyclic hypoxia. Intriguingly, the in vivo compatible tracer was found to remain at relatively high and stable levels during the scanning periods, suggesting that unstable red blood flux is the direct cause of cyclic hypoxia. In addition to intratumor factors, other extratumor pathologies may cause hypotonic cyclic hypoxia in cancer patients. For instance, obstructive sleep apnea is associated with increased cancer mortality, due to hypoxia-induced aggressive behaviors of cancers (Almendros et al., 2012a,b, 2013; Nieto et al., 2012). Collectively, there is a substantial amount of direct and/or indirect evidence to support the pervasive presence of cyclic hypoxia in solid tumors. For therapeutic purposes, new strategies and approaches are necessary to develop the proper manipulation of each corresponding portion of different types of cyclic hypoxia.

Cyclic hypoxia of solid cancers centers on H/R circulation

Cyclic hypoxia is a recurrent process of prolonged continuous hypoxia interrupted by an episode of recovery to a normal oxygen supply. Unlike normoxic conditions, hypoxia followed by reoxygenation resembles the process of ischemia/reperfusion and exerts stressful stimulation on tumor cells. Being exposed to cyclic hypoxia-induced stress, cancer cells exhibit more aggressive and malignant properties. As indispensible components, frequent alternations between hypoxia and reoxygenation dominate the entire course of cyclic hypoxia. Therefore, a better understanding of the molecular and biological aspects of cyclic hypoxia necessitates further investigations on the most intriguing parts of H/R.

In response to cyclic hypoxia, cancer cells activate protective pathways to accommodate micro-environmental changes and to avoid cell death, facilitating aggressive and malignant formations.

Exposure of cancer cells to cyclic hypoxia-induced stress induces the production of reactive oxygen species (ROS). It is believed that ROS play critical roles in regulating cell death induced by H/R stress. Thioredoxin (Trx) and glutathione (GSH) are two major small molecular weight thiol-containing compounds that scavenge harmful ROS. Roudier et al. (2007) demonstrated that hepatocellular carcinoma has a great requirement for pyruvate, a precursor for the biosynthesis of GSH under hypoxic conditions, which is necessary for cancer cells to maintain their antioxidative capacities in a way that is independent of GSH. Although the level of GSH decreases during hypoxia, it is restored after reoxygenation, leading to powerful antioxidant effects against ROS induced by cyclic hypoxia. The cyclic hypoxia-induced alterations of antioxidative capacities have also been observed in some other tumors in different facets.

In lung cancer, adrenomedullin (ADM) is upregulated by hypoxia-inducible factor-1α (HIF-1α), a major transcription factor activated under hypoxic conditions, and participates in the regulation of GSH and Trx pathways to attenuate H/R-induced cell death. It has been proposed that ADM interacts with redox regulation systems through several associated signaling pathways pertaining to activation of the cAMP-protein kinase A, inhibition of nicotinamide adenine dinucleotide phosphate oxidase, and upregulation of c-glutamate-cysteine ligase (c-GCL), a rate-limiting synthetic enzyme of cGMP (Kim et al., 2010).

Peroxiredoxin 1 (Prx1) is another peroxide-detoxifying enzyme and even functions as a growth-promoting factor independent of its antioxidant capacity (Jung et al., 2001; Mu et al., 2002). Kim et al. (2007) demonstrated that Prx1 is upregulated by H/R in lung cancer. It was observed that H/R facilitates nuclear localization of nuclear factor erythroid-related factor 2 (Nrf2) and its binding to the electrophile-responsive elements allocated at the regions of the Prx1 promoter. Moreover, decreased expression of Kelch-like ECH-associated protein (Keap1), known as a suppressor of the nuclear accumulation of Nrf2, might be responsible for the increased nuclear translocation and activation of Nrf2 in response to cyclic hypoxia.

In addition to direct upregulators of scavengers to ROS, some other proteins serving as sensors and transducers of DNA damage contribute to preventing injuries caused by H/R. For instance, ataxia telangiectasia and Rad3-related factor (ATR), an ATM kinase mutated or inactivated in ataxia telangiectasia (AT) patients, is an important member of the phosphatidyl inositol-3-kinase-like kinase (PIKK) family. Upon activation by ATR, ATM is recruited to sites of DNA breaks (Andegeko et al., 2001) and phosphorylates downstream targets, such as p53, Chk2, and BRCA1 and 2, which participate in DNA repair, cell cycle control, and apoptosis regulation (Bencokova et al., 2009). Importantly, ATR has been shown to respond to DNA damage induced by H/R through initiating Chk 2- rather than Chk 1-dependent cell cycle arrest in the G_2 phase (Bencokova et al., 2009), and is particularly activated under severe hypoxic conditions in the presence of single-stranded DNA at stalled replication forks, without noticing comet-detectable NDA damage (Hammond et al., 2004; Bencokova et al., 2009). Since inhibition of either ATR or Chk1 increases the sensitivity of tumor cells to H/R-induced injury (Hammond et al., 2004), activation of ATR could prevent cancer cells from DNA damage caused by cyclic hypoxia at least in part due to the regulation of downstream Chk1.

Cyclic hypoxia and cancer stem cells (CSCs)

CSCs are a heterogeneous subgroup of tumor cells with particular biological characteristics (Sun et al., 2011). Typical of normal stem cells, CSCs can self-renew to proliferate rather than differentiate to undergo programmed death. In particular, the heterogeneous subset exhibits powerful resistance to conventional treatment modalities such as chemotherapy and radiotherapy (Philip et al., 2013). Therefore, it is now believed that treatment failures are attributed to the regrowth of remnant CSCs that survive therapeutic interventions. From a clinical perspective, the recurrence of a cancer may arise from the regrowth of surviving CSCs after a certain period of quiescence following chemoradiation therapy, although tumor bulk shrinkage or even complete disappearance is noticed at the primary stage of treatment.

Like normal stem cells, CSCs survive in a specific microenvironment, also called a niche, to maintain stemness and homeostasis. In solid tumors, the so-called niche is usually characterized by hypoxia. The interaction between hypoxia and CSCs has been associated with induction of stemness-related genes, such as Oct-4 and Notch (Keith and Simon, 2007, Garcion et al., 2009), both of which are targets regulated by HIF-1α under hypoxic conditions (Keith and Simon, 2007). We postulated that CSCs are mostly maintained by cyclic hypoxia, based on the following facts: i) the malignant features of cancer sustained by cyclic hypoxia resemble the behaviors of CSCs; ii) some CSCs co-opt host vessels for their survival; and iii) cyclic hypoxia is pervasive in cancers (Sun et al., 2011).

In a recent study, Louie et al. (2010) provided direct evidence to the above hypothesis. They harvested nonadherent breast cancer cells from original parental adherent tumor cells exposed to hypoxia, nutrient deprivation, and reoxygenation. These newly isolated cells formed colonies readily, showed highly tumorigenic properties in immune-deficient mice, and exhibited stem-like, highly metastatic, and epithelial-mesenchymal transition (EMT) properties. More recently, Bhaskara et al. (Bhaskara et al., 2012) found that neuroblastoma cells exposed to 10 cycles of H/R [hypoxia (1% O_2, 24 h); reoxygenation (normoxia, 24 h)] showed upregulation of stem cell-related factors CD133 and Oct-4, and exhibited immature neural-crest-

line phenotypes. Our previous studies demonstrated that chronic hypoxia could enrich a $CD133^+$ CSC-like cell population in human laryngeal carcinoma Hep-2 cells (Wang et al., 2013) and that cyclic hypoxia [hypoxia (1% O_2, 12 h); reoxygenation (normoxia 12 h)] could enrich CD133+ CSC-like cells more than chronic hypoxia (unpublished data). The intimate relationship between cyclic hypoxia and CSCs may depend on the significant induction of HIF-1α expression by cyclic hypoxia; it has been confirmed that downstream genes of HIF-1α include some important stemness-maintaining genes, such as Oct-4 and Notch (Keith and Simon, 2007). The exact details of how cyclic hypoxia maintains CSC's properties of self-renewal remain to be determined.

Targeting cyclic hypoxia to prevent the malignant progression of cancer cells

The effects of chronic hypoxia on tumor cells, especially their impacts on metastasis and therapeutic resistance of cancers, have been well investigated (Noman et al., 2009; Selvendiran et al., 2009; Luo et al., 2011; Hu et al., 2012). Under chronic hypoxic conditions, although the global inhibition of both transcription and translation occurs, some specific factors are paradoxically overexpressed to potentiate angiogenesis, degradation of unfolded proteins, DNA repair, and anti-apoptosis properties, which promote the adaptation of cancer cells to hypoxia stress. As a result, tumor cells acquire more malignant properties, with resultant invasiveness, metastasis, therapeutic resistance, and poor prognosis (Rademakers et al., 2008). However, some conflicting results demonstrate that chronic severe hypoxia increases radiosensitivity (Zolzer and Streffer, 2002) and is not associated with a poor prognosis (Yu and Hales, 2011). Unlike chronic hypoxia, cyclic hypoxia facilitates more malignant phenotypes including invasion, migration, metastasis, and therapeutic resistance in a variety of cancers through different underlying mechanisms (Table 1).

Cyclic hypoxia promotes aggressive and malignant formation of cancers by several intracellular routes (Fig. 1). First, induction of ROS by hypoxia stress is an unquestionable entity. The roles of cyclic hypoxia in inducing ROS, HIF-1α, angiogenesis, and radio-

Table 1. A summary of different H/R conditions, their effects on cancer cells and corresponding mechanisms by which associated genes are activated in different solid cancers.

Cancer types	H/R conditions	Results	Mechanisms	References
Colon cencer, lung cancer, breast cancer, melanoma, osteosarcoma	0% O_2 24h/ reoxygenation 2h	Reoxygenation increased adhesion among platelet, epithelial cells and tumor cells facilitating metastasis	Not defined	Zhang et al., 2011
Pancreatic cancer	0% O_2 6h/ reoxygenation 18h	Cells exposed to H/R had increased MMP-2 levels with elevated ability of invasiveness	PI3K activates Rac-1 to generate ROS by NAPDH-mediated activation of MMP-2	Binker et al., 2010
Ovarian cancer	Hypoxia (150 μM $CoCl_2$ 16h)/ reoxygenation 24h	Reoxygenation reversed inhibition of proliferation, invasion and adhesion induced by continuing $CoCl_2$	Not defined	Shi et al., 2008
Hepatocellular carcinoma	Anaerobic condition 6h/ reoxygenation 1h or 2h	H/R protected DNA damage though higher requirements of pyruvate	Pyruvate promotes the biosynthesis of glutathione through increasing oxidative metabolism after reoxygenation	Roudier et al., 2007
Breast cancer	1% O_2 24h/ reoxygenation 6h	Poorly invasive breast cancer cells displayed a marked increase in and cell migration following H/R	H/R induces LOX-dependent FAK/Src activation	Postovit et al., 2008
Lung cancer	0.2% O_2 12h/ reoxygenation 6h	Cancer cells required ADM to attenuate H/R-induced cell death	ADM maintained GSH and Trx/TrxR levels as opposed to ROS	Kim et al., 2010
Neuroblastoma	Anoxia15 h / reoxygenation 6 h in the presence or absence of glucose	Higher HIF-1α expression was seen under anoxia in the presence of glucose, while reduced HIF-1α degradation observed under both oxygen and glucose deprivation (OGD)	Altered levels of Kreb's cycle metabolites contributes to defects in the hydroxylation reaction of HIF-1α catalyzed by PHD2 under OGD	Serra-Perez et al., 2010
Lung cancer	Less than 0.05% O_2 4h/ reoxygenation 24h	Prx1 was upregulated by H/R	Nrf2 is the key transcription factor for Prx1 gene expression, and Keap1, an Nrf2 suppressor, is downregulated by H/R	Kim et al., 2007
Breast cancer	0.5% O_2 24h/ reoxygenation 24h	Downregulated NDRG1 potentiate migration of cancer cells during reoxygenation	H/R stimulates expression of functional miRNAs that donwnregulates NDRG1 expression	Lai et al., 2011
Cervical adenocarcinoma	0.02% O_2 / reoxygenation with or without glucose	Reoxygenation plus reglucose stabilized and accumulated HIF-1α	An increase in glucose availability induces Akt phosphorylation under reoxygenation with resultant up-regulated HIF-1α translation	Harada et al., 2009

resistance have been elegantly elucidated elsewhere (Dewhirst et al., 2008, Dewhirst, 2009). Second, under hypoxia stress, mRNAs are silenced by microRNAs and transferred into the cytoplasm along with the associated RNA-binding proteins (RBPs) known to repress target gene translation and promote the formation of stress granules (SGs). Once hypoxia stress is relieved, such as by reoxygenation, silenced mRNAs are released from SGs, resulting in amplified effects on the translation of certain proteins that facilitate metastasis, invasion, and therapeutic resistance. As demonstrated by Moeller et al. (2004), translation inhibition of HIF-1α-regulated downstream proteins by SGs was observed in tumor cells treated under hypoxic conditions, and reoxygenation led to robust translation of HIF-1α downstream effectors both in vitro and in vivo. Third, during the period of H/R, the presence of glucose also participates in maintaining the stability of HIF-1α, through the regulation of Kreb's cycle metabolites and induction of AKT by glucose availability (Harada et al., 2009; Serra-Perez et al., 2010). Finally, hypoxia-evoked changes in components of the blood clotting system, which have been reported to be related to tumor cell migration, contribute to cyclic hypoxia-induced malignant formation and progression.

Previous studies revealed that patients suffering from cancers are accompanied by aberrant blood coagulation and are susceptible to thrombosis (Lerner et al., 2007; Tomita et al., 2008). Zhang et al. (2011) showed that hypoxia/reoxygenation increases adhesion among platelets, epithelial cells, and tumor cells, which is an initial step required for distant metastasis. They demonstrated that tumor cells exposed to cyclic hypoxia in the presence of platelets are far more prone to distant metastasis than those exposed to chronic hypoxia in xenograft animal models.

The components of the plasminogen activation system have been associated with a poor prognosis of cancers (Schmitt et al., 1997; Duffy, 2004). The urokinase-type plasminogen activator (uPA), a component of this system, is secreted by normal and abnormal tissues as an inactive proenzyme (pro-uPA) and activated though cleavage by proteases (Duffy, 2004). By binding to the specific membrane-associated receptor [urokinase-type plasminogen activator receptor (uPAR)], uPA functions to covert the plasma proenzyme plasminogen to plasmin, which acts either directly by degrading extracellular matrix (ECM) components or indirectly by activating pro-matrix metalloproteinases, facilitating cell migration and spread (Fitzpatrick and Graham, 1998; Harbeck et al., 2001). Its inhibitor, the plasminogen activator inhibitor type-1 (PAI-1), interacts with uPA-uPAR and plays a key role in cell signaling and proliferation (Schmitt et al., 1997; Fitzpatrick and Graham, 1998; Harbeck et al., 2001; Duffy, 2004; Sprague et al., 2007). In addition, Sprague et al. (Sprague et al., 2007) studied the effects of reoxygenation on the expression of uPA, PAI-1, and uPAR in head and neck carcinoma cells, and they showed that H/R enhances the expression of mRNAs and proteins of the three components, although no uniform correlation pattern was found between the mRNA and protein levels. More recently, Gupta et al. (2011) confirmed the anti-tumor efficacy of uPA or uPAR downregulation. The interaction between H/R and the alteration of blood coagulation remains to be determined.

Cyclic hypoxia also induces invasiveness and migration of cancer cells through other intracellular pathways. Binker et al. (2010) showed that H/R upregulates matrix metalloproteinase-2 (MMP-2) secretion, which induces pancreatic cancer cell invasion, as a consequence of ROS production mediated through PI3K-dependent activation of the upstream signaling protein Rac1, a member of the Rho family of small GTPases. In addition, Postovit et al. (2008) showed that lysyl oxidase (LOX), a copper-dependent amine oxidase that was previously thought to function only in the extracellular milieu by cross-linking collagens or elastin to increase extracellular matrix tensile strength, participates in cyclic hypoxia-induced cell migration. They also demonstrated that poorly invasive breast cancer cells display a marked increase in LOX-dependent FAK/Src activation and cell migration

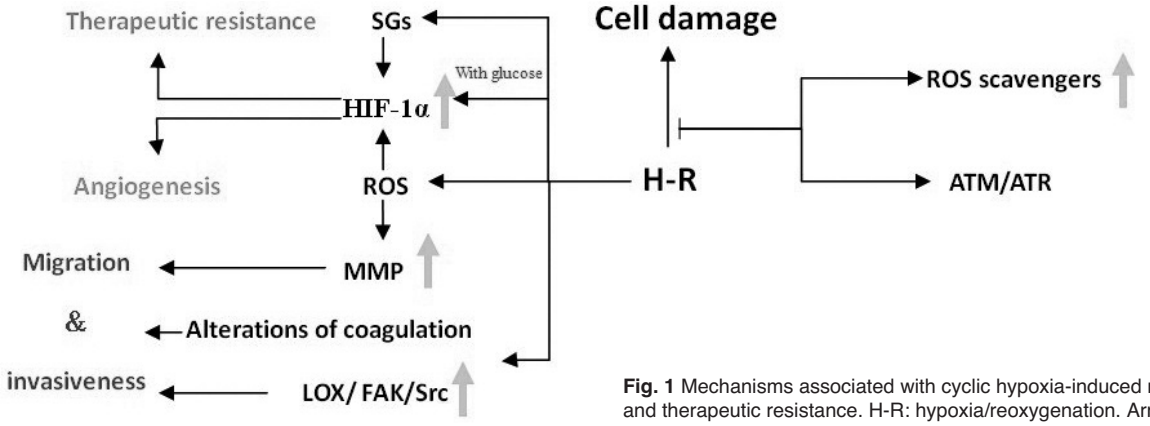

Fig. 1 Mechanisms associated with cyclic hypoxia-induced malignant progression and therapeutic resistance. H-R: hypoxia/reoxygenation. Arrow: upregulation.

following H/R treatment. Similarly, Shi et al. (2008) revealed that reoxygenation could reverse the inhibition of proliferation, invasion, and adhesion induced by hypoxia. Moreover, Lai et al. (2011) found that reoxygenation could downregulate NDRG1 to promote cancer cell migration. More recently, Chaudary et al. (2013) showed that exposure of tumor-bearing mice to cyclic hypoxia promotes the chance of distant metastasis via activation of the CXCR4 or Hedgehog gene. Collectively, the underlying mechanisms of malignant cancer cell progression induced by cyclic hypoxia are much more sophisticated than one can imagine. Interruption of these intriguing processes can only be obtained by targeting some key regulators of cyclic hypoxia-induced intracellular responses.

Targeting cyclic hypoxia to prevent therapeutic resistance in cancers

Notwithstanding, investigations on the effects of regular episodes of H/R on cancer cells in labs disclose the severity of cyclic hypoxia. As early as 1996, Kang et al. (1996) demonstrated that the human colon cancer cell line HT-29 and its MMC-resistant subline HT-29R13 were more resistant to MMC under cyclic hypoxia than under continuous hypoxia. The corresponding mechanism involves decreased topoisomerase IIα (topo II) activity incurred from the reduced expression of topo II protein under cyclic hypoxia.

Estrogen receptor (ER)-α is a primary target for both chemoprevention and endocrine therapy of breast cancer. Reduced levels of ER-α are associated with the progression of malignancy (Lapidus et al., 1998). Cooper et al. (2004) found that breast cancer cells exposed to H/R rather than continuous chronic hypoxia acquired a feature of persistent proteasome-dependent downregulation of ER-α. In addition to downregulating factors that facilitate chemosensitivity, cyclic hypoxia induces advantageous upregulation of HIF-1α in a cyclic H/R-dependent manner to promote therapeutic resistance.

Some investigators (Martinive et al., 2006) found that when preconditioned in 3-4 cycles of hypoxia/reoxygenation, tumor endothelial cells exhibit progressive accumulation of HIF-1α, increased migration ability, and augmented resistance to chemotherapeutic agents. They demonstrated that activation of the PI3K/AKT pathway and stimulation of mitochondrial respiration by cyclic hypoxia mediate the stabilization and accumulation of HIF-1α, while the eNOS pathway acts oppositely to prevent cyclic hypoxia-driven induction of HIF-1α. The responses of endothelial cells to cyclic hypoxia depend on either the cell type or the severity of hypoxia (Toffoli and Michiels, 2008). Interestingly, AKT and eNOS are both highly activated in the reoxygenation phase, suggesting that HIF-1α is not induced only under hypoxia. As demonstrated by Moeller et al. (2004), during the entire course of cyclic hypoxia, SGs store functional mRNAs under hypoxia and release them upon reoxygenation, which amplifies the expression of HIF-1α regulators. This may partly explain why HIF-1α is paradoxically overexpressed and activated under cyclic hypoxia.

Studies by Malec et al. (2010) provided another explanation for the specific regulation pattern of cyclic hypoxia-induced HIF-1α expression. They demonstrated that NADPH oxidase 1 subunit (NOX1) induces the production of ROS and stimulates the expression of Nrf2 under cyclic hypoxia rather than under continuous chronic hypoxia in lung adenocarcinoma A549 cells. As a result, targeting Nrf2 and Trx1 could upregulate HIF-1α expression under cyclic hypoxia. Apart from this, tumor cells subjected to cyclic hypoxia have a high metabolic activity to exclude hypoxia toxins, such as tirapazamine (TPZ), leading to a reduced drug concentration reaching the severely hypoxic tumor cells located at distant sites remote from vessels (Cardenas-Navia et al., 2007). A better understanding and further dissection of cyclic hypoxia in solid cancers and tumor endothelial cells may provide novel insights into therapeutic resistance and shed light on the development of molecular target therapies (Fig. 2).

Potential strategies to target cyclic hypoxia

Hypoxia is a major cause of tumor resistance to conventional therapies, such as chemotherapy and radiotherapy. Some groups have tried using inhalation of Carbogen (95% O_2 and 5% CO_2) and hyperbaric oxygen chambers to improve the hypoxic microenvironment within tumors, and thus therapeutic resistance, but the results are far from satisfactory (Hoskin et al., 2009; Rademakers et al., 2013; Janssens et al., 2014). Therefore, it is mandatory to make modifications to these traditional approaches and to explore new ways of oxygen delivery to hypoxic tumors.

It is recognized that the tumor vasculature is very different from its counterpart in normal tissues, the former of which exhibits architectural distortion, higher permeability, and irregular infusion. This peculiar characteristic of the tumor vasculature not only facilitates cyclic hypoxia, but also provides a specific target for therapeutic purposes. In this sense, cyclic hypoxia in tumors would be effectively rectified whenever the intratumor vasculature could be properly targeted.

Vascular disrupting agents (VDAs) serve as a novel class of vascular-targeting anti-cancer drugs. Unlike angiogenesis inhibitors (AIs) that prevent neoformation of the vascular structure, VDAs directly block or damage existing blood vessels to cause necrosis. To date, VDAs are in clinical trials, and small molecular VDAs have been mostly studied (Gridelli et al., 2009). There are two classes of small molecular VDAs, including tubulin-binding agents and flavonoids. The tubulin-binding agents bind to tubulin molecules of the endothelium and cause tubulin depolymerization, which disrupts the structures of actin and tubulin, leading to

endothelial cell damage. The flavonoids function to induce TNF secretion by tumor cells to cause apoptosis of endothelial cells constituting microvessels (Patterson and Rustin, 2007; Gridelli et al., 2009, Siemann, 2011). VDAs have been believed to induce intratumoral necrosis, leaving the remaining periphery oxygenated. In this regard, the combination of VDAs with AIs may cut both cyclic and chronic hypoxia from the "root". Unexpectedly, phase III clinical trials announced that the promising small molecular VDA ASA404 failed to improve frontline efficacy in advanced non-small cell lung cancer (Gridelli et al., 2009). Therefore, further insights into the biological effects of VDAs and the development of more potent VDAs are absolutely necessary.

SGs exist in cancer tissues suffering from hypoxic stress rather than in nonstressed normal tissue. Therefore, the strategies for specific targeting of cancers may focus on either direct intervention to SGs or improvement of the stressful tumor microenvironment conditions. Because the formed SGs consist of functional miRNAs and mRNA-binding proteins, the identification of key miRNAs and binding proteins is necessary for targeting SGs. For instance, SG-associated protein CUGBP1 participates in the upregulation of P21 following bortezomib treatment, and consequently prevents bortezomib-mediated cell death (Gareau et al., 2011). Therefore, targeting CUBP1 could potentiate bortezomib-mediated apoptosis. Further studies must concentrate on identifying key miRNAs and the associated binding proteins that regulate critical factors, such as HIF-1α, in cyclic hypoxia.

ROS generated by H/R, an intimate process of cyclic hypoxia, promotes both tumor growth and aggressive progression via genetic instability. Efficient antioxidant therapies improve the stressful conditions found in the cancer cell microenvironment and eliminate ROS. Therefore, it is imperative to develop novel strategies for more efficient delivery of antioxidant drugs to the cyclic hypoxia-affected regions in solid cancers.

Conclusion

Cyclic hypoxia exists in most solid tumors and is related with the malignant progression and therapeutic resistance of cancers. When the tumor microenvironment is under cyclic hypoxic conditions, multiple intracellular pathways participate in the regulation of biological behaviors of cancer cells, including CSCs. Targeting some key factors in cell signaling and special cell populations in the tumor microenvironment may have potential implications in preventing cyclic hypoxia-driven malignant progression and therapeutic resistance, as well as the effective treatment of cancers. The future direction for managing cyclic hypoxia is to concentrate on the development of more potent VDAs, specific SG-targeting agents, and efficient antioxidant therapies.

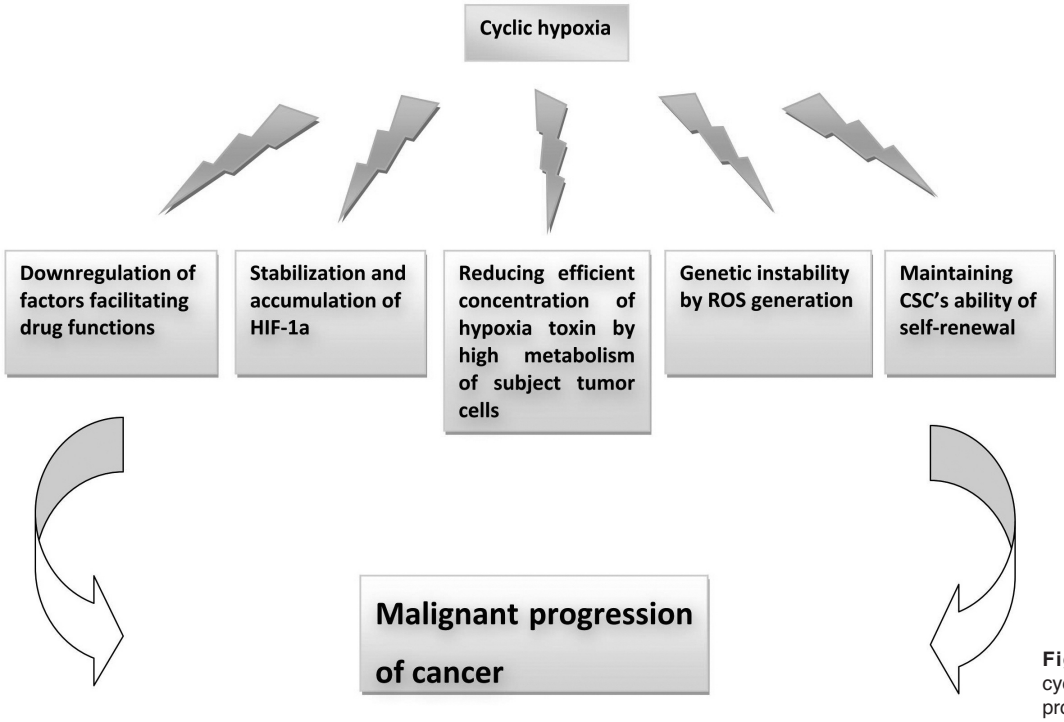

Fig. 2. Putative pathways for cyclic hypoxia-induced malignant progression of cancers.

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References

Almendros I., Montserrat J.M., Torres M., Bonsignore M.R., Chimenti L., Navajas D. and Farre R. (2012a). Obesity and intermittent hypoxia increase tumor growth in a mouse model of sleep apnea. Sleep Med. 13, 1254-1260.

Almendros I., Montserrat J.M., Ramirez J., Torres M., Duran-Cantolla J., Navajas D. and Farre R. (2012b). Intermittent hypoxia enhances cancer progression in a mouse model of sleep apnoea. Eur. Respir. J. 39, 215-217.

Almendros I., Montserrat J.M., Torres M., Dalmases M., Cabanas M.L., Campos-Rodriguez F., Navajas D. and Farre R. (2013). Intermittent hypoxia increases melanoma metastasis to the lung in a mouse model of sleep apnea. Respir. Physiol. Neurobiol. 186, 303-307.

Andegeko Y., Moyal L., Mittelman L., Tsarfaty I., Shiloh Y. and Rotman G. (2001). Nuclear retention of atm at sites of DNA double strand breaks. J. Biol. Chem. 276, 38224-38230.

Bayer C., Shi K., Astner S.T., Maftei C.A. and Vaupel P. (2011). Acute versus chronic hypoxia: Why a simplified classification is simply not enough. Int. J. Radiat. Oncol. Biol. Phys. 80, 965-968.

Bencokova Z., Kaufmann M.R., Pires I.M., Lecane P.S., Giaccia A.J. and Hammond E.M. (2009). Atm activation and signaling under hypoxic conditions. Mol. Cell. Biol. 29, 526-537.

Bhaskara V.K., Mohanam I., Rao J.S. and Mohanam S. (2012). Intermittent hypoxia regulates stem-like characteristics and differentiation of neuroblastoma cells. PLoS One 7, e30905.

Binker M.G., Binker-Cosen A.A., Richards D., Gaisano H.Y., de Cosen R.H. and Cosen-Binker L.I. (2010). Hypoxia-reoxygenation increase invasiveness of panc-1 cells through rac1/mmp-2. Biochem. Biophys. Res. Commun. 393, 371-376.

Brown J.M. (1979). Evidence for acutely hypoxic cells in mouse tumours, and a possible mechanism of reoxygenation. Br. J. Radiol. 52, 650-656.

Cardenas-Navia L.I., Secomb T.W. and Dewhirst M.W. (2007). Effects of fluctuating oxygenation on tirapazamine efficacy: Theoretical predictions. Int. J. Radiat. Oncol. Biol. Phys. 67, 581-586.

Cardenas-Navia L.I., Mace D., Richardson R.A., Wilson D.F., Shan S. and Dewhirst M.W. (2008). The pervasive presence of fluctuating oxygenation in tumors. Cancer Res. 68, 5812-5819.

Chaudary N., Mujcic H., Wouters B.G. and Hill R.P. (2013). Hypoxia and metastasis in an orthotopic cervix cancer xenograft model. Radiother. Oncol. 108, 506-510.

Cooper C., Liu G.Y., Niu Y.L., Santos S., Murphy L.C. and Watson P.H. (2004). Intermittent hypoxia induces proteasome-dependent down-regulation of estrogen receptor alpha in human breast carcinoma. Clin. Cancer Res. 10, 8720-8727.

Dewhirst M.W. (2009). Relationships between cycling hypoxia, hif-1, angiogenesis and oxidative stress. Radiat. Res. 172, 653-665.

Dewhirst M.W., Cao Y. and Moeller B. (2008). Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. Nat. Rev. Cancer 8, 425-437.

Duffy M.J. (2004). The urokinase plasminogen activator system: Role in malignancy. Curr. Pharm. Des. 10, 39-49.

Durand R.E. and Aquino-Parsons C. (2001). Clinical relevance of intermittent tumour blood flow. Acta Oncol. 40, 929-936.

Fitzpatrick T.E. and Graham C.H. (1998). Stimulation of plasminogen activator inhibitor-1 expression in immortalized human trophoblast cells cultured under low levels of oxygen. Exp. Cell Res. 245, 155-162.

Garcion E., Naveilhan P., Berger F. and Wion D. (2009). Cancer stem cells: Beyond koch's postulates. Cancer Lett. 278, 3-8.

Gareau C., Fournier M.J., Filion C., Coudert L., Martel D., Labelle Y. and Mazroui R. (2011). P21(waf1/cip1) upregulation through the stress granule-associated protein cugbp1 confers resistance to bortezomib-mediated apoptosis. PLoS One 6, e20254.

Gridelli C., Rossi A., Maione P., Rossi E., Castaldo V., Sacco P.C. and Colantuoni G. (2009). Vascular disrupting agents: A novel mechanism of action in the battle against non-small cell lung cancer. Oncologist 14, 612-620.

Gupta R., Chetty C., Bhoopathi P., Lakka S., Mohanam S., Rao J.S. and Dinh D.E. (2011). Downregulation of upa/upar inhibits intermittent hypoxia-induced epithelial-mesenchymal transition (emt) in daoy and d283 medulloblastoma cells. Int. J. Oncol. 38, 733-744.

Hammond E.M., Dorie M.J. and Giaccia A.J. (2004). Inhibition of atr leads to increased sensitivity to hypoxia/reoxygenation. Cancer Res. 64, 6556-6562.

Harada H., Itasaka S., Kizaka-Kondoh S., Shibuya K., Morinibu A., Shinomiya K. and Hiraoka M. (2009). The akt/mtor pathway assures the synthesis of hif-1alpha protein in a glucose- and reoxygenation-dependent manner in irradiated tumors. J. Biol. Chem. 284, 5332-5342.

Harbeck N., Kruger A., Sinz S., Kates R.E., Thomssen C., Schmitt M. and Janicke F. (2001). Clinical relevance of the plasminogen activator inhibitor type 1--a multifaceted proteolytic factor. Onkologie 24, 238-244.

Hoskin P., Rojas A. and Saunders M. (2009). Accelerated radiotherapy, carbogen, and nicotinamide (arcon) in the treatment of advanced bladder cancer: Mature results of a phase ii nonrandomized study. Int. J. Radiat. Oncol. Biol. Phys. 73, 1425-1431.

Hu Y.L., DeLay M., Jahangiri A., Molinaro A.M., Rose S.D., Carbonell W.S. and Aghi M.K. (2012). Hypoxia-induced autophagy promotes tumor cell survival and adaptation to antiangiogenic treatment in glioblastoma. Cancer Res. 72, 1773-1783.

Janssens G.O., van Bockel L.W., Doornaert P.A., Bijl H.P., van den Ende P., de Jong M.A., van den Broek G.B., Verbist B.M., Terhaard C.H., Span P.N. and Kaanders J.H. (2014). Computed tomography-based tumour volume as a predictor of outcome in laryngeal cancer: Results of the phase 3 arcon trial. Eur. J. Cancer 50, 1112-1119.

Jung H., Kim T., Chae H.Z., Kim K.T. and Ha H. (2001). Regulation of macrophage migration inhibitory factor and thiol-specific antioxidant protein pag by direct interaction. J. Biol. Chem. 276, 15504-15510.

Kang Y., Greaves B. and Perry R.R. (1996). Effect of acute and chronic intermittent hypoxia on DNA topoisomerase ii alpha expression and mitomycin c-induced DNA damage and cytotoxicity in human colon cancer cells. Biochem. Pharmacol. 52, 669-676.

Keith B. and Simon M.C. (2007). Hypoxia-inducible factors, stem cells, and cancer. Cell 129, 465-472.

Kim Y.J., Ahn J.Y., Liang P., Ip C., Zhang Y. and Park Y.M. (2007). Human prx1 gene is a target of nrf2 and is up-regulated by hypoxia/reoxygenation: Implication to tumor biology. Cancer Res. 67, 546-554.

Kim S.M., Kim J.Y., Lee S. and Park J.H. (2010). Adrenomedullin protects against hypoxia/reoxygenation-induced cell death by

suppression of reactive oxygen species via thiol redox systems. FEBS Lett. 584, 213-218.

Kimura H., Braun R.D., Ong E.T., Hsu R., Secomb T.W., Papahadjopoulos D., Hong K. and Dewhirst M.W. (1996). Fluctuations in red cell flux in tumor microvessels can lead to transient hypoxia and reoxygenation in tumor parenchyma. Cancer Res. 56, 5522-5528.

Lai L.C., Su Y.Y., Chen K.C., Tsai M.H., Sher Y.P., Lu T.P., Lee C.Y. and Chuang E.Y. (2011). Down-regulation of ndrg1 promotes migration of cancer cells during reoxygenation. PLoS One 6, e24375.

Lapidus R.G., Nass S.J. and Davidson N.E. (1998). The loss of estrogen and progesterone receptor gene expression in human breast cancer. J. Mammary Gland Biol. Neoplasia 3, 85-94.

Lerner D.L., Walsh C.S., Cass I., Karlan B.Y. and Li A.J. (2007). The prognostic significance of thrombocytosis in uterine papillary serous carcinomas. Gynecol. Oncol. 104, 91-94.

Louie E., Nik S., Chen J.S., Schmidt M., Song B., Pacson C., Chen X.F., Park S., Ju J. and Chen E.I. (2010). Identification of a stem-like cell population by exposing metastatic breast cancer cell lines to repetitive cycles of hypoxia and reoxygenation. Breast Cancer Res 12, R94.

Luo W., Hu H., Chang R., Zhong J., Knabel M., O'Meally R., Cole R.N., Pandey A. and Semenza G.L. (2011). Pyruvate kinase m2 is a phd3-stimulated coactivator for hypoxia-inducible factor 1. Cell 145, 732-744.

Malec V., Gottschald O.R., Li S., Rose F., Seeger W. and Hanze J. (2010). Hif-1 alpha signaling is augmented during intermittent hypoxia by induction of the nrf2 pathway in nox1-expressing adenocarcinoma a549 cells. Free Radic. Biol. Med. 48, 1626-1635.

Martinive P., Defresne F., Bouzin C., Saliez J., Lair F., Gregoire V., Michiels C., Dessy C. and Feron O. (2006). Preconditioning of the tumor vasculature and tumor cells by intermittent hypoxia: Implications for anticancer therapies. Cancer Res. 66, 11736-11744.

Matsumoto S., Yasui H., Mitchell J.B. and Krishna M.C. (2010). Imaging cycling tumor hypoxia. Cancer Res. 70, 10019-10023.

Minchinton A.I., Durand R.E. and Chaplin D.J. (1990). Intermittent blood flow in the kht sarcoma--flow cytometry studies using hoechst 33342. Br. J. Cancer 62, 195-200.

Moeller B.J., Cao Y., Li C.Y. and Dewhirst M.W. (2004). Radiation activates hif-1 to regulate vascular radiosensitivity in tumors: Role of reoxygenation, free radicals, and stress granules. Cancer Cell 5, 429-441.

Mu Z.M., Yin X.Y. and Prochownik E.V. (2002). Pag, a putative tumor suppressor, interacts with the myc box ii domain of c-myc and selectively alters its biological function and target gene expression. J. Biol. Chem. 277, 43175-43184.

Nieto F.J., Peppard P.E., Young T., Finn L., Hla K.M. and Farre R. (2012). Sleep-disordered breathing and cancer mortality: Results from the wisconsin sleep cohort study. Am. J. Respir. Crit. Care Med. 186, 190-194.

Noman M.Z., Buart S., Van Pelt J., Richon C., Hasmim M., Leleu N., Suchorska W.M., Jalil A., Lecluse Y., El Hage F., Giuliani M., Pichon C., Azzarone B., Mazure N., Romero P., Mami-Chouaib F. and Chouaib S. (2009). The cooperative induction of hypoxia-inducible factor-1 alpha and stat3 during hypoxia induced an impairment of tumor susceptibility to ctl-mediated cell lysis. J. Immunol. 182, 3510-3521.

Patterson D.M. and Rustin G.J. (2007). Vascular damaging agents. Clin. Oncol. (R. Coll. Radiol). 19, 443-456.

Philip B., Ito K., Moreno-Sanchez R. and Ralph S.J. (2013). Hif expression and the role of hypoxic microenvironments within primary tumours as protective sites driving cancer stem cell renewal and metastatic progression. Carcinogenesis 34, 1699-1707.

Postovit L.M., Abbott D.E., Payne S.L., Wheaton W.W., Margaryan N.V., Sullivan R., Jansen M.K., Csiszar K., Hendrix M.J. and Kirschmann D.A. (2008). Hypoxia/reoxygenation: A dynamic regulator of lysyl oxidase-facilitated breast cancer migration. J. Cell. Biochem. 103, 1369-1378.

Rademakers S.E., Span P.N., Kaanders J.H., Sweep F.C., van der Kogel A.J. and Bussink J. (2008). Molecular aspects of tumour hypoxia. Mol. Oncol. 2, 41-53.

Rademakers S.E., Hoogsteen I.J., Rijken P.F., Oosterwijk E., Terhaard C.H., Doornaert P.A., Langendijk J.A., van den Ende P., Takes R., De Bree R., van der Kogel A.J., Bussink J. and Kaanders J.H. (2013). Pattern of caix expression is prognostic for outcome and predicts response to arcon in patients with laryngeal cancer treated in a phase iii randomized trial. Radiother. Oncol. 108, 517-522.

Roudier E., Bachelet C. and Perrin A. (2007). Pyruvate reduces DNA damage during hypoxia and after reoxygenation in hepatocellular carcinoma cells. FEBS J. 274, 5188-5198.

Schmitt M., Harbeck N., Thomssen C., Wilhelm O., Magdolen V., Reuning U., Ulm K., Hofler H., Janicke F. and Graeff H. (1997). Clinical impact of the plasminogen activation system in tumor invasion and metastasis: Prognostic relevance and target for therapy. Thromb. Haemost. 78, 285-296.

Selvendiran K., Bratasz A., Kuppusamy M.L., Tazi M.F., Rivera B.K. and Kuppusamy P. (2009). Hypoxia induces chemoresistance in ovarian cancer cells by activation of signal transducer and activator of transcription 3. Int. J. Cancer 125, 2198-2204.

Serra-Perez A., Planas A.M., Nunez-O'Mara A., Berra E., Garcia-Villoria J., Ribes A. and Santalucia T. (2010). Extended ischemia prevents hif1alpha degradation at reoxygenation by impairing prolyl-hydroxylation: Role of krebs cycle metabolites. J. Biol. Chem. 285, 18217-18224.

Shi J., Wan Y. and Di W. (2008). Effect of hypoxia and re-oxygenation on cell invasion and adhesion in human ovarian carcinoma cells. Oncol. Rep. 20, 803-807.

Siemann D.W. (2011). The unique characteristics of tumor vasculature and preclinical evidence for its selective disruption by tumor-vascular disrupting agents. Cancer Treat. Rev. 37, 63-74.

Sprague L.D., Tomaso H., Mengele K., Schilling D., Bayer C., Stadler P., Schmitt M. and Molls M. (2007). Effects of hypoxia and reoxygenation on the expression levels of the urokinase-type plasminogen activator, its inhibitor plasminogen activator inhibitor type-1 and the urokinase-type plasminogen activator receptor in human head and neck tumour cells. Oncol. Rep. 17, 1259-1268.

Sun Q., Li X., Lu X. and Di B. (2011). Cancer stem cells may be mostly maintained by fluctuating hypoxia. Med. Hypotheses 76, 471-473.

Toffoli S. and Michiels C. (2008). Intermittent hypoxia is a key regulator of cancer cell and endothelial cell interplay in tumours. FEBS J. 275, 2991-3002.

Tomita M., Shimizu T., Hara M., Ayabe T. and Onitsuka T. (2008). Prognostic impact of thrombocytosis in resectable non-small cell lung cancer. Interact. Cardiovasc. Thorac. Surg. 7, 613-615.

Wang M., Li X., Qu Y., Xu O. and Sun Q. (2013). Hypoxia promotes radioresistance of cd133-positive hep-2 human laryngeal squamous carcinoma cells in vitro. Int. J. Oncol. 43, 131-140.

Yasui H., Matsumoto S., Devasahayam N., Munasinghe J.P., Choudhuri R., Saito K., Subramanian S., Mitchell J.B. and Krishna M.C. (2010). Low-field magnetic resonance imaging to visualize chronic and cycling hypoxia in tumor-bearing mice. Cancer Res. 70, 6427-6436.

Yu L. and Hales C.A. (2011). Long-term exposure to hypoxia inhibits tumor progression of lung cancer in rats and mice. BMC Cancer 11, 331.

Zhang N., Zhang W.J., Cai H.Q., Liu H.L., Peng L., Li C.H., Ye L.Y., Xu S.Q., Yang Z.H. and Lou J.N. (2011). Platelet adhesion and fusion to endothelial cell facilitate the metastasis of tumor cell in hypoxia-reoxygenation condition. Clin. Exp. Metastasis 28, 1-12.

Zolzer F. and Streffer C. (2002). Increased radiosensitivity with chronic hypoxia in four human tumor cell lines. Int. J. Radiat. Oncol. Biol. Phys. 54, 910-920.

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