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Cellular and molecular mechanisms in the hypoxic tissue: role of HIF-1 and ROS

Andrea B. Zepeda[1], Adalberto Pessoa jr.[2], Rodrigo L. Castillo[3], Carolina A. Figueroa[1], Victor M. Pulgar[4,5] and Jorge G. Farías[1]*

[1]*Facultad de Ingeniería, Departamento de Ingeniería Química, Ciencias y Administración, Universidad de La Frontera, Casilla 54-D, Temuco, Chile*
[2]*Department of Biochemical and Pharmaceutical Technology, School of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 580-Bloco 16, São Paulo, SP, 05508-900, Brazil*
[3]*Pathophysiology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile*
[4]*Biomedical Research Infrastructure Center, Winston-Salem State University, Winston Salem, NC, USA*
[5]*Department of Obstetrics and Gynecology, Wake Forest School of Medicine, Winston Salem, NC, USA*

Reactive oxygen species such as superoxide anion radicals (O_2^-) and hydrogen peroxide (H_2O_2) have for long time been recognized as undesirable by-products of the oxidative mitochondrial generation of adenosine triphosphate (ATP). Recently, these highly reactive species have been associated to important signaling pathways in diverse physiological conditions such as those activated in hypoxic microenvironments. The molecular response to hypoxia requires fast-acting mechanisms acting within a wide range of partial pressures of oxygen (O_2). Intracellular O_2 sensing is an evolutionary preserved feature, and the best characterized molecular responses to hypoxia are mediated through transcriptional activation. The transcription factor, hypoxia-inducible factor 1 (HIF-1), is a critical mediator of these adaptive responses, and its activation by hypoxia involves O_2-dependent posttranslational modifications and nuclear translocation. Through the induction of the expression of its target genes, HIF-1 coordinately regulates tissue O_2 supply and energetic metabolism. Other transcription factors such as nuclear factor κB are also redox sensitive and are activated in pro-oxidant and hypoxic conditions. The purpose of this review is to summarize new developments in HIF-mediated O_2 sensing mechanisms and their interactions with reactive oxygen species–generating pathways in normal and abnormal physiology.

KEY WORDS—oxidative stress; hypoxia-inducible factor (HIF-1); nuclear factor κB (NF-κB); hypoxic tissue; reactive oxygen species

INTRODUCTION

Many organisms depend on oxygen for their survival,[1] then they developed cardiovascular and respiratory systems able to capture and appropriately distribute oxygen to the tissues. This oxygen in turn serves as an electron acceptor in the mitochondria to generate ATP and for other biochemical reactions, through physiological mechanisms that have been kept through the evolution.[2] The oxygenation of any tissue relies on the balance between oxygen delivery and consumption,[3,4] and any decrease in oxygen availability requires to be quickly detected to generate the appropriate changes and thus to avoid potentially harmful hypoxic episodes. An oxygen sensor system is needed, which varies from organ to organ and from cell to cell according to the threshold of activation.[2] A basic mechanism of oxygen detection at the cellular level comprises the transcription factor, hypoxia-inducible factor (HIF), the enzymes prolyl hydroxylases (PHD), the ubiquitin ligase protein von Hippel Lindau (pVHL) and the proteosome 26S.[5] The cellular responses to hypoxia can be presented as a three-level system: (a) detection or oxygen sensor, (b) regulation, which controls genetic expression, and (c) multiple effects.[6,7]

HIF-1 promotes the up-regulation of genes inducible for hypoxia such as genes that control erythropoiesis, glycolysis and pro-angiogenic factors, such as vascular endothelial growth factor (VEGF).[3–8] As one example of the interactions between reactive oxygen species (ROS) and the transcriptional response to hypoxia, recent studies indicated that an increase in ROS production contributes to the stabilization of HIF-1α and to the hypoxia-induced VEGF expression.[4,5,9,10]

Intracellular oxidative stress is produced in normal conditions by the formation of ROS as the result of the normal mitochondrial respiration, also during reperfusion in hypoxic tissue and in association with infection and inflammation.[11]

*Correspondence to: Jorge G. Farias, Facultad de Ingeniería, Departamento de Ingeniería Química, Ciencias y Administración, Universidad de La Frontera, Casilla 54-D, Temuco, Chile.E-mail: jorge.farias@ufrontera.cl

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In addition, ROS have been involved in a wide range of vascular diseases associated with the functional properties of the endothelial cell barrier.[10]

Several reports support the idea that many diseases are generated as a result of the hypoxic condition, or on the contrary, some illnesses generate a hypoxic microenvironment, thereby inducing the activation of hypoxia-responsive markers. Thus, oxidative stress can be used as a biomarker for the detection of many illnesses and as a basis of treatment selectively directed toward the altered intracellular pathways. The aim of this review is to summarize some of the hypoxia-responsive molecular mechanisms involved in the generation of cellular oxidative stress.

Hypoxia

Hypoxia is defined as the threshold where the oxygen concentration is a limiting factor for the normal cellular processes; this is because oxygen is an essential factor required for metabolism, including the production of ATP. Thus, an inadequate oxygen supply would change the metabolic status as well as other important functions in the affected cells,[1] making hypoxia a paradigm of responses involving the whole organism.[6]

The earth's atmosphere consists of 20.9% oxygen, but tissues normally experience much lower concentrations, typically in the order of 2%–9%.[7] This level of oxygen still represents an appropriate environment for the cells even when the cells are close to a hypoxic threshold. Thus, oxygen levels need to be closely are monitored and those intracellular mechanisms involved in O_2 homeostasis are implicated in many modern diseases such as heart attack, cancer, diabetic retinopathy and rheumatoid arthritis.[1] Conditions that induce transitory hypoxia include exercise, in sepsis or in traumatized cells, whereas systemic chronic hypoxia may be observed during exposure to high altitude or in less oxygenated tissues zones, such as the renal marrow.[6]

Because of its clinical relevance in radiation therapy, tumor hypoxia has been one of the most studied hypoxic microenvironments.[9] Some of the typical characteristics of carcinogenic cells such as accelerated proliferation and abnormal metabolism cause an imbalance between oxygen supply and consumption, leading to tissue hypoxia, with the consequent alterations in cellular function.[12] Because this is a dynamic process resulting from changes in the rate of delivery or oxygen consumption, the term "cyclic hypoxia" has been suggested The kinetics of this hypoxia cycle is complex, involving cycle time between a few cycles per hour to many hours per day.[3] This intermittent hypoxia induces a series of cellular, molecular and pathophysiological responses, which result in adaptation and survival or injury and cellular death. This is because it leads to an oxidative stress and induces lipid peroxidation, increasing the production in stress responding proteins, and apoptosi.[13]

When the metabolism of the tissue exceeds the metabolic supply, like in the case of most tumors, the hypoxic response leads to a series of changes in protein expression to promote defense factors against cellular death,[14] with greater probability of generating metastasis. This association is explained with the increase in the expression of genes associated to metastasis such as CXCR4, uPAR, VEGF and osteopontin in tumors of rats exposed to a cyclic hypoxia regime. Other studies established that the hypoxia cycle increases oxidative stress in mammary tumors of MMTV-PyMT transgenic rats, producing DNA damage and lipid peroxidation.[15] In addition, there are also differences in the efficiency of the metastasis between cyclic and chronic hypoxia, which could be caused by substantial changes in the HIF-1-dependent genetic expression.[3]

Oxidative stress

Oxidative stress (arising either from mitochondrial electron transport chain or excessive stimulation of enzymatic sources) results in a deleterious process that can damage cell structures, including lipids, membranes, proteins and DNA. In contrast, beneficial effects of the highly reactive compounds produced [e.g. O_2^-, and nitric oxide (NO)] occur at low/moderate concentrations and involve physiological roles in cellular responses to anoxia, as for example in defense against infectious agents, cellular signaling pathways and the induction of a mitogenic response. Various ROS-mediated actions in fact protect cells against ROS-induced oxidative stress and re-establish or maintain a "redox balance," also called "redox homeostasis." For example, a growing body of evidence shows that intracellular ROS act as secondary messengers in intracellular signaling cascades, which induce and maintain physiological responses to hypoxia and inflammation. A coordinated oxygen-dependent gene expression sevems to modulate inflammatory responses to hypoxia,[15] with the involvement of the transcription factors HIF and nuclear factor κB (NF-κB).

Hypoxia-inducible factor 1α

Since the 1950s, hypoxia has been understood as a factor limiting the efficacy of radiation therapy, which has been partially explained by the reduced formation of oxygen species in hypoxia, although other mechanisms of resistance to therapy-induced cell death are certainly involved. Recent evidence indicates that ROS and nitrogen reactive species are generated not only in conditions of increased oxygen levels, hyperoxia, but also from hypoxia. The mitochondria exposed to a combined intermittent hypoxia, and hypercapnia presented an increased production of superoxide in presence of depressed respiratory activity, suggesting that ROS are produced by alterations in the activity of the electrons transport chain caused by exposure to combined intermittent hypoxia and hypercapnia.[16] This in turn would correlate with the stabilization of HIF-1α *in vivo* as well as *in vitro*.[16,17] PHDs are enzymes of great importance regarding the regulation of hypoxia mechanisms and HIF-1α-dependent oxidative stress. PHDs sense the intracellular oxygen tension and give the signal to HIFs to induce adaptive processes to hypoxia such as angiogenesis.[18] Several studies showed that their

control mechanisms consist of hydroxylation of HIF-1α, which induce ubiquitination and signals HIF-1α for its degradation via proteosome.[17] It has also been reported that PHD2 suppresses N-terminal transcriptional activity of HIF-1α. This implies that PHDs could also operate as HIF suppressors in hypoxia and refine HIF activity by preventing excessive gene activation. Because PHDs hydroxylate different prolyl amino acids in HIF, some are possibly located in the HIF-1α control center, where only some of them would be related specifically with the responses upon changes in the oxygen tension, or simply some of them are more sensitive to these signals generating some minimal structural change in HIF-1α, but would affect its DNA binding capacity, which can indicate that the transcription's induction would be regulated by specific structural bond in where the active site is possibly being affected. Likewise, it would demonstrate that certain prolyl amino acids are the one to be hydroxylated to allow ubiquitination by pVHL and the subsequent degradation. In addition, it must be considered that other factors exist that regulate PHD's expression, such as growth factor and hormones.[19] Similarly, other molecules are involved in the regulation of HIF-1α degradation, such as receptor for activated c kinase 1 (RACK1). The overexpression of RACK1 leads to an increase in HIF-1α degradation independently from O_2, proline hydroxylation or binding to pVHL. RACK1 competes with HSP90, a molecular chaperone in charge of HIF-1α stabilization in a hypoxic environment through binding to residues 81–200 in HIF-1α, comprising the PAS-A domain.[20,21]

HIF-1α and ROS

HIF-1 is formed by two subunits: HIF-1α and HIF-1β were identified as proteins containing a basic helix–buckle–helix and a Per/ARNT/Sim (PAS) domain, which were responsible for the hypoxic induction of erythropoietin.[2,6,19] Under reduced oxygen levels, these two proteins heterodimerize in the nucleus and induce the transcription of genes involved in angiogenesis, metabolic and hypoxic adaptation and resistance to oxidative stress and increase invasive properties.[10] Among the various regulatory routes of HIF-1, the most important is the degradation mediated by the PHD family, which hydroxylates proline residues in the oxygen-dependent degradation domain via the proteosome. Under normal oxygen conditions, HIF-1α is degraded; whereas under hypoxic conditions, PHD cease its proper function, as they require molecular oxygen for the hydroxylation, causing HIF-1α levels to increase, to heterodimerize with HIF-1β and to enter the nucleus where binding to hypoxia response elements (HRE) in the promoter regions of several genes occurs.[15,22]

Because each oxygen atom contains two free unpaired electrons in the outer layer, the complete reduction of the biradical molecule O_2 to H_2O requires four electrons. The sequential donation of electrons to oxygen during this process can generate ROS as intermediaries, and the "electron draining" can also contribute to the formation of ROS. The donation of one electron to O_2 results in the formation of the radical superoxide (O_2^-). The donation of a second electron produces peroxide, which then undergoes a protonation to produce hydrogen peroxide (H_2O_2). The donation of a third electron, as in the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\cdot}OH + OH^-$), results in the production of the highly reactive radical hydroxyl (${}^{\cdot}OH$). Finally, the donation of a fourth electron produces H_2O. The oxygen Singlet ($^1O_2$), a reactive form of molecular oxygen of short life span, where the outer electrons are raised to a very high-energy state, can be formed by a variety of mechanisms including the Haber–Weiss reaction ($H_2O_2 + O_2^- \rightarrow {}^{\cdot}OH + OH^- + {}^1O_2$). The superoxide ($O_2^-$) as well as the hydrogen peroxide (H_2O_2) fulfills an important modulatory role of HIF-1 activity. This role is performed in two different manners. In one case, NADPH oxidase ($O_2 \rightarrow O_2^-$) is responsible for producing ROS, which under normoxia induces HIF-1α degradation. A second situation indicates that the mitochondrial production of ROS (cyt c1 $\rightarrow O_2^- \rightarrow H_2O_2$) under hypoxia induces the stabilization of HIF-1α.[6] Genetic and pharmacological inhibition of mitochondrial electron transport prevents ROS formation and diminishes the hypoxic induction of HIF, suggesting that mitochondrial ROS modify the response of PHDs to hypoxia. In addition, the oxidation of Fe (II) could positively modulate the transcriptional regulation of HIF-1α and increase its protein levels.[5] It is possible that hypoxia leads to an increase in the ROS from different sources such as NADPH oxidase, mitochondrial transport of electrons, xanthine oxidase and eNOS. The several sources of H_2O_2 in hypoxia and their interactions with HIF-1α stabilization remain to be determined.[9]

Moderated levels of ROS, especially O_2^- and H_2O_2, have shown to activate a cascade of signals that mediate responses to diverse physical and chemical factors.[23] One great example of physical stress that relates to hypoxia is radiation. It has been observed that the lung is inevitably exposed to radiation during the treatment of many tumors in the thorax region. Early hypoperfusion generated by vascular changes induced by radiation and escalated oxygen consumption, a consequence of the increasing cellular metabolism, have been attributed to the generation of tissue hypoxia, which exacerbates the damage. At a molecular level, it is suggested that the sustained chronic oxidative stress, a mediator through which radiation stabilizes HIF-1α, could be associated with increased HIF-1α expression in the lungs radiated.[4]

Different mechanisms have been used to explain how radiation leads to the development of hypoxia in normal tissues. All of them shared a common point: the induction of HIF-1α through the not hypoxic pathway induced by radiation or the hypoxic pathway by HIF-1α. In the "not hypoxic" activation of HIF-1α signaling, free radicals directly or indirectly regulate stabilization, translocation and activation of HIF-1 via cytokines in ROS-dependent mechanisms.[24] A second mechanism of HIF-1α stabilization involves the effects of reoxygenation on the structure of stress granules. These are mRNA–protein complexes formed in cells during periods of stress and avoid these to use the energy for the transduction of proteins. The formation of stress granules coincides with *in vivo* and *in vitro*

hypoxia, being broken up during reoxygenation releasing HIF-1-regulated transcripts. A third stabilization mechanism of HIF-1α coincides with the infiltration of macrophages inside the tumors by the production of NO, where the specific target of NO is the cysteine residue in the oxygen-dependent domain of HIF-1. However, if HIF-1α expression induced by radiation in pulmonary tissue is a consequence of the tissue hypoxia or a result of oxidative stress or both has not yet been determined.[4]

HIF-1-regulated genes are among those retained in stress granules, and once they break up, in few hours are translated into proteins. Given the evidences of increased oxidative stress because of tumor cyclic hypoxic,[3] it would be expected that formation and break up of stress granules occur in response to the hypoxia cycle. Another group of genes that is up-regulated by HIF-1 acts compensating the metabolic effects of low oxygen levels. The increased expression of glucose transporters and glycolic enzymes under hypoxic conditions allows the oxygen-independent generation of ATP. When oxygen levels fall to a critical point, oxidative phosphorylation and mitochondrial electron transport stop, and oxygen-independent energy production is induced via glycolysis. In the glycolic route, four ATP molecules are produced when one molecule of glucose is metabolized to two molecules of pyruvate with two molecules of ATP consumed during this process; this leaves a net production of two ATP. Compared with an aerobic condition where the pyruvate is oxidized in the Krebs cycle and the net production is in the order of 31 molecules of ATP, anaerobic glycolysis is much less efficient. Hypoxia influences many other transcriptional pathways, such as those mediated by the transcription factors AP-1, ets-1, fos and jun, NF-κB and p53. Thus, it is possible that a variety of cellular responses to hypoxia are mediated by HIF-independent mechanisms.[1]

HIF-2 and HIF-3 are proteins highly homologues to ARNT, and the three are implicated in forming dimers with diverse HIF-α subunits. HIF-1α has two closely related homologues: HIF-2α and HIF-3α. HIF-2α (also known as endothelial protein of PAS or EPAS1 domain) is identical to HIF-1α, is induced by hypoxia and binds to HIF-1β to activate gene transcription in response to hypoxia. HIF-3α seems to be a negative dominant regulator of HIF; it dimerizes with HIF-1β to generate a heterodimer transcriptionally inactive. Under normoxic conditions, HIF-1α is expressed in low levels in all organs, whereas HIF-2α is expressed abundantly in lungs, heart, brain, liver and others. Despite similarities in mediating transcriptional response to hypoxia, HIF-1α and HIF-2α have nonredundant different functions.

HIF-1α and VEGF

HIF-1α promotes growth and angiogenesis by the overexpression of angiogenic factors like VEGF.[25] VEGF is a powerful angiogenic factor, also known as vascular permeability factor (VPF) based on its ability to induce vascular hyperpermeability.[9] VEGF belongs to a family of growth factors that includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placenta-like growth factor. VEGFs are secreted as homo dimers linked by disulfides bonds; however VEGF-C and VEGF-D completely processed are secreted as dimers not covalently bound. The principal functions of these molecules are to promote cell survival, to induce proliferation and to enhance migration and permeability of endothelial cells, which contribute to angiogenesis. VEGF's biological effects require trans phosphorylation of tyrosine residues located on the cytoplasmic domain of their receptors (VEGFRs) VEGFR-1, VEGFR-2 and VEGFR-3. Ligand–receptor interactions occur in an overlapped manner: VEGF-A, VEGF-B and placenta-like growth factor bind to VEGFR-1; VEGF-A and VEGF-E bind to VEGFR-2, and VEGF-C and VEGF-D bind to VEGFR-3. The proximity of the intracellular tyrosine kinase domains of the receptors induces their trans phosphorylation in tyrosine residues.[1]

VEGF directly regulates vascular permeability in various organs, and this could have a role in clinical conditions such as acute respiratory syndrome, sepsis, acute mountain sickness and pulmonary edema due to high altitude exposure. In hypoxic human pulmonary artery endothelial cells, increases in H_2O_2, nuclear HIF-1α, cytosolic VEGF and monolayer permeability were observed, whereas increased HIF-1α, VEGF and vascular leaking were observed *in vivo* in pulmonary vessels from hypoxic mice.[10] Interestingly, treatment with the soluble VEGF receptor sFlt-1, that upon binding inhibits VEGF biological effects, decreases human pulmonary artery endothelial cell permeability.[10] VEGF, as well as sFlt-1, contributed to the malfunction of the endothelial barrier in human and rodent models and correlated with the severity in the sepsis sickness and acute respiratory syndrome.[26]

The suppressor tumor gene p16 is an inhibitor of cyclin D kinase, and a negative regulator of the cellular cycle, p16 expression, is correlated with the inhibition of VEGF and angiogenesis in human gliomas; however, the mechanisms used by p16 to regulate VEGF has not been explored. It is believed that p16-HIF-1 interactions play an important role in the modulation of p16 and VEGF expression, which contribute to the angiogenesis and tumor progression.[25] For this reason, VEGF and other angiogenic factors have been recognized as therapeutic targets for sicknesses such as cancer.[23] VEGF is also overregulated by conditions associated with the generation of free radicals and reactive oxygen intermediaries; these mechanisms are still unknown but are associated with reductions in antioxidant systems.[27]

HIF-1α and NO

NO, a key regulator of endothelial function, derives from the endothelium and is generated by endothelial NO synthase (eNOS). Vascular NO relaxes blood vessels, prevents platelet aggregation and adhesion, limits oxidation of low-density lipoprotein (LDL), inhibits proliferation of vascular smooth muscle cells and reduces the expression of pro-inflammatory genes that favors the atherogenesis.[28] Hypoxia shares the ability to control vascular homeostasis with NO. The induction of angiogenesis leads to an increase in vascular density and thus

decreases the distance for oxygen diffusion, whereas NO contributes to control vascular diameter. Hypoxia as well as NO affects HIF-1α stability, and HEK-293 cells overexpressing a mutant form of HIF-1 unable of undergo hydroxylation (P402A/P564A HIF-1α) have shown that reactive nitrogen intermediaries inhibit hypoxia-induced HIF-1α accumulation and VEGF synthesis.[29] The use of mitochondrial respiration inhibitors such as DETA-NO showed that they shared the actions of NO in regard to hypoxic HIF-1α stabilization, suggesting that NO is able to redistribute oxygen toward nonrespiratory oxygen-dependent processes such as PHDs, making them unable to sense the cellular hypoxia.[5,29]

A dominant mechanism in the reduction of NO bioavailability in vascular tissue is its rapid oxidative inactivation by the ROS superoxide ($O_2^{-}\cdot$). Thus, an increase in the production or damage in the inactivation of ROS, such as during oxidative stress, lead to a reduction in NO bioactivity. Moreover, evidences indicate that a persistent oxidative stress may generate a dysfunctional eNOS that not produce NO, but $O_2^{-}\cdot$ [30]. There exists the notion that NO and O_2^{-} react by controlled diffusion, in a reaction that competes with the enzymatic removal of O_2^{-} by superoxide dismutase. Because NO y O_2^{-} are important signal messengers, they could affect the accumulation of HIF-1α.[5]

HIF-1α and NADPH oxidase (Nox)-4

NADPH oxidase (Nox)-4, originally identified in the kidney and called renox (renal oxidase), is a new isoform of NADPH oxidase expressed in nonphagocytic cells such as vascular endothelial and smooth muscle cells.[9] NADPH oxidase is one of the principal sources of ROS production. This multicomponent enzyme group generates superoxide anion radicals in a regulated manner to allow electron transfer from NADPH through a catalytic nuclear protein (NOX), which contains flavin and heme fractions for the molecular oxygen. NOX4 has been associated with systemic function and in smooth pulmonary muscle.[31] It has been observed that Nox4 levels increase significantly in hypoxia where the generation of intracellular ROS is also significantly increased, suggesting that hypoxia could activate multiple routes that contribute to the up-regulation of ROS levels and the proliferation of hypoxic pulmonary artery smooth muscle cells (PASMC).[29–32] The hypoxic induction of NOX4 mRNA and protein *in vitro* and *in vivo* is abolished by depletion of HIF-1α. HIF-1α binds to an HRE in NOX4 promoter and in this way enhances NOX4 expression. The HIF-1α-dependent regulation of NOX4 contributes to the restoration of ROS levels after prolonged hypoxia, whereas the induction of NOX4 by HIF-1α promotes the proliferative activity of PASMCs in response to hypoxia.[32]

HIF-1α and NF-κB

NF-κB is a transcriptional regulator that consists of homo- and heterodimers of members of the Rel family. Five members of this family have been identified to date and include p65 (RelA), p50/p105, c-Rel, p52/p100 and RelB. These proteins share structural features and contain a conserved N-terminal sequence called Rel-homology domain (RHD) consisting of 300 amino acids. RHD is necessary for homo- and heterodimerization, DNA binding, nuclear localization and interaction with the inhibitory IκB proteins.[33] NF-κB is maintained as a latent form in the cytoplasm where it is complexed to IκB proteins. Seven members of the IκB family of proteins have been identified and include IκB-a, IκB-b, IκB-e, Bcl-3, p100/IκB-d, p105/IκB-g and IκB-R. IκB proteins contain multiple copies of 30–33 amino acid sequences called ankyrin repeats that mediate the interaction with Rel proteins. The interaction of NF-κB with IκB masks the nuclear localization signal and retains NF-κB in the cytoplasm in a latent form. Upon activation of NF-κB, IκB is phosphorylated by IκB kinases, at two conserved serine residues in the N-terminus, which targets the protein for ubiquitination and degradation by the 26S proteosome.[33] The degradation of IκB unmasks the nuclear localization signal of NF-κB, which allows for a rapid translocation of NF-κB into the nucleus where it binds to DNA.

NF-κB is primarily a regulator of inflammatory and anti-apoptotic gene expression, and it is likely that the physiological reason for its activation in hypoxia lies in the inhibition of apoptosis, thus enabling cellular survival to a hypoxic period. However, it may also play an important role in driving hypoxic inflammation through the expression of cytokines, adhesion molecules and pro-inflammatory enzymes. Although the signaling events linking cellular hypoxia to transcriptional activation are less well understood for NF-κB than for HIF, it has recently been suggested that the same oxygen-sensing hydroxylases responsible for conferring hypoxic sensitivity to the HIF pathway may also regulate important components of the NF-κB pathway. However, it is important to note that although hypoxia is a strong activator of HIF-dependent pathways, it is a milder and possibly modulatory stimulus for NF-κB signaling.[34]

HIF-1α and molecule target of rapamycin

As a component of the mTORC complex, the molecule target of rapamycin (mTOR) is a serine/threonine protein kinase involved in the regulation of gene transcription and protein synthesis associated primarily to changes in nutrients, energy status or cellular oxidative stress.[31] This signaling route also includes PI3K and protein kinase B, which regulate processes related to cellular proliferation, motility, differentiation and survival.[35]

In tumor therapy, it has been reported that oxygen distribution from tumor blood vessels to hypoxic tumor cells is substantially recovered after radiation as the result of the death of tumor cells oxygenated and a subsequent decrease in oxygen consumption, which is known as tumor reoxygenation.[36] It is believed that HIF-1 and mTOR are involved in a feedback circuit mediated by hypoxia, where mTOR is regulated downstream by an indirect mechanism

mediated by HIF-1-regulated proteins, such as Redd1, Redd2 and Bnip3.[3]

ROS generation due to radiation-induced tumor reoxygenation inhibits PHD activity and thus stabilized HIF-1.[37] Similarly, it has been observed that Akt/mTOR pathway plays an essential role in the accumulation of HIF-1α, in both glucose- and reoxygenation-dependent manner in irradiated tumors.[12] An intriguing possibility is that tumor sensitivity to the antitumor drug rapamycin is partially attributed to the suppression of the HIF-1 function and the adaptive response to hypoxia.[38]

HIF-1α and the unfolded protein response

During their transit through the endoplasmic reticulum (ER), secreted proteins undergo posttranslational modifications required to reach the "native" state.[39] These modifications are controlled by an array of enzymatic activities highly regulated,[40,41] and alterations on this process generate misfolded polypeptides that are exported to the cytoplasm and ultimately degraded. This unfolded protein response (UPR) is generated when there are changes in the cellular function, such as when the cells are stressed. The consequence of its activation includes changes in the production of proteins and maturity, cellular metabolic response and fate and has been recently associated with several diseases.[42] Hypoxia causes ER stress and activation of UPR, and although the mechanisms are not yet elucidated they are probably related to the fact that some of the modifications that happen in the ER are oxygen dependent, such as disulfide bond formation,[43] making hypoxia and oxidative stress powerful inducers of UPR.[44] UPR has also been associated to hypoxic stress in tumors.[45] One hallmark of UPR is the formation of stress granules, which are complexes formed by ribosomal subunits, mRNA and proteins, these complexes block mRNAs access to the ER and thus regulate protein synthesis.[3]

There exists growing evidence that suggests that the dependent response of UPR-HIF in hypoxia acts in an integrated manner, influencing each other and the common downstream routes that affect gene expression, metabolism, cell survival, tumorigenesis and tumor growth.[46] Although the exact mechanisms of interaction between UPR and hypoxic responses are being investigated, recent data suggest that UPR affects tumor growth through the protection from apoptosis and could influence angiogenic-signaling ways.[47]

HIF-1α as a molecular target of experimental conditions associated with hypoxia

Ischemia reperfusion injury. The current view of reperfusion is that it is essential to salvage ischemic tissue. However, reperfusion has the potential to cause further irreversible cell injury, largely dependent on the duration of preceding ischemia, and this is closely linked to the extent of mitochondrial permeability transition pore opening in early reperfusion. None of the recent information detracts from the proven therapeutic value of reperfusion, but it has prompted a reassessment of reperfusion injury and its mechanisms and the potential for therapeutic intervention to maximize the benefits of reperfusion in acute occlusion such as in the myocardial infarction.[48]

In addition to ROS release after heart ischemia, the early reperfusion phase is characterized by enhanced cytokine and adhesion molecule expression. During the first hour of reperfusion, superoxide triggers neutrophil infiltration, which increases cardiac damage by further release of ROS, inflammatory mediators and proteases. Myeloperoxidase, an inflammatory marker accounting for neutrophil accumulation, is also able of increasing ROS production via hypochlorous acid breakdown.[49] Indeed, the chemokine response in ischemic tissues may be induced by various factors, including ROS, cytokines (e.g. tumor necrosis factor α), complement and NF-κB activation. Local sources of ROS (e.g. NADPH oxidase, xanthine oxidase and eNOS) could be stimulated during reperfusion and probably modulated by time reperfusion.[50]

Intermittent systemic hypoxia occurs in many common physiological and pathophysiological conditions in human life, caused by environmental factors (e.g. high altitude exposure), cardiopulmonary disorders (e.g. heart failure, chronic obstructive pulmonary disease and sleep apnea) and hematological diseases (e.g. anemia). In fact, intermittent exposure is much more frequent than chronic exposure to hypoxia. Interestingly, a few controlled protocols of intermittent hypoxia can induce protective effects against myocardial infarction via a signaling mechanism that depends of improvement the NO availability.[51] Because the iNOS gene contains an HRE in its promoter region and HIF-1 is essential for the hypoxic regulation of iNOS gene expression in cardiomyocytes, it is logical to speculate a role for HIF-1 in intermittent hypoxia-induced cardioprotection. Cai *et al.*[52] showed that in heterozygous HIF-1α-deficient mice, the acute cardioprotection induced by either single- or multicycle ischemic preconditioning was absent, suggesting that HIF-1α is necessary for the early window of ischemic preconditioning. The same research group reported that intermittent hypoxia exposure induces HIF-1α-dependent increases in kidney and plasma erythropoietin levels, which leads to the delayed window of cardioprotection in wild type but not in heterozygous HIF-1α-deficient mice.[53]

Hypobaric hypoxia. In high altitude locations (>2500 m above sea level) where atmospheric pressure exponentially descends in function of altitude, oxygen availability is reduced. This lower partial pressure of oxygen (PO_2) consequently originates a decrease in the quantity of oxygen transported by the blood stream to all cells of the organism.[54,55] Thus, exposure to hypobaric hypoxia either intermittent or chronic gives rise to a series of physiological responses aimed to compensate for the imbalance between demand and supply of cellular oxygen.

Effects of hypobaric hypoxia exposure on the endocrine system include higher levels of follicle stimulating hormone during the first days of exposure, decreased levels of luteinizing hormone (LH) and plasma testosterone compared with normoxic controls. The continuous decrease of

LH has been related with the effect of hypoxia on the activity of the hypothalamic–pituitary–gonadal axis and the diminished levels of testosterone could be related to spermatogenesis.[56] This condition also affects the cardiovascular system, where it induces a significant decrease in cardiac α-adrenoceptors and calcium levels and increases the expression of muscarinic receptors; however, this condition also acts increasing the hematological constants and producing an elevation of systolic blood pressure.[57]

The effects of hypobaric hypoxia in human visitors depend not only on the actual elevation but also on the rate of ascent. There are increases in sympathetic activity resulting in increases in systemic vascular resistance, blood pressure and heart rate. Pulmonary vasoconstriction leads to pulmonary hypertension, particularly during exercise. The sympathetic excitation results from hypoxia, partly through chemoreceptor reflexes and partly through altered baroreceptor function.[58] Systemic vasoconstriction may also occur as a reflex response to the high pulmonary arterial pressures. Many communities live permanently at high altitude and most dwellers show excellent adaptation although there are differences between populations in the extent of the ventilatory drive and erythropoiesis. Despite living all their lives at altitude, some dwellers, particularly Andeans, may develop a maladaptation syndrome known as chronic mountain sickness. The most prominent characteristic of this is excessive polycythemia, the cause of which has been attributed to peripheral chemoreceptor dysfunction. The hyperviscous blood leads to pulmonary hypertension, symptoms of cerebral hypoperfusion and eventually right heart failure and death.[59]

In ischemia induced by hypobaric hypoxic, HIF-1 transcriptionally activates hundreds of genes vital for cell homeostasis and angiogenesis. Although potentially beneficial in ischemia, the up-regulation of the HIF-1 transcription factor has been linked to inflammation and oxidative stress occurrence. Considering HIF-1's function, HIF-1α protein and its hydroxylation cofactors look increasingly attractive as therapeutic targets independently; antioxidants have shown promise in lowering the risk of organ dysfunction and improving neurological and cardiac function after ischemia.[60]

Conclusions and perspectives. The hypoxic conditions such as high altitude-induced hypobaric hypoxia and ischemia reperfusion injury involve a series of adaptive changes in the cells and organs physiology systems. These pathophysiologic responses conduce to establish different models of study and assess various control points in the signaling pathways to generate a pharmacological target or simply a detection strategy.

In this process, multiple factors have been investigated; however, our work focuses in the core of discussion, the events associated with the occurrence of oxidative stress, which is defined as an imbalance antioxidant/pro-oxidant of cellular system that disrupt a "oxidative tone." The relation of the activated mechanisms in response to these processes in different species, cellular types, organs and tissues could be a necessary tool to determine the molecules with larger implication and that can generate a more enclosed regulation system that permits to generate further biomarkers for different sicknesses. According to the compiled information, further studies could be performed in areas connected directly with HIF-1α and NF-κB, being of greater importance to know and determine some mechanism that permit maintain the PHDs stable so it can perform its function and in occasions, as in cancer, ischemia-reperfusion injury or infertility by hypoxia, can be maintained active and counteract the effect in the generation of oxidative stress as that in hypoxic micro environment.

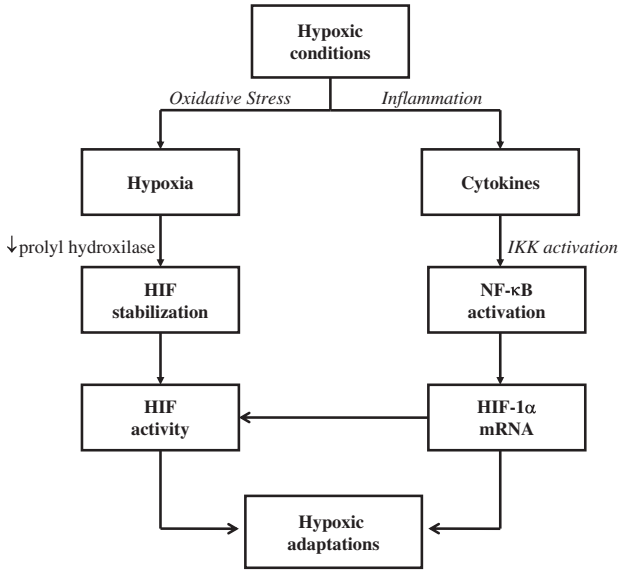

Figure 1. Regulation of the HIF and NF-κB pathway in hypoxic conditions. PHD, prolyl hydroxylase; NF-κB, nuclear factor κB; HIF, hypoxia-inducible factor.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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