Review

Science review: Redox and oxygen-sensitive transcription factors in the regulation of oxidant-mediated lung injury: role for hypoxia-inducible factor-1α

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Abstract

A progressive rise of oxidative stress due to altered reduction–oxidation (redox) homeostasis appears to be one of the hallmarks of the processes that regulate gene transcription in physiology and pathophysiology. Reactive oxygen species and reactive nitrogen species serve as signaling messengers for the evolution and perpetuation of the inflammatory process that is often associated with the condition of oxidative stress, which involves genetic regulation. Changes in the pattern of gene expression through reactive oxygen species/reactive nitrogen species-sensitive regulatory transcription factors are crucial components of the machinery that determines cellular responses to oxidative/redox conditions. The present review describes the basic components of the intracellular oxidative/redox control machinery and its crucial regulation of oxygen-sensitive and redox-sensitive transcription factors within the context of lung injury. Particularly, the review discusses mechanical ventilation and NF-κB-mediated lung injury, ischemia-reperfusion and transplantation, compromised host defense and inflammatory stimuli, and hypoxemia and the crucial role of hypoxia-inducible factor in mediating lung injury. Changes in the pattern of gene expression through regulatory transcription factors are therefore crucial components of the machinery that determines cellular responses to oxidative/redox stress.

Keywords antioxidant, hypoxia-inducible factor-α, injury, lung, oxygen, redox, transcription factors

Altering gene expression is the most fundamental and effective way for a cell to respond to extracellular signals and/or changes in its environment, in both the short term and the long term [1]. In the short term, transcription factors are involved in mediating responses to growth factors and a variety of other extracellular signals [2]. In contrast, the long-term control of gene expression induced by growth factors and the changes in gene expression, which occur during development, is generally (with few exceptions) irreversible.

During development, the expression of specific sets of genes is regulated spatially (by position/morphogenetic gradients) and temporally. Regulation of the signaling responses is governed at the genetic level by transcription factors that bind to control regions of target genes and alter their expression [1,2]. Transcription factors are endogenous substances, usually proteins, that are effective in the initiation, stimulation or termination of the genetic transcription process. While in the cytoplasm, the transcription factor is incapable of promoting transcription. A signaling event occurs, such as a change of the state of phosphorylation, which results in protein subunit translocation into the nucleus [3,4]. Transcription is a process in which one DNA strand is used as a template to synthesize a complementary RNA. Signal transduction therefore involves complex interactions of multiple cellular pathways [1,2].

AM = alveolar macrophage; CF = cystic fibrosis; EPO = erythropoietin; HIF-1 = hypoxia-inducible factor-1; HS = hemorrhagic shock; IL = interleukin; NF-κB = nuclear factor-κB; pO_2 = partial pressure of oxygen; redox = reduction–oxidation; ROS = reactive oxygen species; SLPI = secretory leukocyte protease inhibitor; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.

In particular, reduction–oxidation/oxygen (redox)-sensitive transcription factors have gained an overwhelming backlog of interest momentum over the years, ever since the onset of the burgeoning field of free radical research and oxidative stress. The reason for this is that redox-sensitive transcription factors are often associated with the development and progression of many human disease states. Their ultimate regulation therefore bears potential therapeutic intervention for possible clinical applications [1–4].

In the present review, I will focus on elaborating a comprehensive overview of the current understanding of redox/oxidative mechanisms mediating the regulation of transcription factors. These transcription factors regulate a plethora of cellular functions that span the range from anoxia and hypoxia to oxidative stress within the context of oxidant-mediated lung injury.

Inflammatory reactions and lung injury

Mechanical ventilation and NF-κB-mediated lung injury

Some unprecedented conditions may occur during the evolution of the inflammatory process, which can eventually lead to dramatic changes in the progression of lung injury. For example, positive-pressure mechanical ventilation supports gas exchange in patients with respiratory failure but is also responsible for significant lung injury.

Pugin and colleagues, for instance, have developed an *in vitro* model in which isolated lung cells can be submitted to a prolonged cyclic pressure-stretching strain resembling that of conventional mechanical ventilation [5]. In this model, cells cultured on a silastic membrane were elongated up to 7% of their initial diameter, corresponding to a 12% increase in cell surface. The lung alveolar macrophage (AM) was identified as the main cellular source for critical inflammatory mediators such as tumor necrosis factor (TNF)-α, the chemokines IL-8 and IL-6, and matrix metalloproteinase-9 in this model system of mechanical ventilation. These mediators were measured in supernatants from ventilated AMs, monocyte-derived macrophages and promonocytic THP-1 cells. In addition, NF-κB was found to be activated in ventilated macrophages. Synergistic proinflammatory effects of mechanical stress and molecules such as bacterial endotoxin were observed, suggesting that mechanical ventilation might be particularly deleterious in pre-injured or infected lungs. Dexamethasone, an anti-inflammatory steroid, prevented IL-8 and TNF-α secretion in ventilated macrophages. Mechanical ventilation also induced low levels of IL-8 secretion by alveolar type II-like cells. Other lung cell types such as endothelial cells, bronchial cells and fibroblasts failed to produce IL-8 in response to a prolonged cyclic pressure-stretching load [5]. This model is of particular value for exploring physical stress-induced signaling pathways, as well as for testing the effects of novel ventilatory strategies or adjunctive substances aimed at modulating cell activation induced by mechanical ventilation.

Furthermore, alterations in AM function during sepsis-induced hypoxia may influence TNF secretion and the progression of acute lung injury. It was proposed that acute changes in partial pressure of oxygen (pO_2) tension surrounding AMs alter NF-κB activation and TNF secretion in these lung cells. AM-derived TNF-α secretion and NF-κB expression were determined after acute hypoxic exposure of isolated Sprague–Dawley rat AMs. Adhered AMs (10^6/ml) were incubated (37°C at 5% CO_2) for 2 hours with 1 μg/ml lipopolysaccharide–endotoxin (*Pseudomonas aeruginosa*) in normoxia (21% O_2–5% CO_2) or in hypoxia (1.8% O_2–5% CO_2). The AMs exposed to lipopolysaccharide–endotoxin in hypoxia had higher levels of TNF-α and enhanced expression of NF-κB than those in normoxia; the predominant isoforms were *Rel*A (p65) and c-*Rel* (p75). Increased mRNA bands for TNF-α, IL-1α and IL-1β were also observed in the hypoxic AMs [6]. This observation demonstrates that acute hypoxia in the lung may induce enhanced NF-κB activation in AMs, which may result in increased production and release of inflammatory cytokines.

Ischemia-reperfusion and transplantation

It has been reported that secretory leukocyte protease inhibitor (SLPI) in mice regulates local and remote organ inflammatory injury induced by hepatic ischemia-reperfusion [7–9]. Intravenous infusion of SLPI reduced liver and lung damage and diminished neutrophil accumulation in both organs. These effects were accompanied by reduced serum levels of TNF-α and macrophage inflammatory protein-2. SLPI also suppressed activation of NF-κB in the liver. Moreover, hepatic ischemia and reperfusion caused increased expression of SLPI mRNA and SLPI protein, which was found specifically in hepatocytes. Furthermore, treatment of mice with anti-SLPI antibodies enhanced serum levels of TNF-α and macrophage inflammatory protein-2, and it increased hepatic neutrophil accumulation and the amount of liver injury and lung injury [7–13]. These data indicate that SLPI has protective effects against hepatic ischemia-reperfusion injury and suggest that endogenous SLPI regulates the hepatic and remote inflammatory responses.

In concert with these observations, attenuation of lung reperfusion injury after transplantation using an inhibitor of NF-κB was achieved [14]. It was hypothesized that NF-κB is a critical early regulator of the inflammatory response in lung ischemia-reperfusion injury and that inhibition of NF-κB activation reduces this injury and improves pulmonary graft function. With the use of a porcine transplantation model, left lungs were harvested and stored in cold Euro-Collins preservation solution for 6 hours before transplantation [14]. Activation of NF-κB occurred 30 min and 1 hour after transplantation, and it declined to near baseline levels after 4 hours. Pyrrolidine dithiocarbamate, a potent inhibitor of NF-κB, given to the lung graft during organ preservation (40 mmol/l), effectively inhibited NF-κB activation and significantly improved lung function. Compared with control lungs

4 hours after transplant, pyrrolidine dithiocarbamate-treated lungs displayed significantly higher oxygenation, lower pCO_2, reduced mean pulmonary arterial pressure and reduced edema and cellular infiltration [14]. This demonstrates that NF-κB is rapidly activated and is associated with poor pulmonary graft function in transplant reperfusion injury. Targeting the NF-κB pathway may therefore be a promising therapy to reduce injury and to improve lung function.

Compromised host defense

Progressive pulmonary infection may be a prominent clinical feature of lung injury, but the molecular basis for this susceptibility remains incompletely understood.

To study this problem, Sajjan *et al*. developed a model of chronic pneumonia by repeated instillation of a clinical isolate of *Burkholderia cepacia*, an opportunistic Gram-negative bacterium, from a case of cystic fibrosis (CF) into the lungs of Cftr (m1unc$^{-/-}$ [Cftr$^{-/-}$]) and congenic Cftr$^{+/+}$ controls [15]. Nine days after the last instillation, the CF transmembrane regulator knockout mice showed persistence of viable bacteria with chronic severe bronchopneumonia, while wild-type mice remained healthy. A mixed population of macrophages and neutrophils characterized the histopathological changes in the lungs of the susceptible Cftr$^{-/-}$ mice by infiltration of a mixed inflammatory cell population into the peribronchiolar and perivascular spaces, by Clara cell hyperplasia, by mucus hypersecretion in the airways and by exudation into alveolar airspaces. An increased proportion of neutrophils was observed in the bronchoalveolar lavage fluid from the Cftr$^{-/-}$ mice that, despite an increased bacterial load, demonstrated minimal evidence of activation. In addition, alveolar macrophages from Cftr$^{-/-}$ mice also demonstrated suboptimal activation [15].

These observations suggest that the pulmonary host defenses are compromised in lungs from animals with CF, as manifested by increased susceptibility to bacterial infection and lung injury. This murine model of chronic pneumonia thus reflects, in part, the situation in human patients and may help to elucidate the mechanisms leading to defective host defense in CF [16–25].

Summary

Acute lung injury therefore occurs as a result of a cascade of cellular events initiated by either infectious or noninfectious inflammatory stimuli. An elevated level of proinflammatory mediators combined with a decreased expression of anti-inflammatory molecules is a critical component of lung inflammation.

Expression of proinflammatory genes is regulated by transcriptional mechanisms. NF-κB is one critical transcription factor required for the expression of many cytokines involved in the pathogenesis of acute lung injury [26–35]. In acute lung injury caused by infection of bacteria, cytokine receptors play a central role in initiating the innate immune system and in activating NF-κB. Anti-inflammatory cytokines have the ability to suppress inflammatory processes via the inhibition of NF-κB, which can interact with other transcription factors, and these interactions thereby lead to greater transcriptional selectivity. Modification of transcription, and particularly of NF-κB, is likely to be a logical therapeutic target for the manipulation and treatment of acute lung injury [36–42].

Hypoxemia

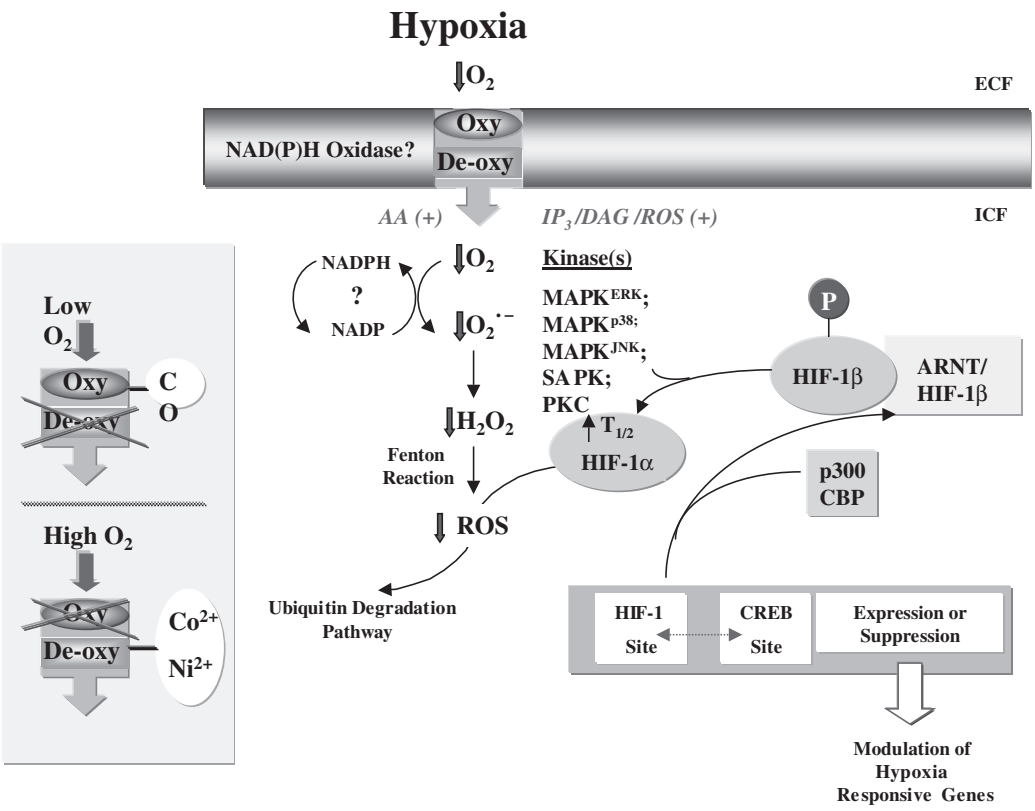
A crucial transcription factor that is a master regulatory element in sensing hypoxic conditions and in integrating an adapted response via gene expression of oxygen-sensitive and redox-sensitive enzymes and cofactors is hypoxia-inducible factor-1 (HIF-1) (Fig. 1) [43–45]. The signal transduction components that link the availability of oxygen to the activation of these transcription factors are poorly defined, but are broadly believed to hinge on the free abundance of oxidants.

HIF-1 consists of two subunits: HIF-1α, which is unique to the oxygen response; and HIF-1β (aryl hydrocarbon receptor nuclear translocator). The stability and activity of HIF-1α, first identified as a DNA-binding activity expressed under hypoxic conditions, increase exponentially when pO_2 is lowered. Whereas HIF-1β is constitutively expressed under normoxic conditions, HIF-1α is rapidly degraded by the ubiquitin–proteasome system. Under hypoxic conditions, however, HIF-1α protein stabilizes and accumulates, thus allowing the heterodimer to translocate to the nucleus and to bind specific promoter moieties of selective genes encoding erythropoietin (EPO), vascular endothelial growth factor (VEGF), glycolytic enzymes and glucose transporters, as well as cytokines and other inflammatory mediators (Fig. 1) [44–46]. It is expected that any reduction of tissue oxygenation *in vivo* and *in vitro* would therefore provide a mechanistic stimulus for a graded and adaptive response mediated by hypoxia-inducible factor (Fig. 2).

Inflammatory stimuli

The role of HIF-1α in oxidant-induced lung injury is less clear, or less prominent, than that of NF-κB. Indirect, but unprecedented and unequivocal, evidence was independently provided by Hellwig-Bürgel and colleagues [47–49] and by Haddad and Land [50,51], however, to indicate HIF-1 as a possible regulator of the evolution and propagation of the inflammatory process. The rate of transcription of several genes encoding proteins involved in oxygen and energy homeostasis is controlled by HIF-1. Since EPO gene expression is inhibited by the proinflammatory cytokines, such as IL-1β and TNF-α, while no such effect has been reported with respect to the VEGF gene, Hellwig-Bürgel *et al*. investigated the effects of these cytokines on the activation of the HIF-1 DNA-binding complex and the amount of HIF-1α protein in human hepatoma cells in culture [47]. Under normoxic conditions, both cytokines caused a moderate activation of HIF-1

Figure 1



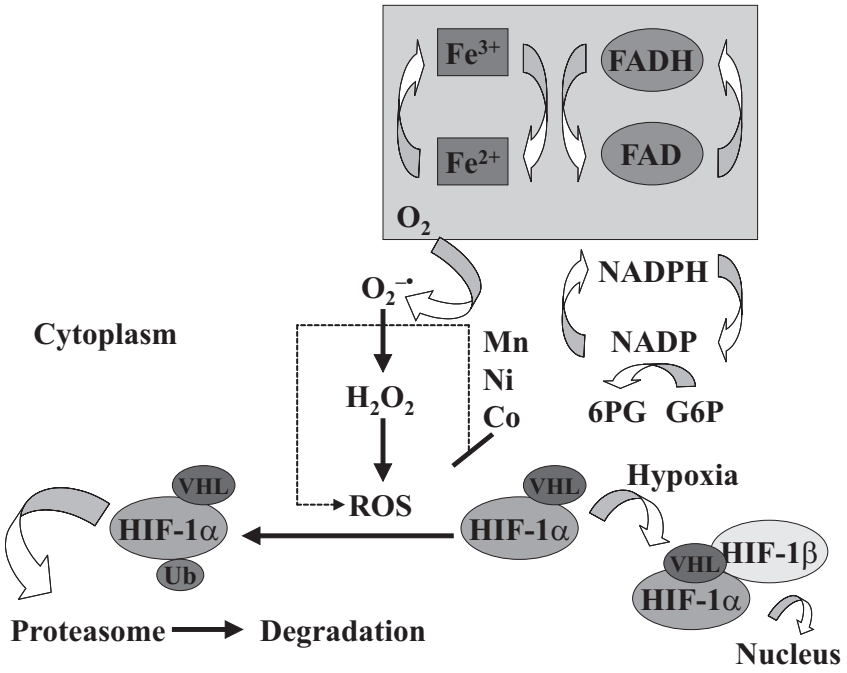
Oxygen-sensing proposed mechanisms for the regulation of gene transcription and the involvement of hypoxia-inducible factor-1 (HIF-1) as a hypoxia-mediated transcriptional activity (see text for further details). AA, arachidonic acid; ARNT, aryl receptor hydrocarbon nuclear translocator; CREB, cAMP-responsive element binding protein; CBP, CREB-binding protein; DAG, diacyl glycerol; ECF, extracellular fluid; ICF, intracellular fluid; IP_3, inositol triphosphate; MAPK, mitogen-activated protein kinase; NADP, nicotinamide dinucleotide oxidized; NADPH, nicotinamide dinucleotide reduced; PKC, protein kinase C; ROS, reactive oxygen species; SAPK, stress-activated protein kinase.

DNA binding. In hypoxia, cytokines strongly increased HIF-1 activity compared with the effect of hypoxia alone. Only IL-1β increased HIF-1α protein levels. In transient transfection experiments, HIF-1-driven reporter gene expression was augmented by cytokines only under hypoxic conditions. In contrast to their effect on EPO synthesis, neither IL-1β or TNF-α decreased VEGF production. The mRNA levels of HIF-1α and VEGF were unaffected. Cytokine-induced inhibition of EPO production may thus not be mediated by impairment of HIF-1 function [47].

Hellwig-Bürgel and colleagues subsequently proposed that HIF-1 might be involved in modulating gene expression during inflammation. Furthermore, since VEGF promotes angiogenesis and inflammatory reactions, in a parallel study VEGF mRNA was found detectable in the proximal tubules of inflamed kidneys but not in normal kidneys [48]. In other organs, VEGF gene expression is induced by hypoxia and by cytokines. To identify the cellular mechanisms in control of tubular VEGF production, the effects of hypoxia and IL-1β on VEGF mRNA levels, on VEGF secretion and on activity of HIF-1 in human proximal tubular epithelial cells were assessed. The human proximal tubular epithelial cells were grown in monolayers from human kidneys, and hypoxia was induced by incubation at 3% O_2. Significant amounts of VEGF mRNA and VEGF protein were measured in human proximal tubular epithelial cell extracts and culture media, respectively. Moreover, stimulation of VEGF synthesis at low pO_2 tension and following IL-1β treatment was detectable at the protein level only. Nuclear HIF-1α protein levels and HIF-1 binding to DNA were also increased under these conditions [48].

VEGF induction appears to increase DNA binding of HIF-1 to hypoxia-responsive elements in the VEGF gene promoter. In inflammatory diseases of the kidney, tubular cell-derived VEGF may therefore contribute to microvascular leakage and to monocyte extravasation. Regarding the mechanisms reported, LY-294002 (an inhibitor of phosphatidylinositol 3-kinase) suppressed HIF-1 activation in a dose-dependent manner irrespective of the stimulus. With respect to target proteins controlled by HIF-1, the production of EPO was fully blocked and that of VEGF reduced following inhibition of the phosphatidylinositol 3-kinase pathway [49]. The role of mitogen-activated protein kinase kinases in this process

Figure 2



Potential oxygen-sensing mechanisms and the role of the transcription factor hypoxia-inducible factor-1 (HIF-1). 6GP, 6-glucose phosphate; 6PG, 6-phosphoglycerate; FAD, flavin adenine dinucleotide oxidized; FADH, flavin adenine dinucleotide reduced; NADP, nicotinamide dinucleotide oxidized; NADPH, nicotinamide dinucleotide reduced; ROS, reactive oxygen species; VHL, von Hippel-Lindau tumor suppressor protein.

remained ambiguous, because PD-98059 and U-0126 inhibitors did not significantly reduce HIF-1α levels at non-toxic doses [49]. It was proposed that phosphatidylinositol 3-kinase signaling is not only important in the hypoxic induction of HIF-1, but that it is also crucially involved in the response to insulin and IL-1.

Furthermore, evidence that reactive oxygen species (ROS) signaling mediates cytokine-dependent regulation of HIF-1α has been postulated by Haddad and Land [50,51]. In the airway epithelium, recombinant human IL-1β and recombinant murine TNF-α induced, in a time-dependent manner, the nuclear translocation of HIF-1α. This translocation is an effect associated with upregulating the activity of this transcription factor under normoxic conditions. In addition, analysis of the mode of action of IL-1β and TNF-α revealed a novel induction of intracellular ROS, including hydrogen peroxide, the superoxide anion ($O_2^{-\bullet}$) and the $^{\bullet}OH$ radical [50,51]. The antioxidants dimethyl sulfoxide and 1,3-dimethyl-2-thiourea, purported to be prototypical scavengers of hydrogen peroxide and $^{\bullet}OH$, attenuated cytokine-induced HIF-1α nuclear translocation and activation in a dose-dependent manner. The NADPH-oxidase inhibitor 4′-hydroxy-3′-methoxy-acetophenone, which may affect mitochondrial ROS production, attenuated cytokine-mediated nuclear translocation and activation of HIF-1α. Furthermore, inhibition of the mitochondrion complex I nicotinamide ADP-dependent oxidase by diphenylene iodonium, which blocks the conversion of ubiquinone to ubiquinol, abrogated IL-1β-dependent and TNF-α-dependent nuclear translocation and activation of HIF-1α. Similarly, interrupting the respiratory chain with potassium cyanide reversed the excitatory effect of cytokines on HIF-1α nuclear translocation and activation [50,51]. These results indicate that a nonhypoxic pathway mediates cytokine-dependent regulation of HIF-1α translocation and activation in a ROS-sensitive mechanism.

Direct evidence implicating HIF-1 in lung injury emerged with VEGF, which has been recognized as a potent mediator of endothelial barrier dysfunction and is upregulated during ischemia in many organs [43–46]. Because ventilated pulmonary ischemia causes a marked increase in pulmonary vascular permeability, it was hypothesized that VEGF would increase during ischemic lung injury.

To test this hypothesis, VEGF expression was measured by northern and western blot analysis in isolated ferret lungs after 45 or 180 min of ventilated (95% or 0% O_2) ischemia [52]. Pulmonary vascular permeability, assessed by measurement of the osmotic reflection coefficient for albumin, was evaluated in the same lungs, as was expression of HIF-1α. The distribution of VEGF as a function of ischemic time and oxygen tension was also evaluated by immunohistochemical staining in separate groups of lungs. VEGF mRNA increased threefold by 180 min of ventilated ischemia, independent of

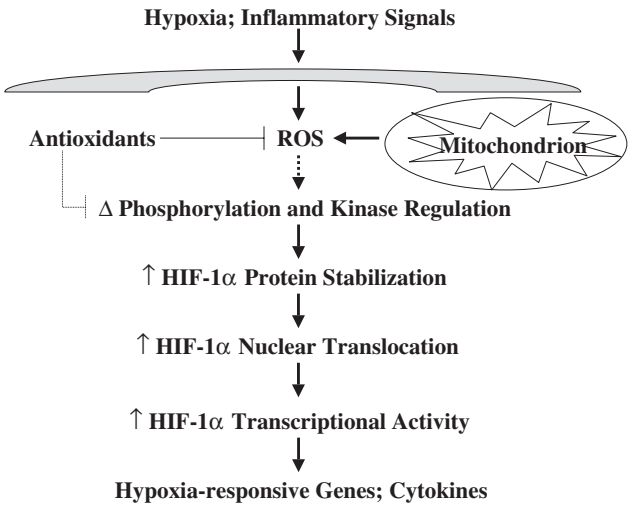
oxygen tension. VEGF protein increased in parallel to VEGF mRNA. Immunohistochemical staining demonstrated the appearance of VEGF protein along alveolar septae after 180 min of hyperoxic ischemia and after 45 or 180 min of hypoxic ischemia. In addition, albumin was not altered by 45 min of hyperoxic ischemia (0.69 ± 0.09 versus 0.50 ± 0.12, respectively), but decreased significantly after 180 min of hyperoxic ischemia and after 45 and 180 min of hypoxic ischemia (0.20 ± 0.03, 0.26 ± 0.08 and 0.23 ± 0.03, respectively) [52]. HIF-1α mRNA increased during both hyperoxic and hypoxic ischemia, but HIF-1α protein increased only during hypoxic ischemia. This implicates VEGF as a potential mediator of increased pulmonary vascular permeability in this model of acute lung injury.

Further elaborating on the mechanisms involving HIF-1 in regulating the inflammatory response, Hierholzer *et al*. reported that hemorrhagic shock (HS) initiates an inflammatory response that includes increased expression of inducible nitric oxide synthase and production of prostaglandins [53]. Induction of inducible nitric oxide synthase during the ischemic phase of HS may involve the activation of HIF-1. Increased expression of cyclooxygenase-2 during HS contributes to prostaglandin production. The lungs of rats subjected to HS demonstrated a twofold increase in HIF-1 activation and a 7.4-fold increase in expression of cyclooxygenase-2 mRNA, as compared with sham controls [53]. It was concluded that the upregulation of inducible nitric oxide synthase and cyclooxygenase-2 during ischemia are two important early response genes that promote the inflammatory response and may contribute to organ damage through the rapid and exaggerated production of nitric oxide and prostaglandins.

Furthermore, in a novel study by Shoshani and colleagues, the identification and cloning of a HIF-1-responsive gene, designated RTP801, was recently reported. Strong upregulation of RTP801 by hypoxia was detected both *in vitro* and *in vivo* in an animal model of ischemic stroke [54]. When induced from a tetracycline-repressible promoter, RTP801 protected MCF7 and PC12 cells from hypoxia in glucose-free medium and from hydrogen peroxide-triggered apoptosis via a dramatic reduction in the generation of ROS. However, expression of RTP801 appeared toxic for nondividing neuron-like PC12 cells and increased their sensitivity to ischemic injury and oxidative stress. Furthermore, liposomal delivery of RTP801 cDNA to mouse lungs also resulted in massive cell death [54]. The biological effect of RTP801 overexpression thus depends on the cell context and may be either protecting or detrimental for cells under conditions of oxidative or ischemic stresses. Altogether, the data suggest a complex type of involvement of RTP801 in the pathogenesis of ischemic diseases.

A hypothetical schematic depicting the role of HIF-1 in lung injury is displayed in Fig. 3.

Figure 3



A schematic overview of the potential signaling pathways involved in cytokine-mediated regulation of hypoxia-induced hypoxia-inducible factor-1α (HIF-1α) translocation and activation. Hypoxia and inflammatory signals induce the intracellular accumulation of reactive oxygen species (ROS), which may cause changes in the phosphorylation state of target kinases, thereby mediating a specific regulatory mechanism. The mitochondrion is a potential source for cytokine-unleashed ROS, whose regulation is selectively mediated by antioxidants. ROS-mediated signaling allows HIF-1α protein stabilization, nuclear translocation and transcriptional activation.

Conclusion and future prospects

The molecular response to oxidative stress is regulated by redox-sensitive transcription factors [55–60]. The study of gene expression and regulation is critical in the development of novel gene therapies [61–70]. Recognition of reactive species and redox-mediated protein modifications as potential signals may open up a new field of cell regulation via specific and targeted genetic control of transcription factors, and can thus provide us with a novel way of controlling disease processes [71–75]. Dynamic variation in pO_2 and redox equilibrium thus regulate gene expression, apoptosis signaling and the inflammatory process, thereby bearing potential consequences for screening emerging targets for therapeutic intervention.

Competing interests

None declared.

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