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# Effect of hypoxia on lung gene expression and proteomic profile: Insights into the pulmonary surfactant response

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## ABSTRACT

Exposure of lung to hypoxia has been previously reported to be associated with significant alterations in the protein content of bronchoalveolar lavage (BAL) and lung tissue. In the present work we have used a proteomic approach to describe the changes in protein complement induced by moderate long-term hypoxia (rats exposed to 10% $O_2$ for 72 h) in BAL and lung tissue, with a special focus on the proteins associated with pulmonary surfactant, which could indicate adaptation of this system to limited oxygen availability. The analysis of the general proteomic profile indicates a hypoxia-induced increase in proteins associated with inflammation both in lavage and lung tissue. Analysis at mRNA and protein levels revealed no significant changes induced by hypoxia on the content in surfactant proteins or their apparent oligomeric state. In contrast, we detected a hypoxia-induced significant increase in the expression and accumulation of hemoglobin in lung tissue, at both mRNA and protein levels, as well as an accumulation of hemoglobin both in BAL and associated with surface-active membranes of the pulmonary surfactant complex. Evaluation of pulmonary surfactant surface activity from hypoxic rats showed no alterations in its spreading ability, ruling out inhibition by increased levels of serum or inflammatory proteins.

Biological significance
This work reveals that hypoxia induces extensive changes in the proteomic profile of lung bronchoalveolar lavage, including the presence of proteins related with inflammation both in lung tissue and lavage, and a significant increase in the synthesis and secretion by the lung tissue of different forms of hemoglobin. The level of specific pulmonary surfactant-associated proteins is not substantially altered due to hypoxia, but hypoxia-adapted surfactant exhibits an enhanced ability to form surface-active films at the air-liquid interface. The increased amount of β-globin integrated into the operative surfactant complexes obtained from hypoxic

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rats is a relevant feature that points to the existence of adaptive responses coupling surfactant function and oxygen availability.

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## 1. Introduction

Provision of oxygen to the tissues, the main function of the lung, is achieved by gas exchange between air and blood, which occurs in the alveoli. Oxygen deficit leads to a severe impairment of tissue function, including alterations of the lung itself. The hypoxia response of the organism depends on severity and exposure time and includes two types of effects. Acute effects (seconds to minutes) are mediated through ion channel regulation, while chronic responses (hours to days), include several effects such as activation of glucose metabolism, erythropoiesis, angiogenesis, pulmonary hypertension (caused by vasoconstriction and vascular hypertrophy) and inflammation [1–4]. Chronic responses to hypoxia are mediated through induction of several transcription factors (hypoxia-inducible factors; HIFs), including the ubiquitously expressed HIF-1 and the tissue limited HIF-2 and HIF-3. HIF-1 binds to hypoxia responsive elements of several gene enhancers, such as vascular endothelial growth factor (VEGF), involved in vascular responses to hypoxia, and hypoxia induced mitogenic factor (HIMF), with angiogenic and vasoconstrictor effects [1,5].

Several in vivo models of hypoxia have been previously described. Exposure of animals to 10% oxygen has been reported to induce several changes in the organism, including alteration of alveolar permeability [6]. Impairment of transalveolar fluid transport has been found to cause edema due to insufficient alveolar fluid clearance, though some authors reported that edema during the first hours decreases at longer exposure times [7]. In the same way, inflammation occurring because of reactive oxygen species (ROS) and albumin extravasation, could be resolved in a few days after exposure when vascular epithelium acclimates [8]. Referring to changes in protein expression induced by hypoxia, different temporal expression patterns have been found in animal models exposed to 10% oxygen, including increased expression of genes involved in immune responses and pulmonary vascular remodeling, occurring between days 1 and 7 of hypoxia exposure [9].

The main metabolically active lung epithelial cells are alveolar type II pneumocytes, which produce and secrete pulmonary surfactant. Interestingly, expression of α and β hemoglobin in type II cells has been reported, indicating potential functions of this protein in lung as an oxygen transporter, oxygen sensor or oxidative stress protector [10,11]. Recently, induction by hypoxia of hemoglobin expression in these cells has been demonstrated in vitro [12], suggesting the existence of an oxygen-sensing pathway in alveolar epithelial cells.

Pulmonary surfactant is a lipid-protein complex that lines the alveolar surface, and reduces surface tension at the air–fluid interface. This function is essential to stabilize the alveoli, prevent their collapse at the end of expiration, and avoid alveolar edema. Composition of surfactant includes about 90% lipids (mainly phospholipids) and 8–10% of surfactant-associated proteins. Pulmonary surfactant is stored in type II alveolar epithelial cells in the form of densely packed bilayers called lamellar bodies that are secreted and efficiently transferred into the interface [13]. Lipid transport into lamellar bodies could be ultimately mediated by the transporter ABCA3. This protein seems to have an important role in lung and surfactant maturation [14,15]. The surface active function of interfacial surfactant films is mainly supported by its major phospholipid dipalmitoyl phosphatidylcholine (DPPC) (40–50%) and the presence of hydrophobic surfactant proteins SP-B and SP-C [13,16]. Hydrophilic surfactant proteins SP-A and SP-D participate in the innate immune response by binding to pathogens and activating alveolar macrophages. Besides its surface activity and defense function, it has been recently proposed that a proper structure of the pulmonary surfactant layer can also be important to optimize oxygen diffusion through the air–water respiratory interface [17]. It remains, therefore, an open question whether there is an optimization of surfactant to conditions of limited oxygen availability, and whether exposure to reduced oxygen partial pressures could affect composition and/or function of pulmonary surfactant.

In the present work we have performed a large-scale characterization of the overall proteomic pattern of lung tissue and bronchoalveolar lavage (BAL) from rats subjected to moderate long term hypoxia (10% oxygen for 72 h) using a two-dimensional difference gel electrophoresis (2D-DIGE) approach. We have also studied and compared mRNA expression of several hypoxia-related factors such as HIF-1α, HIMF, α-globin and β-globin, and surfactant-related proteins SP-A, SP-B, SP-C, SP-D, and ABCA3, in lung tissue from normoxic and hypoxic rats. Subsequently we compared the presence of these proteins in BAL from both groups of animals. Finally, to detect possible hypoxia-promoted changes in the surface activity of pulmonary surfactant we analyzed and compared the interfacial absorption capabilities of surfactant.

## 2. Experimental procedures

### 2.1. Sample collection

Rats were maintained either under normoxic conditions (21% $O_2$) or in a hypoxia incubator for 72 h at 10% $O_2$. Animals were then anesthetized and perfused with saline, to remove circulating cells and serum proteins in order to avoid accidental blood contamination during the subsequent collection of BAL. After clamping the right mainstem bronchus the left lung was lavaged with 0.9% NaCl. The right lung was removed and cut into four pieces that were immediately frozen in liquid nitrogen. BAL was centrifuged at 150 *g* for 10 min at 4 °C to remove cells and the supernatants were frozen.

To obtain whole native rat surfactant, BALs from both lungs were centrifuged for 1 h at 100,000 *g* and membranes were cleaned to remove potential blood contaminants by centrifugation on a sodium bromide gradient, as previously described [18].

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