Physiol. Res. 73 (Suppl. 2): S557-S568, 2024 *https://doi.org/10.33549/physiolres.935436*

REVIEW

Influence of Hypoxia on the Airway Epithelium

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Received May 25, 2024
Accepted June 26, 2024

Summary

The necessity of oxygen for metabolic processes means that hypoxia can lead to serious cell and tissue damage. On the other hand, in some situations, hypoxia occurs under physiological conditions and serves as an important regulation factor. The airway epithelium is specific in that it gains oxygen not only from the blood supply but also directly from the luminal air. Many respiratory diseases are associated with airway obstruction or excessive mucus production thus leading to luminal hypoxia. The main goal of this review is to point out how the airway epithelium reacts to hypoxic conditions. Cells detect low oxygen levels using molecular mechanisms involving hypoxia-inducible factors (HIFs). In addition, the cells of the airway epithelium appear to overexpress HIFs in hypoxic conditions. HIFs then regulate many aspects of epithelial cell functions. The effects of hypoxia include secretory cell stimulation and hyperplasia, epithelial barrier changes, and ciliogenesis impairment. All the changes can impair mucociliary clearance, exacerbate infection, and promote inflammation leading to damage of airway epithelium and subsequent airway wall remodeling. The modulation of hypoxia regulatory mechanisms may be one of the strategies for the treatment of obstructive respiratory diseases or diseases with mucus hyperproduction.

Keywords

Secretory cells • Motile cilia • Epithelial barrier • Oxygenation • Obstructive respiratory diseases

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Introduction

The continuous cellular need for oxygen requires the maintenance of oxygen homeostasis. While in very simple microscopic organisms diffusion is enough to distribute oxygen sufficiently, in vertebrates, including humans, the increase of body mass led to the development of a complicated system for oxygen distribution [1,2]. Lack of oxygen in the environment or failure of the oxygen distribution system can lead to hypoxia.

Hypoxia is defined as deprivation of oxygen level in a tissue. The term was probably used for the first time in 1938 [3]. In his recent review, Sargon divided hypoxia into four groups: hypoxemic hypoxia, ischemic hypoxia, anemic hypoxia and histotoxic hypoxia [4]. The relationship between the terms hypoxia and ischemia should be mentioned, too. Whereas hypoxia is the result of disproportion between oxygen supply and demand, regardless of the cause, ischemia is defined as reduction or interruption of the blood flow leading to hypoxia of perfused tissue [5]. The necessity of oxygen for metabolic processes means that hypoxia, due to its severity and duration, can lead to serious cell and tissue damage. On the other hand, in some situations, hypoxia occurs under physiological conditions and in this case, hypoxia serves as an important regulation factor, e.g. in the intrauterine period [6,7], in the intestinal epithelium [8] or in renal medulla, bone marrow, lymphoid tissue and placenta [9]. General hypoxia affecting the whole organism can be caused by high altitudes, by various pathological conditions including respiratory, circulatory, hemato-logical or infectious disorders, or by artificial conditions in animal experiments. Local hypoxia can develop in many situations of local failure of natural oxygenation [10].

In the respiratory system, hypoxia influences many processes. They include pulmonary vascular remodeling, shifts in immune response and changes in the

airway and alveolar wall. However, the airway epithelium is specific in that it gains oxygen not only from the blood supply but that the majority of oxygen transported into epithelial cells is provided directly by the luminal flowing air [11–13]. Many respiratory diseases are associated with airway obstruction or excessive mucus production and thus with epithelial hypoxia. The main goal of this review is to point out how the airway epithelium reacts to hypoxic conditions.

Cellular hypoxia

The need for oxygen homeostasis led to the evolution of many regulative mechanisms. The quick adaptation involves increasing respiration, blood flow, and survival responses. Longer hypoxia activates mechanisms either increasing the oxygen supply or allowing the adaptation to hypoxia. The effect of hypoxia on cells is an important research field. In 2019 Nobel Prize in Physiology or Medicine "for their discoveries of how cells sense and adapt to oxygen availability" [14] was awarded to G. Semenza, P. J. Ratcliffe and W. G. Kaelin.

Cells detect low oxygen levels using molecular mechanisms involving hypoxia-inducible factors (HIFs) [15]. These mechanisms are linked to oxygen-sensing prolyl hydroxylase domain proteins (PHDs), which hydroxylate proline in α subunits of HIFs [16], and are summarized in Fig. 1. HIFs are heterodimeric transcription factors, consisting of α (1α or 2α) and β subunits [1]. Under normoxic conditions, the α subunit is hydroxylated by PHDs using oxygen as a substrate. Hydroxylated HIFα interacts with Von Hippel-Lindau protein (VHL) and this complex undergoes ubiquitination. Under hypoxic conditions, the hydroxylation is arrested. This leads to stabilization of the α subunit, transport to the nucleus, and dimerization with the β subunit. This HIF complex then acts as a transcription factor for many genes [17], with VEGF, leptin and TGF-β3 among the regulated genes. The number of genes known to be directly or indirectly regulated by HIF-1 gradually increases - more than 800 genes had been described by 2015 [18], and they accounted for over 2 % of known genes in 2017, respectively [19]. Main HIF-2 targets are EPO (erythropoietin) and EDN1 (endothelin 1). HIF-2 also plays a role in NO metabolism [17]. Currently, in the HIF family, three HIFs are described - HIF-1, HIF-2 and HIF-3. HIF-3 was for some time considered to be only a negative mediator of HIF-regulated genes [20] but later findings have shown different functions of HIF-3 [21]. HIF-3 is particularly important in chronic hypoxia [22,23].

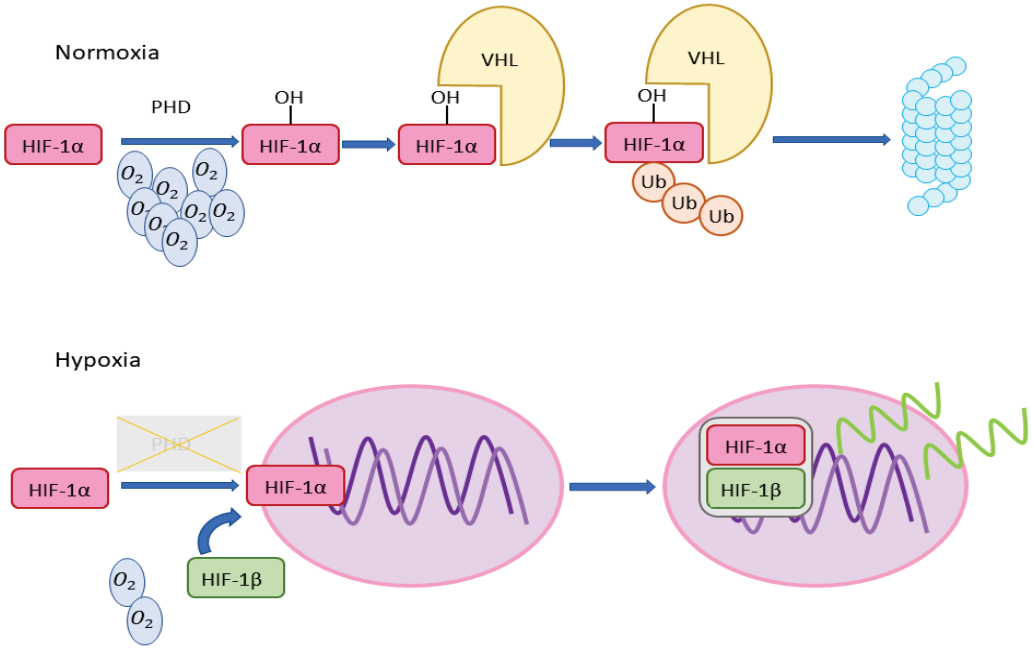

Fig. 1. In normoxia, prolyl hydroxylase domain proteins with O_2 as a substrate hydroxylate HIF-1α prolines. Hydroxylated HIF-1α subunit forms a complex with VHL and this complex is subjected to ubiquitination and proteasome digestion. In hypoxia, HIF-1α gets not hydroxylated and it leads to stabilization of this subunit and its transport to the nucleus. In the nucleus, the α subunit dimerizes with the β subunit and this HIF complex then functions as a transcription factor. HIF-1α - α-subunit of the hypoxia-inducible factor 1; PHD - prolyl hydroxylase domain proteins; VHL - Von Hippel-Lindau protein; Ub - ubiquitin; HIF-1β - β-subunit of the hypoxia-inducible factor 1

Stabilization of HIFs is the main way cells detect low oxygen levels. However, low oxygen levels can be also detected in other stress pathways as well as changes in metabolite levels and the generation of reactive oxygen species by mitochondria [10]. Some genes are influenced by hypoxia without the HIFs [24].

Under normoxic conditions, HIF-1α is the target of VHL-mediated degradation [25]. VHL protein is the main regulation factor of HIFs in normoxia [26]. The importance of VHL for this regulation can be shown in a genetic condition called Von Hippel-Lindau disease. Von Hippel-Lindau disease is a tumor predisposition characterized by the occurrence of highly vascularized tumors in the CNS, retina and visceral organs. The VHL protein (pVHL), is the product of the VHL gene. Protein pVHL influences many cellular processes, especially the cellular adaptive response to hypoxia [27]. Highly vascularized tumors in Von Hippel-Lindau disease overproduce angiogenic polypeptides such as VEGF. The situation when the pVHL protein is non-functional (as here due to genetic condition in Von Hippel-Lindau disease) has analogous consequences as physiological hypoxia [18]. And vice versa, hypoxia can show tumorigenic effects [28–30].

VHL also interferes with other processes. It has an impact on extracellular matrix organization [31–33]. It also affects microtubules [34–36] and through the control of microtubule growth orientation and stabilization influences the ciliogenesis of primary cilia [37,38]. Hypoxia, which stabilizes HIFs, weakens the primary cilia [39] and induces their elongation [40]. The mechanism connecting hypoxia and primary cilia formation is probably VHL regulation of primary cilia formation [41]. Primary cilia are important for embryogenesis, too, because they are deeply involved in the mediation of intercellular [42] and intracellular signaling [43]. A relationship exists between the ciliogenesis of primary cilia and that of motile cilia, as seen in some types of ciliopathies where the gene mutation affects both primary and motile cilia [44].

The activity of HIF-1 is regulated by many genes [45,46]. Besides hypoxia, HIF-1 can be activated by other mechanisms, e.g. by lipopolysaccharide [47,48]. In tumor tissue, HIF-1 activation can occur due to mutations in oncogenes and tumor suppressor genes [49]. Rohwer and her co-workers described increased levels of HIF-1 even in cells without hypoxia in intestinal tumorigenesis, showing that non-canonical HIF-1 stabilization through oncogenes exists [50].

Genetic selection as an adaptation to hypoxia at high altitudes was described. The principles of adaptation are lower levels of hemoglobin and the absence of polyglobulia (which otherwise are linked to chronic mountain sickness - Monge's disease) [51]. This adaptation in Tibet is represented by single nucleotide polymorphisms in the endothelial Per-Arndt-Sim domain protein 1 (EPAS1) gene, coding for the HIF-2α subunit, involved then in the stimulation of red blood cell production [52]. Chronic mountain sickness occurs more frequently in Andeans than in Tibetans and the search for this genetic adaptation in Andeans was unsuccessful at first [51]. It may be a consequence of the fact that the population lives in the Andes for a shorter period than in Tibet [53]. However, later the EPAS1 adaptation was found in Andeans as well. Furthermore, a genetic adaptation affecting the G protein-coupled receptor 126 (GPR126) gene was revealed in the Andean population [54]. The Andean EPAS1 adaptation results in a hypomorphic allele [17]. Another genetic adaptation localized in the PHD2 gene was later discovered in both Tibetans and Andeans [55,56]. The relationship between HIF-2α and erythropoietin levels can be also illustrated by inherited HIF-2α mutation in a large pedigree, accompanied by erythrocytosis and an increase of erythropoietin in serum [57].

The relationship between hypoxia and inflammation exists [58–60]. Indirectly, in people with mountain sickness, an increased level of inflammation markers was observed [16,61]. Hypoxia activates HIFs influencing many aspects of immune cells' function [9,62]. HIFs can be stabilized also under normoxic conditions during inflammation and regulate then the metabolism and expression of immune genes, thus HIFs can be seen not only as homeostasis regulators under hypoxic conditions but as well as specific regulators of immune and inflammatory genes [63]. Hypoxia induces airway epithelium to produce compounds influencing innate immunity in surrounding areas and this mechanism can then contribute to chronic pulmonary disease pathogenesis [64,65]. This process might be related to HIF stabilization in myeloid cells [62].

Hypoxia and airway epithelium

The airway epithelium lines the conductive portion of the respiratory tract from the nasal cavity to the smallest bronchioles. Although several cell types are present in both, the airway epithelium differs in large and

small airways. According to classic morphological descriptions, the large airways like nasal cavity, larynx, trachea, and bronchi are lined with a pseudostratified columnar epithelium containing mostly ciliated cells, goblet cells, and basal cells, while the small terminal and respiratory bronchioles are lined with a simple columnar to cuboidal epithelium where mucus-secreting goblet cells are gradually replaced by multifunctional club cells and ciliated and basal cells decrease in number and density [66]. However, further studies and mainly novel single-cell RNA sequencing (scRNA-seq) have uncovered enormous cellular heterogeneity within the airway epithelium. For example, ion-transporting pulmonary ionocytes, several types of tuft cells, variable pulmonary neuroendocrine cells, hillock cells, and pulmonary microfold cells were described [67–69]. Basic cell types and their prospective quantitative changes under hypoxic conditions are visualized in Fig. 2.

Hypoxia in airways can, due to various causes, affect the airway system globally as well as locally. Local hypoxia can arise as the result of obstruction (e.g. the obstruction of nasal sinuses) or the aggregation of mucus (e.g. cystic fibrosis). Mucus hyperproduction and disruption of epithelial barrier function by the production of VEGF and down-regulation of junctional proteins caused by local obstruction of nasal sinuses led to overexpression of HIF-1 in epithelial cells. Moreover, hypoxia-induced inflammation by high-mobility group box 1 (HMGB1) protein translocation into the cytoplasm resulted in the release of IL-8 through a ROS-dependent mechanism in upper airway epithelium [70]. In patients with cystic fibrosis, the thick mucus not only led to partial obstructive luminal hypoxia, but also created particularly hypoxic niches in the airway epithelium. Epithelial hypoxia in this case was potentiated by increased epithelial oxygen consumption associated with increased epithelial Na^+ channel (ENaC) mediated Na^+ absorption [71].

The general hypoxia can influence the airway epithelium from both luminal and basal sides. In our previous studies, we tested the effect of inhalation of a 10 % hypoxic atmosphere on the epithelium of large and small airways in rabbits at the level of transmission electron microscopy [72,73]. After four-day exposure, the most affected cells were tracheal goblet cells, which were stimulated to mucus release. After rapid mucus discharge,

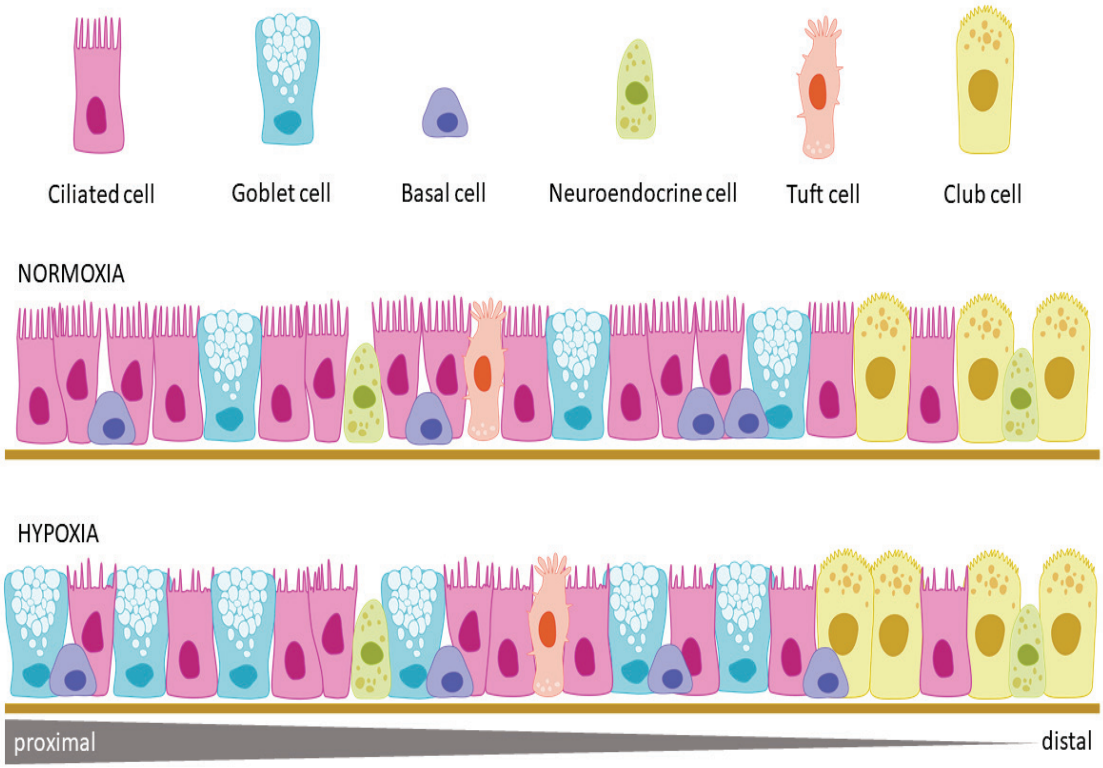

Fig. 2. The normal airway epithelium lining the tracheobronchial tree contains more cell types. The main cell types in the upper part of the tree are represented by ciliated cells, goblet cells and basal cells, in lower branches these types are gradually replaced by club cells. Among main cells the minor cell types are irregularly interspersed. Hypoxia influences both cellular proliferation and cilia formation. The proliferation was observed mainly in the population of secreting cells - goblet cells and club cells. Cilia of ciliated cells under hypoxic conditions become shorter and sparse.

the overstimulated goblet cells mostly did not take part in further secretory cycles but they degenerated and gradually sloughed off. We have demonstrated that a high level of stimulation of secretory cells in the airway epithelium accompanied by degeneration of about 50 % of goblet cells induced a massive differentiation of new secretory elements [74,75]. As the differentiating goblet cells retained the ability to divide [76], the result of this process was hyperplasia of secretory elements followed by changes in their distribution in the epithelium [74,75]. Indeed, differentiating goblet cells represented almost one-fifth of secretory elements and the formation of intra-epithelial mucous glands was recorded [72]. Ultrastructural changes of the epithelium in terminal bronchioles were not so prominent, but they corresponded with the findings described in the tracheal epithelium. Cytoplasmic alteration was found both in ciliated and club cells, but degenerative changes were observed only in some club cells. Observed significant increase of club cell relative number in hypoxic rabbits could reflect their compensatory proliferation [73].

Secretory cells

Although the airway epithelial cells' gross morphology in the light microscope can be described as intact in hypoxic conditions, many metabolic pathways are up- or downregulated [13], which can explain the discrete changes in the cellular ultrastructure found in our studies [72,73]. The secretory cell stimulation and hyperplasia seem to be a common response of the airway epithelium to the hypoxic conditions. The goblet cell hyperplasia mediated by HIF-1α was described in human bronchial epithelial (HBE) cell cultures of patients with chronic obstructive pulmonary disease (COPD) [77]. Hypoxia in mouse bronchial epithelium activated FoxM1 (proliferative factor for club cells) and bronchial cell growth factors RELMα and RELMβ *via* HIF2α. This activation led to a proliferation of bronchial epithelial cells (Ki67 staining has shown proliferation activity in 10-15 % of cells). Detailed analysis revealed that 78 % of them were club cells, 8 % ciliated cells, and the remaining portion was probably represented by basal cells [78]. Hypoxia-induced HIF-1 stabilization also increased the mucin 5AC (MUC5AC) expression in HBE and this mechanism led to the elevation of its secretion [79]. In a recent study, chronic hypoxia of HBE was associated with an increase in mucus concentration and MUC5AC transcription. The high mucus concentration could be also explained by ion-transport dysregulation *via* an epithelial Na+ channel (ENaC) beta and gamma subunits hyperexpression, which is HIF dependent [13]. Accumulation of mucus in airways can cause harm in more ways: by aggregation of pollution and immunomodulatory effect [80], by induction of inflammation and epigenetic regulation of macrophages [81].

Neuroendocrine cells

Shivaraju et al. were concerned with the neuroendocrine (NE) cell population in airways. The authors of this study filtered possible other influences that could mimic or modify the effect of pure hypoxia. Hypoxia led to significant proliferation of NE cells. Although some new NE cells could be derived from solitary NE cells, most newly differentiated NE cells arose from basal stem cells. Moreover, some basal stem cells displayed NE-specific vesicles [82]. On the other hand, NE cell proliferation as a reaction to hypoxia was not observed in the later study [13] and our personal observation did not reveal any increase in NE cell density, either [72,73].

Epithelial barrier

Epithelium represents a complicated barrier between luminal content and tissues that can be influenced by hypoxia. HIFs contribute to the expression of barrier-related genes and act in the regulation of barrier-adaptive responses within the mucosa [83]. Although more was described for the hypoxic effect on epithelial barrier function in the gastrointestinal tract [84], the general principles would probably be similar in epithelia, including the airway epithelium [83]. HIF-1 in the intestinal mucosa impacts two ways of barrier maintenance: 1) continual proliferation and epithelium renewal (*via* expression of regulation genes as WNT/β-catenin and Notch), 2) tight sealing of the barrier (*via* expression regulation of genes involved in mucus composition, permeability and integrity of tight junctions) [8]. Airway epithelium barrier impairment due to hypoxia affecting various mechanisms here described was observed [85–87]. On the other hand, if the barrier function was impaired by other conditions, then hypoxia-activated HIF-1 improved the epithelial barrier function [58].

Cilia

Oxygen is needed for motile cilia differentiation in airway ciliated cells. If cultivated in submersion, hypoxia blocks the cilia growth through impact on Notch signaling pathway activation and influences the expression of multicilin and Forkhead box J1 (FoxJ1) genes, both strongly involved in cilia formation [88] - multicilin in the early regulation stage of ciliogenesis, FoxJ1 gene in the later one [89]. The importance of the Notch signaling pathway for ciliogenesis can be illustrated also in tumors derived from multiciliated cells. Notch activation led to reduced multiciliation in choroid plexus tumors because of Notch inhibition of Geminin Coiled-Coil Domain Containing (GMNC) and multi-ciliate differentiation and DNA synthesis associated cell cycle protein (MCIDAS) [90]. GMNC and MCIDAS were previously described as having a crucial role in the ciliogenesis of multiciliated cells [89,91]. As described above, hypoxia can impact the organization and growth of cytoskeletal elements, including microtubules. Centrosomes, too, as microtubular complexes, can be influenced by hypoxia. Hypoxia plays an important role in centrosome amplification through the polo-like kinase 4 (PLK4) receptor, leading to their abnormal size, shape, number and position [92,93]. As PLK4 plays an important role in the early stages of centrosome duplication [94], there is a possibility that motile cilia could be affected by this mechanism under hypoxic conditions in multiciliated cells. Indeed, a significant decrease in the number of kinocilia/mm^2, an increase in the percentage of altered cilia and morphological signs of impairment of the vital self-cleaning ability were recorded in the rabbit tracheal epithelium after 4-day normobaric hypoxia [72]. In human airway epithelial cells the decrease of kinocilia number due to hypoxia was described in ALI (air-liquid interface) cell cultures derived from two COPD patients [87].

Conclusion

Hypoxia arouses significant interest in the scientific community, as seen in the increasing number of publications concerning hypoxia published in the last decades. Particularly the COVID-19 pandemic focused the interest of the scientific community not only on hypoxia in general [95] but specifically on the hypoxia of the respiratory system, as visualized in Fig. 3.

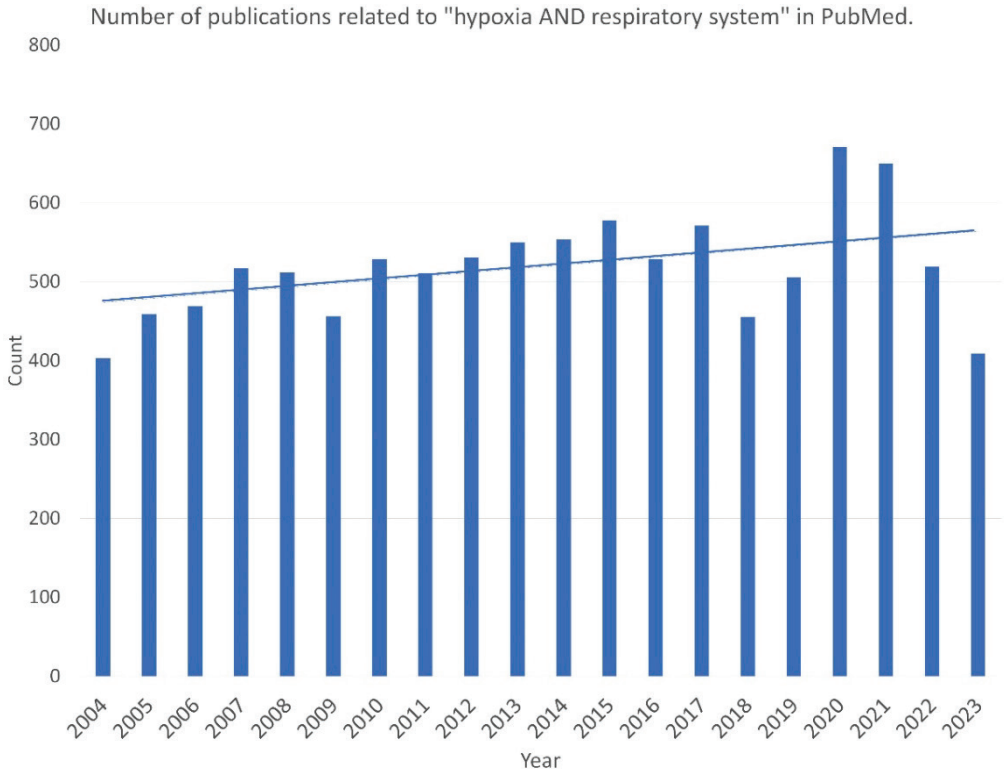

Fig. 3. Number of publications related to "hypoxia AND respiratory system" in the last two decades. Particular increase can be observed in the time of the COVID-19 pandemic.

The unique position of the airway epithelium at the interface between ambient and vascular oxygen supply makes it interesting for the comprehensive understanding of the hypoxia influence on the respiratory system. Therefore, we attempted to review the literature and summarize the current understanding of this topic. The common effects of the hypoxic conditions, either local or general, seem to be 1) secretory cell stimulation, hypersecretion and subsequent proliferation, 2) epithelial barrier functional changes, and 3) ciliogenesis impairment. All the described changes can exacerbate the cycle of impaired mucociliary clearance, infection, and inflammation leading to damage of airway epithelium and subsequent airway wall remodeling. The modulation of hypoxia regulatory mechanisms may be one of the strategies for the prevention of airway remodeling changes and the treatment of obstructive respiratory diseases or diseases with mucus hyperproduction.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

The work was supported by the Charles University Grant Agency, grant No. 239123. We sincerely thank Mgr. Vladimír Šmilauer for his help with the graphic design of the figures.

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