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REVIEW ARTICLE

Hypoxia, HIF-1α, and COVID-19: from pathogenic factors to potential therapeutic targets

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The pandemic of coronavirus disease 2019 (COVID-19) and its pathogen, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have become the greatest current threat to global public health. The highly infectious SARS-CoV-2 virus primarily attacks pulmonary tissues and impairs gas exchange leading to acute respiratory distress syndrome (ARDS) and systemic hypoxia. The current pharmacotherapies for COVID-19 largely rely on supportive and anti-thrombi treatment and the repurposing of antimalarial and antiviral drugs such as hydroxychloroquine and remdesivir. For a better mechanistic understanding of COVID-19, our present review focuses on its primary pathophysiologic features: hypoxia and cytokine storm, which are a prelude to multiple organ failure and lethality. We discussed a possible link between the activation of hypoxia inducible factor 1α (HIF-1α) and cell entry of SARS-CoV-2, since HIF-1α is shown to suppress the angiotensin-converting enzyme 2 (ACE2) receptor and transmembrane protease serine 2 (TMPRSS2) and upregulate disintegrin and metalloproteinase domain-containing protein 17 (ADAM17). In addition, the protein targets of HIF-1α are involved with the activation of pro-inflammatory cytokine expression and the subsequent inflammatory process. Furthermore, we hypothesized a potential utility of so-called "hypoxic conditioning" to activate HIF-1α-induced cytoprotective signaling for reduction of illness severity and improvement of vital organ function in patients with COVID-19. Taken together, we would propose further investigations into the hypoxia-related molecular mechanisms, from which novel targeted therapies can be developed for the improved management of COVID-19.

Keywords: hypoxia; HIF-1α; ACE2; COVID-19; cytokine; hypoxic conditioning

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INTRODUCTION

In December 2019, a new outbreak of a lethal disease with the characteristics of severe acute respiratory syndrome (SARS) first occurred in Wuhan, a major city of 10 million population in central China. This highly contagious disease was identified to be caused by a novel coronavirus, which was later termed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the World Health Organization, whereas the disease was termed as coronavirus disease 2019 (COVID-19). Due to the remarkably high person-to-person transmission rate of SARS-CoV-2, COVID-19 has rapidly spread in the first quarter of 2020 to about 200 countries around the world and caused a global pandemic. Despite that the medical knowledge and molecular and cellular mechanisms of COVID-19 remain largely speculative, our present review article attempts to provide an overview of the potential importance of hypoxia in the pathogenesis as well as progression of COVID-19 and, accordingly, to propose hypoxia-related signaling pathways as possible prophylactic and/or therapeutic targets for intervention in this devastating viral disease that results in severe harm to public health.

SARS-CoV-2 is highly infectious and pathogenic and primarily transmitted via respiratory droplets and direct contact with infected patients, as well as potentially transmitted through the fecal-oral route [1]. Individuals who are asymptomatic carriers or patients who are within the incubation period have also been identified possible sources of infection [2]. The SARS-CoV-2 infected individuals who develop severe disease are more common in patients with a history of preexisting conditions or chronic diseases, such as hypertension or chronic obstructive pulmonary disease (COPD) [3]. A reported disease progression timeline was within 5 days from the onset of symptoms to dyspnea and 7 days to admission to hospital, and 8 days to acute respiratory distress syndrome (ARDS) that is often associated with severe hypoxemia [4].

Factors associated with mortality have included preexisting illness (e.g., diabetes, coronary disease), increased age, higher Sequential Organ Failure Assessment scores, and laboratory abnormalities such as lymphopenia, leukocytosis, and elevated d-dimer [5]. Cytokines are typically part of a normal immune response to infectious agents in a healthy host. However, the patients with COVID-19 who progress to severe illness typically have an immune system overreaction known as "cytokine storm", which results in healthy tissues being damaged by an upregulated host immune response [6]. The pro-inflammatory cytokines leading to cytokine storm include tumor necrosis factor α (TNFα), interleukin 6 and 1β (IL-6 and IL-1β) [7]. The high volume of swiftly increasing cytokines can cause pulmonary injury through the infiltration of inflammatory agents (such as neutrophils and monocytes) into pulmonary interstitial tissues [8].

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High morbidity and mortality found in a subset of COVID-19 patients may have resulted from a high viral load and strong immune-mediated inflammatory response [8], which also contributes to the development of venous thromboembolism (VTEs) and in turn aggravates ventilation/perfusion (V/Q) mismatch leading to worsened clinical findings [7]. In addition, COVID-19 patients who develop thromboses are at risk for more severe disease and higher mortality [6]. Klok et al. reported a 31% incidence of thrombotic complications in 184 patients with confirmed COVID-19 pneumonia, despite the fact that they were already receiving standard pharmaceutical VTE prophylaxis [9].

Currently, there are no FDA-approved drug therapies for COVID-19, whereas a few existing common drugs have been repurposed to treat COVID-19 patients. For example, chloroquine and hydroxychloroquine have long been used as antimalarial drugs, and also in the treatment of autoimmune conditions such as rheumatoid arthritis and systemic lupus erythematosus. Their immunomodulatory effects, as well as antiviral activity, by inhibiting glycosylation of host receptors were recognized and both drugs demonstrated in vitro antiviral activity against SARS-CoV-2 [10]. In addition, chloroquine or hydroxychloroquine are well-known lysosome inhibitors, which are capable of inducing transient lysosomal abnormalities that may interfere with intracellular viral trafficking and fusion, as recently hypothesized by Ballout et al. to be one of the mechanistic explanations of hydroxychloroquine against SARS-CoV-2 infection [11]. Moderate dosing regimens of chloroquine/hydroxychloroquine appeared to have better outcomes in clinical studies [12]. Gautret et al. found an association of hydroxychloroquine treatment and viral load reduction; co-treatment with azithromycin further increased efficiency of virus elimination [13].

Azithromycin is a commonly prescribed macrolide antibiotic with broad spectrum activity and a favorable safety profile. In vitro antiviral activity of azithromycin against SARS-CoV-2 was demonstrated [14]. Gautret et al. demonstrated a significant difference in viral elimination in the patients receiving both azithromycin and hydroxychloroquine when compared to the group receiving only hydroxychloroquine or the control group [13]. A recent multi-center retrospective observational study by The Henry Ford Health System in 2541 hospitalized patients with COVID-19 reported a 66% hazard ratio reduction of in-hospital mortality in the hydroxychloroquine alone group and a 71% reduction in the hydroxychloroquine + azithromycin group as compared to the standard care control group [15]. The observed synergistic therapeutic efficacy of azithromycin in combination with hydroxychloroquine may be due to their similar lysosome-mediated antiviral activities and independent inhibition of endosomal–lysosomal fusion and lysosomal proteases, which are keys for successful viral fusion of SARS-CoV-2 [11].

Remdesivir is a nucleotide analog prodrug that inhibits RNA polymerases in viruses and has broad-spectrum activity against several viral families, including coronaviruses. It was granted an emergency use authorization by the United States Food and Drug Administration for use in severe cases of confirmed COVID-19 on May 1, 2020. Preliminary results revealed more than 50% of 400 patients with severe COVID-19 recovered within 2 weeks of treatment; however, this study did not have a control group [16]. In vitro testing has shown efficacy of remdesivir against SARS-CoV-2 [17]. An improvement in oxygen support was noted in 68% of a group of critically ill and hypoxic COVID-19 patients with 64% on invasive mechanical ventilation and 8% on extracorporeal membrane oxygenation (ECMO) [17].

Overall, the current pharmacologic treatments for COVID-19 are complicated by several factors including small size of patient cohorts in studies, limited duration of follow-up, and difficulties in performing randomized controlled trials regarding treatment strategies for COVID-19. There is an ongoing urgent need for further understanding of cellular and molecular mechanisms to identify effective targets for COVID-19. To this end, we have focused on the cellular signaling mechanisms in response to hypoxia—a key clinical feature of COVID-19 in our subsequent discussions.

HYPOXIA CONTRIBUTES TO THE PATHOGENESIS AND PROGRESSION OF COVID-19

Hypoxia is a primary pathophysiologic feature and main cause of mortality in patients with severe COVID-19 and it accompanies all the stages of the disease. The pathogenic determinants of hypoxia may act at all systemic, organ and cellular levels, and the hypoxia-triggered factors may have aggregate effects on each other. Severe cases of COVID-19 have been observed to progress to ARDS, which can ultimately lead to end organ dysfunction and failure. Clinical findings of ARDS are often marked by respiratory distress with reduced compliance. Classic diagnostic criteria for ARDS include acute lung injury, noncardiogenic respiratory failure, decreased PaO_2:FiO_2 ratio, and bilateral infiltrates on radiologic imaging. However, a subset of COVID-19 patients with ARDS had hypoxemia with relatively good lung compliance [18]. This atypical form of ARDS is characterized by a significant pulmonary shunt and higher than expected compliance, which could be secondary to hypoxic pulmonary vasoconstriction and a hypercoagulable state, which leads to microvascular thromboses in the pulmonary circulation of COVID-19 patients [19]. Therefore, COVID-19 patients with severe respiratory failure likely have widespread inflammation, leading to thrombogenesis and increased cytokine release, and subsequently end organ damage [18].

At the cellular level, once cells are infected with SARS-CoV, a coronavirus that shares 79.5% identity to SARS-CoV-2, accumulation of hypoxia inducible factor 1α (HIF-1α) may occur due to increased expression as well as inhibited proteasome degradation [20]. In addition, a possible secondary bacterial infection during the later phase of COVID-19 may result in the stabilization of HIF-1α in macrophages via the activation of toll-like receptor 4 (TLR4) and decrease in prolyl hydroxylase mRNA, a pathway which was indirectly demonstrated in a recent study on aortic dissection, a severe inflammatory vascular disease [21]. Subsequent local hypoxia events may also occur when leukocytes are activated in response to secreted interferon as well as to accumulation of pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs). These hypoxia-related complex pathophysiological pathways are hypothetically illustrated in Fig. 1.

Beside the tissue inflammation-disturbed oxygen supply, severe hypoxemia in COVID-19 often results from pneumonia with bilateral interstitial infiltrates causing serious alterations in ventilation-perfusion ratio and severe ARDS [22], which exacerbates hypoxemia [23]. Cardiac complications may also be partially responsible for hypoxemia in patients with COVID-19 [24]. Heart failure could be caused by direct viral myocardial damage, hypoxia, hypotension, or enhanced inflammatory status [25] as well as secondary hemophagocytic lymphohistiocytosis [26].

ACE2 AND TMPRSS2 IN SARS-COV-2 CELL ENTRY: POTENTIAL TARGETS OF HIF-1α SIGNALING?

SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) receptor and transmembrane protease serine 2 (TMPRSS2) for its entry into the host cell. ACE2 acts as the host cell receptor for SARS-CoV-2 by binding to the spike protein on the viral capsid, whereas TMPRSS2 primes the viral spike protein as previously shown in SARS-CoV [27]. Consequently, SARS-CoV-2 can enter only the cells expressing both ACE2 and TMPRSS2 on cellular membrane surfaces. Hence, ACE2 is the primary target for pharmaceutical approaches in diminishing the availability of molecular docking sites for SARS-CoV-2 and, in turn, slowing

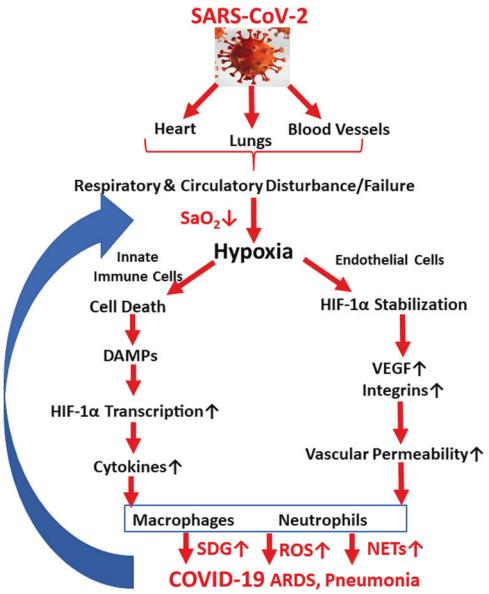

Fig. 1 Proposed pathological mechanisms and disease outcomes of COVID-19 involving hypoxia and HIF-1α-dependent detrimental cell signaling pathways. SARS-Cov-2—severe acute respiratory syndrome coronavirus 2, HIF-1α—hypoxia inducible factor 1α, ARDS—acute respiratory distress syndrome, DAMPs—damage associated molecular patterns, VEGF—vascular endothelial growth factor, NETs—neutrophil extracellular traps, ROS—reactive oxygen species, SDG—secretory degranulation

down the viral entrance into the cells. This notion has indirect support by the fact that the human ACE2 gene is located on the X chromosome region (Xp22). Given that males lack the second X chromosome, females appear to be less susceptible to SARS-CoV2 infection [28]. Several polymorphisms of the human ACE2 gene may affect both the susceptibility of people to SARS-CoV-2 infection and the outcome of COVID-19 disease [29]. In addition, ACE2 is a well-known vasodilator, which antagonizes adverse vasoconstrictor and profibrotic effects of angiotensin, which may be a critical determinant of cardiovascular complications of COVID-19.

The activity of ACE2 can be modulated through RNA or protein expression levels as well as cleavage from the cell membrane, which is mediated by disintegrin and metalloproteinase domain-containing protein 17 (ADAM17). Therefore, ADAM17 activation or ACE2 antibodies are considered to serve as possible remedies for SARS-CoV-2-caused COVID-19. Interestingly, previous evidence obtained from a study on SARS-CoV shows that HIF-1α may transcriptionally target all the proteins controlling cell entry of SARS-CoV-2, i.e., ACE, ACE2, TMPRSS2, and ADAM17 [30]. It was further demonstrated in a model of hypoxic pulmonary hypertension that HIF-1α exerts its inhibitory effect on ACE2 through microRNA let-7b, which directly targets the coding sequence of ACE2 [31]. It is postulated from previous studies under other nonviral conditions that HIF-1α also upregulates expression of ADAM17, which cleaves ACE2 from the alveolocyte surface and consequently may shut down the cell entry gates for SARS-CoV-2 [32, 33]. On the other hand, in castrate-resistant prostate cancer cells, HIF-1α suppressed TMPRSS2, which is necessary for the priming of coronavirus spike-protein [34]. Taken together, as illustrated in Fig. 2, we would speculate that the activation of the HIF-1α signaling pathway would decrease ACE2 and TMPRSS2 and increase ADAM17 levels on the surface of alveolocytes and therefore decrease the invasiveness of SARS-CoV-2.

Coincident to our above-mentioned postulation, a notion of possible decreased severity of infection by SARS-CoV-2 under hypoxic conditions at high altitudes was recently discussed by

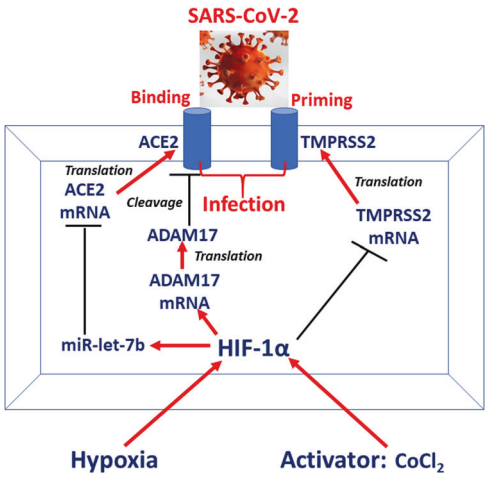

Fig. 2 HIF-1α participates in the regulation of protein expression of key molecules of SARS-Cov-2 entrance: ACE2 and TMPRSS2 on the cell surface. Spike protein of SARS-Cov-2 binds to the host membrane protein ACE2. This process becomes possible after spike protein priming by another membrane protein enzyme TMPRSS2. ACE2 shedding into the extracellular space provided by membrane peptidase ADAM17 diminishes the rate of SARS-Cov-2 infection. HIF-1α transcriptionally stimulates ADAM17, inhibits TMPRSS2, and activates miR-let-7b, which in turn inhibits protein expression of ACE2. It suggests that activation of HIF-1α with hypoxia or chemical activator may decrease the rate of SARS-Cov2 entry to the cells. SARS-Cov-2—severe acute respiratory syndrome coronavirus 2, ACE2—angiotensin-converting enzyme 2, TMPRSS2—transmembrane protease serine 2, ADAM17—ADAM metallopeptidase domain 17, HIF-1α—hypoxia inducible factor 1α, miR-let-7b—Let-7b micro RNA

Arias-Reyes et al., who proposed a cellular mechanism for high altitude-induced environmental protective factor in decreasing the half-life of the virus and downregulating ACE2 pulmonary epithelium via the cellular response to hypoxia [35]. Therefore, individuals under chronically hypoxic conditions at higher altitudes could be less vulnerable to widespread infection by SARS-CoV-2 [35]. These findings have prompted our hypothesis toward developing "hypoxic conditioning" as a potentially new therapeutic modality for management of COVID-19 patients, which will be discussed further in the following sections.

HYPOXIA AND CYTOKINE STORM

As abovementioned briefly and summarized in Fig. 1, cytokine storm is characterized by the fulminant activation of large numbers of white blood cells, which release inflammatory cytokines including IL-1β and IL-6, eventually leading to multiple organ failure [8, 26]. The cytokine profile associated with disease severity of COVID-19 has been characterized by increased IL-2, IL-7, granulocyte colony stimulating factor (GCSF), interferon-γ inducible protein 10 (IP10), monocyte chemoattractant protein 1 (MCP1), macrophage inflammatory protein 1α (MIP1α), and TNFα. The COVID-19 patients in Wuhan, China, who subsequently needed intensive care, had higher levels of IL-2, IL-7, IL-10, GCSF, IP10, MCP1, MIP1α, and TNFα than those of non-ICU patients [36]. High levels of IL-1β, IL-6, TNFα, and CC chemokines (CCLs) were previously detected in the late stages of SARS-CoV and MERS-CoV infections [37, 38]. Serum cytokine and chemokine levels in patients with less severe MERS were detected to be significantly lower than in patients with severe disease [39]. The infected epithelial cells produce IFN-α/β. The activation of mononuclear macrophages IFN-α/β receptors causes the production of monocyte chemoattractants (such as CCLs), recruiting the next pool of mononuclear macrophages which elevates the

production of pro-inflammatory cytokines (TNFα, IL-6, IL-1β). The tissue damage attracts neutrophils, which infiltrate together with monocytes to the lungs, intestine, or heart in large amounts. The peculiar aspect of SARS-CoV and MERS-CoV, as well as COVID-19 infections is that mononuclear macrophages and dendrite cells infected by virus induce delayed but elevated levels of pro-inflammatory cytokines and chemokines [8]. Activation of T-helper type 17 plays a role in neutrophil production and recruitment in COVID-19-associated cytokine storm [40]. Inflammatory cytokines may also activate T-helper type 1 cell response as well as T-helper type 2-derived cytokines in COVID-19 patients [36].

The roles of hypoxia and HIF-1α in regulating cytokine expression have been controversial and depend upon various conditions. Hypoxia and HIF-1α can either stimulate or inhibit cytokine-mediated inflammatory response. For example, hypoxia and HIF-1α stabilization may trigger or enhance cytokine storm, since vascular endothelial growth factor (VEGF) is transcriptionally upregulated by HIF-1α and accumulates under hypoxia. Endothelial cells are essential contributors to the initiation and propagation of severe COVID-19 [41]. The migration of circulating inflammatory cells into tissues depends on a number of adhesive molecules and chemoattractants that are produced by endothelial cells and increased vascular permeability. VEGF and VEGF receptors play a role in this process [42]. VEGF-A can bind to VEGFR-2 and increase cytokine expression and pulmonary vascular permeability in cystic fibrosis patients undergoing lung transplantation and in experimental animals [43–45].

DAMPs and PAMPs cause the activation of mast cells, macrophages, dendritic cells and neutrophils, release of reactive oxygen species and proteases, and formation of neutrophil extracellular traps (NETs). The accumulation of activated neutrophils in the microcirculatory bed of the lungs, alveoli, and interstitium is one of the characteristics of ARDS and is considered as one of the causes of local damage of lung tissue [46]. An important aspect in inflammation is the elimination of neutrophils through apoptosis and through clearance by macrophages. Neutrophil turnover is known to be impaired in ARDS [47]. On the other hand, hypoxia reduces neutrophil apoptosis through HIF-1α via nuclear factor κB and MIP1α [48]. Thus, it can be speculated that the activation of HIF-1α is involved in the disruption of neutrophil apoptosis in ARDS.

Nevertheless, under ischemia, a decrease in cytokine expression is achieved by stabilization of HIF-1α. Under ischemia in brain tissue, HIF-1α stimulates vascular growth and helps ischemic organs survive hypoxia. Stabilization of HIF-1α by systemic administration of ML228, an activator of HIF-1α, significantly attenuated the levels of IL-6 and TNFα. HIF-1α stabilization caused the decrease of expression of both caspase-3 and cleaved caspase-3 [49]. In cell cultures (human bronchial epithelial cells), exposure to hypoxia or prolyl hydroxylase inhibitor administration resulted in a significantly decreased expression of inflammatory mediators (IL-6, IP10) in response to ligands for TLRs. HIF-1α knockdown resulted in increased expression of inflammatory mediators. It was suggested that hypoxia suppresses the innate immune response via HIF-1α [50].

One possible mechanism for the anti-inflammatory effect of HIF-1α is activation of the adenosine receptor-dependent pathway. ATP in extracellular matrix as a DAMP signalizes cell injury and death and serves as a potent activator of inflammation, whereas adenosine, on the contrary, has potent, and well documented, anti-inflammatory effects via one or more of its four G protein-coupled adenosine receptors [51]. HIF-1α switching to anaerobic glycolysis promotes the accumulation of adenosine, turning on this pathway which can be lifesaving when it protects inflamed tissues of vital organs from collateral damage by overactive antipathogen immune cells or enables the differentiation of cells of adaptive immunity [52].

Such a duality of HIF stabilization depends on cellular conditions. If a healthy cell in a state of homeostasis falls into hypoxic conditions, then the activation of HIF will lead to inhibition of oxidative phosphorylation and trigger adaptation reactions. If the cell is already under stress at the time of hypoxic exposure, activation of HIF and a decrease in ATP synthesis will suppress important adaptive mechanisms, such as UPR, autophagy, proteasomic proteolysis, leading to induction of necrosis and cytokine production. Therefore, the effect of hypoxia for an extended duration or on cells with an altered pH, impaired membrane transport, or stress of the endoplasmic reticulum will be damaging.

The other possible explanation can be in peculiarities of HIF-1α targeting gene regulation. Dengler et al. suggested that HIF-1α targeting genes as a group might be regulated at the pause-release stage, rather than by chromatin remodeling or transcription initiation [53]. Importantly, genome-wide analysis of both total and transcriptionally active RNAPII revealed that hypoxia-inducible genes are predominantly bound by active but paused RNAPII prior to their induction by hypoxia. This demonstrates that HIF-1α preferentially binds to permissive loci and activates genes with basal transcriptional activity. We can speculate that HIF-1α will potentiate cytokine expression in tissues predisposed to inflammation.

Another possible key molecule for HIF-1α-dependent cytokine expression activation can be the membrane metalloproteinase ADAM17, which insures the shedding of some membrane proteins into the extracellular matrix or blood stream. Cleavage of ACE2 from the membrane increases the effectiveness of conversion of angiotensin II into angiotensin and reaching vasodilatation. Since ACE2 serves as a molecule for SARS-Cov-2 cell entry, ADAM17 activation would decrease the rate of COVID-19 spread in an organism. On the other hand, ADAM17 is known as an enzyme which is involved in processing TNFα [54]. Proteolytic liberation of soluble TNFα significantly increases its effectiveness in the attraction of innate immune cells and development of inflammation. ADAM17 also plays an important role in IL-6 signaling. As IL-6 receptor (IL-6R) can be bound to the cell membrane or act in a soluble state, the complex of IL-6 with IL-6R and gp130 initiate intracellular signaling. Interestingly, the complex with the membrane-bound IL-6R turns on a mainly protective and regenerative reaction, whereas IL-6 acting via the sIL-6R is rather pro-inflammatory. ADAM17 is responsible for IL-6R cleavage and its pro-inflammatory action [55]. We suggest that chronic hypoxia under SARS-CoV-2 infection may activate HIF-1α pathways, including an increase in ADAM17 transcription that leads to increased processing of TNFα-driven signaling as a lever arm of the cytokine storm.

ROLE OF HIF-1α IN ACTIVATION OF INNATE IMMUNE CELLS

As a key molecule for oxygen sensing and adaptive response to hypoxia, HIF-1α plays a role in glycolysis via the induction of hexokinase 2, glucose-6-phosphate isomerase, and triosephosphate isomerase and pyruvate kinase M2 [56]. In inflammatory cells, HIF-1α accumulation in the nucleus can be reached both in the PHD-dependent pathway and by direct transcriptional activation through TLR4-mTOR. Hypoxia-independent HIF stabilization or so-called “pseudo-hypoxia” becomes possible in immune cells because of their specific metabolism. Through being activated by TLR ligands or pro-inflammatory cytokines, innate immune cells switch their metabolism from oxidative phosphorylation to aerobic glycolysis. This metabolic change happens after TLR4 activation and depends on the phosphatidyl inositol 3′-kinase/AKT pathway [57, 58]. PHDs utilize α-ketoglutarate as a co-substrate to split molecular oxygen to hydroxylate HIF-1α and, in parallel, to oxidize and decarboxylate α-ketoglutarate to succinate. After the inhibition of the tricarboxylic acid (TCA) cycle, excess

succinate is transported out of the mitochondria into the cytosol and creates product inhibition of PHD. The process of OXPHOS inhibition is also upregulated by HIF-1α itself through the induction of pyruvate dehydrogenase kinase 1 and 3 [59].

HIF-1α stabilization in inflammatory cells depends on reactive oxygen species (ROS) formation; pharmacologic inhibition of NADPH oxidase causes downregulation of HIF protein expression [60]. Enhanced expression of HIF-1α was accompanied by increased ROS levels. H_2O_2 treatment enhanced HIF-1α protein expression through modulation of PHD activity [61]. α-ketoglutarate dehydrogenase (αKGDH) is a key enzyme of TCA cycle and is highly sensitive to ROS and, therefore, a target of oxidative stress [62]. The inhibition αKGDH by NADPH oxidase derived ROS can cause α-ketoglutarate accumulation and modulation of PDH activity. Cobalt chloride induced a hypoxia-like response and cardioprotection via HIF-1α accumulation in the nucleus and the protective effect was blocked by an antioxidant, diethyldithiocarbamate, suggesting a trigger role of ROS in the cardioprotective effects of $CoCl_2$ [63]. Thus, the application of antioxidants in COVID-19 therapy may be effective in providing tissue protection against ROS generated by activated immune cells and also inhibit HIF-1α mediated cytokine production and NETs formation.

There is growing data that NETs formation is induced with participance of HIF. NETs stimulation by REDD1 was accompanied with higher HIF-1α expression [64]. HIF-1α stabilization by the iron chelating HIF-1α-agonist desferoxamine, or AKB-4924, enhanced the release of phagocyte extracellular traps [65]. Moreover, HIF-1α activation by lipopolysaccharides causes NETs formation and NET deployment was inhibited after pharmacologic and genetic knockdown of HIF-1α expression and activity [66].

As shown in a simplified illustration in Fig. 3, HIF-1α has special pro-inflammatory targets. It activates such potent chemo-attractants such as MCP-1, chemical chemokine 2, and IL-1β directly via hypoxia response elements [67]. HIF-1α activation in inflammatory cells can enhance immune response. ADAM17 is known as an enzyme that provides processing of TNFα and IL-6R [54].

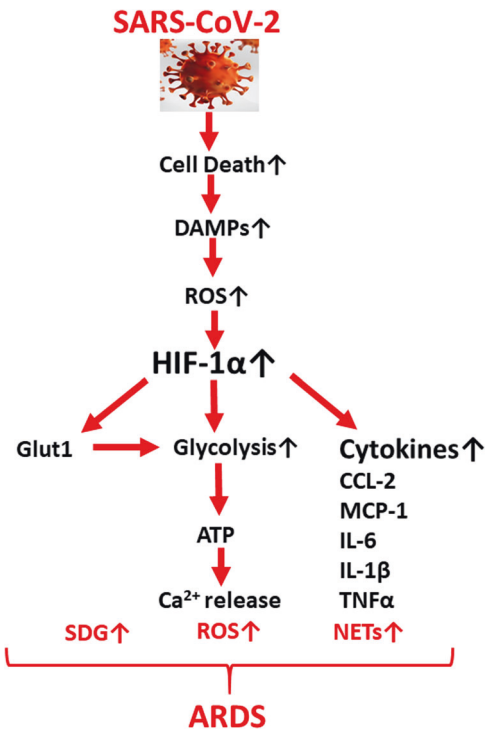

Fig. 3 HIF-1α-centered hypothetical pathogenic mechanism of ARDS in the cytokine storm phase of COVID-19. HIF-1α—Hypoxia inducible factor 1α, ARDS—acute respiratory distress syndrome, NETs—neutrophil extracellular traps, DAMPs—damage associated molecular patterns, TLR—toll-like receptor, CCL-2—CC chemokine ligand 2, MCP1—monocyte chemoattractant protein 1, IL-6—interleukin 6, IL-1β—interleukin 1β, TNFα—tumor necrosis factor α, ROS—reactive oxygen species, SDG—secretory degranulation

PROPHYLACTIC AND THERAPEUTIC POTENTIAL OF HYPOXIC CONDITIONING AGAINST COVID-19: ROLE OF HIF-1α

Although hypoxia may trigger a vicious cycle of events including mitochondrial dysregulation, acidosis, altered mitochondrial membrane permeability, and eventually insufficiency of ATP biosynthesis [68], certain moderate types/degrees of hypoxia can induce an adaptive phenomenon called "hypoxic conditioning" or "hypoxic preconditioning", which would act as a vaccine to protect against hypoxia-induced lethal damages in various organs and cells. Such hypoxic signals can be generated via a well-controlled intermittent hypoxia-normoxia or hypoxia-hyperoxia training program by means of (1) inhalation of low oxygen gas mixtures; (2) staying in hypobaric chambers, and (3) recurrent sojourn at high altitudes [69–72].

Adaptive reaction on the lack of oxygen by the cell occurs through the transcriptional complex HIF-1, which is a heterodimer composed of an alpha and a beta subunit. HIFs are regulated by HIF prolyl-4-hydroxylases (PHDs) and HIF asparaginyl hydroxylase (FIH), which in normoxic conditions, mark HIF for ubiquitination and rapid degradation by the ubiquitin–proteasome pathway. Under hypoxia, PHDs and FIH are inhibited and HIF-1α becomes stabilized and transcriptionally active. Since its first discoveries in 1995 [73–75], new cell signaling pathways involving HIF-1α have been increasingly identified as a universal bio-sensor and regulator in various living cells under various hypoxic conditions. For example, It causes upregulation of the expression of many target genes e.g., erythropoietin, VEGF, and antioxidant enzymes that afford beneficial effects such as cardioprotection [76]. In addition, recent studies also revealed the roles of autophagy [77], unfolded protein response [78], and mitochondrial biogenesis [79] among other known and unknown factors. For instance, VEGF is transcriptionally regulated by HIF-1α and plays a key role in angiogenesis in response to hypoxia, activating vessel growth and improving oxygen supply, which in turn diminishes tissue hypoxia, prevents cell death, migration of macrophages, and inflammation [80]. At the same time, VEGF increases vessel permeability and, thus, migration of immune cells into tissues, which would exacerbate inflammation [44].

Hypoxic conditioning diminishes chronic inflammation and cytokine expression. In healthy humans, exposure to four cycles of 5-min 10% O_2 and 5-min room air daily for 14 days suppressed pro-inflammatory mediators such as TNF-α and IL-4 by more than 90%. The beneficial effects of hypoxic conditioning were long-lasting and persisted beyond 7 days post-training [81]. A recent study demonstrated that the course of IHHT decreases neutrophil activation in patients with Alzheimer's disease (AD) [71]. In addition to secretory degranulation, reactive oxygen species production, and phagocytosis, neutrophils can also produce neutrophil extracellular traps. NETs represent chromatin with inserted proteases released by activated cells. This DNA network may serve as a trap for bacteria and other antigen carriers. NETosis is activated in brains of AD patients. The presence of proteases in NETs initiates blood vessel inflammation, which leads to blood–brain barrier destruction and neuronal degradation, further activating platelets. At baseline, in AD patients, the NETs exceeded more than three times than those of healthy people. Intermittent hypoxia training decreased the formation of NETs by 53% in AD patients and such benefits were sustained or even more pronounced 1 month after IHHT termination [71]. Thus, IHHT

may be useful as a tool for diminishing the activation of neutrophils and pro-inflammatory cytokine formation under chronic inflammatory pathologies. It is postulated that the individuals who received IHHT would be better prepared to encounter COVID-19.

Furthermore, this hypothetical notion is also indirectly supported by the published works demonstrating hypoxia conditioning/preconditioning is beneficial against pro-inflammatory pulmonary diseases such as COPD [82, 83] and asthma [83, 84] in humans. Hypoxic preconditioning or chemical activation of HIF-1α with $CoCl_2$ also alleviated severe hypoxia induced pulmonary edema and lung injuries in rodent models [85–87]. Nonetheless, we should emphasize that we are not suggesting the use of hypoxia conditioning as a therapy during the acute and severe phases of COVID-19. The prevention and correction of severe hypoxemia become more critical for managing COVID-19 patients, by means of oxygenation, mechanical ventilation, as well as ECMO, depending on the individual levels of oxygenation and pulmonary function, and medical equipment availability for the patients. Rather, we speculate that hypoxic conditioning may be useful as a noninvasive and nonpharmacological modality in preconditioning certain biological or social-economic vulnerable populations. This may potentially reduce their rate of SARS-CoV-2 infection and exacerbation with COVID-19, which, in turn, can prevent and/or alleviate the pathologic consequences of COVID-19.

CONCLUDING REMARKS

Despite the tremendous global human efforts and financial resources which have been devoted to controlling and treating the COVID-19 pandemic since its first outbreak in late 2019, full understanding of the cellular and molecular mechanisms of this novel coronavirus pathogen remains to be gained and the current therapeutic strategies are limited in terms of efficacy and accessibility. In this article, we attempted to look through an unexplored angle with an in-depth analysis of the role played by hypoxia, a common feature of COVID-19, as an important determinant of its pathological progression and clinical outcomes (Fig. 1). We have speculated that the activation of the HIF-1α signaling pathway under mild hypoxic conditions would decrease ACE2 and TMPRSS2 and increase ADAM17 levels on the surface of alveolocytes and, therefore, decrease the invasiveness of SARS-CoV-2 (Fig. 2). To the contrary, the protein targets of HIF-1α are involved in the severe hypoxia-induced activation of pro-inflammatory cytokine expression and the subsequent inflammation process and cytokine storm phase of COVID-19 (Fig. 3). Meanwhile, moderate doses of intermittent hypoxia could lessen the pro-inflammatory process. Therefore, we have hypothesized the potential utility of "hypoxic conditioning" to activate HIF-1α-induced cytoprotective signaling for reduction of illness severity and improvement of vital organ function in patients with COVID-19. Taken together, we would propose further investigations into the hypoxia-related molecular mechanisms, from which novel targeted therapies can be developed for the improved management of COVID-19. Finally, it will be our privilege to dedicate this review work to all the healthcare workers on the frontlines in Wuhan, Milan, Seattle, and New York among other cities for their extraordinary bravery and humanity in fighting against this COVID-19 pandemic and saving lives.

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AUTHOR CONTRIBUTIONS

All authors made substantial intellectual contributions on various topics discussed in this review and approved the final version of the manuscript. ZOS, EYC, and LX wrote the manuscript; TVS and LVT provided valuable expert suggestions to improve the work.

ADDITIONAL INFORMATION

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