Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Review

Hypoxia-inducible factor (HIF): The link between obesity and COVID-19

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ABSTRACT

The COVID-19 death toll has involved to date more than 1 million confirmed deaths. The death rate is even higher in the obese COVID-19 patients, as a result of hypoxia, due to the interplay between adipose tissue hypoxia and obstructive sleep apnea. The discrepancy of manifestations seen in COVID-19 seems to be mediated by a differential immune response rather than a differential viral load. One of the key players of the immune response is HIF. HIF-1β is a stable constitutively expressed protein in the nucleus; and under hypoxic changes, its activity is unaffected, whereas the HIF-α subunit has a short half-life and because of its degradation by an enzyme known as propyl hydroxylase; under hypoxic conditions, propyl hydroxylase gets deactivated thus leading to the stabilization of HIF-1α. As mentioned before, HIF-1α expression is triggered by hypoxic states, this crippling condition will aggravate the pro-inflammatory characteristics of HIF-1α. The vast majority of decompensated COVID19 cases manifest with drastic lung injury and severe viral pneumonia, the infection-induced hypoxia will the existing hypoxia in obesity. This will additionally augment HIF-1α levels that will provoke the already existing cytokines' storm to fulminant. Consequently, this will directly correlate the effect of a hypoxic environment with the increase of HIF-1α level. HIFα exists in two main isoforms HIF-1α and HIF-2α. HIF-1α and HIF-2α act in distinct ways in how they work on different target genes. For example, HIF-2α may act on hemopoietin genes (heme-regulating genes); while HIF-1α acts on EPO. HIF-1α release seems to be markedly augmented in obesity due to adipose tissue hypoxia and obstructive sleep apnea resulting in cyclic hypoxia. HIF-1α can also be secreted by direct viral proteolytic effects. Whereas, HIF-2α is stimulated by chronic hypoxia. HIF-1α exerts detrimental effects on the immune system, characterized by unopposed pro-inflammation at the macrophages, dendritic cells, T cells, and complement levels resulting in cytokines' storm, which is linked to the poor outcomes of COVID-19. On the other hand, HIF-2α role is regulatory and largely opposes the actions mediated by HIF-1α. In view of this, inhibiting HIF-1α release or switching its production to HIF-2α by natural products such as resveratrol or by synthetic drugs, offer a good therapeutic strategy that can prevent COVID-19 worst outcome in infected patients. The approach of breaking the vicious circle between lung damage-induced hypoxia and HIF-1α

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pro-inflammatory stimulant through drugs is considered to be extremely promising as a therapeutic manner to combat further deterioration of COVID19 cases.

Abbreviations

ALI	Acute Lung Injury
ARDS	Acute Respiratory Distress Syndrome
ATH	Adipose Tissue Hypoxia
C5a	Central Complement Protein 5a
CRP	Complement Regulatory Proteins
CXCLs	Chemokine (C-X-C motif) Ligand
DC	Dendritic cells
EBV	Epstein- Barr Virus
FIH-1	Factor inhibiting HIF-1
FOXP3	Forkhead Box P3 Protein
HBx	Hepatitis B Virus X Protein
HCV	Hepatitis C Virus
HIF	Hypoxia Induced Factor
HPV	Human Papilloma Virus
H-R	Hypoxia-Reoxygenation
HS-1793	4-(6-hydroxy-2-naphtyl)-1,3-benzenediol
IL	Interleukin
MCP-1	Monocyte Chemoattractant Protein 1
OSA	Obstructive Sleep Apnea
PHD	Prolyl Hydroxylase Domain-Containing Protein
PO2	Partial Pressure of Oxygen
ROS	Reactive Oxygen Species
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
TGF-β	Transforming Growth Factor Beta
Th	T-helper cells
TNF-α	Tumor Necrosis Factor Alpha
Treg	Regulatory T cell
VEGF	Vascular endothelial growth factor

1. Introduction

The COVID-19 pandemic is one of the largest turning points of the century, generating more than 37 million confirmed cases worldwide and counting. Nevertheless, this respiratory virus's manifestations have varied significantly between, mild and moderate symptoms (affecting 80.9% cases), to severe and potentially fatal critical cases, claiming more than 1 million deaths presently (Johns Hopkins Coronavirus Resource Center 2020).

Critical cases could even progress to extreme manifestations of Acute Respiratory Distress Syndrome (ARDS), where 10%–25% of hospitalized patients may require ventilation for several weeks, creating a significant load on hospitals internationally. They may also potentially develop dire consequences such as the deterioration to multiorgan system complications such as liver injury, cardiomyopathy, acute kidney failure, gastrointestinal, and, central and peripheral nervous system affection. (L. Li et al., 2020)

Increasing evidence suggests that this is caused by the detrimental inflammatory response of several aspects of the innate and specific immunity rather than the viral load itself. With that being said, the presence of such a spectrum of COVID manifestations can be explained by the existence of 2 main immune-types: Immunotype 1 related to severe disease, and Immunotype 2 related to the mild form. This was shown by Matthew et al., and confirmed by Liao et al., who successfully demonstrated pro-inflammatory macrophage subtypes, depleted dysfunctional resident T lymphocytes, and a cytokine storm in the lung milieu of severe COVID cases, in contrast with mild cases (Liao et al., 2020; Mathew et al., 2020).

Several aspects have been involved in the variation of this inflammatory response from a favorable to injurious one, such as; obesity, race, age and sex, and thus have been a target, with strategies currently focusing on identifying these aspects and targeting them to prevent the worst disease outcomes in COVID patients (AbdelMassih et al., 2020; Kopel et al., 2020).

Meanwhile, Hypoxia Induced Factor (HIF), a common byproduct of the continuous or intermittent hypoxia stimulated in inflammatory tissues and has several isoforms have been implicated in similar immune-regulatory effects (Kumar and Choi 2015).

Accordingly, this study not only highlights the potential role of HIFs, particularly the antithetical actions of the favorable HIF-2α and the injurious HIF-1α but also strives to demonstrate the conditions by which they are secreted. Furthermore, this study outlines the potential applications of naturally occurring and experimental drugs targeting HIFs in promoting hopeful COVID manifestations.

2. HIF mode of action and different targets of HIF isoforms

2.1. Mechanism of secretion of HIF

It is well known that HIF is a hetero-dimeric transcription factor consisting of an oxygen-regulated α-subunit and a constitutively expressed ß-subunit. HIF-1β is expressed in the nucleus and, under hypoxic changes, its activity is unaffected, whereas the HIF-1α subunit has a short half-life (5 min) and is influenced by oxygen tension. For instance, intracellular HIF-1α is unstable under normoxic conditions, being rapidly degraded by prolyl hydroxylase enzymes. Moreover, these enzymes are inactivated under low oxygen levels, demonstrating why HIF-1α is indeed induced by hypoxia. However, some small molecules such as dimethyloxalylglycine (DMOG) can stabilize HIF levels under normoxic conditions by inhibiting prolyl hydroxylase. (Chu and Jones, 2016; Al Okail, 2010).

It was previously thought that HIF alongside other transcription factors is exclusive to the cell nucleus, and cannot play a role in cell-to-cell paracrine communication. According to recent data, the possibility of the transfer of nuclear transcription factors to affect remote cells and tissues through extracellular microvesicles has become evident (Zonneveld et al. 2019).

2.2. How different HIF isoforms can act differently?

HIF-1α and HIF-2α subunits function together in activating HRE-dependent gene transcription. However, they were each separately identified. The affinity purification detected HIF-1α as the original HIF isoform from EPO locus using oligonucleotides, while HIF-2α including HIF-3α by homology searches or screens for interaction partners with HIF-1β. On the other hand, definitive studies in mice revealed that HIF-1α and HIF-2α work in two separate lines. Therefore, the inactivation of each subunit results in a distinctly different phenotype (Koh and Powis 2012).

This may result from two different poorly understood mechanisms:

Firstly, the differences in tissue-specific induction of each isoform: As concluded by Sowter et al., in the breast carcinoma and endothelial cell lines, the major HIF required for induction of a set of well-

characterized hypoxic genes is HIF-1α (Sowter et al., 2001). However, for hypoxia-induced cell migration, both subunits are necessary, which leads us to a similar conclusion as Blancher et al. who stated that while HIF 1 alpha is responsible for the regulation of a broad spectrum of hypoxia-inducible genes, HIF 2 alpha inhibits the growth of breast cells (Blancher et al., 2000). This shows a strong relationship between the pattern of expression of both subunits wherein six human breast cancer cell lines, HIF 1 alpha was expressed at various levels, whereas, HIF 2 alpha was low or not even present in more aggressive cell lines. On the other hand, HIF 2 alpha overexpression is especially important in the development of renal carcinoma in patients with the von Hippel Lindau syndrome. In this setting, HIF 2 alpha may act as a renal cancer oncogene. Moreover, HIF 2 alpha is highly expressed in embryonic vascular endothelial cells and activates the expression of target genes whose products modulate vascular function and angiogenesis (Blancher et al., 2001). Recently, Skuli et al. introduced a model of mice with HIF-2α-deficient endothelial cells and found that although they developed normally, such animals are characterized by increased vessel permeability, aberrant endothelial cell ultrastructure, and pulmonary hypertension. Moreover, these animals exhibited defective tumor angiogenesis associated with increased hypoxic stress and tumor cell apoptosis (Skuli and Simon 2009).

Other studies proved that HIF-1α and HIF-2 α have different transcriptional targets; despite sharing common targets such as VEGF. HIF-1 α regulates the transcription of genes that convert enzymes responsible for glycolysis. In the liver, erythropoietin release is maintained by HIF-2α. However, the glycolytic enzyme, phosphoglycerate kinase (Pgk), and the proapoptotic gene, BCL2/adenovirus E1B–interacting protein 1 (NIP3/Bnip3), are identified as better HIF-1α targets (Rankin et al., 2009). Moreover, HIF-2 α regulates transcription of cyclin D1 and transforming growth factor. The difference between the two subunits has been responsible for iron homeostasis. A study using knockout mice that don't have HIF-1α and HIF-2α in the intestinal epithelium has proven that only HIF-2α maintains the transcription of the gene responsible for divalent metal transporter 1, which is the main intestinal iron transporter (Mastrogiannaki et al. 2013).

3. Different types of hypoxia and the differential release of HIF isoforms

3.1. Acute vs. chronic hypoxia

The occurrence of hypoxia at sites of inflammation is a potent microenvironmental feature of innate immunity. Thus, hypoxia exists in severe pneumonia and respiratory distress following SARS-CoV-2 infection. There is growing evidence that hypoxia acts as an inflammatory stimulant through HIF. Three HIF-α proteins have been recognized; HIF-1α, HIF-2α, and, more recently, HIF-3α. Despite sharing 48% of their amino acid sequence and having a similar protein structure, HIF-1α and HIF-2α have dissimilar target genes and are expressed differently. Cells acclimate to hypoxia by tailoring a cascade of responses mediated by HIF-1α and HIF-2α, depending upon the span and pattern of hypoxia. In their review, Saxena and Jolly distinguishably analyzed the pattern of hypoxia responsible for the switch between the two main isoforms of HIF: acute hypoxia mainly stimulates HIF-1A release, while chronic hypoxia stimulates HIF-2α release. Moreover, the review added additional insights on the third type of hypoxia, an intermittent one. Brown et al. were the first to identify the presence of this type of hypoxia in mice tumors, which occurred because of the intermittent closure in the tumor blood vessels. This inefficient blood perfusion occurs due to increased oxygen demands in the growing tumor that leads to angiogenesis, forming structurally, and functionally abnormal blood vessels (Saxena and Jolly 2019).

3.2. Obesity dual relationship with HIF-1α

3.2.1. Cyclic hypoxia of obstructive sleep apnea (OSA)

3.2.1.1. The relationship between obesity & OSA. Obesity is considered a significant risk factor for the development and progression of Obstructive Sleep Apnea (OSA). In obese or highly obese patients, the incidence of OSA is almost twice that of an average weight adult. Furthermore, in patients with mild OSA who gain 10 percent of their baseline weight, the risk of progression of OSA is six-fold-increased, and an equivalent weight loss will result in a more than 20 percent improvement in OSA severity. In addition, the higher prevalence of OSA in obese subjects is not confined to adults; recent statistics indicate that, relative to children seen in a general pediatric clinic, obese children have a 46% prevalence of OSA (33%) (Schwartz et al., 2008).

This increased prevalence may be attributed to the deposition of fat at specific sites. Fat deposition in the tissues surrounding the upper airway, for instance, tends to result in a smaller lumen and increased upper airway collapsibility, predisposing to apnea. In addition, fat deposits around the thorax (truncal obesity) decrease chest compliance and residual functional capacity, and can increase the demand for oxygen (3%) (Romero-Corral et al., 2010).

Visceral obesity is also prevalent in patients with OSA. However, the relationship between OSA and obesity is complex. In other words, in spite of compelling evidence showing that obesity, as well as visceral obesity, can predispose to OSA, and that losing weight contributes to OSA improvement, recent studies suggest that OSA can cause weight gain itself. This finding may be attributed to OSA causing reduced activity levels and increased appetite, particularly for refined carbohydrates. Whether OSA predisposes to preferential accumulation of visceral fat is still unclear. However. as assessed by abdominal CT scanning, CPAP treatment of OSA has been shown to decrease the amount of visceral fat even in patients without significant loss of weight (Kritikou et al., 2013).

Finally, some studies show that there is a substantial overlap in genetic substrates between OSA and obesity. For instance, Popko et al. showed that leptin receptor polymorphisms involved in the regulation of body weight and energy homeostasis are significantly associated with both OSA and obesity compared to healthy controls. In other words, these studies show that genetic polymorphism may be substantially correlated with the development of these conditions (Popko et al., 2007).

3.2.1.2. OSA as a drive for cyclic hypoxia and release of HIF-1α. OSA is characterized by episodic upper airway obstruction causing systemic intermittent hypoxia, which, in turn, results in a cyclical nocturnal hypoxia/reoxygenation pattern. The most severely affected patients may show blood oxyhemoglobin saturation as low as 50% of normal and have $\leq$60 episodes/hour. Although this can vary depending on the tissue type, partial pressure of oxygen (Po2) at tissue level generally reflects arterial blood oxygen desaturation (Martinez et al., 2019; Saxena and Jolly 2019). Recent studies show that cyclic hypoxia-driven pathways, unlike chronic hypoxia, tend to **increase HIF-1α and to reduce HIF-2α.**

3.2.2. Adipose tissue hypoxia

3.2.2.1. How obesity induces ATH. Ischemia/reperfusion induces adipose tissue hypoxia (ATH). It is strongly evident in obese mice under the influence of reduced PO2. Studies on human tissue vascularization and O2 tension support this finding. The increased ATH can be explained by the fact that, as obesity develops: 1- Tissue mass expands 2- Clusters of adipocytes become hypertrophic and distant from the vasculature. The hypertrophic adipocytes overgrow in diameter beyond the limit of oxygen perfusion. White adipose tissue depots in turn become hypoxic. ATH is then confirmed by the use of an endogenous marker (elevated

lactate) and an exogenous marker, detected with a chemical hypoxia probe, (pimonidazole hydrochloride). (Trayhurn 2013).

3.2.2.2. Adipose tissue hypoxia as the driving force of low-grade inflammation in obesity. After ATH, two key transcription factors, HIF-1α and NF-κB are upregulated. HIF-1α is the master regulator of O2 homeostasis. It accumulates during hypoxia and rewires cellular energy metabolism in adipose tissue macrophages. This reprogramming regulates the macrophages' number, phenotype, and function. As such, macrophages are more prevalent in the adipose tissue of obese people than in that of leaner ones. Macrophage infiltration and activation in adipose tissue is involved with inflammation, and subsequently with measures of insulin resistance. ATH increases the number of ATM and induces their inflammatory phenotypes via HIF-1α dependent mechanisms. Activation of NF-κB enables the adipose tissue macrophages to survive in an obesogenic environment. ATH thus creates a state of low-grade inflammation by stimulating ATM and changing their function to secrete pro-inflammatory cytokines; such as tumor necrosis factor (TNF)-α, adiponectin, leptin, (MCP-1), and retinol-binding protein-4.

Despite inadequate PO2 supply, adipocytes' leptin production increases, and adiponectin production decreases. These changes potentiate the vicious cycle of low-grade inflammation and subsequent insulin resistance (Engin 2017; Ye 2009).

3.2.3. Systemic hypoxia of COVID-19 meets cyclic hypoxia and ATH of obesity

Acute systemic hypoxia is a primary pathophysiologic feature and the main cause of mortality in patients with severe COVID-19. It accompanies all stages of the disease. The pathogenic determinants of hypoxia act at all levels (systems, organs, and cells), and the hypoxia-triggered factors may act synergistically. As stated above, ATH upregulates HIF-1α isoform, by mediating ATH, or through OSA. In obese patients with COVID-19 the interplay between systemic hypoxia (due to lung injury), ATH, and cyclic hypoxia lead to a canonical activation of HIF-1α. The subsequent effect of HIF-1α (explained in section 3) might explain why obesity increases the risk of cytokine storm in COVID-19 infected patients (Jahani, Dokaneheifard, and Mansouri 2020a).

3.3. Viral infection as an independent stimulus for HIF-1α

Different types of viruses have been shown to implement various molecular mechanisms to increase and/or stabilize HIF-1α resulting in the survival of the infected cells.

It has been shown that prolonged expression of Hepatitis C Virus (HCV) protein depresses mitochondrial oxidative phosphorylation. Despite this, the infected cells survived. This was attributed to HIF-1 stabilization by HCV proteins, which in turn causes upregulation of the HIF-1α-controlled genes, including those coding for glycolytic enzymes, compensating for the energy shortage caused by mitochondrial damage. Several studies have argued that viral infections could enhance the activity of HIF-1α through the diversity of mechanisms, these include increasing its expression, stabilization, and/or decreasing its degradation (Ripoli et al., 2010).

Hepatitis B virus was shown to boost the activity of HIF-1α via Hepatitis B-encoded X protein (Yoo et al., 2003).

Some Epstein- Barr virus (EBV) associated proteins were proven to increase the breakdown of prolyl HIF-hydroxylases-1and 3 (PHD1 and PHD3), which catalyzes the degradation of HIF-1, thus, increasing its availability (Kondo et al., 2006).

Moreover, Influenza viruses cause stabilization of HIF-1α via hindering proteasomal functions. Also, they cause downregulation in the expression of factor inhibiting HIF-1α (FIH-1). (Guo et al., 2017).

Another study has demonstrated that the presence of HPV oncogenes causes an elevation of HIF1a level under hypoxic conditions. It has also been shown that HPV-16 E6 and E7 oncoproteins can cause the progression of non-small cell lung cancer probably via HIF-1alpha/VEGF mediated angiogenesis (G. Li et al., 2011).

To date, there is no study investigating the cellular or proteomic effect of coronaviridae on HIF-1α; however, given the aggregated data from RNA viruses, it could be hypothesized that coronaviridae might increase HIF-1α, independent of the hypoxia-induced by lung injury.

4. Differential effects of HIF isoforms on immune system activation

Next Generation Sequencing (NGS) showed the presence of multiple different immune responses against COVID-19 infection. These variations link to the severity of COVID manifestations. As such, it is important to develop strategies aimed at switching to a more favorable immune response, with a better disease outcome. The main variations, characterized by Matthew et al., are Immunotype 1 (associated with severe outcomes of the disease) and Immunotype 2 (associated with milder outcomes).

Our working group observed an inflammatory state similar to immunotype 1 present in severe COVID cases (AbdelMassih et al., 2020). Liao et al. further corroborated the characterization above by demonstrating the presence of pro-inflammatory macrophage subtypes, dysfunctional dendritic cells, cytokine storms, and the predominance of Th17 cells in the lungs of severe COVID cases; as opposed to mild cases (Liao et al., 2020; Mathew et al., 2020).

This section displays that HIF 1 induces changes similar to those seen in severe COVID-19 cases, while HIF-2 induces changes seen in milder forms.

4.1. Macrophage phenotypic switching

Macrophages can change their phenotype in response to different stimuli; a process called Polarization.

The two main phenotypes are

1 ***Inflammatory macrophages (M1):*** these are characterized by the expression of high levels of pro-inflammatory cytokines, the promotion of Th1 response, and the production of high levels of reactive nitrogen and oxygen species. In addition, they have strong microbicidal and tumoricidal actions.
2 ***Wound-healing macrophages (M2):*** these are involved in tissue remodeling, parasite clearance, and inflammatory resolution. However, they also facilitate tumor development and suppress effector T cells.

NGS shows that the lungs of COVID19 patients are predominated by M1 macrophages. Takeda and colleagues simulated a model of both HIF-1α and HIF-2α to determine the mRNA expression in different macrophage phenotypes. They demonstrated that M2-polarized macrophages express HIF-2 exclusively, whereas M1 macrophages express HIF-1α abundantly. This could lead to the conclusion that HIF-1α expression is predominant in the lung milieu of severe COVID cases, causing an uncontrolled destructive inflammation of the lung tissue (Choe et al., 2014).

4.2. T-regulatory cells vs. Th17 cells

Serious COVID-19 patients have had a critical diminishing in Treg cell levels and expanded degrees of Th17 cells, with a resulting decline in the Treg/Th17 cell proportion. The Treg/Th17 balance plays a significant role in:

1. The severity of lung injury.
2. The uncontrolled systemic inflammation is characteristic of acute lung injury.

Treg and Th17 cells are parts of the complex immune system. The differentiation of Th17 and Treg from naïve CD4+T cells requires TGF-β. Cytokines (IL6/IL22) and TGF-β induce naïve $CD4^+$ T cells to differentiate into Th17 cells. Treg and Th17 cells have 2 completely different functions:

1 ***Th17 cells*** are mainly characterized by the production of inflammatory cytokines such as IL-17, hence the name.

IL-17 activates target cells and induces chemokine (C–X–C motif) ligands (CXCLs).

44 CXCLs attract myeloid cells (ex: neutrophils) to the infected tissues.

2 ***Treg cells*** produce anti-inflammatory cytokines (IL-4, IL-10, and TGF-β) and regulate immune responses. They are classified into:

HIF-1α binds to Foxp3 and promotes its degradation, this results in the inhibition of Treg differentiation that leads to the loss of Treg's suppressive function. **Whereas,** according to an in-vitro experiment, they found a contradictory and unexpected role for HIF-2α in Treg cells by which the Treg cells were normal with unchanged suppressive function. On the other hand, Dang and colleagues found that TH17 differentiation is enhanced by hypoxia under the effect of HIF-1α-dependent manner.

Therefore, it is likely that HIF-1α activity represents a major mechanism by which the hypoxic conditions associated with inflammation can promote TH17 differentiation, while HIF-2α induces CD-4 cell polarization towards the Treg phenotype and diminishes CD-4 cell polarization towards the TH17 cells (Hsu et al., 2020; Tao et al. 2015).

4.3. Dendritic cells

Dendritic cells (DC) are bone marrow-derived leucocytes and are the most potent type of antigen-presenting cells. They initiate the adaptive immune responses of the immune system and hence they are known as the watchmen of the immune system. The tree-like cytoplasmic processes of DC allows it to act as an antigen-presenting cell and consequently linking the innate and adaptive immune systems. In COVID-19 patients, the delayed T cell immune response has been attributed to the rapid loss of the DC function, which was claimed to be resulting from steroid administration. Zhou et al., however, proved that assumption wrong. It is becoming widely accepted that inflammatory hypoxia is crucial for normal DC function with HIF-1α and HIF-2α as the key transcription factors regulating DC adaptation. In vivo studies by Hamammi et al. and Fluck et al. concluded, that DC lacking HIF-1α were the most potent inducers of inflammatory response. On the other hand, prolonged DC hypoxia has shown higher expression of CD40. Hypoxia has also caused higher T cell stimulation of DCs. Inhibition of the HIF pathway could block these changes (Hammami et al., 2018).

4.4. Complement system

The complement system is a network of 30 proteins, included in both the innate and the acquired immune system, where they have opsonization properties as well as promoting the function of antibodies and phagocytic cells. The anaphylatoxin C5a, a central complement protein, is considered a culprit in Acute Lung Injury (ALI) mediated by CC-chemokine receptor 5. This is thought to occur via an exaggerated inflammatory response in the form of cytokine release. This increases vascular permeability and activates phagocytic cells with the subsequent release of histones and Reactive Oxygen Species (ROS), leading to endothelial injury and thrombosis, followed by a drop of the innate immune system and multiorgan failure. Marchetti argued that the complement system plays a relevant role in the impact of HIF-1α on the COVID-19 pathogenesis and ARDS (Marchetti, 2020).

In a study about mouse orthotropic tracheal transplantation, Khan et al. found that hypoxia-induced activation of HIF-1α favors a cell-mediated immune response ($CD4^+$, $CD8^+$, and proinflammatory cytokines, IL-2 and TNF-α). This proportionally brings about the down-regulation of Complement Regulatory Proteins (CRP), especially CD55, thus, augments the uncontrolled release of active-C3, and Caspase-3 deposition on graft endothelial cells. These molecular changes were associated with pathological microvascular and airway epithelial adjustments and injuries (Khan et al. 2015).

Fig. 1 summarizes the stimuli releasing each isoform of HIF F and their differential effects on the immune system.

4.5. HIF and cytokine storm

Accumulating evidence indicates that increased levels of inflammatory mediators including cytokines and chemokines such as interleukin (IL-2, IL-7, IL-10, tumor necrosis factor (TNF), granulocyte colony-stimulating factor (G-CSF), monocyte chemoattractant protein-11 are associated with severity of COVID-19. Of note, Fajgenbaum and colleagues highlighted that the amount of blood IL – 6 is strongly associated with disease mortality among the elevated inflammatory mediators when COVID-19 survivors and non-survivors are compared. This indicates that fatal COVID19 is characterized by a cytokine release syndrome (CRS) caused by a high mortality cytokine storm (Fajgenbaum and June 2020).

It has been shown that hypoxia and HIF-1α roles in regulating cytokine expression is so controversial and depend upon various conditions. Hypoxia and HIF-1α can affect the cytokine-mediated inflammatory response by either stimulating or inhibiting it. For instance, the stabilization of hypoxia and HIF-1α may cause the enhancement or trigger the cytokine storm, as the vascular endothelial growth factor (VEGF) is transcriptionally upregulated by HIF-1α and accumulates under hypoxia. Endothelial cells are essential contributors to severe COVID-19 initiation and propagation. Circulating inflammatory cells migration into tissues is decided by a number of adhesive molecules and chemo-attractants produced by endothelial cells and increased vascular permeability. VEGF and VEGF receptors are participants in this process. When VEGF-A binds to VEGFR -2, an elevation in the expression of cytokine and pulmonary vascular permeability was noticed in cystic fibrosis patients undergoing lung transplantation and in experimental animals (Krenn et al., 2007).

HIFs cause a series of cell activation including mast cells, macrophages, dendritic cells, and neutrophils, causing the release of reactive oxygen species, the release of proteases, and neutrophil extracellular traps (NETs) formation. The accumulation of activated neutrophils in the microcirculatory bed of the lungs, alveoli, and interstitium, is not just one of ARDS characteristics, it's also considered to be one of the causative factors of lung tissue damage due to a local factor. Neutrophil elimination through apoptosis and macrophage clearance is thought to be a principal feature in the inflammatory process. ARDS affects neutrophils by turnover impairment. On the other hand, hypoxia decreases neutrophil apoptosis through HIF-1α and nuclear factor κB and MIP1α. According to Cramer and colleagues, HIF-1α is crucial for the glycolytic capacity regulation in myeloid cells: when HIF-1α is absent, the cellular ATP pool is dramatically decreased. This role for HIF-1α manifests its direct regulation of survival and function in the inflammatory microenvironment. That's why we can conclude that HIF-1α activation is not only included in the genesis of cytokine storm but also in the neutrophil apoptosis disruption in ARDS (Thorsten Cramer, Yuji Yamanishi, Björn E. Clausen, Irmgard Förster, Nigel Mackman, Volker H. Haase, Rudolf Jaenisch, Maripat Corr, Victor Nizet, and Firestein, Hans-Peter Gerber, Napoleone Ferrara 2015).

5. Development of therapeutic targets for HIF switch

In view of the above, HIF-1α and HIF-2α have antagonistic effects on

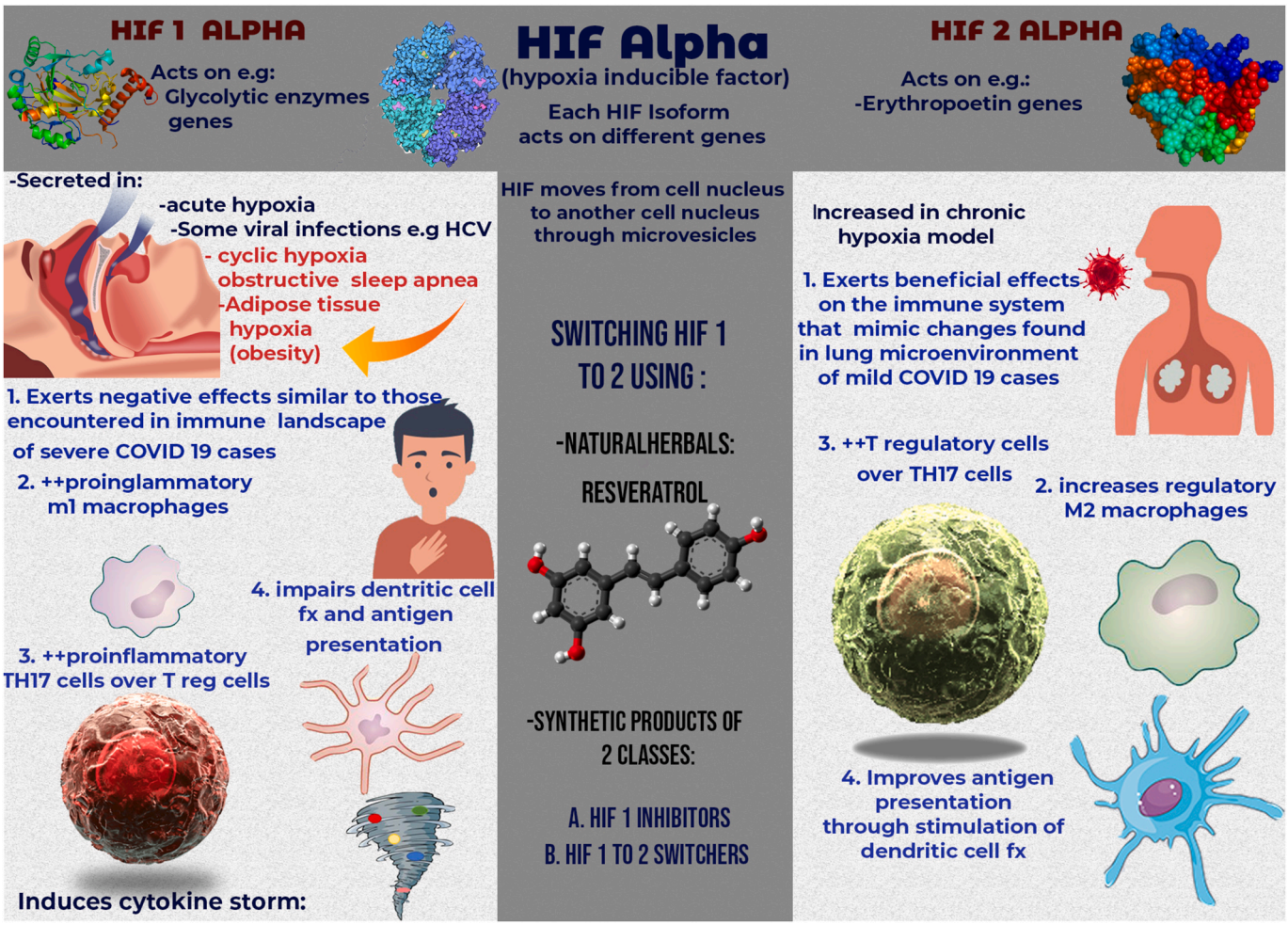

Fig. 1. HIF-1α and HIF-2α releasing stimuli and differential effects on the immune system. Abbreviations Fx: Function, HIF: Hypoxia induced factor, TH: T helper cells, T Reg: T regulatory cells.

the immune response with HIF-1α favoring an immune response resembling the immunotype 1 observed in COVID-19 complications. On the other hand, HIF-2α distorts the immune response and drives it closer to the immunotype 2 commonly seen in mild cases. HIF class switching can therefore be tested as a potential therapeutic strategy for COVID-19.

Recent studies have revealed the existence of some natural agents capable of switching HIF-1α to HIF-2α, as well as several more experimental synthetic drugs of similar actions as well as specific HIF-1α inhibitors:

5.1. Resveratrol: a naturally available HIF-1α inhibitor

Resveratrol is a stilbenoid (a type of natural phenol) produced by several plants in response to injury. While a fair amount of resveratrol is readily found in food such as; grapes, blueberries, raspberries, mulberries, peanuts, and dark chocolate, 4-(6-hydroxy-2-naphtyl)-1,3-benzenediol (HS-1793), a synthetic analog of resveratrol with improved photosensitivity and stability profiles, has been recently reported to exhibit anticancer activity on various cancer cells. This was achieved through the inhibition of hypoxia-induced HIF-1α expression at the protein level. Furthermore, HS-1793 also reduces the secretion and mRNA expression of vascular endothelial growth factor (VEGF), a key mediator of HIF-1-driven angiogenesis, without affecting cell viability (Zhang et al., 2014).

5.2. Cancer model and the development of synthetic HIF-1α inhibitors

A number of HIF inhibitors have been synthesized or discovered for treating cancer, particularly for advanced and refractory solid tumors, on the basis that they inhibit the expression and/or functions of HIF-1α, through direct and indirect mechanisms. Based on the recognized mechanisms of action, these HIF-1α inhibitors can operate through six different mechanisms:

1) Modulating RNA expression;
2) Interfering with protein synthesis;
3) Inducing protein degradation;
4) Encouraging dimerization and subsequent loss of action;
5) Inhibiting the transcriptional activity;
6) Switching HIF-1α expression to HIF-2α.

The names and uses of the medications acting at each level have been discussed in Table 1 (Jahani, Dokaneheifard, and Mansouri 2020b). Despite the encouraging results of such drugs, there are no ongoing trials about their repurposing for the treatment of COVID-19 treatment.

6. Conclusion

Hypoxia is a direct consequence of the lung injury seen in COVID-19. On a molecular level, it acts by stabilizing HIF-1α by inactivating its degrading enzymes. Furthermore, the obesity-coronaviridae complex seems to upregulate the release of HIF-1α, which acts to increase

Table 1
Potential HIF-1α inhibitors and switchers to HIF-2α.

Main Action	Drug Name	Additional actions	Pharmaceutical use
HIF-1 Inhibitory drugs			
Modulate expression	EZN-2208		Anti-cancer agent
	EZN-2968		
	PX-478	Inhibits HIF-1α mRNA translation	Anti-cancer agent
	SIRT1		
Protein synthesis	PX-478	Inhibits HIF-1 α mRNA expression	Anti-cancer agent
	2-methoxyestradiol	Inhibits nuclear translocation and transcriptional activity	Experimental
	KC7F2		Experimental
	Glyceollins		Experimental
	CAY10585	Inhibits transcriptional activity	Experimental
	Topotecan		Anti-cancer agent
Protein accumulation and degradation	PX-12	Inhibits transcriptional activity	Experimental
	YC-1	Inhibits transcriptional activity	Anti-cancer agent
	NSC 607097	Inhibits HIF-1α transcriptional activity	Experimental
	PHD1-3		Experimental
	pVHL		Experimental
	RACK1		Experimental
	HAF		Experimental
	Hsp70/CHIP		Experimental
	17-AAG		Anti-inflammatory and anti-cancer agent
	17-DMAG		Anti-inflammatory and anti-cancer agent
	Bisphenol A		Experimental
	BAY 87-2243		Experimental
	Cryptotanshinone		Experimental
	Vorinostat		Experimental
	LW6		Anti-cancer agent
Dimerization	TAT-cyclo-CLLFVY		Experimental
	Acriflavine	Inhibits transcriptional activity	Antiseptic and anti-bacterial agent
DNA binding	Echinomycin		Antiseptic and anti-bacterial agent
Transcriptional activity of HIF	PX-12	Inhibits HIF-1α protein accumulation	Experimental
	Acriflavine	Inhibits HIF dimerization	Antiseptic and anti-bacterial agent
	Indenopyrazole 21		Experimental
	Fm19G11		Experimental
	YC-1	Inhibits HIF-1α protein accumulation	Anti-cancer agent
	NSC 607097	Inhibits protein accumulation	Experimental
	2-Methoxyestradiol	Inhibits HIF-1α protein synthesis	Experimental
	CAY10585	Inhibits HIF-1α protein synthesis	Experimental
HIF 1 α to 2 α Switchers			
	SIRT1	Promotes HIF-2 α and inhibits HIF-1a	Experimental
	HAF		Experimental
	Ets-1		Experimental

Abbreviations: HIF= Hypoxia-inducible factors, HAF= Human Chorionic Gonadotropin Associate Factor, Hsp70/CHIP= Heat shock protein 70/Carboxy terminus of Hsp70 binding protein, PX-12 = 1-methylpropyl 2-imidazolyl disulfide, PHD1-3 = Prolyl hydroxylase, PVHL= Von Hippel Lindau Protein, RACK1 = Receptor of activated protein C Kinase 1, SIRT1 = Sirtuin 1, YC-1 = Guanylyl cyclase activator, 17-AAG = Allylaminogeldanamycin, 17-DMAG = 17Dimethylaminoethylamino-17-demethoxygeldanamycin.

unopposed inflammation at various levels of the immune system, potentially resulting in the cytokine storm responsible for the devastating mortality seen by COVID-19. Despite being a transcription factor, its action is not limited to the nucleus alone, where it also acts in a paracrine manner through extracellular vesicles to stimulate pro-inflammatory mechanisms in nearby cells. In contrast, HIF-2α opposes the pro-inflammatory detrimental effects of HIF-1α. The opposing actions of both isoforms are attributed mainly to their actions on different target genes. In view of the above, we urge working groups all over the world to initiate clinical trials involving HIF-1α inhibitors or HIF-1α switchers to HIF-2α to guard against the generation of cytokine storms and by default against COVID-19's systemic and pulmonary complications.

Declaration of competing interest

Authors declare that there is no conflict of interest.

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