Indian Journal of Biochemistry & Biophysics
Vol. 57, December 2020, pp. 670-675

A Review

# HIF-1α contributing to COVID-19 infections and poor prognosis in cancer patients – A hypothesis

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*Received 19 June 2020*; *revised 06 August 2020*

In 2019, a new coronavirus (SARS-CoV-2) infecting Humans first identified in Wuhan, China, has caused the worst pandemic of the 21st century. This virus infection leads to the clinical symptoms that may range from asymptomatic condition to life-threatening illness. The insights from the recent studies suggest that SARS-CoV-2 requires a host enzyme, Furin to activate receptor-binding domain (RBD) of its S protein. Upon binding of RBD to host cell membrane-bound Angiotensin Convertase Enzyme 2 (ACE2), it facilitates the entry of virus in the host cell. Evidence from the literature also suggests that HIF-1α (Hypoxia-inducible factor 1-α) is one of the factors regulating the expression of Furin. In addition, it is also well documented that the interior of solid tumours, which grow very fast, leads to the hypoxic tumour microenvironment, resulting in overexpression and release of HIF-1α. The SARS-CoV-2 infected patients with severe tissue damage and inflammatory injury also suffer from tissue hypoxia. So, we hypothesize that hypoxic condition due to tumour microenvironment in cancer patients upregulates the HIF-1α, leading to increased expression of Furin. Upon infection of cancer patients with SARS-CoV-2 having increased Furin expression in the cells due to upregulation of HIF-1α, leads to the entry of a greater number of SARS-CoV-2 virus in these cells resulting in severe infection. The vicious cycle of the virus infection in which virus is more easily invaded into surrounding tissue leads to the involvement of multiple organs and ultimately poor prognosis in the disease outcome. Therefore, we suggest evaluating the expression of HIF-1α in SARS-CoV-2 infections at an early phase of infection particularly in patients with comorbidities like solid malignancies as well as patients having signs and symptoms of hypoxia. It is also suggested that continuous monitoring of the $SpO_2$ level and early institution of preventive $O_2$ therapy at an early stage in these patients may lead to lesser morbidity as well as mortality in COVID-19 patients.

**Keywords**: Furin, Hypoxia-Inducible factor-1α, Metallocarboxy-peptidase, Tumour Hypoxia

## Introduction

### COVID-19

In December 2019, a series of patients with symptoms resembling viral pneumonia presented in Wuhan, Hubei province, China emerged[1]. Later the disease has spread to more than 200 countries throughout the world and infected more than eighteen million people leading to more than 7,00,000 deaths. The whole-genome sequencing and the phylogenetic evaluation revealed that the newly emergent virus is more closely related to SARS coronavirus[2]. So, the novel coronavirus was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the resulting disease termed as COVID-19.

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*Abbreviations*: ACE2, Angiotensin-converting enzyme 2; ARDS, Acute respiratory distress syndrome; COVID, Coronavirus disease; HIF-1α, Hypoxia-inducible factor 1-α; MERS-CoV, Middle East respiratory syndrome coronavirus; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2

SARS-CoV-2 disease symptoms resemble SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV). COVID-19 primarily spread through the saliva or the discharge from the nose of the patient[3].

Coronaviruses belong to the Coronaviridae family. It is enveloped, single-stranded, and possess positive-sense RNA. The total length of the genome is 30 Kb, consisting of a 5′-terminal noncoding region, an open reading box (ORF) 1a/b-coding region, an *s* region encoding the spike glycoprotein (S protein), an *e* region encoding the envelope protein (E protein), an *m* region encoding the membrane protein (M protein), an *n* region encoding the nucleocapsid protein (N protein), and a -3′-terminal noncoding region[4].

### Role of Furin in SARS-CoV-2 Infection

Like all other coronaviruses, SARS-CoV-2 also needs its S protein cleaved to facilitate its entry to host cells. Unlike SARS-CoV, the S1/S2 site in SARS-CoV-2 contains multiple arginine residues (multi-basic)[5,6]. The presence of polybasic residues at the S1/S2 site requires a different cleavage strategy for SARS-CoV-2

for efficient proteolytic processing in human tissues and also confers better cleavability. While a monobasic site is sufficient for SARS-CoV to be cleaved by TMPRSS2 (Transmembrane Serine Protease 2), a multi-basic S1/S2 site cleavage is essential for efficient SARS-CoV-2 entry into human lung cells[7].

The cleavage of S protein at the S1/S2 cleavage site in SARS-CoV-2 is catalysed by a host proprotein convertase Furin (Fig. 1), a calcium-dependent membrane-bound protease which is expressed ubiquitously as a 794 amino acid zymogen that undergoes autocatalytic cleavage to become fully active. S1 is essential for the attachment to receptor *i. e.* angiotensin-converting enzyme 2 (ACE2) receptors present on the cells, while S2 is required for the fusion[5,8,9].

ACE2, a type I transmembrane metallocarboxy–peptidase mainly found attached to the outer surface of the lungs, arteries, heart, kidney, and intestines, acts as a functional receptor for SARS-CoV-2 S-mediated entry into cells (Fig. 1). Increase affinity between S1 and hACE2 (human angiotensin-converting enzyme 2) may be a key for the efficient transmission of SARS-CoV-2 in humans[5].

## Regulation of Furin by HIF-1α in Cancers

HIF-1α (Hypoxia-inducible factor 1-α), is a subunit of a heterodimeric transcription factor hypoxia-inducible factor 1- α, encoded by the HIF1A gene. Significant overexpression of HIF-1α has been reported in most solid tumours studied, which include cancers of the colon, ovary, breast, prostate, pancreas, kidneys, brain, bladder, *etc.* (Fig. 1)[10]. Increased HIF-1α levels have been associated with aggressive tumour progression and reported as a predictive and prognostic marker for therapy resistance to radiotherapy, chemotherapy. Increased mortality in several solid cancers, including cervical cancer, non-small-cell lung carcinoma, breast cancer, ovarian cancer, endometrial cancer, head, and neck cancer[11].

In most of the solid tumours, the rate of growth of tumour outpaces the neovascularization. As a result, there is a relatively hypoxic microenvironment inside the tumour. HIF-1α is induced due to this low oxygen concentration in the tumour microenvironment. It is reported that HIF-1α positively correlated with the expression of Furin in the case of solid tumours *i. e.* the expression of Furin is upregulated upon HIF-1α stimulation[12]. Hence the cancer patients are already

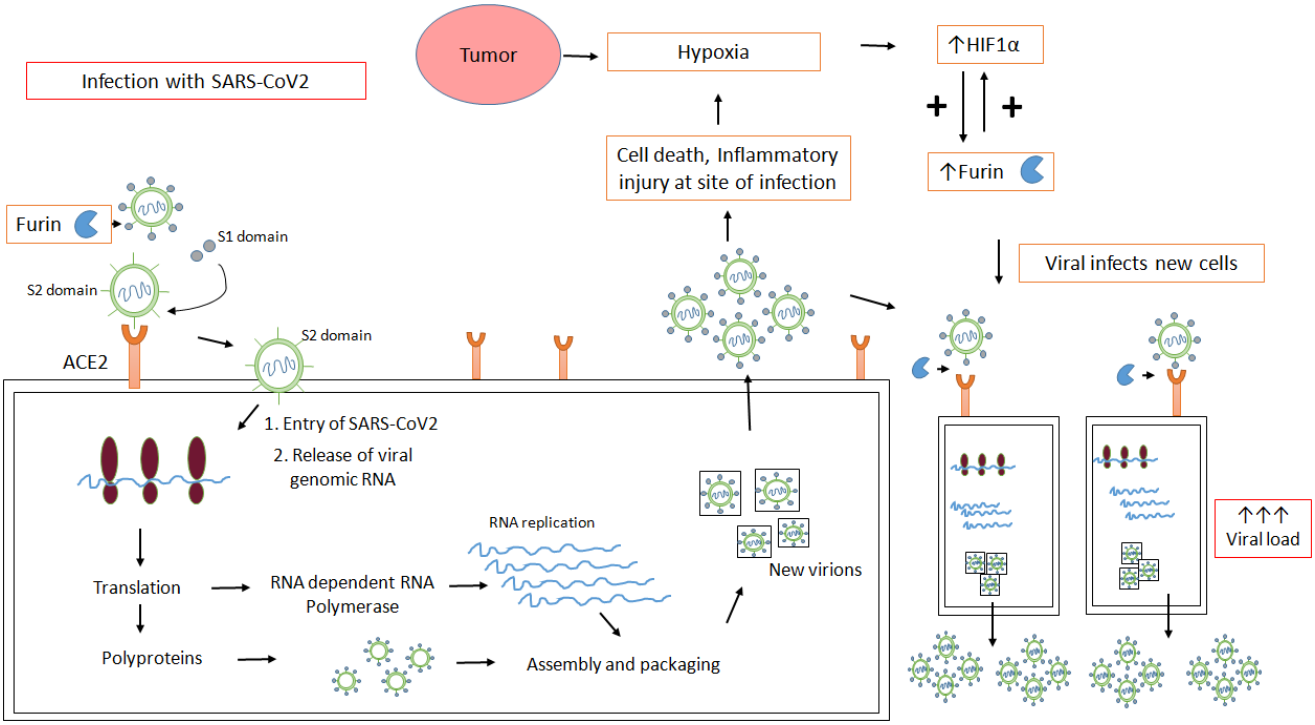


Fig. 1 — Tumour hypoxia induces increased viral load through HIF-1α-Furin mediated pathway. Intratumor hypoxia, a common phenomenon in solid tumours, leads to stabilization of HIF-1α, which in turn induce the upregulation of furin. Furin catalyses the formation of S1 and S2 fragments in S protein. S2 remains attached to SARS-CoV-2, acts as Receptor Binding Domain (RBD) for ACE2, constitutively expressed on epithelial cells of the upper aerodigestive tract, facilitating the increasing entry of the virus into the host cell

expected to have upregulated expression of Furin. Also due to increased Furin expression, it was hypothesised that oral cancer patients have a higher risk of SARS CoV-2 infection as well as the increased severity of the disease (Fig. 1). The plausible mechanism behind the Furin upregulation is that HIF-1α binds to the hypoxia-responsive elements present on the promoter region of Furin[14]. It is also reported that hypoxia further favours relocalisation of intracellular Furin to the cell surface[15]. On the other hand, Furin also positively regulates the HIF-1α in squamous carcinoma cells[16] (Fig. 1).

**Hypoxia and Inflammation in COVID-19**

ACE2, which acts as a functional receptor for host-virus interaction in SARS-CoV-2 infection, is highly expressed on the cell surface of many tissues and organs including the lungs and the myocardium, hence the early involvement of lung and the severity of the respiratory syndrome are explained[17,18]. The local damage caused by the ARDS (Acute Respiratory Distress Syndrome) to the tissue of lungs leads to inflammatory changes and thus the inflammation can also lead to the hypoxic condition at the site of viral infection[19,20]. The tissue damage is also responsible for hypoxic conditions that can upregulate HIF-1α which in turn upregulate Furin expression[21]. Furin, then enhance the process for the SARS-CoV-2entry resulting in the severity of the disease. Thus, it suggests that the hypoxic microenvironment in COVID-19 contributes to the disease worsening.

**Hypothesis**

As it is evident from the literature that SARS-CoV-2 requires host enzymes Furin to activate the receptor binding domain of its S protein and ACE2 is required as the functional binding receptor, facilitating the entry of the virus in the host cell. Intra-tumor hypoxic conditions in solid tumours like breast cancer, lung cancer *etc*. induce the HIF-1α in tumour and its neighbouring tissues that ultimately upregulate proprotein convertase Furin expression. Again, at the early stage of infection with SARS-CoV-2, there is an insult to the respiratory system leading to hypoxic conditions and an increase in HIF-1α. Therefore, a hypothesis arises which states that COVID-19 infection in patients with solid tumour causes the upregulation in HIF-1α. Then HIF-1α upregulates the expression of Furin in neighbouring cells that further facilitates the entry of SARS-CoV-2 virus in neighbouring tissues resulting in the vicious viral cycle and more chances of multiple organ involvement in the patients. Also, as the cancer patients already have the upregulated HIF-1α in the tumour microenvironment. The possibility of poor prognosis in the cancer patients infected with SARS-CoV-2 is much higher due to pre up-regulated HIF-1α.

**Discussion**

It has been reported that the infection rate in cancer patients due to SARS-CoV-2 was higher than the general population, deteriorating conditions, and poor outcomes[22]. As it is evident that Furin expression is upregulated in cancer patients, So the infection of cancer patients with SARS-CoV-2 may result in poor prognosis. A serious matter of concern has been raised by the oncologists regarding increased chances of infection due to the immunocompromised nature of the cancer patients due to cancer itself or due to the nature of anticancer therapy, making them utmostly vulnerable with the very adverse outcome as reported by Kuderer *et al*. by a multicentric study of Cancer Consortium (CCC19) registry database[23].

Hypoxia is a physiological as well as a pathological condition associated with a large number of diseases such as in cancer and bacterial and viral infections. The expression of protein hypoxia-inducible factor 1-α is regulated by the pro-inflammatory cytokines, growth factors, and infections. In SARS-COV-2 infection, it may be presumed that the viral infection is leading to the tissue deprivation of oxygen. In response to oxygen deprivation, hypoxia-inducible factor 1-α is stabilized. Upon stabilization, it binds to HIF-responsive elements (HREs) in the gene promoter region that responds to the hypoxic condition[24]. In Cancer, the expression of Furin was increased when the HIF-1α expression was induced. Thus in SARS-COV-2 infection, the stimulation of HIF-1α might be upregulating the Furin expression. Hence, leading to the severity of the infection.

Again, it is a well-documented fact that the Furin gene is constitutively expressed. However, the regulation of the expression of Furin is yet not well explored. But few *in vitro* studies reported that the expression of Furin is upregulated when the expression of HIF-1α and SOX9 is upregulated. Thus the expression of Furin is not only upregulated by HIF-1α[25]. To date, the positive feedback loop involving HIF-1α and Furin expression is studied in cancer only, to the best of our knowledge. Overall solid cancer leads to the hypoxic environment, which in turn leads to the activation of HIF-1α. Thus the expression of furin is stimulated in response to oxygen deprivation, as all three FUR promoters have the binding sites for the hypoxia-inducible factor 1-α.

So if the expression of HIF-1α is induced in SARS-CoV-2 infection, then it might be possible that HIF-1α is stimulating Furin expression.

Interestingly, there is no study reporting the expression level of HIF-1α in tissues or serum of COVID-19 patients to substantiate our theory. However, there is indirect evidence of a possible increase in HIF-1α as many studies have reported hypoxic conditions in SARS-CoV-2 infection either due to inflammation or viral pneumonia. Moreover, the immune response against the viral infection leads to the positive regulation of the apoptotic pathway in the infected cells. The exaggerated inflammatory response often results in tissue damage. The molecular pathways involved during the viral infection, mediating the increased cell damage and inflammation are the areas of active research. But it has been reported that the cell death and inflammatory injury, at the site of virus infection, can also lead to hypoxia. The hypoxic environment also plays the important role in the regulation of various genes such as HIF-1α, HIF-2α, NF-κB (nuclear factor-kappa B), CREB (cAMP-response Element-binding Protein), Nrf (NF-E2-related factor), STATs (signal transducers and activators of transcription)[26]. In case of severe infection of SARS-CoV-2, the patients showed tissue damage and inflammatory response. So, the hyperinflammatory response might results in the hypoxic environment at the infected site. There is a possibility that in response to SARS-CoV-2, the expression of HIF-1α will be higher in COVID-19 patients, due to the hypoxic environment, which further results in the upregulation in the expression of Furin. Furin plays a key role in the entry of the virus into the host cell. Therefore, it might be possible that HIF-1α is regulating the entry of SARS-CoV-2 into the host cells by regulating the expression of Furin. This will result in a vicious cycle where the hyper inflammation and tissue damage due to viral infection leads to increased expression of HIF-1α, which further enhances SARS-CoV-2 entry by upregulating the expression of Furin. Therefore, HIF-1 expression most probably is the key player that upregulates the Furin, further increasing the viral entry in the neighbouring tissues of COVID-19 patients and multiple organ involvement. On the other hand, patients with solid tumours already have increased HIF-1α levels due to intra-tumour hypoxia. Along with it the expression of Furin is also upregulated in cancer patients. Thus suggesting that these may be the factors that cancer patients are more susceptible to infection due to SARS-CoV-2. Thus, in our opinion, early signs of even a very small degree of hypoxia may be considered an ominous sign in the cancer patients because it can predict early involvement of multiple organ involvement and ultimately poor prognosis in COVID-19. In the patients having hypoxia at earlier stages of infection, an earlier institution of $O_2$ as a preventive measure may lead to a better outcome in Cancer patients infected with SARS-CoV-2. Confirmation of the hypothesis can help to improve the prognosis in patients with COVID-19 infections in normal as well as in cancer patients. Thus, targeting HIF-1α can be a plausible way to decrease the COVID-19 pathogenesis in cancer patients.

HIF-1α inhibitors are categorized as direct inhibitors and indirect inhibitors. The direct inhibitors target HIF-1α whereas the indirect inhibitors target the molecules upstream or downstream the HIF-1α signalling axis. In SARS-CoV-2 infection, we are more interested to specifically target the function of HIF-1α so it will be better to use the direct inhibitors. The direct inhibitors can be used as a single agent or the agent combined with other molecules. 2ME2 NCD (Panzem), 17-AAG (Tanespimycin), Vorinostat (SAHA, Zolinza), EZN-2208 (Pegylated SN-38), CRLX101 are some of the drugs targeting HIF-1α. These drugs are FDA approved and are in their Clinical trials for treating the cancers. So these agents may be of potential use to specifically target the HIF-1α in SARS-CoV-2 infection[27].

However, Furin cannot be targeted to be a potential molecule in COVID-19 infection as this is a very important proprotein convertase, physiologically catalysing a lot of very important molecules to their biologically active products *e.g*. proalbumin, proparathyroid hormone, TGFβ, MMPs, β subunit of pro-nerve growth factor, von-Wille brand factor *etc*. Thus, targeting Furin may result in loss of physiological homeostasis.

On the contrary, it has been hypothesized in one of the studies that HIF-1α is playing the opposite role as proposed by ours in the prognosis of SARS-CoV-2 patients[28]. Though it is the hypothesis stating the protective role of HIF-1α in SARS-CoV-2 infections by downregulation of ACE2 in high altitude acclimatized individuals, at the same time they also suggested the positive correlation with hypoxemia and related ACE2 upregulation.

## Conclusion

The role of HIF-1α in SARS-CoV-2 infections is one of the definitive concern to understand the pathogenesis. Although HIF-1α also plays role in the

pathogenesis of other diseases particularly in patients with solid tumours like Head and Neck Squamous Cell Carcinoma, Breast Cancers etc., has been elucidated in details, the definitive role and crosstalk between the HIF-1α mediated pathway and COVID-19 pathogenesis is yet to be explored. In our opinion particularly in context with the cancer patients, where the HIF-1α, is a major key player associated with tumour biology, the SARS-CoV-2 infection becomes more of a concern.

## Acknowledgement

Council of Scientific and Industrial Research, India for fellowship to Rajandeep Kaur [09/141(0210)/2019-EMR-1] and Anshika Chauhan [09/141(0209)/2019-EMR-1]. Figure has been drawn using Microsoft Power Point.

## Conflict of interest

All authors declare no conflict of interest.

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