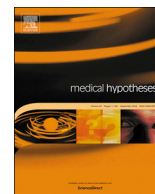




Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Increased susceptibility of SARS-CoV2 infection on oral cancer patients; cause and effects: An hypothesis

Anshika Chauhan^a, Sushmita Ghoshal^b, Arnab Pal^{a,*}

^a Department of Biochemistry, Post Graduate Institute of Medical Education and Research, Chandigarh, India

^b Department of Radiotherapy, Post Graduate Institute of Medical Education and Research, Chandigarh, India



ABSTRACT

In 2019, a new coronavirus (SARS CoV2) infecting humans has emerged in Wuhan, China which caused an unprecedented pandemic involving at least 185 countries infecting 2.5 million people till date. This virus is transmitted directly or indirectly through the upper aerodigestive tract. As it is evident from the recent studies that SARS-CoV-2 requires host enzyme Furin to activate receptor binding domain of its S protein and host Angiotensin Convertase Enzyme 2 (ACE2) is required as binding receptor, facilitating the entry of virus into the host cell. Evidence from literature shows that oral cancer tissues as well as paracarcinoma tissue exhibit higher expression of both Furin and ACE2, giving rise to the hypothesis that patients with oral cancer have higher chances of SARS CoV2 infection. It is also hypothesised that there will be increased severity of disease due to facilitated entry of the virus into the cells. Therefore, we suggest oral cancer patients require extra attention during COVID-19 pandemic and re-evaluation of current treatment paradigms in oral oncology is also needed.

Background

COVID-19

A novel coronavirus emerged in December 2019, which has spread rapidly across the world causing a pandemic which was never observed in history with the viruses of this family [1,2]. The virus is a new member of the lineage b of the genus *Betacoronavirus* and it also has similarity with the 2002 severe acute respiratory syndrome virus SARS-CoV. Therefore, it is named as SARS-CoV-2 by WHO. The respiratory disease caused by the virus was designated as coronavirus disease 2019 (COVID-19) Fig. 1.

SARS-CoV-2 is transmitted directly through cough, sneeze, droplet inhalation and indirectly through contact of oral, nasal, and eye mucous membranes via hand, contaminated with live virus [3]. This virus can also be transmitted directly or indirectly through saliva. This suggests that oral cavity is one of the sites which are more susceptible to be exposed to SARS-CoV-2.

Role of ACE2 and Furin in COVID-19

Coronaviruses (CoVs) are a family of enveloped, single-stranded positive-sense RNA viruses and are able to infect a wide range of mammalian and avian species, causing respiratory or enteric diseases [4]. CoVs have a major surface protein denoted as the spike (S) protein, which facilitates the infection by binding to host cellular receptors and

ultimately leading to the fusion of the viral lipid envelope with host cellular membranes. Before binding to the host receptor, the S protein is activated by host proteases [5].

Various structural studies and experimental data revealed two most important features of S protein in SARS-CoV-2:

- i. It has receptor-binding domain (RBD) which is optimized for binding to human receptor ACE2 (Angiotensin-converting enzyme 2) [6–9]. ACE-2 is a type I transmembrane metalloprotease mainly found attached to the outer surface of the lungs, arteries, heart, kidney, and intestines. It plays a key role in the Renin-Angiotensin System (RAS) and is targeted for the treatment of hypertension [10]. ACE2 degrades Angiotensin II to generate Angiotensin 1–7, thereby, negatively regulating RAS [11,12].
- ii. It also has a functional polybasic cleavage site (RRAR) at the junction of S1 and S2, the two subunits of the S protein [7]. This site allows effective activation of S protein by a host proprotein convertase Furin. Furin is a calcium dependent membrane bound protease which is expressed ubiquitously as a 794 amino acid zymogen that undergoes autocatalytic cleavage to become fully active [13,14].

It suggests that host ACE-2 and Furin are the most crucial proteins involved in COVID-19 infection.

* Corresponding author.

E-mail addresses: anshikapu@gmail.com (A. Chauhan), rtsushmita@gmail.com (S. Ghoshal), pal.arnab@pgimer.edu.in (A. Pal).

<https://doi.org/10.1016/j.mehy.2020.109987>

Received 22 April 2020; Received in revised form 1 June 2020; Accepted 7 June 2020

Available online 09 June 2020

0306-9877/ © 2020 Elsevier Ltd. All rights reserved.

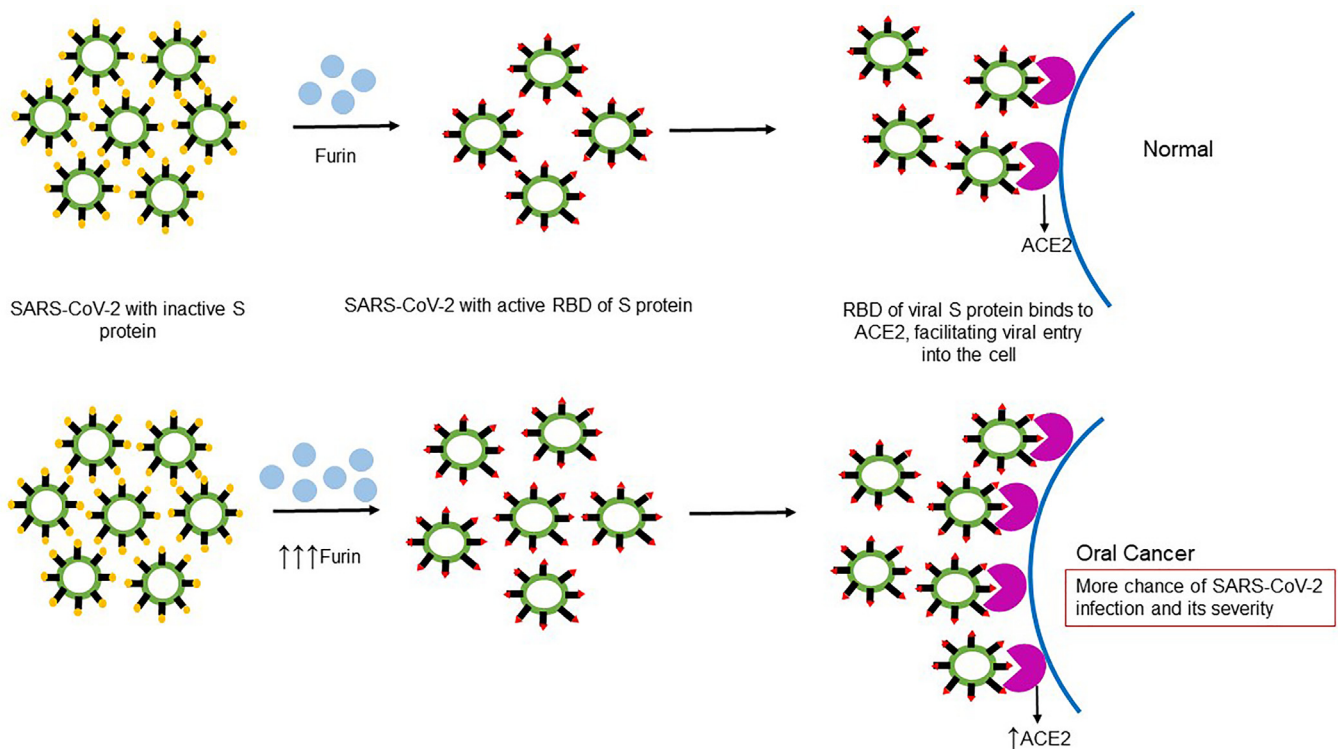


Fig. 1. Under normal conditions, host enzyme Furin activates receptor binding domain (RBD) of SARS-CoV-2 S protein, and this activated RBD binds to host cell membrane receptor ACE2, ultimately leading to fusion of viral particles with host cell membrane, facilitating viral entry into host tissue. In case of oral cancer, increased expression of Furin leads to activation of S protein RBD of a greater number of SARS-CoV-2 and increased expression of ACE2 facilitates binding of a greater number of SARS-CoV-2, ultimately increasing the chances of infection and its severity.

Overexpression of Furin in oral cancer

Increased expression of Furin was documented in various cancer types and Furin is involved in most cancer hallmark processes like cell proliferation, migration and invasion or neo-vascularization. Overexpression of Furin was correlated with aggressiveness of cancer and proposed marker for advanced or high-grade disease. Cicco et al. confirmed increased expression of Furin in approximately 90% of oral squamous cell carcinoma (OSCC) and in all squamous cell carcinoma of the oesophagus analyzed using microarray technology [15]. Furin expression was found to be significantly higher in most precursor lesions and OSCC than in normal epithelia [16]. Also, in post radiotherapy recurrent laryngeal cancers, it was observed that Furin expression was higher in samples obtained after radiation, this suggests radiation induced overexpression of Furin in recurrent or resistant cancers [17]. In addition to these, various other studies have demonstrated increased expression of Furin in oral cancer [18,19].

Expression of ACE2 in oral cancer

While analysing RNAseq data from the public database, Xu et al. [20] reported in a recent article that paracarcinoma and normal tissues express high ACE2 in oral buccal and gingiva tissue. Single-cell RNAseq data from patients' oral tissue by them also suggested that ACE2 were highly enriched in epithelial cells of tongue. Liu, L. previously demonstrated in animal model that epithelial cells layer of salivary gland ducts are early target cells of severe acute respiratory syndrome coronavirus infection in the upper respiratory tracts through their over expressed ACE2 [21]. Featherston et al. observed that ACE expression, which is homologous to ACE2, was higher in cancer stem cells of moderately differentiated buccal mucosal squamous cell carcinoma [22]. In laryngeal cancer, ACE was also found to be upregulated [23].

Hypothesis

As it is evident from the recent studies that SARS-CoV-2 requires host enzyme Furin to activate receptor binding domain of its S protein and ACE2 is required as binding receptor, facilitating the entry of virus in the host cell. It is also recited in literature that oral cancer tissue express higher level of Furin and ACE2 and oral cavity is one of the most exposed tissue for SARS CoV2 infection. Therefore, an hypothesis arises which states that oral cancer patients may be more susceptible to SARS CoV2 infection. Also, they have higher expression of Furin and ACE2, allowing entry of a large number of viruses at a time, leading to increased viral load in the patients with oral cancer. This ultimately leads to poor prognosis of COVID-19 in these patients.

Evaluation of the hypothesis

Multiple studies suggested that there is overexpression of host Furin in oral cancer, which is a key enzyme to activate the Furin like cleavage site present in the S spike of SARS CoV2 leaving to gain of function in attaching the viral particles with its receptors like ACE2 [24,25]. However, there is limited data on overexpression of Furin in oral cancers of different geographical regions. Another limitation is that Furin overexpression was analyzed in a relatively less number of samples. Again, there is very limited scientific literature available regarding oral cancer and COVID19. So, it is evident that studies with larger sample size is required to substantiate the overexpression of Furin in oral cancer tissue along with its mechanistic reasoning.

ACE (homologue for ACE2) overexpression was demonstrated in oral cancers. There is also evidence from available database that ACE 2 mRNA is over-expressed in para-carcinoma as well as normal cells in oral mucosa. However, studies showing ACE2 expression at mRNA as well as protein level in cancer cells are not available in literature. Hence, studies are required to analyze the expression of ACE2 in oral

cancer tissue.

Observational as well as epidemiological studies are mandatory which can reveal the frequency of oral cancer patients infected with COVID-19 and how the prognosis of these patients differ from the control population.

Furthermore, in one of the studies, it was observed that radiotherapy induced the increased expression of Furin [18]. Therefore, more extensive studies are needed to analyze the susceptibility of oral cancer patients undergoing radiotherapy for COVID-19 infection.

Overall, till now, there is limited data to evidently prove this substantial hypothesis. However, in our opinion, evidence of our hypothesis will be soon substantiated with the more data published in scientific literature.

Consequences of hypothesis and discussion

More serious infection

Oral cancer tissues express higher levels of Furin, while paratumour oral tissue expresses high ACE2, key proteins mandatory for SARS CoV2 infection. Therefore, oral cancer patients are more susceptible to this infection as compared to normal population as over expression of Furin will increase more conversion of S protein of the virus particle, which will attach in greater number to overexpressed ACE2 and finally increased and easy entrapment of the viral particles into the cells. Moreover, these patients are more prone to SARS CoV2 reinfection leading to viremia which is also a challenge to treat. Furthermore, most oral cancer patients are older males which themselves are two independent risk factors for COVID19. Again, older age is associated with reduced immune state, further increasing the chances of SARS CoV2infection and its severity.

Multilevel risks COVID-19 on oral cancer patients

In our opinion, during the COVID-19 pandemic, it would be more practical to give extra attention to oral cancer patients during COVID-19 pandemic and re-evaluation of current treatment paradigms in oral oncology is also needed as suggested by Day et al in a recent article [26]. They very aptly suggested that there are increased multilevel risks to patients, surgeons, health care workers (HCWs), institutions and even to society due to positive COVID19 oral patients. It is suggested that the nonsurgical management outweighs the surgical management under this unprecedented pandemic situation, even though there is a chance of radiotherapy induce Furin expression in resistant cancers. Thus, comprehensive risk benefit ratio needs to be developed in managing the Oral Cancer patients with SARS-CoV2 infection.

Sources of support in the form of grants

Council of Scientific and Industrial Research, India for fellowship to Anshika Chauhan [Grant no: 09/141(0209)/2019].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [1] Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020;579(7798):270–3.
- [2] Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. *Nature* 2020;579:265–9.
- [3] Lu CW, Liu XF, Jia ZF. 2019-nCoV transmission through the ocular surface must not be ignored. *Lancet* 2020;395(10224):e39.
- [4] Weiss SR, Navas-Martin S. Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. *Microbiol Mol Biol Rev* 2005;69(4):635–64.
- [5] Belouzard S, Chu VC, Whittaker GR. Activation of the SARS coronavirus spike protein via sequential proteolytic cleavage at two distinct sites. *Proc Natl Acad Sci USA* 2009;106(14):5871–6.
- [6] Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by the novel coronavirus from wuhan: an analysis based on decade-long structural studies of SARS coronavirus. *J Virol* 2020;94(7):e00127–220.
- [7] Alexandra & Park, Young-Jun & Tortorici, M. & Wall, Abigail & McGuire, Andrew & Veessler, David. (2020). Structure, function and antigenicity of the SARS-CoV-2 spike glycoprotein., *Cell* 2020;180, 281–292.
- [8] Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 2020;367(6483):1260–3.
- [9] Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. *Nat Microbiol* 2020;5(4):562–9.
- [10] Riordan JF. Angiotensin-I-converting enzyme and its relatives. *Genome Biol* 2003;4(8):225.
- [11] Kuba K, Imai Y, Ohto-Nakanishi T, Penninger JM. Trilogy of ACE2: a peptidase in the renin-angiotensin system, a SARS receptor, and a partner for amino acid transporters. *Pharmacol Ther.* 2010;128(1):119–28.
- [12] Tikellis C, Thomas MC. Angiotensin-converting enzyme 2 (ACE2) is a key modulator of the renin angiotensin system in health and disease. *Int J Pept.* 2012;2012:256294.
- [13] Seidah NG, Day R, Marcinkiewicz M, et al. Precursor convertases: an evolutionary ancient, cell-specific, combinatorial mechanism yielding diverse bioactive peptides and proteins. *Ann N Y Acad Sci* 1998;839:9–24.
- [14] Anderson ED, VanSlyke JK, Thulin CD, et al. Activation of the furin endoprotease is a multiple-step process: requirements for acidification and internal propeptide cleavage. *EMBO J* 1997;16:1508–18.
- [15] López de Cicco R, Bassi DE, Page R, Klein-Szanto AJ. Furin expression in squamous cell carcinomas of the oral cavity and other sites evaluated by tissue microarray technology. *Acta Odontol Latinoam.* 2002;15(1–2):29–37.
- [16] Lopez de Cicco R, Watson JC, Bassi DE, et al. Simultaneous expression of furin and vascular endothelial growth factor in human oral tongue squamous cell carcinoma progression. *Clin Cancer Res* 2004;10:4480–8.
- [17] Lee M, Ryu CH, Chang HW, et al. Radiotherapy-associated furin expression and tumor invasiveness in recurrent laryngeal cancer. *Anticancer Res* 2016;36:5117–25.
- [18] Bassi DE, Mahloogi H, Al-Saleem L, et al. Elevated furin expression in aggressive human head and neck tumors and tumor cell lines. *Mol Carcinog* 2001;31:224–32.
- [19] Bassi DE, Mahloogi H, Lopez De Cicco R, et al. Increased furin activity enhances the malignant phenotype of human head and neck cancer cells. *Am J Pathol* 2003;162:439–47.
- [20] Xu H, Zhong L, Deng J, et al. High expression of ACE2 receptor of 2019-nCoV in the epithelial cells of oral mucosa. *Int J Oral Sci.* 2020;12(1):8.
- [21] Liu L, Wei Q, Alvarez X, et al. Epithelial cells lining salivary gland ducts are early target cells of severe acute respiratory syndrome coronavirus infection in the upper respiratory tracts of rhesus macaques. *J Virol.* 2011;85(8):4025–30.
- [22] Featherston T, Yu HH, Dunne JC, et al. Cancer stem cells in moderately differentiated buccal mucosal squamous cell carcinoma express components of the renin-angiotensin system. *Front Surg.* 2016;3:52.
- [23] Han CD, Ge WS. Up-regulation of angiotensin-converting enzyme (ACE) enhances cell proliferation and predicts poor prognosis in laryngeal cancer. *Med Sci Monit.* 2016;22:4132–8.
- [24] Coutard B, Valle C, de Lamballerie X, Canard B, Seidah NG, Decroly E. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. *Antiviral Res.* 2020;176:104742.
- [25] Li, X. D. et al. A Furin Cleavage Site was Discovered in the S Protein of the Wuhan 2019 Novel Coronavirus. www.chinaxiv.org/abs/202002.00004 (2020).
- [26] Day AT, Sher DJ, Lee RC, et al. Head and neck oncology during the COVID-19 pandemic: Reconsidering traditional treatment paradigms in light of new surgical and other multilevel risks. *Oral Oncology.* DOI: 10.1016/j.oraloncology.2020.104684.