In silico analysis of the Key Receptors of SARS-CoV-2: ACE2 and TMPRSS2 in Head and Neck Cancer

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Research Article

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Abstract

Background

It is reported that cancer patients are more susceptible to SARS-CoV-2 infection due to immunocompromised immunity. To understand the long-term impact of SARS-CoV-2 in HNSC cancer, the current study is designed to assess the status of two vital host molecules (ACE2 & TMPRSS2) for the entry of virus in HNSC patients using computational methods.

Methodology

We used public databases such as TIMER, UALCAN, and GEPIA2 to assess the mRNA expression and association of ACE2 and TMPRSS2 in HNSC. cBioPortal was used to assess the genetic alterations in the ACE2 and TMPRSS2 protein sequences that are involved in HNSC development. The CTD database was also used to identify the genes associated with COVID-19 and HNSC. Finally, the PANTHER online platform was used to investigate the protein-protein interaction between COVID-19 and HNSC progression genes collected from the CTD database.

Results

A differential expression of ACE2 (downregulated) and TMPRSS2 (upregulated) was noted in HNSC those with human papilloma viral infections. A strong genetic alteration in the protein sequence of ACE2 and TMPRSS2 supports their significant role in HNSC disease progression. In addition, the protein-protein interaction network revealed that genes associated with COVID-19 and HNSC are strongly involved in binding activity, catalytic activity, and various cancer signaling pathways.

Conclusion

From our in silico analysis, we would like to conclude that ACE2 and TMPRSS2 are strongly associated with disease progression in both pathological conditions (HNSC and COVID-19). So, it could serve as potential biomarkers for the early prediction of disease outcomes in cancer patients with COVID-19 disease. Thus, further investigation is needed to evaluate the long-term impact of SARS-CoV-2 infection on HNSC patients.

1. Introduction

Recently, Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) caused a fierce mobilization of clinicians and scientists to explore the etiology of Coronavirus Disease-19 (COVID-19). However, because of the rapid spreading of different strains over the world, we still don’t know the serious consequence for those who have been infected and recovered from SARS-CoV-2 (Martelli-Júnior et al. 2020). Despite the

fact that COVID-19 vaccination is available, clinical outcomes of the patients with and without comorbidities, as well as immunocompromised persons, must be monitored (Mahmood et al. 2021). According to early research on COVID-19, patients with comorbidities such as cardiovascular disease, carcinomas, diabetes, hypertension, and chronic kidney disease, are more prone to infections and disease severity (Yang et al. 2020). Individuals with cancer, on the other hand, are thought to be more vulnerable to COVID-19 than those with underlying complications due to suppressed and compromised immune responses (Dai et al. 2020). While SARS-CoV-2 mediated morbidity and fatality are strongly associated with respiratory and cardiovascular complications, oral complications have been reported in COVID-19 positive patients, with oral lesions as well as dysgeusia being the main clinical symptoms of recurrent SARS-CoV-2 infection and leading to the development of head and neck carcinomas (HNSC) (Aranda Romo et al. 2021; Carrillo-Larco and Altez-Fernandez 2020; Chen 2019). This may be related to the binding of SARS-CoV-2 to angiotensin-converting enzyme-2 (ACE2), a known component of the renin-angiotensin system (RAS), which is extensively expressed on the tongue surface and oral cavity (Xu et al. 2020). In addition, TMPRSS2 is involved in proteolytic cleavage and subsequent priming of spike (S) proteins of SARS-CoV-2 (Sacconi et al. 2020). Thus, these molecules serve as a gateway for SARS-CoV-2 infection. Moreover, RAS components have been previously reported to be associated with oral cancer, a type of HNSC.

HNSC is the eighth most frequent cause of cancer death and the sixth leading cancer by incidence worldwide (Bonomi et al. 2014). Tumors in the head and neck region quickly double in volume within 1–3 months, irrespective of their size and site of origin (Jensen, Nellemann, and Overgaard 2007). Typically, HNSC is characterized by a higher incidence of local recurrences, which occurs in 60% of the cases and is a common cause of death in HNSC (Argiris et al. 2008; Sacconi et al. 2020). Though reports suggested the possibility of suspending surgeries for a few cases, the current standard of care is surgery followed by radiotherapy or chemotherapy (Brody et al. 2020). Perhaps, a delay in diagnosis and/or treatment of HNSC impacts the overall survival rate of patients (Schutte et al. 2020). In support of this, recent investigations suggest that the clinical outcome and survival rate are expected to be very adverse due to delayed presentation, diagnosis, and treatment of HNSC patients during and after the COVID-19 pandemic (Tevetoğlu et al. 2021; Werner et al. 2020; Schutte et al. 2020; Kiong et al. 2021). Despite the advancement in new medical and surgical therapies that have improved the quality of patients' life, the survival rate is still 40–50%. Regrettably, it also failed to improve the overall clinical outcomes of the disease, and thus, HNSC remains one of the major challenges for clinicians and physicians. Therefore, the overall impact of COVID-19 on the HNSC needs further exploration. In this sense, we investigated ACE2 and TMPRSS2 by *in silico* analysis in terms of expression, function, and mutations in HNSC to assess their impact on the disease severity of COVID-19 with HNSC utilizing several freely available public and online platforms/databases for effective screening of various genes and proteins associated with these diseases at a molecular level.

2. Methodology

2.1 Expression Analysis of ACE2 & its co-expressed Genes (TMPRSS2)

2.1.1 Expression profile in HNSC

To explore the transcriptional profile of ACE2 and its co-expressed genes (TMPRSS2), we utilized a public web tool Tumor Immune Estimation Resource (TIMER2.0) server (http://timer.cistrome.org/), a comprehensive resource for the clinical relevance of tumor-immune filtrations across various cancer types. This comprehensive resource comprises six principal analysis catalogs which allow the users to analyze the expression of genes and their interconnection with the immune-suppressed cancer cells (Li et al. 2017).

2.1.2 In-depth Analysis of Gene expression in HNSC

We assessed the mRNA expression data of ACE2 and its co-expressed gene in HNSC through the UALCAN website (http://ualcan.path.uab.edu/) using the TCGA (The Cancer Genome Atlas) datasets. UALCAN is a user-friendly online platform used to study the gene expression of query genes and their association with the clinical outcomes of 31 types of cancer using TCGA data (Chandrashekar et al. 2022).

2.1.3 Dependence Test of the targeted genes in HNSC

Gene Expression Profiling Interactive Analysis (GEPIA) 2 is a web-based tool widely used to evaluate the transcriptional profile of different human cancer and normal tissue, via the TCGA database and the Genotype-Tissue Expression (GTEx) projects. Besides this, it can be used to evaluate the gene-specific correlation between various cancer types (Tang et al. 2017). So, we have assessed the correlation impact of ACE2 with TMPRSS2 in HNSC through GEPIA2 2 web portal (http://gepia2.cancer-pku.cn/#index).

2.2 Functional Analysis of the Targeted Genes

2.2.1 Evaluation of Mutations and Copy Number Alterations (CNAs) in the Targeted proteins

The genetic alterations of the targeted molecules were explored by cBioPortal (https://www.cbioportal.org/), an interactive online portal for assessing the diverse datasets of cancer genomics. This database is extensively used for molecular profiling of different types of cancer tissues and cell lines, identification of mutation frequencies, and other genomics alterations from various cancer studies (Gao et al. 2013).

2.2.2 Protein-Protein Interaction Analysis

Genes associated with COVID-19 disease and HNSC were retrieved from the Comparative Toxicogenomics Database (CTD) (http://ctdbase.org/). CTD is a public database used to understand the chemical-gene/protein-interaction, chemical-disease, and gene-disease relationship (CJ et al. 2006). To check whether the proteins are associated with COVID-19 disease and HNSC, we created a protein-protein

interaction (PPI) among the targeted proteins via STRING software (https://string-db.org/) (Szklarczyk et al. 2021).

2.2.3 Interpretation of functional role of Associated Genes

The list of genes retrieved from CTD was subject to the integrative and molecular assessment using Protein Analysis Through Evolutionary Relationship (PANTHER) (http://www.pantherdb.org/), a tool that categorizes the genes in terms of biological function, molecular function, and pathway (Mi and Thomas 2009).

3. Results

3.1 Expression analysis of ACE2 & its Co-expressed genes

We investigated the expression level of ACE2 and TMPRRS2 in a variety of patients' malignancies using the TIMER 2.0 web application. The findings illustrated that ACE2 expression was unaltered in both HNSC (N-520) and normal tissues (N-44). Its expression was also reduced in HNSC with a positive status of human papillomavirus (HPV) infection (Fig. 1A). In the case of its co-expressed gene, a significant downregulation of TMPRSS2 was noticed in HNSC when contrasted with healthy control in the TCGA dataset. However, when compared to non-infected (N-421) HNSC, HNSC with HPV infection (N-97) revealed a substantial elevation in TMPRSS2 mRNA expression (Fig. 1B). This suggests that during viral infection, virus and/or viral factors may differentially influence the expression of ACE2 and TMPRSS2 in cancer patients.

3.2 Expression dynamics and correlation analysis of ACE2 & its co-expressed gene in HNSC

An interactive web-based platform called UALCAN was used to assess the mRNA expression of ACE2 and TMPRSS2 based on TGCA datasets in terms of distinct study factors like gender, individual cancer stages, variable age groups, and tumor grades. Compared to healthy tissues, the expression of ACE2 was substantially upregulated in female HNSC cases (Fig. 2A). Although there were no significant changes in the ACE2 mRNA levels across the individual cancer stages, there was a significant rise in the ACE2 expression in grade 2 tumors of HNSC (Fig. 2C & G). Furthermore, people aged 61–80 years old had higher levels of ACE2 expression than other age groups and healthy controls (Fig. 2E). In all the study parameters, the expression level of TMPRSS2 was entirely tapered off in HNSC when compared to healthy control in the TGCA datasets (Fig. 2B, D, F & H).

3.3 Correlation Analysis of the targeted Genes

A scatter plot was developed using GEPIA 2 web tool, which depicts the association between ACE2 and TMPRSS2 in HNSC based on their expression score. We found that ACE2 was positively correlated with TMRPSS2 ($R = 0.11$; $P = 0.014$) in the HNSC condition (Fig. 3A). In addition to this, we created a heat map

based on the expression score of targeted genes and found that all the targeted genes showed differential expression when they were expressed on tumor cells in comparison with normal cells (Fig. 3B).

3.4 Genetic alterations in ACE2 and TMPRSS2 associated with HNSC

Using cBioPortal, we investigated genetic variations of ACE2 and TMPRSS2 to determine their functional significance in HNSC development. A meta-analysis of the ACE2 protein sequence from 12 studies (2 overlapping studies were not included) with a total sample size of 1130 HNSC patients pointed out 5 missense mutations with a somatic mutation frequency of 0.4% in the 805 amino acid long human ACE2 protein (Fig. 4A). We also looked into the frequency of genetic alterations in ACE2 and found that ACE2 was largely altered in HNSC, with a frequency of 3.21%, and a low rate of alteration in adenoid cystic carcinoma (Fig. 4B). Further, we went on to analyse the expression level of unique types of genetic alterations as part of our study. From this analysis, we discovered that the shallow deletion type in HNSC causes the largest level of copy number alteration in ACE2 (Fig. 4C & D).

The genetic changes in TMPRSS2 in HNSC were investigated using a similar method. We found three missense and one nonsense type mutation in TMPRSS2, with a somatic mutation frequency of 0.4% (Fig. 5A). The mutations were located at 4 different locations throughout the 492 amino acid long TMPRSS2 protein sequence (Fig. 5A). In the TMPRSS2 mRNA, the highest rate of genetic alteration frequency was reported to be 1.7% (Fig. 5A). In TMPRSS2, shallow deletion was the most common kind of copy number alteration, similar to ACE2 (Fig. 5C & D). Together, the functional characterization of ACE2 and TMPRSS2 by utilizing various head and neck cancer studies uncovered some of the most relevant evidence of their interconnection with head and neck cancer development.

3.5 Interaction of proteins associated with the progression of COVID-19 and HNSC severity

Various numbers of genes are involved directly or indirectly in COVID-19 and HNSC progression. Using CTD, we identified 9,880 and 16,991 genes associated with COVID-19 disease and HNSC based on their inference score. Each of these genes was either curated or associated with the disease (marker/mechanism and/or therapeutic) or inferred associated via curated chemical interactions. From this huge dataset, a set of 21 and 19 curated genes was determined as the biomarker or therapeutic targets for COVID-19 and HNSC treatment, in which ACE2 and TMPRSS2 were included (Table 1). The translated protein sequence of these genes was utilized for generating the PPI network through the STRING database. Though the predicted edges were 20, 32, and 107 for COVID-19, HNSC, and both (COVID-19 & HNSC) according to the information provided by the database itself, we found 123, 95, and 336 connecting edges among these selected proteins (Fig. 6A-C). This PPI indicates that proteins are functionally interconnected more strongly than expected outcomes. We also found that ACE2 and

TMPRSS2 proteins are interlinked with other protein molecules associated with COVID-19 as well as HNSC disease progression.

Table 1
List of Genes Directly associated with COVID-19 & HNSC

Gene Directly Interconnected with COVID-19 Disease			
S. No.	Gene Name	Association	Inference Score
1	CCL2	Biomarker	28.70
2	IL6	Biomarker	24.36
3	TNF	Biomarker	23.78
4	CXCL8	Biomarker	22.61
5	IL10	Biomarker	21.97
6	IL1B	Biomarker	20.74
7	IL2	Biomarker	18.05
8	AGT	Biomarker	17.04
9	CCL3	Biomarker	12.08
10	CXCL10	Biomarker	11.73
11	IL7	Biomarker	8.38
12	MUC1	Biomarker	7.59
13	CCR2	Biomarker	6.96
14	ACE2	Biomarker & Therapeutic Target	6.74
15	CSF3	Biomarker	6.21
16	TMPRSS2	Biomarker	5.04
17	IL2RA	Biomarker	4.75
18	CD209	Biomarker	3.55
19	IFNAR2	Biomarker	2.68
20	ICAM5	Biomarker	2.66
21	IL10RB	Biomarker	2.54
Genes Directly Interlinked with HNSC			
1	TP53	Biomarker	46.99
2	ABCC1	Biomarker	39.48
3	AKT1	Biomarker	38.38
4	CASP8	Biomarker	37.88

Gene Directly Interconnected with COVID-19 Disease			
5	CDK2	Biomarker	35.23
6	ABCB1	Biomarker	34.58
7	CDK4	Biomarker	33.12
8	STAT3	Biomarker	32.84
9	CTNNB1	Biomarker	32.03
10	CDK6	Biomarker	29.92
11	PTEN	Biomarker	29.87
12	MGMT	Biomarker	26.08
13	IL1A	Biomarker	24.95
14	TNFRSF10B	Biomarker	24.36
15	IGF1	Biomarker	22.65
16	PIK3CA	Biomarker	13.72
17	IL1RAP	Biomarker	11.83
18	YAP1	Biomarker	4.79
19	ING1	Biomarker	2.73

3.6 Functional Role of Associated Genes

To assess the functional role of genes associated with HNSC and COVID-19, we used the list of previously retrieved genes (CTD) using the PANTHER database. First, we run a query using various parameters to determine the molecular activity of genes associated with COVID-19 and HNSC. The majority of the genes (33.9%) were involved in binding activity, according to the molecular function. Furthermore, 19% of the genes were found to be involved in catalytic activity, particularly acting on the proteins (Fig. 7A). In terms of biological processes, 18.74% of the genes were identified to be associated with cellular processes. We observed that the majority of the genes are involved in cell growth and cell proliferation after a thorough investigation (Fig. 7B). In addition to this, we also found that these genes were associated with cancer development and endothelial signalling pathway (Fig. 7C).

4. Discussion

SARS-CoV-2 infection become a new public health crisis across the world with the emergence and rapid spread of coronavirus disease 2019 (COVID-19). Since cancer patients are known to be immunocompromised, they are considered a distinct vulnerable population with a high risk of COVID-19-

associated complications (Yang et al. 2020; Desai et al. 2021). For instance, SARS-CoV-2 infection led us to reduce elective surgeries due to a lack of knowledge on the impact of COVID-19 disease on cancer patients (COVIDSurg Collaborative 2020b). However, a major problem put forth by physicians/clinicians is that a delay or cancellation of surgery may directly or indirectly result in poor outcomes and increased fatality. For instance, reports have suggested that a delay in proper care for a patient with mild or less aggressive forms of HNSC resulted in pulmonary complications associated with the SARS-CoV-2 virus (COVIDSurg Collaborative 2020a). Though studies suggested the possibility of suspending surgery in a few cases, the main therapeutic strategy for these tumors is surgery (Brody et al. 2020). In this view, the potential impact of COVID-19 on the pattern of presentation of HNSC patients needs further exploration to provide optimal care with efficient treatment. Thus, we hereby investigated the possible explanations for the increased susceptibility and mortality rate in HSNC patients infected with SARS-CoV-2.

First, we targeted the ACE2 and TMPRSS2 expression levels because they both play a vital role in viral host entry and disease pathogenesis of SARS-CoV-2. Indeed, ACE2 and TRMPRSS2 are associated with influenza, SARS-CoV, as well as SARS-CoV-2 in regulating viral entry into the host cell (Heurich et al. 2014; Simmons et al. 2013). To note, it is demonstrated that the affinity of the S1 spike protein of SARS-CoV-2 is 10–20 times higher towards ACE2 (Wrapp et al. 2020) and it is cleaved by TMPRSS2, thereby activating the endocytic route of SARS-CoV-2 (Hoffmann et al. 2020). Numerous studies have recently suggested that ACE2 and TMRPSS2 could be potential biomarkers for the early prediction of COVID-19 disease severity (Skarstein Kolberg 2020; Fagyas et al. 2022; Strope, PharmD, and Figg 2020; Rahbar Saadat et al. 2021). With this background, we evaluated the mRNA expression of ACE2 and TMPRSS2 in HNSC utilizing multidisciplinary parameters. We found that ACE2 expression was downregulated in HNSC with HPV infection, suggesting higher shedding of membrane ACE2 from head and neck region, particularly in sinuses, vocal cords, salivary gland and oral cavity, since these regions are known to express higher levels of ACE2 (Descamps et al. 2020). On the other hand, though we observed reduced expression of TMRPSS2 in HNSC, its mRNA levels were found substantially elevated in HNSC associated with HPV. In reference to this, a recent study reported that the reduction of TMPRSS2 was more evidence in HNSC patient with shorter survival as well as those with HPV negative status. (Sacconi et al. 2020). This indicates the virus or any viral factors/viral-induced host mediators could be involved in modulating TMPRSS2 expression, which leads to higher expression of TMPRSS2 and aids in priming of S protein for host viral entry as well as increased disease severity.

Since both the genes mediate sex-specific effects (Baratchian et al. 2021), we explored their expression levels in terms of gender. A differential expression of ACE2 in males and females indicates sex different expressions of ACE2 due to the effect of sex hormones, thereby contributing toward gender disparity in morbidity and mortality from COVID-19 disease. In contrast, TMPRSS2 expression was completely downregulated in both male and female HNSC cases. Thus, studies regarding the impact of sex hormones on the sex-specific expression of these genes need further validation as suggested by Majdic, 2020 (Majdic 2020). Furthermore, amplified expression of ACE2 expression in individual cancer stage-2 and age group (61–80) suggests that this patient needs effective management during SARS-CoV-2 infection because early studies have reported that people over the age of 40 are at high risk for HNSC

(Schantz and Yu 2002). In alignment with this, our results showed an amplified expression of ACE2, particularly in individual cancer stage-2 and age group (61–80) compared to normal. However, the lack of TMPRSS2 expression in all cancer stages and age groups suggests that other host proteases may be involved in the proteolytic cleavage of the S protein of SARS-CoV-2 infection. In support of this, new scientific findings have reported the involvement of various other host proteases (furin, matriptase, cathepsin B, cathepsin L) in proteolytic cleavage of coronavirus spike proteins (Jaimes, Millet, and Whittaker 2020; Papa et al. 2021; Strope, PharmD, and Figg 2020). To note, furin, as well as cathepsin, are a few upregulated proteins associated with carcinoma invasion and progression in oral cancer patients (López de Cicco et al. 2002; Kawasaki, Kato, and Mizuno 2002). In reference to this, an in silico analysis by Zhong et al 2020 demonstrated that SARS-CoV-2 could invade the oral mucosal cell via the ACE2 receptor and interact with the cell membrane through the activation of furin protease (Zhong et al. 2020). From this, we could speculate that oral mucosa tissue is more susceptible to SARS-CoV-2, thereby enhancing the disease severity in HNSC patients. Though ACE2 and TMPRSS2 expression were downregulated in HNSC cases compared to normal, our correlation analysis showed a significant positive association between ACE2 and TMPRSS2 in HNSC patients, which emphasizes the importance of these molecules in the disease severity of HNSC and/or COVID-19 patients. This initial assessment provides a piece of primary evidence that differential expression of ACE2 and TMPRSS2 is one of the plausible explanations for the higher susceptibility and mortality rate of HNSC patients with COVID-19.

To provide strong evidence, we further investigated the functional assessment of ACE2 and TMPRSS2. In this, we developed a protein-protein interaction network among the topmost significant 40 proteins associated with COVID-19 as well as HNSC, which includes ACE2 and TMPRSS2. We observed 40 nodes of interaction, where the major interaction of nodes was noted with ACE2 and TMRPSS2. This indicates the functional involvement and substantial role of ACE2 and TMPRSS2 in the disease progression of HNSC patients with SARS-CoV-2 infection. Besides, we also characterized the mutation and copy number alteration in their respective protein sequences based on 12 studies. In total, we noted 5 mutations at five different locations of the ACE2 protein, where the highest frequency of alteration is 3.21%. Whereas, 4 mutations at 4 different locations were determined against the TMPRSS2 protein sequences, where the maximum level of alteration frequency was 1.7%. Importantly, shallow deletion was the most frequent type of CNA observed in both protein sequences. This result supports the significant involvement of ACE2 and TMRPSS2 in HNSC and COVID-19 disease progression.

Finally, we assessed the functional and molecular activities of various genes associated with HNSC and COVID-19. We found that 19% of genes were associated with catalytic activity, indicating TMPRSS2 and other host protease enzymes may be involved in proteolytic cleavage of S protein of SARS-CoV-2 for host viral entry as well as active shedding of membrane/cellular bound ACE2. This could be a possible explanation for direct release and elevated levels of ACE2 in circulation, which is shown to be strongly associated with COVID-19 disease severity (Fagyas et al. 2022). Besides TMPRSS2, other enzymes such as TMPRSS11D, HNP/TMPRSS1, ADAM17, and ADMA10 are also shown to cleave the ACE2 receptor (SENAPATI et al. 2021; Jia et al. 2009). In terms of cellular function and pathway, most of the genes were associated with cell growth, cell proliferation, inflammation, and various cancer signaling (P53, PI3K, RAS,

EGFR, JAK/STAT, angiogenesis, interleukin mediated, Wnt signaling) pathways, indicating the higher possibility of HNSC progression. This was ascertained in early studies, which reported the potential involvement of underlying signaling pathways in HNSC (de Bakker et al. 2021; Thomas et al. 2015; Alamoud and Kukuruzinska 2018; Mock et al. 2021). Additionally, studies have described pro-inflammatory mediators as a crucial factor for HNSC development (Bonomi et al. 2014; Astradsson et al. 2019). Moreover, genes associated with inflammation and vascular functions show that elevated levels of pro-inflammatory mediators accompanied by hyper-inflammatory responses may be directly or indirectly involved in epithelial/endothelial dysfunction, which ultimately destroys the pulmonary endothelial cells as well as the oral cavity, leading to multi-organ failure and death, as observed in COVID-19 patients (Pelaia et al. 2020; Jin et al. 2020; Fodor et al. 2021). Hence, further investigations are required to ascertain the role of ACE2 and TMPRSS2 as biomarkers or therapeutic targets for HNSC patients to provide effective treatment and triage.

5. Conclusion

Based on our *in silico* analysis, we would like to summarize that both ACE2 and TMPRSS2 are generally downregulated in HNSC but their expression levels are differentially regulated during the pathological condition, particularly during the viral infection. In addition, our mutation and PPI analysis revealed a strong functional role of ACE2 and TMPRSS2 in HNSC development with SARS-CoV-2 infection. Therefore, ACE2 and TMPRSS2 could serve as robust biomarkers for early prediction of clinical outcomes in HNSC patients infected with SARS-CoV-2. Thus, further investigations are urged to study the long-term effect of SARS-CoV-2 in HNSC patients.

Declarations

Declaration of Competing Interest

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

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Figures

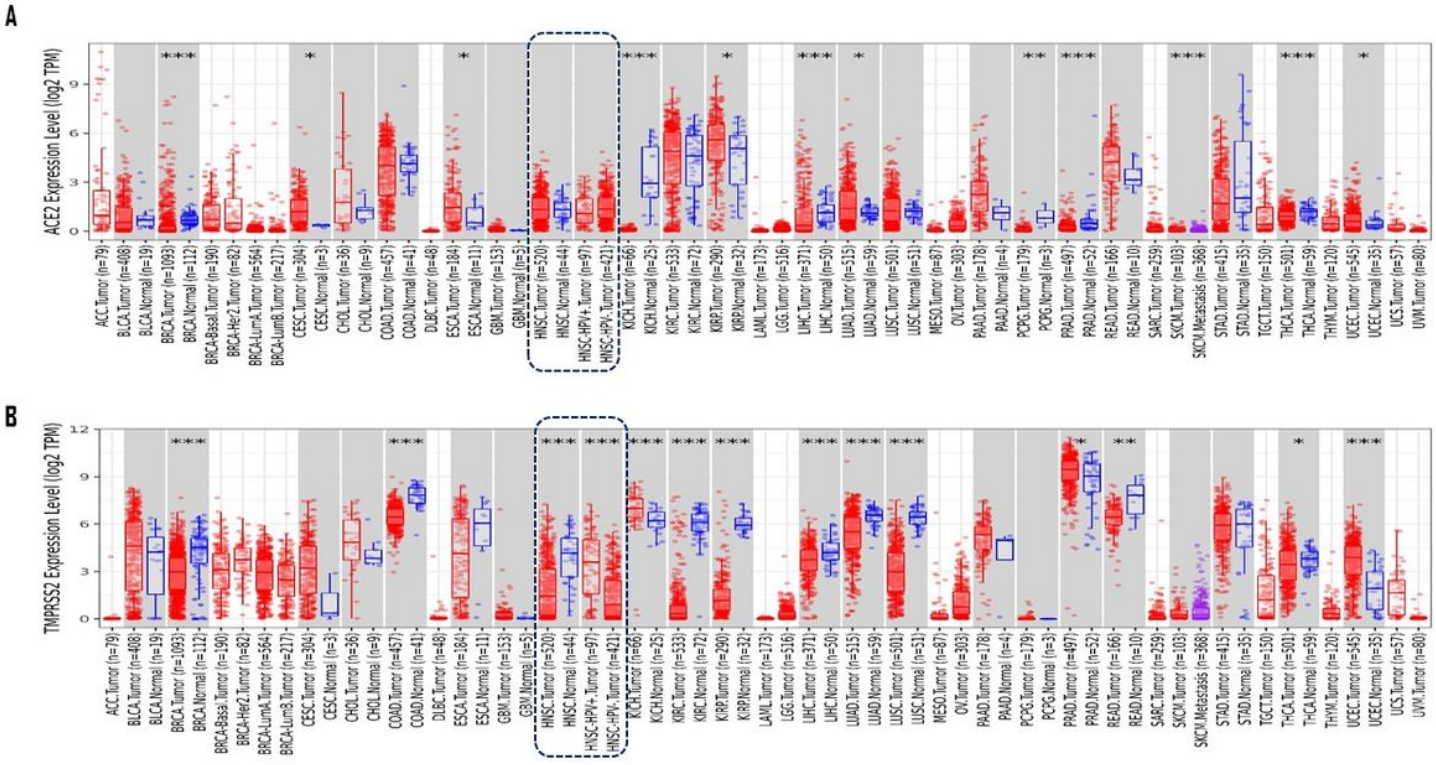

Figure 1

Expression Level of ACE2 and TMPRSS2 in HNSC and other cancers. (A) Expression Level of ACE2 (B) Expression level of TMPRSS2. *** Indicates P ≤ 0.001 compared to Normal

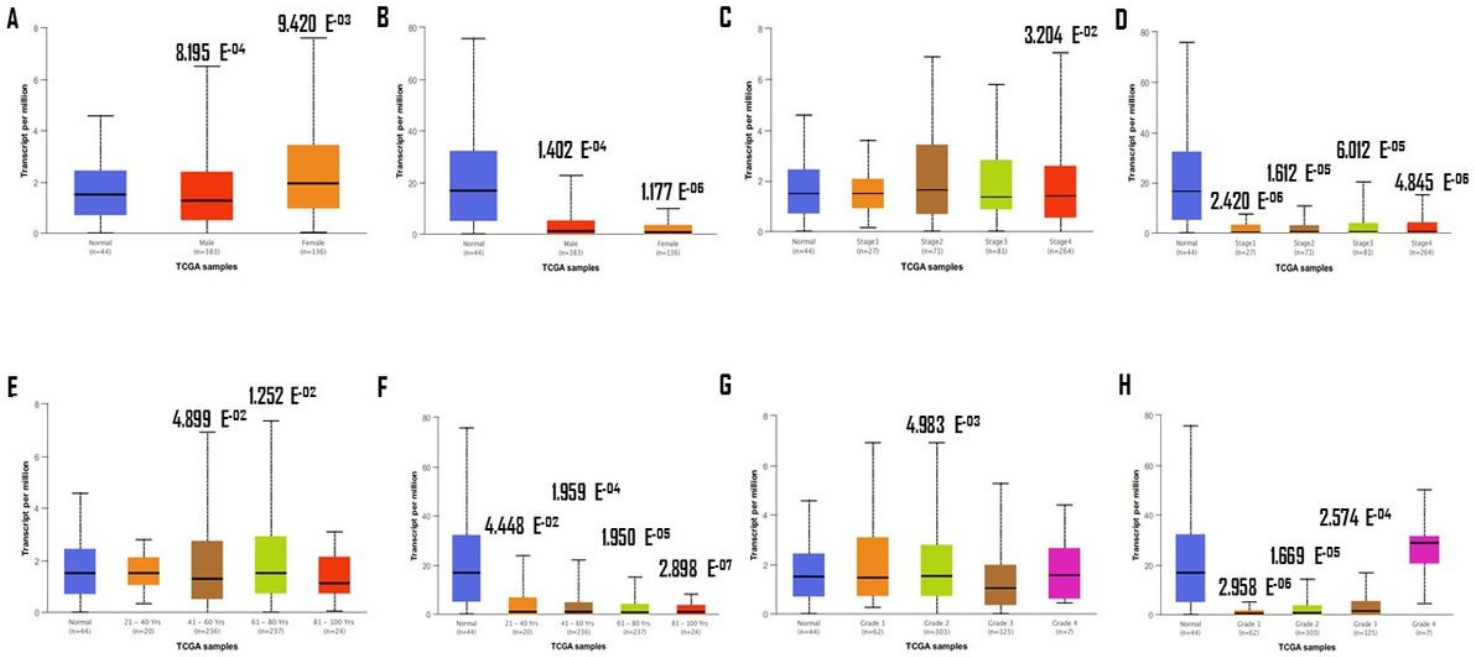

Figure 2

mRNA levels of ACE2 and its co-expressed genes in terms of Gender, Individual Cancer Stage, Age group, and Tumor grade. (A & B) mRNA levels of ACE2 and TMPRSS2 based on Gender. **(D & C)** mRNA levels of ACE2 and TMPRSS2 based on the individual cancer stage. **(E & F)** mRNA levels of ACE2 and TMPRSS2 based on different age groups. **(G & H)** mRNA levels of ACE2 and TMPRSS2 based on tumor grades.

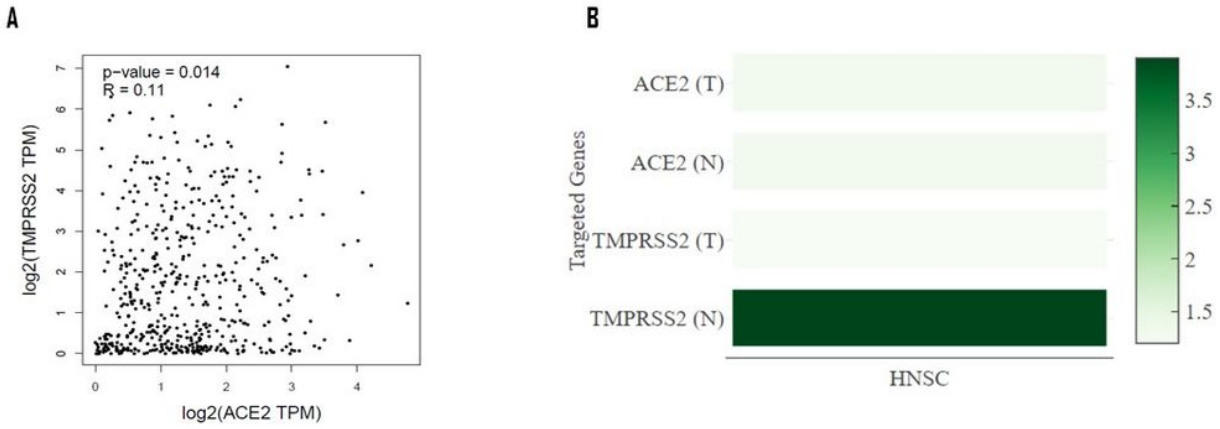

Figure 3

Association between ACE2 and TMPRSS2. (A) Correlation between ACE2 and TMPRSS2. Spearman's Rho Correlation was performed to assess the association between ACE2 and TMPRSS2. **(B)** Heatmap based on the expression score of ACE2 and TMPRSS2. $P \leq 0.05$ is considered statistically significant.

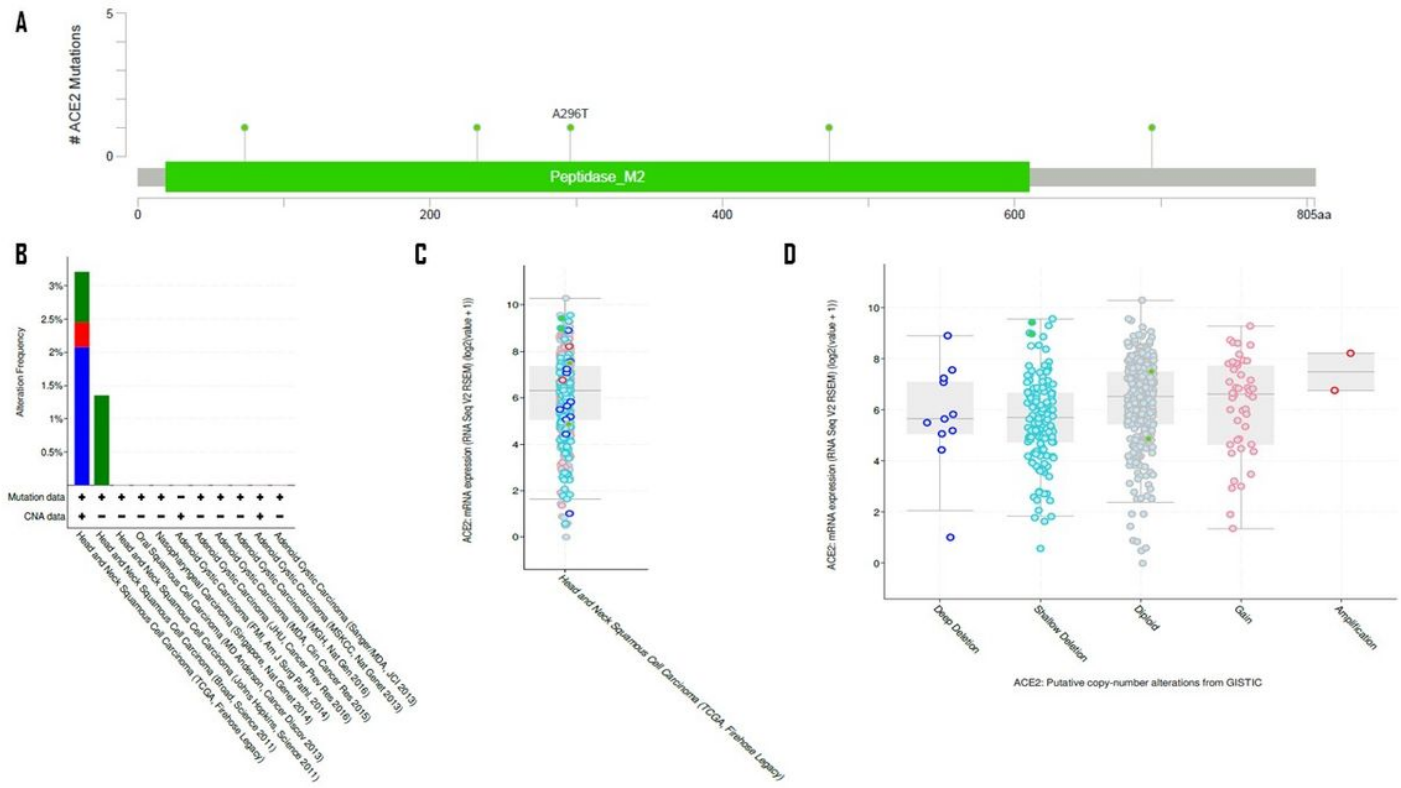

Figure 4

Mutation and CNAs in ACE2 Protein Sequence. (A) Types of Mutations in ACE2. **(B)** Frequency of Genetic Alterations in ACE2. **(C & D)** Various types of copy number alterations in ACE2.

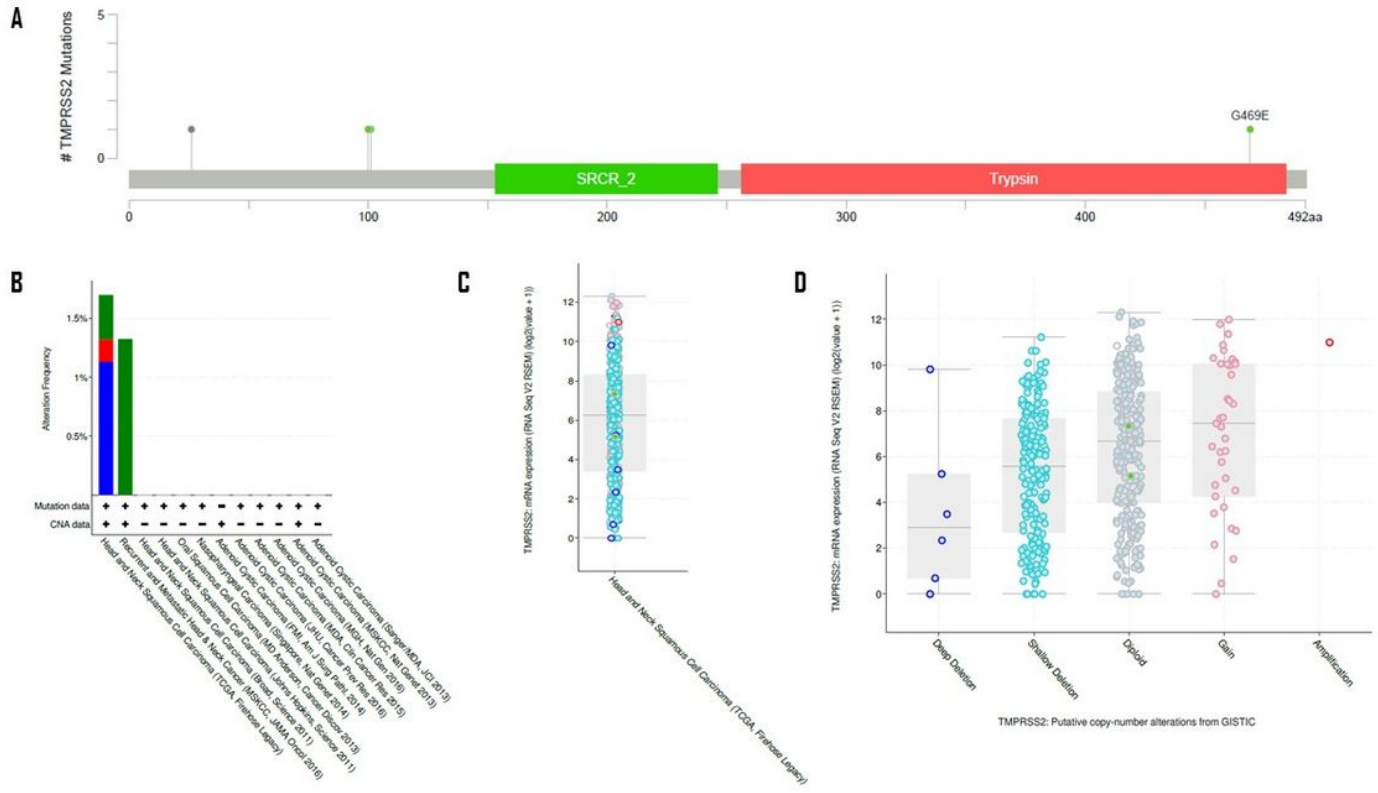

Figure 5

Mutation and CNAs in TMPRSS2 Protein Sequence. (A) Types of Mutations in TMPRSS2. **(B)** Frequency of Genetic Alterations in TMPRSS2. **(C & D)** Various types of copy number alterations in TMPRSS2.

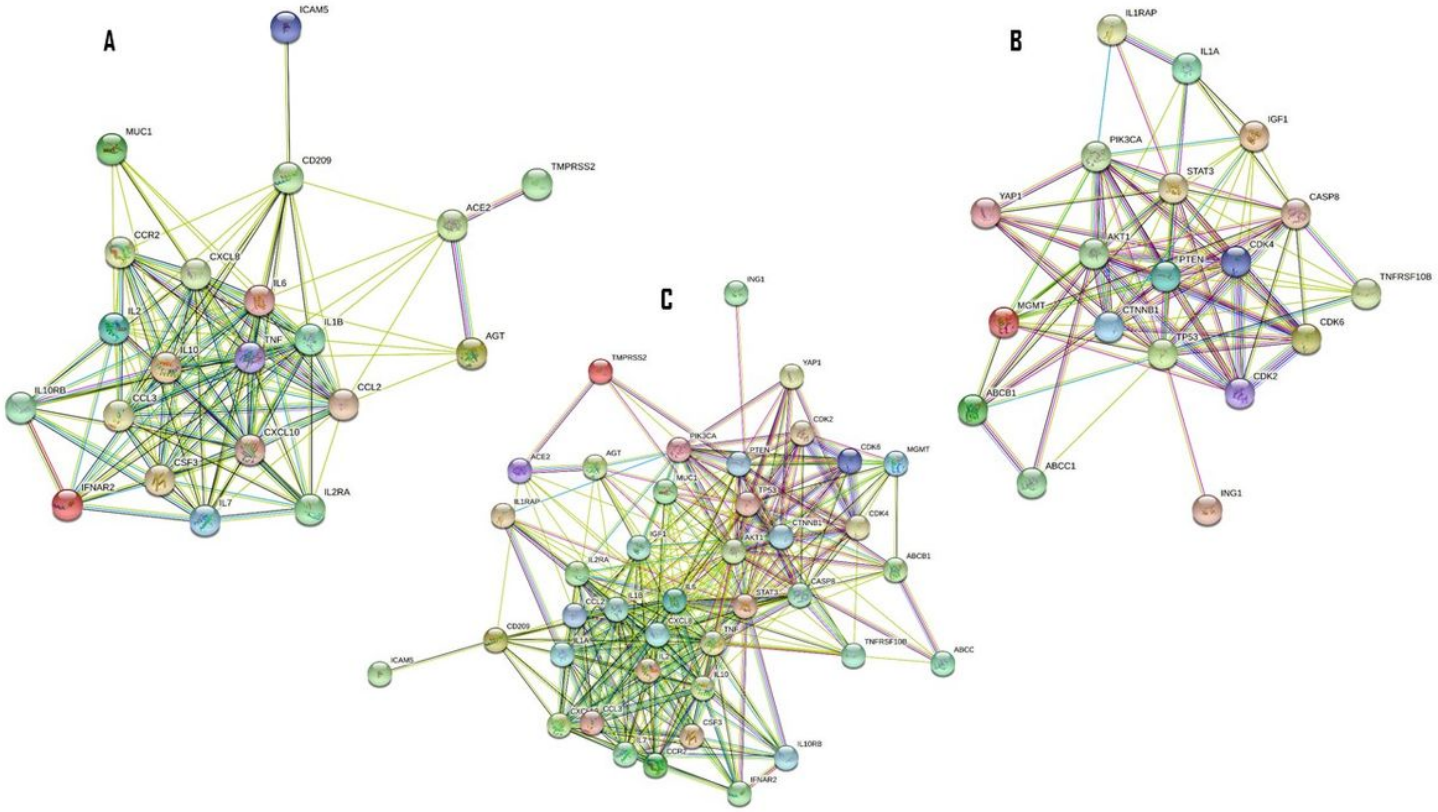

Figure 6

Protein-Protein Interaction Network with various proteins associated with COVID-19 and HNSC. (A) PPI analysis among the genes associated with COVID-19. **(B)** PPI analysis among the genes associated with HNSC. **(C)** PPI analysis among the genes associated with COVID-19 & HNSC.

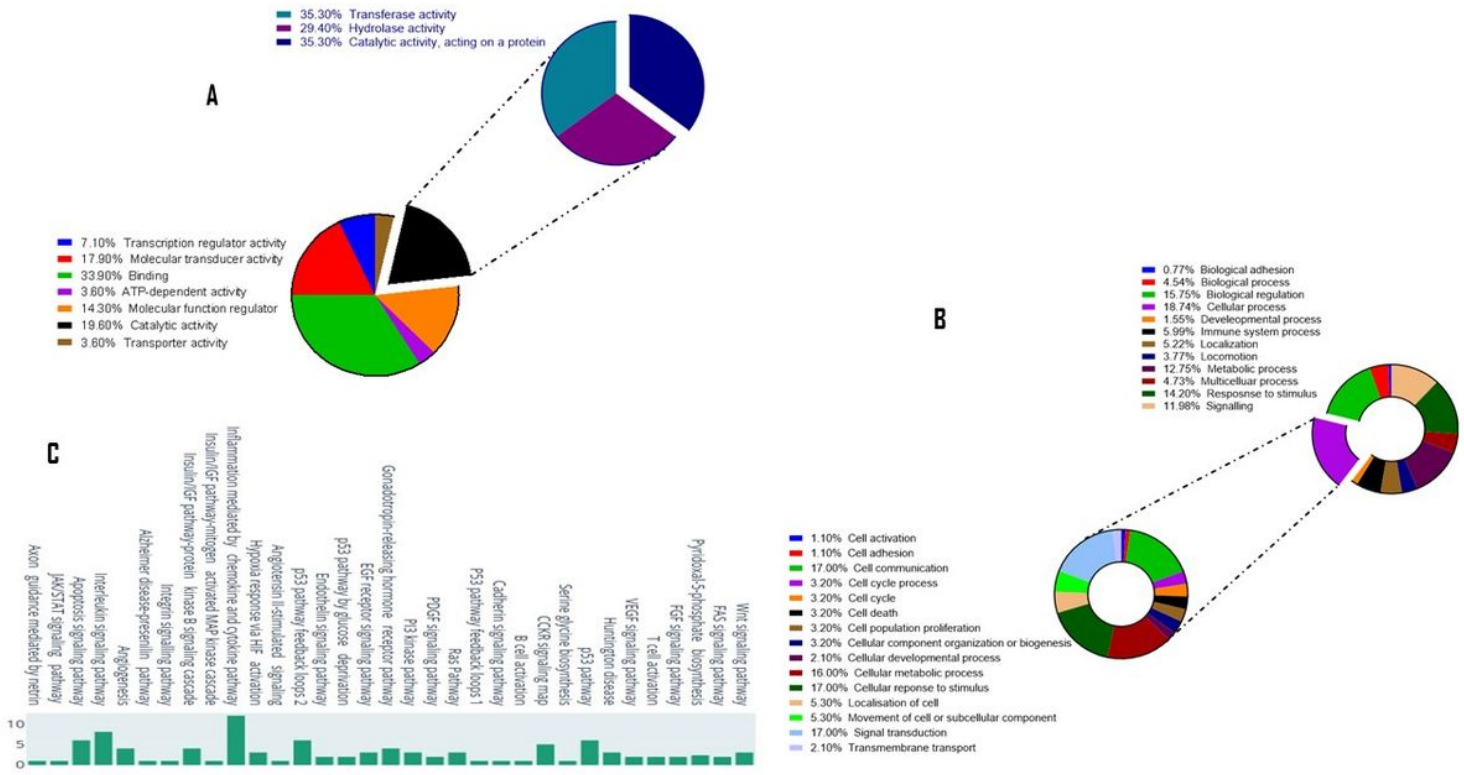

Figure 7

Functional role of genes associated with COVID-19 and HNSC. (A) Molecular Activity of genes associated with COVID-19 and HNSC. **(B)** Biological processes of genes associated with COVID-19 and HNSC. **(C)** Signalling pathways of genes interconnected with COVID-19 and HNSC.