Review Article

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New Emerging SARS-CoV-2 Virus; Structure, Function, and Bioinformatics Analysis

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Abstract

The novel coronavirus, known as a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), is a single-stranded RNA virus, from which structural and some accessory proteins are encoded. It seems that this newly emerged virus uses the ACE2 receptor to enter the cells in the human body. SARS-CoV-2 undergoes an intense immunological pressure in humans, and this generates mutations to bypass the immune system. Some mutations have been detected in this virus genome, which can induce change in viral potency. By performing pathway enrichment analysis over those genes and identifying relevant protein-protein interactions (PPIs), we were able to list essential pathways affected in infected cells. In this review, we mainly discuss genetic, intracellular mechanisms also diagnosis and feasible therapeutic targets of this novel coronavirus.

Keywords: Genomic Structural Variation; SARS-CoV-2; Signal Transduction; Mutation.

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Introduction

The family Coronaviridae comprised of a broad spectrum of animal and human viruses. Constituents of the subfamily Coronaviridae are categorized into four genera: 1-Alphacoronavirus (α-CoV) 2-Betacocronavirus (β-CoV) 3-Gammacoronavirus (γ-CoV) 4-Deltacoronavirus (δ-CoV) (1). Beta-coronaviruses are essential to consider as they have produced overwhelming outbreaks in recent decades. In 2002, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) was first seen in the Guangdong province, China. The origin of this virus was a bat and transmitted to humans by an intermediary host (palm civet) (1, 2).

Ten years after, Middle East Respiratory Syndrome Coronavirus (MERS-CoV) emerged in Saudi Arabia, in which patients underwent from pneumonia.

This coronavirus, as like as SARS-CoV, originated the bat and crossed over to humans by the intermediary host (camels) (3, 4).

SARS-CoV and MERS-CoV are beta-coronaviruses that lead to a broad spectrum of clinical features from asymptomatic to the rapidly lethal disease; especially in patients with multiple comorbidities (5).

In December 2019, a number of patients with severely ill disease were referred to hospitals with an early diagnosis of progressive pneumonia in an unrecognized manner.

These were epidemiologically linked to Huanan Seafood Wholesale Market, Hubei Province, China.

The infection began to spread from the Huanan Seafood Market, while the precise infection track of the first case remains obscure. However, the virus originated from bats, but the intermediary host is not yet known, although scientists suspect the Pangolins (6, 7). Soon, this novel coronavirus, which is known as SARS-CoV-2, extend quickly throughout the world, and the number of infected cases has been increased. Based on the large number of affected patients that were exposed to the seafood market in Wuhan city where live wild animals are routinely sold make physicians thought it might be a zoonotic viral organism (8). Current reports reveal that person-to-person transmission is the most common route of spreading for COVID-19 infection. This theory is supported by cases that arise within families and people who did not visit the market. Usually person-to-person transmission occurs via direct contact or through respiratory aerosol spread by coughing or sneezing from an infected patient (9).

Structure

Coronaviruses are spherical, enveloped single-strand (positive) RNA viruses, which are 120-160 nm in diameter. They have the largest genome (26.4 to 31.7 kb in length) compared with other known RNA viruses (10). Because of their spike proteins on their surface, they have a crown appearance under an electron microscope or as they say corona in Latin. Coronavirus genome contains both 5' UTR and 3' UTR, and at least six open reading frames (ORFs). The first gene from 5' end have ORF1a and ORF1ab, and they interpreted into Poly-Protein 1a (PP1a) and Poly-Protein 1ab (PP1ab). These two proteins are processed by viral proteases, Chymotrypsin-Like protease (3CLpro) or Main protease (Mpro), and papain-like proteases, into 16 non-structural proteins. These non-structural proteins (naps) form viral replicase–transcriptase complexes. The genome of these viruses encodes four structural proteins (ORF 10 and ORF11, near to 3'End), including; Spike (S), Envelope (E), Membrane (M), and Nucleocapsid (N) proteins. (Figure 1) Furthermore, different coronaviruses encode other structural and ancillary proteins such as; Hem Agglutinin-Esterase proteins (HE) protein, 3a/b protein, 4a/b protein, and 9b protein (11-15). It had been revealed that coronaviruses membrane contains 3 or 4 viral proteins; which includes membrane (M), spike (S), small envelope (E), and in some Hemagglutinin-Esterase (HE) proteins (16). The S proteins are presented on the coronaviruses surface and form palmers. This protein is a type 1 membrane glycoprotein with a short C-terminal transmembrane domain, and they have the most variable sequences in the coronaviruses genome. Sequence comparison among SARS-CoV-2, SARS-CoV, and MERS-CoV revealed different S protein sizes; 1273 amino acid (aa), 1255 aa, and 1270 aa, respectively. (Figure 2) Coronaviruses bind to Angiotensin-Converting Enzyme 2 (ACE2) via S protein; as a cell entry receptor in human SARS-CoV too (10, 17, 18). Following that, in order to coronavirus fully enter into a host cell, a cellular protease called Transmembrane Protease Serine 2 (TMPRSS2) should be primed the spike protein. It is noteworthy that in this process, SARS-CoV-2 (similar to SARS-CoV) uses the same protease (19).

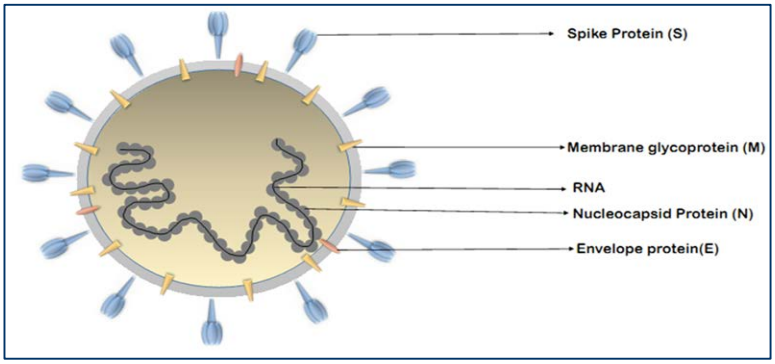

Figure 1. Schematic structure of coronavirus. Coronavirus are enveloped single stranded RNA viruses. Their membrane possesses, Membrane (M), Spike(S), Envelope (E) structural proteins. Other structural protein, Nucleocapsid (N), encapsidate viral RNA genome and forms Ribonucleoprotein(RNP) complexes (known as a capsid).

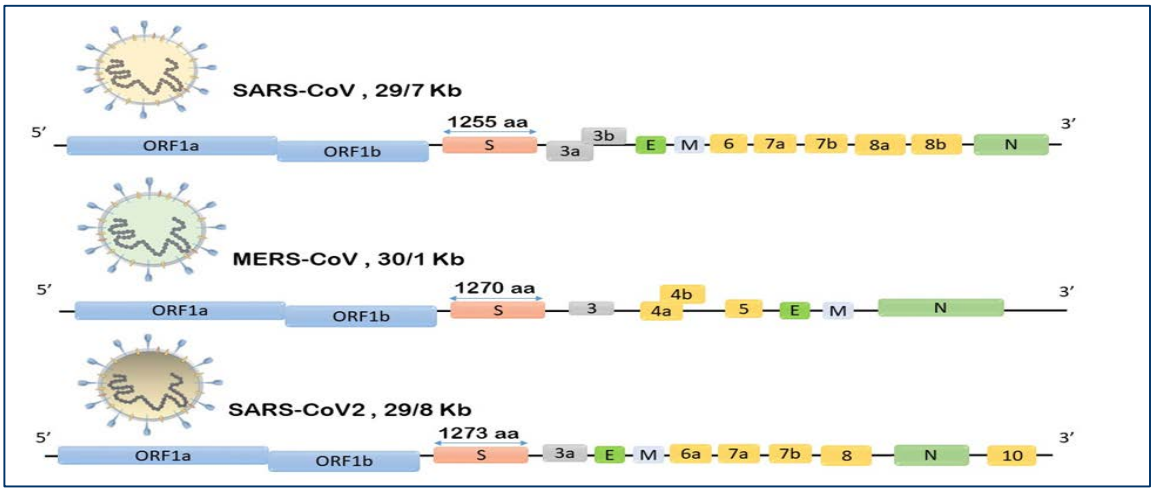

Figure 2. Comparison of SARS-CoV, MERS-CoV, and SARS-CoV2 genome structure. Different open-reading frames exist in the viral genome. ORF1a and ORF1b are translated into large poly-proteins (PP1a, PP1ab). Particular protease such as 3CLpro and PLpro cleaves these poly-proteins proteolytically into 16 Non-structural proteins, including proteases, polymerase and helicase. These coronaviruses contain four structural proteins, which are encoded by S, E, M, and N genes, and other accessory proteins. Among them, the S gene has the most variable sequence in the viral genome. Accessory proteins (e.g. 3a, 3b, 6, 7a, 7b, 8a, 8b, 10), which are interspersed throughout the viral genome, and involved in host innate immune response and may have other unknown functions.

Another membrane protein known as (HE) exists in human coronavirus OC43 (HCoV-OC43) and HKU1 (HCoV-HKU1). HE has neuraminate O-acetyl esterase activity. It has been believed that HE gene probably acquired from influenza C virus, by the mechanism called heterologous recombination, in which new genes acquires from another non-coronaviruses(20). One of the matchless features of coronaviruses is exoribonuclease (ExoN) function which is encoded by non-structural protein 14 (nsp14). This protein has a proofreading role in maintaining sizeable viral genome from the accumulation of harmful mutations (21-23).

Coronaviruses pathophysiology, as well as SARS-CoV-2, depends on the function of non-structural and structural proteins. For example, investigations revealed that the host innate immune response could be inhibited by viral non-structural proteins (24).

Intracellular mechanisms

Studies have shown that SARS-CoV-2 and SARS-CoV both act similarly in their pathogenicity, clinical spectrum, and epidemiology. They also suggest that SARS-CoV-2 uses the same receptor to enter the cells in the human body as SARS-CoV (25). In both cases, spike proteins which are anchored into the envelope bind to the ACE2 receptors (26). SARS-CoV spike protein (SARS-S) and SARS-CoV-2-S are 76% identical (19). Spike proteins have two different subunits S1 at the C-terminal, which binds to the ACE2 receptor, and S2 subunit at the N-terminal that mediates membrane fusion (27, 28). It has been proven that two trimmer of S proteins simultaneously binds to a dimer of the ACE2 receptor (29). To date, it has not been confirmed whether COVID-19 acts the same as SARS after the cell attachment or not. After attachment, SARS-S will be primed for two cleavages at the S1/S2 and the S2' sites by cellular proteases. Then, the S2 subunit will induce the virus to fuse into the host cell. In 2020, Hoffmann et al. demonstrated that SARS-CoV-2 uses the same serine protease for priming the S protein as SARS virus called Transmembrane Protease Serine 2 (TMPRSS2) (19),consistent with Glowacka et al study (30). In the infected cells, the S proteins are synthesized in the constitutive secretory pathway, and during this path, they become trimeric and undergo extensive modifications by N-glycans (31). After the entrance, the virus genome will be uncoated and transcribed into two main proteins called PP1a and PP1ab (32), which forms Replication-Transcription (RT) complex in a vesicle (33). Furthermore, produce different kinds of RNAs (34) which are going to be transcribed into the accessory and structural proteins that are necessary for packaging new viruses. 3-Chymotrypsin-Like cysteine protease (3CLpro) and

Papain-Like protease (PLpro) are two proteases that make this proteolysis process possible. To replicate the viral replicase –RNA dependent RNA polymerase- is produced. (35) Finally, the newly formed RNAs and proteins assemble and move towards the cytoplasmic membrane through a vesicle and release by exocytosis (36-38). After maturation, the newly released virus will induce the inflammatory pathway by interacting with Toll-Like Receptors (TLRs) (39). In severe cases, high levels of pro-inflammatory cytokines such as IL-6 and IL-1 will happen which as a result a massive amount of cytokines will be released and the "cytokine storm" will occur (40).

There are two forms of ACE2, the full-length form and the soluble form. The former has a transmembrane domain which is anchored in the cell membrane and an extracellular domain which is the receptor for S proteins of SARS-CoV and SARS-CoV-2 (17, 41). The latter is mostly present in the blood circulation in small amounts rather than being anchored in the cell membrane (42). One of the reasons that SARS-CoV-2 is highly contagious is that its S protein affinity to ACE2 is 10-20 fold higher than SARS-CoV.

Interestingly, Jiang Gu et al. managed to prove that ACE2 expression does not necessarily correspond with the degree of infection since they found some ACE2 expressing cells without any SARS-CoV infection while the virus infected the non ACE2 expressing cells. So they concluded that probably some additional co-factors are needed for productive infection (43). ACE2 is a part of the renin-angiotensin system, and its physiological role is to make angiotensin 1-7, which is a vasodilator, either indirectly by cleaving the angiotensin I and producing angiotensin 1-9 or directly by processing angiotensin II (44, 45). Angiotensin is a hormone which regulates blood pressure and the constriction of blood vessels (46). ACE2 gene is mapped on the X chromosome and has a ubiquitous expression in the body (47).

In February 3rd, 2020 Li Y et al. showed the risk for different organs to be attacked by SARS-CoV-2 by analyzing the transcriptome of human tissues and found out that ACE2 is expressed in heart, kidney, testis, colon and gut (48). In February 23rd 2020, Xiaodong Jia et al. suggested that the obese and those affected by specific cancers are more liable to COVID-19 infection due to the higher level of ACE2 expressions on their cells (48).

In February 25th 2020, Ying Chen et al. indicated that there is no difference between the ACE2 expression rate in different races and genders (49). However, Yu Zhao et al. achieved the opposite results and proposed the hypothesis that ACE2 expression is higher in Asians than whites or African Americans (50). In February 27th 2020, Pei-Hui Wang et al. found high expression of ACE2 in spermatogonia, leydig and sertoli cells of the testis. They also reported that ACE2 expression would be upregulated in cells infected by various viruses (51). In March 2nd 2020, Guoshuai Cai proved that smokers are more prone to infection by SARS-CoV-2 since those with a high level of expression are more vulnerable to be infected by SRAS-CoV-2 and that ACE2 expression rate in lungs, increases by smoking (52).

SARS-CoV-2 Variants

SARS-CoV-2 undergoes an intense immunological pressure in humans, and this generates mutations to bypass the immune system. These alterations could result in changes in viral potency such as virulence, infectivity, and transmissibility (53, 54). A recent study shows that SARS-CoV-2 has no more alterations and is relatively conserved. The regions which were not found mutations are E, 6, 7b regions. However, this virus has some hotspot areas such as ORFs 1a, S, 8, and the N region, which cause alterations in the amino acid sequences of viral proteins; therefore, these mutations can affect viral replication and transmission (17, 55).

In an investigation that has been taken place from December-2019 until 05 of April-2020, they analyzed 95 SARS-CoV-2 complete genome sequences available in GenBank, National Microbiology Data Center (NMDC), and NGDC Genome Warehouse. They revealed that there are three most common mutations in SARS-CoV-2, including 8782C>T in ORF1ab gene, 28144T>C in the ORF8 gene, and 29095C>T in the N gene (56). In another study, they performed meta-transcriptome sequencing for the broncho-alveolar lavage fluid sample from eight SARS-CoV-2 patients, 25 community-acquired pneumonia (CAP) patients, and 20 healthy controls. Their findings represent that the number of intrahost variants ranged from 0 to 51, with a median number of 4, suggesting a high evolution rate of the virus.

By investigating a person-to-person spread event, they found no evidence for the transmission of intra-host variants (57).

To analyze genetic variants, scientists collected 86 complete or near-complete genomes of SARS-CoV-2 from GISAID [https://www.gisaid.org/]. These strains were detected in infected patients from different countries such as China (50), USA (11), Australia (5), Japan (5), France (4), Singapore (3), England (2), Taiwan (2), South Korea (1), Belgium (1), Germany (1), and Vietnam (1). These observations represented the genetic diversity and rapid evolution of this novel coronavirus. Their genetic analysis discovered three deletions within the genomes of SARS-CoV-2 from Japan (24 nucleotides deletion in ORF1ab poly-protein), USA (3 nucleotides deletion in ORF1ab poly-protein), and Australia (10 nucleotides deletion within the 3′ end of the genome). Furthermore, nucleotide sequence alignment also revealed 93 mutations over the whole genomes of SARS-CoV-2 (58).

The spike surface glycoprotein plays a vital role in binding to receptors on the host cell and determines host tropism (59). It is also the main target of neutralizing antibodies (60).

Mutations within the spike surface glycoprotein might induce its conformational changes, which probably led to alteration of its antigen potential. Three mutations (including D354, Y364, and F367) within the receptor-binding domain of the spike surface glycoprotein have been located (58).

Phylogeny analysis of SARS-CoV-2

Phylogenetic analyses were done to determine the developmentaly relationships between SARS-CoV-2 and pangolin-CoV, and previously identified coronaviruses based on the nucleotide sequences of the whole-genome sequence, RNA-dependent RNA polymerase gene (RbRp), non-structural protein genes ORF1a and ORF1b, and main structural proteins encoded by the S and M genes.

The most intriguing finding is that in all sequences, pangolin-CoV, RaTG13, and SARS-CoV-2 showed high similarity, so clustered in a well-supported group. S1 protein of pangolin-CoV is less closely related to RaTG13 than to that of 2019-CoV. Furthermore, the analysis showed that the pangolin-CoV and SARS-CoV-2 were highly conserved in RNA binding motif (RBD motif), except for one amino acid change (500H/500Q), which is not one of the critical residues involved in the interaction with human ACE2 (27, 60).

Mutation around the globe

The SARS-CoV-2 virus uses error-prone RNA polymerase for replication so that its genome will build up mutations during every copying cycle. Because the copying cycle can come about in hours, a single infected host can build a diverse virus population. Fantastic capacity to mutate that changes how a virus is transmitted or its virulence, it will not spread to high frequencies only if it is selectively advantageous (61).

Similar work has also been pursued, in which 53 full genomics 2019-nCoV sequences from the Gisaid database plus the GenBank-deposited sequence from the Wuhan seafood market pneumonia virus isolate Wuhan-Hu-1 and two partial sequences from Italian isolates. The analysis showed two core positions of high variability remaining have low variability (>99% sequence identity) within the 56 2019-nCoV genomes available.

One in ORF1ab locus is demonstrating in a silent variant and the other in ORF8, resulting in one of its two variants, ORF8-L and ORF8-S. They claim ORF8-L and ORF8-S can change protein structure individually, the 83-89 amino acidic region. Based on structural analyses, ORF8-S isoform is more affinity and pathogenic (62).

The other phylogenetic study conducted using a total of 145 sequences were obtained from databases, 50 sequences were excluded from the study according to predetermine criteria result, 95 sequences. In a homology analysis of sequence, they found 99.99% (99.91%-100%) homology among full-length genomics sequences, and 15 (15.79%) clinical isolates were identical to the reference strain. Interestingly in encoding regions, the homology in ORF1a was 99.99% (99.88%-100%); the majority of other regions showed 100% homology; no variation was found in E6 and 7b regions.

The homology among full length sequences at amino acid level was 99.99% (99.79% to100%), with homology among most isolates in each region being 100% overall variation in both nucleotide and amino acid levels were rare, mutation in:

1a (nt2662,8782,11083, 1b (nt17373,18060,

S (nt21707,2403, 3a（nt26144, M（nt26729,

8（nt28077,28144, N（nt28854, 29095) showed in more than three strain phylogeny result in tight cluster strain for SARS-CoV-2 strains (55).

Diagnosis

The diagnosis of viral types of pneumonia, such as caused by SARS-CoV-2, involves collecting the correct specimen from the patient at the proper time. The sample can be obtained through respiratory sources such as nasal, throat, nasopharyngeal, bronchial fluid swabs, and sputum (63). However, the US centers for disease control and prevention (CDC) recommends collecting the nasopharyngeal swab. (https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html) accessed March 16th 2020). Laboratory characteristics of patients infected with 2019-nCoV normal or decreased white blood cell counts, and lymphocytopenia especially with CD_4^+, CD_8^+ reduction, in those severe patients which consistent with the main characteristic of viral infection (64). Although the prothrombin time, lactate dehydrogenase (LDH), D-Dimer, alanine transaminase, C-reactive protein (CRP), creatinine kinase (CPK), and erythrocyte sedimentation rates (ESR) are elevated. Several reports, interestingly, suggested that some indications such as prothrombin time and D-dimer level, cytokines including IL2, IL7, IL10, GSCF, IP10, MCP1, MIP1A, and tumor necrosis factor-α(TNF-α) were higher in ICU patients compared with non-ICU (63). Laboratory findings were likely to those observed in patients with MERS-CoV and SARS-CoV infections (65).

Specific molecular tests can achieve a specific diagnosis of respiratory specimens. The Real-time PCR assay is a molecular test that can be used for the SARS-CoV-2 infection diagnosis (66).

Various molecular tests have been designed and established for 2019-nCoV diagnosis, based on their detection efficiency, the N gene RT-PCR is the recommended test for screening, and the ORF1b assay is suggested as an affirmative. If the positive PCR results occurred, sequence analyses of positive amplicons might be helpful for results confirmation and differential diagnosis between 2019-nCoV and other coronaviruses such as SARS-CoV (49).

Although the real-time reverse transcription-polymerase chain reaction (RT-PCR) is a reference standard test for the detection of viral nucleic acid, some scientific reports demonstrate that this technique is impotent to detect in those with false-negative RT-PCR or without symptoms. In order to puzzle out of this, recent studies recommend using the chest computed tomography (CT) examination (67). Diagnosis of COVID-19, despite its usage, may have some challenges because the laboratory findings and radiographic features are not always compatible with clinical characteristics, and COVID-19 disease manifestations are variable and changed rapidly, therefore deciding on these may be fall in trouble. Laboratory detection methods included genomic sequencing, reverse-transcription polymerase chain reaction (RT-PCR), and serological practice (51, 68).

As we know, the RT-PCR method, as like other examination, may have false positive and false negative results, especially in SARS-CoV-2 detection (69, 70).

According to recent diagnosis and treatment guidelines for COVID-19, if one person is tested twice by RT-PCR, and the results were negative, it means that person is recovered and can be discharged from the hospital. However, some studies indicated that some patients who have discharged from hospitals their RT-PCR test result is positive (71).

Among patients admitted to hospital due to COVID-19, patients with symptoms of pneumonia and fever who do not have COVID-19, but have other coronavirus diseases such as influenza, or even patients who have been hospitalized, are found to be unaffected and have no disease. Because RT-PCR could generate false-positive results due to sample contaminations or other reasons (69).

By technical difficulties, deletions, and mutations in the SARS-CoV-2 genome that occurred during viral evolution may also contribute to false results produced by RT-PCR.

Possible targets for treatment

Possible treatments can interfere with the virus mechanisms of host responses. By virtual screening, Canrong Wu et al. predict that although ACE2 inhibitors bind to the ACE2, they only inhibit its enzymatic function and do not hinder infection because they do not bind to the contact surface of ACE2–Spike complex (69). However, Han Y et al. claimed that base on the classical molecular dynamics simulations; peptide inhibitors extracted

from ACE2 could be very promising for SARS-CoV-2 infection blocking (70).

A soluble form of ACE2 can inhibit virus replication in monkey kidney cell lines, Vero-E6, under *in vitro* circumstances (72). To achieve this, drugs that can impede virion's (virus) RDRP like remdesivir and favipiravir may be a possible treatment for this infection (73).

Another possible treatment can be drugs like Lopinavir and Ritonavir, which inhibit the cellular proteases and thus prevent the virus assembly (70, 73, 74). Other possible approaches may be the inhibition or reduction of cytokine production or any other component of the inflammatory pathway (69).

For instance, Chloroquine and Hydroxychloroquine can prevent TLR responses by changing the endosomal pH. These drugs can also affect the inflammatory pathway and cytokine release and can even adjust the inflammatory pathway by upregulating anti-inflammatory components (75, 76).

Based on the bioinformatics analysis, Baricitinib can play a role in SARS-CoV-2 infection reduction by inhibiting AP-2-associated protein kinase 1 (AAK1), which acts in the viral cellular entry or dampen the inflammatory pathway by blocking Janus kinases (JAK) 1 and 2, which involved in the inflammatory pathway (76).

Scientist believes that maybe even immunosuppressive agents such as Tocilizumab (IL-6 blockade) and Anakinra (IL-1 blockade) can be helpful in severe cases of COVID-19 (77).

In conclusion, we would like to mention that Canrong Wu et al. managed to provide 21 targets for possible treatments including virus coding proteins and human components functioning in immunological responses by performing target-based virtual ligand screening (69).

Three major types of vaccines suggested are the whole vaccine; sub unites vaccine, nucleic acid vaccines. Subunit vaccines for both SARS coronaviruses rely on eliciting an immune response against the S-spike protein to prevent its docking with the host ACE2 receptor (50).

Although multiple genes regulate the viral mode of transmission and virulence, the chance to make more virulent virus decrease, mutations are not indicative of outlandish and devastating new viral characteristics. Instead, they can inform our understanding of emerging outbreaks (63).

COVID-19 related genes

We first identified the candidate genes which could be involved in COVID-19 by reviewing articles and UniProt database (https://www.uniprot.org/). 62 regulated genes have been recognized (Table 1). Because COVID-19 is not yet a catalogued disease in this database, we searched for gene products associated to the following available key terms: ("coronavirus infectious disease" OR "severe acute respiratory syndrome" (SARS) OR "SARS Cov" OR "MERS Cov") AND "Homo sapiens".

Pathway enrichment and protein-protein interaction (PPI) analyses

For our goal to identify major cellular pathways putatively affected by the viral infection, we performed pathway enrichment analysis with our gene-list by using String database (https://string-db.org/).

After we identified all direct PPI among the proteins in the gene-list, we found the most important genes in the network, by using network analysis (MCODE plug-in) in Cytoscape which include:

PTGS1
MT-CO3
MT-CYB
MT-ND1
MT-ND6
MT-ATP6
MT-ND2
POLG
MT-CO2
MT-ND3
MT-ND4L
MT-CO1
and *MT-ATP8*.

The Molecular Complex Detection (MCODE) plug-in (http://apps.cytoscape.org/apps/mcode) Used to screen PPI network hub gene modules with cut-off degree =10, haircut on, node score cut-off = 0.2, k-core=5, and max. Depth=100.

Among the statistically enriched pathways ($p < 0.001$), our pathway enrichment analysis identified numerous signal transduction pathways involved in the immune response, respiratory electron transport, cytokine signal transduction, oxidative phosphorylation, metabolic pathways and thermogenesis (Table 2).

Table 1. Gene-list obtained from articles and UniProt database

NLRP3	DDX58	HLA-A	COX3
ACE2	MDA5	MT-CO1	ATP8
TMPRSS2	IFIH1	TMPRSS11D	ND3
FTO	CCL2	DDX1	ND1
MT-ND6	MBL	SPIDR	ATP6
CD209	SMAD3	ABCA3	CLEC4G
MT-ATP6	TLR3	ADAM17	IFITM2
SDHD	CD55	SGTA	IFITM1
POLG	CD4	HPN	IFITM3
SFTPB	CD8	COX1	MPP5
CLEC4M	CPK	ND2	ITGAL
OPA1	IL2	COX2	KPNA4
MT-CO3	IL7	CYTB	CYTB
SFTPC	IL10	ND3	ND4L
HADHA	MCP1	ND6	TNF
LHX4	MIP1A		

Table 2. Pathway enrichment of gene list

ID	term description	observed gene count	background gene count	false discovery rate	matching proteins in the network (labels)
hsa00190	Oxidative phosphorylation	12	131	5.89E-13	MT-ATP6,MT-ATP8,MT-CO1,MT-CO2,MT-CO3,MT-CYB,MT-ND1,MT-ND2,MT-ND3,MT-ND4L,MT-ND6,SDHD
hsa163200	Respiratory electron transport, ATP synthesis by chemiosmotic coupling, and heat production by uncoupling proteins.	11	123	2.45E-11	MT-ATP6,MT-ATP8,MT-CO1,MT-CO2,MT-CO3,MT-CYB,MT-ND1,MT-ND2,MT-ND3,MT-ND6,SDHD
hsa04714	Thermogenesis	12	228	9.78E-11	MT-ATP6,MT-ATP8,MT-CO1,MT-CO2,MT-CO3,MT-CYB,MT-ND1,MT-ND2,MT-ND3,MT-ND4L,MT-ND6,SDHD
hsa01100	Metabolic pathways	16	1250	5.55E-06	HADHA,MT-ATP6,MT-ATP8,MT-CO1,MT-CO2,MT-CO3,MT-CYB,MT-ND1,MT-ND2,MT-ND3,MT-ND4L,MT-ND6,PIK3C2A,POLG,PTGS1,SDHD
hsa168256	Immune System	24	1925	5.79E-09	ADAM17,CCL2,CCL3,CD209,CD4,CD55,CD8A,CLEC4G,DDX58,HLA-A, IFIH1, IFITM1, IFITM2,IFITM3,IL10,IL2,IL7,ITGAL,KPNA4,MBL2,NLRP3,SMAD3,TLR3,TNF
hsa1280215	Cytokine Signaling in Immune system	15	654	1.50E-08	ADAM17,CCL2,CCL3,CD4,DDX58, HLA-A, IFITM1, IFITM2, IFITM3,IL10,IL2,IL7,KPNA4,SMAD3,TNF

Conclusion

In conclusion some mutations have been detected in this virus genome, which makes its viral potency change. By performing pathway enrichment analysis over those genes and identifying relevant protein-protein interactions (PPIs), we were able to list essential pathways affected in infected cells.

Conflict of Interest

The authors declare no conflicts of interest.

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Authors Contributions

Not declared.

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