COVID-19

Novel Coronavirus (nCoV): a Bitter Old Enemy in a New Avatar

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Abstract

Currently, pandemic coronavirus disease 2019 (COVID-19) is the biggest threat to all human beings globally. Till June 8, 2020, it has infected 6,931,000 people and caused 400,857 deaths worldwide. The first case was identified in a patient with influenza-like symptoms along with severe acute respiratory syndrome in Wuhan, China, in December 2019 and now it has spread in more than 200 countries. Since there is no approved cure for this disease until now, there is a lot of mass fear, apprehensions, and questions globally regarding (i) genetic origin and history of the novel coronavirus, (ii) what are the first-line therapies for those who contract this disease, and (iii) what could be the potential vaccine targets. In this short review, we have tried to address these queries in the simplest manner and compiled the history of previous coronaviruses, recent developments in the COVID-19 research, potential future therapeutics, and possible targets to cure the disease.

Keywords COVID-19 · Novel coronavirus · Pandemic · nCoV · Pneumonia · ACE2 · Plasma therapy

Introduction

The novel coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a public health emergency, posing a serious threat to people's health as well as the medical and health system worldwide. On February 11, 2020, World Health Organization named disease caused by a novel coronavirus (2019-nCoV) as coronavirus disease 2019 (COVID-19) [1, 2]. Initially, this was identified in three patients, who were hospitalized in Wuhan, China, from December 2019 to January 2020. The ailment which had likely been caused by this novel coronavirus (nCoV) was named "Novel Coronavirus-Infected Pneumonia" (NCIP). Genome sequence analysis revealed that nCoV falls into the genus *Betacoronavirus* and subgenus *Sarbecovirus* which also includes coronaviruses (SARS-CoV, bat-SARS-like CoV, and others) discovered in humans, bats, and other wild animals [3–7].

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Coronaviruses contain RNA as genetic material and are found predominantly in humans, other mammals, and birds. There are primarily six coronavirus species known to cause different types of respiratory, neurological, and hepatic diseases [8, 9]. Out of six, four species NL63, OC43, HKU1, and 229E predominantly infect humans and usually cause moderate respiratory tract illness such as common cold [10]. The two other strains which cause severe acute respiratory syndrome (SARS-CoV) and Middle East Respiratory Syndrome (MERS-CoV) are zoonotic in origin and have been related to deadly infections [11–14]. SARS-CoV was the causal agent of the severe acute respiratory syndrome outbreaks in 2002 and 2003 in Guangdong Province, China [14–17].

Although so far there are controversies about the source of the COVID-19 and its intermediate host, the evolutionary analysis showed that the coronavirus was most similar to bat coronavirus isolate RaTG13 (GenBank No. MN996532). It has a linear single-stranded RNA genome ~30 kb [5, 7, 18] being 96.2% homologous to the nucleotides in the whole genome of the same. Also, it is interesting to note that sequences from the number of recent patients are strikingly identical and show 79.6% sequence identity to SARS-CoV [7, 18, 19]. Sequence alignment of 2019-nCoV, RaTG13, and SARS-CoV showed no proof of any kind of recombination in the genome of nCoV [19].

Along with flu and respiratory problems, people of all ages are susceptible to COVID-19, and there was no significant

gender bias in terms of viral infection [2]. Recent researches have revealed that COVID-19 is less lethal in children than adult patients, though some infants were vulnerable to infection [20]. Also, prominent changes were seen in the blood coagulation of patients with SARS-CoV-2 infection. The coagulation function in patients with SARS-CoV-2 is significantly unstable in comparison to healthy people [21]. It is also reported that the patients with pre-existing cardiovascular metabolic diseases such as hypertension, cardia-cerebrovascular diseases, and diabetes are at a greater risk of developing severe conditions and these pre-existing ailments can also greatly affect the prognosis of the COVID-19 [22].

WHO on March 11, 2020 declared COVID-19 a pandemic, pointing to over 118,000 cases of the coronavirus illness in over 110 countries and territories around the world and the sustained risk of further global spread [23]. From December 12, 2019, when the first patient was admitted to hospital, to June 8, 2020, there are 6,931,000 confirmed cases and 400,857 confirmed deaths in more than 200 countries. European region with 2,286,560 positive cases and 184,120 deaths and regions of America with 3,311,387 positive cases and 181,804 deaths are the most affected regions worldwide. In Europe, the UK is the worst affected country with 40,542 deaths to date, followed by Italy, Spain, France, and Germany [24, 25].

Genetics and Molecular Structure of nCoV and Previous Coronaviruses

The virus genome of nCoV consists of six major open reading frames (ORFs) like all other coronaviruses. The 3′-end of ORF1a, the ORF1b, and most of the spike regions of nCoV and RaTG13 were similar to a distant lineage within the *Sarbecovirus* branch. In this region, nCoV and RaTG13 are distantly related to the bat-SARS-like coronavirus sequences which were also confirmed by phylogenetic analyses, emphasizing that nCoV may have originated in bats [19, 26]. nCoV is distinct from SARS-CoV in terms of the phylogeny of the complete RNA-dependent RNA polymerase (RdRP) gene, reiterating that it is a novel coronavirus from the subgenus *Sarbecovirus*. The 3-D structure of nCoV revealed that the nCoV receptor-binding domain, composed of a core and an external subdomain which is similar to that of SARS-CoV suggesting that nCoV might as well be thriving by using angiotensin-converting enzyme 2 (ACE2) as a cell receptor. Interestingly, several key residues responsible for the binding of the SARS-CoV receptor-binding domain to the ACE2 receptor were variable in the nCoV receptor-binding domain (Asn439, Asn501, Gln493, Gly485, and Phe486). Past reports confirm that coronavirus contains four key structural proteins: spike (S), membrane (M), envelope (E), and nucleocapsid (N). These are encoded in the 3′-end of the genome (Fig. 1). The S

protein utilizes an N-terminal signal sequence to gain access to the endoplasmic reticulum (ER) [27, 28]. The M protein is the most abundant one with 3 transmembrane domains which gives the shape to virion [29–31]. The E protein is the least abundant one and is highly divergent. It is known for facilitating the assembly and release of the virus [32]. The N protein is the only protein found in the nucleocapsid of virus particles and binds the viral genome in a beads-on-a-string type conformation. N protein is also reported to bind to replicate complex and the M protein, and these protein interactions tether the viral genome to the replicase-transcriptase complex and package the encapsulated genome into viral particles [33–38].

The S gene coding for receptor-binding spike protein which is crucial for membrane fusion and transmission capacity is not very similar to pre-existing CoVs, nonetheless having an exceptional similarity to RaTG13 (93.1%) [39–41]. In nCoV, there are three novel insertions in the N-terminal domain and key residues in the receptor-binding motif which are not present in SARS-CoV [7] and possibly, these insertions have a pivotal role in conferring sialic acid-binding activity which is the key factor in determining the pathogenicity of coronaviruses [7, 42–44].

First-Line Therapeutics Devised from Existing Antiviral Drugs

The first step in the diagnostic and therapeutic approach is the detection and distinction of nCoV from other CoV strains. Therefore, timely detection of nCoV infection cases is very important to stop the community spread. Real-time PCR (RT-PCR) has been deployed in diagnostic virology as a powerful weapon. Conventionally, the preferred targets of coronavirus RT-PCR assays include the conserved and abundantly expressed genes such as the structural S, E, and N genes; the non-structural RdRP; and replicase open reading frame (ORF) 1a/b genes [45, 46]. Until now, E gene and N gene assays are being used as the first-line screening and RdRP gene assay is being used as a confirmatory test. The RdRP assay is highly sensitive and specific [7, 47–49].

Till now, there are no specific US Food and Drug Administration, USA (FDA)-approved drugs for the treatment of nCoV patients. At present, treatments and control measures for severely ill patients include intensive care, supplementary oxygen, and mechanical ventilatory support. Several new drugs are being studied in more than a hundred clinical trials across the globe. FDA issued an emergency use authorization for the investigational antiviral drug remdesivir and antimalarial drug hydroxychloroquine for the treatment of suspected or laboratory-confirmed COVID-19 in adults and children hospitalized with severe disease, though the mechanism of actions is still unknown, they showed improved recovery in some severely ill patients. Remdesivir is administered

Fig. 1 Novel coronavirus genome and structure. **a** The viral genome consists of two partially overlapping replicase open reading frames (OFR1a and 1b) and several downstream ORFs that encode viral functional structural proteins and other proteins. **b** Schematic representation of the virus
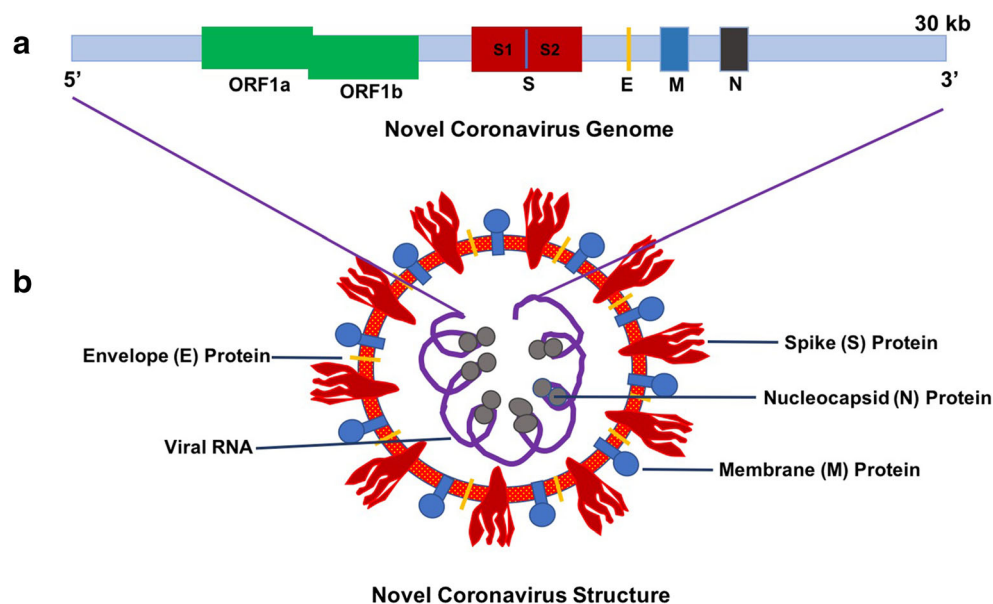
intravenously. It has a broad antiviral activity and is known to inhibit viral replication through premature termination of RNA transcription. There are reports of its in vitro activity against SARS-CoV-2 and in vitro and in vivo activity against beta coronaviruses [50, 51]. One other combination of drugs which has been tested in recent months is lopinavir-ritonavir, but it did not show promise for the treatment of hospitalized COVID-19 patients with pneumonia in a recent clinical trial in China [52].

Recently, hydroxychloroquine and chloroquine had been reported to be of importance in relevance to COVID-19. These are oral prescription drugs that have been used for the treatment of malaria and certain inflammatory conditions like rheumatoid arthritis, systemic lupus erythematosus, and porphyria cutanea tarda. Both of these drugs have in vitro activity against SARS-CoV, SARS-CoV-2, and other coronaviruses, with hydroxychloroquine having relatively higher potency against SARS-CoV-2 [51, 53, 54]. A study in China reported that chloroquine treatment of COVID-19 patients had the clinical and virologic benefit and they recommended this as an antiviral drug or the treatment of COVID-19 in China [55]. In the USA, several clinical trials of hydroxychloroquine for prophylaxis or treatment of SARS-CoV-2 infection are going on. Although its temporary use on patients in hospitals during the COVID-19 pandemic has been authorized by the FDA, still it has not been certified as safe and successful for treating or preventing COVID-19.

One therapy, which is giving a positive hope in the current crisis is plasma therapy. In this therapy, the blood is collected from patients who have recovered from COVID-19 and plasma is separated from that. Convalescent plasma which contains antibodies to nCoV is then administered to patients with COVID-19. The use of convalescent plasma has been studied in previous epidemics like the SARS-CoV-1 epidemic, the H1N1 pandemic, and the MERS-CoV epidemic. This treatment has its roots from the first time use of convalescent plasma for the cure of diphtheria in 1901 by Nobel Prize winner (Physiology and Medicine) Emil von Behring. A number of clinical trials with convalescent plasma are going on worldwide with some showing promising results for the prophylaxis of severe cases of COVID-19 (USA Food & Drug Administration https://www.fda.gov/media/136470/download).

Ongoing Approach and Targets Under Investigation

We have been under the continuous invasion of deadly virus infections like SARS-CoV, Ebola, Nipah, and Zika in recent years. This year, the aggressive outbreak of nCoV has emerged as a brutal pandemic taking the lives of thousands. The repeated surfacing of coronaviruses at repeated intervals is a grave threat to health and economy.

The major concern here is that after decades of research on coronaviruses, there is no first-line defined defense therapeutics or vaccines. This potentiates the utmost need for developing potent vaccines or prophylaxis drugs to put a full stop to the possibility of future outbreaks. Several clinical, genetic, and epidemiological features of nCoV resemble SARS-CoV infection. Hence, the research advancements on SARS-CoV treatment might help the scientific community in a quick understanding of this virus pathogenesis and develop effective prophylactic drugs to treat and prevent this infection.

Some potential drug targets which could be crucial in therapeutic design to prevent COVID-19:

The Spike Protein

The spike (S) protein from coronaviruses has been reported to be a crucial viral antigen which stimulates a strong humoral and cell-mediated immune response in humans. It is also known to mediate receptor binding and virus entry during host infection [14]. The role of S protein in receptor binding and membrane fusion marks it a perfect target for vaccine and therapeutic development. Previous studies on SARS-CoV reveal that vaccines based on the S protein can induce antibodies to block virus binding and fusion or neutralize virus infection [56]. Although to date, there are no certified vaccines available against prior coronaviruses [57, 58]. Since there is a similarity between S protein of MERS-CoV and nCoV, it would be great to target nCoV S protein for therapeutic research. Also, MERS-CoV relies on the binding between its RBD region and the hCD26 receptor to initiate infection [56, 59, 60], interrupting this interaction could abrogate virus invasion and hence could be a great prospective site for drug design.

ACE2

Protein structure modeling studies have shown that (ACE2) is the receptor for the S protein of SARS-CoV. The complex structure of ACE2 with the RBD of S protein of SARS viruses has been resolved and it demonstrated tight interaction among them. Recent studies have confirmed that nCoV binds to ACE2. Structural analysis of potential interactions between RBD of S protein from the human SARS virus and ACE2 protein depicted several interaction points [61, 62]. Interestingly, the interaction between RBD of 2019-nCoV and human ACE2 depicted only one potential hydrophobic interaction between ACE2 (L79, M82) and RBD (F486) and one cation-π interaction ACE2 (K353)/RBD (Y492) (N Dong - 2020 F1000). This gives definitive leads that ACE2 has a pivotal role in virus infectivity and pathogenicity and opens up doors for specific and targeted drug design for combating the pathogenicity and lethality of the nCoV which elicited worldwide pandemic.

GRP78

A spike binding site, host cell receptor glucose-regulated protein 78 (GRP78) which is specific to nCoV has been predicted using docking and bioinformatics analysis. Upon stress, GRP78 is known to act as a chaperone for the misfolded proteins and escape ER to translocate to the cell membrane. It can mediate virus recognitions and entry in the cell by its substrate-binding domain [63–65]. GRP78 should bind to nCoV, as it does in the case of the MERS-CoV coronavirus. Four regions of the spike protein were predicted to be the binding site to GRP78, based on sequence and structural similarity [66]. Inhibiting the interaction between GRP78 and nCoV spike protein would probably decrease the rate of viral infection and could be a strong target for therapeutic designs. Also, vaccine designed against the nCoV spike protein could be a potent preventive measure to bring down the mortality rate due to COVID-19.

Sialic Acid-Binding Residues

There are reports which show sialic acid serves as a ligand for the binding of a transmissible gastroenteritis virus (TGEV), which is an enteropathogenic porcine coronavirus to erythrocytes [67, 68]. Sialic acid-binding activity may increase the stability of the virions by the binding of the virus to sialoglycoconjugates. Increased stability would help virions to survive the passage through the gastrointestinal tract [42]. In nCoV, there are three novel insertions in the N-terminal domain unlike that of SARS-CoV [7]. These insertions are thought to confer sialic acid-binding activity and could be very potent targets for future vaccines. Also, sialic acid-binding activity is sensitive to certain competitive inhibitors which could be a good start point for further research and therapeutic targeting [43, 69].

Conclusion

Control measures for COVID-19 are majorly based on the understanding of previously existing coronaviruses, mode of their infections, and consideration of potential modes of transmission. Many labs are working hard in collaboration for the development of therapeutics and vaccines to put a full stop to thousands of deaths occurring worldwide every day. Few of them have cleared phase I trials and there is an anticipation of a potential vaccine by the end of the year 2020. Clinicians are advised to see the Centers for Disease Control and Prevention (CDC), USA, website for changes in guidance for testing, treatment, and infection control. Response actions against the ferocious nCoV must be aggressive. Until a new vaccine against nCoV becomes available, avoidance of viral spreading is the most appropriate way to flatten the curve for the number of people getting infected and dying due to COVID-19.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Informed Consent No patient or clinical sample used in this study.

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