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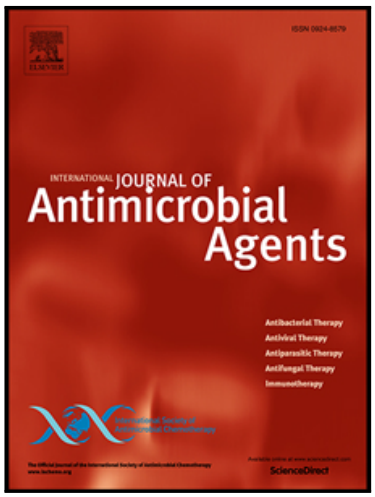

Recent Progress in understanding 2019 Novel Coronavirus associated with Human Respiratory Disease: Detection, Mechanism and Treatment

Shuntong Kang , Wenyao Peng , Yuhao Zhu , Shiyao Lu , Min Zhou , Wei Lin , Wenfang Wu , Shu Huang , Liping Jiang , Xuan Luo , Meichun Deng

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Highlights

- 2019-nCoV has spread many provinces in China, and multiple countries are affected.
- The viral outbreaks have stirred panic and emergency on public health around the world.
- But the cause and consequence of the pneumonia still remain unknown.
- The clinical manifestations, detection methods and treatment options for 2019-nCoV.
- And propose potential strategies for preventing the infection.

Recent Progress in understanding 2019 Novel Coronavirus associated with Human Respiratory Disease：Detection, Mechanism and Treatment

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Running title: Recent Progress in 2019-nCoV and COVID-19

Abstract

Viral respiratory diseases such as the severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), always pose severe threat to people. First identified in the late December 2019, in Wuhan, China, a novel coronavirus (2019-nCoV) has rampantly spread to lots of provinces in China, and multiple countries are affected. The viral outbreaks have stirred panic and emergency on public health around the world and the number of infections continues to rise. But the cause and consequence of the pneumonia still remain unknown. In order to effectively implement the epidemic prevention, early identification and diagnosis is critical to disease control. Herein we scrutinize a series of available studies by global scientists on the clinical manifestations, detection methods and treatment options of 2019 novel coronavirus pneumonia, also propose potential strategies for preventing the infection.

Keywords: novel coronavirus; mechanism; treatment; detection

Importance

The viral outbreaks have stirred panic and emergency on public health around the world and the number of infections continues to rise. But the cause and consequence of the pneumonia still remain unknown. In order to effectively implement the epidemic prevention, early identification and diagnosis is critical to disease control. Herein we scrutinize a series of available studies by global scientists on the clinical manifestations, detection methods and treatment options of 2019 novel coronavirus pneumonia, also propose potential strategies for preventing the infection.

Introduction

By the end of 2019, several patients were diagnosed with pneumonia of unknown reason, epidemiologically associated with a same seafood market in Wuhan, Hubei province, central China. Along with the Spring Festival Exodus, the outbreaks seemed unavoidable. This condition called the attention of the Chinese Center for Disease Control and Prevention (CDC) who launched an emergency response immediately. The World Health Organization (WHO) also responded promptly, and declared the outbreak a global health emergency (PHEIC). The causative agent of the unidentified pneumonia had been confirmed as a novel coronavirus by sequencing and etiological investigations by several independent laboratories in China. After the isolation of the new coronavirus, it was found to be distinct from both MERS-CoV and SARS-CoV[1,2]. Coronaviruses are single-stranded RNA viruses belonging to the family Coronaviridae, which can cause various disease with enteric, respiratory, hepatic and neurological symptoms[3]. The new coronavirus denoted as the 2019 novel coronavirus (2019-nCoV) or coronavirus disease 2019(COVID-19) quickly becomes of tremendous worldwide concern. It leads to a significant outbreak in many regions in China and expands globally, including Asia, Europe, North America, South America, Africa and Oceania. The disease is potentially zoonotic with estimated 2-5% mortality rate. Person-to-person transmission may occur through contact and respiratory transmission or probable fecal-oral route. Currently, the number of ascertained infection cases has been daily increasing, but there is no definite treatment for the pneumonia although some potential drugs are under investigation. For the last two decades, the outbreaks of coronaviruses and intermittent worldwide public health emergences remind us that CoVs are still a severe global

health threat which can't be ignored. According to the latest research, useful information needed urgently for the control of the disease is highly essential.

1. Virology

Coronaviruses (CoVs) are named for the crown-like (or corona in Latin) spikes of the virus protruding to the periphery with a diameter of 60-160nm under electron microscopy. Each particle is enveloped containing a single-stranded positive-sense RNA (+ssRNA) genome of 27-32 kb with 5'-cap structure and 3'-poly A tail which interacts with the nucleoprotein. All these coronaviruses have similarities in the organization as well as expression of the genome, and the genome size of CoV is largest among all RNA viruses. 16 nonstructural proteins (nsp1 to nsp16), encoded by open reading frame (ORF) 1a/b at the 5' end, are followed by the structural proteins Nucleocapsid (N), Spike (S), Envelope (E), and Membrane (M), which are encoded by other ORFs at the 3'end[4]. The envelope includes three proteins: M protein binds nucleocapsids and enhances viral assembly and budding; E protein is involved in viral morphogenesis, release as well as pathogenesis. And S protein contributes to homotrimeric spikes which recognize the receptor of the cell, thus helping virus invade target cells[5]. Since the outbreaks of SARS in 2002 and the MERS in 2012, the possibility of CoVs transmission from animals to human has been proved. CoVs are ubiquitous pathogens in nature for human and animals, usually causing gastrointestinal and respiratory infections, sometimes involving important organs such as liver, kidney, heart and brain. Sensitive to ultraviolet rays and heat. Lipid solvents such as ether, 75% ethanol, chlorine-containing disinfectant, peracetic acid, and chloroform can effectively inactivate the virus.

Coronaviruses consist of the largest group belonging to the Nidovirales order, which

contains Coronaviridae, Arteriviridae, and Roniviridae families. The family Coronaviridae is composed of large, single, plus-stranded RNA viruses which are isolated from several species and previously known to induce common colds and diarrheal illnesses in humans[5].

The Coronavirinae is one of two subfamilies in the Coronaviridae family, and the other one is the Torovirinae. The Coronavirinae is further subdivided into four groups, the alpha, beta, gamma and delta coronaviruses. The viruses were sorted into the groups according to serology initially but are now labelled by phylogenetic clustering[6]. Only alpha and beta coronavirus are of interest for human and clinical virologists[7]. Based on epidemiological data before 2019, only six CoVs proved to cause human respiratory diseases: i) HKU1, HCoV-NL63, HCoV-OC43 and HCoV-229E only lead to mild upper respiratory disease, but rarely bring about severe diseases in people; ii) SARS-CoV and MERS-CoV attack lower respiratory tract and always induce severe respiratory syndrome. Sequence analysis shows that 2019-nCoV has a typical genome structure of coronavirus and belongs to the cluster of beta coronaviruses that includes SARS-CoV and MERS-CoV and it forms a clade within the subgenus Sarbecovirus, Orthocoronavirinae subfamily. It has been the seventh member of the family of coronaviruses that infect humans so far[8].

2. Potiential hosts

Evolutionary models and phylogenetic analysis deserve attention for helping estimate genetic variability and the evolutionary rate so that in turn to provide important implications for disease progression, drug trial and vaccine development. Zhou et al.[2] report the full-length genome sequences acquired from patients at the early outbreak are almost identical to each other and shared 79.5% sequence similar to SARS-CoV. In addition, 2019-nCoV is 96%

identical at the whole genome level to a bat coronavirus. Wu et al.[1] almost simultaneously and independently discover the novel RNA virus from the family Coronaviridae. Phylogenetic analysis of the complete viral genome reveals that the coronavirus is most closely related (89.1% nucleotide similarity) to a group of SARS-like coronaviruses previously sampled from bats in China. Benvenuto et al.[9] build a phylogenetic tree including the whole genome sequences of 2019-nCoV and highly similar available whole genome sequences in gene bank. The phylogenetic tree shows that 2019-nCoV clustered with Bat SARS-like Coronavirus sequence isolated in 2015 while The Fast Unconstrained Bayesian Approximation (FUBAR) analysis reveals mutation in Spike Glycoprotein and nucleocapsid protein. Therefore 2019-nCoV probably transmits from bats or other hosts from where it gets the ability to infect humans. Ramaiah et al.[10] speculate the novel virus closely relates to bat-CoV, which reminds us this 2019-nCoV strain might be evolved from the bat-CoV by accumulating favorable genetic changes for human infection. Epitopes in 2019-nCoV structural proteins are differentially recognized by HLA-DR alleles, which suggests that a subunit of vaccine including the 8 immunodominant HLA-DR epitopes can induce effective antiviral immune responses in various populations. Paraskevis et al.[11] find the 2019-nCoV closely resembles bat-CoV RaTG13 sequence throughout the genome (similarity 96.3%). The latter does not provide the exact variant that caused the outbreak in humans, but the hypothesis that 2019-nCoV has originated from bats is possible. Xu et al.[12] also report that the 2019-nCoV shares with the SARS/SARS-like coronaviruses a common ancestor that relates to the bat coronavirus HKU9-1. They conclude that the 2019-nCoV S protein and SARS-CoV S protein share an almost identical 3-D structure in the RBD domain, thus maintaining similar van der

Waals and electrostatic properties in the interaction interface of human ACE2 molecules despite the sequence diversity。

Although many scientists believe that bats are intermediate hosts, most species of bats live in tropical or subtropical rain forests and caves far from the population, the probability of bats transmitting the virus directly to humans is not enough. It is generally known that bats may deliver the viruses to intermediate hosts such as wild animals or livestock, then the viruses are transmitted to humans by these intermediate hosts. Ji et al.[13] carry out using relative synonymous codon usage (RSCU) bias among different animal species. The result suggests that the 2019-nCoV appears to be a recombinant virus between the bat coronavirus and an origin-unknown coronavirus. They analyze that snake is a most probable wildlife animal reservoir, but the conclusion is controversial. Guo et al.[14] introduces the VHP (Virus Host Prediction) to dig out the potential hosts of viruses by deep learning algorithm and predicts bat coronaviruses are assigned with more similar infectivity patterns with 2019-nCoV. The consequences illustrate that bat and mink may be two candidate reservoirs of 2019-nCoV. Tsan-Yuk Lam et al.[12] identify pangolin associated CoVs belonging to two sub-lineages of 2019-nCoV related coronaviruses include one closely resemble 2019-nCoV in the receptor-binding domain by metagenomic sequencing. Hence, pangolins should be considered as possible intermediate hosts and should be removed from markets to prevent such zoonotic transmission

3. Clinical manifestation

More than 80,000 cases have been confirmed as of February 21, 2020, and most of them had a history of close contact with the epidemic area in Wuhan or confirmed patients. Major

initial symptoms include fever, most of which are high fevers that occur within several days and are not alleviated by routine anti-infective drugs, additionally coughing, headache and muscle pain or fatigue[15]. Other clinical symptoms observed at low frequency include elevated troponin levels, diarrhea, myalgia and myocarditis[15]. It should be emphasized that some asymptomatic patient are diagnosed as 2019-nCoV[16], so the presence of asymptomatic carriers requires due attention. Nearly 20% of the patients appeared comorbidities with regard to the dysfunction of other organs, primarily renal impairment and patients with underlying cardiovascular diseases often demonstrated comorbid heart failure[13]. They gradually develop initial symptoms in the cardiovascular system, digestive system, and nervous system, which increased the difficulty of diagnosis[15]. The median interval from the start of initial symptoms to significant symptom aggravation like dyspnea or appearance of acute respiratory distress syndrome is 7 days, ranging from 1 day to 20 days, which was consistent with previous reports[13]. According to the newly released pneumonitis diagnosis and treatment plan for new coronavirus infection, severe patients often have dyspnea and/or hypoxemia one week after the onset of the illness. Serious cases can quickly progress to acute respiratory distress syndrome, septic shock, irreformable metabolic acidosis, coagulopathy and multiple organ failure. Nearly 80% of the patients have normal or decreased white blood cell counts, and 72.3% hav lymphocytopenia[15]. Lung involvement was present in all cases[17,18], with most chest computed tomography (CT) scans showing lesions in multiple lung lobes, some of which are dense. Ground-glass opacity co-existed with consolidation shadows or cord-like shadows are observed. Since respiratory supports are administered to most of the patients, oxygen saturation can be maintained at above 90% as indicated by pulse oximetry

monitoring[15]. It is reported that severe and critically ill patients have moderate to low fever, even without obvious fever. Mild patients show just low fever, mild fatigue, and no pneumonia. Judging from the current cases, most patients have a good prognosis but poor for the elderly and those with chronic underlying disease. Symptoms in children are relatively mild.

As for the current situation, the source of infection is mainly the infected patients, but the possibility of asymptomatic infection should not be ignored. Respiratory droplets and close contact are the key routes of transmission. The possibility of aerosol transmission in a relatively closed environment for a long-time exposure to high concentrations of aerosol also exists, but still needs scientific evidence. Recently, 2019-nCoV RNA was detected in the feces of some confirmed patients with the pneumonia, indicating that 2019-nCoV is likely to be transmitted through fecal-oral pathway. Zhang et al.[19] find ACE2 is highly expressed in type II alveolar epithelial cells, esophageal epithelium, stratified epithelial cells, even in absorptive intestinal epithelial cells from ileum and colon. Their study of bioinformatics analysis with single-cell transcriptomes suggests that the digestive tract may serve as an infection pathway for 2019-nCoV. The spread and infection of the virus are complex problems requiring cooperation from multiple perspectives such as medicine, biology and fluid mechanics to give a complete answer.

4. Detection

4.1 General examination

Mild patients may not have positive signs, while severe patients may have shortness of breath with moist rales of both lungs, weakened breath sounds, dullness percussion, enhanced or weakened vocal fremitus palpation[20].

4.2 Chest imaging examination

Suspected or confirmed cases should undertake chest X-ray examination as early as possible and chest CT scan is required when necessary[21]. In the early phase of the disease, chest images show interstitial changes and multiple small plaques, especially in the lung periphery. Then the changes further deteriorate to the bilateral and are mainly distributed in the middle and outer zones of the lung with multiple infiltrating shadows and/or ground-glass opacity. Patients may have a single lobe or multiple lobes involved. When the condition gets better, a little fibrous stripe may appear[22]. Conversely, lung consolidation may occur in severe cases whose pleural effusions are rarely seen.

4.3 Laboratory examination

4.3.1 Hematologic examination

At the early stage, white blood cell counts are normal or decreased, with decreased lymphocyte counts. If the absolute value of lymphocyte is less than 0.8×10^9/L, or the CD4 and CD8 T cell counts are significantly decreased, it needs high attention. It is generally recommended to recheck the blood routine changes after 3 days[20]. In some patients, muscle

enzymes, liver enzymes, and myohemoglobin levels are increased. Otherwise, the troponin is increased in some critical patients. Most patients display elevated erythrocyte sedimentation rate and C-reactive protein level, and normal procalcitonin levels. Severe cases show progressively decreased blood lymphocytes counts and high D-dimer levels. Inflammatory factors are often increased in severe and critical patients.

4.3.2 Molecular diagnosis

RT-PCR Samples of suspected 2019-nCoV patients collected from the upper respiratory tract (nasopharyngeal and oropharyngeal), the lower respiratory tract (expectorated sputum, endotracheal aspirate, or bronchoalveolar lavage), blood and feces can be diagnosed by real time reverse-transcription–polymerase chain- reaction (RT-PCR)[23]. Two sequence regions (ORF1b and N) designed based on the first public access sequence in Genbank are selected for primer and probe designs, which are highly conserved amongst Sarbecoviruses. In detecting positive clinical specimens, the N gene assay is about 10 times more sensitive than the ORF-1b gene assay[24]. The existing PCR methods have very good specificity but low sensitivity, which means that the negative test results cannot exclude the presence of 2019-nCoV in patients. Moreover, the laboratory sample contamination caused by the lack of control can lead to the false positive results. Additionally, RT-PCR tests may be falsely negative due to insufficient viral materials or operational error. Some patients with negative results of RT-PCR may present with positive chest CT findings for 2019-nCoV, which means PCR results can assist clinical diagnosis and evaluation but the possibility of disease cannot be confirmed or ruled out. For individuals with high clinical suspicion but

negative RT-PCR screening, a combination of CT scanning and repeated swab tests may be helpful[25].

SHERLOCK technique The CRISPR-based SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing) technique, termed specific high-sensitivity enzymatic reporter unlocking , allows portable, multiplexed, and ultra-sensitive detection of RNA or DNA from clinically relevant samples. SHERLOCK assays are set up with recombinase-mediated polymerase pre-amplification of DNA or RNA and subsequent Cas13- or Cas12-mediated detection via colorimetric readouts and fluorescence that provide results in less than 1 h with a setup time of less than 15 min[26]. Based on the RNA sequence of the New Coronavirus, the researchers carefully designed two guide RNAs, one that recognizes the S gene of the new coronavirus, and the other that recognizes the Orf1ab gene. In order to maximize the accuracy of the detection, scientists have selected the sequences that are most specific for the new coronavirus. In this way, interference from other respiratory virus genomes can also be minimized. Theoretically, as long as the RNA corresponds to the new coronavirus in the sample, the guide RNA can accurately recognize it and activate the Cas13a protein to bind to it. Cas13a is a very interesting enzyme, once activated, it will indiscriminately and insanely cut any other RNA molecules it encounters. In this way, by confirming whether these molecules have been cut off, it can detect the present of the new coronavirus in the original sample. They consistently detect COVID-19 target sequences in a range between 20 and 200 aM (10-100 copies per microliter of input) by using synthetic COVID-19 virus RNA fragments. The test can be read out using a dipstick in less than an hour, without requiring elaborate instrumentation, but still needs to be confirm valid with

patient samples.

5. Pathogenic mechanism

Lu et al. [13] sequence the 2019-nCoV and phylogenetic analysis reveals that the 2019-nCoV belonged to beta coronaviruses with 79.0% nucleotide identity to SARS-CoV and 51.8% identity to MERS-CoV which means 2019-nCoV is closer to SARS-CoV than MERS-CoV. Importantly, the homology modelling revealed that the receptor-binding domain structure of 2019-nCoV is similar to that of SARS-CoV[21]. From the previous reports, the interaction with Angiotensin Converting Enzyme 2 (ACE2) is responsible for SARS-CoV entering human cells[27,28]. Dong et al.[29] compare the structural similarity of the protein ACE2 in various viruses and speculate that the 2019-nCoV could most likely use the same receptor as SARS-CoV. Zhou et al.[30] conduct virus infectivity studies using Hela cells with or without ACE2 proteins obtained from humans or animals and find that 2019-nCoV can invade all ACE2 protein-expressing cells but the cells from mouse. In short, 2019-nCoV uses ACE2 as an entry receptor in the ACE2-expressing cells, but not cells without ACE2, and 2019-nCoV is likely to bind to ACE2 receptor in humans just like SARS-CoV. Dimitrov et al. [31]reported that SARS-CoV infects the host cells in human by a basic interaction of its S glycoprotein and the receptor ACE2 on human cells. Though the sequence of 2019-nCoV is a little different from the SARS-CoV, its functionally important ORFs and major structural proteins especially the Spike protein are well annotated, of which the original identity is 76%[32]. The S protein has two regions, S2 and S1, which holds stronger affinity with the receptor binding domain (RBD) in the S1 region interacting with ACE2[24]. Similar to SARS-CoV, the 2019-nCoV may also engage the RBD to bind ACE2 in order to enter the human host cells, but since many residues

in S1 and S2 have been replaced in 2019-nCoV, the differences in the interactions with ACE2 on host cells exist[29,33]. Daniel et al. [34] get the cryo-EM structure of the 2019-nCoV S protein and the structure is presented in the Fig 1, 2D class averages of this particles are shown in the left picture. These structures are used to calculate the reconstruction of S protein of 2019-nCoV. The right picture contains the side and top views of the prefusion structure of the 2019-nCoV S protein with a single RBD in the "up" conformation in ribbons and the two RBDs "down" protomers are shown as cryo-EM density in either white or gray, which are colored corresponding to the schematic in Fig 1.The result suggests that ACE2 bound to 2019-nCoV S protein ectodomain with 15 nM affinity, which is around 10 to 20 fold higher affinity than SARS-CoV, and the result is very surprising.

However, if the SARS-CoV trys to invade the host cells, it's not as simple as ACE2 interacting with S protein for some pathways and cytokine included. Chemokine (C-C motif) ligand 2 (CCL2) is an important cytokine in Spike-ACE2 signaling pathway. CCL2 is a CC chemokine and it attracts memory T lymphocytes, monocytes and basophils. The receptor of CCL2 is CCR2, both of which are involved in inflammatory reaction[35], therefore they are associated with some lung inflammatory disorders. Referring to previous reports, the patients who infect SARS-CoV would have pulmonary fibrosis which is responsible to CCL2. Cheung et al. [36] take an experiment to observe the expression of CCL2 in SARS-CoV patients and they find the expression of CCL2 is upregulation in the sera of patients. To work out the relationship between Spike-ACE2 signaling pathway and CCL2, Chen et al. [37] get a conclusion and showed it in Fig 2 showing the interaction between S protein of SARS-CoV with ACE2 on the human cells. The infected lung epithelial cells induce casein kinase II (CK

II) which is able to phosphorylate ACE2 and involves ERK1/2 activation. CK II phosphorylates ACE2 at Ser-787, by which SARS-CoV binds to its ACE2 receptor and causes the conformational change of ACE2. It also activates the ACE2 downstream signal transduction pathways including ERK1/2 and AP-1[38]. The change will lead to the activation of ERK1/2 and AP-1 and upregulates CCL2. ACE2 also participates in an intracellular signaling pathway. It activates ERK1/2 via interacting with upstream factors such as Ras, and PKC. In the end, the elevated level of CCL2 protein in the sera of SARS-CoV-infected patients accounts for the development of lung fibrosis.

6. Therapeutic target

2019-nCoV is characterized by strong contagion, high morbidity and high mortality, but no specific drugs of 2019-nCoV have been developed so far. Many researchers are trying to find the therapeutic targets of the virus to work out high-efficiency and low toxicity targeted drugs. A number of epidemiological studies have shown that the transmission characteristics of 2019-nCoV appear to be a similar of SARS [39-42]. The series of similarities give doctors and scientists a clue to look for targeted drugs. Although coronaviruses are subject to extensive mutations, some key proteins, particularly replication-related enzymes, are highly conserved[2], so the drugs that target conserved proteases are usually able to block replication and proliferation of the virus and exhibit a broad spectrum[22]. Specific inhibitors of key proteases involved in viral replication and proliferation are effective ways of killing viruses. Candidate compounds include RNA proteases, membrane proteins, spike glycoproteins, polymerases, and viral envelope that act directly on the virus, as well as targets on the host, such as receptors and proteases for virus entry and endocytosis [24,43,44]. Recently, scientists screen out 4

small molecule drugs (Prulifloxacin, Tegobuvir, Nelfinavir and Bictegravir) with strong binding ability to the main protease of SARS-CoV[22]. The first case of the 2019-nCoV infected pneumonia in the United States experienced significant improvement in clinical symptoms after receiving an intravenous infusion of Remdesivir on the seventh day of hospitalization[45]. Remdesivir is a novel nucleotide analogue prodrug under development that inhibits viruses by inhibiting RNA-derived RNA polymerase (RdRp), which was originally used to treat Ebola but didn't work. Amazingly, it is effective against SARS and MERS.[46-49]

In addition to this, 2019-nCoV is unable to infect cells without ACE2 and cannot bind to other common receptors of coronavirus (APN, DPP4, etc.) [2,8,50]. After replacing four out of five important interface amino acid residues, 2019-nCoV S protein maintains the core structure and interacts perfectly with human ACE2 molecule[12]. These studies show that ACE2 targeted drugs are expected to be used to treat 2019-nCoV. The S2 subunit of SARS-CoV spike protein plays a key role in mediating the fusion of virus and host cell and entering the host cell. Heptapeptide repeat 1 (HR1) and heptapeptide repeat 2 (HR2) can interact to form six helix bundles (6Hb), which makes the virus and cell membrane tightly bound[51]. A variety of effective fusion inhibitors against SARS coronavirus and Middle East respiratory syndrome (MERS) coronavirus have been developed by using S-HR1 and S-HR2，such as HR2P peptide[52]. Researchers have found that the HR1 and HR2 regions of the 2019-ncov could also interact to form 6hbs, and they have designed a pan-coronavirus fusion inhibitor denoted as EK1, which can significantly inhibit 2019-nCoV pseudo virus infection in a dose-dependent manner[53].

7. Treatment strategy

7.1 Antiviral therapy

At present, different institutions and organizations in China have issued several guidelines for the diagnosis and treatment of novel coronavirus pneumonia, none of which pointed out specific drugs for 2019-nCoV. According to the latest treatment protocol (Pilot Version 6) issued by the Chinese health commission, antiviral therapy can be tried with interferon, lopinavir/litonavir, chloroquine phosphate and arbidol. These drugs and other potentially effective drugs are described here.

7.1.1 Nucleoside analogs

Remdesivir is a nucleoside analogue with antiviral activity developed by Gilead Scientific. Its anti-RNA virus activity has been confirme, such as Ebola, Marburg virus, Nipa virus, Hendra virus[54-56]. What's more, Remdesivir has exhibited preventive and therapeutic effects on MERS-CoV and SARS-CoV in vitro [57,58]. Remdesivi is detected to have the ability to reduce MERS-CoV, improve pulmonary function and reduce lesions of pulmonary in Calu-3 cells and mouse models[48]. It has been reported that in the Vero E6 cell model, Remdesivir exhibits the effect of inhibiting the infection of 2019-nCoV virus, with the EC50 at 0.77 micrometers and the SI more than 129.87[34]. Since 2019-nCoV and MERS-CoV possess similar structure belonging to the coronavirus[2,8], Remdesivir has a great potential of anti-2019-nCoV. As reported, the first case of new coronavirus pneumonia treated in the United States only received supportive treatment for nausea, vomiting and other symptoms at the initial stage of admission, while the patient's symptoms improved significantly after being given the intravenous injection of Remdesivir for one day[45]. However, Remdesivir has not

approved listed in any country, its safety and effectiveness must be confirmed. Thus , two randomized, controlled, double blind Phase III trials have been addressed in February 2020 (NCT04252664 and NCT04257656).

Ribavirin is a broad-spectrum antiviral nucleoside analogue which has been used in the treatment for HCV and RSV. The antiviral mechanism is interacting with virus RdRp to inhibit RNA synthesis[55]. In vitro experiments, it's shown that Ribavirin inhibits replication of MERS-CoV and HCoV-OC43, but the dosage that produces significant effects is not within the range of typical human therapies[59]. Using interferon simultaneously can reduce the dose of Ribavirin[60]. In a primate model, the clinical symptoms of MERS could be improved by the combination of Ribavirin and type I interferon[61]. However, the side effects of ribavirin, such as anemia, limit its widespread use[62]. In addition, two meta-analyses of SARS and MERS case studies exhibited limited efficacy in treating patients with coronavirus respiratory syndrome [63,64]. Therefore, further studies are needed to determine whether ribavirin can effectively treat novel coronavirus pneumonia (COVID-19), and Ribavirin and interferon are still in clinical trials (ChiCT R2000029387).

7.1.2 Protease inhibitor

Lopinavir/Ritonavir (Kaletra/Aluvia) is a protease inhibitor used in combined therapy for HIV[65]. Lopinavir inhibits the decomposition of gag-pol protein, while Ritonavir inhibits the decomposition of gag-pol protein precursor and lopinavir metabolism to increase the concentration of Lopinavir[66]. It has been shown that Lopinavir/Ritonavir could inhibit replication of MERS-CoV and SARS-CoV in vitro. In primate models, animals treated with

Lopinavir/Ritonavir for MERS have a better prognosis than those not treated[67]. A non-critical 2019-nCoV infected patient in South Korea received Lopinavir/Ritonavir (Kaletra, AbbVie) on the eighth day of admission, after which the clinical symptoms improved and the coronavirus load began to decrease until undetectable[68]. Animal models suggest that TMPRSS2 (type II transmembrane serine proteases) plays an important role in coronavirus transmission[69]. TMPRSS2 activates the S protein of highly pathogenic human coronavirus, which binds to ACE2 receptor and enters host cells[38,69-72]. Thus, TMPRSS2 inhibitors are also considered as drugs for the treatment of novel coronavirus pneumonia[73].

7.1.3 Broad-spectrum antiviral

Interferon is a kind of glycoprotein and triggers the antiviral immune response in patients infected with virus[74]. In animal models, interferon inhibits the replication of SARS-CoV and MERS-CoV. In addition, combination therapies of interferon with other antiviral drugs have been used to treat SARS or MERS patients and show synergistic effects[60,74].

Chloroquine also has a strong antiviral effect on SARS-CoV infected cells. It interferes with virus-receptor binding by ACE2 terminal glycosylation[75,76]. In vitro, chloroquine can enhance the effects of other antiviral drugs[34].

7.2 Immunity therapy

Due to the similar RBD structures of 2019-nCoV and SARS-CoV, screening the anti-SARS-CoV antibodies will facilitate rapid development of monoclonal antibodies and vaccines against 2019-ncov[33]. Li et al.[33] reported that CR3022, SARS-CoV specific human

monoclonal antibody can effectively bind to 2019-nCoV RBD. CR3022 has the potential to be used alone or in combination with other neutralizing antibodies for the prevention and treatment of 2019-nCoV infection[33]. However, the dose of needs to be screened before applying monoclonal antibodies[77]. Using convalescent plasma to treat critically ill patients has been included by the latest Chinese treatment protocol (Pilot Version 6). One meta-analysis exhibits glucocorticoids reduces the risk of ARDS[78]. But different studies suggest that glucocorticoids slow viral clearance[79]. Clinical data are still needed to demonstrate the value of glucocorticoid use for 2019-nCoV[66].

7.3 Other methods

Studies have shown that, similar to SARS-CoV, S protein receptor binding domain (RBD) of 2019-nCoV and human angiotensin conversion enzyme Ⅱ (ACE2), which is one of the reasons for the 2019-nCoV to infect humans[12,33]. Zhou et al.[2] report that ACE2 is the receptor of 2019-nCoV through cell experiments: 2019-nCoV could bind ACE2 receptors of civet, bat, pig and human origin, and cannot infect cells without ACE2. Therefore, the application of ACEI and AT1R inhibitors under the condition of close monitoring of blood pressure is likely to reduce the body damage in patients with 2019-nCoV infection[80]. Xuebijing is a traditional Chinese medicine injection commonly used to treat inflammation in severe cases. A randomized controlled trial shows that on the basis of western medicine treatment, xuebijing injection could significantly reduce the fatality rate of community acquired pneumonia[81]. Further reliable evidence needs accumulating as a potential Chinese medicine for COVID-19.

8. Prevention

8.1 Traveler screening

Following its recent emergence, traveler screening is a way used to limit further spread of 2019-nCoV and the purpose is to curtail geographic spread of the infection[82]. The traveler symptom screening depends on the natural history of the infection. The individuals who were infected are likely to show detectable symptoms with the increasing time since exposure. However, the traveler screening is also limited. Katelyn et al. [83] perform tests to estimate the effectiveness of traveler screening and they find within the narrow range of the tests, traveler screening outcomes are sensitive to the short incubation period mean. However, for the longer incubation periods, the larger proportions of departing travelers will not exhibit symptoms. The point at long incubation periods they still feel healthy enough to travel and do not realize they have been exposed to 2019-nCoV which is simultaneously difficult to detect[84].

8.2 Sesame oil

A folk method expresses that the adding of sesame oil into nostrils can prevent the spread of 2019-nCoV. In order to find the theoretical reasons to support this method for preventing viral infection of 2019-nCoV, Fan et al.[85] make discussions based on colloid and interface science pure and point out that sesame oil has a low surface tension and it is incompatible with water[86]. They investigate the epidemiological features of 2019-nCoV and find because of the low intermolecular attraction between adjacent two sesame oil molecules, the pure sesame oil has a good wettability which can readily wet the surface of various solid and aqueous phases. In view of this feature, the sesame oil might prevent the spread of 2019-nCoV. Unfortunately, the mechanism of this method remains uncertain and it is not

approved and verified by experimental and clinical studies. In the future, scientists need to pay more scientific attention to these potential useful clues from the folk medical methods.

8.3 Natural compounds

Traditional Chinese medicine herbs have been practiced for thousand years. In 2003, glycyrrhizin, a traditional Chinese medicine, was suggested to be promising for treating SARS[87] and it was considered to be effective and a valuable pool for the availability and the low toxicity. Searching the active compounds from Chinese herbal medicine to prevent the 2019-nCoV could be a potential strategy. Chen et al. [88] used the molecular docking to find natural compounds, they proposed the five candidates including Scutellarin, baicalin, Hesperetin, Nicotianamine and glycyrrhizin which are the potential compounds targeting the ACE2 receptor and exert anti-virus effects to prevent 2019-nCoV infection.

9. Conclusion

2019-nCoV is driving China's urgent public health actions, as well as international concern. We collect the recent progress of 2019-nCoV in the hope of providing potential interventions. The spread is fast with increasing infected patients nationwide and the future development of the disease is unclear but the public should pay attention to the virus since it may be very contagious.

Declarations

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Author Contributions

Shuntong Kang, Wenyao Peng, Yuhao Zhu, Shiyao Lu, Min Zhou, Wei Lin, Wenfang Wu, Shu Huang, Liping Jiang, Xuan Luo, and Meichun Deng contribute to the material collection and manuscript writing. All authors read and approved the final manuscript.

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Figure Legends

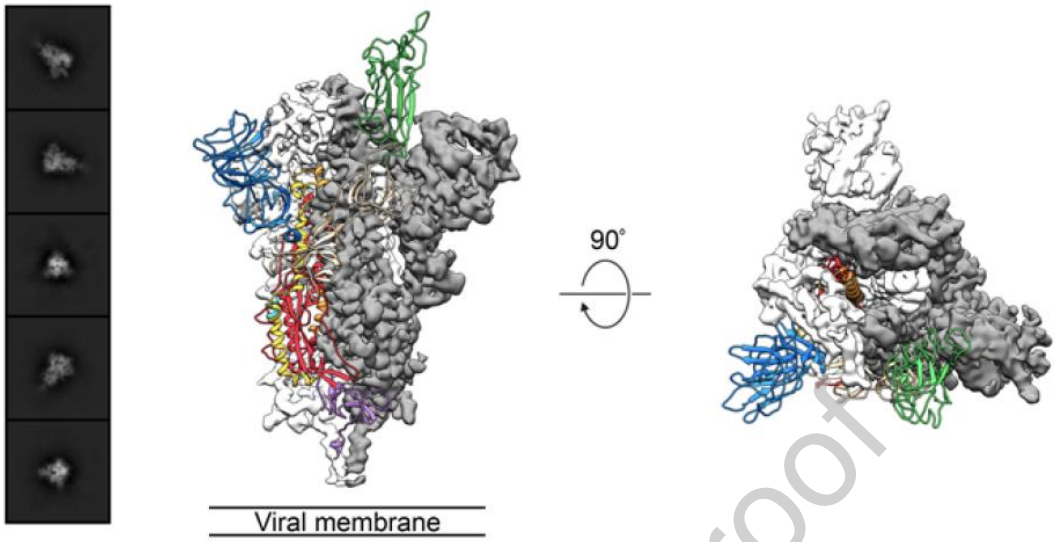

Fig 1. Structure of 2019-nCoV S in the prefusion conformation. Select 2D class averages of the particles that were used to calculate the 2019-nCoV S reconstruction (left). Side and top views of the prefusion structure with a single RBD in the "up" conformation (right). The two RBD "down" protomers are shown as cryo-EM density in either white or gray and the RBD "up" protomer is shown in ribbons, colored corresponding to the schematic in Fig 1.

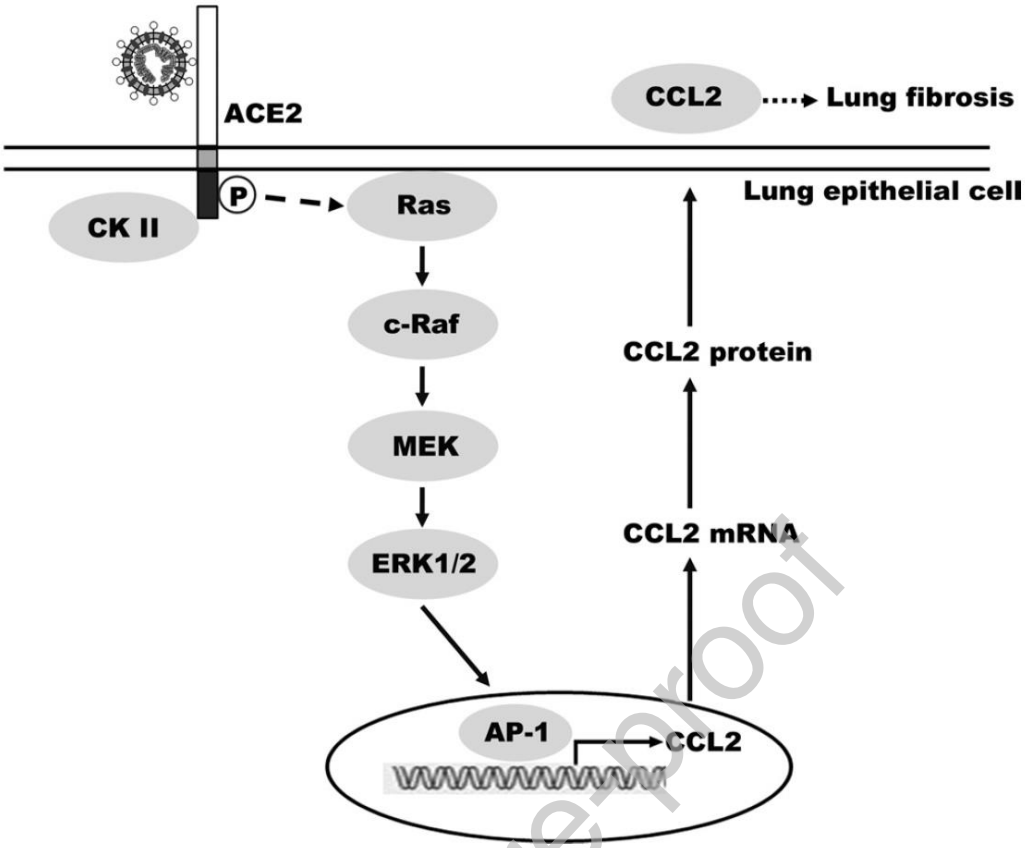

Fig 2. A model of the ACE2 signaling pathway involved in SARS-CoV-induced CCL2 expression. Infection of lung epithelial cells with SARS-CoV induces casein kinase II (CK II)-mediated phosphorylation of the ACE2 receptor, leading to the activation of ERK1/2 and AP-1 and the upregulation of CCL2. The elevated level of CCL2 protein detected in the sera of SARS-CoV-infected patients may account for the development of lung fibrosis.