

## Possible inhibitors of ACE2, the receptor of 2019-nCoV

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## Abstract

Most recently, an outbreak of severe pneumonia caused by the infection of 2019-nCoV, a novel coronavirus first identified in Wuhan, China, imposes serious threats to public health. Many important aspects about 2019-nCoV remain largely unknown, among which, the limitation of antiviral therapies represents one of the most critical problems. More recently, it was confirmed that human ACE2 is the receptor for the entry of 2019-nCoV into lower respiratory tract epithelial cells. Give this observation, it is thus expected that the virus could be inhibited if we decrease the expression of ACE2. Here by screening two databases, Connectivity Map (CMap) and our JeaMoon Map (JMap), we identified a number of candidate agents that decrease ACE2 expression. CMap analysis identified 5 compounds, among which, Azathioprine is a possible therapeutic strategy for anti-2019-nCoV. Moreover, JMap analysis revealed a number of comounds, biologics, and traditional Chinese medicine, among which, Andrographis, Urtica, Sambucus, Astragalus, valproic acid, butyrate, and epoxomicin represent the most significant and possible strategies for anti-2019-nCoV therapies. This study provides a number of clues and possible therapeutic strategies for 2019-nCoV prevention and treatment.

## Introduction

In the end of 2019, some patients with severe febrile respiratory illness were admitted to a hospital in Wuhan, Hubei Province, China. However, no known pathogens were detected in the clinical specimens from these patients[1]. Quickly, sequencing to the RNA extracted from bronchoalveolar-lavage fluid from the patients revealed a novel coronavirus, designated as

2019-nCoV, which belong to the lineage B of the genus betacoronavirus[1]. The virus seems spreads rapidly. As of January 24, 2020, more than 800 cases have been reported and the mortality rate is about 3%[2]. China made a quick response including active case finding, improve public awareness, and sharing sequence information, which provides helps in the patient monitoring, diagnostics and prevention of 2019-nCoV[3,4]. However, a number of key questions remain to be explored[4], for example the virus's origin and cross-species transmission, its ability of spread, the extend of interhuman transmission, and the spectrum of clinical disease.

To address these important questions and issues, the global scientific community also has a quick response. For example, the detection of 2019-nCoV has achieved good advances[5]. In addition, clinical features of patients infected with 2019-nCoV were revealed[6]. Moreover, a number of computational analysis and modeling have been performed to explore the origin, infectivity, and cross-species transmission[7,8,9], evolution[10], transmission patterns and rules[11,12,13,14,15]. These studies provided helps in better understanding the origin, evolution, and transmission of 2019-nCoV.

However, one of the most important questions is that current treatments for 2019-nCoV are empirical[6] and there are still no efficient antiviral therapies[2]. Moreover, we also failed to find publications for the development of therapeutic strategies for 2019-nCoV. Recently, a team led by Profs. Zihe Rao and Haitao Yang at ShanghaiTech University reported the structure of 2019-nCoV proteinase ( $M^{\text{pro}}$ , also called  $3CL^{\text{pro}}$ , PDB ID: 6LU7). Based on the structure, ~20 research teams from Shanghai Institute of Materia Medica (SIMM), Chinese Academy of Sciences (CAS) and ShanghaiTech University together quickly identified 30

potential chemical compounds and natural products with anti-2019-nCoV activity using virtual screening and enzymology, as stated in a News at the SIMM web site on January 25, 2020 ([http://www.simm.ac.cn/xwzx/kydt/202001/t20200125\\_5494417.html](http://www.simm.ac.cn/xwzx/kydt/202001/t20200125_5494417.html), in Chinese). However, before the identification of efficient drugs, the development of more possible therapeutic strategies is still emergently needed. Like SARS-CoV, 2019-nCoV also belongs to the lineage B of the genus betacoronavirus. It thus appears that as SARS-CoV, the human angiotensin-converting enzyme 2 (ACE2) is also the receptor for 2019-nCoV's entry into lower respiratory tract epithelial cells[2]. This was recently confirmed by Letko and Munster[16]. Given that ACE2 is a key human gene for 2019-nCoV' infection to human, it is thus expected that approaches decreasing ACE2 expression could has anti-2019-nCoV activities.

Based on the above observations, here we screened potential agents decreasing ACE2 expression using two databases, Connectivity Map (CMap) developed by Broad Institute and JeaMoon Map (JMap) developed by Co., Ltd of JeaMoon Technology. As a result, CMap analysis identified 5 compounds and JMap analysis identified a number of agents including compounds, traditional Chinese medicine (TCM), and biologics that can functionally decrease the expression of ACE2, suggesting that these agents could be possible possible therapeutic strategies for the prevention and treatment of 2019-nCoV infection.

## Methods

### Datasets of gene expression profiles induced by drugs and other agents

From Connectivity Map[17] (CMap, version 2.0), we obtained the gene expression profiles

induced by ~1000 compounds. We obtained nearly 2000 gene expression profiles induced by a variety of agents including compounds, traditional Chinese medicine, environmental factors, exercise etc from JeaMoon Map (JMap), which is a commercial database developed by Co., Ltd of JeaMoon Technology.

### **Screening agents decreasing ACE2 expression**

We used the normalized gene expression datasets. A cutoff of fold change (FC) 2.0 was selected to determine whether ACE2 is significantly decreased by a putative agent or not. Then, we screened CMap and JMap using a single-gene screening module in the JeaMoon DrugMine software platform.

## **Results**

### **The CMap compounds that decrease ACE2 expression**

As a result, CMap analysis identified 5 compounds that functionally decrease the expression level of ACE2 (**Fig 1**), including Fulvestrant (FC=3.16), Pirinixic Acid (FC=3.13), AG-013608 (FC=2.72), Azathioprine (FC=2.08), and Staurosporine (FC=2.01), suggesting that these compound could relatively block the entry of 2019-nCoV into lower respiratory tract epithelial cells. After a literature search, it seems that only Azathioprine is possibly used for anti-2019-nCoV. Azathioprine is used for the prevention of organ rejection and rheumatoid arthritis by weakening the immune system as an immunosuppressant. The severe pneumonia could be an outcome of extreme immune response of our body to 2019-nCoV. The information for AG-013608 is quite limited and we have no idea about its possibility to be an anti-2019-nCoV therapy. The possibility of the other three compounds is low because of

potential health hazard, toxicity, and side effects.

### **The JMap agents that decrease ACE2 expression**

As a result, JMap analysis identified a number of agents that functionally decrease the expression level of ACE2 (**Fig 2**). These agents include traditional Chinese Medicine (TCM), compound, and biologics (**Fig 2**). The 4 TCM showed significant ability to decrease ACE2 expression, especially Andrographis (FC=21.57), Urtica (FC=11.59), and Sambucus (5.99). Indeed, some studies reported potential effects of anti-pneumonia for active ingredients from Andrographis[18,19], Urtica[20], Sambucus[21], and Astragalus[22]. This finding suggests that the four TCM could be possible anti-2019-nCoV therapies. For the small molecules, we found literature that some predicted compounds can mitigate pneumonia or lung injury, including valproic acid[23], butyrate[24], epoxomicin[25], and emetine[26]. Because arsenic trioxide shows significant toxicity, it should be carefully to test its possibility. Interestingly, nicotine shows an ability of decreasing ACE2 expression, suggesting a possible role of anti-2019-nCoV. This finding may be consistent with our previous report that smoking-inhibited miRNAs are significantly associated with SARS[27]. For the biologics, previous reports supported the prediction for lipopolysaccharide[28]. We previously found oxLDL has protective roles in influenza virus infection[29], suggesting that oxLDL could be a protective factor for 2019-nCoV infection, as predicted here.

## **DISCUSSION**

The recent outbreak 2019-nCoV infection brings a new severe problem for public health.

The unavailability of efficient therapeutic strategies is one of the most important questions at

present. To address this issue, here we identified a number of possible therapeutic strategies by screening CMap and JMap for potential agents decreasing ACE2 expression based on the observation that ACE2 is one key gene for the entry of 2019-nCoV into human cells. This study provides clues for possible therapeutic strategies to fight 2019-nCoV. However, when trying these therapeutic strategies in cases with 2019-nCoV infection, some issues should be considered. Firstly, it is difficult to predict a suitable dosage for the identified agents. Therefore, the dosage should be explored in practice in human cases. Moreover, people in different stages of infection may be sensitive and safe to different dosages. Secondly, the modes of drug delivery should be explored too. Different drugs may take different modes of drug administration for anti-2019-nCoV. In addition, the diseased location is in the respiratory tract, atomization aspirate could get better outcome. Thirdly, combinations of two or more of the identified agents could achieve better therapeutic outcome. Finally, the present study is to provide clues for 2019-nCoV therapy. Practice and operations based on the results of this study should be performed according to specific conditions.

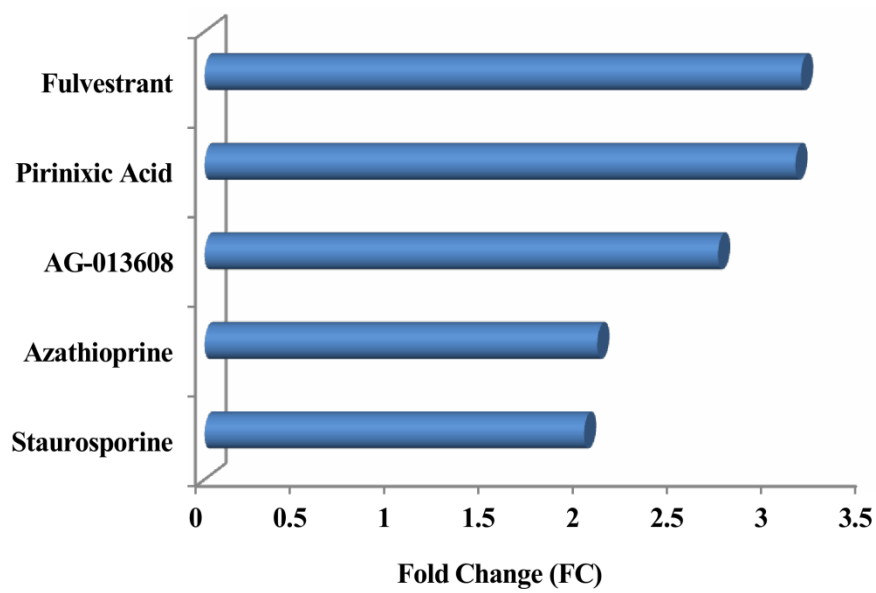
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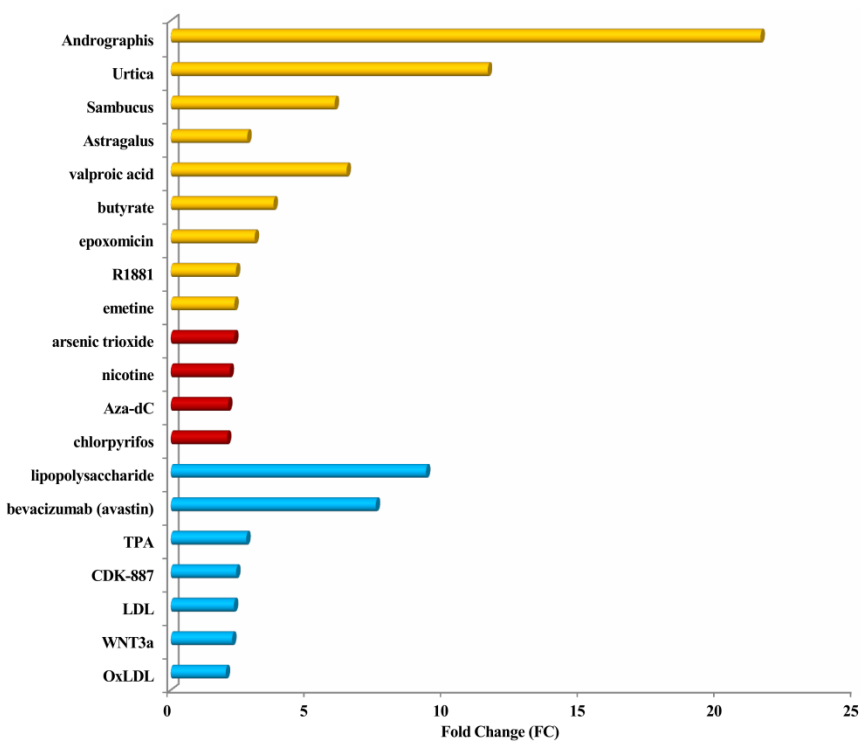
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**Figure 1. CMap analysis identified five compounds that decrease ACE2 expression.** The length of each bar represents the fold change (FC) of ACE2 induced by the corresponding compound. Because ACE2 is one key gene for the entry of 2019-nCoV into human cell, compounds that can decrease ACE2 expression are thus considered to be possible therapeutic strategies for anti-2019-nCoV.



**Figure 2. JMap analysis identified 36 agents that decrease ACE2 expression.** The length of each bar represents the fold change (FC) of ACE2 induced by the corresponding agent. Because ACE2 is one key gene for the entry of 2019-nCoV into human cell, agents that can decrease ACE2 expression are thus considered to be possible therapeutic strategies for anti-2019-nCoV. The agents include traditional Chinese medicine (TCM), Compound, and Biologics, which are shown in different colors.