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Activating Organ's Immunizing Power Against COVID–19–Learning from SARS

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Research Article

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ABSTRACT

Background: Coronaviruses cause respiratory diseases in many animals, including humans. The disease such as SARS has a fatality rate of 15 percent in patients before the age of 60 and more than 40 percent in older patients. The spike–ACE2 complex mediates their entry to host cell and independent from drug therapy inhabit the formation of such kind of complex and people can have complicated immunocompetence to against virus.

Result: Spike protein is an important component of coronavirus structure. Molecular docking experiment revealing the potential capacity for C–type lectin to interact with spike protein obstructs the formation of spike–ACE2 complex. Based on the expression profile of C–type lectin family during infection, we predicting certain member of this kind of protein as potential therapeutic target such as Clec7a, Clec12a and Clec11a, corresponding immune cell types such as CD_4/CD_{28} T–cell, antigen adjuvant with similar C–type lectin receptor–TDM and some immune–boosting drugs–radix sophorae, lactoferrin and *Astragalus membranaceus* for future testing.

Conclusion: C-type lectin-dependent immune cell regulation network may be the potential therapeutic targets for the disease caused by Coronaviruses infecting.

Keywords: C-type lectin; Spike protein; Coronavirus; COVID–19; TDM (Trehalose 6,6′-dimycolate)

INTRODUCTION

Coronaviruses cause respiratory diseases in many animals, including humans [1]. Until the global outbreak of Severe Acute Respiratory Syndrome (SARS) in 2002, the coronavirus "threat" to humans was not

taken seriously enough [2-5]. The disease has a fatality rate of 15 percent in patients before the age of 60 and more than 40 percent in older patients. Nearly 40 percent of patients suffer from respiratory decline requiring assisted ventilation [6]. A decade later, MERS broke out in the Middle East with also coronavirus, Middle East Respiratory Syndrome Coronavirus (MERS-COV) as pathogen.

SARS-CoV combines with type II angiotensin converting enzyme to infect bronchial epithelial ciliary cells and type II lung cells; MERS-COV can bind Dipeptidyl Peptidase 4 (DPP4) and infect undifferentiated bronchial epithelial cells and type II lung cells [7-11]. Four other coronaviruses that infect people and cause respiratory disease are named: HCOV-NL63, HCOV–229E, HCOV-OC43 and HKU1. In this article, investigated the phylogenetic position of COVID–19 with 7 coronaviruses mentioned above. Then, the pathogenesis of the most closely related coronavirus can be borrowed to provide help for the treatment of the disease caused by COVID-19. Meanwhile, C–type lectin family are widely distributing on the surface of human cells and have been shown to activate the immune system [12,13]. Studies have also shown that C–type lectins increase the susceptibility of host cells to coronavirus [14-16], which can be inhibited by mannose lectin or seven repeat small peptide [17,18]. In the case of C–type lectin family members are very rich and different family members may also play different functions during coronavirus infection. We first simulate the interaction between spike protein and one member of C–type lectin to inferring this docking probably inhabiting the interaction between spike protein and ACE2. Then we inspect the expression pattern of C–type lectin family in mouse infected by SARS-CoV, to find out is there any chance for C–type lectin family members participate in way the body's resistance to the coronavirus infection? Suppose if it is possible, appropriate changing in C–type lectin expression profile is an effective response to viral infection which will lead us to propose number of potential drugs for future testing. To further expand our prediction, model C–type lectin–dependent CD_4/CD_{28} T cell network and the presumed indirectly regulator C–type lectin being verified by experiment data.

MATERIALS AND METHODS

Alignments are inferred by MAFFT V7 (G-ins-i, Blosom). Maximum likelihood analyses and bootstrap test carried out by RAXML V8.2 ML+BP online platform. Protein structure is predicted by Swiss modelling online platform. Molecular docking experiment is carried out by Z–dock Version 3.0.2. C–type lectin–dependent CD4/CD28 T–cell Network is modeled by EMT theory.

RESULTS

COVID-19 being closed to SARS

Genome nuclear acids data is used to reconstruct the phylogenetic relationship between COVID–19 and other 7 coronaviruses. The sequences are alignmented by program MAFFT, strategy G–INS–1, scoring matrix for amino acid sequences is BLOSUM. The optimal tree under the popular Maximum Likelihood (ML) criterion is found by RAXML in this work. The phylogenetic position of spike protein of COVID–19 is analysed with the same strategy and based on amino acid sequence. Their tree topology lead to the prediction that either the COVID–19's nuclear acids sequence or the spike protein amino acid sequence is much closer to SARS–CoV'S than with any other species included in this analysis (BP=100, BP=100) (Figures 1a and 1b).

Figure 1a: COVID–19 is closed to SARS. Animal phylogeny based on genome nuclear acid sequences reconstructed using GTR+I+gamma under a Maximum likelihood analyse. COVID-19 is close to SARS-CoV (BP=100).

a

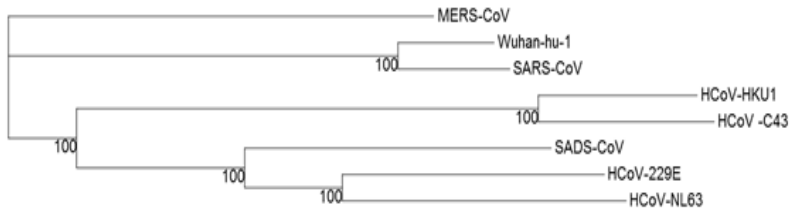
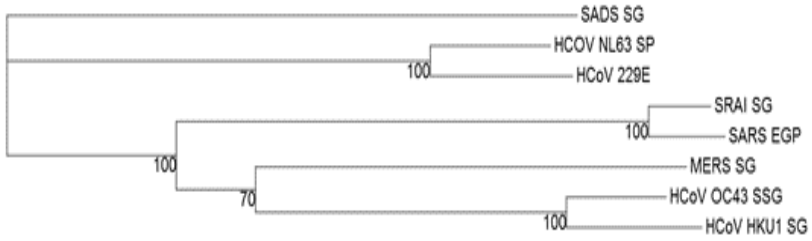


Figure 1b: COVID–19 is closed to SARS. Spike protein's phylogeny based on amino acid sequences reconstructed using WAG+I+gamma under a Maximum likelihood analyse. COVID–19's spike protein is closed to SARS–CoV (BP=100).

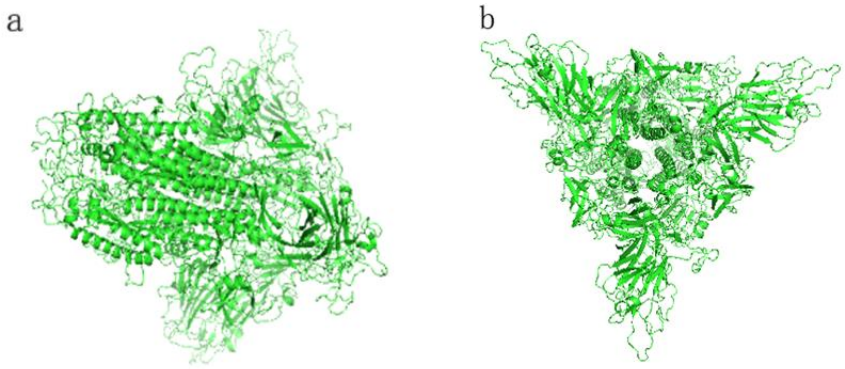
b



Modeling COVID–19 spike protein

Homologous modeling was done for simulating the 3D structure of spike protein of COVID–19 using SWISSMODEL. The similarity between the modeling protein sequence and the template protein sequence is 76.47% and the template sequence is from SARS-CoV (spike protein). The predicted structure passed the test of PROVE, but failed the test of VERIFY, ERRAT and PROCHECK (GMQE=0.73, QMEAN=–3.63) (Figure 2). The other predicted models is either GMQE or QMEAN unqualified.

Figure 2. Homologous modeling for simulating the 3D structure of spike protein of COVID–19 using SWISSMODEL. The similarity between the modeling protein sequence and the template protein sequence is 76.47% and the template sequence is from SARS-CoV (spike protein). The predicted structure passed the test of PROVE, but failed the test of VERIFY, ERRAT and PROCHECK (GMQE=0.73, QMEAN=–3.63). The other predicted models are either GMQE or QMEAN unqualified. a) The vertical axis; b) The horizontal angle of view.



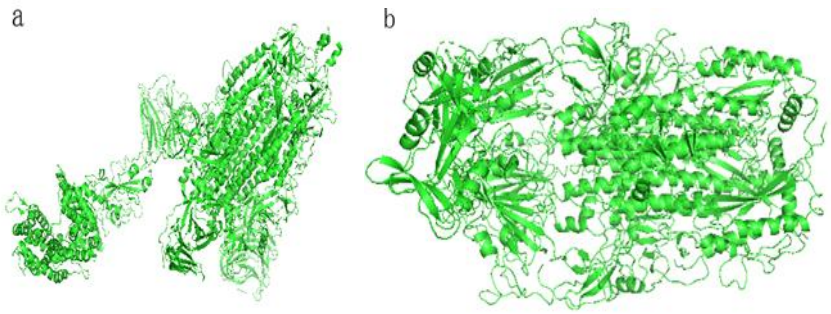
Due to the modeling result cannot pass three independent tests, we can only use the spike protein structure of SARS–CoV to carry out the protein molecular docking experiment.

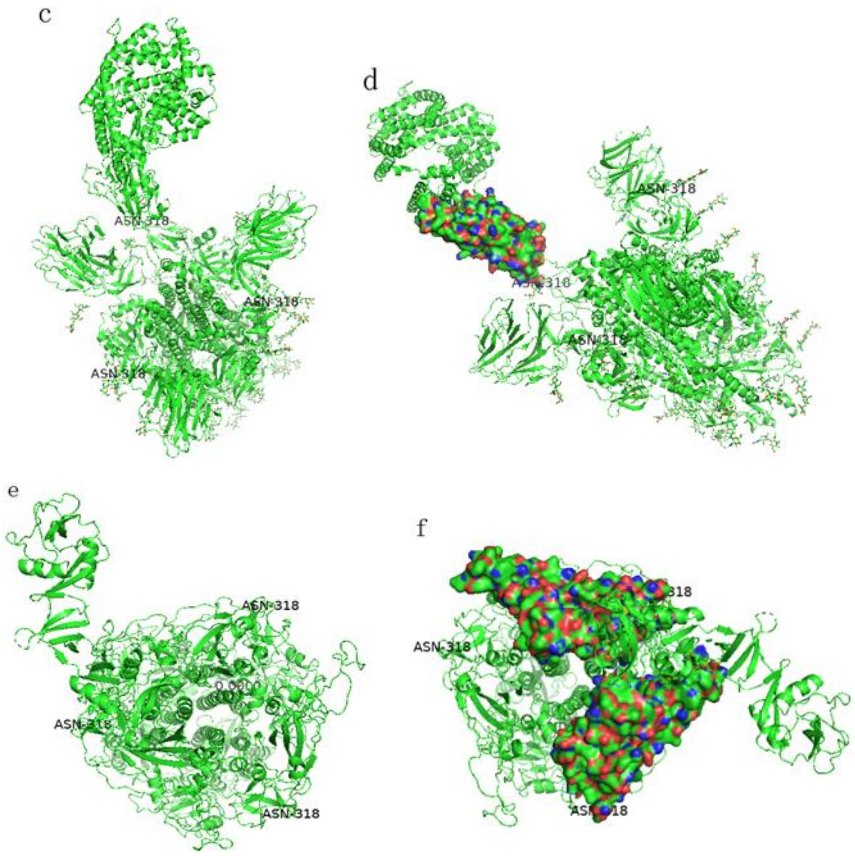
C-type lecin interact with spike protein inhabiting the formation of ACE2–spike complex

We selected the spike protein (PD id=5wrg) of SARS–CoV with known crystalline structure (sequence similarity up to 75.4% and ratio coverage up to 99%) for protein molecular docking experiment. The published protein structures of spike protein (PD id=5wrg) and C–type lectin (macrophage C–type lectin, CELC4D PD id=3whd) were used for this experiment. The online software Z–dock was used to simulate the docking and filtering the predictions with its built–in scoring matrix. The prediction is shown in Figures 3a and 3b.

ACE2 mediates the entry of SARS–CoV to the host cells by binding to virus's spike protein. The binding site is within the RBD (Receptor Binding Domain, N318-V510) [19], as shown in Figures 3c and 3d (PD id=6cs2). The molecular docking result shows that the binding site of C–type lectin also within RBD of spike protein and the docking of C–type lectin shows spatially obstruction for the ACE2–Spike complex formation (Figures 3c-3f).

Figure 3. The simulating of protein interaction. a,b) two perspective of one member of C–type lectin family interact with spike protein (PD id=3whd id=5wrg), using Z–dock to do the docking; c,d) two view of published SARS spike glycoprotein–human ACE2 complex structure (PD id=6cs2); e,f) C–type lectin is docked within the RBD domain. The Spherical surfaces show RBD and the ASN 318 is the first amino acid of RBD, either defines the C–type lectin or the spike protein as the receptor does not change the docking domain under Z–dock simulation.





Changing of expression profile of C–type lecin family indicating potential therapeutic targets

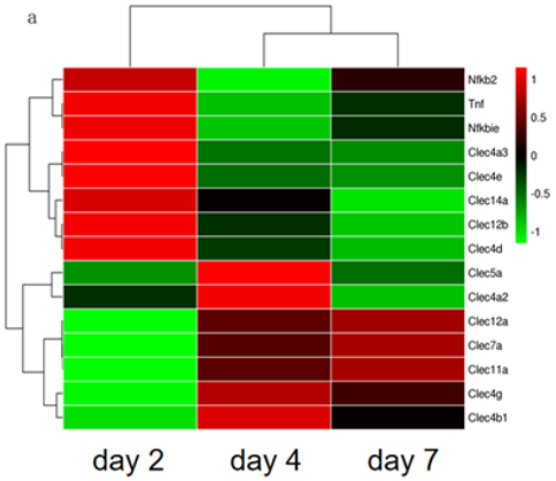
To further test whether C–type lectin family participate in the resistance of virus, mining transcriptome data of mouse response to virus infection [20]. We find that the expression profile of C–type lectin family being significantly changed during first seven days after infected by SARS–CoV in mouse (Figure 4a). Nfkb2, Tnf, Nfkbie, Clec4a3, Clec4e, Clec1 4a, Clec1 2b,Clec4d's expression rates make the peak in the same day the weight of mouse meeting their minimum level [20]. The expression rates of Clec12a, Clec7a and Clec11a rise with the mouse recovering from SARS, indicating their potential roles against virus. While the Clec4a3, Clec4e, Clec1 4a, Clec1 2b and Clec4d has similar expression trend with Nfkb2, Tnf and Nfkbie, indicating that they participate in the C–type lectin–dependant immunological mechanism in the first two days and the real roles of C–type lectin family members shall be further functionally tested.

The immune cell cooperate with C–type lectin during infection. CD (Cluster of Differentiation) is a class of cell surface molecules that are expressed in various types of immune cells [21]. We often use these molecules as cell markers to identify different types of immune cells. We then expand our datasets for clustering analysis adding all CD markers identified in Waters' transcriptome data to predict the cell types participate in the C–type lectin–dependant manner.

The expression rate of Cd59b and Cd209f are positively correlated with Clec12a, Clec7a and Clec11a, indicting the cell type they represent carry out the roles against virus mediated by Clec12a, Clec7a and Clec11a. All Cd28, Cd3d, Cd6, Cd247, Cd27, Cd3g, Cd8a, Cd48, Cd226, Cd8b1, Cd3e, Cd2, Cd19, Cd5, Cd4, Cd160, Cd79b and Cd209a show negative correlation with inflammatory reaction and their expression rates meet their peak when most of mouse recover from SARS indicating the cell type they represent having potential function against virus. Cd80,

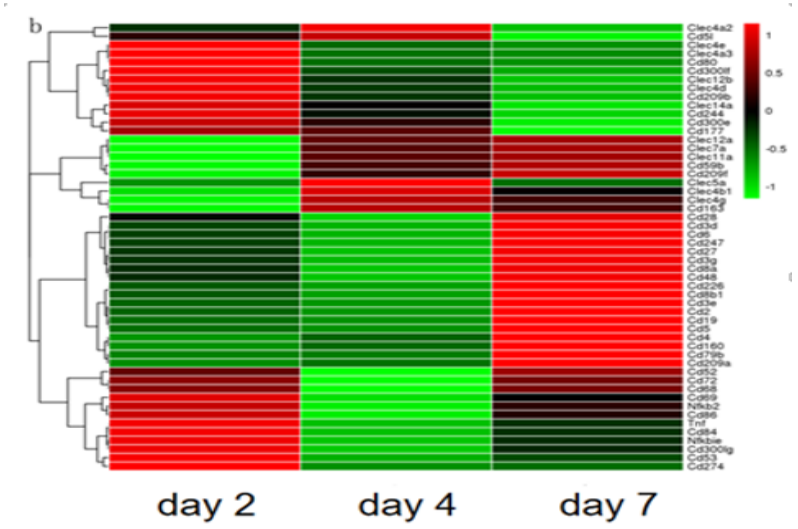
Cd300lf, Cd209b, Cd244, Cd300e and Cd177's expression rates decreasing maybe caused by the cell types they represent are susceptible to virus (Figure 4b).

Figure 4a. Clustering analysis based on Water's wild type mouse infected SARS–CoV transcriptome data.



The expression profile of C–type lectin family being significantly changed during first seven days after infected by SARS–COV in mouse (Figure 4a). Nfkb2, Tnf, Nfkbie, Clec4a3, Clec4e, Clec1 4a, Clec1 2b, Clec4d's expression rates make the peak in the same day the weight of mouse meeting their minimum level [20]. The expression rates of Clec12aClec7a and Clec11a rise with the mouse recovering from SARS, indicating their potential roles against virus. While the Clec4a3, Clec4e, Clec14a, Clec12b and Clec4d has similar expression trend with Nfkb2, Tnf and Nfkbie, indicating that they maybe participate in the C–type lectin–dependant immunological mechanism in the first two days and the real roles of C–type lectin family members shall be further functionally tested.

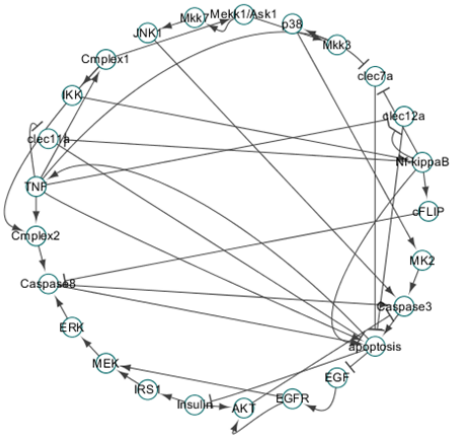
Figure 4b. The expression rate of Cd59b and Cd209f are positively correlated with Clec12a, Clec7a and Clec11a, indicting the cell type they represent maybe carry out the roles against virus mediated by Clec12a, Clec7a and Clec11a. All Cd28, Cd3d, Cd6, Cd247, Cd27, Cd3g, Cd8a, Cd48, Cd226, Cd8b1, Cd3e, Cd2, Cd19, Cd5, Cd4, Cd160, Cd79b and Cd209a show negative correlation with inflammatory reaction and their expression rates meet their peak when most of mouse recover from SARS indicating the cell type they represent having potential function against virus. Cd80, Cd300lf, Cd209b, Cd244, Cd300e and Cd177's expression rates decreasing maybe caused by the cell types they represent are susceptible to virus. Data is normalized with Z–score.



Modeling CD4/CD28 T-cell network to verify the potential indirectly regulation roles of C-type letin during infection

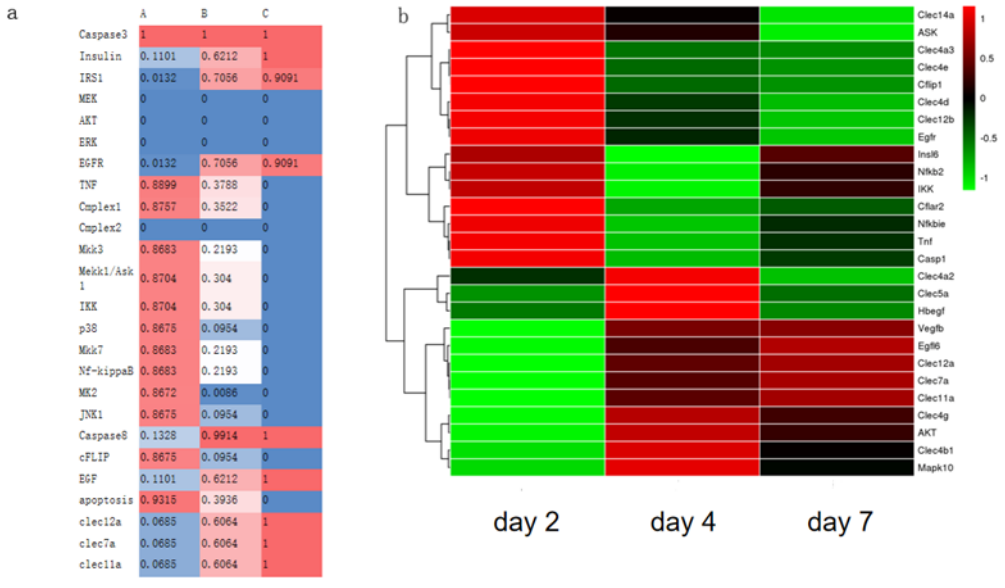
modeled the CD4 and CD 28 regulation network [22], presuming that Clec7a, Clec12a and Clec11a which positively correlate with CD4 and CD28 while negatively correlating with TNF and Nf-kippa B participate in the network as the correlation showed (Figure 5).

Figure 5. C-type lectin-dependent CD4/CD28 T cell network. **Note:** The arrows "→" in the network indicate "activate" and the other type "⊣" indicate "inhibit". The nodes and relationship between each nodes details in reference [22].



Using EMT (Endogenous Molecular Network Theory) to provide a general framework to quantify the network and transformed it into a nonlinear dynamic system, there are three attractors underlying the T-cell Endogenous Molecular Network (TEMT) being found [23]. The presumed roles of Clec7a, Clec12a and Clec11a been verified by experimental data while the expression trend of EGF, IKK, AKT, ASK, and cFLIP in attractors (A-B-C) all in good agreement with experiment data (day 2-day 4-d 7).

Figure 6. The prediction of roles of Clec7a, Clec12a and Clec11a within T cell survival network being verified by experiment data. a. Three attractors predicted by EMT (Endogenous Molecular Network Theory) methods [23]. b. Experiment data of mouse infected by SARS-COV which shows EGF, IKK, AKT, ASK, and cFLIP all in good agreement with prediction. Meanwhile the real C-type lectin-dependent T-cell regulation network may be far more complicated than our idealized model.



Above all, the COVID–19's nuclear acids sequence or the spike protein amino acid sequence has much closer relationship with SARS–COV than with any other species included in this analysis. Using the spike protein of SARS–COV, to do molecular docking finds out C–type lectin may inhibit the interaction between ACE2 and spike protein. The expression profile of C-type lectin family changes significantly during infection and the correlation between C–type lectin, Tnf, NF-kippa B and some CD markers meets the logic of C–type lectin activate immunological mechanism to against virus–The activation of NF-kippa B and TNF are important to the host immune response during infection [20] . The activation of NF-kippa B signaling can alleviate SARS pathological characterization [20,24] C–type lectin can activate NF-kippa B signaling [20,25,26]-NF-kippa B and TNF have an indirect regulatory relationship after coronavirus infection [20,27] indicating C–type lectin and related immune cells shall be the potential therapeutic target of SARI and the changing of expression profile of C-type lectin family maybe an effective way for mouse against to coronavirus. Modeling C–type lectin–dependent T-cell network and the modeling result being verified by experiment data.

Studies have shown that macrophage derived C-type lectin can recognize Trehalose Dimycinate （rehalose 6,6'–dimycolate, TDM） and activate NF–kippa B signaling [20,25,27]. TDM is a surface antigen of bacteria such as mycobacterium, which can be recognized by C–type lectin and induce the immune response of type 1 macrophages [28,29]. TDM can also induce pneumonia and activate the immune function of Th cells [30,31]. So it meets logic to try the TDM–aqueous solution as antigen adjuvant to activate the adaptable immunology of organ against COVID–19 [26,31,32]. This prediction has been testified to some extent by other studies such as: CELC4d (PD id=3whd) can activate NF-kippa B dependenting CARD9/Bcl10/Malt1 for TDM induced Mincle expression and activating NF–kippa B signaling can alleviate SARS pathological characterization [20, 24, 26], whose expression rate positively correlates with NF-kippa B and interacting with spike protein to inhabit Spike-ACE2 complex formation which we has illustrated above in this work. We also noticed that CD209 is highly expressed in day seven and one of its role is facilitating SARS–COV spike protein bearing pseudo type driven infection of permissive cells *in vitro*, but SARS patients with CD209 does not show significant chance of having poorer prognosis (60% is not a persuasive data) [33], which claims for further elucidating the function of corresponding cells during virus infection.

Meanwhile, to alleviate symptoms of SARI, we suppose some drugs that are effective in treating TDM induced pneumonia considering the antigen structure similarity: radix sophorae [34]; lactoferrin [35]. Also, drugs that increase the number of immune cells and activate cytokines such as TNF and IL6, shall be taken into consideration: *Astragalus membranaceus* [36-39].

DISCUSSION

Coronavirus genes have been known to evolve in a variety of ways. Spike protein also went through the complicated process of adaptive evolution [40]. So the phylogenetic analysis under current methodology this work shall only be the evidence to learning from SARS-CoV related immunological mechanism.

We already know that innate and acquired human immunity can play an important role in the response to coronavirus infection. The fact that SARS patients recovering spontaneously which has been widely reported is circumstantial evidence. Which drives us to improve the body's immunity to against coronavirus.

CONCLUSION

Some particular members of C-type lectin and related immune cells shall be the potential therapeutic target of SARI and the changing or expression profile of C-type lectin family effective way for mouse against to coronavirus. Based

on the prediction we suppose using TDM–aqueous solution as antigen adjuvant; radix sophorae, lactoferrin and *Astragalus membranaceus* for adjuvant therapy.

It must be clarified that the *in vivo* mechanism of SARI shall be far more complicated and further test of our prediction is imperative.

COMPETING INTERESTS

The authors declare that they have no competing interests.

ACKNOWLEDGEMENTS

Thanks to my sister Chuanxin Xia's for her kind help and favorable assistance. Hoping this work can inspire fellow scientists for related experiment design. I hope to improve people's confidence in overcoming SARI. Although the specific mechanism related to SARI, SARS or MERS has not been made all clear, this work can at least suggest that mammalian can fight against coronavirus itself.

AUTHORS' CONTRIBUTION

Yi Wang create the project, designed the experiment and wrote the manuscript.

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