Activating organ's immunizing power against COVID–19–learning from SARS

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Research

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Abstract

Background Coronaviruses cause respiratory diseases in many animals, including humans. Spike protein is an important component of coronavirus structure and the formation of ACE2 (angiotensin converting enzyme 2)–spike complex mediates virus entry to host cells. C–type lectin family are widely distribute on the surface of human cells and have been shown to activate the immune system. In this article, we first illustrate why we can “learn from SARS” with phylogenetic analysis. Then, we use SARS spike protein structure, to inferring our molecular docking experiment, revealing the potential capacity of C–type lectin to directly interact with spike protein obstructs the formation of spike–ACE2 complex. Considering the expression profile of C–type lectin family changing significantly during infection, we predict certain members of this kind of protein as potential therapeutic target and verify their assumed function by inferring an C–type lectin–dependent CD4/CD28 T cell survival molecular network with endogenous molecular network theory (EMT) and comparing the predicted expression trend corresponding to each molecular with experiment data.

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 SWISSMODELLING 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 inferred by EMT theory.

Result Our molecular docking experiment revealing the potential capacity of C–type lectin to directly 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 CD4/CD28 T cell simulated by EMT theory and verified by experiment data, antigen adjuvant with similar C–type lectin receptor–TDM and some immune–boosting drugs–radix sophorae, lactoferrin and Astragalus membranaceus, for future testing.

Conclusions C–type lectin and their corresponding immune cells predicted in this work may be the potential therapeutic targets for the disease caused by COVID–19. C–lectin with the capacity of directly interact with spike protein inhibiting the formation of ACE2–spike complex may be the way they execute anti–virus function. The corresponding cell type such as CD4/CD28 T cell may participate and against virus while Clec7a, Clec12a and Clec11a presumed capacity for facilitating CD4/CD28 T cell survival during infection being verified by EMT combining with experiment data. Our prediction at least suggest the possibility of activating organ’s immunizing power to prevent from COVID–19 and the drugs we suggested are all need to be further tested. Trial registration Retrospectively registered. Keywords C–type lectin, spike protein, coronavirus, COVID–19, TDM.

Background

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 ACE2 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, we first investigated the phylogenetic position of COVID–19 with 7 coronaviruses mentioned above included. 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 members are widely distribute 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]. We also known that C–type lectin family members are very rich and different member may also play different roles during coronavirus infection.

In this work, we first simulate the interaction between spike protein and one memeber 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 it were possible, appropriate changing in C–type lectin expression profile is an effective response to viral infection which lead us to propose a number of potential drugs for future testing. To further verified our prediction, we model C–type lectin–dependent CD4/CD28 T cell network and the simulation results are in good agreement with experiment data .

Results

COVID–19 being closed to SARS

We use genome nuclear acids data to reconstructed the phylogenetic relationship between COVID–19 and other 7 coronavirus. 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 postion 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 to any other species included in this analysis(BP = 100, BP = 100) (Fig. 1a,b).

Modeling COVID−19 spike protein

We then do 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) (Fig. 2a,b). The other predicted models is either GMQE or QMEAN unqualified.

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

Type lecin interacting with spike protein inhabiting the formation of ACE2−spike complex.

We select 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) are used for this experiment. The online software Z−dock are used to simulate the docking and filtering the predictions with its built−in scoring matrix. The prediction is shown in Fig. 3a,b.

ACE2 mediates the entry of SARS−Cov to the host cells by binding virus's spike protein. The binding site is within the RBD (receptor binding domain, N318−V510) [19], as shown in Fig. 3c,d (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 (Fig. 3c, d, e, f).

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, we 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 (Fig. 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 have similar expression trends with Nfkb2, Tnf and Nfkbie, indicting 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.

We also wondering, which type of 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 McDermott's transcriptome data to predicting 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 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 recovered from SARS indicating the cell type they represent having potential function against virus. Cd80, Cd300lf, Cd209b, Cd244, Cd300e and Cd177's expression rates decreasing the whole time may be caused by the cell types they representing are susceptible to virus (Fig. 4b).

b.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 recovered 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 representing are susceptible to virus. Data is normalized with Z–score for clustering analysis.

Inferring C–type lectin–dependent CD4/CD28 T cell survival network

We infer the C–type lectin–dependent CD4/CD28 T survival cell network (detailed in reference [22]), presuming the roles of Clec7a, Clec12a and Clec11a, which positively correlating with CD 4 and CD 28 while negative correlating with TNF and Nf–kippa B (Fig. 5) as the inhibitor of apoptosis, comparing the network dynamic landscape with experiment data to verify the predicted function of these C–type lectin family members.

Using EMT to provide a general framework to quantify the network and transformed it into a nonlinear dynamic system, there are three states underlying the T cell survival endogenous molecular network (TEMT) being found–state A, B and C; B is a saddle point and the network dynamics constructed by introducing stochastic fluctuation.) [23]. The presumed roles of Clec7a, Clec12a and Clec11a been verified while the predicted expression trends of EGF, IKK, AKT, ASK, and cFLIP (A–B–C) all in good agreement with experiment data (day 2–day 4–d 7) (Fig. 6a,b).

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. We also inferring C–type lectin–dependent T cell network and the modeling results being verified by experiment data.

Studies have shown that macrophage–derived C–type lectin can recognize TDM(trehalose 6,6'–dimycolate) 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 macrophages [28, 29]. TDM also can induce pneumonia and activate the immune function of Th cells [30, 31]. So it also meets logic to try the TDM–aqueous solution as antigen adjuvant to activate the adaptable immunology of organ to 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. We also notice that CD209 is highly expressed in day seven and one of its role is facilitating SARS–Cov spike protein–bearing pseudotype 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 in 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 recovered spontaneously which has been widely reported is circumstantial evidence. This is what drives us to improve the body's immunity to against COVID–19.

The particular members of C–type lectin and related immune cells predicted above shall be the potential therapeutic target of SARI and the changing of expression profile of C–type lectin family may be an effective way to against 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.

Above all, C–type lectin and their corresponding immune cells predicted in this work may be the potential therapeutic targets for the disease caused by COVID–19. C–lectin with the capacity of directly interact with spike protein inhibiting the formation of ACE2–spike complex may be the way they execute anti–virus function. Our prediction at least suggest the possibility of activating organ’s immunizing power to prevent from COVID–19 and the drugs we suggested are all need to be further tested.

Conclusions

The phylogenetic analysis inferring the logic to “learn from SARS” and the molecular docking experiment revealing the potential capacity of C-type lectin to directly interact with ACE2 to obstruct the formation of spike–ACE2 complex. Considering the expression profile of C–type lectin family changing significantly from infection to recovery spontaneously, we predicting certain members of this kind of protein as potential therapeutic target such as Clec7a, Clec12a and Clec11a, the potential function of C–type lectin and corresponding immune cell types such as CD4/CD28 T cell simulated by EMT theory and verified by experiment data, antigen adjuvant with similar C–type lectin receptor–TDM and some immune–boosting drugs–radix sophorae, lactoferrin and Astragalus membranaceus. Our prediction at least suggest the possibility of activating organ’s immunizing power to prevent from COVID–19, with Clec7a, Clec12a and Clec11a which have postivtive correlation expression rates with recovering and presumed capacity for facilitating CD4/CD28 T cell survival during infection being verified by EMT combining with experiment data; the significantly changing of expression file of C–type lectin family from infection to recovery combining with the capacity of interacting with spike protein inhibiting the formation of ACE2–spike complex. Meanwhile the in–vivo mechanism of C-type lectin–dependent T cell network shall be far more complicated than our idealized one claiming for further investigation and the drugs we suggested are all need to be further tested.

Abbreviations

Cov: coronavirus; SARS: severe acute respiratory syndrome; MERS: Middle East respiratory syndrome coronavirus; Wuhan-Hu-1: Wuhan seafood market pneumonia virus isolate Wuhan-Hu-1; SADS: Swine acute diarrhea syndrome; HCoV-NL63: Human Coronavirus NL63; HCoV-229E: Human coronavirus 229E; HCoV-HKU1: Human coronavirus HKU1; HCoV-OC43: Human coronavirus OC43; TDM: trehalose 6,6'–dimycolate; TEMT: T cell endogenous molecular network; EMT: endogenous molecular network theory; CD: cluster of differentiation; ACE2: angiotensin converting enzyme 2. DPP4: dipeptidyl peptidase 4.

Declarations

Ethics approval and Consent for publication

Written informed consent for publication was obtained from all participants and Ethics approval is not applicable.

Availability of data and materials

8 species of coronavirus and their spike protein from GenBank used in this study is detailed in supplement table 1. Top 10 predicted C–type lecin–Spike complex showed in supplement 1.

Competing interests

The authors declare no competing interests.

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Authors' Contributions

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

Chuanxin Xia wrote the manuscript. All authors read and approved the final manuscript.

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Hoping this work can inspiring fellow scientists for related experiment design and improving people's confidence in overcoming SARI.

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Figures

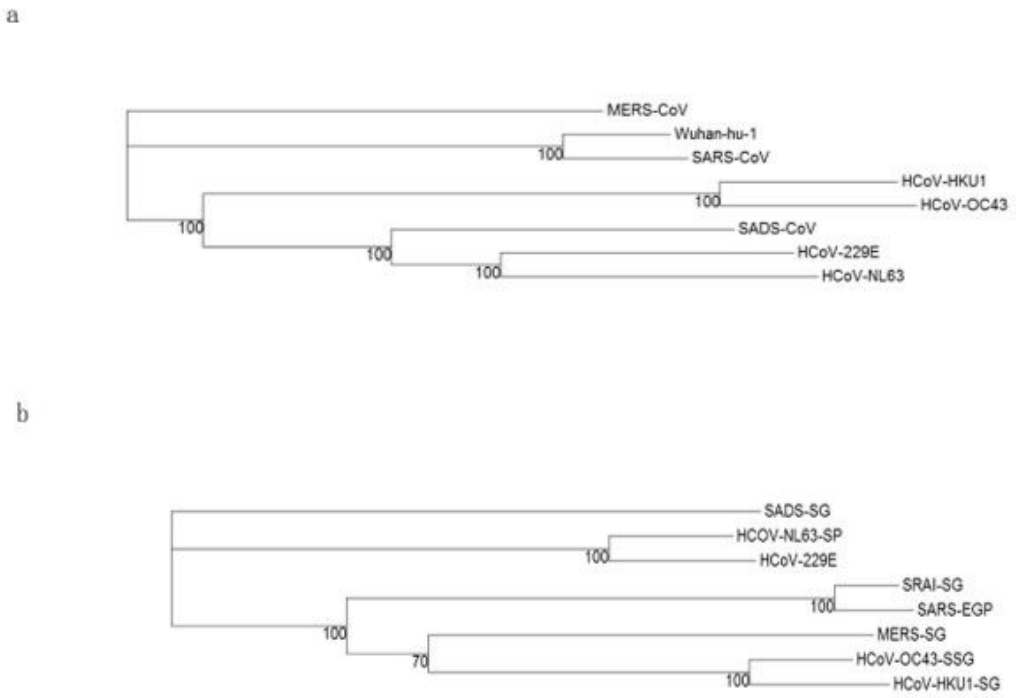

Figure 1

COVID–19 is close to SARS. a. 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). b. 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 close to SARS–Cov (BP=100).

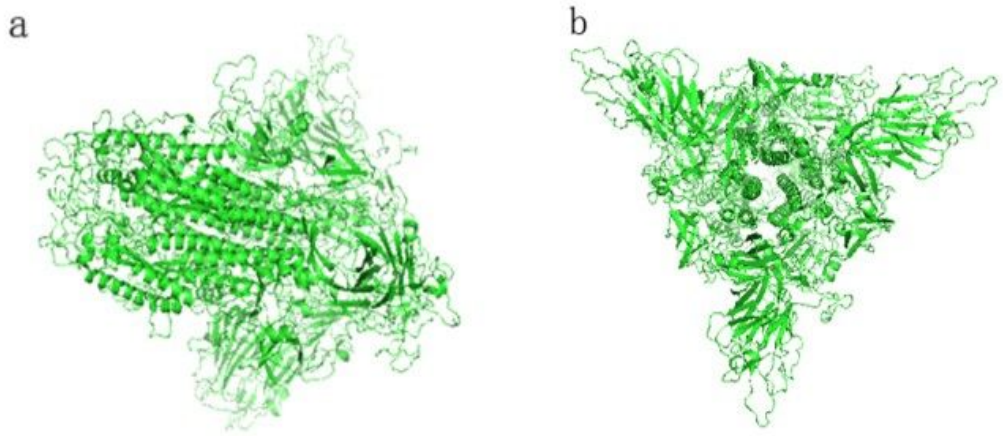

Figure 2

Homologous modeling 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 is either GMQE or QMEAN unqualified. a. The vertical axis. b. The horizontal angle of view.

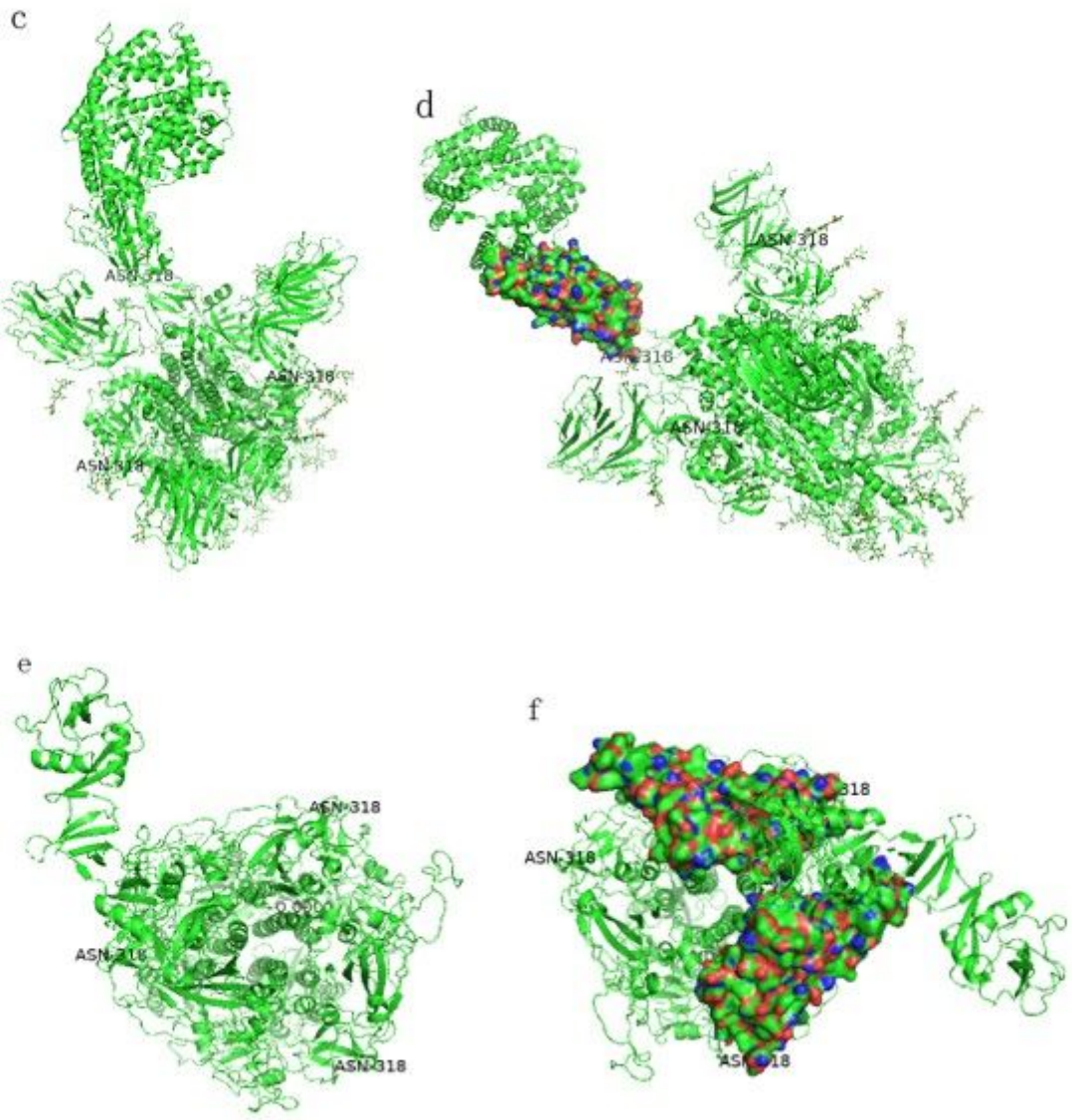

Figure 3

The simulating of protein interaction. a,b two perspective of one member of C–type lectin family (PD id= 3whd) interact with spike protein (PD id = 5wrg), using Z–dock to do the docking. c,d two view of published SARS spike–ACE2 complex structure (PD id =6cs2). e,f C–type lectin is docked within the spike's RBD which spatially obstruct the formation of spike–ACE2 complex. 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 result under Z–dock simulation.

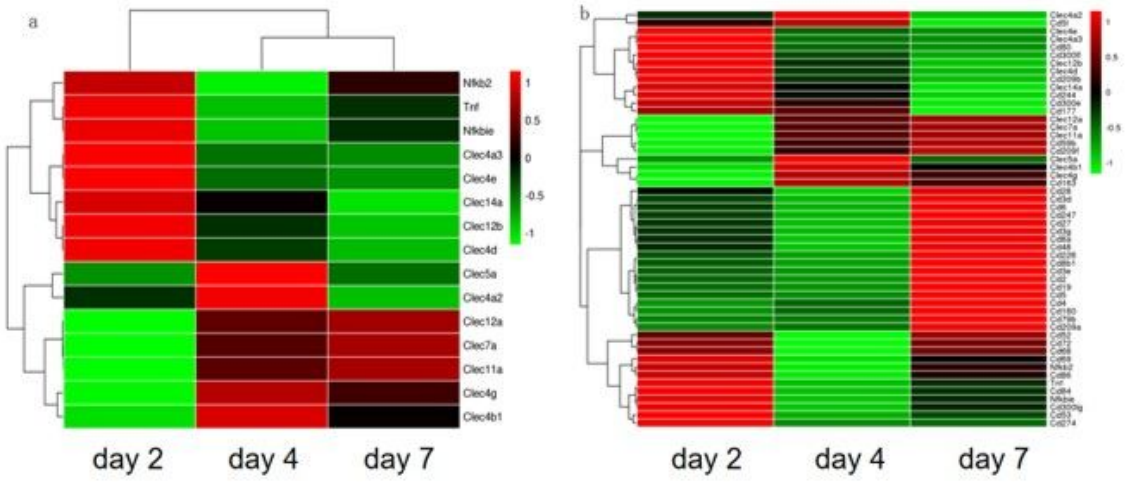

Figure 4

Clustering analysis based on McDermott's transcriptome data. a. The expression profile of C–type lectin family is significantly changed during first seven days after infected by SARS–Cov in mouse (Fig 4 a). Nfkb2, Tnf, Nfkbie, Clec4a3, Clec4e, Clec1 4a, Clec1 2b,Clec4d's expression rates make their 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 have similar expression trends with Nfkb2, Tnf and Nfkbie, indicting 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.

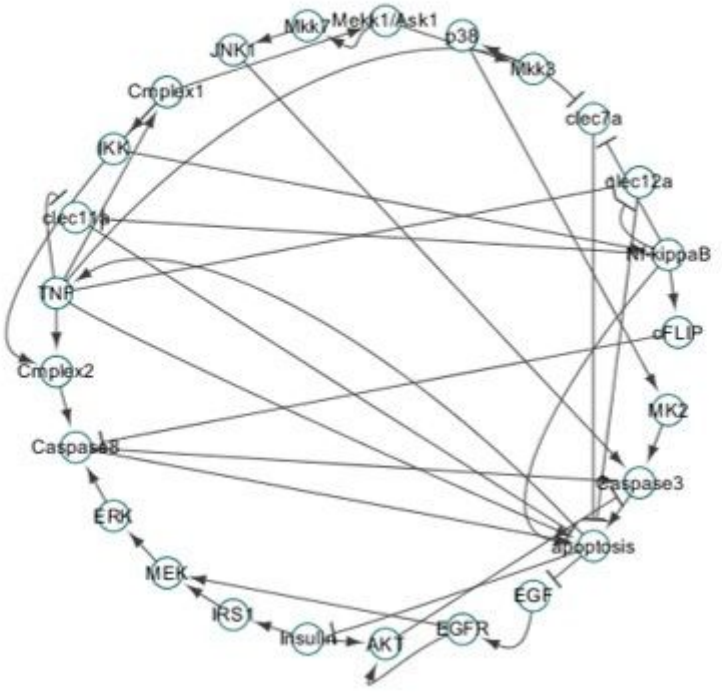

Figure 5

C–type lectin–dependent CD4/CD28 T survival cell network. The arrows “ ” in the network indicate “activate” and the other type “ ” indicate “inhibit”. The molecules and interaction between each molecule are detailed in reference 22.

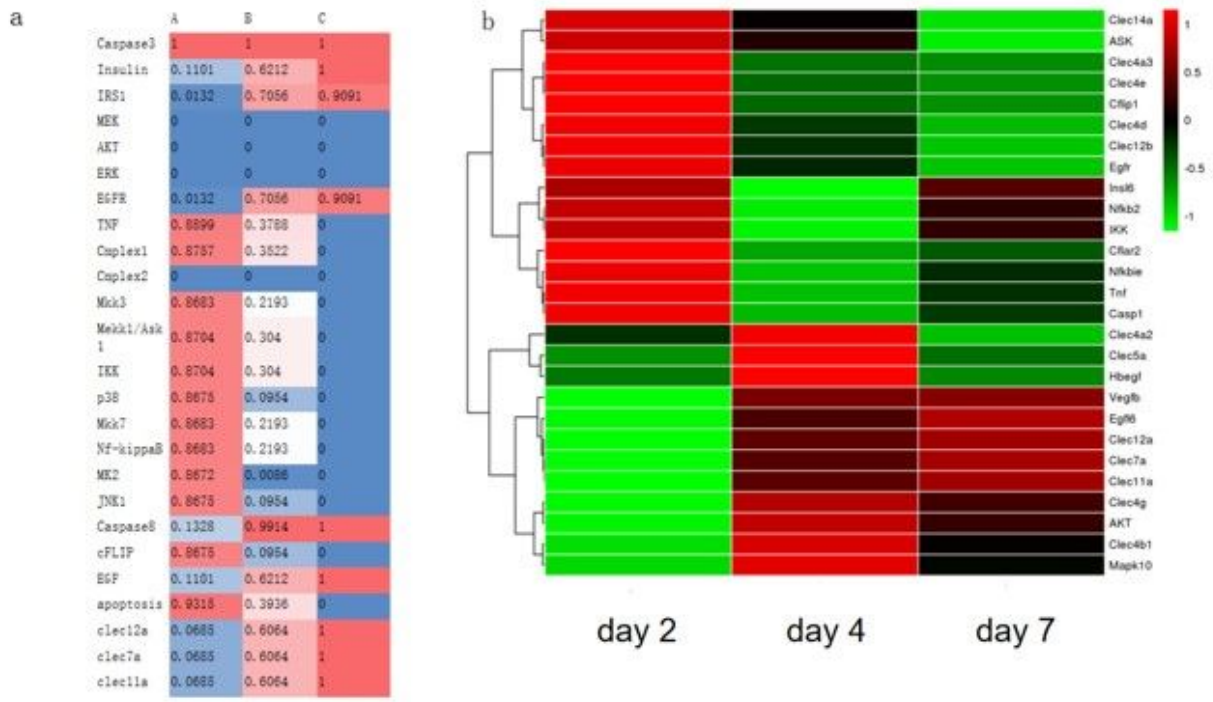

Figure 6

The prediction of roles of Clec7a, Clec12a and Clec11a within T cell survival network. a. Three states predicted by EMT (endogenous molecular network theory) methods (details in reference [23]). B. Experiment data of mouse infected by SARS–Cov shows EGF, IKK, AKT, ASK, and cFLIP's expression trends all in good agreement with simulation results. Meanwhile the real C–type lectin–dependent T cell regulation network maybe far more complicated than our idealized network model.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementtable1.docx
- supplement1SPIKECL.7z