Discovery of potential drugs for COVID-19 based on the connectivity map

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Research

Abstract

Background: Corona virus infective disease 19 (COVID-19) is the disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and spreads very rapidly, which become a worldwide public healthy crisis. Until now, there is no effective antivirus drugs or vaccines specifically used for its treatment. So it is urgent to discover efficient therapeutic methods. The same as SARS-CoV, SARS-CoV-2 also invades organism by combining with Angiotensin-converting enzyme 2 (ACE2). Recently, there are reports about SARS-CoV-2 infected host not only through the respiratory tract, but also gastrointestinal tract. However, it is proved that ACE2 plays a key role in protecting subjects from lung injury and resisting the inflammation caused by intestinal epithelial damage. Interestingly, the expression of ACE2 protein is reduced after SARS-CoV infection.

Methods: According to the dataset of genes co-expressed with ACE2 in the colonic epithelial cells, we established a protein-protein interaction (PPI) Network and selected hub genes from them. The cluster analysis was performed to find out the dense region of the PPI Network. Then, gene ontology (GO) and pathway enrichment analysis were performed to explore the main function of genes co-expressed with ACE2. Finally, we predicted the potential drugs for the treatment of COVID-19 based on the connectivity map (Cmap) .

Results: We constructed a PPI network containing 125 hub genes of genes co-expressed with ACE2 in the colonic epithelial cells and obtained two modules through cluster analysis. The GO analysis and the KEGG pathway revealed these genes were aggregated in ribosome, exosomes, extracellular cellular components; structure constituent of ribosome, G-protein coupled receptor activity, MHC class I and II receptor activity biological processes; immune response, protein metabolism, signal transduction biological processes; and ribosome, graft-versus-host disease, viral myocarditis pathways. The result from Cmap indicated ikarugamycin, molsidomine had highly correlated scores with the query files.

Conclusion: We found out that ikarugamycin and molsidomine were the potential drugs for the treatment of COVID-19.

Background

The outbreak of SARS-CoV-2 in 2019, originated from Wuhan, Hubei Province in China, reached multiple continents in merely a month, which has been declared to be a Public Health Emergency of International Concern by the World Health Organization (WHO). And the disease caused by SARS-CoV-2 is named as corona virus infective disease 19 (COVID-19). It is reported that compared with SARS-CoV, although SARS-CoV-2 has lower case fatality rates[1], it has higher transmissibility and is prone to affect older patients with comorbidities[2]. The main transmission route is through respiratory tract, however there is lots of evidence supporting the infection by gastrointestinal tract. For example, some cases had diarrhea[3, 4] or detecting positive SARS-CoV-2 from the stools of a patient[5].

After comparing the genome of SARS-CoV-2 and SARS-CoV, it showed that SARS-CoV-2 had 82% nucleotide identity with SARS-CoV[6]and used ACE2 as its receptor just like SARS-CoV[7]. ACE2 is a carboxymonopeptidase catalyzing vasoactive angiotensin II to angiotensin-[1–7],which acts like the antagonist of angiotensin and balances the ACE/Ang II/Ang II type I receptor axis[8]. Paradoxically, ACE2 is protective against a variety of pulmonary diseases, including acute respiratory distress syndrome, acute lung injury, pulmonary hypertension, asthma, and chronic obstructive pulmonary disease[9]. Besides, ACE2 also plays important role in maintaining the normal function of intestinal tract, such as regulating dietary amino acid homeostasis, gut microbial ecology and innate immunity. Deficiency in ACE2 increased susceptibility to intestinal inflammation for the reason of epithelial damage[10]. SARS-CoV downregulated ACE2 protein expression after infecting host[11]. Considering the homology of SARS-CoV-2 and SARS-CoV, SARS-CoV-2 may interfere the expression of ACE2 as well. Given that SARS-CoV-2 can spread through gastrointestinal tract, it is reasonable to speculate that SARS-CoV-2 can inhibit intestinal ACE2 expression, which leads to dysfunction of intestinal tract and the development of COVID-19.

In view of the above, Jun Wang and his colleagues analyzed the expression of ACE2 in single-cell RNA sequencing dataset from healthy subjects and patients with colitis or IBD and found that ACE2 was highly expressed in colonocytes[12]. After that, they analyzed genes co-expressed with ACE2 in the colonic epithelial cells and found 3420 positively correlated, 2136 negatively correlated genes. Using this dataset, we constructed a PPI network and found out hub genes which were used for the GO analysis including cellular components, molecular functions and biological processes as well as the KEGG pathways. The Cmap is a database including gene expression profiles of varies of human cell lines which are disposed in different small molecules[13]. Comparing the genes co-expressed with ACE2 and gene expression profiles in Cmap, we preliminarily speculated the potential existing drugs for the therapy of decreased ACE2 expression in COVID-19. It was a process of drug repositioning which resolved the troubles in new drug research and development.

Methods

Genes Co-expressed with ACE2

We obtained the genes co-expressed with ACE2 from the gene list shared by Jun Wang[12]. It includes 3420 positively correlated, 2136 negatively correlated genes.

The Construction and Analyzing of PPI Network

The PPI network was constructed by STRING database with combined score > 0. 4 (Version 11.0, ELIXIR, Europe, https://string-db.org/)[14] based on the top 1000 positively and negatively correlated co-expressed genes of ACE2 in the colonic epithelial cells. The PPI network was shown in Cytoscape (Version 3.6.1, Cytoscape Consortium, U.S) and the unconnected nodes were discarded, which had 952 nodes and 4824 edges. Centiscape[15], the app of Cytoscape, was used to calculate the degree measure of each node. Referring to the previous study of others[16, 17], we determined the nodes whose degree

were more than twice the mean degree value as hub genes. In order to explore more specific regulatory relationship in the above PPI network, the cluster analysis was performed by MCODE [18]. Data parameters was set with thresholds of K-Core > 5.

GO and KEGG Pathway Enrichment Analysis

To investigate the main functional mechanisms of the genes co-expressed with ACE2 in the colonic epithelial cells, the GO analysis dividing into cellular components, molecular functions, biological processes and the KEGG Pathway enrichment analysis were performed by FunRich[19] and Webgestalt (ORA method) (http://www.webgestalt.org)[20].

The Potential Drugs Based on The Cmap Database

We divided the genes into two groups: positively regulated genes and negatively regulated genes and uploaded files to to the CMap Web Service (Update 12 September 2017, https://portals.broadinstitute.org/cmap/index.jsp). In the permuted result, small molecules with a score > 0.4 were highly positively correlated with genes co-expressed with ACE2 and were taken as potential drugs for the treatment of COVID-19.

Results

Genes Co-expressed with ACE2 in The Colonic Epithelial Cells

We got 3420 positively correlated and 2136 negatively correlated genes co-expressed with ACE2 in the colonic epithelial cells from the supplement material of the study reported by Jun Wang and his colleagues[12](Supplementary table).

Protein−Protein Interaction

The top 1000 genes of positively correlated and negatively correlated genes co-expressed with ACE2 in the colonic epithelial cells were selected to construct the PPI network through STRING database, involving 952 nodes and 4824 edges. CentiScape was used to calculate the degree of each nodes. The node whose degree was more than twice the mean degree value was identified as hub gene. According to this, 125 genes were considered to be hub genes. And a PPI network was performed to the hub genes (Fig. 1).

In order to analyze the main subunits and their interactions of the complex, cluster analysis was performed using MCODE. Two modules were extracted from the PPI network with K-Core > 5 (Fig. 2). One cluster included 36 nodes and 396 edges (cluster rank 1; Score 22.629) and the other cluster included 22 nodes and 231 edges (cluster rank 2; Score 22.000).

The GO and KEGG Pathway Analysis

The network of hub genes consisted of 125 nodes and 807 edges. Three topologically features (degree, betweenness, closeness) were calculated to find out the most important genes from the hub genes. We

kept 113 genes whose scores of the three items mentioned above were more than the mean values of all hub gene nodes. In order to further explore the functions of co-expressed genes with ACE2, enrichment analysis was performed and we obtained cellular components related with ribosome, exosomes, extracellular, integral to plasma membrane, centrosome, cytosol, and molecular functions related with structure constituent of ribosome, G-protein coupled receptor activity, MHC class I and II receptor activity, and biological processes related with immune response, protein metabolism, signal transduction, cell communication (Fig. 3 and Table I). The KEGG pathway were ribosome, graft-versus-host disease, viral myocarditis, allograft rejection, antigen processing and presentation (Fig. 4 and Table II).

Potential COVID-19 Drugs Predicted by The Cmap

To find out the potential drugs that are capable of treat COVID-19, the above 113 genes were uploaded to the Cmap database. The most promising candidate drugs were revealed based on the rank of positive connectivity scores, which may counteract the gene expression change caused by the decreased expression of ACE2 after infected by SARS-CoV-2.Ultimately, we found there were two drugs, ikarugamycin and molsidomine, being supposed to be potential drugs (Table III).

Discussion

COVID-19 broke up in 2019 at Wuhan, Hubei Province of China and spreads over the world at a horrific speed. The pathogen of COVID-19 is named as SARS-CoV-2, who had 82% nucleotide identity with SARS-CoV. From the clinical data, COVID-19 manifests with fever, nonproductive cough, dyspnea, myalgia, fatigue, normal or decreased leukocyte counts, and severe lung injury. The sereve or death cases also showed organ dysfunction, including shock, acute respiratory distress syndrome (ARDS), acute cardiac injury, acute kidney injury, liver dysfunction and secondary inflammation[3, 21–23]Therefore, it is urgent to find out effective strategies to protect the organ and reduce the mortality rate.

The same as SARS-CoV, SARS-CoV-2 also invades into host by combining with ACE2. It is reported SARS-CoV can inhibit the expression of ACE2 after infection. ACE2 is a carboxymonopeptidase which acts like the antagonist of angiotensin. The renin-angiotensin system (RAS) exacerbates pulmonary hypertension, acute lung injury and experimental lung fibrosis[24]. So ACE2 plays an protective role in lung infection.

Recently, many studies reported that SARS-CoV-2 could not only infect host through respiratory tract,but also gastrointestinal tract. ACE2 protects intestinal from inflammation induced by epithelial damage. It is a key regulator of dietary amino acid homeostasis, innate immunity, gut microbial ecology. SARS-CoV-2 may reduce the expression of ACE2 when infected through intestinal tract just like SARS-CoV. So it is reasonable to find drugs recovering the function of genes co-expressed with ACE2, by which we can prevent the development and infection of COVID-19.

The genes co-expressed with ACE2 in the colonic epithelial cells was acquired from Jun et al. We selected 125 hub genes from them and constructed a PPI network. The cluster analysis was performed to figure out the main network and correlation between these genes. Two clusters were obtained. Genes of cluster

1 were mainly about immune response such as CCR10, GPR31, F2RL1 and neurotransmission such as PNOC, NPFFR2, NPY. Genes of cluster 2 were mainly about ribosome assembling. It supported the view that ACE2 played an important role in defending against virus infection by means of intensifying immune response or interfering ribosome normal function to decrease the replication of virus[25]. The GO analysis and the KEGG pathway revealed these genes were aggregated in ribosome, exosomes, extracellular cellular components; structure constituent of ribosome, G-protein coupled receptor activity, MHC class I and II receptor activity biological processes; immune response, protein metabolism, signal transduction biological processes; and ribosome, graft-versus-host disease, viral myocarditis pathways.

After uploading the genes co-expressed with ACE2 in the colonic epithelial cells to the Cmap database, we got two potential drugs (ikarugamycin and molsidomine) which positively correlated with our gene profiles. Ikarugamycin is a previously discovered antibiotic, however it has been found to inhibit clathrin-mediated endocytosis[26]. Most virus depend on endocytic uptake to get into cells, one way of which is the internalization involving clathrin-mediated endocytosis[27]. Surprisingly, there is a report about SARS-CoV invading into host cells depending on clathrin-mediated endocytosis[28]. So ikarugamycin is possibly to treat COVID-19. Molsidomine is an orally active, long-acting vasodilator[29]. It is a nitric oxide (NO) donor and there is a case that inhalation of NO alleviated the symptom of severe acute respiratory syndrome (SARS)[30]. It is also reported that NO inhibited the replication cycle of SARS-CoV[31]. Inhaled NO achieves selective vasodilation of the pulmonary circulation, which contributes to the improvement of ventilation-perfusion matching and oxygenation in patients with acute respiratory distress syndrome[32]. Moreover, inhibition of AngII receptor type 1 attenuated acute severe lung injury and pulmonary edema caused by the protein of SARS-CoV[11]. The same as SARS, COVID-19 also showed progressive dyspnoea and lung field shadowing. According to the pathology of COVID-19, the lung tissue displayed pulmonary oedema and desquamation of pneumocytes and hyaline membrane formation, indicating acute respiratory distress syndrome[33, 34]. Therefore, molsidomine is potential to alleviate the symptom of COVID-19.

Conclusion

Drug prediction by the Cmap database is an efficient way to reuse known drugs, which avoids the difficulty to support expenditure in researching novel drugs and long-term clinical trials, especially in this time when SARS-CoV-2 spreads so quickly. Our research find out two drugs for the treatment of COVID-19 based on the genes co-expressed with ACE2 in the colonic epithelial cells. Nonetheless, the effect of potential drugs based on Cmap prediction should be further investigated using experimental evidence. Although this is only a simple step towards success, the above results are still very useful for illuminating the mechanism of the development of COVID-19 and providing important information for further animal and clinical trials to prove the efficacy of ikarugamycin and molsidomine on COVID-19.

Abbreviations

COVID-19, corona virus infective disease 19; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ACE2, angiotensin-converting enzyme 2; PPI, protein-protein interaction; GO, gene ontology; Cmap, the connectivity map; ARDS, acute respiratory distress syndrome

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The dataset generated during the current study are available in the http://dx.doi.org/10.1101/2020.02.05.20020545.[12].

The dataset supporting the conclusions of this article is included within the supplement material in this article.

STRING database : Version 11.0, ELIXIR, Europe, https://string-db.org/.

Cytoscape : Version 3.6.1, Cytoscape Consortium, U.S

FunRich : http://www.funrich.org

Webgestalt: http://www.webgestalt.org

the CMap Web Service : Update 12 September 2017, https://portals.broadinstitute.org/cmap/index.jsp.

Competing interests

All authors confirm that there are no conflicts of interest.

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Author contribution statement

Li performed the experiments and drafted the manuscriptand with the help of Bai and XH. Hou, L.Yang is responsible for the concept, study design and revised the manuscript.

Acknowledgements

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Tables

Table I. Signifcant GO terms for each GO category enriched by Funrich

Categories	GO terms	Percentage	-log10(P value)	Fold
Cellular components	Ribosome	17%	14.91	17.2
	Exosomes	46.4%	12.99	3.3
	Cytosolic large ribosome	8%	8.37	32.5
	Extracellular	37.5%	7.97	3
	Cytosolic small ribosome	7.1%	6.72	28.9
	Integral to plasma membrane	25%	6.64	3.9
Molecular function	Structure constituent of ribosome	18%	18.29	21.5
	G-protein coupled receptor activity	18%	5.37	4.4
	MHC class I receptor activity	5.4%	4.69	26.5
	MHC class II receptor activity	5.4%	4.61	25.8
Biological process	Immune response	16.2%	5.64	5.1
	Protein metabolism	24.3%	5.46	3.3
	Signal transduction	39.6%	2.60	1.8
	Cell communication	37.8%	2.47	1.8

Table II. Significant KEGG pathways enriched by Webgestalt

Gene Set	Description	Size	P value
hsa03010	Ribosome	134	0
hsa05332	Graft-versus-host disease	41	2.72E-09
hsa05416	Viral myocarditis	59	5.00E-09
hsa05330	Allograft rejection	38	3.10E-08
hsa04940	Type I diabetes mellitus	43	8.69E-08
hsa05320	Autoimmune thyroid disease	53	4.75E-07
hsa04080	Neuroactive ligand-receptor interaction	277	8.35E-07
hsa04612	Antigen processing and presentation	77	8.36E-07
hsa04145	Phagosome	152	8.71E-07
hsa04062	Chemokine signaling pathway	189	1.43E-06

Table III. Top 2 small molecules with complete P value and specificity score ranking based on positively connectivity scores

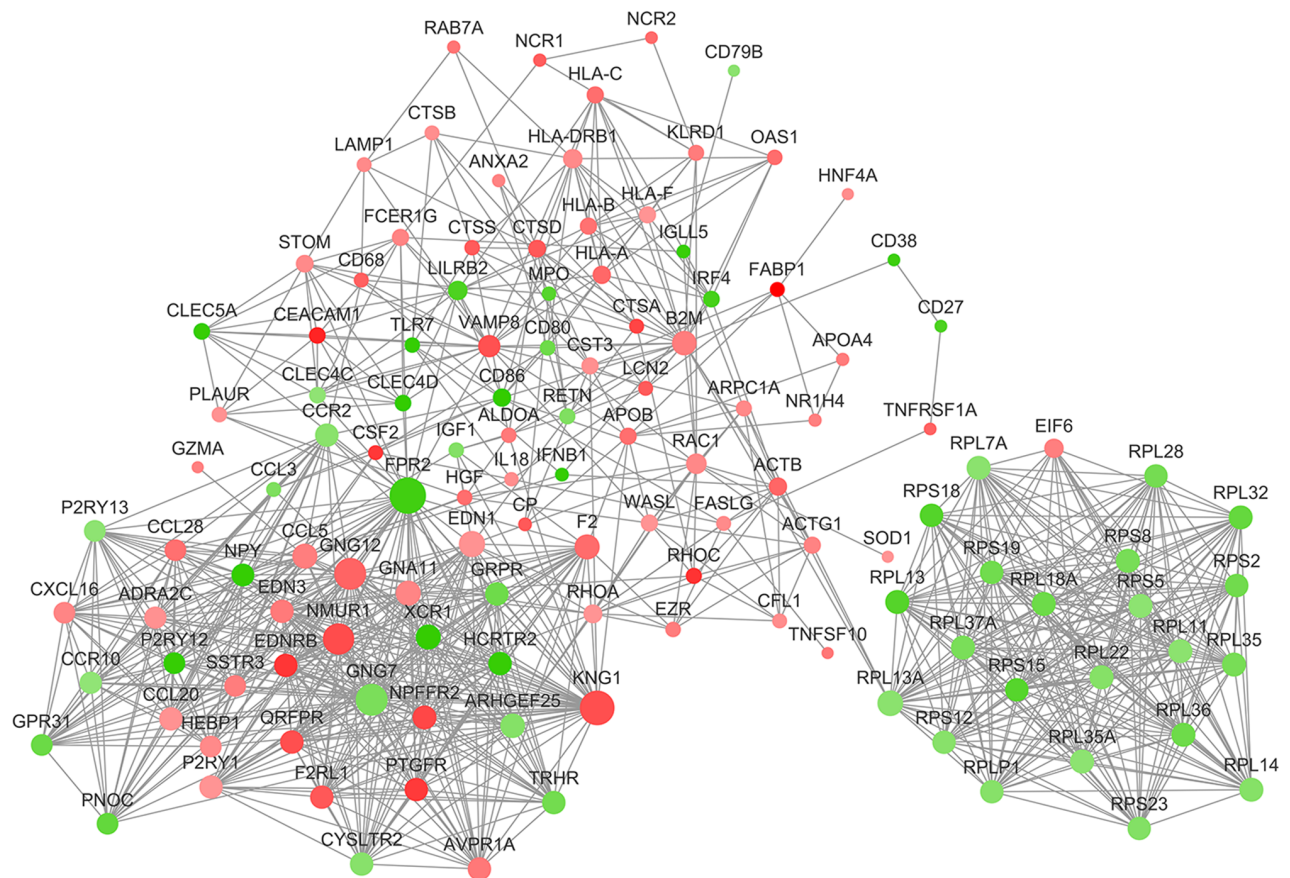| Rank	Compound Name	Mean CMap Score	n	P value
1	ikarugamycin	0.662	3	0.00072
2	molsidomine	0.416	4	0.00167

Figures

Figure 1

The nodes representing positively correlated genes are shown as red circles and the negatively correlated genes are presented as green circles. The colors of the nodes are illustrated from red to green (white in the middle) in descending order of r values. The sizes of the nodes are illustrated from small to big in ascending order of degree values.
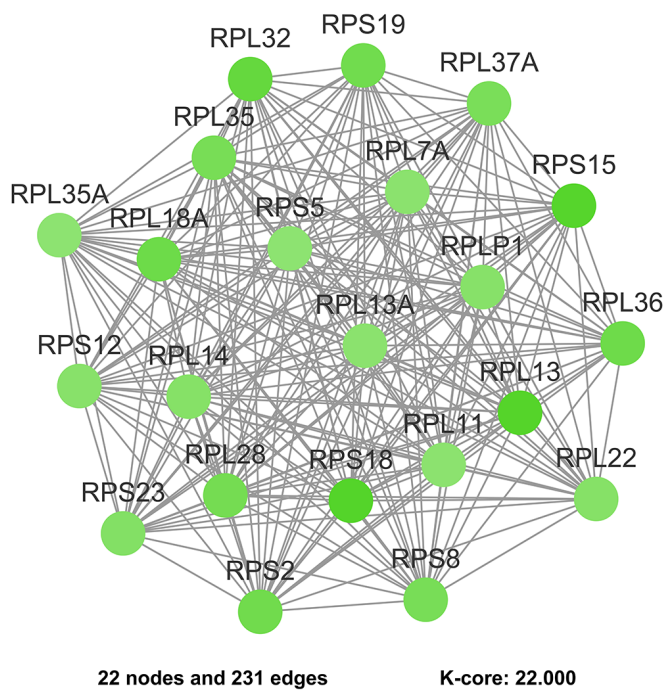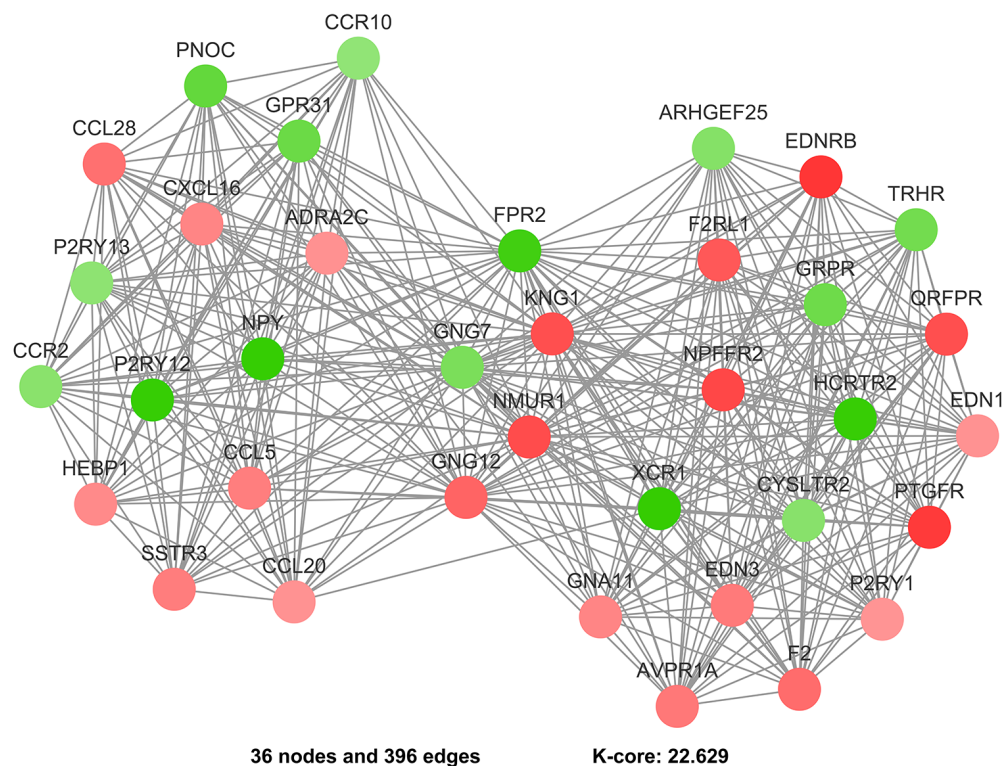
Figure 2

The modules were extracted from the PPI network through MCODE analysis. The colors of the nodes are illustrated from red to green (white in the middle) in descending order of r values. The sizes of the nodes are illustrated from small to big in ascending order of degree values. K-Core >5.
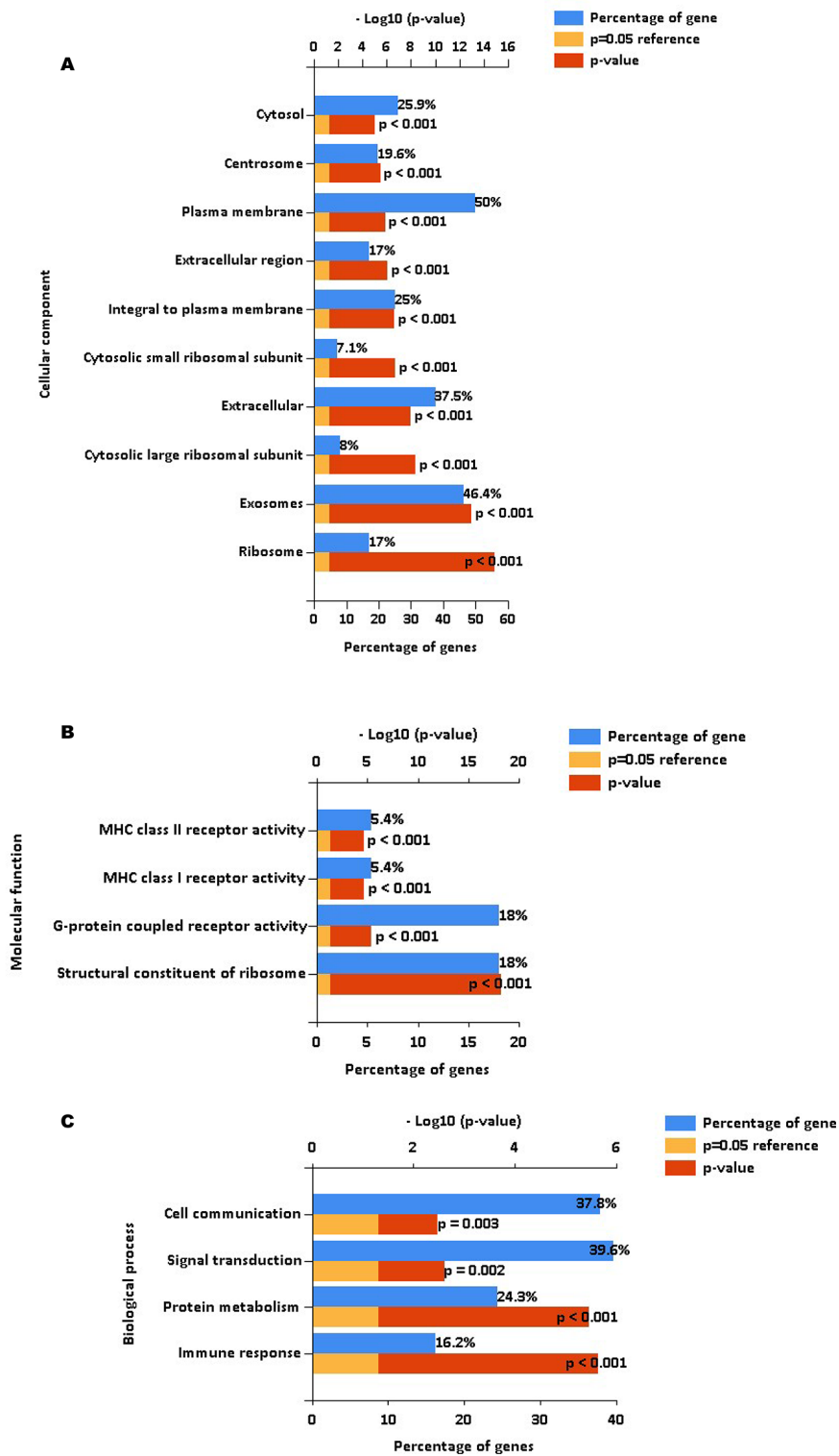
Figure 3

The GO analysis of 113 genes co-expressed with ACE2 in the colonic epithelial cells. (A) cellular components (B) molecular functions (C) biological processes.
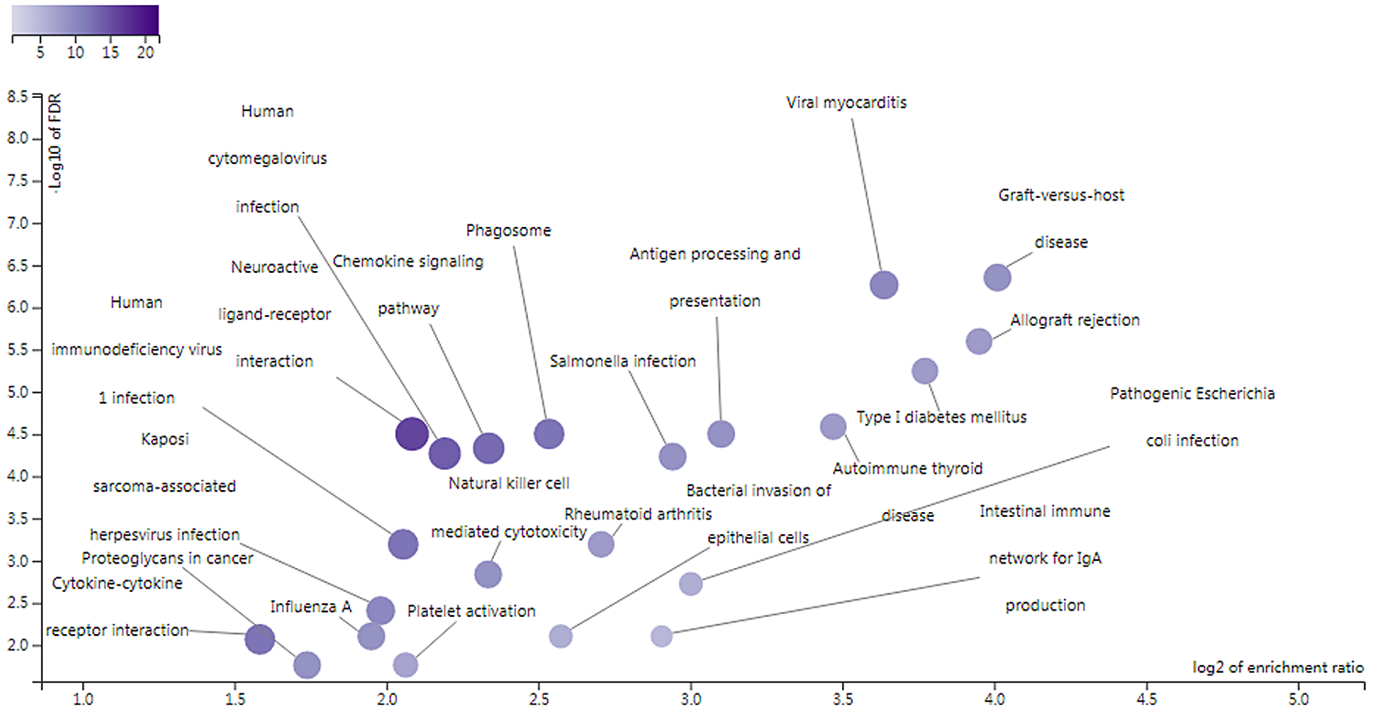
Figure 4

The KEGG pathway analysis of 113 genes co-expressed with ACE2 in the colonic epithelial cells. The X axis is log2 of enrichment ratio, the Y axis is log10 of FDR, and the different shades of purple colour are used to distinguish the number of genes enriched in each pathway, and the sizes of the nodes are illustrated from small to big in ascending order of P values.

Supplementary Files

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- Supplementarytable.xls