Transcriptional analysis of lung epithelial cells using WGCNA revealed the role of *IRF9* and *IFI6* genes in SARS-CoV-2 pathogenicity

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Abstract

The coronavirus disease 2019 (COVID-19) outbreak is an ongoing global health emergence, but the pathogenesis remains unclear. Here, we applied weighted gene co-expression network analysis to comprehensively characterize transcriptional changes in bronchial epithelium cells (NHBE and A549 cells) during SARS-CoV-2 infection. Our analysis identified a network highly correlated to COVID-19 pathogenicity based on *MX1*, *IFIT1*, *ISG15*, *IFI6*, *DDX60*, *IRF9*, *PARP9*, *PGLYRP4*, *IL36G*, *SAA2* and *IL-8* hub genes. The results also indicated a unique transcriptional signatures of infected cells including *IFI6* and *IRF9* as novel gene candidates and suggested their prospective mechanism in COVID-19 pathogenesis. The result of hub genes enrichment showed that the most correlation topic in biological process and KEGG were type I interferon signaling pathway, IL-17 signaling pathway, cytokine mediated signaling pathway, and defense response to virus categories which all play significant roles in restricting viral infection. Also according to the drug-target network, we recognized 54 FDA-approved drug candidates for other indications could potentially use for the treatment of COVID-19 patients through regulation of six hub genes of the co-expression network. Our findings also showed that the 19 experimentally validated miRNAs regulated the co-expression network through 5 hub genes (*SLC19A3*, *FAM13A*, *PLA2G16*, and *HRASLS5*). In conclusion, these hub genes had potential roles in the translational medicine and might become promising therapeutic targets further in vitro and in vivo experimental studies are needed to evaluate the role of above mentioned genes in COVID-19.

1. Introduction

The SARS-CoV-2 pandemic has affected millions of people globally since December 2019. As of 23rd April 2020, more than 2,650,000 people are reported to be infected, with ~7% mortalities (<https://coronavirus.jhu.edu/map.html>). The WHO has declared this outbreak as a currently worldwide public health emergency and named its disease as the coronavirus infected disease-19 (COVID-19). This novel pathogen is a single-stranded RNA virus belongs to the Coronaviridae family and can infect mammals and birds [1]. Clinical research has defined COVID-19 as an acute respiratory tract infection with diverse symptoms including fever, dry cough, fatigue, myalgia, conjunctivitis, and pneumonia. However, some patients develop severe illnesses including pneumonia, acute respiratory distress syndrome, pulmonary edema, acute kidney injury, or multiple organ failure quickly [2-4]. Despite the high infection and mortality rates, the host's immune response to infection with Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) remains poorly understood [5, 6].

In multiple cases, involvement of both lungs suggests viral dissemination after the initial infection. Viral RNA has been identified in symptomatic patients in the upper airways, with greater viral loads in nasal swabs opposed to those collected from the throat [7]. Comparable viral loads have been observed in an asymptomatic patient, suggesting that the nasal epithelium is a significant portal for initial infection, and may act as a main reservoir for viral dissemination across the respiratory mucosa and an essential viral

transmission mediating locus. To improve diagnostic and therapeutic approaches, it is important to recognize the cells and mechanisms that host viruses and enable viral replication as well as those which contribute to inflammation and pathogenesis of the disease.

Dysregulated host immune response and inflammatory cytokine production are believed to correlate with severity of the disease and poor prognosis in two other corona virus related infections, SARS coronavirus (SARS-CoV) and Middle East respiratory syndrome-related coronavirus; MERS-CoV (MERS-CoV) [8, 9]. Nevertheless, the underlying molecular mechanisms of the anomalous inflammatory responses under SARS-CoV-2 infection are still not clear.

Transcriptomic study of cells when infected with viruses is remarkably useful in determining the dynamics of the host immune response as well as genes regulatory networks [10, 11]. In this regard, Xiong et al., conducted a transcriptome sequencing of the RNAs isolated from the bronchoalveolar lavage fluid as well as peripheral blood mononuclear cells (PBMC) specimens of COVID-19 patients. Their results reveal showed host inflammatory cytokine profiles to SARS-CoV-2 infection in patients, and highlight the association between COVID-19 pathogenesis and excessive cytokine release such as chemokine (C-C motif) ligand 2 (CCL2)/ monocyte chemoattractant protein 1 (MCP-1), C-X-C motif chemokine 10 (CXCL10)/IP-10, CCL3/MIP-1A, and CCL4/MIP1B [11]. In another study, Xu et al., used Single-cell RNA sequencing (scRNA-seq) analysis to evaluate the expression and distribution of Angiotensin-converting enzyme 2 (ACE2) and Transmembrane Serine Proteases (TMPRSSs) genes in kidney cell components. They found that podocytes and proximal straight tubule cells were potential host cells targeted by SARS-CoV-2, resulting in Acute kidney injury (AKI) caused by the SARS-CoV-2-induced cytopathic effect [12].

In this study, weighted gene co-expression network analysis (WGCNA) was used to investigate the transcriptional changes in primary human lung epithelium (NHBE) and transformed lung alveolar (A549) cells cell lines after SARS-CoV-2 infection hope to shed a light in the pathogenesis of this virus.

2. Materials And Methods

2.1. Gene expression dataset processing

The RNA-seq gene expression dataset GSE147507 was obtained and downloaded from the Gene Expression Omnibus (GEO) database which is based on Illumina NextSeq 500 platform. In total, this dataset contains raw read count of 20 samples of human lung epithelial cells including independent biological triplicates of primary human lung epithelium (NHBE) and transformed lung alveolar (A549) cells. The NHBE cells were mock treated or infected with SARS-CoV-2 (USA-WA1/2020) and independent biological duplicates of A549 cells were mock treated or infected with human respiratory syncytial virus (RSV) or seasonal influenza A virus (IAV). The raw data were modified and standardized using R 3.6.1 affy package in Bioconductor [13, 14]. Following the mapping of the probe to gene IDs, the expression coefficient of variance was calculated to obtain a list with decreasing coefficient of variance for all processed expression data and the first 4000 genes with large coefficient of variance expression were selected.

2.2. Identification of differentially expressed genes

Differential expressed genes (DEGs) were considered by using the DESeq2 package v1.18.1. Genes were considered as DEGs when they met the following criteria: p -value < 0.05, and $|\log_2\text{FC}| \geq 2$.

2.3. WGCNA network Construction and identification of significant modules

Gene co-expression network of treated and control groups reconstructed using WGCNA [13]. Briefly, the matrix of the gene expression profile was converted into the matrix of pairwise gene similarity according to the Pearson test, followed by conversion into the matrix of adjacency. According to already represented scale-free gene co-expression topological algorithm, a minimum possible of β value was considered in order to adjacency matrix met the scale-free topology criteria. For the next step, topological overlap matrix (TOM) and dissimilarity TOM (dissTOM) were created using TOM similarity and dissimilarity modules. Finally, module identification was performed by the dynamic tree cut, while the minimum of module size was defined as 30. Modules with high similarity score were merged with a threshold of 0.25.

2.4. Feature vectors in WGCNA network

The Module Membership (MM) was defined as the correlation between individual gene expression and module eigengenes and Gene significance (GS) was defined as the absolute value of the correlation between genes and SARS-CoV-2 infection. Thus, the genes with high GS and MM have been identified as intra-modular hub genes that are highly related to the trait.

2.5. Functional enrichment

The ClueGO (version 2.2.5) Plug-in tool on Cytoscape (version 3.6.0) was used to identify and visualize the enriched gene ontology (GO), KEGG pathway and biological pathways in interesting gene modules (kappa score = 0.4).

2.6. mRNA-miRNA network reconstruction

The experimentally validated miRNA regulatory network was constructed for identified hub genes using miRWalk database (http://mirwalk.umm.uni-heidelberg.de/search_genes/).

2.7. Identification of candidate regulatory drugs

The well-firmly established Drug-Gene Interaction Database (DGIDB) (<http://www.dgidb.org/>) was used to predict functional and drug-able hub-genes with the list of commercially available or clinical trial drugs [15].

3. Results

3.1. Preprocessing and DEGs analysis

We performed quantile normalization to reduce the effects of technical noises. No outliers were observed in dataset samples by sample clustering; thus, all samples were included in the analysis (Fig. S1). Based on DEG analysis between SARS-Cov-2 treated and mock-treated cell lines, a total of 62 (42 up-regulate, 20 down-regulate) and 167 (96 up-regulate, 71 down-regulate) genes were identified as DEGs in A549 and NHBE subsets, respectively. These DEGs were then selected for subsequent analysis. The GO and KEGG pathway analysis of DEGs are represented in figure 1.

3.2. Identification of WGCNA modules

Based on the variance of expression values, a total of 4000 genes were included in WGCNA. Afterward, β value equal to 12 and 24 was considered for A549 and NHBE cells, respectively and weighted gene co-expression network of treated and mocked samples reconstructed (Fig. S2). The result of dynamic tree cut gave a total of 20 co-expressed modules recognizing by hierarchical clustering dendrogram with range size of 30 (mediumpurple2) to 1313 (brown4) in A549 dataset (Fig. S3) and 24 co-expressed modules with range size of 33 (yellow4) to 841 (darkolivegreen) in NHBE dataset.

3.3. Module-trait association analysis

Evaluation the relationship between each module eigengenes and the trait and module-module association recognized that Turquoise module ($r = 1$, p -value = 1.00×10^{-5}) in A549 cell type and Yellowgreen ($r = 1$, p -value = 2.00×10^{-5}) in NHBE cells were positively correlated with the SARS-CoV-2 trait (Fig. 2).

3.4. Functional annotation analysis of interesting modules

Gene Ontology (GO) and functional and KEGG pathway enrichment analysis were conducted to explore meaningful biological relevance of the interesting modules using ClueGO (Fig. 3). As shown in figure 3, functional annotations of the Turquoise and Yellowgreen modules showed similar enrichments mainly focused on immune responses against viral infection. Common pathways are as follows: regulation of viral life cycle, negative regulation of viral genome replication, type 1 interferon signaling pathway, NOD-like receptor signaling pathway, defense response to viruses such as influenza A, measles, Herpes simplex 1 virus, and Epstein-bar virus.

3.5. Hub gene identification and network analysis of interesting modules

Focusing on the Turquoise and Yellowgreen modules, genes with high GS and MM named as Hubgenes are important for the integrity and proper functioning of the entire network. Co-expression networks were reconstructed for each module using hubgenes with logarithm of fold changes $|\log_2 FC| \geq 2$. The two hubgene sets which met these criteria (Fig. 4) were then separately imported to GeneMANIA database to visualize interactions between the genes in each co-expression module. The resulted co-expression networks are visualized as red circles in figure 5.

The top 15 Hubgenes of Turquoise module with GS and MM > 0.9915 were listed in table 3. Moreover, the transcriptional changes of these Hubgenes were compared to that of RSV to determine unique key transcriptional responses to SARS-CoV-2 infection and are listed in Table 3. Finally, the top 15 Hubgenes of Yellowgreen module with GS and MM > 0.94 are as follows: *Peptidoglycan Recognition Protein 4 (PGLYRP4)*, *Interleukin-36 gamma (IL36G)*, *Serum Amyloid A2(SAA2)*, *Interleukin-1 Receptor-Associated Kinase-2 (IRAK2)*, *Complement Factor B (CFB)*, *Interleukin 8 (IL8)*, *Hephaestin like 1(HEPHL1)*, *S100 calcium binding protein A8 (S100A8)*, *TNF Alpha Induced Protein 3(TNFAIP3)*, *Interferon Regulatory Factor 9 (IRF9)*, *LIF Interleukin 6 Family Cytokine (LIF)*, *Heparin Binding EGF Like Growth Factor (HBEGF)*, *Chromosome 15 Open Reading Frame 48 (C15orf48)*, *B Cell Linker(BLNK)*, and *Zinc Finger Protein 773 (ZNF773)*.

3.6. Identification of candidate regulatory miRNAs

In order to find the potential molecular mechanisms of the hub-genes, their predicted miRNAs were analyzed by the miRWalk database. The experimentally validated miRNAs were shown in figure 5. According to the figure 5, 4 hub genes of interesting modules regulated by these miRNAs were *Interleukin-1 β (IL-1 β)*, *Interleukin 8 (IL-8)*, and *Serum Amyloid A1(SAA1)*.

3.7. Drug-target network construction

Drug repositioning analysis was performed on the same co-expressed Hub genes to identify potential agents suitable for combating to COVID19. As illustrated in fig. 5, hub genes of which are target of detected FDA-approved drugs were Interferon Induced Protein with Tetratricopeptide Repeats 1 (*IFIT1*), ISG15 Ubiquitin Like Modifier (*ISG15*), *CXCL2*, *IL-1 β* , *SAA1*, *IL-8*.

4. Discussion

The infection of SARS-CoV-2 can cause severe pneumonia as well as other complications with significant morbidity and mortality. Currently, there is no suitable cure or appropriate medication for this fatal lung involvement. Our knowledge about the host immune interaction with SARS-CoV-2 is restricted, making it more difficult for management and developing new therapies. Viral infection typically induces massive changes in the transcriptome of the host, resulting in aberration of host cells' metabolism and modulation of immune response, toward enhancing viral replication [16, 17]. Using several samples expression data from human lung epithelial cells including independent biological triplicates of primary human lung epithelium (NHBE) and transformed lung alveolar (A549) cells, we performed transcriptome analysis to better understand the molecular basis of the COVID-19 and identify putative markers. We found that 62 and 167 genes were differential expressed (p -value<0.05, and $|\log_2FC| \geq 2$) when A549 and NHBE samples of COVID-19 cells were compared to mock treated control, respectively. As expected for GO functional and KEGG pathway enrichment analysis, these genes belongs to type I interferon signaling pathway, IL-17 signaling pathway, cytokine mediated signaling pathway, and defense response to virus categories which all play significant roles in restricting viral infection.

We found that the expression level of *IL-6* was increased several folds in both studies cells' lines. In parallel, Systemic inflammation and cytokine storms with elevated levels of CXCL family, *IL-8*, and *IL-6* are reported among SARS patients [16, 18]. Latest laboratory findings from Wuhan patients also showed that COVID-19 mild patients had elevated levels of *IL-1B*, Interferon gamma (*IFN γ*), *CXCL10* and *CCL2*, while in severe cases, Granulocyte-colony stimulating factor (*G-CSF*), *CXCL10*, *CCL2* and *CCL3* were elevated [2]. Consistent with our results, a significant elevated expression of a large number of cytokines was observed in SARS-CoV-2 treated samples compared to mock-treated control. These findings show that infection with the SARS-CoV-2 virus can resulted to a cytokine storm that associated with severity of the disease. In another study, *IL-6* was increased in patients with SARS disease, which is needed to regulate inflammatory response, B-cell differentiation and antibody development [19].

Use of WGCNA for co-expression analysis does not depend on particular contrasts (differential expression) and may establish associations in the study design between the co-expressed genes and important factors. Based on our analysis of data in the convenient models of bronchial epithelium cells (NHBE and A549 cells), we created gene co-expression network using WGCNA and recognized two associated modules. The Turquoise module from A549 samples with 196 gene and Yellowgreen module from NHBE samples with 194 gene were the most correlated modules with SARS-CoV-2 infection. KEGG and GO enrichment analysis of genes of these two modules further confirmed multiple viral infection-related processes both enriched in acute inflammatory response and defense response to virus categories which are biologically rational and associated with COVID-19 pathogenicity. One of remarkable pathways enriched in interested modules is regulation of viral life cycle. The genes in this category have a wide range of roles in SARS-CoV-2 life cycle like facilitating survival, attachment, entry as well as replication of the virus particles are so clear as candidate targets of antiviral drugs [20].

As mentioned in results section, the first 10 Hubgenes of Turquoise and Yellowgreen modules with GS and MM > 0.9915 and 0.94 respectively with $|\log_2FC| \geq 2$ were selected including *MX1* (Myxovirus (Influenza) Resistance 1), *IFIT1*, *ISG15*, *DDX60* (DExD/H-Box Helicase 60), *IRF9*, *PARP9* (Poly(ADP-Ribose) Polymerase Family Member 9), *PGLYRP4*, *IL36G*, *SAA2* and *IL-8* which had a high significance for immune related COVID-19 response. The important role of these genes in host response to various viruses has been described by many studies [21-31]. In line with this, some of these Hubgenes has been recently reported to be greatly related to COVID-19. For example, functional overexpression of *MX1* was significantly mediates antiviral response to SARS-CoV as well as probably SARS-CoV-2 [21, 32]. Elevated expression levels of *IL-8* and *IL-36G* have been declared to be closely related to COVID-19 severity [33, 34].

Transcriptional changes of well-characterized Hub-genes of Turquoise module in table 3 shows direct effectors of the *IFN*-stimulated antiviral response including *MX1*, *IFIT1*, *ISG15*, *IFI6*, *DDX60*, *OAS1-3* and Signal transducer and activator of transcription 1 (*STAT1*) in response of SARS-Co-2.

In order to compare differences between SARS-CoV-2 and RSV molecular pathogenicity, we performed DEG analysis on A549 cell which infected with RSV. Interestingly, the pattern of gene expression between two lists revealed diminished antiviral response to SARS-CoV-2 discriminating this response from

common robust interferon induction of antiviral genes which reported in other studies after viral infections [35]. However, considering obvious suppressed expression levels of *IFN-1* and *IFN-3* by means of SARS-CoV-2, the underlying mechanism of this unexpected antiviral response remains to be ascertained. In line with this, *IRF9* and *IFI6*, amongst these hub genes are so attracting due to unabated transcriptional response to SARS-Co-2 as compared to RSV.

IRF9 is a well-known essential *IFN* regulatory factor acts as a part of interferon-stimulated gene factor 3 (*ISGF3*) complex consisting of *STAT1*, *STAT2* and *IRF9* that mediates expression of several *IFN*-inducible genes including *MX1*, *OAS1-3*, *ISG15*, *IFIT1*, *IFI6* as well as transcription factors of *IFN-1* expression, interferon regulatory factor 3 and 7 (*IRF3* and *IRF7*) [35, 36]. The pivotal role of *IRF9* in antiviral responses against various common viruses including respiratory viruses has been well demonstrated [36, 37]. Importantly, emerging data suggest that various viruses such as human papillomavirus (HPV), reovirus, hepatitis B virus, adenovirus, human cytomegalovirus and RSV evolved mechanisms to evade from *IRF9*-mediated antiviral response [38-42]. Strikingly, *IRF9* overexpression amplifies the interferon response to viral infections. Interestingly, more recent study declared that initial concentration of *IRF9* is a substantial determinant regulating amplitude, speed as well as strength of IFN α -mediated antiviral signaling [38].

Taking together, these findings strengthen the idea that unique unmuted expression of *IRF9* in response of SARS-Co-2 infection would responsible for observed significant up-regulation of at least a subset of ISGs to escape from robust control over *IFN-1* and *IFN-3* expression. Therefore, the early increase of *IRF9* expression levels during early stages of SARS-CoV-2 infection is a crucial regulator of early, enhanced and accelerated initiation immune response despite of suppressed expression of *IFN* avoiding presumed route used by coronavirus to escape from immune response during early phase of infection. Thus, variations in either *IRF9* basal levels or the amount of *IRF9* induction rate may has a robust predictive value not only for disease severity and clinical outcomes providing a putative potential target for COVID19-related therapy, but also may be a reason for patient-to-patient differences in disease development.

Whereas, *IRF9* hub gene is a pathway activator/regulator, *IFI6* is known as an antiviral effector and its expression can be induced by *IFN-1* [43]. Recently, *IFI6* has been purposed as a gene expression signature distinguishes between viral and bacterial infections of respiratory tract [25]. Importantly, some studies reported an essential role of *IFI6* overexpression in pro-survival signaling by subverting *TRAIL*-mediated mitochondrial loss, cytochrome C release and subsequent apoptosis [44]. In this regard, RSV has been revealed to suppressed *IFN*-induced up-regulation of *IFI6* in a time dependent manner at early phase of infection to escape from *IFN*-mediated antiviral response [45].

Remarkably, recent study suggests that diffuse alveolar damage and selective viral-mediated type 2 pneumocytes apoptosis would be as the major pathological basis of COVID-19, culprit for both decreased capacity of air exchange and fluid leakage result in acute respiratory distress syndrome (ARDS) and even death [46]. These findings support a model in which *IFI6* up-regulation could serve as a possible antiviral

strategy to protect healthy respiratory epithelial cells from early apoptosis elicited by surrounding pro-apoptotic cytokines leading to enhanced *IFN* production. Therefore, IFI6 could serve as an appealing target for innovate new therapies in combating COVID-19.

On the other hand, it is identified that *IFI6* up-regulation potentiates the antiviral activity of *IFN* α and restricts replication of several RNA viruses including dengue, Zika, West Nile and yellow fever viruses (Dengue virus (DENV), Zika virus (ZIKV), West Nile virus (WNV) and Yellow fever virus (YFV) respectively) as shown by its ability to interfere in YFV replication indirectly via preventing viral invagination into endoplasmic reticulum (ER) [47-49]. However, this antiviral mechanism of *IFI6* localized in ER seem to be specific to flaviviruses due to lack of inhibitory effect on neither HCV nor coronavirus replication in COS-7 cells that organized in double-membrane ER structures [48]. Moreover, *IFI6* is also identified to improve *IFN*-induced antiviral response as a result of activation of *STAT3* signaling [45]. Given this findings, one can speculate that perhaps localization of *IFI6* at either mitochondria or ER membrane in various cell types might be an important determinant of IFI6 activity against viruses.

As depicted in figure 5, further network-based drug repurposing is calculated and identified novel and potent drugs targeting SARS-CoV-2. However, some of predictions have been showed by recent literature to be beneficial for COVID-19. For instance, recent computational studies offer Cidofovir, Deferoxamine, Cytarabine and Clarithromycin as potent drugs for COVID-19 treatment [50-53]. *ACE2*-associated network analysis suggest a crucial role of Fluticasone propionate for COVID-19 treatment [54]. Moreover, Cyclosporine exhibited antiviral efficacy (with the half maximal inhibitory concentration (IC₅₀) value of 5.82 μ M) against SARS-CoV-2 [55]. Notably, considering immunomodulatory and anti-oxidative properties as well as beneficial roles of melatonin in reducing vessel permeability, anxiety, and ameliorating sleep quality, strongly suggest potential adjuvant use of melatonin for critical care patients infected by COVID-19 [56]. Importantly, emerging data supporting the beneficial role of Dipyridamole, a convenient antiplatelet agent, against broad spectrum of viruses mainly the positive-stranded RNA viruses as well as COVID-19 which stimulate type I interferon responses, decrease lung injury and suppressed in vitro replication of COVID-19 [46]. Moreover, corticosteroids such as Hydrocortisone, Beclomethasone and Fluticasone have been suggested to might have beneficial effects for the sever patients with pulmonary edema in combination with ventilator to prevent COVID-19 associated-ARDS development [46].

Additionally, Naproxen is a familiar non-steroid anti-inflammatory drug (NSAID) currently used as a part of triple combination therapy (Azithromycin, Naproxen and Prednisolone) that largely administered by Iranian's physicians to treat patients with COVID-19 that supposed to reduce duration of hospitalization [57].

According to figure 5, Ribavirin and PEG interferon α -2b are shown to interact with *IFIT1* which its vital role in the *IFN*-induced host defense against RSV, IAV, HBV and vesicular stomatitis virus (VSV) has been reported by several studies [23, 24]. Ribavirin is a well-known directly acting antiviral drug against hepatitis C virus (HCV) with a broad-spectrum of antiviral [58]. It has been reported that ribavirin exhibits an inhibitory effect against zoonotic paramyxoviruses leading to severe respiratory infection such as

Hendra virus (HeV) and Nipah virus (NiV) [59]. Moreover, ribavirin has been reported to effectively prevents RSV reproduction [60]. Surprisingly, recent modeling and docking analysis suggests the effectiveness of ribavirin as a potent drug for COVID-19 treatment [58]. PEG interferon α -2b is another broad-acting antiviral drug used for hepatitis treatment that its inhibitory effect on coronaviruses has been demonstrated by in vitro studies [61]. Interestingly, both *IFN- α* and ribavirin therapy of HCV infection could lead to up-regulation of *IFI6* [62]. Consistent with our results, Ribavirin and PEG interferon α -2b have been suggested in the guidelines for the Prevention, Diagnosis, and Treatment of COVID-19 issued by National Health Commission (NHC) of the People's Republic of China [61]. Taking together, Ribavirin and PEG interferon α -2b may offer a potential combination therapy for COVID-19 that emerged with promising results by synergistically targeting *IFIT1* and *IFI6* hub genes.

5. Conclusion

In summary, we used weighted gene co-expression network analysis to construct a gene co-expression network for COVID-19 and identified network highly correlated hub genes including *MX1*, *IFIT1*, *ISG15*, *IFI6*, *DDX60*, *IRF9*, *PARP9*, *PGLYRP4*, *IL36G*, *SAA2* and *IL-8* as well as unique transcriptional signatures of this virus including *IFI6* and *IRF9* as novel gene candidates and suggested their prospective mechanism in COVID-19 pathogenesis. Moving forward, these hub genes had potential roles in the translational medicine and might become promising therapeutic targets further in vitro and in vivo experimental studies are needed to evaluate the role of above-mentioned genes in COVID-19.

List Of Abbreviations

coronavirus disease 2019; COVID-19, Severe acute respiratory syndrome coronavirus 2; SARS-CoV-2, SARS coronavirus; SARS-CoV, Middle East respiratory syndrome-related coronavirus; MERS-CoV, peripheral blood mononuclear cells; PBMC, chemokine (C-C motif) ligand 2; CCL2, monocyte chemoattractant protein 1; MCP-1, C-X-C motif chemokine 10; CXCL10, Single-cell RNA sequencing; scRNA-seq, Acute kidney injury; AKI, Angiotensin-converting enzyme 2; ACE2, Transmembrane Serine Proteases; TMPRSSs, weighted gene co-expression network analysis; WGCNA, Gene Expression Omnibus; GEO, respiratory syncytial virus; RSV, Differential expressed genes; DEGs, topological overlap matrix; TOM, dissimilarity TOM; dissTOM, Module Membership; MM, Gene significance; GS, gene ontology; GO, Drug-Gene Interaction Database; DGIDB, *MX1*; Myxovirus (Influenza Resistance 1), *IFIT1*; Interferon Induced Protein with Tetratricopeptide Repeats 1, *ISG15*; ISG15 Ubiquitin Like Modifier, *IFI6*; Interferon alpha-inducible protein 6, *DDX60*; DExD/H-Box Helicase 60, *IRF9*; Interferon Regulatory Factor 9, *PARP9*; Poly (ADP-Ribose) Polymerase Family Member 9, *PGLYRP4*; Peptidoglycan Recognition Protein 4, *IL36G*; Interleukin 36 Gamma, *SAA2*; Serum Amyloid A2, Interleukin-1 β ; IL-1 β , Interleukin 8; IL-8, Serum Amyloid A1; SAA1, Peptidoglycan Recognition Protein 4; *PGLYRP4*, Interleukin-36 gamma; *IL36G*, Serum Amyloid A2; *SAA2*, Interleukin-1 Receptor-Associated Kinase-2; *IRAK2*, *Complement Factor B*; *CFB*, Hephastin like 1; *HEPHL1*, S100 calcium binding protein A8; *S100A8*, TNF Alpha Induced Protein 3; *TNFAIP3*, Interferon Regulatory Factor 9; *IRF9*, LIF Interleukin 6 Family Cytokine; *LIF*, Heparin Binding EGF Like Growth Factor;

HBEGF, Chromosome 15 Open Reading Frame 48 ;*C15orf48*, B Cell Linker; *BLNK*, Zinc Finger Protein 773 ; *ZNF773*. human papillomavirus; HPV, Dengue virus; DENV, Zika virus; ZIKV, West Nile virus; WNV, Yellow fever virus; YFV, half maximal inhibitory concentration; IC50, respiratory syncytial virus; RSV, Influenza A virus; IAV, *DDX60* (DEXD/H-Box Helicase 60), *IRF9* (Interferon Regulatory Factor 9), *PARP9* (Poly(ADP-Ribose) Polymerase Family Member 9), vesicular stomatitis virus ;VSV, hepatitis C virus ;HCV, Hendra virus; HeV, Nipah virus; NiV, non-steroid anti-inflammatory drug ;NSAID.

Declarations

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Tables

Table 1: Module colors characterization of A549 dataset.

Module Colors	Number of Genes	correlation	<i>p</i> -value
Turquoise	196	1	1.00E-05
Grey60	138	0.72	0.1
Brown4	1313	0.53	0.3
Darkturquoise	111	0.44	0.4
Thistle2	41	0.4	0.4
Red	217	0.32	0.5
Skyblue3	47	0.22	0.7
Salmon4	91	-0.1	0.9
Darkolivegreen	216	-0.16	0.8
Saddlebrown	56	-0.16	0.8
Mediumpurple2	30	-0.19	0.7
Coral2	118	-0.28	0.6
Lightpink4	35	-0.3	0.6
Cyan	201	-0.31	0.5
Palevioletred3	82	-0.33	0.5
Maroon	37	-0.54	0.3
Plum	188	-0.67	0.1
Darkslateblue	233	-0.77	0.07
Lightsteelblue1	529	-0.86	0.03
Darkorange2	121	-0.89	0.02

Table 2: Module colors characterization of NHBE dataset.

Module Colors	Number of Genes	correlation	p-value
Yellowgreen	198	1	2.00E-05
Violet	57	0.72	0.1
Black	175	0.65	0.2
Cyan	68	0.4	0.4
Coral1	68	0.26	0.6
Darkred	112	0.19	0.7
Plum1	242	0.14	0.8
Floralwhite	425	0.1	0.8
Darkorange2	46	0.075	0.9
Indianred4	88	0.058	0.9
Yellow4	33	-0.066	0.9
Lightcyan1	48	-0.075	0.9
Darkseagreen4	74	-0.18	0.7
Sienna3	53	-0.28	0.6
Thistle2	132	-0.36	0.5
Lavenderblush3	38	-0.38	0.5
Blue	154	-0.47	0.4
Bisque4	214	-0.56	0.2
Darkgrey	120	-0.6	0.2
Darkolivegreen	841	-0.65	0.2
Brown2	184	-0.74	0.09
Pink	206	-0.76	0.08
Darkgreen	186	-0.82	0.05
Coral2	238	-0.92	0.01

Table 3: Top 15 Hubgenes of Turquoise module based on GS and MM >0.9915 and their corresponding transcriptional changes in response of SARS-CoV-2 and RSV.

Turquoise Hubs	COVID19	RSV
MX1	5.06329244	5.547139361
IFI6	4.33611129	2.924256784
IFIT1	4.29571052	5.097280379
ISG15	3.76605567	4.524538215
DDX60	2.30034197	2.334733725
IRF9	2.16374635	1.336146317
PARP9	2.07827268	3.027196369
IFIT3	1.96971947	4.987533314
OAS1	1.54861258	3.101263907
OAS3	1.39394763	2.984430543
STAT1	1.32978587	2.561269876
IFITM3	0.85820618	1.973212927
APOL1	0.7532857	2.401081657
MLXIPL	0.477235316	na
PRRT3	-0.408394138	na

Table 4: Potent drugs with literature-derived antiviral evidence for COVID-19 drug repositioning.
(see Table 4 in the Supplementary Files)

Figures

Figure 1

A549 (A) and NHBE (B) datasets DEGs enrichment.

Figure 2

Module-trait relationship. Each row corresponds to a module eigengene and column corresponds to SARS-CoV-2 trait. Numbers in each cell represent the corresponding correlation and p value. A) A549 dataset and B) NHBE dataset.

Figure 3

Biological processes and pathways detected within the A) Turquoise module from A549 dataset and B) Yellow-green module from NHBE dataset. Gene ontology and pathway analysis was performed using significant genes across all datasets. Node size represents the counts (number of genes that are part of each pathway), and node color corresponds the statistical significance. The darker the pathway node, the

more statistically significant it is, with a gradient from red (p-value 0.05–0.005) to black (p-value < 0.0005).

Figure 4

Similarity assessment between DEGs and hub genes of the interested module from A549 (A) and NHBE (B) cells using Venn diagram. A total of 7 and 4 hub genes which were similar in both list were chosen and then imported to GeneMANIA to construct a co-expression network.

A

B

Figure 5

Co-expression network and related miRNA and drugs of Turquoise (A) and Yellowgreen (B) modules.

Supplementary Files

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

- [Supplementary20200423.docx](#)

- [Table4.pdf](#)