A comprehensive transcriptome analysis reveals broader but weaker host response of SARS-CoV-2 than SARS-CoV

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Abstract

COVID-19, which has resulted a worldwide health crisis with more than 74.9 million confirmed cases worldwide by December 2020, is caused by a newly emerging coronavirus identified and named SARS-CoV-2 in February in Wuhan, China. Experiences in defeating SARS, which infested during 2002-2003, can be used in treating the new disease. However, comparative genomics and epidemiology studies have shown much difference between SARS-CoV and SARS-CoV-2, which underlies the different clinical features and therapies in between those two diseases. Further studies comparing transcriptomes infected by these two viruses to uncover the differences in host responses would be necessary. Here we conducted a comprehensive transcriptome analysis of SARS-CoV and SARS-CoV-2-infected human cell lines, including Caco-2, Calu-3, H1299. Clustering analysis and expression of ACE2 show that SARS-CoV-2 has broader but weaker infection, where the largest discrepancy occurs in the epithelial lung cancer cell, Calu-3. SARS-CoV-2 genes also show less tissue specificity than SARS-CoV genes. Furthermore, we detected more general but moderate immune responses in SARS-CoV-2 infected transcriptomes by comparing weighted gene co-expression networks and modules. Our results suggest a different immune therapy and treatment scheme for COVID-19 patients than the ones used on SARS patients. The wider but weaker permissiveness and host responses of virus infection may also imply a long-term existence of SARS-CoV-2 among human populations.

Key words: SARS-CoV, SARS-CoV-2, transcriptome, host response, tissue-specificity

Introduction

Coronavirus Infectious Disease 2019 (COVID-19) has caused 74.9 million confirmed infections worldwide including more than 1.41 million deaths by December 18th, 2020, evolving to a threatening pandemic (Dong, Du, & Gardner, 2020). The etiological pathogen of the emerging pneumonia was at first identified as 2019-nCoV in Wuhan by genome sequencing and epidemiological investigations (Tan et al., 2020), and was later officially named as "SARS-CoV-2" on February 11th, 2020 by ICTV (Gorbalenya et al., 2020).

Coronaviruses (CoVs) are critical strains of virus for human and vertebrates, within the family of Coronaviridae under the order of Nidovirales (Hu, Ge, Wang, & Shi, 2015). Mostly hosted by bats, they readily infect cardiovascular, respiratory, and gastrointestinal system of human and a large proportion of wild animals (L. F. Wang et al., 2006) (Chen & Guo, 2016). Since 21st century, outbreaks of the Severe Acute Respiratory Syndrome (SARS) in 2003 and the Middle East Respiratory Syndrome (MERS) in 2012, along with the current COVID have provided evidence for the possibility of human-to-human transmission of CoVs, which signifies potential future outbreaks (Poletto et al., 2014) (Cui, Li, & Shi, 2019). From a genomic perspective, SARS-CoV from 2003 is the most related human CoV to SARS-CoV-2 with a 79.5% similarity in nucleotides, with merely 380 amino acid substitution mostly in accessory open reading frames (ORFs) 8a, 8b, and 3b, then followed by MERS-CoV at about 50% similarity. (Zhang & Holmes, 2020) (H. Wang et al., 2020). The essential structural spike protein has no amino acid substitution in its receptor-bind motif (RBM) (Wu et al., 2020), but there may be a functional difference associated with the six mutations in other counterparts of RBD (Wu et al., 2020). Further comparison between SARS-CoV and SARS-CoV-2 may provide more insights into understanding of COVID.

Despite the viruses' genomic similarity and common respiratory signs like fever, dry cough, and dyspnea similar to SARS and MERS, COVID-19 patients display fewer common features such as diarrhea, nausea, vomiting, and abdominal discomfort (Booth et al., 2003). In terms of clinical outcome, COVID patients with gastrointestinal (GI) symptoms (nausea, vomiting or diarrhea) are oftentimes severe and associated with family clustering (Jin et al., 2020). Ackermann et al. (2020) demonstrated that COVID patients and SARS patients have atypical pneumonia regarding pulmonary vasculature, where histological analysis among COVID-19 patients shows extensive

thrombosis and microangiopathy relative to acute respiratory distress syndromes (ARDS). COVID-19 patients also have higher incidence of alveolar capillary microthrombi and new tissue growth (Ackermann et al., 2020).

SARS-CoV and SARS-CoV-2 are similar to certain degrees on their pathogenesis and molecular mechanism. Amino acid sequence of their spike proteins(-S) shares the essential residues to effective binding to human angiotensin-converting enzyme 2 (hACE-2), allowing SARS-2-S and SARS-S to employ the same entry site with the same cleavage protease TMPRSS2 (Hoffmann et al., 2020). Furthermore, SARS-CoV-2 likens SARS-CoV in terms of cell tropism and infection spectrum: most common cell lines of humans are readily susceptible to SARS-S and SARS-2-S mediated entry (Hoffmann et al., 2020) (Hui et al., 2020), and they target identical cell types in lung infections (types I and II pneumocyte and alveolar macrophage) (Chu et al., 2020). However, some of the SARS-CoV-2's mechanisms may account for its distinctive epidemiological and clinical characteristics. A faster viral replication (3.2-fold higher than SARS-CoV) is observed in SARS-CoV-2 infected human organs samples (Chan, Zhang, et al., 2020). The underlying reason may be attributed to the difference in host immune response pattern. SARS-CoV-2 does not significantly trigger any upregulation in interferons; activations of proinflammatory cytokines are also significantly infrequent in COVID-19 patients (Chu et al., 2020). The combinations of these traits may lead to remarkably high viral load present at upper respiratory tracts long before symptoms emerge (To et al., 2020).

SARS-CoV and SARS-CoV-2's difference at host transcriptome level can create a discrepancy in host immune-responses, which leads to differentiated symptoms among patients. Although research on pathogenesis and clinical records of COVID-19 are abundant, comprehensive studies on human subjects' post-infection transcriptome are still largely lacking. In this study, we focused on human cell lines challenged by SARS-CoV and SARS-CoV-2, computed permissiveness, tissue-specificity, and constructed a weighted correlation network (WGCNA) of gene expression profile as well as the subsequent gene ontology analysis. With bioinformatic approach, the study may give elemental understanding to COVID-19's characteristics of all kind. While nowadays, there are few treatment options for COVID-19 (Dong, Hu, & Gao, 2020) (Mehra, Desai, Kuy, Henry, & Patel, 2020), and serums of recovered SARS patients have only exhibited limited efficacy in reducing SARS-2-S mediated entry (Hoffmann et al., 2020, analysis on their transcriptome would have

significant clinical and pharmaceutical implications, especially among target drugs and immunotherapy development.

Results

SARS-CoV-2 manifests lower permissiveness for most cell lines

Transcriptomes of host cells with virion reveals the hosts' responses, which differ across various virus, tissue, and infection time. To uncover those differences between the infected cell responses of the two coronaviruses, SARS-CoV and SARS-CoV-2, we collected the RNA-seq data of 42 samples of three cell lines (non-small cell lung carcinoma (NSCLC) cell line H1299, epithelial lung cell line Calu-3 and epithelial colorectal adenocarcinoma cell line Caco-2) at different hpi (hours post infection, 4h, 12h and 24h for Calu-3 and Caco2 while 4h, 12h, 24h and 36h for H1299) from Emanuel et al (Series Accession Number: GSE148729, Emanuel et al., 2020). We mapped those RNA-seq reads to the combined genome of human and SARS-CoV-2 using HISAT2 (version 2.1.0), then measured the proportion of viral reads in the transcriptomes by cell lines and infection time. As shown, the proportion of viral reads varies among different cell types, and generally, that of perfect viral reads from SARS-CoV were higher than those from SARS-CoV-2 in both Caco-2 and Calu-3 (Students' t test, $p < 0.05$, Figure 1), showing a severer infection of SARS-CoV than SARS-CoV-2. However, almost no infection was detected in H1299. Besides the increasing trend of viral reads along infection time, we also detected a decrease of SARS-CoV-2 reads from 12 hpi to 24 hpi in Calu-3, which may imply the recovery of host cells from SARS-CoV-2 infection (Figure 1). Our results are consistent to those reported by Emanuel et al (2020).

Figure 1. Proportion of viral reads detected in Caco-2 (left), Calu-3 (middle), and H1299 (right)

cell lines at 4, 12, 24, and 36 hpi. Means and standard errors of two biological replicates were shown by dots and error bars. Two-way Student's t-test examines statistical significance. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Abbreviations: hpi, hours post-infection.

Calu-3 cells respond differently against the infection of SARS-CoV-2 from that of SARS-CoV

To briefly elucidate the differences of transcriptomes between SARS-CoV and SARS-CoV-2 infected cells, we also measured the transcripts per million (TPM) as expression level of both human and viral genes by StringTie (version 1.3.3b, -e -A). The k-means cluster analysis is applied to determine the host transcriptome similarity across samples of cell lines with various treatments and time points (Figure 2A). Caco-2 and H1299 cells show almost the same topologic structure and clustered well together in the trees, but Calu-3 cells not. In SARS-CoV-infected transcriptomes, three time points of Calu-3 cells clustered together and located the outmost of the tree, while the 12 hpi and 24 hpi of SARS-CoV-2-infected Calu-3 cells clustered with Caco-2 cells. The results show that Calu-3 cells responded differently than other cells in infection of SARS-CoV-2 from that of SARS-CoV (Figure 2A).

The expression level of ACE2 gene is deemed decisive to the magnitude of viral permissiveness, because both SARS-CoV and SARS-CoV-2 employ ACE2 as the only site for spike protein mediated entry into human cells (Hoffmann et al., 2020). Hence the expression abundance of ACE2 can present the magnitude of viral permissiveness. To further illustrate the differences in Calu-3 cells resulted by these two coronaviruses, we compared the expression level of ACE2 among cell lines infected by SARS-CoV and SARS-CoV-2 along time series. Among all, ACE2 are expressed much higher at 12 hpi in SARS-CoV-infected Calu-3 cells than SARS-CoV-2-infected ones (Students' t test, $p = 0.032$, Figure 2B). Our results show a wider permissiveness of SARS-CoV-2 in cell types, and host may respond differently from 12 hpi to 24 hpi in Calu-3 cells, an epithelial lung cell line.

To further showcase the differences among cell types, we employed a method from Kryuchkova-Mostacci and Robinson-Rechavi (2016) to calculate the tissue-specificity index (τ) based on the expression level of viral genes, measured in transcripts per kilobase million (TPM), among three cell types and 3-4 time points. Genes of SARS-CoV-2 have lower τ values than SARS-

Comparison of tissue-specificity measure by τ between SARS-CoV and SARS-CoV-2 genes. Mann-Whitney U test, * $P < 0.05$.

SARS-CoV-2 induces broader immune responses than SARS-CoV does

Next, we compared the detailed host responses between SARS-CoV- and SARS-CoV-2-infected cells, by constructing weighted gene co-expression networks through R package “WGCNA” (Langfelder P. and Horvath S., 2008). The soft-thresholding power indicates the scale-free topology of weighted gene co-expression networks (Zhang and Horvath, 2005). In SARS-CoV-infected network, power 5 is the lowest power to fit the scale-free topology while power 9 in SARS-CoV-2-infected network, showing quite different network topologies (Sup Fig 1A, B). Then we detected co-expression modules in these two networks, respectively (Sup Fig 1A, B). Most of the 24 modules detected in SARS-CoV-infected network can be related to modules in SARS-CoV-2-infected network, but only 7 of 28 modules in SARS-CoV-2-infected network can be related to modules in SARS-CoV-infected network (Figure 3A).

To relate modules to biological functions and illustrate host responses, we applied functional annotation to each module of the two networks by R package “anRichment” (supplementary table1,2, ref), finding that the module denoted as black in SARS-CoV-infected network and the one denoted as red in SARS-CoV-infected network both enriched with Gene Ontology (GO) terms of immune responses (Figure 3B, C). 55 of those terms are overlapping between two viral-infected networks, with higher enrichment ratio in SARS-CoV-2-infected network. Most of them are essential non-specific immune response pathways and responses (Figure 3B). These fundamental functions include the reception, response, and signaling pathways of interferons and phagocytic leukocytes. There were only 3 uniquely enriched term in SARS-CoV-infected network, but 98 in SARS-CoV-2-infected network (Figure 3B, C). Our functional annotations show that SARS-CoV-2 induced broader immune responses.

Figure 3. Module detection and functional annotations. (A) Correspondence of modules detected in SARS-CoV-infected and SARS-CoV-2-infected transcriptomes. X-axis lists modules in SARS-CoV-2 transcriptome while Y-axis lists modules in SARS-CoV transcriptome. Modules sizes (numbers of genes) are shown following module colors. Color bar shows the $-\log_{10}$ p-values of fisher exact test on overlapping genes between modules. (B, C) Enriched Gene Ontology (GO) terms in black module of SARS-CoV and red module of SARS-CoV-2 shown in (A). Unique and overlapping GO terms were shown respectively. Abbreviations: S1, SARS-CoV; S2, SARS-CoV-2.

Common immune responses induced by SARS-CoV-2 are more tissue-/time-specific but weaker expression than SARS-CoV

Here we focused on the 55 overlapping GO terms annotated as immune responses in Figure 3B, which are reasonably inferred as crucial immediate antagonists against acute infections of these two coronaviruses. We calculated the tissue-/time-specificity of genes involved in those GO terms, showing that SARS-CoV-2-infected transcriptome has a higher tissue-/time-specificity than that of

SARS-CoV-infected transcriptome (Mann-whitney U test, $p < 0.001$) (Figure 4A). Furthermore, we compared their expression levels in Calu-3 cells as most the transcriptome differences occur on Calu-3 cell line at 12 hpi and 24 hpi (Figure 2). Surprisingly, the genes involved in immune responses exhibit significantly lower expression in SARS-CoV-2-infected transcriptomes than SARS-CoV-infected ones (Mann-whitney U test, $p < 0.001$), despite that it happened solely at 12 hpi (Figure 4B). Those results indicate that the common immune responses to these two coronaviruses are weaker but more specific in SARS-CoV-2 transcriptomes than in SARS-CoV transcriptome.

Figure 4. Expression pattern of genes in overlapping GO terms. (A) Comparison of tissue-specificity (τ) between SARS-CoV black and SARS-CoV-2 red modules showed in figure 3A. (B, C) Gene expression levels of SARS-CoV black and SARS-CoV-2 red modules (shown in figure 3A) in Calu3 cells. Mann-whitney U test, *** $P < 0.001$. Abbreviations: S1, SARS-CoV; S2, SARS-CoV-2.

Discussion

Based on percentage of viral poly-A RNA transcript in human cell line samples of Caco-2, Calu-3, and H1299, we determined that SARS-CoV-2 is less capable of infecting and infesting host cells. Upon infection broadness, SARS-CoV-2 infections are more ubiquitous across all tissue with a significantly lower (0.72-fold) tissue-specificity compared to SARS-CoV. These findings may

explain the universal low case fatality rate (CFR) of 3.2% among COVID-19 confirmed cases (Dong, Du, & Gardner, 2020), compared to a CFR of 11% in the short outbreak of SARS in 2003 (Chan-Yeung & Xu, 2003). The clustering pattern of SARS-CoV-2 (Figure 2) also matches an aforementioned clinical distinction, the co-exhibition of GI and respiratory symptoms, of COVID-19, frequently leading to worse prognosis (Jin et al., 2020) (Garland, Kumar, & Borum, 2020). Our results might demonstrate a broader infection spectrum of SARS-CoV-2, leading to frequently documented complications and complication-induced mortality (Guo et al., 2020) (Babapoor-Farrokhran et al., 2020).

More importantly, SARS-CoV-2 transcriptome is found to have more annotated (2.86-fold) non-specific immunity related functions, including cell pathways of interferons, leukocyte activities, and specific lymphocyte activations. However, the expression level of those overlapped functions is lower in SARS-CoV-2 transcriptomes compared to SARS-CoV. As innate immune responses being the first and crucial response against viral infection, COVID-19 patients are speculated of having a wider range of signs and symptoms triggered by multiple inflammation but to a mild extent. Therefore, unlike SARS patients manifesting few but stark and easily identifiable symptoms like abnormally high fever and readily progressive pneumonia (P. K. S. Chan et al., 2006), the mild and cold-mimicking symptoms may account for relatively low alertness and awareness of emerging COVID patients, catalyzing invisible chains of transmission in community and nosocomial settings due to testing inefficiency, which leads to an rapidly-spreading and uncontrolled global pandemic.

Our findings provide implications in preventative and treatment strategies. As evidenced by our study that COVID-19 has a further potential for wide-range spreading, universal testing and masking would be crucial to identify asymptomatic and incubated patients and cut off invisible spreads within communities, which is recommended in early onset countries and regions such as China and Hong Kong (K. H. Chan & Yuen, 2020). Furthermore, applications of large dose anti-inflammatories like corticosteroids shall be prudent and to an extent with simultaneous uses antiviral drugs out of already weak innate responses to SARS-CoV-2 infections, in order to to achieve an optimal clinical outcome for COVID patients with especially mild to moderate symptoms.

There are some limitations to our study. First, the RNA-seq data on which all subsequent analyses are based on is sequenced from cultured samples of three human cell line, which is in lack of genetic variations and versatile cell process and systemic immune response functions facilitated

by partilar lymphocytes. Second, the differential characteristics of SARS-CoV and SARS-CoV-2 infected transcriptome may not be able to apply to all strains of SARS-CoV and SARS-CoV-2 due to limited inclusion of viral strains in cell line treatments. Further investigations on SARS-CoV-2 transcriptome are recommended to perform on a larger range of live tissues or organoids of cardiovascular and urinary systems to elucidate a more well-rounded host response and viral infection profiling.

Materials and Methods

Data Source

RNA-seq data of SARS-CoV and SARS-CoV-2 infected human cell lines used in this publication were retrieved from NCBI's Gene Expression Omnibus (Edgar *et al.*, 2002) and are accessible through GEO Series Accession Number GSE148729 (Emanuel W. et al, 2020). (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE148729>).

Mapping, quantification, and clustering

RNA-seq reads were mapped to a combined genome of human (gencode, v34) and SARS-CoV (GCF_000864885.1) or SARS-CoV-2 (GCF_009858895.2) using HISAT2 (version 2.1.0) (Kim et al. 2015). Reads mapped to human and viral genome were considered as the effective libraries and are used to calculate proportions of viral reads and expression levels. Expression levels were measured as transcripts per million (TPM) using StringTie (version 1.3.3b, -e -A) (Pertea et al. 2015). TPMs of human genes were used to do the clustering of samples using R function “hclust” based on their square Euclidean distance.

Measuring tissue specificity (τ)

A measurement of tissue-specificity denoted as τ were used as Kryuchkova-Mostacci N. and Robinson-Rechavi M. (2016), except that TPM values were used instead of RPKM.

Constructing weighted gene co-expression networks and module detection

To compare the transcriptome difference between SARS-CoV and SARS-CoV-2 infected human cell lines, we constructed the weighted gene co-expression networks and detected modules of SARS-CoV and SARS-CoV-2-infected transcriptomes using step-by-step method according to the R package “WGCNA” (Langfelder P. and Horvath S., 2008). Corresponding modules between SARS-CoV and SARS-CoV-2 were detected by performing Fisher Exact test on overlapping genes of any two modules in each transcriptome.

Gene Ontology (GO) enrichment analysis

R function “enrichmentAnalysis” from package “anRichment” was performed on modules of SARS-CoV and SARS-CoV-2 infected transcriptomes to detected module functions (<https://horvath.genetics.ucla.edu/html/CoexpressionNetwork/GeneAnnotation/>). Only GO terms of biological process were selected. GO terms with Bonferroni adjusted p-value ≤ 0.05 and enrichment ratio ≥ 2 were used in further analysis.

Supplementary materials

Supplementary Figures.

Supplementary Table S1. Expression levels of SARS-CoV genes with expression specificity (τ).

Supplementary Table S2. Expression levels of SARS-CoV-2 genes with expression specificity (τ).

Supplementary Table S3. GO terms that enriched in SARS-CoV modules.

Supplementary Table S4. GO terms that enriched in SARS-CoV-2 modules.

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