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Research paper

Gene signatures of SARS-CoV/SARS-CoV-2-infected ferret lungs in short- and long-term models

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ABSTRACT

Coronaviruses (CoVs) consist of six strains, and the severe acute respiratory syndrome coronavirus (SARS-CoV), newly found coronavirus (SARS-CoV-2) has rapidly spread leading to a global outbreak. The ferret (*Mustela putorius furo)* serves as a useful animal model for studying SARS-CoV/SARS-CoV-2 infection and developing therapeutic strategies. A holistic approach for distinguishing differences in gene signatures during disease progression is lacking. The present study discovered gene expression profiles of short-term (3 days) and long-term (14 days) ferret models after SARS-CoV/SARS-CoV-2 infection using a bioinformatics approach. Through Gene Ontology (GO) and MetaCore analyses, we found that the development of stemness signaling was related to short-term SARS-CoV/SARS-CoV-2 infection. In contrast, pathways involving extracellular matrix and immune responses were associated with long-term SARS-CoV/SARS-CoV-2 infection. Some highly expressed genes in both short- and long-term models played a crucial role in the progression of SARS-CoV/SARS-CoV-2 infection, including *DPP4, BMP2, NFIA, AXIN2, DAAM1, ZNF608, ME1, MGLL, LGR4, ABHD6*, and *ACADM*. Meanwhile, we revealed that metabolic, glucocorticoid, and reactive oxygen species-associated networks were enriched in both short- and long-term infection models. The present study showed alterations in gene expressions from short-term to long-term SARS-CoV/SARS-CoV-2 infection. The current result provides an explanation of the pathophysiology for post-infectious sequelae and potential targets for treatment.

1. Introduction

Coronaviruses (CoVs) contain club-shaped spikes that project from their surface. They constitute a group of enveloped positive-sense single-stranded RNA viruses, which infect humans and animals through the respiratory and central nervous systems (Lam et al., 2020; Morens et al., 2020). Various strains of CoVs have been identified, including human CoV (HCoV)-OC43, HCoV-229E, HCoV-NL63, and HCoVHKU1.

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These low-pathogenic strains can infect humans without causing severe symptoms. However, the severe acute respiratory syndrome (SARS)-CoV has caused two large-scale pandemics with more than 8422 SARS cases worldwide, including approximately 916 related deaths (Desforges et al., 2019; Ashour et al., 2020; Ou et al., 2020; Schwartz and Graham, 2020). Outbreaks of CoVs indicate that this virus had zoonotic origins, and some of their strains are highly pathogenic, especially during transmission from animals to humans(Wong et al., 2020; Yu et al., 2020).

Therefore, it is crucial to develop effective therapeutic strategies against CoVs. In late 2019, a novel CoV, namely 2019-nCoV/SARS-CoV-2 emerged that led to a severe CoV disease in 2019 (abbreviated COVID-19) and caused a large global outbreak. As of June 10, 2020, according to statistical reports from World Health Organization (WHO) (https://www.who.int/emergencies/diseases/novel-coronavirus-2019) there have been over 7,145,539 confirmed cases of COVID-19, including approximately 408,025 deaths (Barton et al., 2020; Rehman et al., 2020; Yip et al., 2020). The mean incubation period for SARS-CoV-2 has been estimated to be in the range of 2.1– 11.1 days (Backer et al., 2020). The age of patients with positive test results ranged from 10 months to 78 years, and different symptoms were displayed in short- and long-term infections (Wang et al., 2020b; Xiao et al., 2020). Further, human-to-human transmission has been observed to occur among close contacts. Although efforts are being made by researchers across the globe to develop vaccines against SARS-CoV-2, no effective medication has yet been identified.

The ferret, *Mustela putorius furo*, displays symptoms and pathological features that resemble to those observed in SARS-CoV-infected *Homo sapiens*, and is susceptible to the unadapted SARS-CoV-2 strain. Therefore, the ferret has been utilized as an animal model to understand SARS-CoV/SARS-CoV-2 infection and develop therapeutic strategies (Siragam et al., 2018; Kim et al., 2020; Shi et al., 2020). Recently, Blanco-Melo et al. describes an antiviral gene signatures induced in human cell lines as well as the ferret models after SARS-CoV-2 infection (Blanco-Melo et al., 2020). SARS-CoV-2 infection evokes a weaker antiviral transcriptional response, lower interferon-I/III levels, and higher cytokine expression to induce severe illness of COVID-19. However, to date, a holistic approach to identify differences in gene signatures between short- and long-term SARS-CoV-2 infections is lacking. Meanwhile, there are numerous studies working on potential messenger (m) RNA candidates associated with CoV infection and their downstream target genes (Nilsson et al., 2019; Abele et al., 2020; Lopez et al., 2020; Pan et al., 2020). Predictive markers and therapeutic strategies for CoVs have not been identified and systematically investigated. A comprehensive approach needs to be developed for thousands of gene expression profiles through the use of high-throughput technology involving functional genomics and biological systems (Liu et al., 2018; Cao et al., 2020; Lan et al., 2020). The current study investigated gene expression profiles in ferret lung model using a bioinformatics approach. Short-term (3 days) and long-term (14 days) SARS-CoV/SARS-CoV-2 infections were compared. The Gene Expression Omnibus (GEO) database was utilized to analyze all available samples associated with SARS-CoV/SARS-CoV-2-infected ferret models. We also predicted a vital regulatory downstream network and evaluated the potential roles of these candidate genes as therapeutic biomarkers for COVID-19. These crucial results provide essential shreds of evidence of the role of novel transcript regulation in SARS-CoV/SARS-CoV-2 infection.

2. Materials and methods

2.1. Bioinformatics and high-throughput database analyses

SARS-CoV/SARS-CoV-2-infected ferret models were acquired from the National Center for Biotechnology Information (NCBI) GEO database. High-throughput data of the GSE11704 and GSE147507 datasets were employed and analyzed. Briefly, castrated and descended ferrets (*M. putorius furo*) were infected with SARS-CoV or a mock control in the GSE11704 dataset(Cameron et al., 2012), and SARS-CoV-2 or mock in the GSE147507 dataset(Blanco-Melo et al., 2020). After 3 and 14 days, the ferrets were sacrificed, and fresh lung tissues were collected using TRIzol reagent. The CLC Genomics Workbench v10.1 (CLC bio, Aarhus, Denmark) was used to analyze the data, and gene symbols were mapped to the Ensembl characteristics by using the package of biomaRt (v. 2.26.1). To perform clustering based on mRNA expression profiles using pheatmap V1.0.12 in the R environment (Gentleman et al., 2004; Durinck et al., 2009), signals were processed and normalized as described previously (Ou et al., 2014; Depeille et al., 2015; Lawson et al., 2015). The Database for Annotation, Visualization, and Integrated Discovery (DAVID) (version 6.8) from the Laboratory of Human Retrovirology and Immunoinformatics (LHRI) is a public web-based database that possesses dominant gene functional classifications (Da Huang et al., 2009a, 2009b). This database includes various processes and pathways, such as Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Genes of interest are categorized into different groups in DAVID, and their related biological functions, signaling pathways, or associated diseases were calculated according to an agglomeration algorithm method. Short- and long-term SARS-CoV/SARS-CoV-2-infected ferrets and mock controls were compared. The top 10% of differentially expressed genes (DEGs) with a higher degree of expression in SARS-CoV/SARS-CoV-2-infected groups were analyzed as described previously (Wang et al., 2017a; Wang et al., 2019; Wang et al., 2020a; Yang et al., 2020). A p value of 0.05 was set, an adjusted false discovery rate (FDR) was followed, and a list of genes with significantly different expressions was used as input to the GO database for constructing biological networks and their associated biological processes and diseases (Ashburner et al., 2000). To identify significantly enriched pathways or groupings of annotated genes, a cutoff of $p < .05$ was used (Subramanian et al., 2005).

We identified DEGs of SARS-CoV/SARS-CoV-2-infected ferret lungs between short- and long-term models. Statistically significantly upregulated mRNA levels were analyzed using various bioinformatics tools, including a Venn diagram for merging different datasets, GO for searching cellular components, MetaCore for finding downstream networks, search tool for the retrieval of interacting genes (STRING) for exploring protein-protein interaction (PPI) networks, and the DAVID database for investigating associated pathways (Fig. 1).

2.2. Pathway and network enrichment analyses

MetaCore (GeneGo, St. Joseph, MN, USA) identifies biological processes associated with gene expression profiles that are used as input to the program. To compare average levels of gene expression in SARS-CoV/SARS-CoV-2-infected ferret models, the upper 10% of highly expressed genes with significant differences in expression were used as input to the MetaCore software. Signal transduction pathways were investigated. $p < .05$ indicated a statistically significant difference.

2.3. Search tool for the retrieval of interacting genes (STRING) analysis

The STRING database contains information about protein-protein networks of about 5090 organisms, 24.6 million proteins, and more than 2000 million interactions (Szklarczyk et al., 2019). In this study, the STRING database (version 11.0) was utilized to analyze PPI networks based on DEGs, and the k-means clustering algorithm was employed to classify proteins into different clusters.

3. Results

3.1. GO analysis of SARS-CoV/SARS-CoV-2-infected ferret models

Results of SARS-CoV-infected ferret lungs from the GSE11704 dataset were compared with the mock treatment groups. DEGs in long-

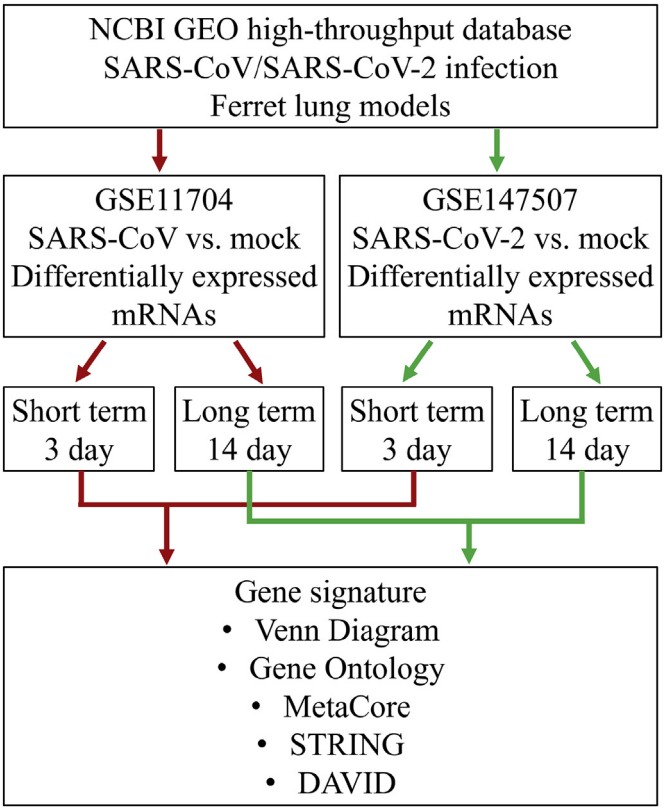

Fig. 1. Schematic illustration of the study design. High-throughput data of severe acute respiratory syndrome coronavirus (SARS-CoV)/SARS-CoV-2-infected ferret lung models were acquired from GEO. Differentially expressed genes in the top 10% of SARS-CoV- and SARS-CoV-2-infected models were determined through a Venn diagram analysis. Results were analyzed using GO, MetaCore, STRING, and DAVID for pathway analyses and functional interpretations.

term infection (14 days) of SARS-CoV were matched with those in short-term infection (3 days). Pathways associated with long-term SARS-CoV infection are shown in Fig. 2A. Cluster 1 (c1) contained pathways with the most significant p values, followed by clusters 2 (c2). We revealed that virus-reproductive (c1) and metabolism-related pathways (c2) were significantly correlated with SARS-CoV infected lung models. Results of SARS-CoV-2-infected ferret lungs from the GSE147507 dataset were compared with the mock controls. DEGs in long-term infection (14 days) of SARS-CoV-2 were matched with those in short-tem infection (3 days). Pathways associated with long-term SARS-CoV-2 infection are shown in Fig. 2B. Cluster 1 (c1) contained pathways with the most significant p values, followed by clusters 2 (c2) and 3 (c3). The extracellular matrix (ECM) part and ECM-associated pathway were the most crucial pathway ($p = 5 \times 10^{-38}$ and 9×10^{-37}, respectively). Pathways in c1 were related to the ECM, plasma membranes, adhesion, development, and catabolism. Pathways in c2 were associated with intracellular contents, metabolism, gene expressions, protein transport, and viral reproduction. Pathways in c3 were correlated with immune responses, transmembrane transport, and cell differentiation. From the ferret lung models, viral reproduction and metabolism-related pathways were activated both in SARS-CoV and SARS-CoV-2 infection. However, ECM-associated pathways were enriched uniquely in c1, and the innate immune response was discovered in c3 of long-term SARS-CoV-2 infection.

3.2. Pathway analysis of SARS-CoV/SARS-CoV-2-infected ferret models

The Venn diagram was utilized to search for regulated genes in common between the SARS-CoV- and SARS-CoV-2-infected ferrets models. For the short-term model, 2589 of the top 10% of mRNAs were highly expressed in SARS-CoV-infected groups compared to those of the mock controls in the GSE11704 dataset, and 2587 mRNAs were highly expressed in SARS-CoV-2-infected ferret models from the GSE147507 dataset (Fig. 3A). We detected 308 overlapping upregulated genes common to both groups, which could serve as potentially regulated genes in SARS-CoV/SARS-CoV-2 infection. These 308 genes were uploaded to the MetaCore platform for a map analysis. We found that several common maps were related to short-term SARS-CoV/SARS-CoV-2 infection. The “development: negative regulation of WNT/β-catenin signaling at the receptor level” and “cytoskeleton remodeling: regulation of actin cytoskeleton organization by the kinase effectors of Rho GTPases” were the most significant maps (Fig. 3C, Table A1).

To further explore potential maps in long-term SARS-CoV/SARS-CoV-2-infected ferret models, 2129 of the top 10% of highly expressed mRNAs in SARS-CoV-infected groups were detected compared to the mock controls from the GSE11704 dataset. In total, 2674 mRNAs were highly expressed in SARS-CoV-2-infected ferret models from the GSE147507 dataset. By comparing the two groups of highly expressed genes, we discovered 419 common genes (Fig. 3B). Through the MetaCore platform, we analyzed these 419 genes and explored several maps associated with long-term infection of ferret lung models with SARS-CoV/SARS-CoV-2. The “cell adhesion: integrin-mediated cell adhesion and migration” and “transport: the role of AVP in the regulation of aquaporin 2 and renal water reabsorption” were the most significant maps (Fig. 3D, Table A2). Maps associated with short-term and long-term SARS-CoV/SARS-CoV-2-infected ferret models differed. In the results of short-term infection, cytoskeletal remodeling-related maps were shown as ranks 2, 11, and 20, and cell adhesion-related maps as ranks 14 and 23. In the long-term infection analysis, the importance of cell adhesion-related maps increased and were ranked numbers 1, 7, and 19. The cytoskeletal remodeling-related maps moved to ranks 46 and 47. The significance of immune response-associated maps was also discovered in long-term infection. Fig. 4 shows the “cell adhesion: integrin-mediated cell adhesion and migration” maps. Collagen, laminin, or fibronectin in the ECM (upper part) interacted with integrin receptors on cell membranes to activate intracellular signaling and module, cell adhesion, migration, or stress fiber assembly. The map revealed potential responses of ferret lungs after long-term infection with SARS-CoV/SARS-CoV-2, which may also reflect the condition of SARS-CoV/SARS-CoV-2-infected patients (Fig. 4). Focal adhesion kinase 1 (FAK1, gene: PTK2) and P21-activated kinase 1 (PAK1, gene: PAK1) proteins were centers of the map.

Meanwhile, we also analyzed the top 10% down-regulated genes in a Venn diagram from the short-term and long-term SARS-CoV-/SARS-CoV-2-infected ferret lung models. The “role of osteoblasts in bone lesions formation in multiple myeloma”, “NF-kB pathway in multiple myeloma”, and “dual function of Treg cells in cancer development” were the most significant suppressed maps in short-term SARS-CoV- and SARS-CoV-2 infected ferret models Fig. A1A/B and Table A3). The “role of Bregs in attenuation of T and NK cells mediated anti-tumor immune responses”, “cell cycle: regulation of G1/S transition”, and “development: WNT/beta-catenin signaling in organogenesis” were the most significant repressed maps in long-term SARS-CoV- and SARS-CoV-2 infected ferret models (Fig. A1C/D and Table A4). We found that “dual function of Treg cells in cancer development” pathway plays a crucial role in short-term SARS-CoV/SARS-CoV-2-infected ferret lung models (Fig. A2).

3.3. Distinguishing co-regulated interactions and cellular component annotations in short- and long-term SARS-CoV/SARS-CoV-2-infected ferret models

In previous sections, results provided a broad set of previously unrecognized genes in response to SARS-CoV/SARS-CoV-2 infection. Further, we compared these highly expressed genes in both short- and

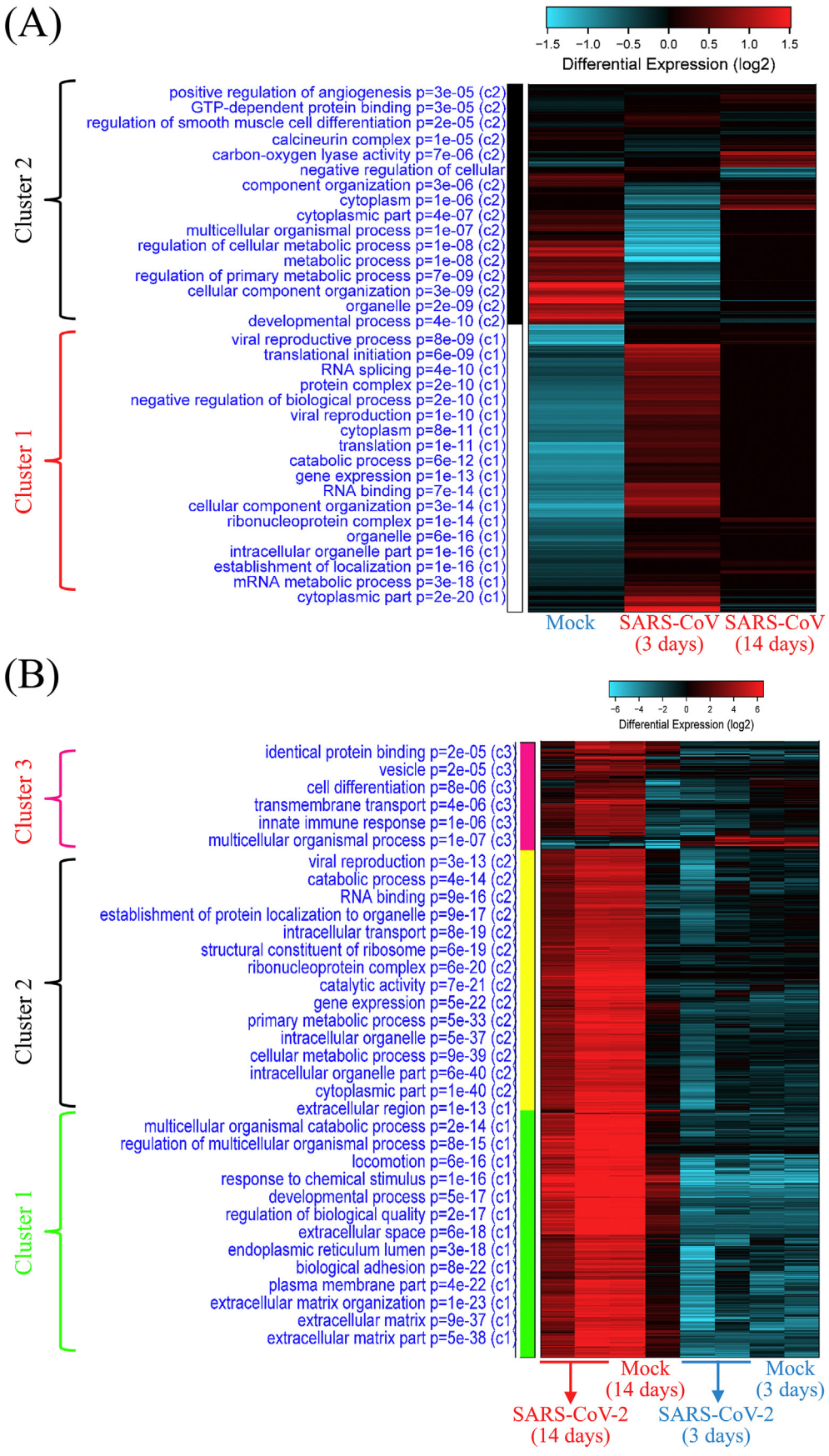

Fig. 2. Gene ontology enrichment analysis and heatmap visualization of SARS-CoV and SARS-CoV-2-infected ferret models. (A) Short-term SARS-CoV-infection (3 days) and long-term infection (14 days) were compared with mock controls. Cluster 1 (c1) were the most significant pathways and followed by the cluster 2 (c2). Viral reproduction and metabolism-related pathways were significantly correlated with SARS-CoV infected lung models. (B) Long-term SARS-CoV-2 infection (14 days) and mock controls were compared with short-term infection (3 days) and mock controls. Cluster 1 (c1) were the most significant pathways, followed by the cluster 2 (c2) and cluster 3 (c3). Extracellular matrix-associated pathways appeared significantly in long-term SARS-CoV-2-infected ferret lungs (c1) as compared to the short-term infection (3 days).

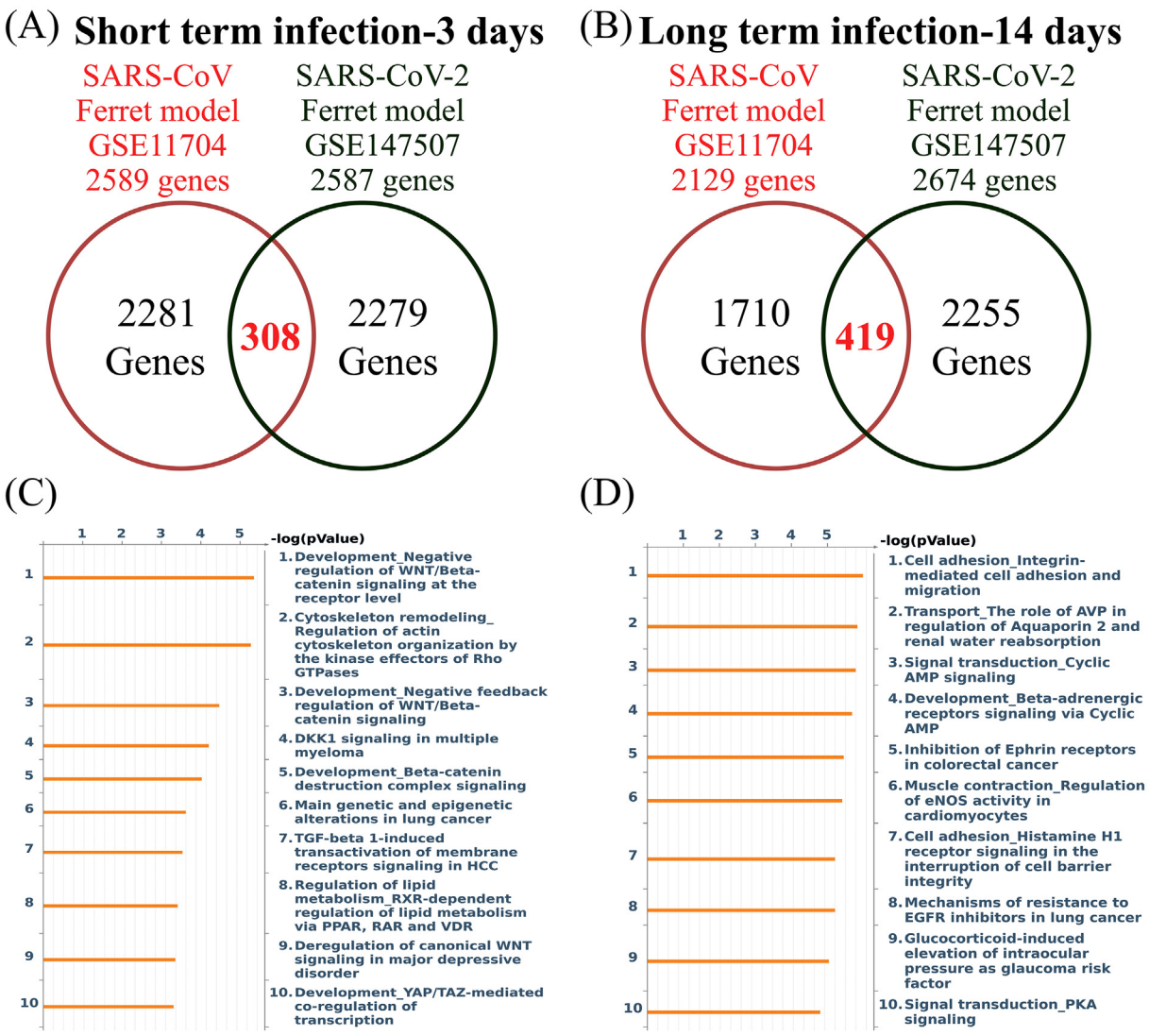

Fig. 3. Venn diagram of overexpressed pathways from severe acute respiratory syndrome coronavirus (SARS-CoV)/SARS-CoV-2-infected ferret lung models. (A) Analysis of the top 10% of differentially highly expressed genes in a Venn diagram from the GSE11704 and GSE147507 datasets in short-term and (B) long-term SARS-CoV/SARS-CoV-2-infected ferret lung models. (C) The MetaCore platform was used to analyze overexpressed genes of SARS-CoV/SARS-CoV-2-infected ferret lung models in the GSE11704 and GSE147507 datasets to build potential maps of short-term and (D) long-term infection.

long-term SARS-CoV/SARS-CoV-2-infected groups in the GSE11704 and GSE147507 datasets. The intersection of top highly expressed 308 genes, which belonged to the short-term SARS-CoV/SARS-CoV-2-infected groups and 419 genes, which belonged to the long-term infection revealed 11 potential targets, including DPP4 (protein: dipeptidyl peptidase 4), BMP2 (protein: bone morphogenetic protein 2), NFIA (prtein: nuclear factor I A), AXIN2 (protein: axis inhibition protein 2), DAAM1 (protein: disheveled associated activator of morphogenesis 1), ZNF608 (protein: zinc finger protein 608), ME1 (protein: malic enzyme 1), MGLL (protein: monoglyceride lipase), LGR4 (protein: leucine-rich repeat-containing G protein-coupled receptor 4), ABHD6 (protein: abhydrolase domain containing 6), and ACADM (protein: acyl-coa dehydrogenase medium chain) (Fig. 5A). These 11 genes were imported into the STRING platform, and the PPI networks were investigated (Fig. 5B). BMP4 (protein: bone morphogenetic protein 4) and AXIN2 were displayed from the interacting networks of the 11 targeted genes. The networks may play crucial roles in clinical manifestations after SARS-CoV/SARS-CoV-2 infection.

Upregulated genes in common between SARS-CoV- and SARS-CoV-2-infected ferrets in Fig. 3A were imported into the GO platform to analyze cellular component annotations for short-term infection (Fig. 6A). These upregulated genes mostly functioned in nucleoli, spectrin, lamellipodia, ribosomes, and mitochondria. The same analytical process was applied to common genes in Fig. 3B to obtain cellular components of long-term infection (Fig. 6B). Genes with the greatest significance functioned in extracellular exosomes, brush borders, bicellular tight junctions, stress fibers, and apical plasma membranes. The intracellular machinery of SARS-CoV/SARS-CoV-2-infected ferret lungs changed at different time points (Fig. 6C).

4. Discussion

Although pharmaceutical research and development of vaccines against viral infection has increased significantly over recent years, the approval of new drugs and treatments by the US FDA is slow and limited. Newer technologies have more-advantageous features than previous methods, such as high-throughput data capacity, improved accuracy and sensitivity, and provision of a higher detection range. Novel discovered transcripts, and various bioinformatics tools could be applied to these data. CoVs were correlated with cytokine storms and inflammation (Di Gennaro et al., 2020; Mehta et al., 2020). A recent study also found that the PIKfyve (phosphoinositide kinase, FYVE-type

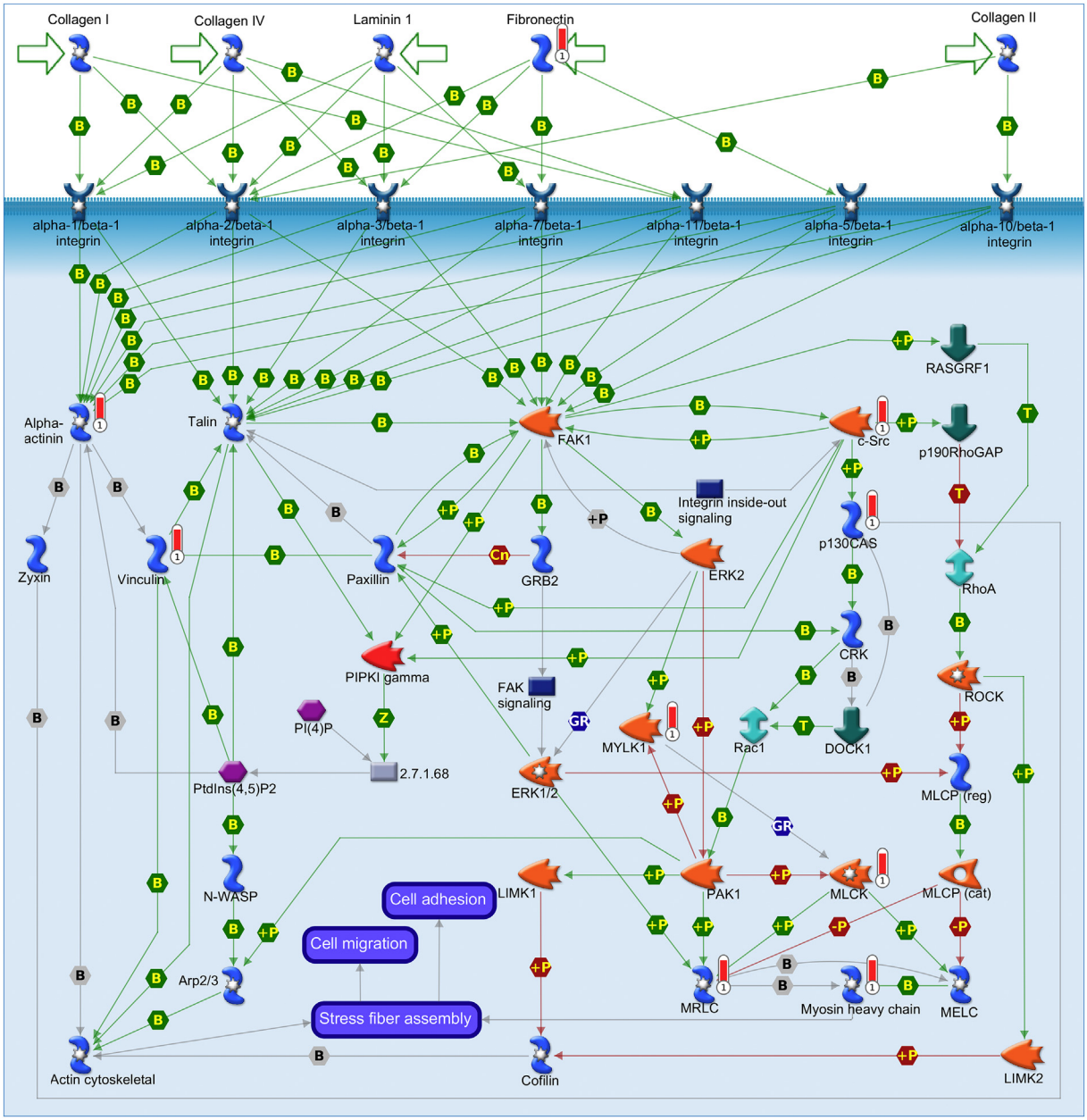

Fig. 4. MetaCore pathway analysis of upregulated gene pathways in severe acute respiratory syndrome coronavirus (SARS-CoV)/SARS-CoV-2-infected ferret lung models. MetaCore pathway and network analyses indicated that the "cell adheison: integrin-mediated cell adhesion and migration" pathway plays a crucial role in long-term SARS-CoV/SARS-CoV-2-infected ferret lung models.

zinc finger containing), TPC2 (two pore segment channel 2), and cathepsin L signaling pathways were activated in SARS-CoV-2 infection and can possibly serve as potential drug targets for therapeutic intervention strategies (Ou et al., 2020). SARS-CoV-2 relies on angiotensin-converting enzyme 2 (ACE2) and transmembrane protease, serine 2 (TMPRSS2) to enter cells (Hoffmann et al., 2020), and therapy with mesenchymal stem cells suppresses the cytokine storm and promotes endogenous repair by reparative properties of stem cells (Ratajczak, 2020). Interleukin (IL) family members, including IL-6, IL-1, IL-1β, tumor necrosis factor (TNF), and C—C motif chemokine ligand 2 (CCL2), serve as over-responders during the cytokine storm syndrome in COVID-19 (Conti et al., 2020; Malavolta et al., 2020). SARS-CoV regulates collagen expression through the transforming growth factor (TGF)-β1 signaling pathway (Wang et al., 2017b) and interacts with host cells through ECM components (Baas et al., 2006). Those results are consistent with our present study. Through the GO analyses of long-term SARS-CoV-2-infected ferret lung models in the GSE147507 dataset, we found that several pathways were enriched, including ECM, biological adhesion, viral reproduction, viral infectious cycle, virion assembly, cellular metabolic processes, exosomes, innate immune responses, mast cell cytokine production, IL-6 production, and immunoglobulin receptor binding (Fig. 2).

A previous study also demonstrated that SARS-CoV and SARS-CoV-2 induce inflammation (Malavolta et al., 2020), and symptoms of the most severe patients can be controlled by low-dose glucocorticoids (Lin et al., 2020). A combination of thalidomide and low-dose glucocorticoids was effective in treating lung injury and immunological stress caused by SARS-CoV-2 (Chen et al., 2020). However, comprehensive preclinical studies to prove the utility of these drugs are still lacking. In the present study, DEG sets of SARS-CoV- and SARS-CoV-2-infected ferret lung models were enriched in cytokines, immune responses, and glucocorticoid-associated maps (Tables A1, A2). The pathway

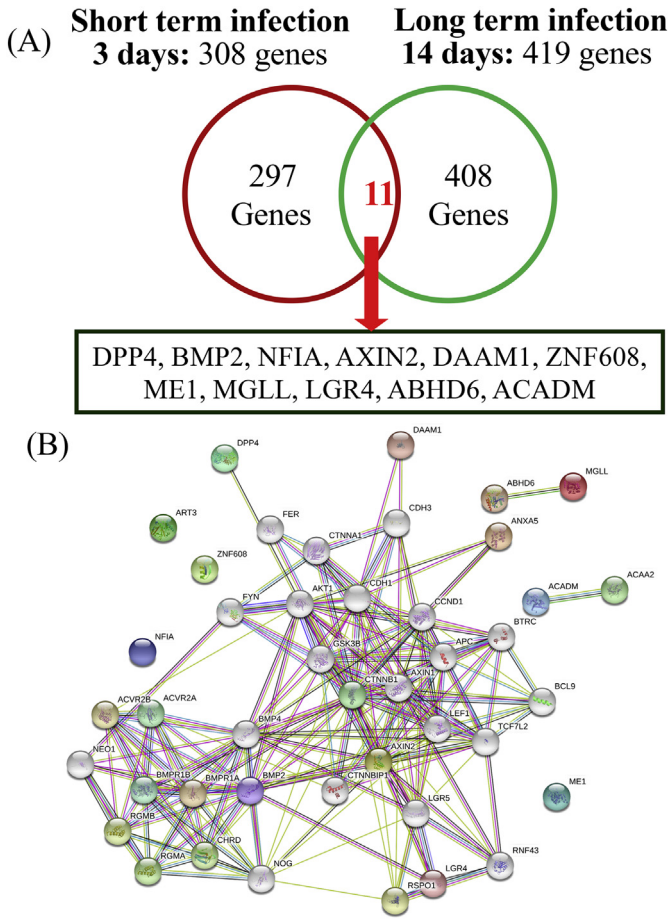

Fig. 5. Protein–protein interacting networks of differentially expressed genes in short- and long-term SARS-CoV/SARS-CoV-2-infected ferret lung models. (A) Data from the GSE11704 and GSE147507 datasets were compared through a Venn diagram. Upregulated genes in common between the SARS-CoV- and SARS-CoV-2-infected ferret lung models were obtained. Genes which overlapped between the 308 genes from short-term infection and 419 genes from long-term infection revealed 11 target genes. (B) The above 11 differentially expressed genes were input to the STRING platform to analyze protein-protein interactions. Using k-means clustering, the networks were separated into different clusters. Colored nodes were 11 target genes of input and grey nodes were proteins connected to target genes.

enrichment analysis through the MetaCore platform showed that several "development/stemness/Wnt/cytoskeletal remodeling-related pathways" were specifically correlated with the short-term SARS-CoV/SARS-CoV-2-infected model (Fig. 3A, C). Enriched pathways in long-term SARS-CoV/SARS-CoV-2-infected models were strongly correlated with ECM/immune/G protein-coupled receptor (GCPR)/cell adhesion-related pathways (Figs. 2, 3B, Table A2). "Cell adhesion: integrin-mediated cell adhesion and migration" was the most important pathway in the long-term infection model (Figs. 3D, 4). Therefore, our enrichment analysis showed that the response of host cells changed during the progression of SARS-CoV/SARS-CoV-2 infection. The development, stemness, Wnt, and cytoskeleton remodeling-related pathways in short-term results explain the viral-reproduction process during the early phase of infection. The ECM, immune response, GCPR, and cell adhesion-related pathways in the long-term models described the potential mechanism of cytokine storms and lung fibrosis during the middle and late phases of infection. On the contrary, down-regulated pathways included "dual function of Treg cells in cancer development" in the short-term and "role of Bregs in attenuation of T and NK cells mediated anti-tumor immune responses" in long-term SARS-CoV-/SARS-CoV-2-infected models. Dysregulation of T cells and B cells is revealed, which implicates the difficulty to developing protective immunity after SARS-CoV-2 infection.

We compared these highly-expressed genes in both short- and long-term SARS-CoV/SARS-CoV-2-infected ferret lung models (Fig. 3). Through the Venn diagram analysis, we detected 11 targeted genes, including *DPP4*, *BMP2*, *NFIA*, *AXIN2*, *DAAM1*, *ZNF608*, *ME1*, *MGLL*, *LGR4*, *ABHD6*, and *ACADM*, which may play crucial roles in SARS-CoV/SARS-CoV-2 infection (Fig. 5A). The interacting networks building from STRING software suggested that BMP4 and AXIN2 were located in the center of protein-protein interactions from 11 targeted genes (Fig. 5B). Some researchers demonstrated that DPP4 is a known receptor for HCoVs, and a DPP4 inhibitor is a glucose-lowering agent for diabetic patients (Drucker, 2020; Qi et al., 2020). GATA4-induced BMP2 signaling was observed to be correlated with the Epstein-Barr virus-infection pathway (Olsavszky et al., 2017). MicroRNA (miR)-370 and miR-200c attenuated hepatitis B virus (HBV) replication by directly targeting the *NFIA* gene (Fan et al., 2017; Tian and He, 2018). Axin2 is one of the response proteins to Wnt signaling in lung disease (Hogan, 2018), and it was also correlated with the Wnt/β-catenin pathway in HBV-associated hepatocellular carcinoma (Kim et al., 2016). MGLL was upregulated in human papillomavirus (HPV)-associated disease (Kaczkowski et al., 2012), and LGR4 was reported to be essential in facilitating vesicular stomatitis viral infection (Zhang et al., 2017). Upregulating BMP4 was correlated with coronary artery disease and had high risk for reactivation of the varicella zoster virus (Watanabe et al., 2017). Recombinant BMP4 increased HCV replication negatively regulated by vascular endothelial growth factor A (VEGF-A) (Rowe et al., 2014). Those reports are consistent with our present studies. Previous studies also demonstrated that exosomes serve as therapeutic biomarkers in SARS-CoV-infected patients (Kang, 2020; Zheng et al., 2020). SARS-CoV-2 regulates signal transduction, metabolism in mitochondria, and apoptosis of host cells (Guzzi et al., 2020). It also manipulates the mitochondrial function of host cells to avoid antiviral innate immunity through ORF-9b signaling (Lippi et al., 2020; Tiku et al., 2020). The findings of the present study are in agreement with those of previous studies. Through GO analyses of cellular components, upregulated genes in short-term infection were located in nucleoli, spectrin, lamellipodia, ribosomes, and mitochondria (Fig. 6A). Gene-associated cellular components changed to extracellular exosomes, brush borders, bicellular tight junctions, stress fibers, and apical plasma membranes in the long-term infection model.

Since the present study focused on the pathways of SARS-CoV/SARS-CoV-2-infected ferret-related signatures, further studies are required to validate the applicability of our findings in treating COVID-19 patients. However, our study revealed crucial roles of several novel genes and regulated pathways in SARS-CoV/SARS-CoV-2-infected ferret lung models. The present research can diminish the translational gap between on-bench research and clinical applications, which is a noteworthy issue in the expeditious development of efficient treatment strategies for the SARS-CoV-2 outbreak. Meanwhile, we identified 11 highly expressed genes in SARS-CoV/SARS-CoV-2-infected ferret models. These genes can potentially be targeted for treating and preventing SARS-CoV/SARS-CoV-2 infection. The results of the present study can guide experimental efforts towards the development of vaccines against SARS-CoV-2 and further combat the pandemic COVID-19.

Author contributions

All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare no conflict of interest.

(A) **Short term infection-3 days**

GO: cellular component	*p*
Nucleolus	2E-04
Spectrin	2E-03
Lamellipodium	3E-03
Ribosome	4E-03
Mitochondrion	7E-03
Golgi apparatus	2E-02
Cytosol	2E-02
Nucleoplasm	2E-02
Focal adhesion	2E-02
Apical plasma membrane	2E-02
Centrosome	3E-02
Early endosome	3E-02
Stress fiber	4E-02
Collagen trimer	4E-02
Microtubule cytoskeleton	5E-02

(B) **Long term infection-14 days**

GO (cellular component)	*p*
Extracellular exosome	4E-18
Brush border	5E-07
Bicellular tight junction	2E-06
Stress fiber	9E-05
Apical plasma membrane	1E-04
Focal adhesion	5E-04
Basolateral plasma membrane	6E-04
Lateral plasma membrane	1E-03
Plasma membrane	2E-03
Terminal web	2E-03
Perinuclear region of cytoplasm	3E-03
Brush border membrane	3E-03
Cell surface	5E-03
Integral component of membrane	9E-03
Desmosome	9E-03

(C)

Fig. 6. Gene Ontology of Cellular Component annotations associated with highly expressed genes in short-term and long-term SARS-CoV/SARS-CoV-2-infected ferret lung models. (A) The most highly enriched GO cellular components by upregulated genes in short-tem SARS-CoV/SARS-CoV-2-infected ferret models. (B) The most highly enriched GO cellular components by upregulated genes in long-term SARS-CoV/SARS-CoV-2-infected ferret models. (C) Summary of enriched maps of the bioinformatic analysis of overexpressed genes from SARS-CoV/SARS-CoV-2-infected ferret lung models.

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Appendix A. Supplementary data

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