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Experimental Strategy to Understand Host Molecular Mechanisms during COVID-19 Infections

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Abstract: ***The neoteric coronavirus, SARS-CoV-2, has been the single most prolific pathogenic threat to humans for nearly a century; thus, a comprehensive study of SARS-CoV-2 at the molecular level is paramount. The proposed research study will employ a safe, non-infectious pseudovirus-based cell entry protocol to elucidate undiscovered gene expression profiles, molecular networks, biologic mechanisms, and protein-protein interactions involved in SARS-CoV-2 entry into human cells.***

Keywords: SARS-CoV-2; pseudovirus; pandemic; RT-PCR; bioinformatics

1. Introduction

The COVID-19 pandemic is responsible for the deaths of millions of men, women, and children worldwide. Currently, the mortality rate in the United States is significantly lower than when the pandemic began due to the success of vaccine administration and natural immunity that has developed over time. Since 2002 the world has faced two epidemics (SARS-CoV-1 and MERS) and one pandemic (SARS-CoV-2) that originated from zoonotic species jumping events. No one will argue the global impact of SARS-CoV-2 and its variants [1]. That there are several thousand bat coronaviruses in nature suggests that this is not the last viral health threat that will severely impact humans. Thus, there is an urgent need for the continual study of coronaviruses.

The current pandemic virus can infect many cells in the human body [2]; however, the impact of viral entry and gene expression modulation in respiratory, renal, and cardiac muscle cells is not fully understood. The spike glycoprotein on the surface of the novel coronavirus interacts with the angiotensin-converting enzyme 2 (ACE2) receptor on the host surface to mediate cell attachment and viral entry. ACE2 expression is prevalent in the lung, kidney, heart muscle, and several other cell types. SARS-CoV-2 preferentially infects lung cells (e.g., type 2 alveolar cells), leading to ARDS [3-4]. Incipient findings also demonstrate the association of harmful renal abnormalities following infection of kidney cells [5-6]. Chen, Li, Chen, Feng, and Xiong [7] examined ACE2 expression in the human heart and the correlation between cardiovascular disease. Recent evidence also explored the link between SARS-CoV-2 infection and heart conditions such as myocarditis related to college athletes [8].

The proposed molecular strategy will resolve sinuous molecular mechanisms of virus entry into the lungs, kidneys, and heart muscle. The proposed methodology will allow scientists to identify gene expression patterns, transcriptional signals, molecular networks, signaling pathways, and protein-protein interactions in human lung cells (A529), kidney cells (HK-2), and heart muscle cells (HASMC) infected with pseudo-SARS-CoV-2 viral particles. Briefly, human cells infected with SARS-CoV-2 pseudoviral particles enriched with the spike glycoprotein will undergo next-generation sequencing (e.g., RNA sequencing) for gene expression profiling. Bioinformatics procedures will precipitate human gene expression characterizations during the SARS-CoV-2 entry process. Protocols will identify unknown host cell factors modulated during viral entry.

The methodology will provide insight into the molecular mechanisms involved in viral-host entry processes.

2. SARS-CoV-2 Cell Entry Assay

The use of viral pseudotypes is a standard method for investigating various virus-host interactions, including viral cell entry mechanisms [9-12]. The proposed pseudotyped viruses are baculoviruses that contain SARS-CoV-2 spike glycoproteins on the envelope surface, so it resembles and behaves like the coronavirus; however, it is replication negative (e.g., PseudoSARS-CoV-2 Green Reporter - Montana Molecular). Thus, while pseudovirions are valuable models for studying coronavirus cell entry, they are non-infectious and pose no threat to humans (e.g., BSL-1).

The baculovirus pseudotypes mimic SARS-CoV-2 external features and transfer green fluorescent proteins into mammalian cells following interaction with the ACE2 receptor and other cellular factors. A detectable green fluorescence in the nuclei of host cells confirms pseudoviral entry.

A fluorescent cell imager, fluorescence microscope, or microplate reader can track intracellular fluorescence (Figure 1).

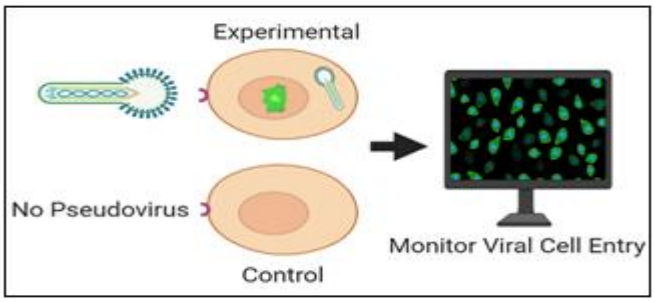

Figure 1: Schematic of viral cell entry assay. Pseudoviral infection facilitates green fluorescent protein expression in the infected cell's nucleus. Spike glycoprotein (blue) binds to the ACE2 receptor (purple) and permits viral entry.

3. Experimental Strategy

The proposed experimental approach is shown in Figure 2. The methodology will include cell culture techniques, viral cell entry assays, nucleic acid extraction, sequencing protocols, bioinformatics, and real-time polymerase chain reaction experiments [13-16].

3.1 Human Cells

Human lung epithelial cells (A549), human kidney cells (HK-2), and primary aortic smooth muscle cells (HASMC) comprise the host cells. A549 cell lines are typically maintained in F-12K Medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin in twelve-well tissue culture plates at 37°C in a 5% CO_2 atmosphere. HK-2 cells are maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 100 U/mlpenicillin, and 100 µg/ml streptomycin in twelve-well tissue culture plates at 37°C in a 5% CO_2 atmosphere. HASMC cells are cultured in vascular cell basal media supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin in twelve-well tissue culture plates at 37°C in a 5% CO_2 atmosphere. Cells can then be analyzed using a microscope to evaluate cell morphology and stringency of culture conditions and enumerated using the trypan blue exclusion method [17-18].

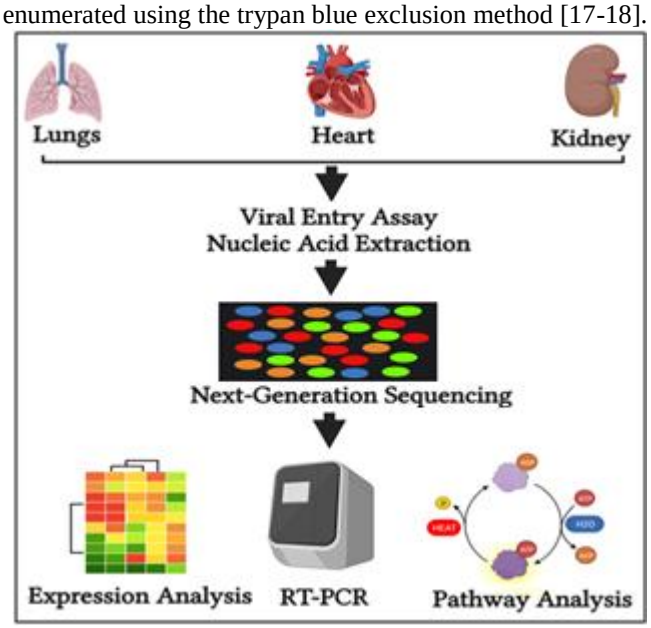

Figure 2: Schematic of proposed research methodology to investigate molecular mechanisms during viral cell entry.

3.2 Viral Cell Entry Assay

In a previous study, pseudovirus particles helped investigators deduce the role of lactoferrin during SARS-CoV-1 infections [19]. In this study, the SARS-CoV-2 pseudovirus will infect human cells according to the manufacturer's specifications [20-21]. SARS-CoV-2 pseudovirus-infected GFP-expressing cells can be observed and measured by fluorescence microscopy using a fluorescent cell imager. Nucleic acid samples extracted from A549, HK-2, and HASMC cells will undergo next-generation RNA sequencing procedures. Control experiments (no virus, cells only) should be performed for each cell type.

3.3 Bioinformatics Analysis

Analysis of the microarray dataset can be performed using open-access and licensed bioinformatics software (e.g., Metascape, Kyoto Encyclopedia of Genes and Genomes [KEGG], Database for Annotation, Visualization, and Integrated Discovery [DAVID], and Ingenuity Pathway Analysis [IPA]) as previously described [22]. Bioinformatics software will identify molecular networks, signal transduction pathways, and functional categories associated with differentially expressed genes attributed to SARS-CoV-2 pseudovirus cell entry [15].

3.4 Real-Time Polymerase Chain Reaction (RT-PCR)

RT-PCR experiments using an appropriate real-time PCR thermal cycler will confirm expression levels of select differentially expressed genes based on the next-generation sequencing data [23].

4. Conclusion

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiological agent responsible for Coronavirus Disease 2019 [24]. COVID-19 is a highly contagious respiratory illness that has caused incalculable human suffering, economic impacts, and a distressing number of deaths worldwide. The proposed research project will investigate the molecular mechanisms involved in viral entry into the intracellular host environment.Identifying regulatory host genes and proteins involved in propagating the coronavirus may elucidate new viral therapies.

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References

[1] Jackson C, Farzan M, Chen B, Choe H. Mechanisms of SARS-CoV-2 entry into cells. Nature Reviews Molecular Cell Biology.2022; 23(1): 3-20.
[2] Koelle K, Martin M, Antia R, Lopman B, Dean N. The changing epidemiology of SARS-CoV-2. Science.2022; 375(6585): 1116-1121.
[3] Morris G, Bortolasci C, Puri B, Olive L, Marx W, O'Neil A et al. The pathophysiology of SARS-CoV-2:

A suggested model and therapeutic approach. Life Sciences.2020; 258: 1-15.
[4] Zhang T, Wu Q, Zhang Z. Probable pangolin origin of SARS-CoV-2 associated with the COVID-19 outbreak. Current Biology.2020; 30(7): 1346-1351.
[5] Farkash E, Wilson A, Jentzen J. Ultrastructural evidence for direct renal infection with SARS-CoV-2. Journal of the American Society of Nephrology.2020; 31(8): 1683-1687.
[6] Puelles V, Lütgehetmann M, Lindenmeyer M, Sperhake J, Wong M, Allweiss L et al. Multiorgan and renal tropism of SARS-CoV-2. The New England Journal of Medicine.2020; 383(6): 590-592.
[7] Chen L, Li X, Chen M, Feng Y, Xiong C. The ACE2 expression in human heart indicates new potential mechanism of heart injury among patients infected with SARS-CoV-2. Cardiovascular Research.2020; 116(6): 1097-1100.
[8] Schellhorn P, Klingel K, Burgstahler C. Return to sports after COVID-19 infection: Do we have to worry about myocarditis? European Heart Journal.2020; 0: 1-3.
[9] Nie J, Huang W, Liu Q, Wang Y. HIV-1 pseudoviruses constructed in Chinaregulatory laboratory. Emerging Microbes & Infections.2019; 9(1): 32-41.
[10] Nie J, Li Q, Wu J, Zhao C, Hao H, Liu H et al. Establishment and validation of a pseudovirus neutralization assay for SARS-CoV-2. Emerging Microbes & Infections.2020; 9(1): 680-686.
[11] Ou X, Liu Y, Lei X, Li P, Mi D, Ren L et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nature Communications.2020; 11(1): 1-12.
[12] Wan Y, Shang J, Sun S, Tai W, Chen J et al. Molecular mechanism for antibody- dependent enhancement of coronavirus entry. Journal of Virology.2020; 94(5): 1-15.
[13] Flowers L. The use of PCR arrays to investigate human immune responses. Biomedical Journal of Scientific & Technical Research.2019; 21: 15847-15851.
[14] Flowers L. Bioinformatics analysis to explore ischemic stroke. International Journal of Biology, Pharmacy and Allied Sciences.2019; 8: 1290-1299.
[15] Flowers L. Comparison of the gene expression profiles of human primary prostate epithelial cells and DU145 prostate cancer cells. Biomedical Journal of Scientific & Technical Research.2020; 28: 21690-21696.
[16] Flowers L. COVID-19 and the cytokine storm. Biomedical Journal of Scientific &Technical Research.2020; 29: 22644-22647.
[17] Flowers L, Subramaniam P, Johnson H. A SOCS-1 peptide mimetic inhibits both constitutive and IL-6 induced activation of STAT3 in prostate cancer cells. Oncogene.2005; 24(12): 2114-2120.
[18] Louis K, Siegel A. Cell viability analysis using trypan blue: Manual and automated methods. Methods in Molecular Biology.2011; 740: 7-12.
[19] Lang J, Yang N, Deng J, Liu K, Yang P, Zhang G et al. Inhibition of SARS pseudovirus cell entry by lactoferrin binding to heparan sulfate proteoglycans. PLoS One.2011; 6(8): 1-11.
[20] Xia S, Liu M, Wang C, Xu W, Lan Q, Feng S et al. Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. Cell Research.2020; 30(4): 343-355.
[21] Zheng Y, Shang J, Yang Y, Liu C, Wan Y, Geng Q et al. Lysosomal proteases are a determinant of coronavirus tropism. Journal of Virology.2018; 92(24): 1-14.
[22] Li Y, Su Z, Wei B, Qin M, Liang Z. Bioinformatics analysis identified MMP14 and COL12A1 as immune-related biomarkers associated with pancreatic adenocarcinoma prognosis. Mathematical Biosciences and Engineering.2021; 18(5): 5921-5942.
[23] Shen H, Liang Z, Zheng S, Li X. Pathway and network-based analysis of genome-wide association studies and RT-PCR validation in polycystic ovary syndrome. International Journal of Molecular Medicine.2017; 40(5): 1385-1396.
[24] Ciotti M, Benedetti F, Zella D, Angeletti S, Ciccozzi M, Bernardini S. SARS-CoV-2 infection and the COVID-19 pandemic emergency: The importance of diagnostic methods. Chemotherapy.2021; 66(1-2): 17-23.