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Bioinformatics Analysis of Significant Host Immune Response Genes as Potential Biomarkers in COVID-19 Infection

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1. Abstract

1.1. Introduction: The Corona virus disease 2019 (COVID-19) pandemic continues to spread across the world. Hereafter, require an urgent handling from health workers start from identification through clinical diagnosis of suspect COVID-19, sample collection and laboratory diagnosis of COVID-19 itself. We aimed to analyse the host immune response genes towards spikes protein as an antigen in COVID-19 infection by using bioinformatics tools.

1.2. Materials and Methods: The Target Scan as bioinformatic tool that was used in the analysis of the host response which recognise the spikes protein as an antigen in COVID-19 infection.

1.3. Results: The bioinformatic analysis result by using bioinformatics tool showed that Angiotensin-Converting Enzyme 2 (ACE 2) associated with has Mir 150-5p and PRRs associated with has-Mir-3064-5p and has-Mir- 6504-5p which able to bind with spikes protein during COVID-19 infection.

1.4. Conclusion: The bioinformatics analysis of the host genes which associated with microRNA as potential biomarkers from selected gene ACE 2 and PRRs by using bioinformatics tools designated that associated with definite miRNAs such as Mir-150 – 5p, has-Mir-3064-5p and has-Mir-6504-5p. The circulating microRNAs associated with panels of significant host genes should be further investigated.

2. Introduction

The Corona Virus Diseases 2019 (COVID-19) is an infectious disease caused by the Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus. First time reported in December, 2019 in Wuhan, China, this virus rapidly spread in China and then many other countries globally (Sidiq et al, 2020) [1,2]. As 19 of August 2022, more 594 million have confirmed in 216 countries in the world. Europe with total confirmed cases 245,915,246, Americas with confirmed cases 173, 998, 701, Western Pacific confirmed cases was 79, 803, 949, South-East Asia total confirmed cases 59, 807, 693, Eastern Mediterranean 22, 890, 125 and in Africa 9, 267, 141 cases were confirmed (https://covid19.who.int).

In COVID-19 infection, the viral particles are inhaled, enter the airways and bind to the receptors on host cell surface. Wan et al 2020 [3] reported that a define receptor - binding domain (RBD) of SARCoV spike specifically recognizes its host receptors angiotensin-converting enzyme 2 (ACE 2). The virus enters through engaging the host receptor and mediating virushost membrane fusion (Yuan et al, 2020) [4-6] is the major antigen coronaviruses. Therefore, when the COVID-19 enters to human body, it causes common symptoms like

illness, cough and headache.

The spikes protein and ACE 2 are a dynamic interaction still incomplete understood. Therefore, authors come up with selected genes such as ACE 2 and PRR consider as candidate genes that expressed become miRNAs and can be served as potential biomarkers in COVID19 infection. Micro ribonucleic acids (miRNAs) are a class of small non-coding endogenous RNA molecules that regulate a wide range of biological processes by post-transcriptionally regulating gene expression (Rangel et al 2020). The regulatory activity of miRNAs is exerted at the post-transcriptional level and it is estimated that they act on up to one-third of the protein coding genes (De lacorta singulani et al., 2017) [7] the binding of miRNAs to target mRNA is critical for regulating the mRNA level and protein expression (George and Mittal., 2010)

This study aims to describe the prognosis of host immune response as potential biomarker of pathologies which associated with COVID-19 infection. The forecasting of the selected genes that associated with particular miRNAs using bioinformatics software's such as Target Scan and miRanda. Furthermore, viral entry, infection process and host immune response, biogenesis of miRNAs, circulation of miRNAs as biomarker in COVID-19 infection described.

2.1. Viral Entry, Infection Processes and Host Immune Response

Different types of pathogens require distinct immune effector cell type to be controlled. In this case of viral infection, especially COVID-19, when viral are enters and binds to the receptors on host cell surface, the spike protein of SARS-CoV binds to the Angiotensin-Converting Enzyme 2 (ACE 2), a metalloproteinase found in huge amounts in epithelial and endothelial that undergoes conformational change to permit the fusion of viral and host cell membrane (Li et al, 2003) [8]. The spikes protein consists of 2 sub units namely subunit 1 (S1) and subunit 2 (S2). When the S1 sub unit attaches to the ACE2 receptor on the host cell, a transmembrane protease serine 2 (TMPRSS2) cleaves the Spikes protein to reveal S2 sub unit, and ACE2 (Huan et al, 2020) [9, 10]. The spikes protein undergoes dramatic conformational change, leading to the fusion of the viral membrane with the host cell (https://web.archive). The expression that high from ACE 2 on the surface of lung alveolar epithelium and enterocytes of the small intestines was proposed to contribute of the viral entry SAR-CoV 2 (Hamming et al, 2004) [11,12] to the human cell and infection started.

The history of COVID-19 infection, from incubation to critical disease it takes from 0

- 14 days (https:// www.who.int/emergencies/diseases/novel-coronavirus-2019) during this period, infection processes can be classified as phase one (1) till phase four (IV). In the phase one (1) recognized as direct cytotoxic effect which causes first clinical symptoms, upper respiratory tract infection (URTI) or lower respiratory tract infection (LRTI) which causes cough, fever, thoracic pain. In the phase two (2), infected blood start caused severity of the disease. In the phase three (3), the phase lead to deterioration caused by cytokines and also can lead to acute respiratory distress (phase 4) and death (Melenote et al, 2020) [13].

The infection of COVID-19 provokes host immune response where monocytes, macrophages, dendritic cells are myeloid cells belong to innate immune system. Innate immune response is the first line of defence against from various pathogen include viral infection. In this case, host cells must recognize specific pathogens associated with molecular patterns such as nucleic acids and proteins via specific pattern recognition receptors (PRRs) (Ricci et al, 2021) [14]. This results in the production of type I interferons, which regulate the expression and induction of pro-inflammatory cytokines and chemokines (Connell and Aldhamen, 2020) [15] as well as a host of antiviral restriction factors. Type I IFN family is a multi-gene cytokine family that encodes 13 partially homologous IFNα subtypes in humans (McNab et al, 2015) [16] and 14 in mice, a single IFNβ and several poorly defined single gene products (IFNε, IFNτ, IFNκ, IFNω, IFNδ and IFNζ) (Pestaka et al, 2004) [17,18].

In pneumonia, the lungs filled with fluid and inflamed which cause difficulty in breathing (https://www.hopkinsmedicine.org). A variety of clinical manifestation characterizes SARS-CoV-2 infection ranging from asymptomatic to mild, moderate, and severe disease.

2.2. Biogenesis of miRNAs

The biogenesis of miRNA started from pri-miRNAs then undergoes processing by two cellular nucleases, Drosha and Dicer, to generate mature miRNAs. In animals, miRNAs mostly bind to complementary sequences in the 3' untranslated region (UTR) of their target mRNAs and negatively regulate gene expression either by translational repression or degradation of the mRNA transcript (Rangel et al., 2020); two processing events lead to mature miRNA formation in animals. In the first, the nascent miRNA transcripts (pri-miRNA) are processed into ~70nucleotide precursors (pre-miRNA); in the second event that follows, this precursor is cleaved to generate ~21–25 nucleotide mature miRNAs. MiRNAs transcripts are then processed after their synthesis (https://www.primeopenaccess.com).

2.3. Circulating miRNAs as Biomarker in COVID-19 Infection

In systemic infections, the presence of a pathogenic agent often induces a significant change in the profile of circulating miRNAs that facilitates their use as biomarkers of disease establishment and progression. In certain cases, such as viral infections, these changes in circulating miRNAs are associated with the targeted cell, as demonstrated in the acute and chronic hepatitis C virus infection. Parasitic flatworms, such as Schistosoma japonicum induce a differential expression of circulating miRNAs (De lacorta singulani et al., 2017)

In the lungs or elsewhere, the immune response following viral infection, with many miRNAs known to control cell growth and pro-

liferation, as well as gene expression and susceptibility to infection. The lungs is a known anti-COVID-19 effector organ, air sacks in the lungs fill with fluids, limiting their ability to take in oxygen and causing shortness of breath, cough and other symptoms (https://www.hopkinsmedicine.org). de Gonzalo-Calvo et al, 2021 [19, 20] reported the circulating microRNAs were compared between patients who isolated in the Intensive Care Unit (ICU) and ward, microRNAs 27a-3p, miR 27b-3p, miR 148a-3p, miR 199a-5p and 491-5p were indicated upregulated in ICU patients compared to ward patients. Therefore, bioinformatics analysis result of this study for further confirmation test in the laboratory related selected Mir-150 – 5p, has-Mir-3064-5p and has-Mir-6504-5p either these microRNAs classified as upregulation or down regulation.

4. Materials and Methods

4.1. Overview of this Work

The workflow of this work displayed in (Figure 1). Firstly, the selected genes of COVID-19 were found from the related references. Using the selected genes belong to Homo sapiens. Secondly, the genes symbol entered into the target scan software and submitted to get the list of miRNAs that associated with linked genes. The human 3'UTR sequence of the genes were acquired from NCBI database (www.ncbi.nim.nih.gov).

4.2. Bioinformatics Analysis of miRNAs

The prediction of miRNAs was investigated using 2 different algorithms that are the most widely used in the updated version as follows: miRanda, RegRNA and Target Scan (Rangel, G., et al., 2020). However, in this work, we use only Target Scan to predict the association of the selected genes with specific miRNAs. On the other hand, Miranda and RegRNA unsuccessfully to be used to predict due to unavailable data in the bioinformatics tool because COVID-19 is a newest disease in the world. The workflow of the computational identification of miRNAs involved in COVID-19 infection was shown in the (Figure 2 and 3).

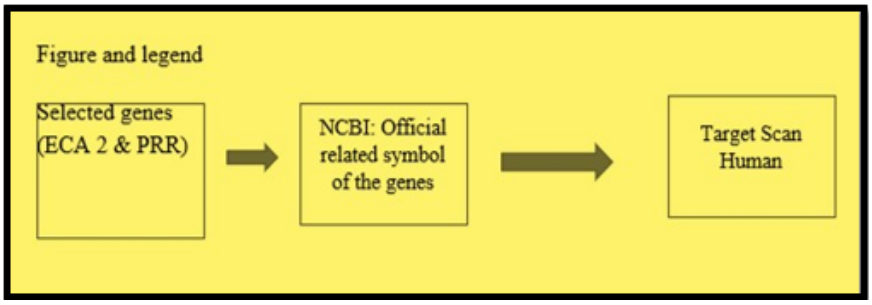

Figure 1: The work flow of processing the selected genes applied in Bioinformatics

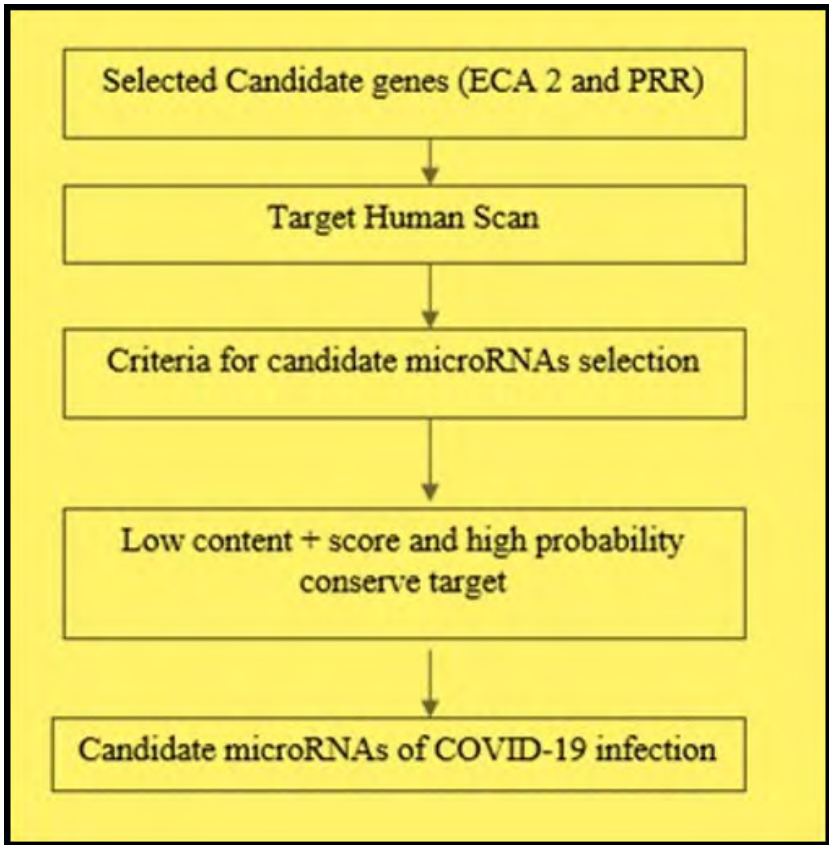

Figure 2: The workflow of bioinformatics analysis of miRNAs

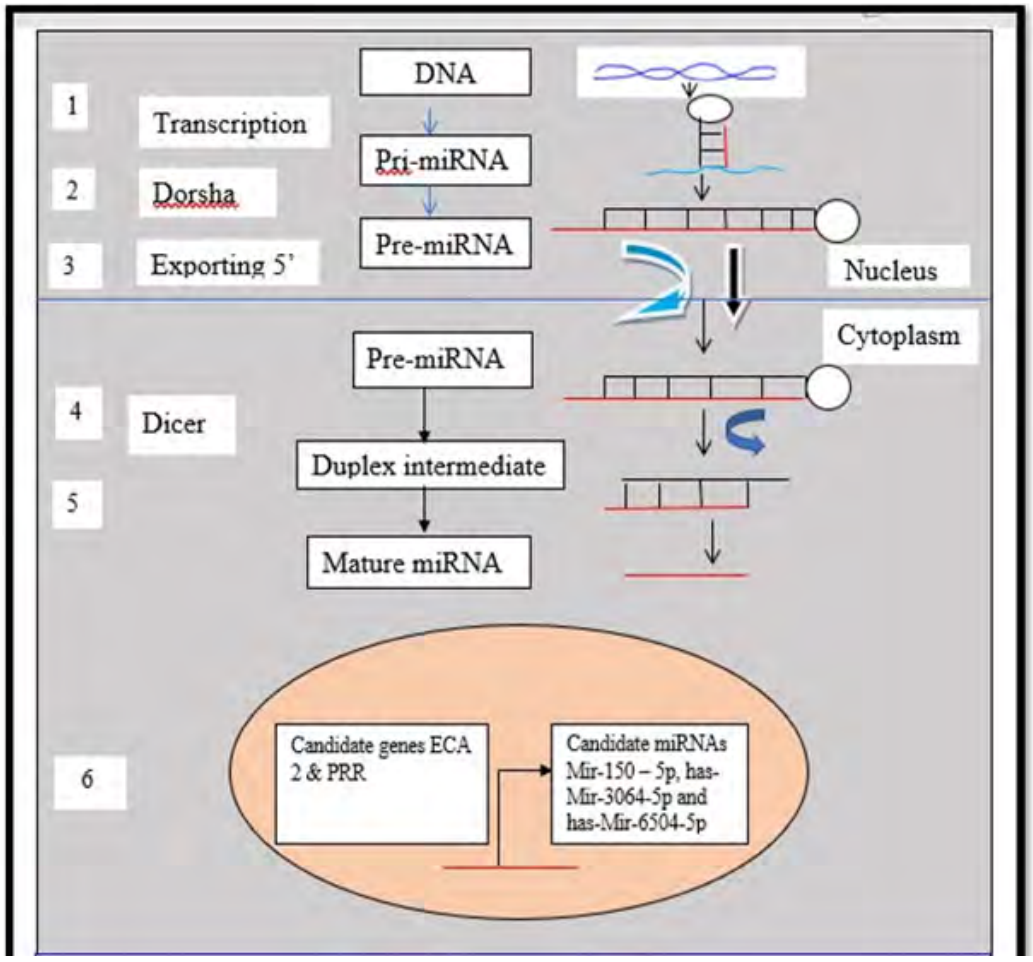

Figure 3: Biogenesis of miRNA. (1) DNA transcription and non-coding genome region. (2) Primary miRNA (Pri-miRNA) transcribed by RNA polymerase II. (3) Dorsha enzyme cut precursor miRNA (Pri-miRNA) and Pri-miRNA transferred out of nucleus into cytoplasam with exporting 5. (4) Dicer cut pre-miRNA convert become duplex intermediate. (5) Duplex transform developed into mature miRNA. (6) Candidate genes translated to be Mir-150-5p, has Mir-3064-5p and has Mir-6504-5p during transcription processses.

4.3. Target Scan

Target Scan, is used to predict miRNA target sites conserved among orthologous 3UTRs of vertebrate (Agarwal et al., 2015) [21]; in mammals, prediction is ranked based on the predicted efficacy of targeting as calculated using the content + scores of the site and their probability of conserved targeting (Seenprachawong et al., 2016) [22]. Thus, Target Scan more defined in the whole genome alignments part.

4.4. MiRNA Selection Criteria

The selection criteria of miRNA based on the association of the genes and miRNAs indicated during the prediction processes. The selection criteria as follows: (1) exhibited greater than or at least 1 prediction tool successfully associated between gene and microRNA; (2) high negative free energy that represented more probable hybridization of miRNA-mRNA duplex; (3) high negative mirSVR score showed a high probability of a target inhibition; and (4) high negative content + score and high probability of conserved target revealed good candidate miRNA for target gene inhibition.

5. Results

We described the prediction of ACE 2 and PRR allied with specific miRNAs using bioinformatics tools. We defined the forecasting of the genes as follows:

5.1. Bioinformatic Analysis of Candidate Genes using Target Scan

The steps of candidate genes (ACE 2 and PRR) bioinformatics analysis explained in materials and methods, to identify the association of the genes correlated with particular miRNAs, we used Target Scan software to analyse the presence of target miRNA as showed in the (Table 1).

Table 1: The bioinformatics analysis result of candidate genes (ECA 2 and PRR) that associated with particular miRNAs using Target Scan.

List of selected candidate genes from Ricci, D et al., 2021 and Huang et al., 2020			
ECA 2		PRR	
miRNA	Content + + score	miRNA	Content + + score
hsa-miR-150-5p	-0. 10	has-Mir-3064-5p	-0.13
		has-Mir-6504-5p	-0.09

6. Discussion and Conclusion

In this study, we identify host immune response genes associated with microRNAs such as Mir-150 – 5p, has-Mir-3064-5p and has-Mir-6504-5p as biomarker in COVID-19 infection. Where the ECA 2 associated with Mir-150 – 5p and PRR associated with has-Mir-3064-5p and has-Mir-6504-5p.

Viral infection stimulates host immune response. Hence, the genes productions were able to use as candidate genes to forecasted using bioinformatics tools. The ACE 2 and PRR were successfully associated with particular miRNAs during the prediction using Target Scan, but unsuccessfully predicted using miRanda.

In summary, this study predicted the host microRNA that related to viral infection and host immune response as potential biomarker from selected gene ACE 2 and PRR using bioinformatics tools with specific miRNAs. Interestingly, miR-150-5p, hsa miR-3064-5p and has-miR-6504-5p could be a further circulating microRNA contributing to the viral infection, pathogenesis and cytokine production during the immune responses associated with particular miRNAs served as biomarkers in viral infection.

6.1. Future Plans

To validated clinical samples which are associated with COVID-19 samples either its RNA samples or fresh samples to realize the existence circulating microRNAs association with the selected genes such as ACE 2 and PRR on candidate Mir-150 – 5p, has-Mir-3064-5p and hasMir-6504-5p should be further investigated.

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7.1. Conflict of Interest

The authors would like to declare that there is no competing interest.

References

1. Sidiq Z, Hanif M, Dwivedi KK, Chopra KK. Benefits and limitations of serological assays in COVID-19 infection. Indian J Tuberc. 2020; 67(4S): S163-S166.
2. Coronavirus disease (COVID-19) pandemic. 2022.
3. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. J Virol. 2020; 94(7): e00127-20.
4. Yuan M, Wu NC, Zhu X, Lee CD, So RTY, Lv H, et al. A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV. Science. 2020; 368(6491): 630-633.
5. Rangel G, Teerawattanapong N, Chamnanchanunt S, Umemura T, Pinyachat A, Wanram S, et al. Candidate microRNAs as Biomarkers in Malaria Infection: A Systematic Review. Curr Mol Med. 2019; 20(1): 36-43.
6. Prime OA Publications.
7. De Lacorte Singulani J, De Fátima Da Silva J, Gullo FP, Costa MC, Fusco-Almeida AM, Enguita FJ, et al. Preliminary evaluation of circulating microRNAs as potential biomarkers in paracoccidioidomycosis. Biomed Rep. 2017; 6(3) :353-357.
8. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003; 426(6965): 450-4.
9. Huang Y, Yang C, Xu XF, Xu W, Liu SW. Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. Acta Pharmacol Sin. 2020; 41(9): 1141-1149.
10. https://web.archive.org/web/20200522053938/https:/www.cebm.net/covid19/coronaviruses-ageneral-introduction/.Accessed August 24, 2022.
11. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, Van Goor H, et al. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol. 2004; 203(2): 631-7.
12. https:// www.who.int/emergencies/diseases/novel-coronavirus-2019. Accessed August 25, 2022
13. Melenotte C, Silvin A, Goubet AG, Lahmar I, Dubuisson A, Zumla A, et al. Immune responses during COVID-19 infection. Oncoimmunology. 2020; 9(1): 1807836.
14. Ricci D, Etna MP, Rizzo F, Sandini S, Severa M, Coccia EM, et al. Innate Immune Response to SARS-CoV-2 Infection: From Cells to Soluble Mediators. Int J Mol Sci. 2021; 22 (13): 2017.
15. O'Connell P, Aldhamen YA. Systemic innate and adaptive immune responses to SARS-CoV-2 as it relates to other coronaviruses. Hum Vaccin Immunother. 2020; 16(12): 2980-2991.
16. McNab F, Mayer-Barber K, Sher A, Wack A, O'Garra A. Type I interferons in infectious disease. Nat Rev Immunol. 2015; 15(2): 87-103.
17. Pestka S, Krause CD, Walter MR. Interferons, interferon-like cytokines, and their receptors. Immunol Rev. 2004; 202: 8-32.
18. https://www.hopkinsmedicine.org/health/conditions-and-diseases/coronavirus/whatcoronavirus-does-to-the-lungs. Accessed August 25, 2022
19. De Gonzalo-Calvo D, Benítez ID, Pinilla L, Carratalá A, Moncusí-Moix A, GortPaniello C, et al. Circulating microRNA profiles predict the severity of COVID-19 in hospitalized patients. Transl Res. 2021; 236: 147-159.
20. www.ncbi.nim.nih.gov. Accessed August 28, 2022.
21. Agarwal V, Bell GW, Nam JW, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. Elife. 2015; 4: e05005.
22. Seenprachawong K, Nuchnoi P, Nantasenamat C, Prachayasittikul V, Supokawej A. Computational identification of miRNAs that modulate the differentiation of mesenchymal stem cells to osteoblasts. PeerJ. 2016; 4: e1976.