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It isn't over 'till it's over: A continuing concern of the SARS-CoV-2 variants, and miRNAs targeting the S protein as a probable absolute cure

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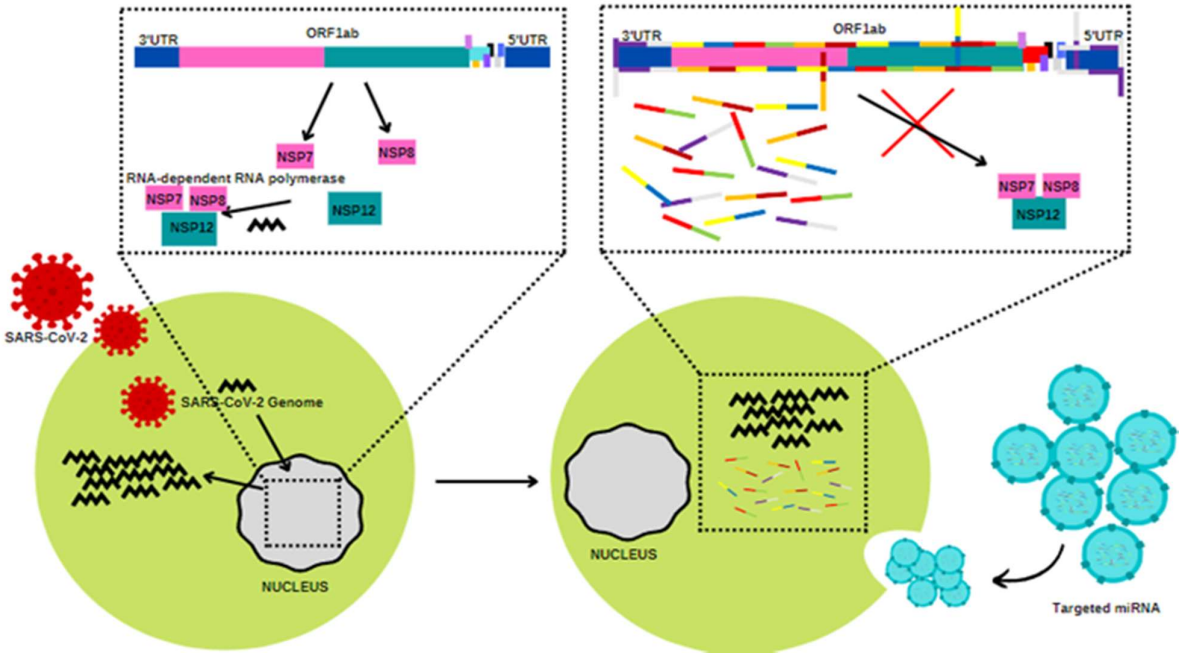
ABSTRACT



The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak which still continues to affect the general population, has mutated day by day and new variants have emerged. More than 40 variants, usually caused by mutations in the spike (S) protein, have been recorded. Observation of S protein mutations in the development of t herapeutic agents will increase success rates. As we identify the three-dimensional (3D) conformation of viruses, it is more and more possible to work on models for understanding molecular interactions. Development of agents for arrays and 3D sequencing of proteins paves the way for potential therapeutic studies against variants. MicroRNAs (miRNAs) seemingly act as a potentially important group of biomolecules in combating uncontrolled cytokine release. Besides antiviral response, miRNAs promise to be

powerful therapeutic agents against infections. Studies have shown that miRNAs are able to inhibit the genome directly by miRNA-based treatments as they are sprecific to the SARS-CoV-2 genome. In order to expose this potential, *in silico* studies before continuing with lab studies are helpful. In our bioinformatics analysis, we proposed to compare the S protein similarities of Delta and Omicron, two of the most common variants, and to detect miRNAs targeting the S protein. The S proteins and coding sequences were compared between the two variants, and differences were determined. Within our analysis, 105 and 109 miRNAs for the Delta and Omicron variants, respectively, were detected.

We believe that our study will be a potential guide for deciding on the miRNAs that may most likely have an effect on the management of the infection caused by both variants.

Key words: Cytokine storm, Delta variant, genome, microRNAs (miRNAs), Omicron variant, SARS-CoV-2, S protein.

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused viral infection and has been a threat to our country and to the world for a long time. Health institutions are making great efforts to prevent, eradicate, and recover from this pandemic. SARS-CoV-2 is a zoonotic, single-stranded positive-sense ribonucleic acid (+ssRNA) virus. The spike (S) protein of the virus binds to the angiotensin-converting enzyme 2 (ACE-2) receptor of the host cell for entry. The ACE-2 receptor acts as a membrane-bound enzyme whereas the S protein is structurally adapted by the transmembrane protease serine-2 (TMPRSS-2). In addition to the SARS-CoV-2 receptor-dependent entry, endocytosis is another path by which the virus can enter the cell [1,2]. Following the entry and the replication of the virus, many symptoms may occur in the patient's body. Fever, dry cough, acute respiratory distress syndrome (ARDS), pulmonary fibrosis, hypertension, thrombosis, and coagulation disorders are among such. One of the causes of the symptoms is the uncontrolled activation of the host's immune system due to the viral infection. This uncontrolled immune response and the increase in the number of neutrophils and macrophages, "cytokine release syndrome (CRS)", which is activated with proinflammatory cytokines in the circulation, causes damage to the organism and may can even lead to multiple organ failure. When the cytokines are emerged at such a high level due to various reasons, the patient enters the CRS [3].

As we have mentioned recently [5], there are more than 40 variants of the virus which have been identified. The mutations that cause the variants occur mostly in the S protein since mutations in the S protein change the ACE-2 affinity of the resulting variant, its infectiousness, and its response to monoclonal antibodies [4]. The number of variants continues to increase as a result of high rate of mutations [5]. Among all, the Delta and Omicron variants attract our attention in terms of their transmission rate and the size of the population which they affect [6].

The Delta variant (B.1.617.2), was born in India in November 2020 [7]. In August 2021, it replaced Alpha as the most transmissible variant [8]. The Delta variant has 60% more spreading

capacity than the Alpha variant. The Delta variant contains 23 different mutations compared to the first identified Alpha variant. Twelve of these mutations occur on the S protein [9]. The Omicron variant (B.1.1.529), on the other hand, first appeared in South Africa in November 2021. It has been observed as the most contagious variant among the variants identified so far [10]. As a result of linear regression analysis, its contagiousness was found to be 2-fold in comparison with the Delta variant [11]. This variant contains 32 amino acid mutations in the S protein [12,13].

There is not yet a clinically approved, absolute treatment for the SARS-CoV-2 infection. Therefore, further studies on the use of new antiviral strategies are still necessary and priority.

microRNAs (miRNAs), are RNA fragments of approximately 18-25 nucleotides length. The miRNAs, designated as miR-5p or miR-3p are named after the region (5' or 3') where the precursor yields the complementary mature single-stranded sequence. miRNA biogenesis begins with its transcription in the nucleus by RNA Polymerase II, and pre-miRNA is extracted from the nucleus to the cytoplasm by exportin-5. By binding with the Ago-Dicer-TRBP complex they act in 3 different mechanisms, i.e. inhibition of translation, messenger RNA (mRNA) storage, and translocation to dendrites/axons [14]. miRNAs have drawn attention after elucidation of their involvement in complex molecular processes such as growth, cycle regulation, proliferation, etc. [15,16]. Therefore, miRNAs have currently been studied as biomarkers and therapeutic targets for systemic illnesses [17], viral [18] and bacterial [19] infections, and cancer [20].

In this study, we aimed to mine the existing literature covering therapeutic potential of miRNAs which have been identified to treat similar viruses in the past and can be used today against SARS-CoV-2 and its variants. Afterwards, bioinformatics analyses were performed to compare S protein similarities and three-dimensional (3D) protein structure of the two most prevalent variants of SARS-CoV-2, and to frame the miRNAs targeting the S protein structures. Our final aim was to identify different and common miRNAs for both variants that have potential for diagnosis and treatment. Thus, our study will be a potential guide for deciding on the miRNAs that may have an effect on the detection and treatment of both variants.

Materials and metods

miRNA detection

miRNAs targeting individual S protein amino acid sequences for the Delta and Omicron variants were detected using miRNA Target Prediction Database (miRDB).In contrast to the case of most other miRNA databases, in the miRDB, mature miRNAs are the primary emphasis.

Candidate transcripts with scores below 50 are displayed in miRDB as anticipated miRNA targets. MİRTargets prediction scores range from 0 to 10. miRNAs with a Target Score of 50 and above were considered, and the common miRNAs were identified.

Data mining

We obtained the potential miRNAs specific to the SARS-CoV-2 genome which was obtained from the National Center for Biotechnology Information (NCBI) database with the keywords "Delta variants", "Omicron variants", "miRNA", "Sars-CoV-2", "spike protein" and "therapeutic".

Data retrieval

S protein sequences were obtained from NCBI (GenBank: UFO69279.1) for Omicron and NCBI (GenBank: QWK65230.1) for the Delta variant. The whole genome sequence and coding

sequences (CDSs) of the S protein of the Omicron (GenBank: OL672836.1) and Delta variants (GenBank: MZ359841.1) were obtained from NCBI. In further analysis to determine the similarity between two 3D protein structures, the S proteins of both variants obtained from Protein Data Bank were used for the Delta and Omicron variants (7W92 and 7WVN, respectively).

Alignment of the genomes

The FASTA files containing S protein sequences and coding CDS were uploaded to the website at EMBL's European Bioinformatics Institute (EMBL-EBI), and the results were analyzed.

Three-dimensional (3D) protein structure

The Phyre2 Protein Fold Recognition Server program was used for protein structure prediction. In order to create 3D models, estimate binding locations, and analyze amino-acid variations in a protein sequence, Phyre2 employs cutting-edge distant homology detection techniques with 4 consecutive steps. First, it gathers homologous protein sequences with HHblits. Then, the multiple-sequence alignment helps to predict the secondary structure with PSPIRED. Afterwards, the secondary structure is scanned against known protein HMMs, Last, the final Phyre2 model is designed by adding the amino acid side chains. Pairwise structure alignment tool from RCSB PDB was used to determine the similarity between two 3D protein structures. The comparison tool enables pairwise comparison of 3D structures and protein sequences. The Smith-Waterman, Needleman-Wunsch, and blast2seq algorithms are offered for sequence comparison. The latest versions of CE and FATCAT, as well as links to some of the external protein structure alignment services, which include Mammoth, TM-align, and Topmatch, provide support for structure comparisons.

Results and Discussion

Spike (S) protein sequence comparison

S proteins, located on the virus and provide cell-virus interactions by taking an active role in the cell entry process, have naturally been targeted in order to interrupt these interactions [21-23]. S proteins penetrate the cell membrane via the ACE-2 receptors and cause many clinical symptoms and the disease progresses [22,24]. Every mutation on the S protein affects this process. In addition, coronavirus variants manifest themselves with varying disease courses and are then detected by molecular and bioinformatics analyses [25]. Therefore, mutations in the S protein change the course of the disease by affecting the cell-virus interaction, and ultimately emerge as a new coronavirus variant [26]. That is, coronavirus variants represent the S protein mutations [24,27]. Variants differ in terms of features such as contagiousness, efficacy, and severity of clinical symptoms, resulting in a change in the course of the disease in the population [25]

In this study, the Delta and Omicron variants were investigated. The Omicron variant is currently the most common variant whereas the Delta variant ranks the second place [28,29]. There are many mutations in variant and the epidemiological effects of the variant differ according to these mutations. Mutations in variants are examined and inferences are made as to what kind of an effect it may have when the same mutation occurs in another variant [25]. As the S proteins of the variants continue to be determined, the success rate of vaccines and therapeutic agents developed against variants will increase. Thus, higher accuracy can be obtained in the studies to end or treat diseases [23,26].

In addition to the S protein sequences, CDS of the S proteins of the variants were also examined.

Table 1. Comparison of S protein sequences of DNA and protein sequences between the Delta and Omicron variants. (S: Spike, CDS: Coding DNA sequence)

	S protein sequences	**CDS**
Length	1276	3831
Identity	1234/1276 (96.7%)	3767/3831 (98.3%)
Similarity	1241/1276 (97.3%)	3767/3831 (98.3%)
Gaps	11/1276 (0.9%)	33/3831 (0.9%)
Score	6448.0	18647.0

With the differences in the sequencing of the genomes of the S proteins it is important to develop model organisms and to speed up studies in case of re-emergence of human pathogens [26].

When the S protein sequences and CDSs of both variants were aligned, the following values were found as shown in Table 1.

The relationship between protein sequence similarity and structural similarity was examined. According to the results of the study, when the structure comparison is examined, they can be defined as homologous pairs since there is more than 70% second structure identity [30,31]. According to the results, there was a high similarity and identity between the S protein sequences of the Delta and Omicron variants with, a low rate of gaps. Consequently, the two sequences showed a high rate of alignment. The lack of gaps in the bioinformatics analysis of S protein sequences indicates that an amino acid is located at the same position across sequences. Thus, the similarity with a high score was significant. This shows that the S proteins of these variants, which dominate at different time spans, have highly similar sequences.

This result can be interpreted in different ways. The fact that the S protein is effective in the way of entry into the organism and that it is similar in two different variants suggests that it may be the best S protein sequence for the virus. In other words, if the S protein continues with mutation at this level and the binding part continues to be similar to the sequence of these variants, perhaps it can dominate the newly emerged variant as much as the Delta and Omicron variants. In addition, the S protein sequence similarity between the two variants will make it possible to conduct studies in the same direction in terms of therapeutic agents to be developed against the S protein. Thus, the work done for one variant can be done for another, with minor modifications. In addition, a true understanding of the sequence of the S protein facilitates profiling for this protein and studying sequence conservation patterns indicate of secondary structure types. Hence, it is important to show such a similarities.

The amino acid sequence alterations of the S proteins of both variants are given in the Table 2, and the coding sequence of the S protein between both variants are given in Table 3.

Sequences of S protein and CDS were compared between the two variants and the differences were determined. Differences in sequences allow us to study the clinical progression of current and future variants. The development of agents via 3D sequencing of proteins lights the way to potential therapeutic studies against variants.

Comparison of 3D S protein structures

In order to better understand the secondary structures of proteins, 3D protein models are constructed. The main objective of studying the 3D structure of proteins is to understand their

Table 2. S protein sequence alterations of the Delta and Omicron variants.

Amino Acid sequence	Amino acid exchange	Type of mutation
19	R→T	semi-conservative
67	A→V	semi-conservative
70	H→-	deletion
71	V→-	semi-conservative
95	T→I	semi-conservative
143	V→-	deletion
144	Y→-	deletion
145	Y→-	deletion
156	-→E	insertion
157	-→F	insertion
158	G-R	semi-conservative
209	N→-	deletion
210	L-I	semi-conservative
213	-→E	insertion
214	-→P	insertion
215	-→E	insertion
337	G→D	semi-conservative
369	S→L	semi-conservative
371	S→P	semi-conservative
373	S→F	semi-conservative
415	K→N	semi-conservative
438	N→K	semi-conservative
444	G→S	semi-conservative
450	R→L	semi-conservative
475	S→N	conservative
482	E→A	semi-conservative
491	Q→R	conservative
494	G→S	semi-conservative
496	Q→R	conservative
499	N→Y	semi-conservative
503	Y→H	conservative
545	T→K	semi-conservative
653	H→Y	conservative
677	N→K	semi-conservative
679	R→H	semi-conservative
762	N→K	semi-conservative
794	D→Y	semi-conservative
854	N→K	semi-conservative
948	N→D	conservative
952	Q→H	semi-conservative
967	N→K	semi-conservative
979	L→F	semi-conservative

R: Arginine, T: Threonine, A: Alanine, V: Valine, H: Histidine, I: Isoleucine, T: Tyrosine, Y: Tyrosine, E: Glutamic acid, F: Phenylalanine, G: Glycine, N: Asparagine, L: Leucine, P: Proline, D: Aspartic acid, S: Serine, K: Lysine, Q: Glutamine.

Table 3. CDS sequence differences of S protein of the Delta and Omicron variants.

DNA sequence	Base exchange	Type of mutation
56	G→C	semi-conservative
200	C→T	semi-conservative
203	T→-	deletion
204	A→-	deletion
205	C→-	deletion
206	A→-	deletion
207	T→-	deletion
208	G→-	deletion
284	C→T	semi-conservative
425	A→-	deletion
426	T→-	deletion
427	G→-	deletion
428	T→-	deletion
429	T→-	deletion
430	T→-	deletion
431	A→-	deletion
432	T→-	deletion
433	T→-	deletion
467	-→A	insertion
468	-→G	insertion
469	-→T	insertion
470	-→T	insertion
471	-→C	insertion
471	-→A	insertion
626	A→-	deletion
627	T→-	deletion
628	T→-	deletion
637	-→G	insertion
638	-→A	insertion
639	-→G	insertion
640	-→C	insertion
641	-→C	insertion
642	-→A	insertion
643	-→G	insertion
644	-→A	insertion
645	-→A	semi-conservative
1010	G→A	semi-conservative
1105	T→C	semi-conservative
1106	C→T	semi-conservative
1111	T→C	semi-conservative
1118	C→T	semi-conservative
1245	G→T	semi-conservative
12314	T→G	semi-conservative
12330	G→A	semi-conservative
1349	G→T	semi-conservative
1424	G→A	semi-conservative
1445	A→C	semi-conservative
1472	A→G	semi-conservative
1480	G→A	semi-conservative
1487	A→G	semi-conservative
1495	A→T	semi-conservative
1507	T→C	semi-conservative
1634	C→A	semi-conservative
1957	C→T	semi-conservative
2031	T→G	semi-conservative
2036	G→A	semi-conservative
2286	C→A	semi-conservative
2380	G→T	semi-conservative
2562	C→A	semi-conservative
2842	A→G	semi-conservative
2856	A→T	semi-conservative
2901	T→A	semi-conservative
2935	C→T	semi-conservative
3432	C→T	semi-conservative

G: guanine, C: cytosine, T: thymine, A: adenine.

biological activity. Since biological activity is dependent on atomic levels, topological expressions alone are inadequate. Seemingly, small editions can greatly affect the activity of the structure [32].

In pairwise structure alignment analysis, residue coverage for both variants is high enough for a correct analysis (7W92: %93 and 7WVN: %95). To establish homological similarity of S protein 3D structures, we simulated a

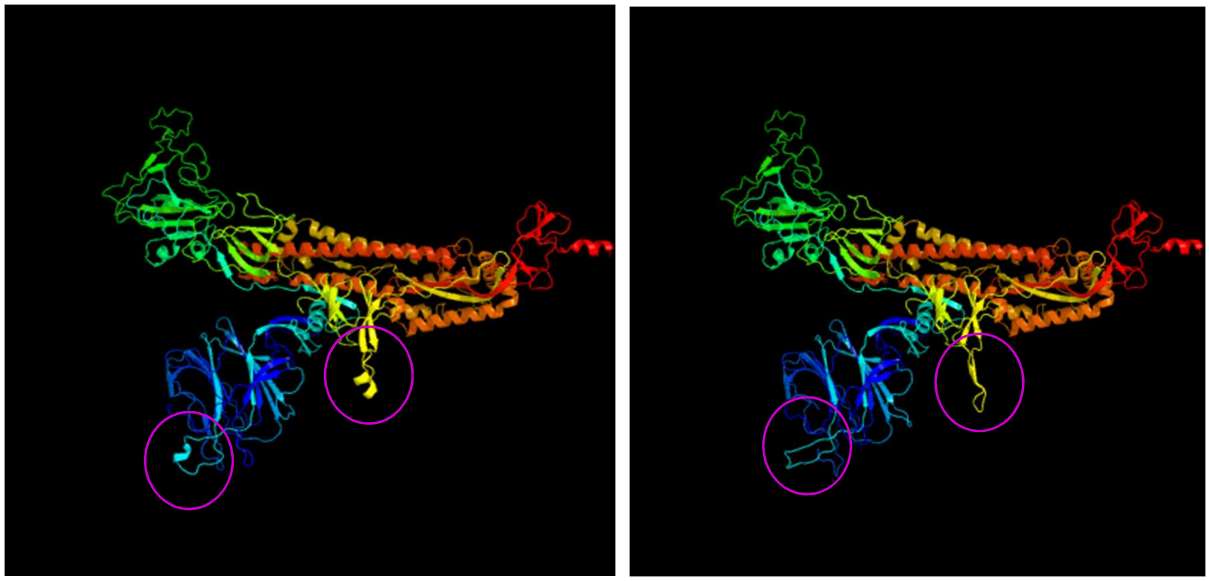

Figure 1. A) The 3D protein structure of the Delta variant **B)** The 3D protein structure of the Omicron variant. The most significant difference in the spike (S) protein between the two variants is marked with a purple circle in shapes of A and B.

In our study, following the sequence comparison of the S proteins, 3D structures of the Delta and Omicron variants were studied (Figure 1). Based on the differences in the 3D structures of the S proteins, we found that there may be differences in the interaction of the variant with the cell. That is, the structure of the S protein determines cell affinity. Studies can also be conducted to determine the connection between an amino acid, its position and epidemiological effects it may cause on 3D structures. In addition, it is useful to know such structural differences in the therapeutic agents developed against the variant. With 3D models, it is possible to work on drug-cell or drug-drug interactions by obtaining information related to the conformation of viruses [21,24,33].

superimposed model of both 7W92 and 7WVN on UCSF Chimera (Figure 2).

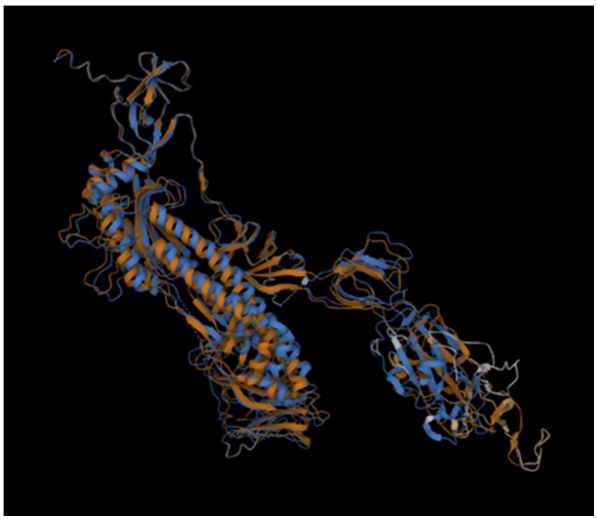

Figure 2. Superimposed view of the 7W92 and 7WVN proteins.

The RMSD score is a measurement of distance between atomic coordinates of two different molecules [34]. Values higher than 3 mean that there is low similarity between two molecules [35]. For the selected protein structures, the RMSD score was found to be 5.5, means these two proteins do not have a statistically significant similarity.

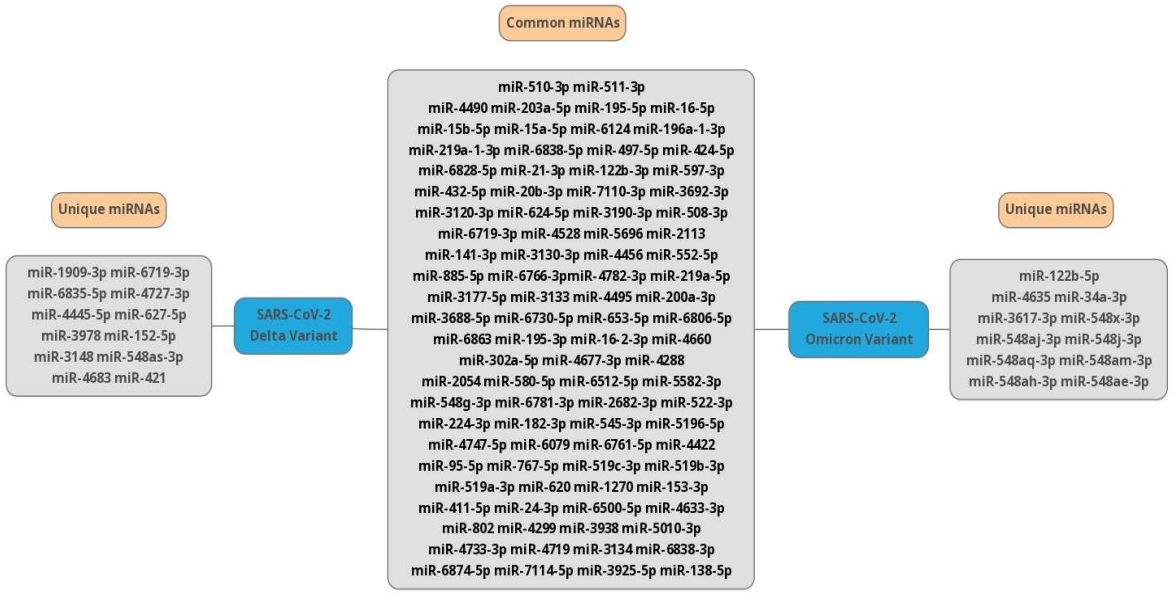

Figure 3. miRNAs targeting the S proteins of the Delta and Omicron variants, and those common to the S proteins of both.

Identification of common miRNAs for both variants

In the past couple of years, both virus-encoded and host miRNAs have been suggested to be significant biomarkers in the said pandemic [36]. In a study, 444 of 2654 hsa-miRs were identified to be associated with different binding sites in the CoV-2 reference genome [37].

It is predicted that miRNAs repress a large portion of all protein-coding genes and participate in the regulation of almost every biological process in the cell. This makes them important as antiviral targets [38]. Understanding biological differences responsible for the severity of SARS-CoV-2 is the key to developing accurate antiviral treatments [37]. miRNAs help to evade or suppress immune response to a number of viruses [39]. Scientific advancements have revealed important functions and pathways involved in host immune responses. Wingless and INT-(Wnt) [40] and mitogen-activated protein kinase (MAPK) signaling [41], T cell-mediated immunity [42], autophagy [43], fibroblast growth factor (FGF) receptor binding [44], transforming growth factor-beta (TGF-β) [45], vascular endothelial growth factor (VEGF) signaling [46], ErbB signaling [47], mammalian target of rapamycin (mTOR) signaling [48] and tumor necrosis factor alpha (TNF-α) signaling [41] are specifically targeted by SARS-CoV-2 [49].

Cellular miRNAs can enhance the host's immune response and aid viral immune avoidance mechanisms [50]. *In silico* study of SARS-CoV-2 encoding miRNA targets the Ca^{+2} signaling pathway, which acts as a key activator influencing other signaling pathways that subsequently branch it [51]. Both cellular

miRNAs and viral encoded miRNAs induced during the SARS-CoV and SARS-CoV-2 infections were envisaged to target cytokine signaling pathways involved in immune responses guiding to enhanced viral pathogenesis [49].

As a result of the analysis, we found that there are 109 predicted miRNAs targeting the 3813 nt-long mRNA sequence sent with a Target Score of 50 or higher for the Omicron variant. Likewise, we found 105 predicted miRNAs targeting the 3816 nt-long mRNA sequence for the Delta variant.

The estimated number of unique miRNAs which target the S protein of the Delta variant shown in Figure 3 is 12, and which 12 are miR-1909-3p, miR-6719-3p, miR-6835-5p, miR-4727-3p, miR-4445-5p, miR-627-5p, miR-3978, miR-152-5p, miR-3148, miR-548as-3p, miR-4683, miR-421. The estimated number of unique miRNAs for the Omicron variant to target the S protein is 11 and these are listed as miR-122b-5p, miR-4635, miR-34a-3p, miR-3617-3p, miR-548x-3p, miR-548aj-3p, miR-548j-3p, miR-548aq-3p, miR-548am-3p, miR-548ah-3p, and miR-548ae-3p.

SARS infections involved in ACE-2 receptor binding results in inhibitory effects on the receptor and decreased receptor expression in infected cells [52]. Here, miR-421 has important modulatory effects on ACE-2 [53]. miR-627-5p was found to be the most down-regulated miRNA in peripheral blood samples of patients compared to the control group [54]. In COVID-19, a decrease in host miR-34a-3p can increase the expression of X-box binding protein 1 (XBP1) by URP, increased endoplasmic reticulum (ER) folding capacity, inhibit lung fibrosis and protect against over activation of the immune system, promotoing survival [55]. The rs3853839 single nucleotide polymorphysm (SNP) of toll-like receptor 7 (TLR 7) can affect miRNA binding capacity and therefore mRNA expression of TLR7. The rs3853839 SNP, on the other hand, might pose a risk on the infection [56]. Conserved regions are predicted by miRanda and mirTarP as a conserved region of the virus. The conserved structured region is assessed as a promising means for the investigation of absolute treatments [57].

Li et al. [58] took peripheral blood from 10 COVID-19 patients and 4 healthy donors. In the blood samples, the exposure to various miRNAs was detected with high efficiency. Compared to healthy donors, 35 miRNAs were upregulated in patient blood whereas 38 were downregulated. For example, the Delta variant-specific miR-421 and the remaining 4 miRNAs are estimated to target 3' of the ACE-2 UTR regions [59] miR-122b-5p, specific to the Omicron variant, was down regulated in severe COVID-19 cases [60].

The estimated number of common miRNAs targeted at the S proteins of the Delta and Omicron variants is 93 (Figure 3). The common miR-195-5p of the Delta and Omicron variants was established by logistical regression analysis that could benefit from early COVID-19 diagnosis [61]. miR-16-5p, which is found to regulate the ACE-2 network with an *in silico* investigation, was investigated [62]. The increase in miR-15b-5p release resulted in a decrease in viral infection and reproduction of SARS-CoV-2 by targeting RNA template component [63] miR-196a-1-3p has been widely linked to members of the Coronavirus family (SARS-CoV, MERS-CoV and SARS-CoV-2) [64]. It has been found that miR-6838-5p is significantly correlated in male individuals carrying COVID-19 [65]. miR-497-5p is estimated to be effective against virus through nucleotide erasure in viral ss-RNA encoding zones [66]. miR-510-3p shows strong binding potential to neuropilin-1 [67]. Neuropilin-1, a host cell receptor, increases its infectiousness and contributes to its tropism [68].

Potentially Therapeutic miRNAs for SARS-CoV-2

miRNAs are known to have antiviral effects, which potentially make them antiviral therapeutic agents. Moreover, miRNAs which have antiviral and anti-inflammatory effects against *Coronaviridae spp.* have been reported. miR-9 accelerates the breakdown of human coronavirus OC43 (HCoV-OC43), a type of coronavirus, by regulating the type I interferon (IFN-1) release [69]. On the other hand, it has been reported that patients who have low circulating miR-146a-5p levels do not respond to tocilizumab, a drug used against the SARS-CoV-2 infection [70]. miR-122 increases the genome stability by binding in the 5' untranslated region (UTR) of the Hepatitis C virus (HCV). Therefore, miR-122 blocks cellular pyrophosphates in the genome of SARS-CoV-2 [71].

Circulating miRNAs may be effective in suppressing the SARS-CoV-2 infection-derived acute respiratory distress syndrome (ARDS) and the CRS. Cytokines have important roles in the emergence of the clinical signs and inflammation development [72]. Significant increase in the levels of cytokines, such as interleukin (IL)-2, IL-6, IL-7, IL-8, IL-10, granulocyte-colony stimulating factor (G-CSF), interferon gamma-induced protein 10 (IP10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1A (MIP1A) and tumor necrosis factor-α (TNF-α), is beheld in patients infected with SARS-CoV-2 [3,73-75]. When patients are confronted with SARS-CoV-2, the CRS occurs due to the body's response and becomes a great source of harm for patients [76]. This uncontrollable increase in the cytokine levels of patients may lead to ARDS and, eventually, to death. miRNAs which are naturally found in mesenchymal stem cell (MSC)-derived exosomes are reported to potentially play an important role in preventing this uncontrolled cytokine release [77]. MSC-derived exosomes also have the potential to repair damage in tissues by stimulating other stem cells [78,79]. For example, patients who are suffering from ARDS have decreased miR-181a-5p (a MSC-derived exosomal miRNA) levels. It has been proclaimed that when miR-181a-5p mimic treatment decreases apoptosis and inflammatory factors via inhibiting metastasis-associated lung adenocarcinoma transcript-1 (MALAT-1) *in vitro*. Another MSC-derived exosomal miRNA, miR-23a-3p, has been reported to suppress acute lung injury and lung fibrosis via inhibiting nuclear factor kappa B (NF-κB) [80]. miR-23a-3p has also been reported to have a potential of binding to IL-8 3'UTR [75]. miRNAs which are not present in MSC-derived exosomes may also play critical roles in prognosis management [81]. For instance, miR-155 acts as a regulator of pro-inflammatory cytokines [82], and it also promotes innate immune responses of miR-155 and miR-128 against respiratory system viruses [83]. miR-146a regulates and modulates the TLR signaling pathway [84]. miR-146a is also associated with angiotensin (1-7)-mediated decrease of IL-6 and with the prognosis of ARDS [85,86]. miR-152 and miR-148 inhibit the production of cytokines such as TNF-α, IL-6 and IL-12 [87]. miR-574-5p suppresses ARDS development via targeting high mobility group box 1 (HMGB1) signaling [88].

The virus is able to stimulate the host's cell synthesis of miRNAs from the viral genome. One of the miRNAs synthesized from the viral genome, miR-8066, is thought to be associated with the cytokine storm, a deadly complication of the infection, due to the cytokine-cytokine receptor pathway activity [89]. miRNAs are associated with the pathogenesis of many diseases–including the SARS-CoV-2 infection–

and host cell responses [90]. When miRNAs showing inhibitory properties by binding to the viral genome are identified, they have the potential to be effective agents in inhibiting the life cycle of the virus. miRNAs which show binding affinity towards the lead proteins of SARS-CoV-2 and which essentially use pathways to infect cells are presented in Table 4.

miRNAs have the potential to bind to the S protein, open reading frame (ORF) 1ab, ORF1a, ORF1b, ORF6, ORF7a and 3' and viral 5'UTR. hsa-miR-203b-3p and hsa-let-7c-5p, previously known for their ability to suppress Influenza A, have the potential to bind to the ORF1ab region [91] and to the ORF6 region of SARS-CoV [92], another type of coronavirus, together with the ORF3b and the nucleocapsid (N) protein [97] which inhibits IFN-1 signaling to prevent and delay host response. hsa-miR-190a-5p binds to ORF6, eliminating the inhibition of type IFN-1 signaling, thereby enabling the development of host response against the virus in the early phase [91]. hsa-miR-4661-3p [90,93], miR-4761-5p and miR-338-3p [69] have the potential to bind to the S protein. hsa-miR-4288, hsa-miR-195-5p, hsa-miR-16-5p, hsa-miR-15b-5p, hsa-miR-15a-5p, miR-6838-5p, hsa-miR-497-5p, hsa-miR-424-5p, hsa-miR-3133 and hsa-miR-21-3p have manifested high integration capacity to the SARS-CoV-2 genome and to the target genes of miRNAs via *in silico* analysis [94]. Five MSC-derived exosomal miRNAs (miR-92a-3p, miR-181a-5p, miR-103a-3p, miR-26a-5p, miR-23a-3p) which are able to bind to the 3 and 5'UTR regions have also been reported [77]. miR-5096, miR-197-5p, miR-3935-5p and miR-18b-5p have the potential to bind to the ORF1a region. miR-1273d and miR-3154 have the potential to bind to the ORF1b region. miR-4436a has the potential to bind to the ORF7a region [69]. Chen and Zhong [96] reported that miR-1307-3p and miR-3613-5p are capable of viral integration, based on the results obtained from miRNA databases [96].

In particular, the rapid mutation of RNA viruses is a critical disadvantage of gene-based therapeutic approaches. If an antiviral miRNA-targeted region in standard viral genome mutates, the miRNA may lose its antiviral properties. However, when the SARS-CoV-2 samples collected from China and Japan were analyzed, the 3'UTR region of the coronavirus genome was found to be very rarely mutated [77]. Therefore, targeting this region would be one of the most appropriate options when trying to target miRNAs. An miRNA that allows the viral genome to degrade by binding to the 3'UTR region will directly stop the life cycle of the virus in the cell.

SARS-CoV-2 replicates inside the cell with RNA-dependent RNA polymerase (RdRp) [98]. RdRp is formed by the assembly of non-structural protein 7 (NSP7) and NSP8, which are both mounted on the skeletal protein, NSP12 [99]. NSP7, NSP8, and NSP12 are formed as a result of transcription and cleavage of polyprotein 1ab and polyprotein 1a by proteases. Genes of polyprotein 1ab and polyprotein 1a are located on ORF1ab. The nucleotide localization on the viral genome of NSP7 (3860-3942), NSP8 (3943-4140), and NSP12 (4393-5324) is clearly specified [100]. miRNAs have the potential to directly inhibit the replication of the virus by targeting the regions of the viral genome that encode proteins which form RdRp. However, mutations must not be overlooked during the course of designing the miRNA selection according to the specific target. Since ORF1ab is the largest region of the viral genome, it is the region with high risk for mutation. Mutations that occur in the SARS-CoV-2 samples examined in the United States concern ORF1ab and even regions where NSP12 is located [101]. The mutated region must be evaluated

Table 4. microRNAs (miRNAs or miRs) with the potential to show antiviral effects by binding to the SARS-CoV-2 genome and miRNA targets according to *in silico* studies.

microRNAs (miRNAs or miRs)	**Where to bind?**	**Potential effect?**	**References**
hsa-miR-203b-3b	ORF1ab	Suppression of Influenza A	[91]
	ORF6	Suppression of SARS-CoV	[92]
hsa-let-7c-5p	ORF1ab	Suppression of Influenza A	[91]
	ORF6	Suppression of SARS-CoV	[92]
hsa-miR-190a-5p	ORF6	Eliminate the inhibition of type 1 interferon (IFN) signal	[91]
hsa-miR-4661-3p	Spike (S) protein	Binding to the S protein	[93]
miR-4761-5p			[69]
miR-338-3p			
hsa-miR-4288	SARS-CoV-2 genome and miRNAs	SARS-CoV-2 genome and miRNA target gene integration	[94]
hsa-miR-195-5p			
hsa-miR-16-5p			
hsa-miR-15b-5p			
hsa-miR-15a-5p			
miR-6838-5p			
hsa-miR-497-5p			
hsa-miR-424-5p			
hsa-miR-3133			
hsa-miR-21-3p			
miR-92a-3p	3'UTR and 5'UTR regions	Down-regulation of SARS-CoV-2 RNA	[77]
miR-181a-5p			
miR-103a-3p			
miR-26a-5p			
miR-23a-3p			
miR-5096	ORF1a	Inhibition of viral replication	[69,95]
miR-197-5p			
miR-3935-5p			
miR-18b-5p			
miR-1273d	ORF1b		
miR-3154			
miR-4436a	ORF7a		
miR-1307-3p	SARS-CoV-2 genome	Prevention of viral replication	[96]

S Protein: Spike protein, ORF1ab: Open Reading Frame-1ab, ORF1a: Open Reading Frame-1a, ORF1b: Open Reading Frame-1b, ORF6: Open Reading Frame-6, ORF7a: Open Reading Frame-7a, hsa-miR: Homo sapiens (human) microRNA.

precisely, and the candidate miRNA should be determined accordingly. An antiviral miRNA targeted precisely and to the correct location will most likely directly stop viral replication.

Conclusions

In this study, miRNAs that are suspected to be involved in arresting viral replication for the two most common variants of SARS-C0V-2, Delta and Omicron, were investigated. For the detection of potential miRNAs, S protein sequences of variants and CDSs were investigated first by using databases. In the analysis, the S protein sequences and 3D protein structures for the two variants were compared and different and common miRNAs were determined for both variants. The miRDB was used for the bioinformatics analysis, and the target score was set as 50 and above. Results revealed that the sequences of the Delta and Omicron variants had a high alignment rate. That is, the S protein sequences of both variants were found to have highly similar sequences. This evident similarity is important in terms of guiding future studies. Furthermore, the comparison of the 3D structures demonstrated that the S proteins of the two variants did not have statistically significant similarity. By the miRNA analysis, 109 and 105 miRNAs were predicted for the Omicron and the Delta variants, respectively, targeting the mRNA sequence. The estimated number of common miRNAs targeting the protein S of both variants is 93. In our study, miRNAs that may play a role in diagnosis and treatment for both variants were investigated. We believe that our study will shed light on future miRNA studies.

Future Perspectives

SARS-CoV-2 is a viral pandemic that the world has been combating for several years. There is not yet a clinically approved, absolute treatment for the SARS-CoV-2 infection. Therefore, further studies and the use of new antiviral strategies are needed.

miRNA content may be one of the most appropriate novel approaches in hope of providing the treatment of the current pandemic. miRNAs promise to be potential therapeutics against infection, as they that can enable the body to give antiviral and anti-inflammatory responses.

They have the ability to bind to the mRNAs which they complement. When the SARS-CoV-2 infection is concerned, they have the potential to act in the earliest phases of the infection and directly target the virus itself. miRNAs can directly inhibit the SARS-CoV-2 genome when administered to the patient. Those that can inhibit this genome by viral genome integration have been identified.

In the future, with the acquisition of new or mutant genome sequences of the S protein, different miRNAs compatible with this region will be discovered. The results of these studies are obtained by processing the data, using *in silico* methods. Increasing the quantity and quality of data added to these databases over time will help the therapeutic methods obtained to be more inclusive.

It has been shown in previous studies that miRNAs have the potential to be used as biomarkers, and with new studies, miRNAs may be an alternative diagnostic tool for COVID-19. It is known that viruses can evade the host's immune response using their own miRNAs. SARS-CoV-2 is thought to exhibit such a response by a similar but not yet fully explained mechanism. Discovery of the Delta and Omicron variant-related miRNAs may also aid in the development of non-miRNA drugs by providing a better understanding of the virus-cell interaction pathway. It can also be used as an

adjuvant to increase the effect of currently used drugs.

The secretion of different miRNA types can give an idea about the course of the disease. The miRNA expression differences between the healthy and patient populations may assist in clinical decision making and treatment administration. As is the case for many diseases, venous thromboembolism is one of the most important causes of morbidity and mortality in COVID-19. Studies showing the relationship between venous thromboembolism and miRNA are limited in the literature. As a result of comprehensive miRNA studies, the use of miRNAs in risk calculation and prophylaxis of venous thromboembolism will perhaps become possible in the future.

Meanwhile, all these therapeutic amenities require further mechanistic evaluation to comprehend how they regulate the virus-host interaction. For this reason, further *in vivo*, *ex vivo* and *in vitro* studies will be required to validate candidate miRNAs for their effects towards the SARS-CoV-2 infection.

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