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Annotation of miRNAs in COVID-19 coronavirus

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ABSTRACT

The COVID-19 coronavirus is a new strain of coronavirus that had not been previously detected in humans. As its severe pathogenicity is concerned, it is important to study it thoroughly to aid in the discovery of a cure. In this study, the microRNAs (miRNAs) of COVID-19 were annotated to provide a powerful tool for the study of this novel coronavirus. We obtained 16 novel coronavirus genome sequences and the mature sequences of all viruses in the microRNA database (miRbase), and then used the miRNA mature sequences of the virus to perform the Basic Local Alignment Search Tool (BLAST) analysis in the coronavirus genome, extending the matched regions of approximately 20 bp to two segments by 200 bp. Six sequences were obtained after deleting redundant sequences. Then, the hairpin structures of the mature miRNAs were determined using RNAfold. The mature sequence on one hairpin arm was selected into a total of 4 sequences, and finally the relevant miRNA precursor prediction tools were used to verify whether the selected sequences are miRNA precursor sequences of the novel coronavirus. The miRNAs of the novel coronavirus were annotated by our newly developed method, which will lay the foundation for further study of this virus.

1. Introduction

Pneumonia associated with the COVID-19 coronavirus, began to erupt in December 2019 [1], and the disease can cause coughing, fatigue, and diarrhea as well as severe respiratory problems, pneumonia and death [2]. COVID-19 coronavirus is primarily transmitted by respiratory droplets and is easily spread, and highly infectious [3]. After entering the human body, a series of processes occur, including viral invasion, viral replication, cell apoptosis, and viral fragments dissemination that triggers the human immune response to promote inflammatory reactions, and even inflammatory storms, leading to pathological damage. Many people have been infected by COVID-19 coronavirus and died in many countries and regions worldwide. Coronaviruses are coated plus stranded single stranded RNA viruses with a diameter of 80 nm to 120 nm. Their genetic material is the largest of all RNA viruses and infects only humans, mice, pigs, cats, dogs, and poultry vertebrates. Coronaviruses are excreted from the body through respiratory secretions, and is transmitted by oral fluid, spray, contact, and airborne droplets. At present, there are no effective drugs against this virus [4,5], and little research has been performed, leading to an urgent need to identify effective therapeutics.

The microRNA (miRNA) is non-coding single-stranded RNA molecules with the lengths of approximately 22 nucleotides and regulate gene expression at the posttranscriptional level [6–8]. The miRNA can regulate many types of cancers [9–12] and can be

proto-oncogenes or tumour suppressor genes that are closely associated with many diseases [9,10,13–21]. Viral miRNA is a newly discovered type of miRNA that regulates the expression of host cells and the target genes of the virus by inducing mRNA cleavage degradation, translation, inhibition, or other mechanisms, causing changes in host cell activity or the replication of the virus [22]. To combat and evade host immune surveillance, the virus must protect itself. Finding viral miRNA molecules and identifying their biological functions has become a research hotspot. Thus, it is important to annotate the miRNAs of the novel coronavirus to better understand its mechanism of action and lay the foundation for basic research and drug development [23–25].

In this study, we obtained 16 novel coronavirus genome sequences and annotated the potential miRNAs. BLASTN was used to identify the mature miRNA sequences of the novel coronavirus, while RNAfold was used to generate secondary structures and miRNA precursor prediction tools were used to predict the miRNA precursor sequences. To improve the accuracy of the results, 5 miRNA precursor prediction methods were used for each sequence. Our results provide a theoretical basis for the study of the novel coronavirus and lay a foundation for drug development.

2. Materials and methods

2.1. Datasets

The 16 novel coronavirus sequences used in this study were downloaded from the web site https://bigd.big.ac.cn/ncov/release_genome and all of the viral miRNA mature sequences were obtained from miRBase (http://www.mirbase.org/).

2.2. Scheme of analysis

As shown in Fig. 1, we first used mature viral miRNAs for the BLAST search in the novel coronavirus genome and then extended the matched regions by 200 bp on either side. The secondary structures were generated by using RNAfold, and the hairpin sequences with a mature miRNA on the arm were observed. Finally, 5 miRNA prediction tools were used to verify whether the selected sequences were miRNA precursor sequences.

2.3. BLAST analysis

Formatdb (version 2.2.20) was used to preprocess the sequences from the genome of the novel coronavirus to ensure that the next search step could be quickly completed. Then BLASTN (version 2.2.20) was used to perform "sequence alignment" of the mature sequences of the virus with the sequences of the novel coronavirus genome. Then the matched sequences of approximately 20 bp were extended on both sides by 200 bp.

2.4. Search for novel coronavirus miRNA precursors

The extended sequences were folded with RNAfold [26–28] to form secondary structures and to identify a hairpin structure sequence containing a mature miRNA sequence on one arm for each different secondary structure. Finally, 4 sequences were identified.

2.5. Prediction of novel coronavirus miRNA precursors

We used 5 miRNA precursor prediction tools to independently verify whether the 4 novel coronavirus sequences were true miRNA precursors. The web sites for these 5 tools are as follows:

● MiPred: http://server.malab.cn/MiPred/predict.jsp
● iMiRNA-PseDPC: http://bioinformatics.hitsz.edu.cn/iMiRNA-PseDPC/
● miRNA-dis: http://bioinformatics.hitsz.edu.cn/miRNA-dis/
● iMcRNA: http://bioinformatics.hitsz.edu.cn/iMcRNA/(This URL contains 2 methods: iMcRNA-PseSSC and iMcRNA-ExPseSSC.)
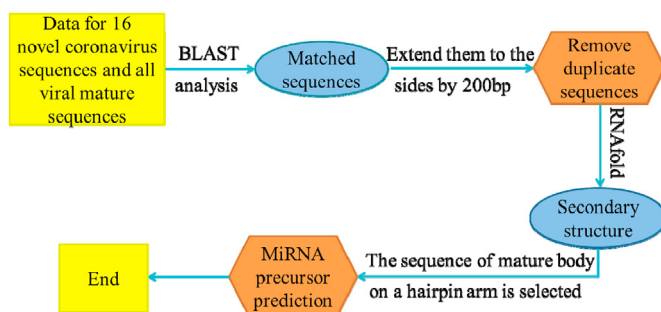

Fig. 1. Main flow chart for the annotation of the novel coronavirus miRNAs. Extension by 200 bp; selection of mature body on the hairpin arm.

Table 1
Matching sequence information.

Name of the sequences	Sequence coded information
MN938384.1 dev-miR-D7-5p MIMAT0028177	CTGTTACAGCGTATAATGGTTA
MN938384.1 hvt-miR-H12–5p MIMAT0012863	TTCTTATGGACCTTGAAGGAAAACA
MN938384.1 mcpv-miR-P1-3p MIMAT0010151 (first)	TTGTCCTTCTCTTAGTCGTTGA
MN938384.1 mcpv-miR-P1-3p MIMAT0010151 (second)	AACAGGAAGAGAATCAGCAACT
MN938384.1 mcpv-miR-P1-3p MIMAT0010151 (third)	AGTTGCTGATTCTCTTCCTGTT
MN938384.1 rlcv-miR-rL1-5-5p MIMAT0003433	TATATTCTTTTCACTAGGTT

3. Results

3.1. BLAST analysis

After the BLAST analysis, we obtained 16 matching regions of approximately 20 bp. When the repeat sequences were removed, 6 sequences remained. The sequence information is shown in Table 1.

3.2. Use of RNAfold to identify miRNA precursors of novel coronaviruses

RNAfold was used to fold the extended sequences and form secondary structures. After identifying the hairpin structure sequence of the mature miRNA sequences on one hairpin arm, 4 sequences remained. The secondary structures are shown in Fig. 2, where the hairpin structure with the mature miRNA is in the red frame and the detail information of the precursor sequences is shown in Table 2.

3.3. Prediction of miRNA precursors of novel coronavirus

We used 5 miRNA precursor prediction tools to verify whether the 4 sequences we identified represent the actual miRNA precursor sequences of the novel coronavirus. The 5 tools used in this study were MiPred, iMiRNA-PseDPC, iMcRNA-PseSSC, iMcRNA-ExPseSSC, and miRNA-dis, and the prediction results are shown in Table 3. The results showed that one of the sequences we identified (named MN938384.1 dev-miR-D7-5p MIMAT0028177) was not a miRNA precursor sequence of the novel coronavirus, while the remaining 3 sequences seemed to be miRNA precursor sequences of the novel coronavirus. The location of the 3 novel coronavirus miRNA precursors and the mature sequences are shown in the gff3 format in Table 4.
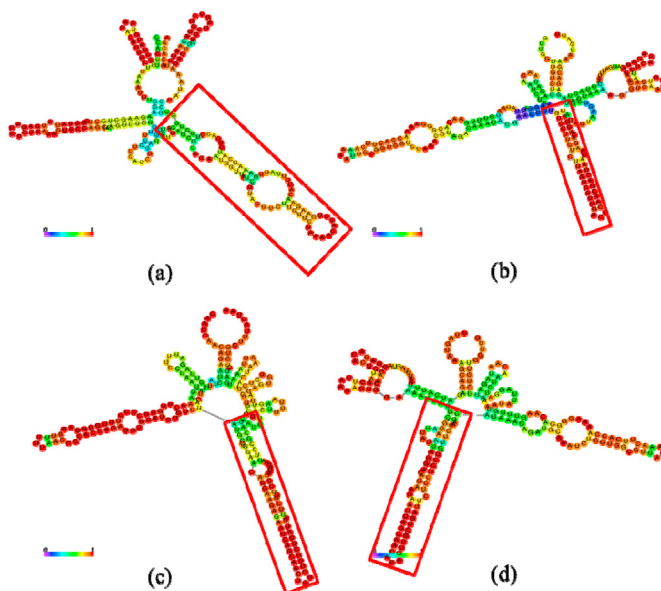

Fig. 2. Secondary structures of the extended sequences: (a) MN938384.1 dev-miR-D7-5p MIMAT0028177, (b) MN938384.1 mcpv-miR-P1-3p MIMAT0010151 (first), (c) MN938384.1 mcpv-miR-P1-3p MIMAT0010151 (second), and (d) MN938384.1 mcpv-miR-P1-3p MIMAT0010151 (third).

Table 2

Information of the 4 precursor sequences.

Name of the sequences	Sequence coded information
MN938384.1 dev-miR-D7-5p MIMAT0028177	CUGUUACAGCGUAUAAUGGUUAUCUUACUUCUUCUUCUAAA
	ACACCUGAAGAACAUUUUAUUGAAACCAUCUCACUUGCUGG
MN938384.1 mcpv-miR-P1-3p MIMAT0010151 (first)	UUGUCCUUCUCUUAGUCGUUGACACAACGACUAAUAAGACAGGAUA
MN938384.1 mcpv-miR-P1-3p MIMAT0010151 (second)	AUGCUUGGAACAGGAAGAGAAUCAGCAACUGUGUUGCUGAUUAUUCUGUCCUAUAUAAUUCCGCAU
MN938384.1 mcpv-miR-P1-3p MIMAT0010151 (third)	GCGGAAUUAUAUAGGACAGAAUAAUCAGCAACACAGUUGCUGAUUCUCUUCCUGUUCCAAGC

Table 3

Prediction results of the four novel coronavirus miRNA precursors.

Name	Method					
	MiPred	iMiRNA-PseDPC	iMcRNA			miRNA-dis
			iMcRNA-PseSSC	iMcRNA-ExPseSSC		
MN938384.1 dev-miR-D7-5p MIMAT0028177	No	No	No	No		No
MN938384.1 mcpv-miR-P1-3p MIMAT0010151 (first)	No	Yes	Yes	Yes		Yes
MN938384.1 mcpv-miR-P1-3p MIMAT0010151 (second)	No	Yes	Yes	Yes		Yes
MN938384.1 mcpv-miR-P1-3p MIMAT0010151 (third)	No	Yes	Yes	No		Yes

Note: "Yes" means that the tool predicts that the sequence is a miRNA precursor sequence, and "No" means that the tool predicts that the sequence is not a miRNA precursor sequence.

Table 4

Location of the novel coronavirus miRNA precursor and mature sequences.

##gff-mature sequence
MN938384.1. miRNA 22611 22590 … Name = mcpv-miR-P1-3p MIMAT0010151 (mature first)
MN938384.1. miRNA 22611 22590 … Name = mcpv-miR-P1-3p MIMAT0010151 (mature second)
MN938384.1. miRNA 22611 22590 … Name = mcpv-miR-P1-3p MIMAT0010151 (mature third)
##gff-precursor sequence
MN938384.1. miRNA 22611 22566 … Name = mcpv-miR-P1-3p MIMAT0010151 (mature first)
MN938384.1. miRNA 22619 22554 … Name = mcpv-miR-P1-3p MIMAT0010151 (mature second)
MN938384.1. miRNA 22645 22584 … Name = mcpv-miR-P1-3p MIMAT0010151 (mature third)

Note: The gff3 format has 9 columns of information. "MN938384.1" indicates the ID of the sequence, and the first point shows the source of the annotation; the third column shows the type of attribute; the fourth and fifth columns show the starting and ending positions of the sequence; the second point shows the score; the third point shows that there was no need to specify a plus or minus chain; the fourth point represents the phase; the last column shows the name of the sequences.

4. Discussion

The virus infection is one of the greatest threats to human health. A virus is a non-cellular organism that is tiny and simple in structure and contains only one kind of nucleic acid (DNA or RNA), which must be parasitized in a living cell and multiplied by replication. Its replication, transcription, and translation capabilities are all carried out in the host cell. When it enters the host cell, it can use the material and energy in the cell to complete life activities.

COVID-19 coronavirus has caused severe infections recently [29,30]. In just one week, more than eight hundred individuals became infected. People infected with the coronavirus exhibit the common signs of respiratory symptoms, fever, cough, and breathing difficulties [31]. In more severe cases, the infection can lead to dyspnoea, metabolic acidosis, and coagulation disorders as well as organ failure and even death. The speed at which the disease spreads is very fast, occurring over a wide area and involving a large proportion of the population [32,33]. COVID-19 coronavirus has become a worldwide epidemic in a short time across provincial and international boundaries [34]. At present, there are infectious persons all over the country and even abroad [35]. However, we do not have a deep understanding of the molecular information of the novel coronavirus, which is needed to promote the development of drugs.

The miRNA is a kind of endogenous noncoding RNA with regulatory functions found in eukaryotes. Mature miRNAs are produced by a series of cleavage of primary transcripts by nuclease, and then assembled into RNA induced silencing complex (RISC). The target mRNA is recognized by base complementary pairing, and the translation of the target mRNA is guided by the different degree of complementation [36]. Recent studies have shown that the miRNA is involved in various regulatory pathways, including development, virus defense, hematopoiesis, cell proliferation, and apoptosis. Viral miRNAs have been shown to induce the degradation of mRNAs or regulate the expression of host cells and viral target genes through other mechanisms [37,38]. Therefore, it is important to annotate the miRNA of the novel coronavirus to gain an in-depth understanding of the virus and provide the basis for drug and vaccine development.

5. Conclusions

In this study, we annotated the miRNAs of the novel coronavirus. We used BLASTN to compare the mature sequences of all viruses with the genome sequences of the novel coronavirus, and RNAfold was subsequently used to generate the secondary structures of the extended sequences. Finally, we identified the precursor sequences and verified them using miRNA precursor prediction tools. Through comparisons using a variety of prediction tools, the accuracy of the prediction was improved, increasing the quality of the data. Finally, we identified 3 miRNA precursor sequences of the novel coronavirus and annotated their mature and precursor sequences. Our results can provide a theoretical basis for use by other researchers to accelerate the study of COVID-19.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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