The miRNA COMPLEXES AGAINST CORONAVIRUSES SARS-CoV-2, SARS-CoV, and MERS-CoV

Aizhan Rakhmetullina (zhanullina1994@gmail.com)
Al-Farabi Kazakh National University https://orcid.org/0000-0002-0117-7110
Anatoliy Ivashchenko
Al-Farabi Kazakh National University
Aigul Akimniyazova
Al-Farabi Kazakh National University
Dana Aisina
Al-Farabi Kazakh National University
Anna Pyrkova
Al-Farabi Kazakh National University

Research

Keywords: SARS-CoV, MERS-CoV, COVID-19, virus, mRNA, miRNA, disease, treatment

Posted Date: June 17th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-20476/v2

Abstract

Background: In the past twenty years humankind has effected from infections caused by SARS-CoV (severe acute respiratory syndrome), MERS-CoV (Middle East respiratory syndrome) and SARS-CoV-2 coronaviruses, which have caused significant harm to human health and resulted in high mortality. The possibility of using miRNA (mRNA-inhibiting RNA) to inhibit infections caused by the coronaviruses SARS-CoV-2, SARS-CoV, and MERS-CoV has been shown.

Methods: The MirTarget program determines the following characteristics of interaction between miRNAs and messenger RNAs (mRNAs): the start of the miRNA binding site on the mRNA; the locations of the miRNA binding sites in the 3'-untranslated region (3'UTR), 5'-untranslated region (5'UTR), or coding sequence (CDS); the interaction free energy (ΔG, kJ/mole); and nucleotide interaction schemes between miRNAs and mRNAs.

Results: Using bioinformatics approaches, completely complementary miRNA (cc-miRNA) complexes were predicted to be able to bind and inhibit the translation of coronavirus proteins and the replication of SARS-CoV-2, SARS-CoV, and MERS-CoV genomes. For complexes of seven completely complementary miRNA of SARS-CoV-2 (cc-miRc), seven completely complementary miRNA of SARS-CoV (cc-miRs), and eight completely complementary miRNA of MERS-CoV (cc-miRm), the interactions with the RNA genomes (gRNAs) of the corresponding coronaviruses was evaluated. The free energy of the interactions of cc-miRNAs with binding sites was significantly higher than the free energy of the interactions with other regions in gRNA, which ensures high selectivity of the binding of cc-miRNAs. Weak binding of cc-miRNAs to the mRNAs of 17508 human genes was shown, which suggests the absence of side effects of the cc-miRNAs in humans. A feature of this method is the simultaneous inhibition of translation and replication by several cc-miRNAs binding from the 5' end to the 3' end of gRNA.

Conclusion: The use of several cc-miRNAs to suppress infections allows each of them to be used at a lower concentration to avoid side effects when one cc-miRNA is introduced into humans at a high concentration.

Background

miRNAs (mRNA-inhibiting RNAs) are involved in the regulation of gene expression at the translational level [1]. These nanosized molecules with a length of 6-8 nm can bind to mRNAs with all 19-28 nucleotides, providing high selectivity for interaction with a single gene or several target genes [2,3]. Based on this property of miRNA, attempts have been made to regulate the expression of viral genes using natural miRNAs or by creating synthetic small interfering RNAs (siRNAs) [4-6]. In some cases, this approach gave a positive result *in vitro* but was not applicable *in vivo* for several reasons, including the lack of evidence that miRNA and siRNA do not have side effects in humans or experimental animals [7-10]. The basis of our approach to the use of miRNAs in the fight against coronaviruses SARS-CoV-2,

SARS-CoV (severe acute respiratory syndrome), and MERS-CoV (Middle East respiratory syndrome) is to create completely complementary miRNA (cc-miRNA) that will highly specifically inhibit the translation of viral proteins by strong interactions with the RNA genomes (gRNA) of viruses. Such cc-miRNAs associated with the gRNA of the virus will inhibit genome replication. Thus, two goals are achieved by using cc-miRNA: to stop the synthesis of proteins of the virus that has entered the cell and to inhibit the reproduction of its genome. Such actions of cc-miRNAs on the virus should be highly specific and not have side effects on any human genes.

Methods

The nucleotide sequences of 2565 miRNAs were downloaded from miRBase (http://mirbase.org, Release 22.1). Another 3307 miRNAs were obtained from the article by Londin et al. [11]. The nucleotide sequences of human genes and coronavirus SARS-CoV-2, SARS-CoV, and MERS-CoV genomes were obtained from GenBank (http://www.ncbi.nlm.nih.gov). A search for the target genes of miRNAs was performed using the MirTarget program [12-14]. This program determines the following binding characteristics: the start of the miRNA binding site on the messenger RNA (mRNA); the locations of the miRNA binding sites in the 3'-untranslated region (3'UTR), 5'-untranslated region (5'UTR), or coding sequence (CDS); the interaction free energy (ΔG, kJ/mole); and nucleotide interaction schemes between miRNAs and mRNAs. The ratio of ΔG/ΔGm (%) was determined for each binding site, where ΔGm is equal to the free energy of the binding of miRNA with its fully complementary nucleotide sequence. The MirTarget program looks for hydrogen bonds between adenine (A) and uracil (U), guanine (G) and cytosine (C), G and U, and A and C [15]. The distances between A and C are equal to 1.04 nanometers, between G and C, and A and U are equal to 1.03 nanometers, between G and U are equal to 1.02 nanometers [14]. The numbers of hydrogen bonds in the G-C, A-U, G-U and A-C interactions were 3, 2, 1 and 1, respectively [13,14].

Results

The first task of this study was to identify human miRNAs that would have the greatest effect on the expression of the genomes of the coronaviruses SARS-CoV-2, SARS-CoV, and MERS-CoV at the translational level. Then, cc-miRNAs were created that could efficiently bind to the gRNA nucleotide sequence at the 5'-end to avoid wasting the resources of the recipient cell on the synthesis of all proteins encoded by the viral genome. Furthermore, cc-miRNAs were also created for other parts of gRNA to enhance translational inhibition by applying two or more cc-miRNAs. To inhibit genome replication, we searched for cc-miRNAs with binding sites at the 3' end of gRNA to block replication at the beginning of the process. To assess the side effects of cc-miRNAs in humans, the characteristics of the interactions of cc-miRNAs with the mRNAs of 17508 human genes in our database were determined. In addition, intramolecular interactions of the cc-miRNA binding site with gRNA regions were taken into account.

Creation of the cc-miRc complex for the gRNA of SARS-CoV-2. Despite the large gRNA of SARS-CoV-2, which is 29903 nucleotides (nt) long, in comparison with human protein-coding genes, only a few human

miRNAs with a ΔG/ΔGm of 90% or more could bind to the SARS-CoV-2 genome. We chose this ΔG/ΔGm value as a performance criterion based on the requirement that different miRNAs with a length of 22 nt are different by two or more nucleotides, which allows them to bind specifically. For example, a decrease in this criterion by 5% leads to an increase in the putative target genes of a particular miRNA by a factor of many, which leads to a large number of false target genes of the miRNA. To create cc-miRc (completely complementary miRNA of SARS-CoV-2), we chose ID02510.3p-miR, ID00448.3p-miR, miR-3154, miR-7114-5p, miR-5197-3p, ID02750.3p-miR, and ID01851.5p-miR, which bind with the gRNA of SARS-CoV-2 with ΔG/ΔGm equal to 89% or more. Furthermore, the length of these miRNAs was increased to 25–27 nt at the 5' and 3' ends of the miRNAs, and noncanonical C-A and G-U pairs were replaced by canonical U-A and G-C pairs to increase the free energy of the interactions of cc-miRc with the gRNA of SARS-CoV-2. Table 1 shows the characteristics of the fully complementary interactions of the nucleotides of seven cc-miRc with gRNA. Lengths of 25–28 nt and more have been found among natural miRNAs (miR-1273a, miR-1273d, miR-1272, miR-1292-5p, miR-3143, miR-1226-5p, miR-7161-3p) and therefore, as part of the RISC (RNA-induced silencing complex), can interact with gRNA. In the gRNA of SARS-CoV-2 intramolecular hydrogen bonds are formed that involve cc-miRc binding sites (bs of cc-miRc), which can impede the interaction of cc-miRc with their bs of cc-miRc. We selected the genome regions that interact with cc-miRc in order to generally suppress translation and replication. The cc-miRc affect subgenomic regions encoding ORF1ab, S, N. The free energy of the intramolecular interactions of bs of cc-miRc is -39 kJ/mole ÷ -43 kJ/mole lower than the ΔG of their interactions with cc-miRc (Table 1), which indicates their weak influence on the binding of cc-miRc with bs of cc-miRc. The created cc-miRc interacted with the mRNAs of 17508 genes with free energy -19 kJ/mole ÷ -22 kJ/mole, that lower than cc-miRc with gRNA (Table 1). This result suggests that each cc-miRc can, when used at adequate concentrations, interact with gRNA without side effects on the human protein-coding genes.

The interaction schemes of the seven cc-miRc complexes with the corresponding bs of cc-miRc on the gRNA of SARS-CoV-2 are shown in Fig. 1. These interaction schemes of cc-miRc with binding sites in gRNA were predicted by the MirTarget program, and quantitative characteristics of interactions are shown in Table 1. In the interaction schemes of bs of cc-miRc with gRNA, there were only completely complementary bindings of all nucleotides, and there were no non-canonical A-C and G-U pairs, which was confirmed by a ΔG/ΔGm value of 100%. Figure 1 shows the completely complementary interactions of the cc-miR1c - cc-miR7c nucleotide sequences with the corresponding bs of cc-miR1c – bs of cc-miR7c binding sites in gRNA of SARS-CoV-2.

Creation of the cc-miRm complex for the gRNA of MERS-CoV. The size of the gRNA of MERS-CoV is 30119 nt, which is several times larger than the average size of the mRNA of human genes. Only a few human miRNAs with a ΔG/ΔGm value of 89% or more could bind with the MERS-CoV genome. To create cc-miRm (completely complementary miRNA of MERS-CoV) for MERS-CoV, we chose miR-3976, ID02684.5p-miR, miR-3591-3p, ID02892.3p-miR, ID02389.3p-miR, miR-1271-3p, and ID00939.5p-miR, which bind with gRNA of MERS-CoV with a ΔG/ΔGm value of 89% or less. Furthermore, the length of these miRNAs was increased to 25–27 nt at the 5' and 3' ends of the miRNAs, and non-canonical A-C and G-U pairs were replaced by canonical U-A and G-C pairs to increase the free energy of the interaction of cc-

miRm with the gRNA of MERS-CoV. Table 2 shows the characteristics of the fully complementary interactions of the nucleotides of seven cc-miRm with gRNA. Lengths of 25–27 nt, as mentioned above, are found among natural miRNAs and therefore, as part of the RISC, these miRNAs can interact with gRNA. Intramolecular hydrogen bonds are formed in the gRNA of MERS-CoV with the participation of bs of cc-miRm binding sites, which can impede the interaction of cc-miRm with their bs of cc-miRm. We selected the genome regions which interact with cc-miRm in order to generally suppress translation and replication. The cc-miRm affect subgenomic regions encoding ORF1ab, S, M. The free energy of the intramolecular interactions of bs of cc-miRm is -30 kJ/mole ÷ -46 kJ/mole, that lower than the ΔG of their interaction with cc-miRm (Table 2), which indicates the weak influence of their intramolecular interactions on the binding of cc-miRm with bs of cc-miRm. The created cc-miRm interacted with mRNA of 17508 genes with free energy -20 kJ/mole ÷ -22 kJ/mole lower than cc-miRm with gRNA (Table 2). This result indicates that each cc-miRm, used at adequate concentrations, can interact with gRNA without side effects on the human protein-coding genes.

The interaction schemes of the complex of seven cc-miRm with the corresponding bs of cc-miRm on the gRNA of MERS-CoV are shown in Fig. 2. These interaction schemes of cc-miRm with binding sites in gRNA were predicted by the MirTarget program, and quantitative characteristics of interactions are shown in Table. 2. Figure 2 shows the completely complementary interactions of the cc-miR1m - cc-miR7m nucleotide sequences with the corresponding bs of cc-miR1m – bs of cc-miR7m binding sites in gRNA of MERS-CoV.

Creation of the cc-miRs complex for the gRNA of SARS-CoV. Despite the large gRNA of SARS-CoV, which is 29751 nt long, compared with that of human protein-coding genes, only a few human miRNAs with ΔG/ΔGm of 89% or more could bind to the SARS-CoV genome. To create cc-miRs (completely complementary miRNA of SARS-CoV), we chose ID00322.5p-miR, miR-20b-3p, miR-497-3p, ID01820.3p-miR, miR-505-3p, ID00749.3p-miR, ID03254.5p-miR, and ID00271.5p-miR, which bound with the gRNA of SARS-CoV with a ΔG/ΔGm value equal to 89% or less. The length of these miRNAs was increased to 25–28 nt at the 5' and 3' ends of the miRNAs, and non-canonical C-A and G-U pairs were replaced by canonical U-A and G-C pairs to increase the free energy of the interaction of cc-miRs with the gRNA of SARS-CoV. Table 3 shows the characteristics of the fully complementary interactions of the nucleotides of eight cc-miRs with gRNA. Intramolecular hydrogen bonds involving bs of cc-miRs are formed in the gRNA of SARS-CoV, which can impede the interaction of cc-miRs with their bs of cc-miRs. We selected the genome regions which interact with cc-miRs in order to generally suppress translation and replication. The cc-miRs affect subgenomic regions encoding ORF1ab, 7a. The free energy of the intramolecular interactions of bs of cc-miRs is -34 kJ/mole ÷ -44 kJ/mole lower than the ΔG of their interaction with cc-miRs (Table 3), which indicates a weak effect of the intramolecular interactions on the binding of cc-miRs to bs of cc-miRs. The created cc-miRs interacted with the mRNAs of 17508 genes with free energy -19 kJ/mole ÷ -24 kJ/mole lower than cc-miRs with gRNA (Table 3). This result suggests that each cc-miRs at concentrations comparable with the concentrations of endogenous miRNA can interact with gRNA without side effects on the human protein-coding genes.

The interaction schemes of the complex of eight cc-miRm with the corresponding bs of cc-miRm on the gRNA of SARS-CoV are shown in Fig. 3. These interaction schemes of cc-miRm with binding sites in gRNA were predicted by the MirTarget program, and quantitative characteristics of interactions are shown in Table. 3. Figure 3 shows the completely complementary interactions of the cc-miR1s - cc-miR8s nucleotide sequences with the corresponding bs of cc-miR1s – bs of cc-miR8s binding sites in gRNA of SARS-CoV.

Synthesis and delivery of cc-miRNAs into humans. The synthesis of cc-miRNAs is an inexpensive procedure - in terms of cost, it corresponds to the synthesis of primers. Our hypothesis can be tested in laboratories with the right and ability to conduct inexpensive and short-term tests proposed by cc-miRNAs as a means of combating SARS-CoV-2, SARS-CoV, and MERS-CoV coronaviruses. Since the size of cc-miRNAs is approximately 9 nm, they can be delivered via circulation to many organs as part of ordinary exosomes in human blood measuring 30–150 nm [16-18]. As part of exosomes, cc-miRNAs can be introduced into the lung by inhalation. The proposed method of combating coronavirus does not have toxicity or side effects. cc-miRNAs are susceptible to degradation by nucleases, similar to all human miRNAs, and the removal of cc-miRNAs from the body is not difficult. In the absence of side effects, cc-miRNAs can be used as a therapeutic agent.

Discussion

We have established complexes of specific cc-miRc, cc-miRm, cc-miRs for each of the SARS-CoV-2, MERS-CoV and SARS-CoV coronaviruses. Using the cc-miRc, cc-miRm, cc-miRs complex to suppress the virus can increase the effect of miRNA and achieve no side effects. A change in the nucleotide sequence by 1 nucleotide leads to a change in the free energy of the interaction by no more than 4%, therefore, point mutations do not significantly affect the interaction of cc-miRc, cc-miRm, cc-miRs with gRNA. The proposed method of inhibiting the infection of the SARS-CoV-2, SARS-CoV, and MERS-CoV coronoviruses is fundamentally different from the existing vaccine-based methods for combating viruses. The basis of our approach is the biological feature of the reproduction of viruses only in the cell through the processes of translation of the genome and the process of replication of the genome without which the reproduction of viruses is impossible. The cc-miRNAs can simultaneously block both processes because they bind to single-stranded RNA of the coronavirus. Due to the use of several cc-miRNAs, it is possible to reduce the concentration of each of them, which reduces their possible toxic effect. Creating a drug containing cc-miRNAs takes less time and the drug can be introduced into the blood of human body, either locally by inhalation or other methods.

Conclusions

The nucleotide sequences of the cc-miRNAs were created based on known miRNAs and on their binding sites in the gRNA of SARS-CoV-2, SARS-CoV, and MERS-CoV. The binding characteristics of cc-miRNAs and their complementary nucleotide sequence (as a pre-miRNA) on mRNAs of human genes were determined to identify side effects of cc-miRNAs on human gene expression. The use of a complex of

several miRNAs to suppress coronavirus infection allows the inhibition of viral protein synthesis and gRNA replication; reduces the toxicity of each cc-miRNA by allowing the use of concentrations equal to those of endogenous miRNA; quickly spreads via circulations to many organs; and is excreted from organisms as are all endogenous miRNAs. The proposed method does not require a large number of reagents and is not time consuming. The cc-miRNAs can be used as a therapeutic agents for coronavirus infections with SARS-CoV-2, SARS-CoV, and MERS-CoV.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

The work was carried out with the financial support of the Ministry of Education and Science of the Republic of Kazakhstan within the framework of the grant №AP05132460.

Authors' contributions

All authors designed the research and wrote the paper.

Acknowledgements

Not applicable

Author details

Rakhmetullina Aizhan Kazievna - PhD-student. al-Farabi Kazakh National University, Almaty, Kazakhstan; e-mail: zhanullina1994@gmail.com; https://orcid.org/0000-0002-0117-7110 Ivashchenko Anatoliy Timofeevich - doctor of biological sciences, professor, chief researcher; al-Farabi Kazakh National University, Almaty, Kazakhstan; e-mail: a.iavashchenko@gmail.com https://orcid.org/0000-0002-7969-2016

Akimniyazova Aigul Nurlankyzy - PhD-student. al-Farabi Kazakh National University, Almaty, Kazakhstan; e-mail: akimniyazova@gmail.com; https://orcid.org/0000-0001-7283-3958

Aisina Dana Yvgenievna – PhD, al-Farabi Kazakh National University, Almaty, Kazakhstan; e-mail: dana.aisina03@gmail.com; https://orcid.org/0000-0002-7137-3408

Pyrkova Anna Yurevna – Professor, al-Farabi Kazakh National University, Almaty, Kazakhstan; e-mail: Anna.Pyrkova@kaznu.kz; https://orcid.org/0000-0001-8483-451X

Abbrevations

3'UTR: 3'-untranslated region; 5'UTR: 5'-untranslated region; bs of cc-miRc: cc-miRc binding sites; cc-miRm: completely complementary miRNA of MERS-CoV; cc-miRs: completely complementary miRNA of SARS-CoV; cc-miRc: completely complementary miRNA of SARS-CoV-2; cc-miRNA: completely complementary miRNA; CDS: coding sequence; mRNA: messenger RNA; gRNA: genomes RNA; MERS-CoV: Middle East respiratory syndrome; miRNA: mRNA-inhibiting RNA; nt: nucleotides; RISC: RNA-induced silencing complex; SARS-CoV: severe acute respiratory syndrome; siRNA: small interfering RNA.

References

1. Bartel DP. Metazoan MicroRNAs. Cell. 2018;173:20-51.
2. Atambayeva S, Niyazova R, Ivashchenko A, Pyrkova A, Akimniyazova A, Labeit S. The Binding Sites of miR-619-5p in the mRNAs of Human and Orthologous Genes. BMC Genomics. 2017:18:428.
3. Kondybayeva AM, Akimniyazova AN, Kamenova SU, Ivashchenko AT. The characteristics of miRNA binding sites in mRNA of ZFHX3 gene and its orthologs. Vavilov journal of Genetics and Breeding. 2018;22:438-444.
4. Ma Y, Wang Ch, Xue M, Fu F, Zhang X, Li L, Yin L, Xu W, Feng L, Liu P. The Coronavirus Transmissible Gastroenteritis Virus Evades the Type I Interferon Response through IRE1α-Mediated Manipulation of the MicroRNA miR-30a-5p/SOCS1/3 Axis. J Virol. 2018;92:e00728-18.
5. Nur SM, Hasan MA, Amin MA, Hossain M, Sharmin T. Design of Potential RNAi (miRNA and siRNA) Molecules for Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Gene Silencing by Computational Method. Interdiscip Sci. 2015;7:257-65.
6. Mallick B, Ghosh Z, Chakrabarti J. MicroRNome analysis unravels the molecular basis of SARS infection in bronchoalveolar stem cells. PLoS One. 2009;4:e7837.
7. Dickey LL, Worne CL, Glover JL, Lane TE, O'Connell, R.M. MicroRNA-155 enhances T cell trafficking and antiviral effector function in a model of coronavirus-induced neurologic disease. J Neuroinflammation. 2016;13:240.
8. Hasan MM, Akter R, Ullah MS, Abedin MJ, Ullah GM, Hossain MZ. A Computational Approach for Predicting Role of Human MicroRNAs in MERS-CoV Genome. Adv Bioinformatics. 2014;2014:967946.

9. Zhang Y, Li T, Fu L, Yu C, Li Y, Xu X, Wang Y, Ning H, Zhang S, Chen W, Babiuk LA, Chang Z. Silencing SARS-CoV Spike protein expression in cultured cells by RNA interference. FEBS Lett. 2004;560:141-6.
10. Lai FW, Stephenson KB, Mahony J, Lichty BD. Human coronavirus OC43 nucleocapsid protein binds microRNA 9 and potentiates NF-κB activation. J Virol. 2014;88:54-65.
11. Londin E, Loher P, Telonis AG, Quann K, Clark P, Jing Y. Analysis of 13 cell types reveals evidence for the expression of numerous novel primate- and tissue-specific microRNAs. PNAS USA. 2015;112;1106-1115.
12. Ivashchenko A, Berillo O, Pyrkova A, Niyazova R, Atambayeva S. MiR-3960 binding sites with mRNA of human genes. Bioinformation. 2014;10:423-427.
13. Kool, E. T. Hydrogen bonding, base stacking, and steric effects in DNA replication. *Annual* Review of Biophysics and Biomolecular Structure. 2001;30;1-22.
14. Leontis NB, Stombaugh J, Westhof E. The non-Watson- Crick base pairs and their associated isostericity matrices. Nucleic Acids Research. 2002;30:3497-3531.
15. Garg A, Heinemann U. A novel form of RNA double helix based on G·U and C·A+ wobble base pairing. RNA. 2018;24:209-218.
16. Zhang Zh, Dombroski JA, King MR. Engineering of Exosomes to Target Cancer Metastasis. Cellular and Molecular Bioengineering. 2020;13:1-16.
17. Herrera-Carrillo E, Liu YP. Berkhout B. Improving miRNA delivery by optimizing mirna expression cassettes in diverse virus vectors. Human Gene Therapy Methods. 2017;28:177-190.
18. Song X, Zhao X, Huang Yo, Xiang H, Zhang W, Tong D. Transmissible gastroenteritis virus (TGEV) infection alters the expression of cellular microRNA species that affect transcription of TGEV gene 7. Int J Biol Sci. 2015;11:913-22.

Tables

Table 1 Characteristics of the interactions of cc-miRc with regions of the gRNA SARS-CoV-2 and mRNAs of human genes

RNA	miRNA/bs of miRNA	Start of site, nt	Region of RNA	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
5’UTR	cc-miR1c	193	5'UTR	-149	100	27
gRNA	bs of cc-miR1c	544	CDS	≤-106	≤71	27
mRNAs	cc-miR1c			≤-126	≤85	27
ORF1ab	cc-miR2c	16390	CDS	-144	100	26
gRNA	bs of cc-miR2c	3157	CDS	≤-104	≤75	26
mRNAs	cc-miR2c			≤-122	≤85	26
ORF1ab	cc-miR3c	17116	CDS	-146	100	27
gRNA	bs of cc-miR3c	253	5'UTR	≤-104	≤71	27
mRNAs	cc-miR3c			≤125	≤86	27
ORF1ab	cc-miR4c	18101	CDS	-146	100	27
gRNA	bs of cc-miR4c	ND	CDS	≤-102	≤69	27
mRNAs	cc-miR4c			≤125	≤86	27
S	cc-miR5c	21893	CDS	-132	100	25
gRNA	bs of cc-miR5c	20296	CDS	≤-93	≤71	25
mRNAs	cc-miR5c			≤-112	≤85	25
N	cc-miR6c	28359	CDS	-140	100	25
gRNA	bs of cc-miR6c	15100	CDS	≤-100	≤71	25
mRNAs	cc-miR6c			≤-119	≤85	25
N	cc-miR7c	28883	CDS	-146	100	27
gRNA	bs of cc-miR7c	23076	CDS	≤-104	≤71	27
mRNAs	cc-miR7c			≤-124	≤85	27

Note. cc-miR1c - cc-miR7c are cc-miRc created on the basis of ID02510,3p-miR (1), ID00448,3p-miR (2), miR-3154 (3), miR-7114-5p (4), miR-5197-3p (5), ID02750.3p-miR (6), ID01851.5p-miR (7), respectively. mRNAs – 17508 mRNAs of human genes.

Table 2 Characteristics of the interactions of cc-miRm with regions of the gRNA of MERS-CoV and mRNAs of human genes

RNA	miRNA/bs of miRNA	Start of site, nt	Region of RNA	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
5’UTR	cc-miR1m	123	CDS	-142	100	27
gRNA	bs of miR1m	24648	CDS	≤-112	≤79	27
mRNAs	cc-miR1m		CDS	≤-120	≤85	27
ORF1ab	cc-miR2m	515	CDS	-144	100	26
gRNA	bs of miR2m	8742	CDS	≤-102	≤71	26
mRNAs	cc-miR2m			≤-122	≤85	26
ORF1ab	cc-miR3m	3164	CDS	-151	100	27
gRNA	bs of miR3m	12492	CDS	≤-105	≤70	27
mRNAs	cc-miR3m			≤-129	≤86	27
ORF1ab	cc-miR4m	10236	CDS	-138	100	25
gRNA	bs of miR4m	236	CDS	≤-99	≤72	25
mRNAs	cc-miR4m			≤-117	≤85	25
ORF1ab	cc-miR5m	13796	CDS	-136	100	26
gRNA	bs of miR5m	12614	CDS	≤-102	≤75	26
mRNAs	cc-miR5m			≤-116	≤85	26
S	cc-miR6m	24307	CDS	-142	100	26
gRNA	bs of miR6m	9997	CDS	≤-103	≤73	26
mRNAs	cc-miR6m			≤-120	≤85	26
M	cc-miR7m	28342	CDS	-146	100	27
gRNA	bs of miR7m	13480	CDS	≤-111	≤79	27
mRNAs	cc-miR7m			≤-126	≤86	27

Note. cc-miR1m - cc-miR7m are cc-miRm created on the basis of miR-3976 (1), ID02684.5p-miR (2), miR-3591-3p (3), ID02892.3p-miR 94), ID02389.3p-miR (5), miR-1271-3p (6), ID00939.5p-miR (7), respectively. mRNAs – 17508 mRNAs of human genes.

Table 3 Characteristics of the interactions of cc-miRs with regions of the gRNA SARS-CoV and mRNAs of human genes

RNA	miRNA/bs of miRNA	Start of site, nt	Region of RNA	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
5’UTR	cc-miR1s	91	5'UTR	-153	100	27
gRNA	bs of miR1s	746	CDS	≤-113	≤74	27
mRNAs	cc-miR1s			≤-133	≤87	27
ORF1ab	cc-miR2s	574	CDS	-157	100	28
gRNA	bs of miR2s	16058	CDS	≤-113	≤72	28
mRNAs	cc-miR2s			≤-133	≤85	28
ORF1ab	cc-miR3s	1849	CDS	-140	100	27
gRNA	bs of miR3s	15403	CDS	≤-106	≤76	27
mRNAs	cc-miR3s			≤-120	≤86	27
ORF1ab	cc-miR4s	2058	CDS	-136	100	26
gRNA	bs of miR4s	11658	CDS	≤-99	≤73	26
mRNAs	cc-miR4s			≤-117	≤86	26
ORF1ab	cc-miR5s	12482	CDS	-144	100	27
gRNA	bs of miR5s	20270	CDS	≤-103	≤72	27
mRNAs	cc-miR5s			≤-123	≤86	27
ORF1ab	cc-miR6s	19145	CDS	-142	100	27
gRNA	bs of miR6s	18037	CDS	≤-99	≤70	27
mRNAs	cc-miR6s			≤-121	≤85	27
ORF1ab	cc-miR7s	21142	CDS	-142	100	27
gRNA	bs of miR7s	17101	CDS	≤-104	≤73	27
mRNAs	cc-miR7s			≤-121	≤85	27
7a	cc-miR8s	27316	CDS	-142	100	27
gRNA	bs of cc-miR8s	21548	CDS	≤-104	≤73	27
mRNAs	cc-miR8s			≤-121	≤85	27

Note. cc-miR1s - cc-miR8s are cc-miRs created on the basis of ID00322.5p-miR (1), miR-20b-3p (2), miR-497-3p (3), ID01820.3p-miR (4), miR-505-3p (5), ID00749.3p-miR (6), ID03254.5p-miR (7), ID00271.5p-miR (8), respectively. mRNAs – 17508 mRNAs of human genes.

Figures

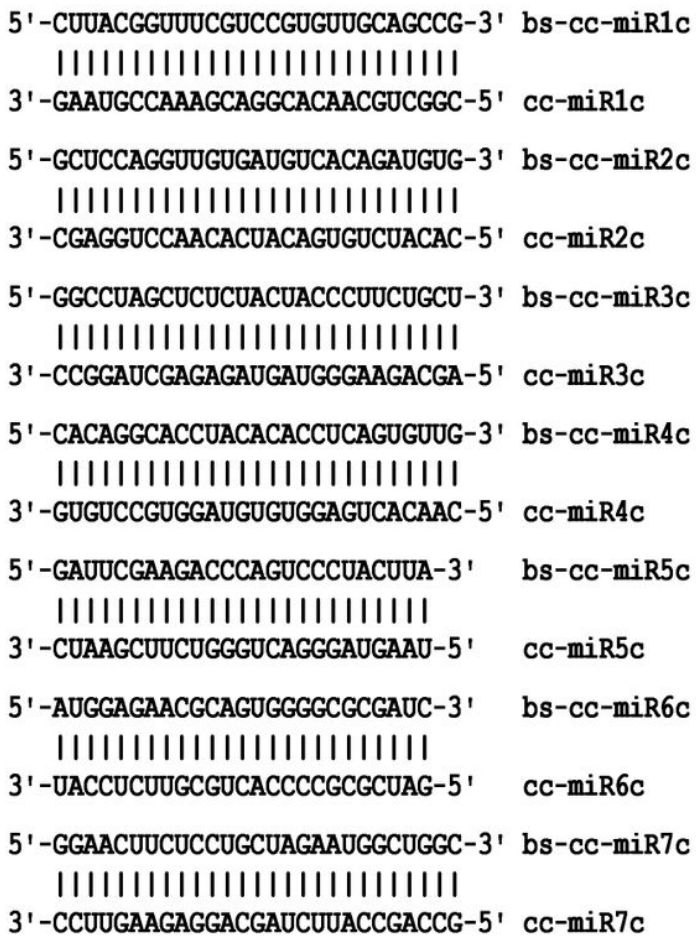

Figure 1

Schemes of the interaction of the cc-miRc complex with the corresponding bs of cc-miRc on the gRNA of SARS-CoV-2. Note: the quantitative characteristics of the interaction of the cc-miRc complex with the corresponding bs of cc-miRc are given in Table. 1.

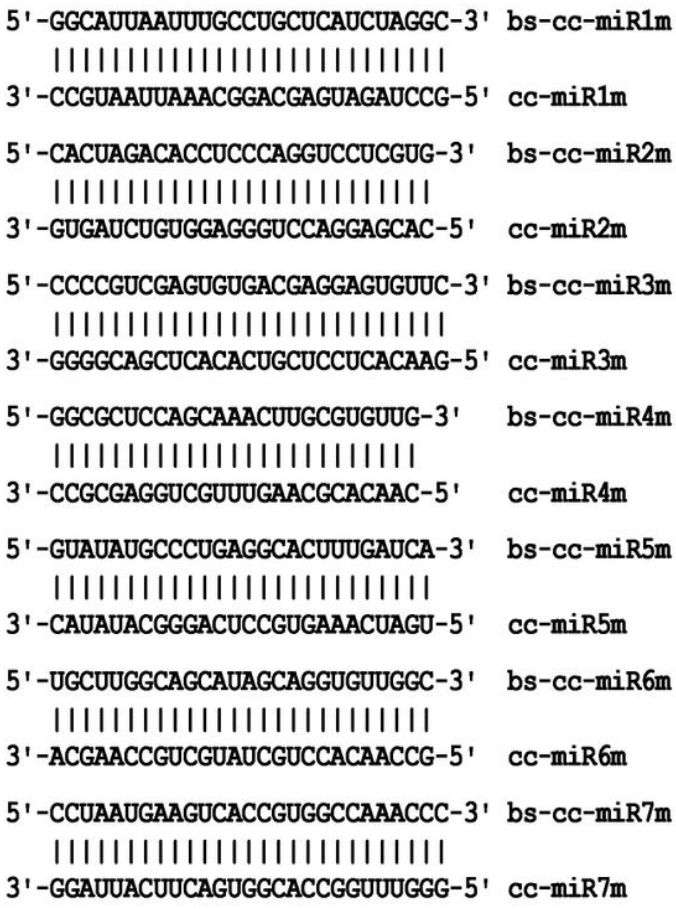

Figure 2

Schemes of the interaction of the cc-miRm complex with the corresponding bs of cc-miRm on the gRNA of MERS-CoV. Note: the quantitative characteristics of the interaction of the cc-miRm complex with the corresponding bs of cc-miRm are given in Table 2.

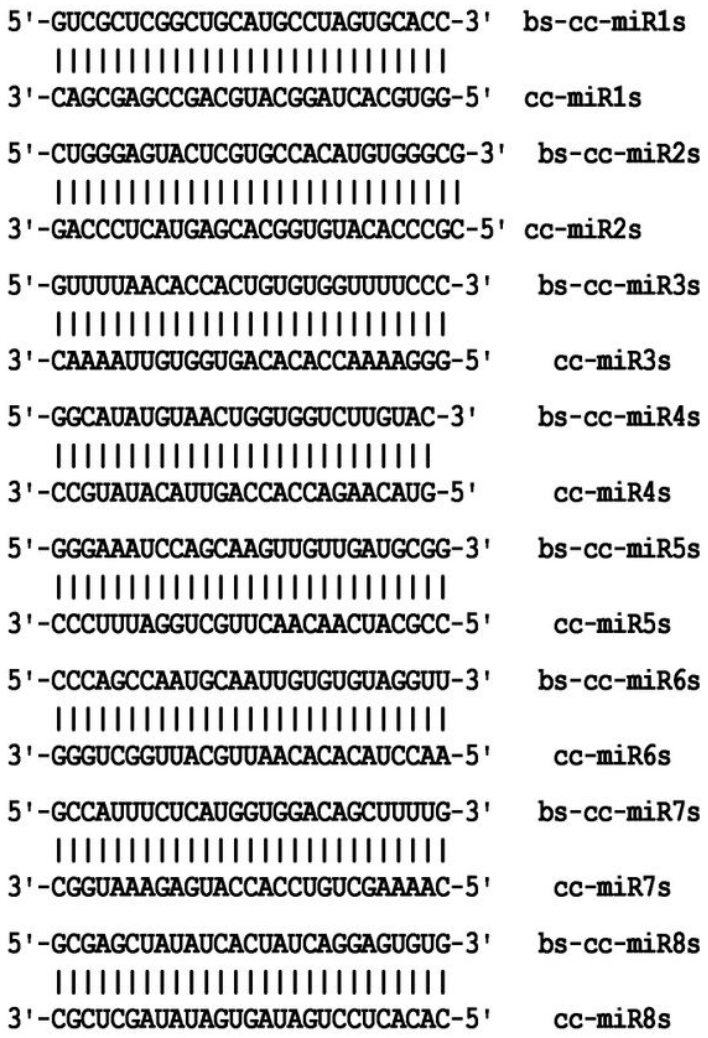

Figure 3

Schemes of the interaction of the cc-miRs complex with the corresponding bs of cc-miRs on the gRNA of SARS-CoV. Note: the quantitative characteristics of the interaction of the cc-miRs complex with the corresponding bs of cc-miRs are given in Table 3.