Non-Coding RNAs and Innovative Therapeutic Strategies to Target the 5'UTR of SARS-CoV-2

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ABSTRACT

After the increasing number of SARS-CoV-2 infections all over the world, researchers and clinicians are struggling to find a vaccine or innovative therapeutic strategies to treat this viral infection. The SARS-CoV infection that occurred in 2002, MERS and other more common infectious diseases such as HCV, led to the discovery of many RNA-based drugs. Among them, siRNAs and antisense LNAs have been demonstrated to have effective antiviral effects both in animal models and humans. Owing to the high genomic homology of SARS-CoV-2 and SARS-CoV (80-82%) the use of these molecules could be employed successfully also to target this emerging coronavirus. Trying to translate this approach to treat COVID-19, we analyzed the common structural features of viral 5′UTR regions that can be targeted by non-coding RNAs and we also identified miRNAs binding sites suitable for designing RNA-based drugs to be employed successfully against SARS-CoV-2.

Keywords: SARS-CoV-2, COVID-19; 5′UTR; miRNAs, RNAi; GapmeRs

Key-points:

- We analyzed the common genomic features of SARS-CoV and SARS-CoV-2 that can be targeted by non-coding RNAs.

- We identified miRNAs binding sites suitable for designing RNA-based drugs to be employed successfully against SARS-CoV-2.

- We suggested potential therapeutic strategies based on non-coding RNAs.

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1. Introduction

After the first reports of a novel severe acute respiratory syndrome coronavirus (SARS-CoV-2) in Wuhan city (Hubei, China) the World Health Organization declared the COVID-19 outbreak a Public Health Emergency of International Concern and more recently a pandemic, as the number of cases all over the world has dramatically increased. As with other acute respiratory illnesses, the clinical presentation may vary including influenza-like symptoms to severe pneumonia or breathing difficulties, and sometimes death, more frequent in older people or individuals with pre-existing diabetes and heart disease conditions (WHO, https://www.who.int/ith/diseases/sars/en/). Similarly to the SARS-CoV outbreak in 2002, the scientific community is struggling to find novel therapies, as neither vaccines or specific treatments are currently available. Among the first therapeutic options that have been employed since the beginning we recall the antiviral therapies [1]. Several antiviral drugs such as Ribavirin (routinely employed to treat hepatitis C) [2], Nelfinavir or a combination of Lopinavir and Ritonavir (widely used as HIV protease inhibitors) [3] and Remdesivir (a monophosphoramidate nucleoside) [4] alone or in combination with chloroquine/hydroxychloroquine [5] are currently being assessed for the treatment of COVID-19. More recently, combinations of drugs (i.e., Bromhexine/Arbidol/Interferon α2b, Hydroxychloroquine/Azithromycin, etc.), biological drugs (i.e., convalescent plasma, MSC and MSC-derived

exsosomes, CAR-NK cells, mRNA-1273, etc.), chinese medicine drugs (i.e., YinHu QingWen decoction, Xiyanping injection) and supplementations with Vitamins C and D are currently under investigation (https://www.clinicaltrials.gov/). However, some results about the lopinavir-ritonavir treatment confirmed that the combined drugs are ineffective [6].

SARS-CoV-2 has a single-stranded positive-sense RNA genome [7] with a ~80% similarity with the human SARS-CoV genome [8,9] although the similarity between genes may vary (i.e., the spike (S) protein of SARS-CoV-2 exhibits ~72% nucleotide sequence similarity with SARS-CoV).

Among all the genomic regions of the SARS-CoV genome that have been studied so far, we think that the 5'UTR region and specific portion of it should be considered crucial when devising novel therapeutic molecules also for SARS-CoV-2 infections.

2. 5'UTR and the leader sequence of SARS-CoV

In the years following the outbreak of SARS-CoV, genomics, phylogeny, antigenic structure, immune response and potential therapeutic interventions have been reviewed [10]. Specific coding regions of the viral genome that encoded proteins fundamental for virus replication and transmission (i.e., replicases, S and M glycoproteins, envelope E protein, etc.) have been investigated as well. Some of these studies focused also on the structure and

functional role of the 5'UTR region in determining coronavirus infection and replication. The 5'UTR (as well as the 3'UTR) genomic sequence is crucial for viral RNA replication and transcription [11]. In particular, a specific portion of 50-100 nucleotides at the 5' end of the genome, referred as the 'leader' sequence, has been found also at the 5' ends of all encoded transcripts (i.e., subgenomic mRNAs). This leader sequence and a *cis*-acting element termed transcription-regulatory sequence (TRS) immediately following the leader sequence, represent a unique feature of coronaviruses and some other viruses of the order Nidovirales [12].

Although the viral transcription mechanism is not fully understood, two major models have been proposed: a leader-primed transcription [13,14] or a discontinuous transcription during minus-strand synthesis [15,16]. Without going into details on these mechanisms, we outline the importance of the 5'UTR region and of the highly conserved leader sequence of coronaviruses especially when prompted to find therapeutic solutions for SARS-CoV-2.

In fact, one of the most interesting aspects reported by Zeng and collaborators in 2003 was the identification of a leader sequence of 63 bp (**Figure 1A**) and an intergenic sequence (IGS) of 9 bp (5'-TAAACGAAC-3') in the 5'UTR region of SARS-CoV HK-39 (NCBI Accession AY278491) [17]. Similarly, Li and collaborators reported a leader sequence of 72 bp for SARS-CoV BJ01 (NCBI Accession AY278488) coincident to that of SARS-CoV HK-39 (**Figure 1A**). [18].

Also in SARS-associated coronaviruses, such as the Tor2 isolate, the presence of a core sequence (5′-CTAAAC-3′) within the IGS region of the 5′UTR has been confirmed [19]. These regions have been indifferently termed IGS or TRS in different works, but the genomic location is the same.

Therefore, all of these data, while emphasizing the conservation of this genomic region among various coronavirus isolates, are useful to address the research toward the development of effective strategies aimed at reducing or abolishing viral activity and replication *in vitro* and *in vivo*. In fact, many strategies that we will describe in the following paragraphs have been adopted to target these viral genomic regions.

3. RNAi against the 5′UTR and the leader region of SARS-CoV

RNA interference (RNAi) was one of the strategies adopted in the past to inhibit the replication of coronavirus [20,21]. Several *in vitro* studies demonstrated that siRNAs targeting different region of SARS-CoV genome were effective in reducing the expression of viral sub-genomic mRNAs, ultimately leading to an inhibition of viral replication. siRNA directed against the Spike region was the most effective although the authors demonstrated that also those siRNAs targeting the leader sequence or the TRS region and the 3′UTR were able to prevent SARS-CoV infection in Vero-E6 cells (**Figure 1A**) [20]. The SARS-CoV Spike protein was considered by many authors the most

obvious candidate owing to its fundamental role in infecting human respiratory epithelial cells by interacting with human receptors (i.e., ACE2) [22]. Similarly, other studies reported the effective targeting of different regions such as the SARS-CoV M gene [23].

However, Li et al. by employing a specific siRNA (outlined in **Figure 1A**) were able to target the SARS-CoV leader sequence and effectively inhibit virus replication in Vero E6 cells [24].

However, a detailed study demonstrated that specific siRNA duplexes (i.e., siSC2 and siSC5), targeting the SARS-CoV Spike and ORF1b (NSP12) regions can significantly suppress SARS-like symptoms also *in vivo* (i.e., in infected macaques)[24]. More importantly, the RNAi approach displayed no adverse effects and was demonstrated to be effective for both prophylaxis and therapy [25].

Although this approach cannot be considered an antiviral approach (as animals developed symptoms), it is surely a valid strategy to reduce the viral load and the severity of the disease. In any case, the idea that RNAi can be used to mitigate the symptoms of new coronavirus infections and other emerging infectious diseases was a debated concept in the past [26], but it is still actual and valuable of further investigations. In fact, we believe that approaches based

on the common features shared between SARS-CoV and SARS-CoV-2 could be employed to block the virus replication.

4. 5'UTR and the leader sequence of SARS-CoV-2

To evaluate whether the concepts already found for 5'UTR of SARS-CoV can be transferred also to SARS-CoV-2, we calculated the similarity between the 5'UTR of the two coronaviruses. We found that the similarity of these two regions is 88.76% (**Figure 1B**) and sequence alignment emphasized the presence of many conserved genomic regions between SARS-CoV and SARS-CoV-2 5'UTRs, especially in the first 90 nucleotides. Interestingly, the regions encompassing the transcription-regulatory sequence (TRS) of both SARS-CoV and SARS-CoV-2 are conserved in all known genome sequences (**Supplementary Material**). Owing to their high similarity, the region that encompasses the TRS (i.e., spanning between 40 and 85 nucleotides) is identical in SARS-CoV and SARS-CoV-2 (**Figure 1B**) and conserved (**Supplementary Material**). More recently, a preliminary work of Taiaroa and his group reports the use of the leader sequence of SARS-CoV-2 for the identification of subgenomic mRNAs [27]. This sequence is very similar to that of SARS-CoV (**Figure 1B**) and this strongly suggests that the RNAi strategy already adopted for SARS-CoV and focused on this specific region would likely lead to inhibition of SARS-CoV-2 replication. However, RNAi is not the only strategy that can be employed to this purpose.

5. Deletion of specific regions in the 5'UTR of SARS-CoV

In 2006 to test the promoter activity of the 5'UTR of SARS-CoV in eukaryotic cells and identify the fundamental regions necessary for viral replication, Zhang and collaborators assessed the function of many 5'UTR clones lacking increasing portions of the SARS-CoV 5'UTR sequence [28]. They found that the *wild type* SARS-CoV 5'UTR has a promoter activity in eukaryotic cells such as A549, HepG2, ECV304, HeLa and Vero E6 and that deletion mutant plasmids with different 5'UTR length have different activities. Interestingly, the mutant plasmid lacking the region 1-36 did not altered the activity, whereas the plasmid lacking almost all the UTR (i.e., the 1-222 region) abolished completely the SARS-CoV promoter activity in human cells. In fact, it is long been known that subgenomic mRNAs lacking the 5' leader sequence are not able to replicate [29,30]. At least four stem loop structures are located in this 5'-end region of the coronavirus genome and these secondary structures are actively implicated in viral replication and transcription [31]. Moreover, the authors identified the initial site of transcription at the 56th nucleotide (see **Figure 1A**) that is proximal to the TRS sequence and to those regions already targeted by siRNAs. It is reasonable to think that this position is among the crucial genomic regions to consider for targeting purposes.

6. Secondary structures of coronaviruses 5'UTR

The RNA synthesis processes of coronaviruses takes place in the cytoplasm and is regulated by proteins of the host cell. Among these proteins, the zinc-finger and RNA binding motif MADP1 was able to interact with the 5′UTR region of the SARS-CoV genome, and this interaction was confirmed also in coronavirus infectious bronchitis virus (IBV) [32]. Interestingly, the interaction seems mediated by the secondary structure of the 5′UTR region and in particular by the presence of several stem-loops in this 5′end of the viral genome. After synthesizing four truncated biotin-labeled mutant RNA fragments of the 5′UTR region, the authors studied the minimal region required for MADP1 binding. Results indicated that even the lack of the first stem loop structure, located within the first 30 nucleotides from the beginning of the 5′UTR, is enough to abolish the binding of MADP1 protein. The absence of this protein determined a defective viral RNA synthesis *in vitro* but outlined the importance of the secondary structure of the 5′UTR region in coronavirus RNA synthesis. The presence of these stem-loops is highly conserved in many coronaviruses derived from all three major CoV groups [33]. Moreover, a couple of papers pointed to the importance of single-nucleotide mutations and deletions that are able to destabilize the first two stem loops in the 5′UTR of coronaviruses, thus inhibiting viral replication [34,35]. By analyzing the first 100-nucleotide region of the 5′UTR of IBV, MERS, SARS-CoV and SARS-CoV-2, we confirmed their similarity (**Figure 2**) and we hypothesized that also their function could be almost identical (i.e., enhancement of viral replication) [11]. However, this

similarity extends also to the entire 5′UTR of SARS-CoV and SARS-CoV-2 (**Figure 3**).

Therefore, all of these data outline once more the importance of 5′UTR when devising therapeutic strategies for coronaviruses.

7. Locked nucleic acid antisense oligonucleotides (LNA)

RNAi is not the only strategy that can be adopted to target the 5′UTR of coronaviruses. A few years ago an innovative antiviral strategy for HCV, another common widespread virus, based on a locked nucleic acid–modified DNA phosphorothioate antisense oligonucleotide, named Miravirsen, has been proposed [36]. This approach was based on a high affinity antisense oligonucleotide complementary to the human mature miR-122, a liver-specific and highly expressed microRNA. This microRNA is able to bind the 5′UTR of the HCV genome in two distinct regions and to promote actively the propagation of HCV RNA [37]. Prolonged subcutaneous administration of Miravirsen (at a dose of 3-7 mg per kilogram of body weight) halted the activity of miR-122 by preventing its binding to HCV 5′UTR and provided viral suppression up to the end of the therapy. This approach is based on two main pillars: the identification of miRNA binding sequences in the 5′UTR, and the presence of the binding miRNA in the target tissue (i.e., the liver in the case of HCV) to boost viral propagation.

To translate this approach into the identification of therapeutic molecules for the potential treatment of COVID-19 infections, we searched for potential miRNAs binding sites targeting the 5'UTR of SARS-CoV-2. Target recognition algorithms such as PITA [38] allowed identifying the accessible genomic binding sites for human miRNAs. Many miRNA binding sites were identified in conserved regions (**Figure 4**) that were filtered according to their score (ddG cutoff< -10) (**Supplementary Material**). Through this approach, up to 41 sites for miRNA binding were identified on the 5'UTR of SARS-CoV-2. Of note, several miRNAs such as miR-4507, miR-638, miR-3150b-3p and miR-602 can bind conserved regions of the 5'UTR of SARS-CoV-2. In HCV infection, miR-122 is highly expressed in the liver and this organ represents the primary target site of the virus. Similarly, we focused to find highly expressed miRNAs in the lungs that are the main target organs for SARS-CoV-2. Therefore, we extracted and analyzed the expression data contained in the human miRNA tissue atlas [39] to gain information about the quantity of these miRNAs in human tissues. We found that miR-4507 was among the most expressed miRNAs (~99th percentile) in the lungs together with miR-638 (~97th percentile) which is highly expressed also in the pleura (~99th percentile). Two distinct binding regions were found in the 5'UTR of SARS-CoV-2 genome for miR-3150b-3p and miR-602. We still do not know if these miRNAs have crucial functions in promoting viral replication or if the binding of other miRNAs in different 5'UTR region has inhibitory functions. If we suppose a similar enhancing mechanism, we

should hypothesize that the design of antisense oligonucleotides similar to Miravirsen could be able to sequester these miRNAs and inhibit viral replication. In any case, these findings prompt the research to validate these targets, explore their function and potentially identify novel drugs.

However, many other miRNAs resulted to bind coding regions and 3'UTR of the coronavirus (see **Supplementary Material**) where other *cis*-acting region are present, although the regulatory mechanisms underlying these interactions are still not completely understood [40]. The RNA-based antisense therapy is of wide applicability and the strategy herein suggested was already assessed in chronic HCV infections with no major adverse events. Based on these findings, we recommend to consider the possibility to employ miRNAs and their antisense analogues as effective candidates for targeting coronaviruses and to identify novel molecules for antiviral therapy.

8. LNA-based oligonucleotides for in vivo applications (GapmeRs)

In the last few years, novel single-stranded antisense oligonucleotides, named GapmeRs, designed to silence mRNA and other long non-coding RNAs *in vitro* and *in vivo* appeared on the market. These oligonucleotides have particular properties imparted by the linking of the 2'-O and 4'-C atoms of the ribose ring that lead to a "locked" conformation, which is ideal for Watson-Crick pairing. The antisense locked (or "bridged") nucleic acid (LNA or BNA, respectively)

GapmeRs can pair more rapidly with a complementary nucleotide strand and the stability of the resulting duplex is increased compared to traditional oligonucleotides. GapmeRs are generally designed to have a DNA portion flanked by LNA (**Figure 5A**). Duplexes of DNA hybridized to RNA generally catalyze RNase H-dependent degradation of the RNA strand, whereas LNA does not activate RNase H. For this reason, antisense LNA GapmeRs are able to cleave efficiently the target RNA. LNA GapmeRs, originally designed for silencing lncRNAs and mRNAs *in vitro* and *in vivo*, have been extended also to study the inhibition of virus replication. In fact, specific LNA gapmers have been designed and employed to target the 5'UTR region of coxsackievirus B3 [41] and the HCV internal ribosome entry site (IRES) containing the distal and proximal miR-122 binding sites [42]. In order to identify GapmerRs able to target the 5'UTR region of SARS-CoV-2 we interrogated the free tool online GeneGlobe (https://geneglobe.qiagen.com) and we identified many regions of the 5'UTR where the putative GapmeRs could be designed (**Figure 5B**). These GapmeRs could elicit RNAse H and induce a degradation of the viral RNA that in some cases would determine a loss of substantial parts of the 5'UTR of SARS-CoV-2 (i.e., GapmeR no.1 in **Figure 5B**).

Therefore, these data emphasize that GapmeRs could be optimized (i.e., proper binding region, sequence length, prevention of off-target effects) and effectively employed to inhibit the replication activity of SARS-CoV-2.

9. Conclusions

We have summarized and discussed here the panorama of non-coding RNA molecules that can be employed to target the 5'UTR region of SARS-CoV-2 with the aim to find novel drugs or innovative therapeutic strategies. The process to commercialize a novel drug is lengthy and costly owing to the regulatory processes related to pre-clinical and clinical studies. Despite this, we think that many of the molecules already studied and developed after the previous SARS-CoV infection in 2002 can be the starting point to develop effective molecules also for the recent SARS-CoV-2 infection. One of the interesting aspects of these molecules, is the possibility to alter not only the functioning of the viral replication machinery but also to impair the secondary structure of the crucial 5'UTR genomic region. Many of the molecules described in this work are natural RNA molecules or already proved to display no toxicity *in vivo* and to enter into cells without the need of delivery vehicles or transfecting agents (i.e., GapmeRs). This is an advantageous property as generally RNA molecules are not very effective *per se.* In fact, they require a mean to reach the cells, such as in the case of the recent phase-I clinical trial on COVID-19 patients that employs mRNA-1273 delivered by lipid nanoparticles (sponsored by the National Institute of Allergy and Infectious Diseases, NIAID, NCT04283461).

Independently by the use of siRNAs, miRNAs or GapmeRs, the arsenal that can be employed to fight SARS-CoV-2 is promising and potentially very

powerful. In principle, some of these therapeutic options could alleviate

COVID-19 symptoms or reduce viral replication. We strongly believe that none

of these possibilities should be left unassessed, as the discovery of an effective

vaccine could be a very long and uncertain path.

Captions to Figures

Figure 1. Alignment of A) the first part (100 nucleotides) of the 5'UTR of three coronavirus isolates that show the similarity of the leader sequence (colored in green and blue) and the intergenic sequence (IGS) depicted in brown. The position 56, considered crucial for viral transcription is represented in red. Blue and black lines indicate the genomic regions where siRNAs reported in the literature have been designed; B) the alignment of complete 5'UTR region showing the similitude of the leader sequences and the high conservation of the transcription-regulatory sequence (TRS) in SARS-CoV and SARS-CoV-2.

Figure 2. Secondary structures of the first 95-100 nucleotides of common coronavirus such as IBV, MERS, SARS-CoV and SARS-CoV-2 showing the structural similarity of the first stem loop structure.

Figure 3. Secondary structures of the 5'UTR region of SARS-CoV and SARS-CoV-2 that emphasize the structural similarity of these two coronaviruses.

Figure 4. Aligned 5'UTR of SARS-CoV and SARS-CoV-2 coronaviruses and the identification of miRNA binding sites. miRNAs targeting one site are depicted in blue, whereas in red if they target two sites (such as miR-3150-3p and miR-602).

Figure 5. Schematic representation of A) the functioning of GapmeRs oligonucleotides made by a DNA oligonucleotide 5'- and 3'-linked to two LNA terminals that is able to activate the degradation of mRNA by RNase H; b) GapmeRs indicated with numbers have been designed starting from the 5'UTR sequence.

References

(1) Zhang L., Liu Y. Potential Interventions for Novel Coronavirus in China: A Systematic Review. J Med Virol 2020 Feb 13.

(2) Te H.S., Randall G., Jensen D.M. Mechanism of action of ribavirin in the treatment of chronic hepatitis C. Gastroenterol Hepatol (N Y) 2007 Mar;3(3):218-225.

(3) Voshavar C. Protease Inhibitors for the Treatment of HIV/AIDS: Recent Advances and Future Challenges. Curr Top Med Chem 2019;19(18):1571-1598.

(4) Sheahan T.P., Sims A.C., Graham R.L., Menachery V.D., Gralinski L.E., Case J.B., et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med 2017 Jun 28;9(396):10.1126/scitranslmed.aal3653.

(5) Wang M., Cao R., Zhang L., Yang X., Liu J., Xu M., et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res 2020 Feb 4.

(6) Cao B., Wang Y., Wen D., Liu W., Wang J., Fan G., et al. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. N Engl J Med 2020 Mar 18.

(7) Chen Y., Liu Q., Guo D. Emerging coronaviruses: Genome structure, replication, and pathogenesis. J Med Virol 2020 Apr;92(4):418-423.

(8) Chan J.F., Kok K.H., Zhu Z., Chu H., To K.K., Yuan S., et al. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microbes Infect 2020 Dec;9(1):221-236.

(9) Zhou P., Yang X.L., Wang X.G., Hu B., Zhang L., Zhang W., et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020 Mar;579(7798):270-273.

(10) Stadler K., Masignani V., Eickmann M., Becker S., Abrignani S., Klenk H.D., et al. SARS--beginning to understand a new virus. Nat Rev Microbiol 2003 Dec;1(3):209-218.

(11) Yang D., Leibowitz J.L. The structure and functions of coronavirus genomic 3' and 5' ends. Virus Res 2015 Aug 3;206:120-133.

(12) Fields BN, Knipe DM, Howley PM, Griffin DE. Fields virology. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001.

(13) Lai M.M., Cavanagh D. The molecular biology of coronaviruses. Adv Virus Res 1997;48:1-100.

(14) Makino S., Stohlman S.A., Lai M.M. Leader sequences of murine coronavirus mRNAs can be freely reassorted: evidence for the role of free leader RNA in transcription. Proc Natl Acad Sci U S A 1986 Jun;83(12):4204-4208.

(15) Sawicki S.G., Sawicki D.L. Coronavirus transcription: subgenomic mouse hepatitis virus replicative intermediates function in RNA synthesis. J Virol 1990 Mar;64(3):1050-1056.

(16) Sawicki S.G., Sawicki D.L. A new model for coronavirus transcription. Adv Exp Med Biol 1998;440:215-219.

(17) Zeng F.Y., Chan C.W., Chan M.N., Chen J.D., Chow K.Y., Hon C.C., et al. The complete genome sequence of severe acute respiratory syndrome coronavirus strain HKU-39849 (HK-39). Exp Biol Med (Maywood) 2003 Jul;228(7):866-873.

(18) Li T., Zhang Y., Fu L., Yu C., Li X., Li Y., et al. siRNA targeting the leader sequence of SARS-CoV inhibits virus replication. Gene Ther 2005 May;12(9):751-761.

(19) Marra M.A., Jones S.J., Astell C.R., Holt R.A., Brooks-Wilson A., Butterfield Y.S., et al. The Genome sequence of the SARS-associated coronavirus. Science 2003 May 30;300(5624):1399-1404.

(20) Wu C.J., Huang H.W., Liu C.Y., Hong C.F., Chan Y.L. Inhibition of SARS-CoV replication by siRNA. Antiviral Res 2005 Jan;65(1):45-48.

(21) Wu C.J., Chan Y.L. Antiviral applications of RNAi for coronavirus. Expert Opin Investig Drugs 2006 Feb;15(2):89-97.

(22) Zhang Y., Li T., Fu L., Yu C., Li Y., Xu X., et al. Silencing SARS-CoV Spike protein expression in cultured cells by RNA interference. FEBS Lett 2004 Feb 27;560(1-3):141-146.

(23) Wang Y., Cao Y.L., Yang F., Zhang Y., Wang S.H., Liu L. Small interfering RNA effectively inhibits the expression of SARS coronavirus membrane gene at two novel targeting sites. Molecules 2010 Oct 18;15(10):7197-7207.

(24) Li B.J., Tang Q., Cheng D., Qin C., Xie F.Y., Wei Q., et al. Using siRNA in prophylactic and therapeutic regimens against SARS coronavirus in Rhesus macaque. Nat Med 2005 Sep;11(9):944-951.

(25) Zheng B.J., Guan Y., Tang Q., Du C., Xie F.Y., He M.L., et al. Prophylactic and therapeutic effects of small interfering RNA targeting SARS-coronavirus. Antivir Ther 2004 Jun;9(3):365-374.

(26) Chang Z., Hu Z. RNAi therapeutics: can siRNAs conquer SARS? Gene Ther 2006 Jun;13(11):871-872.

(27) Taiaroa G., Rawlinson D., Featherstone L., Pitt M., Caly L., Druce J., et al. Direct RNA sequencing and early evolution of SARS-CoV-2. bioRxiv :https://doi.org/10.1101/2020.03.05.976167.

(28) Zhang J.J., Huang A.L., Shi X.L., Zhang X.F. Promoter activity of SARS coronavirus 5' UTR sequence in eukaryotic cells. Sichuan Da Xue Xue Bao Yi Xue Ban 2006 Jan;37(1):5-9.

(29) Sawicki S.G., Sawicki D.L., Siddell S.G. A contemporary view of coronavirus transcription. J Virol 2007 Jan;81(1):20-29.

(30) Cameron CE, Götte M, Raney KD. Viral genome replication. New York, NY: Springer; 2009.

(31) Brian D.A., Baric R.S. Coronavirus genome structure and replication. Curr Top Microbiol Immunol 2005;287:1-30.

(32) Tan Y.W., Hong W., Liu D.X. Binding of the 5'-untranslated region of coronavirus RNA to zinc finger CCHC-type and RNA-binding motif 1 enhances viral replication and transcription. Nucleic Acids Res 2012 Jun;40(11):5065-5077.

(33) Liu P., Li L., Millership J.J., Kang H., Leibowitz J.L., Giedroc D.P. A U-turn motif-containing stem-loop in the coronavirus 5' untranslated region plays a functional role in replication. RNA 2007 May;13(5):763-780.

(34) Madhugiri R., Karl N., Petersen D., Lamkiewicz K., Fricke M., Wend U., et al. Structural and functional conservation of cis-acting RNA elements in coronavirus 5'-terminal genome regions. Virology 2018 Apr;517:44-55.

(35) Li L., Kang H., Liu P., Makkinje N., Williamson S.T., Leibowitz J.L., et al. Structural lability in stem-loop 1 drives a 5' UTR-3' UTR interaction in coronavirus replication. J Mol Biol 2008 Mar 28;377(3):790-803.

(36) Janssen H.L., Reesink H.W., Lawitz E.J., Zeuzem S., Rodriguez-Torres M., Patel K., et al. Treatment of HCV infection by targeting microRNA. N Engl J Med 2013 May 2;368(18):1685-1694.

(37) Machlin E.S., Sarnow P., Sagan S.M. Masking the 5' terminal nucleotides of the hepatitis C virus genome by an unconventional microRNA-target RNA complex. Proc Natl Acad Sci U S A 2011 Feb 22;108(8):3193-3198.

(38) Kertesz M., Iovino N., Unnerstall U., Gaul U., Segal E. The role of site accessibility in microRNA target recognition. Nat Genet 2007 Oct;39(10):1278-1284.

(39) Ludwig N., Leidinger P., Becker K., Backes C., Fehlmann T., Pallasch C., et al. Distribution of miRNA expression across human tissues. Nucleic Acids Res 2016 May 5;44(8):3865-3877.

(40) Bruscella P., Bottini S., Baudesson C., Pawlotsky J.M., Feray C., Trabucchi M. Viruses and miRNAs: More Friends than Foes. Front Microbiol 2017 May 15;8:824.

(41) Dutkiewicz M., Grunert H.P., Zeichhardt H., Lena S.W., Wengel J., Kurreck J. Design of LNA-modified siRNAs against the highly structured 5' UTR of coxsackievirus B3. FEBS Lett 2008 Sep 3;582(20):3061-3066.

(42) Laxton C., Brady K., Moschos S., Turnpenny P., Rawal J., Pryde D.C., et al. Selection, optimization, and pharmacokinetic properties of a novel, potent antiviral locked nucleic acid-based antisense oligomer targeting hepatitis C virus internal ribosome entry site. Antimicrob Agents Chemother 2011 Jul;55(7):3105-3114.
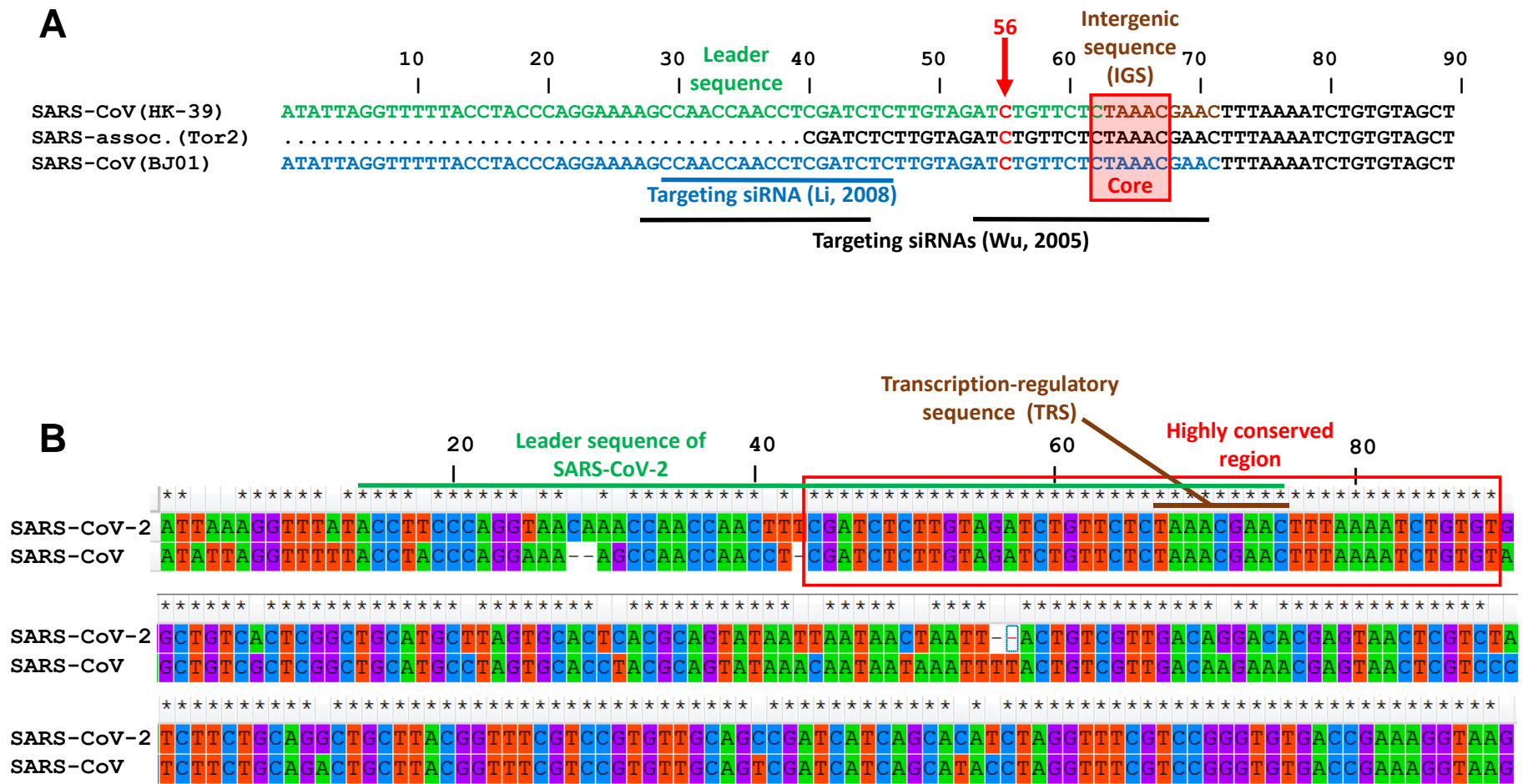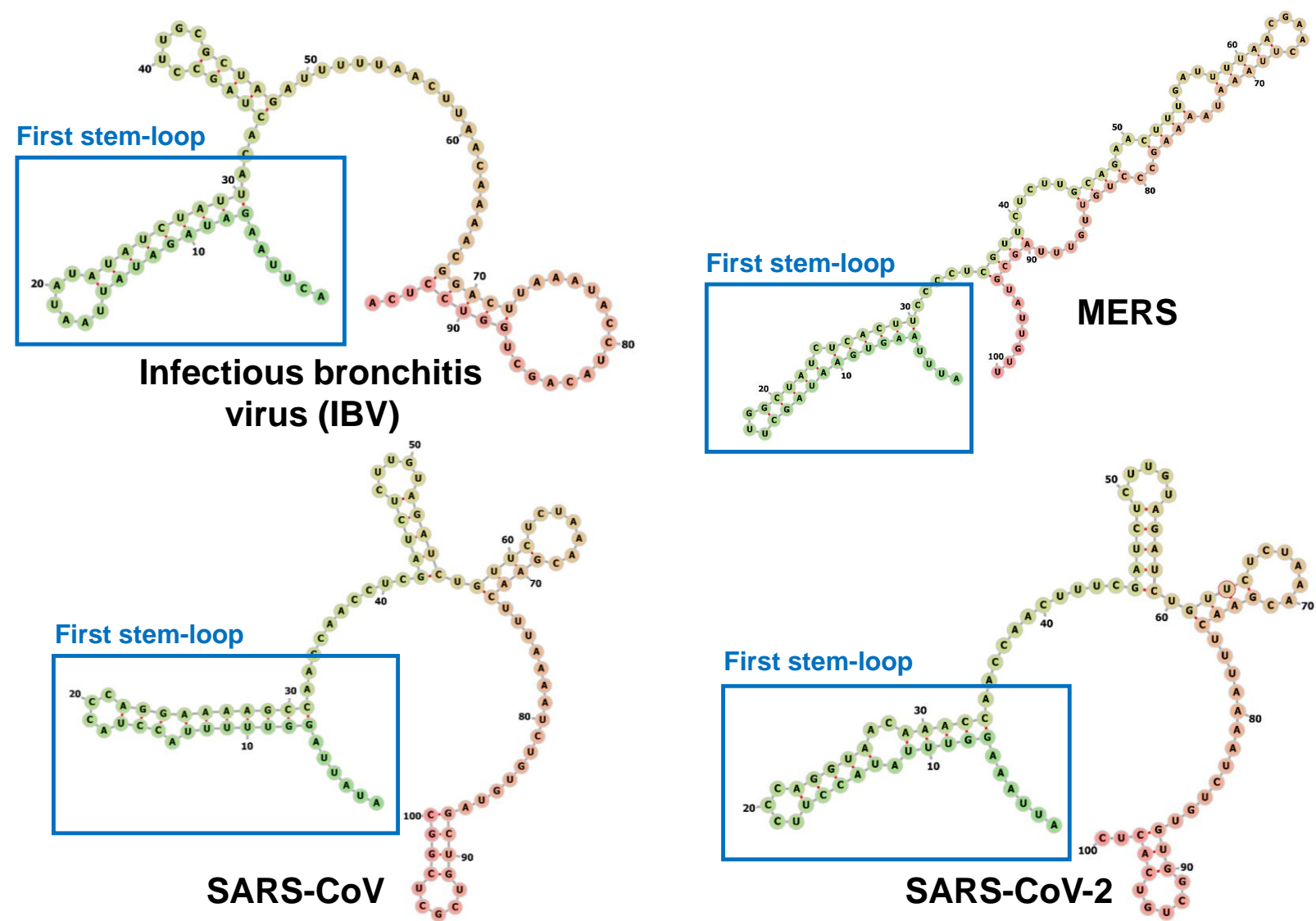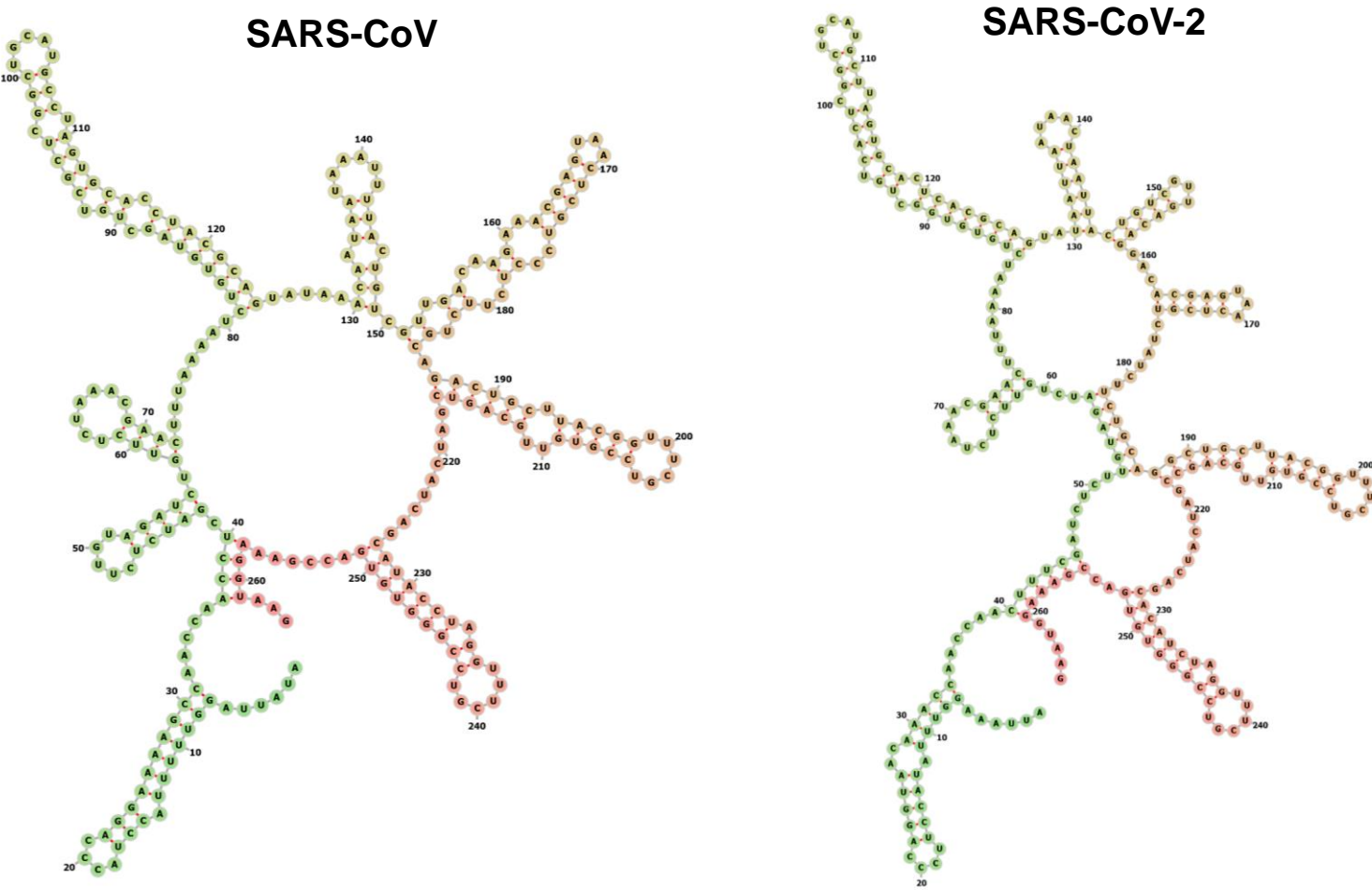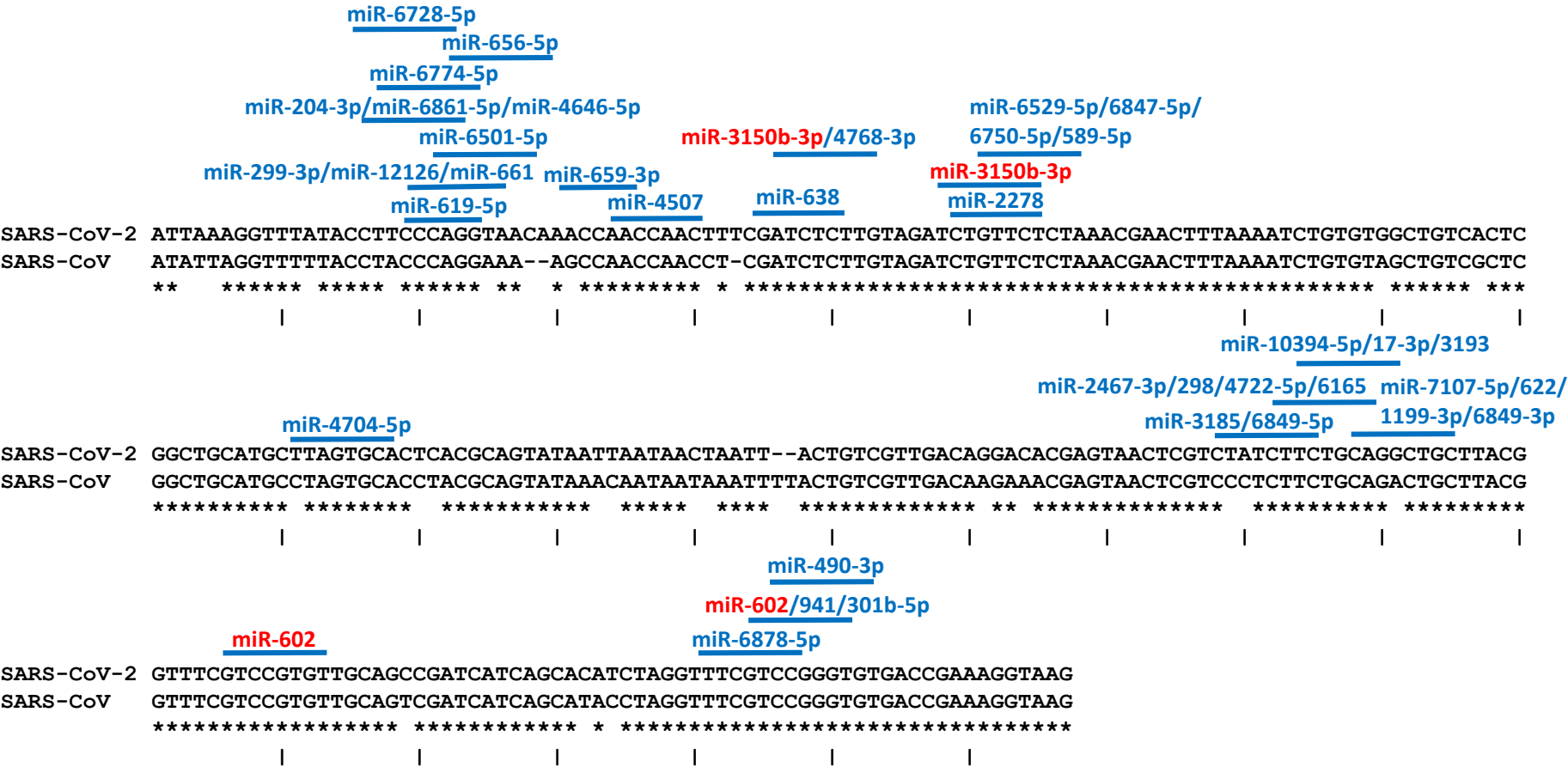
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