PERSPECTIVES | The Pathophysiology of COVID-19 and SARS-CoV-2 Infection

SARS-CoV-2 may regulate cellular responses through depletion of specific host miRNAs

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INTRODUCTION

Coronaviruses (CoVs) are single-stranded RNA viruses that infect a wide variety of animals including humans (HCoV). They have generally been considered to be relatively innocuous until the outbreaks of severe acute respiratory coronavirus (SARS-CoV) in 2002 (18, 31, 108), the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 (120), and the recent coronavirus disease 2019 (COVID-19) caused by severe acute respiratory coronavirus 2 (SARS-CoV-2), which resulted in a global pandemic (130). Each of these dangerous human coronaviruses (HCoVs) has been reported to differ significantly in their pathomechanisms of infection, despite the similarities in their RNA sequences (17). SARS-CoV-2’s active viral replication occurs in the upper respiratory tract (111), and its severe presentation requires a virus receptor expressed in lung epithelial cells (39, 46). Furthermore, 75% of people with severe COVID-19 disease develop evidence of pneumonia (102) and evidence of a strong inflammatory response in the lungs. Unfortunately, a fraction of COVID-19 patients can further develop into severe acute respiratory distress syndrome (20, 36, 77, 110). Previous studies indicate that both SARS-CoV and MERS-CoV infections are accompanied by a delayed/deregulated interferon response in primary human airway epithelial cells. This indicates that these HCoVs have developed the ability to attenuate innate immunity (58, 67), whereas harmless HCoVs seem to lack this capability (117). Similar to SARS-CoV and MERS-CoV, infections, elevated levels of proinflammatory cytokines, the so-called “cytokine storm,” have been reported in COVID-19 patients (47). These findings stress the critical roles of the expression of lung cell-specific receptors for viral entry and the importance of innate immunity in determining the mechanisms controlling the inflammatory responses during SARS-CoV-2 infections.

Given that there is no vaccine currently available for COVID-19, although there are promising candidates, research into the pathogenesis of this viral infection has focused on understanding the mechanisms involved in viral entry and replication, and importantly, in restoring and enhancing innate and adaptive immunity. Following virus attachment and entry into host cells, the viral particle is uncoated and its positive-sense single-stranded RNA genome is released into cytosol where it serves as a matrix for the host translation machinery to produce viral proteins (33). A virus replication cycle such as this potentially exposes its RNA to an antiviral cellular defense that relies on the host’s endogenous microRNAs (miRNAs). These miRNAs could prevent viral protein translation and/or directly degrade its RNA and prevent viral protein translation, such as has been reported for the influenza virus (82). Furthermore, miRNAs, through their role in posttranscriptional gene regulation, modulate cellular signaling that regulates immune responses (100). Notably, a recent study proposed that bats, considered as the SARS-CoV-2 host of origin, have tolerance to potentially deadly viruses because of specific miRNAs (25). Furthermore, viruses can also take advantage of either their
host’s or their own miRNAs to facilitate viral replication or to inhibit the host’s antiviral responses (3). miRNA regulation or manipulation could therefore provide a novel basis for antiviral drug therapies.

miRNA expression profiles are often cell-type specific and differ between individuals, and importantly, can be affected by cellular stress responses (11, 12). In our recent study, we demonstrated that exposure of human lung epithelial cells to endoplasmic reticulum stress (ER stress) results in a global reduction of miRNA expression levels (37) and may also lead to activation of a miRNA-based reduction of antigen presentation (7). Notably, ER stress and the downstream activation of stress responses has been reported during coronavirus infections (16, 35), and has been proposed to facilitate SARS-CoV replication (16). Thus, the individual and epigenetic differences in the miRNA profiles during an infection in human lung epithelial cells could affect the effectiveness of the antiviral responses and the disease severity.

Although underappreciated, miRNAs expressed in human lung cells may be an important factor determining the COVID-19’s severity. Using a bioinformatic analysis of human miRNA potential interactions with the SARS-CoV-2’s genome, we examined the potential miRNA target sites in seven coronavirus genomes that include SARS-CoV-2, MERS-CoV, SARS-CoV, and four nonpathogenic coronaviruses.

COMPARISON OF THE miRNA TARGET SITES IN HUMAN CORONAVIRUSES

To examine whether SARS-CoV-2 could be differentially targeted by human endogenous miRNAs compared with other HCoVs, we analyzed and compared the potential microRNA target sites (MTSs) within a set of seven HCoVs RNA genomes. Our approach was to examine and compare three pathogenic and four nonpathogenic strains of HCoVs. The HCoV RNA genomes of pathogenic strains were SARS-CoV-2 (NC_045512.2), SARS-CoV (NC_004718.3), and MERS-CoV (NC_019843.3). The nonpathogenic strains were HCoV-OC43 (KU131570.1), HCoV-229E (NC_002645.1), HCoV-HKU1 (KF686346.1), and HCoV-NL63 (NC_005831.2). These coronaviruses were tested against the set of 896 confident mature human miRNA sequences that were obtained from the mirBase v2.21 (54, 55) using the RNA22 v2 microRNA target prediction tool (70). The results of the MTS predictions, the strictest parameters were applied to the default computation workflow using a specificity of 92% versus a sensitivity of 22% (70). The results of the MTS mapping to viral RNAs are provided in Supplemental Data Set S1 (see https://doi.org/10.5281/zenodo.3966446).

This analysis illustrates the relative genome size for all seven HCoVs (Fig. 1A, white bars), and indicates that the potential number of MTSs was elevated in the pathogenic strains compared with the nonpathogenic strains (Fig. 1A, black bars). Interestingly, there were no major differences in miRNA numbers when normalized to the genome size (Fig. 1B). The observed differences in MTS number between pathogenic and nonpathogenic HCoVs could be an artifact of small experimental numbers since only seven unique viral genomes were analyzed. Given that concern, we next examined the number of miRNAs that were common or different that could potentially bind to each of the coronavirus RNAs. As shown in Fig. 1C, the pathogenic HCoVs attract a set of miRNAs that differ from the nonpathogenic HCoVs. A detailed analysis defines a set of 28 miRNAs that are unique for SARS-CoV-2, as well as the set of another 21 and 24 miRNAs that are unique for SARS-CoV and MERS-CoV, respectively (Fig. 1D).

On the basis of the above analysis, we envision three different possibilities.

Host miRNAs Serve as an Antiviral Shield

In this possibility, the miRNAs that are specific for nonpathogenic HCoVs are abundant in bronchial epithelial cells and thus provide one type of defense mechanism for viral infection. Whereas, the levels of miRNA specific for the pathogenic coronaviruses are either low or lowered during these viral infections. To test this hypothesis, we compared miRNA abundances between the miRNA specific for pathogenic and nonpathogenic HCoVs. Our approach was to use miRNA expression profiles of normal human bronchial epithelial (HBE) cells. For these studies, we analyzed data from a previous next-generation sequencing analysis of 16HBE14o-cells and primary HBEs. The deep sequencing data are deposited in the Gene Expression Omnibus (GEO) at accession number GSE117629 (37).

As shown in Fig. 1, E and F, the abundance of miRNAs that could recognize pathogenic versus nonpathogenic HCoVs were similar, and the vast majority of miRNAs were well expressed in 16HBE14o- and primary HBE cells (bronchial epithelial cells differentiated at an air-liquid interface). This is despite the fact that the next-generation sequencing depths for these two sample sets were different [12 million (M) reads per sample (primary HBE cells) versus 31 M reads per sample (16HBE14o-cells)]. The miRNAs that potentially could target SARS-CoV-2 were well expressed and similar in both primary HBE and 16HBE14o- cells (Fig. 1G). Hence, the hypothesis that the lung cells lack specific miRNA directed against pathogenic HCoVs seems rather unlikely, although it still cannot be excluded for specific miRNAs. This observation questions the use of miRNA overexpression (miRNA mimics) as a therapeutic approach against the COVID-19 infection since siRNAs directed against viral RNA would be more specific and therefore less prone to have off-target effects (12).

HCoVs RNAs Serve as microRNA Sponges

Given the observation that the pathogenic HCoVs have more potential MTSs in their RNA than the nonpathogenic ones, we speculate that these viruses work like specific microRNA sponges that reduce cellular miRNA levels and therefore modulate the host’s cellular processes to facilitate viral replication. Viral infections result in high abundance of viral RNA and the higher potential miRNA binding sites these pathogenic viruses harbor could offer a very effective defense by lowering the cellular miRNA levels during the early stages of an infection.

How likely is it that a viral RNA can act as a miRNA sponge? Host miRNAs constitute ~0.01% of total cellular and tissue RNA (81), while the viral RNA in infected lung cells can reach more than 50% of total cellular RNA (13). Furthermore, evidence that viral RNA sponges are capable of being efficient in removing host miRNAs is demonstrated in studies on Epstein-Barr virus which illustrates that EBV miRNA sponges regulate virus infection as well as oncogenesis by downregu-
lating a number of target miRNAs (85). Viral RNA sponge activity has also been reported for Herpes virus (15) and hepatitis C virus (64).

Additionally, the pathogenic HCoVs could remove host-specific miRNAs to modulate specific gene expression to suppress immunity or prevent activation of unfolded protein response (UPR)-related apoptosis. To follow this hypothesis, we focused on the potential role of the 28 miRNAs that were uniquely specific for SARS-CoV-2 (Fig. 1D). As shown in Table 1, the majority of these miRNAs are well expressed in bronchial epithelial cells, and their dysregulation has been reported for various human lung pathologies that include lung cancers, chronic obstructive pulmonary disease, cystic fibrosis, and tuberculosis. Furthermore, many of these miRNAs have been proposed to act as tumor suppressors that target apoptosis-related pathways, and thus their reduction has been associated with poor cancer prognosis. Hence, SARS-CoV-2, by its potential reduction of the host’s miRNA pool, may promote infected cell survival and thus continuity of its replication cycle.

Another possibility is the enrichment of MTSs in the virus RNA that are sites for specific miRNAs. The 10 miRNAs that have the highest MTS number in SARS-CoV-2’s RNA genome are listed in Table 2. Although they are not unique for this virus, the MTSs for them are more frequent in the pathogenic HCoVs. Again, some of these miRNAs are either UP trigger regulators (miR-34c-5p; miR-34a-5) or modulators of immune responses (miR-149-3p).

Viruses proteins require and extensively use the endoplasmic reticulum (ER) and Golgi system for proper protein folding and assembly. Except for the N protein, all coronavirus structural proteins are transmembrane proteins synthesized in the ER (35). Furthermore, ER membranes are crucial for the formation of virus double-membrane vesicles (DMVs) (28) that require both virus transmembrane proteins and massive morphological rearrangement of the ER (52, 86). Both the increased ER membrane and the protein folding demand can result in coronavirus-related ER stress and activation of a dedicated signaling pathway, the unfolded protein response (UPR). Importantly, the UPR has the ability to promote cellular survival by increasing ER membranes and folding capacity, but also has the ability to induce cell death if the stress is persistent (5, 8, 10, 37, 45, 51, 104).

During the UPR, the increased demand for chaperone proteins in ER results in the dissociation of the glucose-regulated protein 78 (GRP78 also known as Bip (binding immunoglobulin protein)] from luminal domains of three ER proteins, protein kinase RNA-like endoplasmic reticulum kinase (PERK), the inositol-requiring enzyme 1α (IRE1α), and activating transcription factor 6 (ATF6) (45). When not associated with Bip, PERK and IRE1α undergo multimerization and transautophosphorylation to become active, whereas ATF6 is proteolytically processed to its transcriptionally active form (90). ATF6 promotes protein chaperone and lipid synthesis and enhances N-glycosylation (59, 122). PERK gains control on the cellular translation processes to reduce ER load (38, 41), and eventually facilitates autophagy. If the stress is persistent, PERK and ATF6 induce cell death via accumulation of CCAAT/enhancer binding homologous protein (CHOP) (2, 8, 49, 80). IRE1α uses its endoribonuclease properties to selectively reduce ER mRNA (40, 72) and to produce the active isoform of the X-box binding protein transcription factor (XBP1s) (118). XBP1s increases the ER’s folding capacity and ER membrane biosynthesis, as well as vesicular trafficking (4, 7, 118). XBP1s can also lead to decreased antigen presentation (7). Importantly, IRE1α activates JNK and this leads to an inflammatory response and apoptosis (40, 101).

Indeed, the pathogenic HCoVs infections lead to ER stress and UPR activation (35). The induction of adaptive ER-stress chaperones such as glucose-regulated protein 94 (GRP94) and Bip was observed in cells infected with SARS-CoV (29, 50, 115), along with increased levels of homocysteine-inducible, ER stress-inducible, ubiquitin-like domain member 1 (HERPUD1), a protein involved in the ER degradation pathway (ERAD) (103). Nevertheless, the HCoV effects on the specific UPR branches remain limited and rather unclear, and differ depending on virus and cell culture model used (reviewed in Ref. 35). Notably, however, NF-κB interplay with both IRE1 and PERK pathways has also been proposed to contribute to the cytokine storm that accompanies SARS-CoV (84, 103). The detailed mechanisms underlying this observation, however, remain poorly understood (30, 106).

Taken together, the viral strategies to increase the ER capacities and ER folding capacity and block UPR-associated translational attenuation, inflammatory responses, and apoptosis are critical components for virus production. This strategy could be achieved through modulation of host miRNAs that are involved in the UPR and/or in inflammatory responses. As shown in Table 1, COVID-19-mediated reduction of host miR-34a-3p and miR-495-5p levels could increase XBP1s and Bip expression, respectively, by increasing the ER folding capacity and promoting survival. Through miR-376b-3p, the virus could also potentially modulate the mTOR and autophagy pathways. Furthermore, some of these miRNAs have been proposed to modulate innate responses that include miR-376a-3p, miR-99b-5p, miR-10a-5p, miR-376a-3p, miR-548av-

Fig. 1. Distribution of human miRNAs and microRNA (miRNA) target sites (MTSs) in human coronavirus (HCoV) RNAs. A: the number of miRNAs is marked in light gray bars, the MTS number is marked in dark gray bars, and the virus RNA length (in kb) is marked in white bars. The results of the MTS mapping to viral RNAs are provided in Supplemental Data Set S1 (https://doi.org/10.5281/zenodo.3966446). C: the Venn diagram (43) represents distribution of miRNAs between the COVID-19, SARS-CoV, MERS-CoV, and the group of nonpathogenic HCoVs is shown. *P value compared with all HCoVs (P = 0.0122 and P = 0.0289). **P value for the pathogenic compared with the nonpathogenic groups (P = 0.0001). A P value less than 0.05 was considered significant. E and F: comparison of the miRNA expression profiles is shown for 16HBE14o- cells (median of raw reads: 31M/sample; n = 10) and for primary human bronchial epithelial cells (median of raw reads: 12M/sample; n = 24).
Although these predictions will require further experimental validation, they support the hypothesis that SARS-CoV-2 and other HCoVs modulate host miRNAs to favorably fine-tune the ER of infected cells and protect themselves from the immune system.

Our initial assessment of virus-controlled miRNA effects was focused on literature reporting miRNA effects; however, miRNAs have multiple mRNA targets and thus a wide range of cellular responsibilities. To address this, using the mirDIP database (99) with only the top 1% of the most probable targets considered, we analyzed the potential targets of miRNA that could be bound to either the pathogenic, the nonpathogenic, or both groups of HCoVs. As shown in Table 1, these predictions will require further experimental validation, they support the hypothesis that SARS-CoV-2 and other HCoVs modulate host miRNAs to favorably fine-tune the ER of infected cells and protect themselves from the immune system.

### Table 1. Human miRNAs that could potentially interact with severe acute respiratory coronavirus 2 and their biological roles

| miRNA      | Abundance in HBE | MTS | Potential Impact on Cellular Processes |
|------------|------------------|-----|---------------------------------------|
| hsa-let-7a-3p | Q3/Q2            | 1   | Reduced expression in human lung cancers is associated with shortened postoperative survival (96). |
| hsa-miR-10a-5p | Q3/Q3            | 1   | Constrains the plasticity of helper T cells (95). |
| hsa-miR-126-3p | Q3/Q3            | 1   | Downregulated in breast cancer (19). |
| hsa-miR-154-3p | Q2/nd            | 1   | Is profibrotic in pulmonary fibrosis (69). |
| hsa-miR-195-3p | Q1/nd            | 2   | Deregulated in cancer (119). |
| hsa-miR-200a-5p | Q3/Q3            | 1   | Suppresses MMP9 expression in lung cancer (23). |
| hsa-miR-345-5p | Q3/Q3            | 1   | Deregulated in colorectal cancer (97). |
| hsa-miR-34a-3p | Q3/Q2            | 1   | Low tissue expression was associated with progression and poor prognosis of lung cancer (22). |
| hsa-miR-3664-5p | Q2/Q1            | 2   | Altered in sputum of patients with active pulmonary tuberculosis (116). |
| hsa-miR-376a-3p | Q3/Q1            | 1   | Targets insulin growth factor 1 receptor (121). |
| hsa-miR-376b-3p | Q3/nd            | 1   | Inhibits lung cancer progression (107). |
| hsa-miR-3939 | Q1/Q1            | 1   | Deregulated in lung cancer (114, 129). |
| hsa-miR-4746-5p | Q2/Q2            | 1   | Deregulated in cancers (24, 48). |
| hsa-miR-495-5p | Q2/nd            | 1   | Targets UPR in lung cancer cells - reduction of these miRNA might be the reason for upregulation of GRP78 in NSCLC patients (1). |
| hsa-miR-513a-3p | Q2/nd            | 1   | Controls starvation and mTOR inhibition-related autophagy by targeting ATG4C and BECN1 (53). |
| hsa-miR-513b-3p | Q3/nd            | 1   | Deregulated in lung cancer (105). |
| hsa-miR-513c-3p | Q2/nd            | 1   | Deregulated in colorectal cancer (88). |
| hsa-miR-514b-5p | Q1/nd            | 1   | Potentially associated with transition from immune tolerance to immune activation of chronic hepatitis B (113). |
| hsa-miR-515-5p | Q3/Q2            | 3   | Suppresses non-small cell lung cancer cells growth and invasion via downregulating NOTCH1 (63). |
| hsa-miR-520-5p | Q3/Q1            | 1   | Mediates p53 related cellular responses to stress (112). |
| hsa-miR-521-3p | Q3/Q1            | 1   | Predicted miRNAs from *Toxoplasma gondii* potentially regulates the hosts' gene expression (89). |
| hsa-miR-525-3p | Q3/Q2            | 1   | A potential biomarker for predicting survival in lung adenocarcinoma (61). |
| hsa-miR-529-3p | Q3/Q1            | 1   | DNA damage response (74). |
| hsa-miR-532-3p | Q3/Q1            | 1   | Mycobacterium tuberculosis controls miR-99b expression in infected murine dendritic cells to modulate host immunity (92). |
| hsa-miR-538-3p | Q3/Q1            | 1   | Suppresses the expression of prostate-specific antigen and prostate cancer cell proliferation (94). |

The miRNA abundance in human bronchial epithelial cells is provided as a quartile (Q) of raw next generation sequencing distribution for 16HBE14o-/primary HBE. nd, not detected. COPD, chronic obstructive pulmonary disease; HCMV, human cytomegalovirus; MTS, miRNA target site; mTOR, mammalian target of rapamycin; NSCLC, non-small cell lung cancer; UPR, unfolded protein response; VEGF, vascular endothelial growth factor; XBP1, X-box binding protein transcription factor.
Table 2. miRNAs having the most miRNA target sites in severe acute respiratory coronavirus 2 RNA

| miRNA        | Abundance in HBE | MTS | Potential Impact on Cellular Processes |
|--------------|------------------|-----|--------------------------------------|
| hsa-miR-449c-5p | Q2/Q3           | 9   | 5 | 3 | 2 | 1 | 3 | 2 | Targets c-Myc and inhibits NSCLC cell progression (68) |
| hsa-miR-3940-5p | Q1/nd.          | 9   | 11 | 5 | 7 | 2 | 3 | 3 | Tumor Suppressor in NSCLCs (87) |
| hsa-miR-34c-5p  | Q3/Q3           | 9   | 5 | 11 | 1 | 6 | 7 | 3 | UPR-dependent and targets XBP1 (4, 6). |
| hsa-miR-34a-5p  | Q3/Q3           | 9   | 8 | 11 | 0 | 4 | 6 | 5 | UPR-IRE1 dependent and targets Caspase 2 (60). |
| hsa-miR-149-3p  | Q2/Q2           | 8   | 4 | 6 | 2 | 4 | 1 | 2 | Deregulated in COPD (32). |
| hsa-miR-92a-1-5p | Q3/Q2           | 8   | 4 | 4 | 5 | 5 | 2 | 0 | Deregulated in colorectal and lung cancers (42, 127). |
| hsa-miR-138-5p  | Q3/Q3           | 8   | 6 | 4 | 0 | 4 | 2 | 1 | Unknown |
| hsa-miR-4433b-3p | Q1/nd.          | 8   | 6 | 13 | 7 | 5 | 3 | 5 | Unknown |
| hsa-miR-766-5p  | Q3/Q1           | 7   | 4 | 3 | 1 | 5 | 4 | 3 | Unknown |
| hsa-miR-6741-5p | Q1/Q1           | 7   | 6 | 9 | 3 | 9 | 7 | 3 | Unknown |

Abundance in human bronchial epithelial cells is provided as a quartile (Q) of raw next generation sequencing reads distribution for 16HBE14o-/primary HBE. HBE, human bronchial epithelial; IRE, inositol-requiring enzyme; nd, not detected; NSCLC, non-small cell lung cancer; UPR, unfolded protein response.

A third possibility is that HCoVs encode in their own pre-miRNA sequences that could mature in human cells by entering the human RISC complex. miRNAs have been shown to be produced by both DNA viruses and positive- and negative-strand RNA viruses (reviewed in Ref. 14). Viral miRNAs have been identified for many human viruses, including influenza (83), EV71 (109), hepatitis A (91), and SARS-CoV (71). Although the abundance of these viral noncoding RNAs was shown to be relatively low in infected tissues (below 0.1%), their role in changing the phenotype of SARS-CoV infected cells was confirmed through the use of specific miRNA inhibitors that reduced inflammation and lung damage in vivo (71). Using the miRNAFold web server (98), we identified 10 pre-miRNA sequences in the SARS-CoV-2 RNA sequence that could potentially enter the human RNAi pathway (Supplemental Data Set S3; see https://doi.org/10.5281/zenodo.3966446). Clearly, however, further experimental research will be required to verify their presence in infected tissues as well as their functional roles.

Concluding Notes and Future Prospects

Despite the difficult and hopefully successful fight with the COVID-19 pandemic, there is no guarantee that other novel HCoV-related dangers will not appear in the future, and thus understanding the mechanisms that determine their pathogenicity will remain medically important. This is especially true with the subject of the host-virus interactions during infections. Here, based on an analysis of the current literature and using bioinformatic approaches, we discussed the potential mechanisms by which pathogenic HCoVs including SARS-CoV-2 could modulate host miRNA levels by acting as miRNA sponges to facilitate their replication and to avoid immune responses (Fig. 3). Because our studies focused on human bronchial epithelial cells (16HBE14o- cells and primary HBEs), it is important to note that there may be differences seen in other cell types such as nasal epithelial cells and alveolar type II epithelial cells. Our hypothesis will require validations starting with the assessment of these miRNA levels in infected tissues and ending with restoring the host miRNA balance with miRNA analogs. Furthermore, completely understanding how viruses take advantage of the ER and UPR pathway may also lead to novel therapeutic strategies. Nevertheless, other questions remain including determining how these viruses control the UPR, analyzing the role of the MTS frequencies in relation to the miRNA profile and infection stage, and finally, deciphering the complicated potential cooperation among numerous miRNAs that are affected by the
virus. Hopefully, noncoding RNAs of virus origin that are easier to target and test with synthetic analogs may turn out to be effective drug candidates as well. Finally, numerous reports have illustrated the large diversity in terms of responses and clinical outcomes to COVID-19 infections. One possible variable that has not been considered is the individual differences in patient’s miRNA profiles or polymorphisms in either MTSs or miRNA sequences (9, 11, 56, 73). In that regard, a recent

**Metabolism**
- Cholesterol biosynthesis
  - $P=0.002$ $Q=0.264$
- Formation of tubulin folding intermediates by CCT/TriC
  - $P=0.014$ $Q=0.264$
- Insulin receptor recycling
  - $P=0.014$ $Q=0.264$
- Netrin mediated repulsion signals
  - $P=0.014$ $Q=0.264$
- Folding of actin by CCT/TriC
  - $P=0.014$ $Q=0.264$
- Nef mediated downregulation of MHC I surface expression
  - $P=0.014$ $Q=0.264$
- Activation of Cα-permeable Kainate Receptor
  - $P=0.014$ $Q=0.264$
- Ionotropic activity of Kainate Receptors
  - $P=0.014$ $Q=0.264$
- Ligand-independent caspase activation via DCC
  - $P=0.014$ $Q=0.264$

**Inositol transporters**
- $P=0.005$ $Q=0.490$

**Na⁺-dependent glucose transporters**
- $P=0.065$ $Q=0.798$

**Catabolism of glucuronate to xylulose-5-phosphate**
- $P=0.065$ $Q=0.798$

**Sulfide oxidation to sulfate**
- $P=0.065$ $Q=0.798
study has suggested that COVID-19 virulence in aged patients may be due to a lower abundance of miRNAs and this may be a contributing factor in disease severity (34). This certainly supports the idea that the normal function of miRNAs is to effectively regulate mRNA levels and thus promote cellular homeostasis. Understanding these types of differences in patients is important for developing personalized antiviral therapies.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

R.B. prepared figures; R.B. and J.F.C. drafted manuscript; R.B., S.M., K.S.H., M.S., and J.F.C. edited and revised manuscript; R.B., M.D., B.J., S.M., K.S.H., M.S., and J.F.C. approved final version of manuscript.

Fig. 2. The impact of the pathogenic human coronaviruses (HCoV)-dependent modulation of hosts miRNA profiles and their potential consequences on cellular mRNA levels. A: Venn diagram (43) represents the potential general distribution of human mRNAs that could be affected by the miRNAs that target the pathogenic (orange), the nonpathogenic (green) HCoVs, and all seven coronaviruses (blue). The hypothesis is that the miRNA sponge effect used by the individual viruses could lead to an increase in these human mRNAs since these are also targets of these miRNAs. B: the Gene Ontology assignment of the cellular functions of mRNAs defines very specific targets for the miRNAs potentially regulated by the pathogenic HCoVs as assigned by the Enrichr web server (21, 57). C: the Gene Ontology assignment of the cellular function of mRNAs defines specific targets for the miRNAs potentially regulated by the harmless HCoVs. D: the Venn diagram represents the general distribution of mRNAs that are targets of miRNAs modulated by severe acute respiratory coronavirus 2 (SARS-CoV-2) (orange) and the nonpathogenic (green) HCoVs, and all 7 HCoVs (blue). E: the Gene Ontology assignment of the cellular functions of mRNAs that are high probability targets for the miRNAs potentially modulated by the SARS-CoV-2, as assigned by the Enrichr web server (21, 57). All of the mRNA targets were predicted with the miRDIP database (99) with only the top 1% of the most probable targets considered. The predicted targets lists are provided in Supplemental Data Set S2 (https://doi.org/10.5281/zenodo.3966446). The red bar color depicts the P value less than 0.05. The longer bars have the lower P values. The false discovery rate is provided as a Q value.

Fig. 3. The hypothesis is that severe acute respiratory coronavirus 2 (SARS-CoV-2) regulates cellular responses through depletion of specific host microRNAs (miRNAs). The SARS-CoV-2 uptake and replication cycle is illustrated and our model is that noncoding viral RNAs serve as sponges for the host miRNAs and this accomplishes two goals. First, it disrupts normal cellular homeostasis by upregulating specific host mRNA levels that are normally controlled by host miRNAs. And second, by downregulating certain host miRNAs, the virus enhances its own viral replication cycle and also attenuates immune responses. ER, endoplasmic reticulum; MTS, microRNA target sites.
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