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Importance of miRNA in SARS-CoV2 infection

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SUMMARY

At the end of 2019, a new pathogen, coronavirus SARS-CoV2, was identified. The virus has infected more than 30 million people worldwide with lethality close to 5 %. SARS-CoV2 is an RNA virus. The viral genome contains 29 891 nucleotides which encode 9 889 amino acids; 5´-replicase (orf1/ab) four main structural proteins [Spike (S) -Envelope (E) -Membrane (M) -Nucleocapsid (N)] and open reading frame proteins. MicroRNAs (miRNA) are potent post-transcriptional regulators of gene expression and hence can control viral infection and replication. In silico and bioinformatics assessments revealed that host miRNA (15b-5p, 15a-5p, 197-5p, 548c-5p, 548d-5p, 409-3p, 30b-5p, 505-3p) may be involved blocking viral replication. Also, viral miRNA are shared with cells miRNA (8066, 5197, 3611, 3934-3p, 1307-3p, 3691-3p, 1468-5p), which may modulate cell response and facilitate SARS-CoV2 infection. Even though, these targets have to be validated with studies in vitro and in vivo, there is a high therapeutic potential involved which has been proposed and tested in other viral infections. More studies on the molecular mechanism of this complex viral infection are required to understand viral pathogenesis.

Key words: *SARS-CoV2, miRNA, gene regulation, exosomes, viral replication.*

RESUMEN

Al final del 2019, un nuevo patógeno, el coronavirus SARS-CoV2, fue identificado. El virus ha infectado a más de 30 millones de personas en el mundo entero con una letalidad cercana al 5 %. El SARS-CoV2 es un ARN virus. El genoma viral contiene 29 891 nucleótidos que codifican para 9 889 aminoácidos; 5´-replicasa (orf1/ab), cuatro proteínas estructurales principales [espiga (S)-Envoltura (E)-Membrana (M)-Nucleocápside (N)] y las proteínas de la región terminal ORF. Los microARN (miARN) son potentes reguladores pos-transcripcionales de la expresión génica y por ende pueden controlar la infección y replicación viral. Estudios in silico y bioinformática revelaron los miARN del huésped que afectan la replicación viral (15b-5p, 15a-5p, 197-5p, 548c-5p, 548d-5p, 409-3p, 30b-5p, 505-3p). Además hay miRNA virales que son compartidos con el hospedero, (8066, 5197, 3611, 3934-3p, 1307-3p, 3691-3p, 1468-5p) y son importantes en la infección por SARS-CoV2. Aún cuando esas moléculas deben ser validadas en estudios in vitro e in vivo, hay un alto potencial terapéutico involucrado que ya ha sido propuesto y usado en otras infecciones virales. Más estudios de los mecanismos

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moleculares de esta compleja infección viral son necesarios para entender la patogénesis viral.

Palabras clave: *SARS-CoV2, miRNA, regulación génica, exosomas, replicación viral.*

Conflict of Interest None.

CORONAVIRUS

Coronaviruses (CoVs) are enveloped single chain RNA viruses. The Coronavirdiae family is formed by four subfamilies Alpha coronavirus (αCoV), Beta coronavirus (βCoV), Delta coronavirus (δCoV) and Gamma coronavirus (γCoV). A total of 7CoVs are known to infect humans, consist of two alpha CoV (HCoV-229E and HKU-NL63) and five beta CoV (HCoVOC43, HCoV-HKU1, SARS-CoV, SARS-CoV2, and MERS-CoV) (1).

Human CoVs have been circulating for a long time infecting humans. The coronavirus infections, characterized by flu-like symptoms, are often observed in the cold season (1). It was considered a benign infection. However, this definition changed in 2002-2003 since there was an outbreak, in China, of coronavirus that caused severe acute respiratory syndrome. The virus then was named SARS-CoV2. The virus was highly contagious, with 8 000 confirmed cases, with a mortality rate of 9.6 % (2). The prompt epidemiological control prevented the spreading of the virus. Then, there was another outbreak, this time in Middle East countries in 2012, and South Korea in 2015. The confirmed cases of MERS-CoV exceeded 2 000, with a mortality rate of ~35 % (1,2). Last year, there was an actual outbreak of SARS-CoV2 which belongs to the same family (1). The virus spread worldwide to the lack of proper control of wildlife animal consumption and erratic epidemiological controls. Up to date, there is no medical treatment approved worldwide, and different types of vaccines have been developed to enhance immune response and control of virus spreading.

The new corona viruses, SARS-CoV2, SARS-CoV2, and MERS, infect, mainly, the cells of the respiratory tract (3). They use the angiotensin-converting enzyme 2 (ACE2) as the primary receptor. SARS-CoV2 has an incubation period between 1-14 days, ranging from 3-7 days. The symptoms most commonly described are mild and moderate fever, weakness, dry cough, headache, nasal congestion, sore throat, muscle pain, gastrointestinal symptoms, and lack of smell and taste. The elderly population is more susceptible to develop severe disease. High mortality is recorded in patients with comorbidities, obesity, hypertension, diabetes, and immunocompromised patients.

This minireview focuses on the role of non-coding RNA in SARS-CoV2 infection, the importance of analyzing viral and cellular miRNA, and the pathways involved which would affect viral replication and host response to viral infection.

Micro RNAs

A microRNA (miRNA) is a short non-coding RNA molecule (18-22 nucleotides long) that bind to the 3'UTR site of messenger RNA (mRNA), found in plants, animals, and some viruses. These non-coding RNA silences post-transcriptional regulation of gene expression by base-pairing with complementary sequences within mRNA molecules (4), shown in Figure 1. Consequently, specific mRNA molecules are silenced, by one or more of the following processes: (a) Cleavage of the mRNA strand into two pieces, (b) Destabilization of the mRNA through shortening of its poly(A) tail, and (c) Less efficient translation of the mRNA into proteins by ribosomes (4-5). MiRNA differs from small interfering RNA (siRNA) since they derive from regions of RNA transcripts that fold back on themselves to form hairpins, whereas siRNA comes from more extended parts of double-stranded RNA. There at least 600 miRNA identified in the human genome and may target close to 60 % of the transcribed genes

MiRNAs play an essential role in cell proliferation, cell differentiation, or cell death. MiRNAs have recently emerged as critical modulators of viral infections. They can function as suppressors of gene expression by targeting cellular or viral RNAs during infection (5). There is a potential for miRNA therapeutics, and several clinical trials are underway (6).

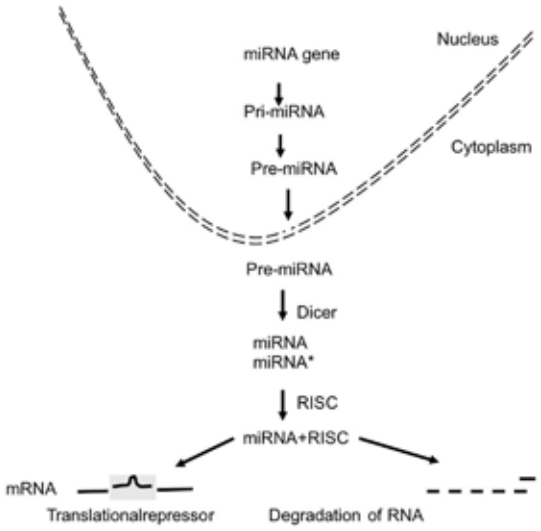

Figure 1. Genesis and function of miRNA in eukaryotic cells. The generation of pre-miRNA occurs in the nucleus and then the molecule. Dicer, also known as endoribonuclease Dicer or helicase with RNase motif, is an enzyme that in humans is encoded by the DICER1 gene.

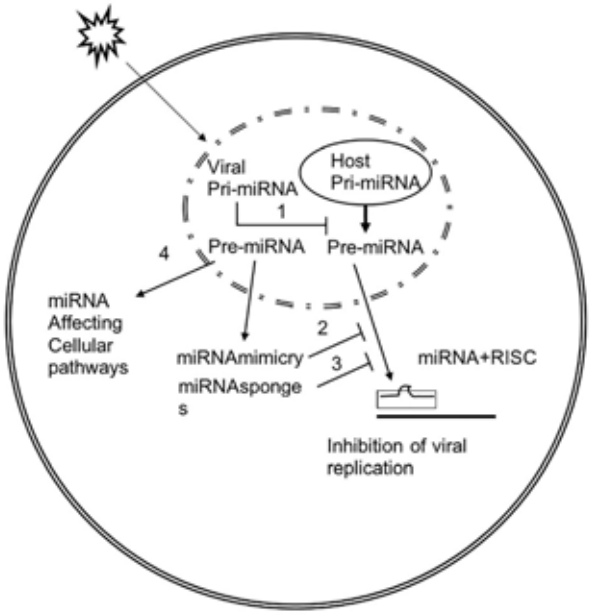

Figure 2. Importance of miRNA on viral infection and replication. Cell miRNAscan inhibits viral replication by binding to viral RNA. However, the virus may block this inhibition by four different mechanisms: 1) inhibition of host's generation of miRNA, Dicer and RISC, 2) viral miRNA mimicry to block cell's miRNA, 3) several miRNAscan block either miRNA or mRNA form the host, 4) viral miRNA can modulate cellular pathways to block or inhibit cell response against the virus.

Effect of host miRNAs on virus infection, replication, and pathogenesis

MiRNA-mediated viral infection regulation has been sufficiently detailed in several hosts, in both DNA and RNA viruses (5). Several events have been described and are illustrated in Figure 2. From the cellular side, miRNAs are directly targeting the expression of host viral receptors, inhibiting cellular processes or responses related to viral replication, inhibition of viral transcripts essential for viral infection, and generation of IFN I-II and or III signaling pathways to protect neighbouring cells from being infected (5). In viral escape viral miRNA or other non-coding RNA block cellular miRNA, in the process of miRNA formation, by miRNA mimicry, and consequently hampering host response, b) inhibition or degradation of cellular transcription of non-coding RNA, and or RNA degradation, RNA sponges (several miRNAs that inhibit host's mRNA), c) inhibition host proliferation and death by modulating cell's pathways (5,6). Therefore, two general outcomes are identified concerning miRNA, a) inhibition of the viral genome and consequently, replication and, b) stabilization of viral RNA, making the virus resistant to the host response (7,8).

There is another possible mechanism of viral escape. Viral of miRNA can be generated upon cleavage of the viral genome by the host. These miRNA will limit cell function, enhancing viral replication (7,8). It follows then that the different pathways of viral escape illustrated in Figure 2, can be generated by viral ARN cleavage which in turn would facilitate viral infection in the target cells. Virus mutations may increase virulence rather than decreasing it.

The two major portals of COVID genetic information https://www.gisaid.org/ and the host COVID host genetics initiative https://www.

covid19hg.org/ have been used for genomic analysis. Several approaches have been used to study, in silico, the different sequences of the virus, the possible viral miRNA, and the types of miRNA from the host that can affect viral infection and replication.

An exciting issue studied by Fulzele and co-workers (9) is to analyze the possible cellular miRNA that would bind to the viral genome affecting elderly individuals as the most prone targets of the virus. They reported the following data 848 miRNA are common in SARS, 873 miRNAs are common in COVID-19, 558 miRNAs are common between SARS and COVID-19, 315 miRNAs are unique to COVID-19, 290 miRNAs are specific to SARS. Upon viral sequence analysis uploaded in the platform, it was clear that virus variability would affect the possible sites of cellular miRNA binding that would eventually control viral replication. A more integrated approach using infected cells and metabolic pathways had to be included.

Fulzele and co-workers, using bioinformatic protocols, (9) were able to do an interactive probability model. This model included viral genomic sequences; cellular derived metabolic pathways, KEGG (bioinformatic acronym used for assessing cell's metabolic pathways and function), and cellular miRNA that could bind to the virus sequence. In SARS-CoV2 infected cells, the most affected pathways are cell signaling pathways that involve organelle, ion binding, cellular nitrogen compound metabolic process, biosynthetic process, and other minor pathways. These pathways were used to do the first screening of cell miRNA. The first matrix generated the following hits for miRNA 15b-5p, 15a-5p, 548c-5p, 548d-5p, 409-3p, 30b-5p, 505-3p.

Then, one important question arises. Are these miRNAs involved in diseases that are part of the comorbidities in adults and could affect viral replication? It is well known that miR-15b-5p is involved in cardiovascular disease (10), asthma overlap syndrome (11), and rhinitis (12) since it affects angiogenesis pathways, cell proliferation, and nuclear factor kappa B (NF-$\varkappa$B) activation. By statistics, this miRNA has 16 different possible binding sites to the viral genome. The binding of this miRNA may be crucial to control viral replication, but at the same time, it is also involved in host pathogenesis. It follows then that, in the tested in silico model, a decrease amount of available human (has) miRNA-15a or miRNA15b-5p will favor viral replication. The other miRNA have different functions but, similarly, its decrease will favor viral infection. The miRNA548c-5p/548d-5p duo is involved in activating pathways involving the Nuclear Factor of Activated T-cells (NFAT) and Retinoid Orphan Receptor-alpha (RORα). The miRNA 409-3p is related to the cAMP-responsive gene element and cAMP. MiRNA 30b-5p regulates cell proliferation and miRNA 505-3p induce the expression of chemokine receptors, CCR3, CCR4, and CXCR1 critical for cell migration and response. Thus, all these miRNAs are involved in the inflammatory reaction, which either eliminates the virus or enhances its chronicity.

According to the Bio project data (PRJNA615032), several SARS-CoV2 miRNAs analyzed upon cell infection, and bioinformatic data several viral miRNAs can target cellular pathways (13). There were seven miRs identified (miRNAs 8066, 5197, 3611, 3934-3p, 1307-3p, 3691-3p, 1468-5p). The miR-8066, bind and activate Nf$\varkappa$B-mediated TLR-8 expression and induce cytokine synthesis. Consequently, miR-8066 may act as an autocrine or paracrine agonist of host cells. It triggers pro-inflammatory cytokines due to their increased NF- $\varkappa$B activity, and consequently, the cytokine storm observed in severe patients could be due to this miRNA. MiRNA8066 has been found in tissue biopsies as well as in exosomes (13). Moreover, this miRNA has been associated with the other six candidates to affect TFG-β signaling, mucin-type O-glycan biosynthesis, and cytokine-cytokine receptor interaction is involved in viral pathogenesis (13).

Out of 2 565 cellular miRNAs, only three critical miRNAs, 5197-3p, 4778-3p, and 6864-5p could interact with complete complementary miRNA (cc-miRNA) and have therapeutic potential since they bind with high-affinity to SARS-CoV2 guide RNA. MiR-5197-3p interacts with the guide RNA of SARS-CoV2, MERS-CoV, and SARS-CoV2 (13). Hsa-miRNA-5197-5p has been already indicated for the treatment of hepatitis B infections (patent WO2018193902A1). Similar to this finding, it was shown that miRNA-5197-3p might be used in vaccine strategies in the herpes simplex virus

(HSV-1) (patent WO2013109604A1).

In a study (14) involving Chronic Obstructive Pulmonary Disease (COPD) patients, healthy volunteers, and miRNA, a significant down-regulation of miRNA-3611 expression was found in COPD patients. This miRNA has been associated with the morphine pathway, which is related to the replication of HIV, hepatitis C virus, and now SARS-CoV2. However, more data is required to ascertain the role of this miRNA in SARS-CoV2 infection.

The miRNA-3934-3p expression profiles have been used to classify cancers into various subtypes, lung, and colon cancer (13). It has been associated with TFG-β signaling, lung fibrosis, and glycosaminoglycan synthesis. Heparan sulphate provides the binding sites for the SARS-CoV2 invasion at the early attachment phase. This pathway, through heparan sulfate, has also been described in hepatitis C and herpes virus infection (13). Blocking this miRNA could be a potential target for viral infection and replication.

Lung-tissue-associated miRNAs

miRNA-1307 is involved in lung and cardiovascular development in newborns. However, it is also involved in cancer since regulates TGF-β and semaphoring signaling (13). It is also involved in inflammatory responses, pulmonary hypertension, systemic scleroderma, and pulmonary fibrosis (13). This mRNA is also associated with the miRNA-3934-3p in some types of tumors.

Another important miRNA related to lung function is miRNA-3691-3p. It is involved COPD, lung injury, and it is significantly reduced in hypoxia (13). MiRNA3691-3ptargets several cell signaling pathways, such as TGF-β signaling, FGF2, and also VCAM-1, which is relevant for lung injury and repair.

MiRNA-1468 activates non-canonical TGF-β1 and MAPKs signaling pathways related to cardiac fibrosis and cell senescence (13). Also, the expression of miR-1468-3p is upregulated in regulatory T cells, which have a significant role in autoimmune disorders, transplant rejection, allergic diseases, and asthma. The link between miR-1468-5 in viral infection and other comorbidities will need to be further investigated.

In another study, using permissive infective cultures, Hosseini Rad and McLelland (14), found an increase in a critical cellular miRNA 197-5p which is a target of viral miRNA. This particular miRNA is involved in cardiovascular disease and could be partially responsible for the cardiovascular abnormalities observed in SARS-CoV2 infected patients. Similar events have been described for other viruses that affect miRNA of the same family (14).

Finally, in a host-pathogen interaction study involving competing endogenous RNAs (ceRNA) (15). This ceRNA is an intrinsic mechanism to regulate biological processes. It has been proposed that dynamic changes of ceRNAs can modulate miRNA activities and consequently changes in ceRNA could be significant in viral infection. Analyzing the predictive data, Arora and co-workers (15) predicted one miRNA (miRNA 124-3p), one mRNA (Ddx58), one long non-coding RNA (lncRNA Gm26917), and two circRNAs (Ppp1r10, C330019G07RiK) in SARS-CoV2 infected cells. The miRNA-124-3p is involved in the mesenchymal transition and is related to the TGF-β pathway. The mRNA Ddx58v is associated with autoimmunity and IFN signaling. The lncRNA Gm26917 is related to cell proliferation, and one of the circular RNA regulates Aurora kinases involved in cell proliferation and the second involved in the IFN I/II signal transduction. Indeed, more research is required to very all the hypothetical models proposed by bioinformatics.

It can be concluded that the modulation of viral infection by the host through miRNA (15b-5p, 15a-5p, 548c-5p, 548d-5p, 409-3p, 30b-5p, 505-3p) and by the virus, shared with host, miRNA (8066, 5197, 3611, 3934-3p, 1307-3p, 3691-3p, 1468-5p) should be further analyzed. Validation *in vivo* of these targets is important. Besides, the assessment of possible polymorphisms will also be important to assess the binding. *In vivo* data using animal models are important to validate the therapeutic options that could be managed with these specific miRNA.

CONCLUSIONS

The non-coding RNA is a mechanism of

protection and regulation (shown in Figure 1). Viral non-coding RNAs control cellular function hampering host response, and managing pathways to generate the required intermediates for viral replication. Viral control of cellular processes upon infection suggests that the virus induces an increase in the inflammatory response, which would, in turn, facilitate viral infection (Figure 2). The lack of cell miRNA to control viral replication in patients with comorbidities would not only contribute to the inflammatory response and viral replication but exhaust the immune response jeopardizing the life of the host.

Studies on non-coding RNA are, therefore, crucial in the SARS-CoV2 model. Most of the proposed in silico models should be tested *in vitro* and *in vivo*. There are important advances on which target to select based on the data presented here. Since there are several groups and clinical trials, shortly we would be able to ascertain the role of these molecules for control of SARS-CoV2 and also for other pathogens.

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