Implications at the ocular level of miRNAs modifications induced by SARS-CoV-2 infection

ADINA-IULIANA MILCU1,2), ANDREI ANGHEL3,4), OVIDIU MUŞAT5), MIHNEA MUNTEANU1), MĂDĂLINA-CASIANA SALAVAT1), ANDREEA IORDACHE5), EMIL UNGUREANU7), DIANA CAMELIA BONŢE3), OVIDIU BORUGĂ1)

1) Department of Ophthalmology, Victor Babeş University of Medicine and Pharmacy, Timişoara, Romania
2) PhD Student, Department of Biochemistry, Victor Babeş University of Medicine and Pharmacy, Timişoara, Romania
3) Department of Biochemistry, Victor Babeş University of Medicine and Pharmacy, Timişoara, Romania
4) Center for Complex Network Science, Victor Babeş University of Medicine and Pharmacy, Timişoara, Romania
5) Department of Ophthalmology, Dr. Carol Davila Central Military Emergency University Hospital, Bucharest, Romania
6) PhD Student, Department of ENT, Victor Babeş University of Medicine and Pharmacy, Timişoara, Romania
7) Department of Ophthalmology, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Abstract

The coronavirus disease 2019 (COVID-19) pandemic determined the use of different research methods and investigations in the management of this novel infectious disease. The impact and development of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) at cellular level is still a challenge and many directions of investigation have been opened, a complex topic that has been explored is the bidirectional interaction between host micro-ribonucleic acids (miRNAs) and viral miRNA. The main point of this study is to analyze the transcriptional modifications induced by the viral infection at ocular level, mediated by miRNAs. It is known that the ocular transmission is a route of infection, and it can cause multiple neuro-ophthalmological manifestations, such as optic nerve dysfunction, eye movement abnormalities, oscillopsia and intracranial hypertension. We have managed to identify more than six miRNAs specifically involved in eye disorders that are strongly dysregulated by the SARS-CoV-2. These miRNAs regulate different pathways, such as optic nerve dysfunction, eye movement abnormalities, oscillopsia and intracranial hypertension. We have managed to identify more than six miRNAs specifically involved in eye disorders that are strongly dysregulated by the SARS-CoV-2. These miRNAs regulate different pathways, such as the nuclear factor-kappa B (NF-κB) pathway, the expression of complement factor H (CFH) gene, the expression of transforming growth factor-beta (TGF-β) fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) genes. In the context of SARS-CoV-2 infection, many more molecular changes at ocular level need to be elucidated to better understanding the COVID-19.

Keywords: miRNA, SARS-CoV-2, ocular modifications.

Introduction

Coronavirus disease 2019 (COVID-19) is an acute respiratory illness brought on by infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes SARS. While COVID-19 was initially discovered in China (in Wuhan, the capital of Hubei Province) in November 2019, the illness quickly spread to become a global pandemic [1]. More than 514 million confirmed cases and more than six million fatalities have been reported worldwide as of May 8th, 2022, but thankfully, the number of new COVID-19 cases is progressively dropping [2]. At the beginning of May, the total number of confirmed infections reached almost three million, in Romania (ranked 38th globally), with the latest surge of daily new cases during the winter months (January–February 2022) [3]. The total number of deaths reached over 65 000 cases, with a mortality rate between 5–25% in those requiring hospitalization [3, 4].

Structure

SARS-CoV-2 virus belongs to a group of related ribonucleic acid (RNA) viruses that causes respiratory tract infections in humans, which can range from mild to severe, and even lethal ones [5]. It has one of the biggest positive-sense, single-stranded RNA genomes (26.4 to 31.7 kb), attached to nucleocapsid (N) proteins, enclosed in a protective inner envelope [6]. The coronavirus is further encircled by an outer membrane envelope comprised of lipids with specific proteins [membrane (M), envelope (E), and spike (S)] inserted to shape the viral envelope, maintain its size, and engage the host’s cells [7]. The primary structural protein of the outer membrane that gives the membrane its overall form is the M protein, a type III membrane protein [8]. The E proteins, which are encased in the lipid layer, are in charge of morphogenesis, intracellular trafficking, and virion assembly [8]. The coronavirus’s S protein, also known as the spike protein, is what gives it...
its distinctive halo-like look. As they are in charge of host cell selectivity, these proteins are also the most changeable parts of the virus [9]. To recognize various protein receptors, such as the angiotensin-converting enzyme 2 (ACE2), aminopeptidase N (APN), and dipeptidyl peptidase 4 (DPP4), the S proteins, C-terminal domains act as receptor-binding domains [10].

ACE2 is the main receptor used by coronaviruses to infect human cells. When the viral S protein binds to the ACE2 receptor on the host cell, infection starts. The viral RNA is then released into the cytoplasm once the virus has been internalized by endocytosis or direct fusion of the viral envelope with the host membrane [9]. The viral RNA molecules enlist the cellular host machinery to produce hundreds of thousands of copies of the viral RNA and direct the cells to produce N, M, E, and S viral proteins. The enzyme in charge of creating fresh copies of viral RNA is known as RNA-dependent RNA polymerase [6]. By exocytosis through secretory vesicles, the new virions are discharged from the host cell, causing the infection to spread to other host cells [6].

Transmission, pathophysiology and diagnosis

Evidence suggests that SARS-CoV-2 attaches to the ACE2 receptor and travels through an aerosol to infect respiratory epithelial cells [11]. SARS-CoV-2 is predominantly spread by respiratory droplets and direct contact between individuals [12]. A person is at risk of having tissues exposed to possibly infectious respiratory droplets when they are in close proximity (1–2 m) to someone who is experiencing respiratory symptoms. As a result, SARS-CoV-2 transmission can happen through direct contact with infected individuals as well as through indirect contact with nearby surfaces or with medical equipment used on the sick individual (such as a stethoscope or thermometer) [13]. There are currently five dominant SARS-CoV-2 variants spreading among populations worldwide as of December 2021: the Alpha variant (previously known as the UK variant), the Beta variant (previously known as the South Africa variant), the Gamma variant (previously known as the Brazil variant), the Delta variant (previously known as the India variant), and the Omicron variant [14].

There is an incubation time before symptoms appear, which for SARS-CoV-2 is between five and six days [15]. This is typical for most infections. One of the factors that facilitated the virus’s rapid spread worldwide has been hypothesized to be pre-symptomatic transmission via aerosol route [15]. COVID-19 illness is frequently described as having two separate phases. The initial viral load, which peaks in the first seven days of sickness, is associated with fever, cough, and other symptoms such as anosmia, ageusia, headaches, nasal congestion, muscle discomfort, or diarrhea. After the initial week of symptoms, many people start to feel better [16]. But in a certain percentage of patients, a second phase comes next. The development of lung inflammation and injury during this second stage is defined by the host’s heightened inflammatory response [17]. A cytokine storm has been described as the inflammatory response in severe COVID-19 cases. This cytokine storm includes increased inflammatory biomarkers like interleukins (IL-6, IL-8), activation of the coagulation pathway, and decreased production of antiviral defense mediators like interferon (IFN), among many other mechanisms [17–19].

True asymptomatic or less symptomatic cases, severe pneumonia, and fatal acute respiratory distress syndrome are all on the disease’s shockingly wide range [20]. Although the upper and lower respiratory tracts are the most common organs and tissues affected by SARS-CoV-2, other tissues and organs, including the central and peripheral nervous system, gastrointestinal tract, cardiovascular system, liver, kidneys, spleen, and even the eyes, may also be affected [21–25].

A nasopharyngeal swab, a nasal swab, or a sample of sputum can be used to capture contaminated secretions, and reverse transcription–quantitative polymerase chain reaction (RT–qPCR) can be used to confirm the SARS-CoV-2 infection [26]. There are also serological assays that can find antibodies [26]. Laboratory testing (complete blood count, inflammatory and coagulation biomarkers), chest computed tomography (CT) or X-ray scans are further diagnostic methods.

microRNAs

A tiny class of non-coding single-stranded RNAs called microRNAs (miRNAs) have a length of 19–25 base pairs. These molecules play a decisive role in the modulation of biological processes by regulating the post-transcriptional modifications of target genes. It is known that a single molecule of miRNA can target hundreds of messenger RNAs (mRNAs), being widely involved in gene regulation. miRNAs contribute to the regulation of both innate and adaptive immune systems, as well as to the differentiation, growth, and apoptosis of the cells. These molecules are involved in the pathogenesis of a wide variety of diseases, such as allergic diseases, malignancies, pathologies related to heart, lungs, and even ocular disorders [27–30].

The mechanisms of miRNA-mediated gene regulation are complex, and they are mainly based on the degradation or translational repression of their mRNA targets, by binding mostly to their 3’ untranslated region (3’UTR). Other miRNAs binding sites, such as 5’UTR, coding sequence and promoter regions, have been reported as potential targets on mRNA. Also, in certain conditions, miRNAs can activate translation, however the exact mechanism still needs to be elucidated in a larger context [31].

In the past few years, miRNAs have drawn the attention of the research community, due to their interaction with viruses. The host miRNAs can attach to a wide range of RNA viruses, modulating the life cycle of the pathogen. Three mechanisms were identified regarding virus–host interaction, either by direct binding to the viral genome or transcripts, or binding to host transcripts. All of them have two main effects: inhibition of viral translation or stimulation of virus replication [32, 33].

Interactions between RNA viruses and host miRNAs are bidirectional, thus they are investigated from two points of view. Firstly, researchers investigated the effect
of direct binding of miRNAs to the RNA virus, leading to different results. One example is the inhibitory effect of Influenza A virus replication by targeting and silencing viral polymerase basic 1 (PB1) gene by miR-3145 [33]. Another decreasing effect was identified in the viral translation of human immunodeficiency virus (HIV), regulated by miR-223. Another example is the increased effect of viral translation of bovine viral diarrhea virus (BVDV) by miR-17. Secondly, some research groups investigated the changes in cellular host miRNA expression after viral infection. Therefore, Influenza virus decreases the miR-24 expression which activates furin protein, resulting in an increased effect of hemagglutinin cleavage. Moreover, the expression of miR-9 was decreased during coronavirus OC43 infection, leading to a higher production of nuclear factor-kappa B (NF-κB) [33, 34].

miRNAs in SARS-CoV-2 infection

The debut of COVID-19 pandemic generated a lot of interest among researchers, especially because the pathogenic agent, SARS-CoV-2, an RNA virus, has generated millions of losses worldwide. This fact determined the research groups to investigate the interactions between the novel coronavirus and the human host. One of the most intriguing subjects was the bidirectional relation between human miRNAs and the pathogenic virus.

Host miRNAs, such as hsa-miR-20b-5p, hsa-miR-17-5p and hsa-miR-323a-5p are involved in reducing the organ damage associated with SARS-CoV-2 infection. Moreover, they play a key role in the inflammatory cascade triggered by the virus [35]. Various host miRNAs, e.g., miR-574-5p, miR-214, miR-17, miR-98, miR-223, and miR-148a bind to SARS-CoV2 encoded transcripts S, E, M, N, and open reading frame 1a (ORF1a), acting as a host defense response [36]. Host defense mechanisms rely on the infected cells apoptosis; however, SARS-CoV-2 uses its own encoded miRNAs to block this process. Therefore, it has been hypothesized that two miRNAs encoded by the virus specifically, MD2-5p and MR147-3p, suppress the activity of pro-apoptotic genes, like RAD9 checkpoint clamp component A (RAD9A) [35].

miRNAs encoded by SARS-CoV-2 have been shown to have inhibitory effects in ribosomal translation of some human proteins, such as hemoglobin, gamma-globin, and olfactory receptor proteins. The hypothesis is that they act on their miRNAs by hybridization. Other effects associated with SARS-CoV-2 infection via miRNA were dysosmia, weak immune response and fluctuations in oxygen levels in vital organs. Moreover, SARS-CoV-2 was reported to alter the genes involved in the modulation of immune response, which are targeted by hsa-miR-146b and hsa-miR-939. Furthermore, the patients addicted to opioids are supposed to be more sensitive to viral infection and their symptoms tend to be more severe, these reactions being corroborated with the expression of hsa-miR-3611. Last but not least, the damage of the heart due to SARS-CoV-2 infection was found to be correlated with the dysregulated expression of hsa-miR-1468-5p [35]. For the following miRNA species: hsa-miR-101, hsa-miR-125a-5p, hsa-miR-126, hsa-miR-222, hsa-miR-23b, hsa-miR-378, hsa-miR-380-5, and hsa-miR-98, significant changes in human miRNA expression linked with SARS-CoV-2. They specifically target viral proteins, such as polymerase basic protein 2 (PB2), polymerase acidic protein (PA), non-structural protein (NS1), nucleoprotein (NP), and major capsid protein in addition to host proteins, such as IFN, adenosine triphosphate (ATP) synthase F1 subunit beta (ATP5B), and erb-B2 receptor tyrosine kinase 2 (ERBB2) (VPI) [37]. Also, other hsa-miRNAs, such as hsa-miR-1307-3p, hsa-miR-8066, hsa-miR-5197-3p and hsa-miR-3691-3p are associated with pulmonary damage caused by oxidative stress, inflammatory response, cytokine–cytokine receptor interaction and transforming growth factor-beta (TGF-β) pathways, which are relevant for the pathogenesis of COVID-19 disease [35].

Implications at ocular level of miRNAs modifications induced by SARS-CoV-2 infection

The speed with which the virus circulates in the communities determined the researchers to investigate more deeply its means of transmission. Therefore, the eye represents an important route of infection. There are two ways of transmission: actively via tears and passively via the nasolacrimal duct. Several studies showed that SARS-CoV-2 detection has been performed using RT-qPCR from samples like tears or conjunctival fluid [38].

The molecular mechanism of SARS-CoV-2 presence in the eye, relies on the interaction between the host ACE2 cellular receptor and the virus S protein. This receptor is located in retina, aqueous humor, retinal pigment epithelium (RPE), choroid, and conjunctival epithelium [38, 39]. The clinical manifestations of SARS-CoV-2 infection at the ocular level involve a wide variety of symptoms frequently associated with keratoconjunctivitis. Some of the most common manifestations are redness, tearing, itching and eye pain. Also, it was shown that patients infected with SARS-CoV-2 present retinal hemorrhages, dilated veins, and cotton wool spots. Moreover, cases of optic neuritis, retinal vaso-occlusion and intracranial hypertension have been observed in positive COVID-19 patients. Oscillopsia, ataxia or myoclonus were also described in several case reports as being associated with SARS-CoV-2 infection [39, 40].

Regarding the expression of miRNAs at the ocular level, researchers have investigated their presence in histopathological tissues like lens, cornea, and retina. Some of the samples were harvested from corpses, to discover the miRNAs roles in the development and functions of the eye and its associated diseases [41]. The variety of eye symptoms and the abundance of miRNAs that can be found in the eye made us question whether the SARS-CoV-2 has a direct impact on eye miRNAs.

Among the miRNAs known to be involved in ocular disorders, we specify the ones that were also found to be associated with COVID-19. The miRNAs associated with age-related macular degeneration (AMD) and COVID-19 are miR-146, miR-125, miR-23, miR-20, miR-17, miR-574 and miR-223 [30].
For instance, miR-146 as a known inflammatory molecule has two possible molecular mechanisms in AMD disease. Specifically, in RPE cells, miR-146 was found to negatively regulate the NF-κB pathway [42] and in retina tissue has been shown to downregulate the expression of complement factor H (CFH) gene modulating the innate immune and inflammatory signals [43]. Moreover, miR-146 was found to be up-regulated also in uveal melanoma and pterygium diseases [30]. miR-125 was found to be localized in the retinal tissue and negatively regulates the expression of CFH gene, leading to the same molecular response as miR-146 in AMD disease [43]. In RPE cells, the expression of miR-23 is downregulated and has a significant role in the early stages of AMD, preventing oxidative damage, by acting upon the Fas cell surface death receptor (FAS) gene as a target [44]. Furthermore, miR-20, miR-17 and miR-223 were shown to have up-regulated expressions in AMD disease, while miR-574 has a decreased expression in this pathology. miR-17-92 cluster was found to be localized in human and murine retinoblastoma (Rb) and Rb cell lines, promoting the tumorigenesis. Last but not least, miR-126 is found in lens epithelial cells at Shumiya cataract rats, acting upon several gene targets, such as TGF-β, fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF). The expression of this mRNA in cataract was found to be downregulated, which leads to the development of this specific disease [30]. The transcriptional modifications of these miRNAs in ocular histopathological samples were also found to be significantly altered by SARS-CoV-2 infection.

**Conclusions**

The main point of this study was the analysis of transcriptional modifications induced by the viral infection at ocular level, mediated by miRNA. Related to this, we have managed to identify more than six miRNAs specifically involved in eye disorders that are strongly dysregulated by the SARS-CoV-2. The molecular mechanisms of these miRNAs regulate different pathways. Downregulations occurred in the NF-κB pathway, in the expression of CFH gene and in the expression of TGF-β, FGF and PDGF genes, last three being involved in cataract. In the context of SARS-CoV-2 infection, many more effects at ocular level need to be elucidated in future experimental studies.

**Conflict of interests**

The authors declare no conflict of interests, financial or otherwise.

**References**

[1] Ghinai I, McPherson TD, Hunter JC, KringHL, Christiansen D, Joshi K, Rubin R, Morales-Estrada S, Black SR, Pacilli M, Frichione MJ, Chugh R, Walilay KA, Ahmed NS, Stoecker WC, Hasan NF, Burdass DP, Reese HE, Wallace M, Wang C, Moeller D, Korpics J, Novosad SA, Benowitz I, Jacobs MW, Dasari VS, Patel MT, Kauerauf J, Charles EM, Eziko NO, Chu V, Midgley CM, Rolles MA, Gerber SI, Lu X, Lindstrom S, Varani JR, Layden JE, Illinois COVID-19 Investigation Team. First known person-to-person transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the USA. Lancet, 2020, 395(10233):1137–1144. https://doi.org/10.1016/S0140-6736(20)30607-3 PMID: 32178678 PMCID: PMC7159555

[2] World Health Organization (WHO). Weekly epidemiological update COVID-19 – 11 May 2022. Emergency Situational Updates, WHO, Geneva, Switzerland, 11 May 2022. https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---11-may-2022

[3] *** World / Countries / Romania. Worldmeter COVID-19 data, last updated: June 28, 2022. https://www.worldometers.info/coronavirus/country/romania/

[4] Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Xue G, Han L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet, 2020, 395(10229):1054–1062. https://doi.org/10.1016/S0140-6736(20)30566-3 PMID: 32171076 PMCID: PMC7270627

[5] Woo PCY, Huang Y, Lau SKP, Yuen KY. Coronavirus genomics and bioinformatics analysis. Viruses, 2010, 2(8):1804–1820. https://doi.org/10.3390/v20818043 PMID: 21994708 PMCID: PMC3185738

[6] Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. Methods Mol Biol, 2015, 1282:1–23. https://doi.org/10.1007/978-1-4939-2438-7_1 PMID: 25270466 PMCID: PMC4639855

[7] Schoeman D, Fielding BC. Coronavirus envelope protein: current knowledge. Virol J, 2019, 16(1):69. https://doi.org/10.1186/s12985-019-1182-0 PMID: 31133031 PMCID: PMC6537279

[8] Lalchhandama K. The chronicles of coronaviruses: the electron microscope, the doughnut, and the spike. Sci Vis, 2020, 20(2):78–92. https://doi.org/10.1080/15376482.2020.1816138

[9] Neuman BW, Kiss G, Kunding AH, Bhella D, Baksh MF, Connolly S, Droese B, Klaus JP, Makino S, Sawicki SG, Siddell SG, Stannou DG, Wilson IA, Kuhn P, Buchmeier MJ. A structural analysis of M protein in coronavirus assembly and morphology. J Struct Biol, 2011, 174(1):11–22. https://doi.org/10.1016/j.jsb.2010.11.021 PMID: 21130884 PMCID: PMC4486061

[10] Simmonds G, Zmora P, Gierer S, Heurich A, Pöhlmann S. Proteolytic activation of the SARS-coronavirus spike protein: cutting enzymes at the cutting edge of antiviral research. Antiviral Res, 2013, 100(3):605–614. https://doi.org/10.1016/j.antiviral.2013.09.028 PMID: 24121034 PMCID: PMC3589662

[11] Li F, Li W, Farzan M, Harrison SC. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science, 2005, 309(5742):1864–1866. https://doi.org/10.1126/science.1116480 PMID: 16166518

[12] World Health Organization (WHO). Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19), 16–24 February 2020. WHO, Geneva, Switzerland, 28 February 2020. https://www.who.int/publications/i/item/report-of-the-who-china-joint-mission-on-coronavirus-disease-2019-(covid-19)

[13] Cng SWX, Tan YK, Chia PY, Lee TH, Ng OT, WONG MY, MARMIMUTHU K. Air, surface environmental, and personal protective equipment contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a symptomatic patient. JAMA, 2020, 323(16):1610–1612. https://doi.org/10.1001/jama.2020.3227 PMID: 32128865 PMCID: PMC7505717

[14] *** New COVID-19 variants. U.S. Department of Health & Human Services, U.S. Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA, 28 June 2021. https://www.cdc.gov/library/sciencevision.org/issue/45/article/296

[15] Gandhi RT, Lynch JB, Del Rio C. Mild or moderate Covid-19. N Engl J Med, 2020, 383(18):1757–1766. https://doi.org/10.1056/NEJMc2009249 PMID: 32329974

[16] Cevik M, Tate M, Lloyd O, Maralo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. Lancet Microbe, 2021, 2(1):e13–e22. https://doi.org/10.1016/S2666-5247(20)30172-5 PMID: 33521734 PMCID: PMC7837293

[17] Del Valle DM, Kim-Schulze S, Huang HH, Beckmann ND, Nirenberg S, Wang B, Lavin Y, Swartz TH, Madduri D, Stock A, Jiang J, Reich D, Sigel K, Cordon-Cardo C, Feldmann M, Parekh S, Merad M, Gnajtis S. An inflammatory cytokine signature predicts COVID-19 severity and survival. Nat Med, 2020, 26(10):1636–1643. https://doi.org/10.1038/s41591-020-1051-9 PMID: 32839624 PMCID: PMC7869028
Implications at the ocular level of miRNAs modifications induced by SARS-CoV-2 infection