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Review Article

MicroRNA: A Novel Approach for the Treatment and Vaccination of COVID-19

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Abstract

MicroRNA (miRNA) is a small non-coding ribonucleic acid (RNA) molecule that executes a vital role in the post-transcriptional gene regulation of messenger RNA. Many messenger RNAs (mRNAs) are regulated by specific miRNAs to mediate cellular proliferation, differentiation, and signaling. SARS-CoV-2 is a novel severe pathogenic coronavirus that causes the coronavirus disease 2019 (COVID-19). Cellular miRNA is a valuable determination point to get the underlying pathological process of COVID-19. Given the possibility of a pandemic, researchers and clinicians have been rushing to comprehend this novel virus and the pathogenesis of this disease in order to develop feasible treatment regimens and efficient therapeutic drugs and vaccinations. Many approaches are being used to manage the irruption of this fatal infectious agent disease. miRNA, being the emerging magnificent signature of response to host-viral interactions, has recently been used to develop therapeutics and vaccine against viral diseases. The aim of this review was to describe the role of miRNAs in the pathogenesis of COVID-19 while also establishing the scholarly foundation for future COVID-19 therapeutic drugs and vaccines development.

Keywords: *COVID-19; Microrna; SARS- Cov-2*

Abbreviation

COVID-19: Corona Virus Disease 19; DNA: Deoxyribonucleic Acid; SARS-CoV-2: Severe Acute Respiratory Syndrome- Coronavirus-2; miRNA: Micro Ribonucleic Acid; ACE-2: Angiotensin-Converting Enzyme-2

Introduction

Coronaviruses are single-stranded RNA (30 kb) viruses with a diameter of 80-160 nm [1-3]. Coronaviruses are divided into four categories: alpha, beta, gamma, and delta. Furthermore, the beta coronavirus class includes three members: severe acute respiratory syndrome virus (SARS-CoV), middle east respiratory syndrome virus (MERS-CoV), and severe acute respiratory syndrome virus-2 (SARS-CoV-2) [4,5]. SARS-CoV-2 is a novel severe pathogenic coronavirus that causes the coronavirus disease 2019 (COVID-19). It is composed of four basic proteins: spike (S), nucleocapsid (N), envelope (E), and membrane (M), as well as a single-stranded viral RNA genome. COVID-19 has sparked international concern due to its global epidemic and healthcare impact [6-8].

Since the beginning of the COVID-19 epidemic, the world has taken enormous measures to battle sickness [9]. It is most typically spread in people by respiratory droplets and close contact. As a result, social separation is seen as a crucial element in the prevention of disease

transmission. Despite this, the disease is spreading at an alarming rate, causing havoc on healthcare, society, and the economy. Despite increased attempts to manage and contain the COVID-19 pandemic, there are still no SARS-CoV-2-specific antiviral agents. Researchers have been working tirelessly to develop potential therapies and vaccinations for future usage [10,11].

MicroRNA (miRNA) is a non-coding RNA with a stretch of 22 nucleotides. Through its partial complementary sequences, it destabilizes and inhibits the translation of target messenger RNA (mRNA). It is well-known for its therapeutic signature in the treatment of many viral diseases [12]. MiRNAs have the ability to control around 30% of human genes. miRNAs have evolved to control the majority of human mRNAs [13,14]. MiRNAs' tight control of genes is primarily responsible for the maintenance of normal physiologic conditions such as cell cycle, differentiation, proliferation, immune response, and resistance to environmental insults such as hypoxia, infection, and DNA damage [15,16]. Extracellular miRNAs are transported to target cells through vesicles or protein-binding. Extracellular miRNAs serve as disease biomarkers and chemical messengers, allowing cells to communicate with one another [17].

Life cycle of SARS-CoV-2

COVID-19 infection primarily affects the lungs, resulting in pneumonia, respiratory failure, severe lung damage, sepsis, multi-organ failure, and death [6,7]. SARS-CoV-2 goes through five stages of life cycle within host cells [18]. Following infection, SARS-CoV-2 attaches to the epithelial membrane of the oral cavity, mucosal membranes of the conjunctiva, or the ear canal. Angiotensin-converting enzyme-2 (ACE2) is important in the internalization of SARS-CoV-2 via membrane fusion [19]. During internalization, spike proteins attach to ACE2 receptors in host cells, and then the serine protease is activated. The spike proteins are primed for internalization via membrane fusion by Transmembrane Serine Protease 2 (TMPRSS2). Following internalization, viral materials are released into host cells. To synthesize viral proteins the viral RNA should enter the nucleus for replication. Finally, newly generated virus particles are discharged into the host cells [20].

The viruses that have been released enter uninfected cells via the endocytic pathway and leave the host cell via direct budding through the membrane. During viral infections, extracellular vesicles incorporate pathogen-derived lipids, nucleic acids, and proteins and serve as a viral material delivery vector [21]. The physiologic benefit of ACE2 is the breakdown of angiotensin-II and the production of angiotensin [1-7], which functions as an ACE-II counter-regulator. After viral replication in the host cell, ACE2 is downregulated, limiting the breakdown of angiotensin-II into angiotensin [1-7]. As a result, the clinical features of COVID-19 are described by a disturbance in the ACE2/angiotensin [1-7] axis [22].

Association of miRNA and COVID-19

Emerging evidence suggests that miRNAs play an important role in the pathogenesis and treatment of a wide range of viral diseases [23]. MiRNAs play a role in many pathologic processes, including inflammatory responses and viral infection. MiRNAs are a type of regulatory mechanism that allows cells to eliminate unwanted or abnormal mRNA [24]. Viruses complete their infectious cycle by relying on a variety of biological factors. Most intriguingly, miRNAs have recently been identified as critical modulators of viral infections [25]. When miRNAs are repressed or in low abundance, the virus can easily replicate, evade immune responses, and increase disease lethality. SARS-CoV-2 pathogenicity reduces cellular miRNA levels, allowing SARS-CoV-2 to become a more dangerous coronavirus to humans [26]. Certain host cell miRNAs control the expression of specific viral genes during the immune system response to protect tissues after infection. Influencing miRNA-RNA virus interaction, miRNA interaction with a viral RNA genome, modulation of host miRNA levels during viral infections, miRNA-facilitated changes in protein expression that alter host responses to infection, and maintenance of miRNA-binding sites in the RNA virus genome are all examples of interactions between viral RNA and cellular miRNAs [27].

MiRNA-based drugs and vaccine development for covid-19

At the present time, the global goal is to develop a vaccine and/or treatment for this virus. There are numerous pre-clinical and clinical trials undertaken to find drugs to treat COVID-19 infection. However, there is little information on the availability of approved treatment options for the newly discovered COVID-19. This situation puts pressure on researchers all over the world to develop new drugs in order to combat this lethal virus [28]. Vital therapeutic approaches for COVID-19 have mainly targeted Spike protein. The spike protein on a viral capsid is essential for host specificity and viral infectivity [29]. After being cleaved by TMPRSS2, the SARS-CoV-2 spike protein activates and attaches to the host cell's ACE2 [30]. Anti-viral miRNAs found in host cells function as a major regulator of immune response during viral infection by targeting viral gene replication and expression. Relative simple structures and predictable mechanisms of miRNAs make it easier to design mimics or anti-miRs as therapeutic targets than conventional chemical drugs [31]. Understanding the deregulated signaling pathways by Spike-ACE2 interaction and the involved miRNAs is useful for the prevention and management of COVID-19. ACE2 and TMPRSS2 have been identified as key elements in the acceleration and binding of SARS-CoV-2, as well as its entrance into the host cell. MiRNAs linked to these ACE2 and TMPRSS2 proteins might be used as a treatment strategy for this virus. For example, the host miRNA-27b controls ACE2, whereas the virus miRNA-147-3P selects TMPRSS2. If these medicinal miRNAs can be administred into cells, the binding of spike proteins and receptors may be blocked, and hence viral infection propagation can be reduced or decreased [29,32].

Furthermore, a genomic analysis of COVID-19 from the United States, Wuhan, Italy, India, and Nepal revealed six anti-viral host tissue miRNAs specific to viral fragments, including has-let-7a and hsa-miRNA-101 (nonstructural protein), hsa-miRNA-126 (nucleocapsid), hsa-miRNA-23b (targets spike protein), hsa-miRNA-378 (targets spike protein) [33]. MiRNAs limit the translations of the target mRNA into the protein during viral protein replication; hence, miRNAs can be used as a treatment modality for viral illnesses [34]. Another strategy for COVID-19 control is to use completely complementary miRNAs (cc miRNAs), which can targeting the viral gene and suppress its posttranscriptional expression. The cc miRNAs (edited to 25-27 nucleotides), including ID02510.3p-miRNA, ID00448.3p-miRNA, miRNA-3154, miRNA-7114-5p, miRNA-5197-3p, ID02750.3p-miRNA, and ID01851.5p-miRNA, exhibited significant interaction with the SARS-CoV-2 viral genome [35]. The izMiR (miRNA prediction software) and PANTHER, bioinformatics-based classification systems, identified the possible mature viral and host cell miRNA candidates that could play a crucial role in SARS-CoV-2 infection [36].

Many COVID-19 candidate vaccines are now being studied, produced, assessed, and reviewed at an exceptional rate. Vaccines are compound biological agents which can only be developed for a diverse spectrum of normal individuals. Therefore, vaccine development and assessment take time since thorough research and surveillance are required to assure the appropriate distribution of any vaccine [37,38]. Since the start of the COVID-19 pandemic, multiple COVID-19 vaccine candidates have entered clinical trials in much less than six months and have been provisionally licensed in less than ten months, setting a new record for vaccine development speed. Multiple vaccine platforms have been explored for COVID-19: conventional whole virus, recombinant viral protein-based, viral vector, and nucleic acid (DNA and RNA) vaccines [39,40]. It has become a well-known technique for silencing/suppressing target genes linked with pathogenicity and pathophysiology since the discovery of regulatory RNAs such as miRNAs. Hence, it is critical to create safe and efficient vaccinations that target miRNAs in order to manage the COVID-19 pandemic, eradicate its transmission, and eventually thwart its reappearance [41].

MiRNA is a regulatory mechanism that cells use to remove unwanted or faulty mRNA. Thus, *in vitro*-created miRNA particular to the SARS-CoV-2 RNA genome would degrade the viral RNA while protecting lung cells from damage. The encoded miRNA is unique to the SARS-CoV-2 genome's conserved region at the 3'-end that encodes for the virus's S, E, M, and N functional proteins. Specific miRNA(s) can be engineered to attack one or all of these segments, and the target sequence will be destroyed utilizing the host cell's RNA-induced silencing complex machinery. This method allows us to create either a vaccination with long-term efficacy or a therapy for individuals who have already caught the illness [42,43].

Several COVID-19 potential vaccines were developed with the SARS-CoV-2 S protein or a portion of it as the immunogen, or an agent effective of eliciting an immune reaction [39,40]. Information from earlier SARS and MERS vaccine research justified the choice of S protein as the immunogen; vaccines that can produce robust antibody responses against the S protein frequently have a substantial effect on preventing viral entry into host cells during natural infection [44]. This finding was supported by investigations that found that the majority of SARS-CoV-2 neutralizing antibodies from COVID-19 recovered individuals were directed either against the S protein or its receptor-binding motif. Other structural proteins, in addition to S protein, have been investigated as vaccine targets. Vaccines based on N protein are unlikely to generate neutralizing antibodies, most probable since N protein isn't really present on the coronavirus membrane. N protein, on the other hand, is more preserved among coronavirus species than S protein, allowing it a possible target for a T-cell-inducing, universal coronavirus vaccines [40,42,44]. On the other side, M protein-based vaccinations can elicit a substantial amount of neutralizing antibodies in vaccinated animals [45]. Nevertheless, no preclinical evidence on neutralizing antibodies or defending immunity of M protein-based vaccines were revealed [46].

The miRNA delivery mechanism

The primary challenges with miRNA-based viral treatment and vaccination are miRNA distribution to destination cells/tissues, low circulation/half-life (since miRNAs are very unstable), and the toxic effects found in traditional delivery particles. A fundamental issue in realizing the full assurance of miRNA vaccines and treatments is maintaining their effective delivery. A basic chemical alteration can act as a method for improving miRNA stabilization, however it may not give *in-vivo* or clinical transformation [47]. Safe and effective delivery of the miRNAs into the target tissues remains a principal challenge in almost all miRNA therapeutics and vaccines. Thus, nanotechnology advances have expedited the development of new delivery strategies for miRNA vaccines and therapies [48]. Various nanoparticle carriers have been suggested for the successful miRNA delivery mainly inorganic nanoparticles, polymeric particles, and lipid nanoparticles [49]. Nanoparticle core shields the uncovered miRNA from degradation; consequently, responsible for improved circulation in the system [50]. The most commonly utilized carriers are lipid nanoparticles composed of synthetic cationic lipids that form nanoscale aggregates with polyanionic nucleic acids. MiRNA is more stable with respect to degradation when coated in positively charged lipids, and it creates self-assembled nanoparticles. When the lipid nanoparticles are endocytosed, they escape the vesicles and transport their payload to the cytosol, where the miRNA is translated into antigenic proteins, causing the defensive system to generate antibodies [51,52].

Concluding remarks and future perspectives

Since the discovery of human coronaviruses in the 1960s, new kinds of coronaviruses have emerged, posing a major danger to worldwide public health. Despite the fact that the first coronavirus epidemic occurred almost two decades ago, the scientific and medical sectors are still lacking in efficient weapons to battle these infections. One lesson we learnt from this is that the existing pharmaceutical market's financial and regulatory mechanisms do not give enough incentive to stimulate vaccine development before a fatal outbreak occurs. To cope, academic institutions and corporations all around the world are already generating an unprecedented number of vaccine candidates with extremely short clinical trial timelines. Furthermore, several treatment possibilities targeting molecules in the SARS-CoV-2 life cycle and the human immune response to COVID-19 have been swiftly investigated. Despite the fact that miRNAs have offered a fresh viewpoint on the regulation of gene expression and a variety of biological processes, many studies have not established all miRNA targets and complex mechanisms. MiRNA plays a crucial role in the pathophysiology of several viral illnesses, such as SARS-CoV-2, because of its post-transcriptional expression control function.

This review offers an overview of published information on worldwide coronavirus-related therapeutic medicines and preventative vaccinations research and development. It covers an overview of coronavirus shape, life cycle, and pathogenesis, with an emphasis on miRNA targeted treatment and vaccine development. The material in this study lays a solid conceptual foundation for ongoing research and development aimed at discovering and developing therapeutic medicines and vaccines for the treatment of COVID-19 and coronavirus-

related illnesses. As COVID-19 demonstrates, emerging infections produced by RNA viruses that are susceptible to mutations and chromosomal rearrangements, as well as cross-species transmission, will remain to be a serious worldwide health problem. Furthermore, the delivery method presents problems for miRNAs with well-defined activity and functioning mechanisms. Continued studies should focus on establishing comprehensive experimental techniques for the practical integration of miRNAs into COVID-19 medications and vaccines.

Availability of Data and Material

N/A.

Competing Interests

The authors of this study declare that they have no competing interests.

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