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UPDATE

Treating COVID-19 using Micro RNA

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Coronavirus disease 2019, Non-coding RNA, Micro-RNA, Treatment for Corona virus

Abstract

Over 7,805,583 people worldwide are susceptible to death because of infection by an encountered microscopic virus known as Coronavirus. Actually, it is a family of infectious viruses that was first discovered for about 50 years by a group of virologists.

By time, COVID-19 has developed and adapted to the surrounding spread prohibitions. That resulted in an incredible increase in the rate of death, in particular for old people who are suffering from other diseases and have frailty in their immunity that permit viruses attacks.

Various dense research is under working, as a way to uncover a cure or a vaccine on the long term. Hence, this paper focuses the lights on treating COVID-19 using a brand-new method of non-coding RNA (ncRNA).

I.BACKGROUNDS

Coronavirus was first identified in 1968 by a group of scientists who conveyed their findings to the journal Nature. Since the virus was pointed out under an electron microscope, it looked as a circular disk. Because of its shape, they have chosen the name Corona, which meant the sun. After various intensive research, it was discovered that Coronaviruses are single-stranded RNA viruses. Furthermore, they have a great susceptibility to mutate and recombine leading to highly diverse copies.

These diversities allowed them to have 40 distinct varieties which permitted infecting bats and wild animals. Therefore, Chinese populations who feed on wild animals' meat, have been contaminated, which resulted in the whole world infection. In consequence, the number of death people reached about 433,107, that stimulated researchers to find anyway to diminish the virus.

For example, transmitting plasma -occupies about 55% of blood components- from cured patients. That is due to the presence of emerged immunoglobulins (antibodies) from the immune system that defeated the virus. Unfortunately, this is a high-cost, inefficient and time-wasting process, as it is not applicable to all the recovered patients and requires exorbitant medical appliances.

Regarding these disadvantages, this research concerns using a new divergent method to deal with Coronavirus using gene expression regulators called non-coding RNA, that is represented in microRNA (miRNA), piwi-interacting RNA (piRNA) and short interfering RNA (siRNA). Since this RNA is non-coding, so it could be synthesized and injected to infected humans without significant interaction or interfering with the body's internal regulation.

II.COVID-19

Coronaviruses are part of Coronavirinae in Coronaviridae and Nidovirales. COVID-19 is a pandemic caused by 2019-nCoV and belongs to Beta-coronavirus genera, closely related to SARS-CoV [**Table 1**]. Due to its similarity to SARS-CoV, it was named SARS-CoV-2 by the Coronavirus Study Group of the International Committee on Taxonomy of Viruses [2-3].

They are spherical particles that contain positive-sense, single-stranded RNA, which acts as mRNA and can be translated by host cells. The RNA is associated with a nucleoprotein within a capsid composed of matrix protein, and the envelope bears club-shaped glycoprotein projections, including the hemagglutinin-esterase protein (HE) in some coronaviruses [4-5].

Coronaviruses have the largest genomes of all known RNA viruses, with varying Guanine and Cytosine contents. The viral genome contains small open reading frames (ORFs) within conserved genes and to the downstream nucleocapsid gene in distinct coronavirus linages. The major structural proteins in coronaviruses occur in the 5-terminus to 3-terminus order as spike, envelope, membrane, and nucleocapsid, with a unique N-terminal fragment within the spike protein [6]. Coronaviruses have six ORFs in their genome, except for Gamma-coronavirus which lacks the first ORFs (ORF1a/b) that encode 16 nsps. ORF1a and ORF1b produce two polypeptides that are developed by encoded proteases into 16 nsps, from which all accessory and structural proteins are translated [7]. The four main structural proteins are membrane (M), spike (S), nucleocapsid (N), and envelope (E), encoded by ORFs 10 and 11. Different coronaviruses code for special accessory and structural proteins responsible for various essential functions.

The M protein plays a sub-dominant role in viral particle origination, while the S protein is the main inducer of neutralizing antibodies and determines the composition and formation of the coronavirus membrane [8]. The absence of the S protein results in noninfectious virions with only the M protein.

The 5-end UTR and 3-end UTR of viruses play important roles in intra and intermolecular interactions, RNA-RNA interactions, and binding viral and cellular proteins. Pb1ab is the first ORF that encodes non-structural proteins in SARS-CoV, COVID-19, and MERS-CoV [9].

The S protein, located at the 3-end, is different in length between the three viruses. COVID-19 is less similar to MERS-CoV and SARS-CoV in terms of the arrangement of the structural proteins N, S, E, and M.

Understanding the replication of COVID-19 is crucial in managing the disease. It binds to ACE2 enzyme using its S protein, which must be activated by the TMPRSS2 protease enzyme. After entry, replication and transcription of its genome occur at cytoplasmic membranes with the help of the viral replicase protein complex consisting of cellular and viral subunits [10].

The virus uses unique processing enzymes, such as RNA helicase, RNA polymerase, protease, 3 to 5 ends exoribonuclease, putative sequence-specific endoribonuclease, 2 end-O-ribose methyltransferases, ADP ribose 1 end-phosphatase, and cyclic phosphodiesterase activities to replicate.

The replication process happens on the cell membrane, and mature particles form by budding from internal cell structures [11].

III.NON-CODING RNA (MICRO-RNA)

The human genome has only 2% for protein translation, while the remaining 98% of non-coding sequences are called non-coding RNA (ncRNA). ncRNAs do not participate in protein translation, but they have essential functions as they bind to distinct types of DNA and RNA in different specific ways leading to changes in their transcription, degradation, editing, processing and aids in translation [12-13]. ncRNAs are classified into two major classes based on transcription length, small (≤200 nucleotides) and long (>200 nucleotides) ncRNAs.

Long ncRNAs lack ORFs and are mostly specific to tissues, whereas small ncRNAs include small interfering RNA (siRNA), microRNA (miRNA), and PIWI-interacting RNA. The regulatory function of miRNA makes it essential in cellular processes [14].

Table 1 shows the most relevant types of viruses with their classification. Note that "ds" means double stranded and "ss" means single stranded

CLASS/FAMILY	ENVELOPE	EXAMPLES THATCAUSE HUMAN DISEASES
Adenovirus(dsDNA)	No	Respiratory viruses;tumor-causingviruses
Papovavirus(dsDNA)	No	Papillomavirus (warts,-cervical cancer);polyomavirus(tumors)
Herpesvirus(dsDNA)	Yes	Herpes simplex I and II(cold sores, genitalsores); varicella zoster(shingles, chicken pox);Epstein-Barr virus(mononucleosis,Burkitt'slymphoma)
Poxvirus(dsDNA)	Yes	Smallpox virus;cowpoxvirus
Parvovirus(ssDNA)	No	B19 parvovirus (mildrash)
Reovirus(dsRNA)	No	Rotavirus(diarrhea); Colorado tick fevervirus
Picornavirus(ssRNA)	No	Rhinovirus (common-cold); poliovirus;hepatitis A virus; otherenteric (intestinal) viruses
Coronavirus(ssRNA)	Yes	Severe acute respiratorysyndrome (SARS)
Flavivirus(ssRNA)	Yes	Yellow fever virus;West Nile virus;hepatitisCvirus
Togavirus(ssRNA)	Yes	Rubella virus; equineencephalitisviruses
Filovirus (ssRNA asmRNA)	Yes	Ebola virus(hemorrhagicfever)
Orthomyxovirus(ssRNAasmRNA)	Yes	Influenzavirus
Paramyxovirus(ssRNAasmRNA)	Yes	Measles virus; mumpsvirus
Rhabdovirus (ssRNAasmRNA)	Yes	Rabiesvirus
Retrovirus (ssRNAforDNAsynthesis)	Yes	Humanimmunodeficiencyvirus (HIV/AIDS);RNAtumorviruses (leukemia)

In 1993, Ambros and Ruvkun discovered that lin-4, which regulates temporal development of C. elegans larvae, was a small non-coding RNA that down-degraded lin-14 post-transcriptionally. miRNA is approximately 22 nucleotides long and mainly interacts with the 3-end UTR of target miRNAs to suppress and degrade their expression, but they also interact with other regions like 5-end RNA and control the rate of translation and transcription [15]. The biogenesis of miRNA involves canonical and non-canonical pathways. In the canonical pathway, pri-miRNA is transcribed and processed into pre-miRNA by the micro-repressor complex, followed by export to the cytoplasm and processing by Dicer. Both strands of the miRNA duplex are loaded into AGO protein, with the guide strand determined by 5-end stability or 5-end U. The unloaded strand is called the passenger and may be degraded by internal machinery.

MiRNA biogenesis involves canonical and non-canonical pathways. The canonical pathway involves transcription of pri-miRNA followed by processing into pre-miRNA, exported to the cytoplasm, cleaved by Dicer, and loaded into AGO. The non-canonical pathway includes Drosha/DGCR8-independent miRNA and Dicer-independent miRNA, both of which are modified and matured differently [16].

miRNAs can bind to the 3-end and 5-end UTR regions of mRNAs to regulate gene expression through miRNA response elements (MREs). The minimal miRNA-induced silencing complex (miRISC) includes AGO protein and the guide strand, which targets mRNA repression and deadenylation based on the degree of complementarity between miRNA and MREs [15-16]. Full complementarity induces AGO2 endonuclease activity leading to targeted mRNA cleavage, while central mismatches and errors prevent endonuclease activity, and AGO2 mediates RNA interference instead. Functional miRNA and MRE interactions occur through the 5-end seed region and pairing at the 3-end, aiding in specificity and stability.

MiRISC complex recruits GW182 family of proteins to recruit effector proteins such as CCR4-NOT and PAN2-PAN3 poly A deadenylase complexes to initiate poly A deadenylation of target mRNA. GW182 interacts with PABPC to stimulate efficient deadenylation. Decapping is done by DCP2, followed by 5-end to 3-end degradation by XRN1 [17].

IV.MODERN TECHNIQUES TO BE APPLIED

It is evident that miRNA serves as an intrinsic mechanism for gene regulation and suppression, particularly when targeting specific mRNAs. As COVID-19 is a single-stranded RNA virus, inhibiting and degrading its mRNA would prevent its replication and disruption in human cells, until a vaccine is developed. Therefore, it is crucial to concentrate research endeavors on using miRNA silencing methods to combat COVID-19. Accomplishing this requires specific technologies and techniques.

To study the virus, its mRNA must be extracted, sequenced, and analyzed for expression. This involves the reverse transcription of RNA to DNA using an enzyme called reverse transcriptase, similar to the process used by HIV. The mRNA is used as a template for the synthesis of a complementary DNA strand using thymine deoxyribonucleotides (dT's) as a primer. After degrading the template mRNA, DNA polymerase synthesizes a second DNA strand, resulting in double-stranded complementary DNA (cDNA) that carries the entire gene coding sequence without introns. The cDNA is produced by the molecular complex spliceosome during pre-mRNA processing.

To ensure that there are sufficient copies of the cDNA strand for experimentation, polymerase chain reaction (PCR) is conducted to amplify the single DNA strand. This method involves denaturing the double-stranded DNA by heating it and then cooling it to form hydrogen bonds between primers and the ends of the target sequences. Subsequently, nucleotides are continuously added in the 5'-end to 3'-end direction by DNA polymerase, and the process is repeated to generate more copies.

To sequence the DNA, the Dideoxy Chain Termination method is utilized. This method involves denaturing the DNA fragment into single strands and mixing it with primers, DNA polymerase, four deoxyribonucleotides, and four dideoxyribonucleotides, each labeled with a specific fluorescent molecule. The synthesis of each strand continues until a dideoxyribonucleotide is randomly incorporated, leading to a set of labeled strands of varying lengths. The resulting strands are separated by electrophoresis based on their lengths, and the sequence of the DNA strand can be determined by reading the pattern from the shortest to the longest.

In the context of miRNA, it has been elucidated that key proteins, such as GW182, play a crucial role in guiding miRNA towards specific mRNA targets. Therefore, modulating these proteins can potentially enable targeted miRNA-mediated regulation of COVID-19 mRNA upon infection. Techniques such as mRNA extraction and gene identification can be employed to study these proteins. Additionally, assessing the expression of their corresponding genes may be necessary, with RT-PCR being a widely used and efficient method. RT-PCR involves reverse transcription using RT enzymes, followed by PCR amplification and gel electrophoresis for gene expression analysis.

Conclusion

In conclusion, the utilization of miRNA for the treatment of COVID-19 is a novel and distinctive technological approach that has the potential to combat the virus by means of guiding proteins for miRNA strands. Further extensive research is required in this field to enhance its efficacy against not only COVID-19 but also other RNA viruses. Moreover, exploring non-coding RNA may provide a secondary immunity against future virus outbreaks.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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