mRNA– *i* TECHNOLOGY- A NOVEL AND UNIQUE APPROACH AND STRATEGY FOR PROPHYLAXIS AND TREATMENT OF COVID 19

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Highlights of Hypothesis

Corona virus disease 2019 (COVID 19) has been pandemic worldwide and still spreading with exceptionally high rate day by day. In such a time effective steps and approaches are urgently approved to control this viral infection. But this task is very intricate and complicated because of the limited effectiveness of accessible anti-viral agents and the high speed of mutation rate of the viral genome. In addition there is no effective drug or vaccine is available to prevent and cure COVID 19.

The present hypothesis emphasizes on application of RNA*i* technology, which is a novel and unique biotechnological method. This RNA interference (RNA*i)* technology may be a promising tool to inhibit Human Corona Virus 2 (HCoV 2) infection by targeting viral or cellular genome. The RNA*i* technology can be used as a possible therapeutic substitute for COVID-19. Though further research is required by geneticists and biotechnologist to develop such an optimized RNA*i* technology to prevent HCoV 2infection and find out siRNA (Small interfering RNA) based antiviral therapy or vaccine.

Abstract

RNA*i* is a natural cellular progression of gene silencing that represents one of the most promising and rapidly advancing frontiers in microbiology and drug invention today. Its discovery has been considered as "a major scientific breakthrough that happens once every decade or so," and was recognized with the award of the 2006- Nobel Prize for Physiology or Medicine. RNA*i* is an adaptive cellular defense mechanism, is a worldwide denominator for the Post-transcriptional Gene Silencing (PTGS) phenomenon observed in a variety of living species including plants and animals. It is a dominant reverse genetic procedure that has been extensively employed to silence gene expression in human cells.

RNA*i*- based gene therapies, especially in viral diseases have become more and more interesting and promising. Recently, siRNA can be used to defend host from viral infection, inhibit the expression of viral antigen and accessory genes, control the transcription and replication of viral genome, obstruct the assembly of viral particles, and display influences in virus-host interactions. Literature and early reports suggest that biologists have applied RNA*i* technology to combat number of viral infections. They have also developed RNA*i* library to study the function of genes in human body, which can smoothen the task of the identification of drug targets against any kind of viral infection. Though further research is necessary to be carried out by geneticists and biotechnologists to develop such a unique and novel RNA*i* technique to prevent HCoV 2 infection and find out siRNA (Small interfering RNA) based antiviral therapy or vaccine for COVID-19.

Keywords: COVID-19; HCoV 2; Post-transcriptional Gene Silencing; RNA*i*; siRNA.

I. INTRODUCTION

1.1 About COVID-19

COVID-19 is an infectious disease caused by Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-HCoV-2) (Mayo Clinic, 2020). The disease was first identified in Wuhan, the capital of China's Hubei province, in December 2019 and has since spread internationally, resulting in the ongoing 2019-20 corona virus pandemic (Hui DS et al, 2019). Common primary symptoms of COVID-19 include fever, cough and shortness of breath (Anonymous, 2020). Other secondary symptoms may include fatigue, muscle pain, diarrhea, sore throat, loss of smell, and abdominal pain (Hopkins & Claire, 2020). The incubation period of infection is characteristically around 5 to 14 days (Velavan & Meyer, 2020). However the majority of cases result in mild symptoms, some progress to pneumonia and gastro-intestinal troubles (WHO, 2020). As of 16 May 2020, more than 4.64 million cases have been reported across 210 countries and territories, resulting in more than 3.08 lakhs deaths (Worldometer, 2020).

1.2 About HCoV 2 and its genomic expression

HCoV 2 are enveloped, positive-stranded RNA viruses. They have a broad host range and are proficient of infecting the tissues of the upper respiratory and gastrointestinal tracts, the liver and CNS (Lai MMC & Holmes KV, 2001). Corona viruses, HCoV-229E and HCoV-OC43, are the major causative agent for 15-30% of common colds in humans each year. Corona virus infections were not previously considered to be serious enough to require treatment by either vaccination or specific antiviral therapy (Holmes KV, 2001). The virion of corona virus is a spherical particle 100-120 nm in diameter and contains a capped, polyadenylated, single-

stranded, positive-sense genomic RNA, which is 27-32 kb in length, and is the largest known RNA virus genome (Lai MMC & Cavanagh, 1997).

For viral RNA synthesis, polypeptides 1-a and 1-b are required and they are the only proteins that are synthesized directly from the original viral (other viral proteins are translated from sub-genomic mRNAs). If once viral RNA synthesis takes place, then more product of gene 1 are translated from the newly synthesized genomic RNA. The primary gene products (ORF 1A and -B), predicted to be 700-800 kDa, undergo post-translational processing into various proteins as a result of their own protease activity. The virus-encoded proteases, Papain-Like cysteine Protease (PLpro) and 3C-Like cysteine Protease (3CLpro), cleave the polypeptide into small polypeptides that are required for replication and transcription (Lee HJ, 1991). RNA-dependent RNA polymerase and helicase are essential components of the replicase complex, which, presumably, contains other viral and cellular proteins. The replicase complex is responsible for transcribing: the full-length negative and positive RNAs; the 3′ co-terminal set of nested sub-genomic mRNAs; and sub-genomic negative RNA strands (Thiel V, 2003).

The invasion of enveloped HCoV 2 indicates the 3 steps of attachment, receptor binding and virus-cell fusion, mediated by viral envelope proteins. The S glycoproteins, which make up large, petal-shaped spikes on the surface of the membrane, bind to a cellular receptor, promoting fusion of the viral and cellular membranes, and this event explains the primary viral invasion (Gallagher T & Buchmeier, 2001). This highly glycosylated protein, with a molecular weight of 150-180 kilo dalton (kDa), can be divided into 3 structural domains: a large external N-terminal domain; transmembrane domain; and short C-terminal cytoplasmic domain (Vennema H, 1991). The MHV receptor is a murine biliary glycoprotein belonging to the carcino-embryonic antigen family of the Immunoglobulin (Ig) superfamily (Williams RK, 1991). The cell membrane-bound metalloproteinase, amino peptidase N (CD13), is probably the receptor for TGEV, HCoV-229E and CCoV (Benbacer L, 1997). CD13 is widely distributed in cells in many tissues, including respiratory, enteric epithelial, neuronal and glial cells. The receptor for SARS- HCoV 2 is considered to be Angiotensin-Converting Enzyme Subtype 2 (ACE2), required for binding to permissive cells and the S1 subunit (Li Wet al, 2003).

The intrinsic membrane proteins, M and E, are the protein units required for virus assembly (Vennema H, 1996). M protein is found not only on the viral envelope but also in the viral internal core, and associates with the Golgi complex in the cell, thereby dictating the site of virus assembly. The expression of M and E proteins may be adequate enough to activate the formation of virus-like particles. When S protein is co-expressed with the M and E proteins, it is incorporated into VLPs with an authentic conformation that is able to infect cells (Bos EC, 1996; Corse E & Machamer CE, 2003). As they do not contain viral nucleic acid, VLPs are an ideal candidate for vaccine preparation. The final structural protein, N protein, with a molecular mass of 50-60 kDa, probably associates with viral RNA to form a long and flexible helical nucleocapsid (Sturman LS, 1980). N protein may also play a role in viral RNA synthesis, as coronavirus RNA polymerase activity is inhibited in vitro by the anti-N protein antibody (Compton SR, 1987).

II. mRNA-*i* TECHNOLOGY

Higher organisms are persistently exposed to viruses and have evolved assorted mechanisms to combat the parasites. Among them one of the important mechanism in a cell inherent fashion, targeting viral genome and their proteins for demolition and causing the premature shutdown of infected cells to prevent them from serving as virus manufacturing unit. Such kind of intrinsic antiviral mechanisms are part of the innate immune system and include RNA*i* and the interferon system. The two systems operate very differently even though they can both be triggered by virally derived long double stranded RNA (dsRNA). DsRNA can derive from the viral genome or from annealing of two strands of complementary RNAs, which are generated as RNA virus replication intermediates or DNA virus convergent transcripts. Highly based paired ssRNAs are found in hairpins within viral genomes or viral transcripts and are generically referred to as dsRNA. Both types of dsRNA are absent from uninfected cells and act as evidence of viral infection to promote innate immune responses against virus.

RNA*i* is a novel biotechnological method that uses short RNAs (20-30 nucleotides) to recognize and manipulate balancing nucleic acids. RNA*i* related pathways have roles in the control of gene expression, epigenetic modification and regulation of heterochromatin, and in the host–parasite interactions. The introduction of long dsRNA into cells can effectively and specifically lead to the degradation of cognate mRNAs in a gene-dependent manner. This powerful technology has been widely employed to manipulate gene expression, elucidate signal pathways and to identify gene functions in a whole-genome scale. Researchers worldwide have used RNA*i* for basic research, and are now developing RNA*i*-based drugs for the prevention and treatment of human diseases such as viral infection, tumors and metabolic disorders (Yin JQ et al, 2003).

III. TOOLS AND MECHANISMS OF mRNA*i* TECHNOLOGY (Fig.1)

Biogenetical studies have revealed the molecular mechanismes, by which dsRNA causes the degradation of target messenger RNA of virus.

RNA*i* technology includes two steps:

1. Initiation step: In the initiation step, Dicer, a member of the RNase III family of ATP-dependent ribonucleases, binds with high affinity to dsRNA containing 2 nucleiotide (nt) 3’ overhangs and chops long dsRNA (introduced directly or via a transgene or virus) into small interfering RNAs (siRNAs) duplexes. Generally, Dicer enzymes contain an N-terminal DEXH-box RNA helicase domain, a Domain of unknown function (DUF283), a PAZ domain, two RIII domains and a dsRNA-binding domain (dsRBD) (Carmell MA, Hannon GJ, 2004). Dicer can cleave dsRNA into siRNAs or microRNAs (miRNAs) from endogenous stem loop precursors (Lee YS et al, 2004). Biochemical studies show that siRNAs are 21-23 nt dsRNA duplexes with 2-nt 3’ overhangs, a 5’- monophosphate and a 3’-hydroxyl group (Elbashir SM, 2001).

2. Effector step: the siRNA duplexes are incorporated into RNA induced silencing complex (RISC). The phosphorylation of siRNA 5'-terminal is required to entry into RISC (Khvorova et al, 2003). A helicase domain of RISC binds to one end of the duplex and unwinds the double-strand in an ATP dependent manner. The thermodynamic stability of the first few base pairs of siRNA can affect the ratio of RISC containing the antisense or sense strands of siRNAs (Drosett Y & Tuschl T, 2004). Dicer with R2D2 (Dcr-2-associated protein) binds siRNA and facilitates its loading onto RISC (Pham JW, 2004). The active RISC then targets the homologous transcript by base pairing interactions and cleaves the mRNA between the 10th and 11th nucleotide from the 5' terminus of the siRNAs (Ahlquist P, 2004). MicroRNAs (miRNAs) are endogenous RNAs consists of ~22 nucleotides that can play important regulatory roles in animals and plants by targeting mRNAs for cleavage or translational repression. miRNAs and endogenous siRNAs have a shared central biogenesis and can perform interchangeable biochemical functions. Therefore, the two classes of silencing RNAs cannot be distinguished by either their chemical composition or mechanism of action. These short RNA species are produced by Dicer cleavage of long (~70 nucleotides) endogenous precursors with imperfect hairpin RNA structure in animals. Mature miRNAs act as a translational repressor by partial base-pairing with 5' or 3' ends of mRNAs while miRNA completely complementary to its target mRNA (endogenous siRNA) can result in the degradation of cognate mRNA. In addition, many other proteins such as eukaryotic translation initiation factor 2C2 (eIF2C2) and Argonaute proteins are likely to function in both pathways. Argonaute proteins are the key components of RNA-induced silencing complex (RISC). They are evolutionarily conserved with two distinguishing domains, PAZ and PIWI. The PIWI domain is restricted to Argonautes while the PAZ domain is shared with Dicer family proteins (Song JJ et al, 2002).

The eventual trigger initiating RNA silencing is dsRNA, which is a replication intermediate generated by viral RNA-dependent RNA polymerases of infecting RNA viruses (Ruiz-Ferrer V & Voinnet O 2009). RNA silencing mechanisms start with preliminary processing or cleavage of a precursor dsRNA into short 21-24 nucleotide small interfering (siRNA) or micro RNA (miRNA) duplexes (Hamilton AJ & Baulcombe DC, 1999) by an RNaseIII-like enzyme called Dicer (DCL) (Baulcombe D, 2011). Double-stranded siRNAs are incorporated into an RISC containing an Argonaute (AGO) protein that has a sRNA-binding domain and an endonucleolytic activity for cleavage of target RNAs (Vaucheret H et al, 2004) The activated RISC subsequently unwinds siRNAs, thereby generating a sense (passenger) and an antisense (guide) strand in an ATP-dependent reaction. While the sense strand is degraded, the RISC containing the antisense strand subsequently targets a complementary mRNA transcript via base pairing interaction, degrades the mRNA and thereby inhibits protein biosynthesis (Ghildiyal M, 2009; Liu Q & Paroo Z, 2010).

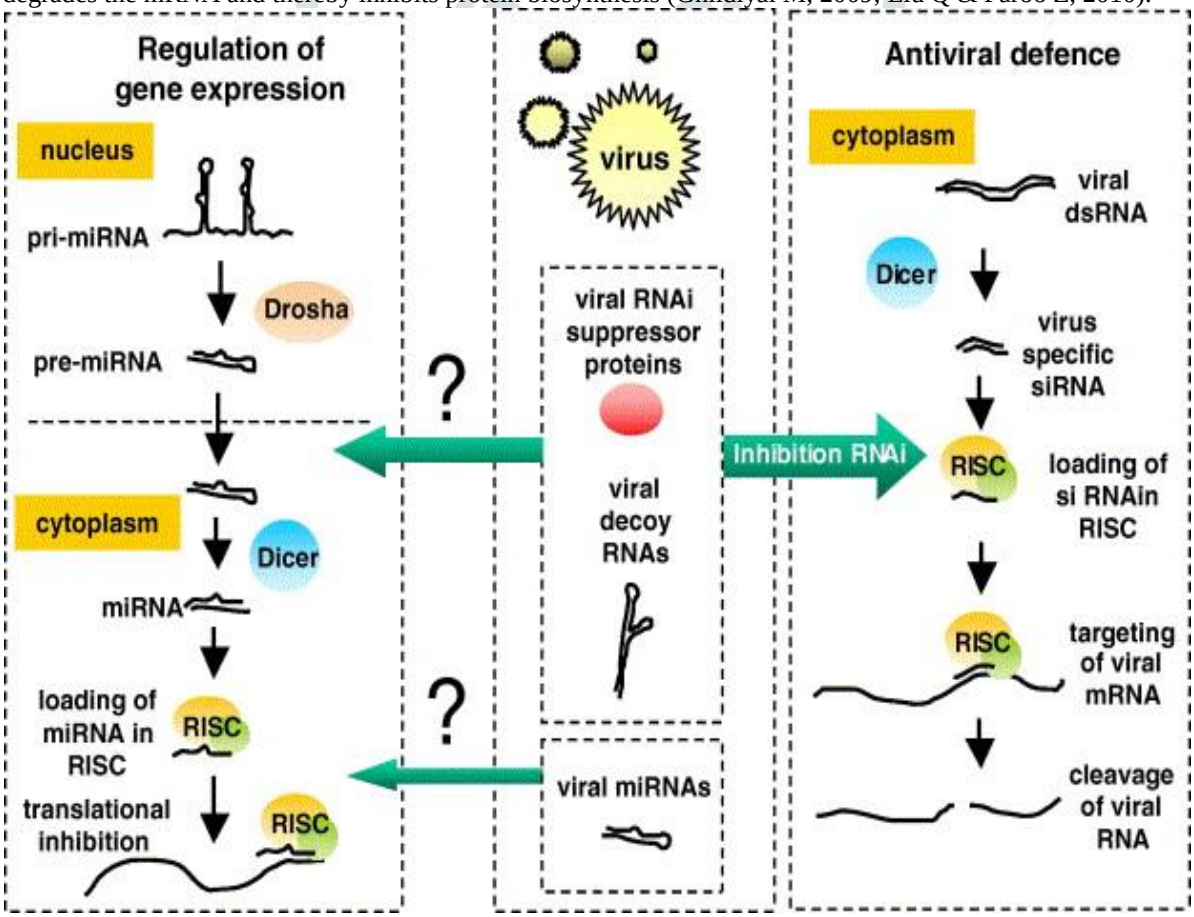

Figure 1 Diagrammatic presentation of working mechanism of mRNA-*i* technology

IV. CLINICAL REPORTS: POSSIBLE TARGETS OF ENDOGENOUS siRNA AS ANTIVIRAL AGENT

- ***RNAi inhibits the expression of viral antigens***.
  Report: Zhang et al, 2004 showed that the DNA vector-driven siRNA against the spike protein of SARS-CoV could electively and specifically silence gene expression of the Spike protein in SARS-infected 293T cells.

Therefore the suppression of expression of viral antigens by RNA*i* will be effective strategies for the therapy of viral infection.

- ***RNAi suppresses the transcription of viral genome.***
  Report: HCoV 2 is a retrovirus. It contains *gag*, *env* and *pol* genes for genome transcription. siRNA may inhibit transcription of these genes.
- ***RNAi blocks viral replication.***
  Report: Sanchez-Vargas I et al, 2004 reported that activation of RNA silencing is thought to be involved in the inhibition of viral replication.
- ***RNAi silences viral accessory genes.***
  The viral accessory genes take part in the viral pathogenesis such as latency and persistence and regulate the expression of other genes.
  Report: Jacque JM et al, 2002 investigated RNAi technique to prevent HIV-1 infection. In his study viral RNA is introduced into the host cell cytoplasm in the form of a nucleoprotein complex. He noted that siRNA duplexes against several regions of the HIV-1 genome, including the viral long terminal repeat (LTR) and the accessory genes *vif* and *nef* were co-transfected with an HIV-1 molecular cloning into CD4-positive HeLa (Magi) cells. Compared with cells not transfected with siRNA duplexes, virus production was reduced 30- fold to 50-fold by homologous siRNAs. These results provided a therapeutic alternative for AIDS by siRNA mediated degradation.
- ***RNAi hinders the assembly of viral particles.***
  Report: Many capsid proteins are involved in the assembly of virus particles including VP4 and matrix proteins. siRNAs targeting to different viral protein genes can be used in the study of the assembly of viral particles. The knockdown of these genes will effectively prevent the viral infection (Barik S, 2004).

- ***RNAi displays roles in virus-host interactions.***
  Viruses can control both viral and cellular gene expression while host cells can also protect themselves against virus infection through immune response. The suppression of these genes by siRNAs can be also used to address the role of such genes in host–virus interaction.
  Report: Bitko et al, 2003 investigated siRNAs-knockdown technology to determine the role of profilin in the RSV life cycle.

V. ADVANTAGES OF mRNA*i* TECHNOLOGY

1. This technique is very easy and flexible to select target sites because target mRNA and siRNA are sequences-specific and complementary. For a given mRNA molecule, the inhibitory effects of siRNAs can be achieved by targeting different regions of target mRNA.
2. To induce silencing of viral gene, only substoichiometric amount of siRNA is enough to significantly decrease homologous mRNA within a day.
3. siRNAs can result in the degradation of cognate mRNA in cells of different organism species.
   siRNAs may be administered prior to infection or during the early phase of infection.
   Thus, it is possible to achieve the prevention of HCoV 2 infection by inhalation of siRNA once per week or even less often. Highly effective inhibition of virus replication by RNA*i* has been achieved both *in vitro* and *in vivo* **(**Ge Q et al, 2004**).**
4. siRNAs do not affect cell control mechanisms. The given length and high homology of siRNA to the target region of cognate transcription ensure the selective destruction of only interested transcript. siRNAs without suitable targets seem to remain inert within cells. This exclusive specificity without adverse side effects is the most attractive feature of RNA*i* as an antiviral approach.
5. siRNAs can silence gene stably. With the application of plasmid vectors and viral vectors, siRNAs can display their long-term biological effects.

VI. SUCCESS STORY OF mRNA-*i* TECHNOLOGY

The utilization of mRNA*i* technology was first of all raised in plant organisms when experimentally induced 'gene silencing' was found to provide resistance to viruses carrying an identical sequence (Lindbo et al, 1993). It was then identified as a natural component of innate antiviral immunity when viruses were found to naturally induce a similar response (Covey *et al.* 1997). For animals, antiviral RNA*i* was first identified in Drosophila cell culture, where the beetle Nodamura virus FHV (flock house virus) acts as both an initiator and a target of the viRNA pathway (Li et al, 2002). Later on it was acknowledged as biologically relevant defense outside of cell culture using the natural host-virus combination of O'nyong-nyong virus (alphavirus; Togaviridae) and Anopheles gambiae mosquitoes (Keene *et al.* 2004). RNA*i* has consequently been recognized as a factor of antiviral immunity in adult Drosophila (Wang *et al.* 2006; Zambon *et al.* 2006) and in nematode worms (Wilkins *et al.* 2005). Recently, RNA*i* has also been investigated as an significant antiviral defense in fungi (Hammond *et al.* 2008). It remains an open question as to whether this RNA*i* pathway might be a component of antiviral immunity in vertebrates (Haasnoot *et al.* 2007). Though vertebrates encode relevant genes (including Argonaute, Dicer and SID family members), there is no physically powerful evidence of a viRNA antiviral pathway, and these RNA*i* genes are used in other RNA*i* pathways that arbitrate host cellular defense mechanism.

VII. CONCLUSIONS

By observing epidemic condition of COVID 19, more efficient approaches are urgently desired to be implemented. Conventional drugs and vaccines used for the treatment of viral diseases may have many adverse effects, such as toxicity, cost and

resistance, and complicated administration protocols. Present hypothesized technology has focused that the application of RNA*i* can inhibit viral infection by targeting viral genes. RNA*i* is considered as a gene-specific therapeutic alternative to combat viral infection. The control of HCoV 2 infection is very difficult and complex because of the limited effectiveness of available anti-viral agents and the high speed of mutation rate of the viral genome. Careful assessments are required for the potential of RNAi as a gene therapy approach for controlling HCoV 2 infection. The challenge of successful siRNA based drugs in the near future is to develop efficient and safe means to deliver siRNAs into affected cells. Scientists have developed many RNA*i* libraries to study the function of genes in and human (Paddison PJ et al, 2004), which can greatly facilitate the identification of drug targets against HCoV 2 infection (Berns K et al, 2004). The current challenge for the success of siRNA-based treatment is the precisely delivery method of siRNAs in an efficient and safe way to the target cells. With the advancement of RNA*i* data bases in human and the refinement of techniques to silence gene, siRNA-based drugs can difinilely make revolution in the prophylaxis and treatment of COVID 19.

VIII. CONFLICT OF INTEREST/ COMPETING INTERESTS

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

IX. ABBREVIATIONS

3CLp : 3C-Like Cysteine Protease
ACE2 : Angiotensin-Converting Enzyme Subtype 2
AGO : Argonaute (AGO)
COVID 19: Corona virus disease 2019
dsRBD : dsRNA-binding domain
dsRNA : double stranded RNA
DUF283: Domain of unknown function
eIF2C2 : eukaryotic translation initiation factor 2C2
HCoV 2 : Human Corona Virus 2
Ig : Immunoglobulin
kDa : kilo dalton
mi RNA: MicroRNAs
PLpro : Papain-Like cysteine Protease
PYGS : Post-transcriptional Gene Silencing
RISC : RNA-induced silencing complex
RNA*i* : RNA interference
SARS-HCoV-2 : Severe Acute Respiratory Syndrome Corona Virus 2
siRNA : Small interfering RNA

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