

An aptamer/ siRNA Chimera against The SARS-CoV-2: A Dual Therapeutic Strategy for The Virus Neutralizing and RNA Interfering

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Method Article

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Abstract

During the ongoing COVID-19 pandemic, besides the vaccines, there is an urgent need for the development of effective therapeutics. Although significant efforts have been made to develop such therapies, there are currently no approved treatments for COVID-19. One of the potential therapeutic targets is the spike (S) protein of SARS-CoV-2, which mediates viral entry into host cells. It has been shown that targeting S protein could neutralize viruses and hinder their binding to the cells. Among known viral neutralizing agents, aptamers' potential in neutralizing the SARS-Cov-2 virus has not yet been revealed. In addition, aptamers could also be used for targeted delivery of drugs and other genetic elements, such as siRNAs, to the cells. Small interfering RNAs (siRNAs) are reliable tools for gene knockdown via RNA degradation. siRNAs have been implemented previously against some viruses, including SARS-CoV, to target its genome. Recently, potential siRNA sequences and their targets in the SARS-CoV-2 genome have been reported, and the efficiency of siRNAs in inhibiting SARS-CoV-2 infection is being revealed. An alternative antiviral approach we propose here relies on an aptamer-siRNA-based system for the treatment of COVID-19. These aptamers could neutralize viruses by hindering their receptor-mediated endocytosis, and siRNAs could suppress the expression of viral genes and halt various aspects of its pathogenesis whenever the aptamers fail to neutralize the virus.

1. Introduction

As of 8 August 2020, the COVID-19 resulted in 19 187 943 confirmed cases, and 716 075 confirmed deaths globally (1). Based on the magnitude of the pandemic, there is an urgent need for the development of safe and effective therapies.

Accordingly, various therapeutic approaches targeting different aspects of SARS-CoV-2 pathogenesis have been proposed since the beginning of the pandemic. These approaches range from dampening SARS-Cov-2 entering host cells to modulating host immune responses (2). Although significant efforts have been made to develop therapies, there are currently no approved treatments for COVID-19 (3). However, biotechnological approaches appear to be promising in the treatment of COVID-19 as well as its prevention (i.e., vaccines) and diagnosis (4–6).

An alternative antiviral approach we propose here relies on an aptamer-siRNA (small interfering RNAs)-based system for the treatment of COVID-19. Aptamers are single-stranded oligonucleotide sequences that specifically bind to target molecules. These aptamers could neutralize viruses by hindering their receptor-mediated endocytosis. siRNAs are small interfering RNAs that suppress the expression of target genes via RNA cleavage. These interfering RNAs could also suppress the expression of viral genes and halt various aspects of its pathogenesis. However, efficient and targeted delivery of siRNAs to target cells remains challenging. A strategy for overcoming the delivery challenge of siRNAs is combining them with aptamers, which have achieved success in cell-specific delivery of siRNAs and subsequent target RNA cleavage (7,8).

The chimeric structure proposed here consists of an aptamer against the SARS-Cov-2 spike protein (S) and an siRNA against the virus genome. This structure is supposed to neutralize the virus and degrade the viral genome within the host cell. Therefore, the proposed chimeric aptamer-siRNA-based system might be auspicious in the treatment of COVID-19.

2. Rationale

The S protein of the SARS-CoV-2 virus mediates viral entry into host cells. This protein, especially by its receptor-binding domain (RBD), interacts with the ACE2 receptor, which is expressed primarily in alveolar epithelial type 2 cells (9,10). It has been shown that targeting S protein could neutralize known coronaviruses (e.g., SARS-CoV) and hinder their binding to the cells. In this regard, monoclonal antibodies (mAbs), single-chain variable fragments (scFvs), etc., have been utilized (6,9–11). Several ongoing clinical trials are employing mAbs to neutralize the SARS-CoV-2 virus as a therapeutic regimen (12–14).

Among established viral neutralizing agents, aptamers' potential in neutralizing the SARS-Cov-2 virus has not yet been revealed. Aptamers are nucleic acids selected from pools of randomly synthesized RNAs according to their high binding affinity to target molecules (15,16). Aptamers' nanomolar binding affinities and exquisite binding specificity have made them eligible tools as targeted therapeutics (16). Using aptamers for targeting proteins has some advantages over using mAbs. First, they can be chemically synthesized, which is cheaper and more precise than endogenous synthesis. Second, aptamers are stable at high temperatures and have a high shelf life; third, they do not generate inadvertent anti-idiotypic responses (15). An aptamer that binds specifically to the SARS-CoV-2 virus nucleocapsid protein has been applied to the early diagnosis of the virus (17). However, aptamers' efficiency in targeting viral S protein for neutralizing the virus and hindering its binding to host cell surface ACE2 receptors has not yet been studied.

Aptamers are also used for targeted delivery of drugs and other genetic elements, such as siRNAs, to cells(18–20). siRNAs are reliable gene knockdown tools. They exploit the intrinsic cell system to become a part of the RNA-induced silencing complex (RISC) and recognize their target RNA. This recognition leads to the repression of translation or degradation of the target RNA (21). Studies have shown high specificity, excessive efficiency, and low toxicity of siRNAs for treating different diseases (22).

RNA interference using siRNAs targeting the viral genome or its key coded proteins has previously been used against some viruses, including SARS-CoV (23–27). Since SARS-CoV-2 is an RNA virus, either its genome or its key protein expression could be targeted via siRNAs (28). Recently, potential siRNA sequences and their targets in the SARS-CoV-2 genome have been reported, and the efficiency of siRNAs in inhibiting SARS-CoV-2 infection has been revealed (29,30).

3. Design

We hypothesized that an anti-S aptamer/siRNA chimera will be a rational drug that employs both viral neutralizing and RNA interfering strategies in one structure (Figure 1). The chimera consists of an anti-S aptamer molecule linked to a siRNA via a UU linker. The anti-S-aptamer part of the chimera could be designed to bind either S1 or S2 segments of the viral S protein. Therefore, this binding can hinder either the interaction between the RBD domain in the S1 segment with its respective receptor or membrane fusion mediated by the S2 segment (6) (Figure 2).

The siRNA part of the chimera is designed against the virus genome and plays its antiviral role whenever the aptamers fail to neutralize the virus. If the virus escapes neutralization through its uncovered S proteins, the chimera will enter the host cell through receptor-mediated endocytosis of the virus (Figure 3). Subsequently, chimeras escape endosomes and release their siRNAs in the cytoplasm via DICER processing (18). Then, siRNA molecules are incorporated into an RNA-induced silencing complex (RISC), and their antisense strand will guide the activated RISC to the cognate RNA. This process will lead to virus RNA cleavage.

Neff et al. experimented with a similar structure that targets viruses against HIV infection. Their anti-gp120 aptamer/siRNA chimera successfully neutralized HIV and simultaneously suppressed the expression of *tat/rev* viral genes. Their chimera showed more extensive inhibition and longer antiviral effects than the aptamer alone in mice (16). Therefore, it can be postulated that the same antiviral effect could be achieved against SARS-CoV-2 using our hypothesized structure.

4. Evaluation Of The Hypothesis

4.1. Identifying the aptamer sequence:

Systematic evolution of ligands by exponential enrichment (SELEX) should be used to identify potential aptamers against the viral S protein. In this strategy, a large pool of variable sequence oligonucleotides that are synthesized *in vitro* will be exposed to the target molecules to allow their binding to the target. Then, molecules that are capable of binding will be separated and further amplified to be used as the new oligonucleotide pool for repeating this procedure. After several cycles of RNAs' artificial selection based on their binding affinity to the viral S protein, the winner molecules will be our desired aptamers (31).

4.2. Identifying the siRNA sequence:

The SARS-CoV-2 genome sequence will be obtained from the NCBI database (32,33). Potential siRNA sequences targeting the viral genome or other viral RNA molecules could be predicted using siRNA prediction programs (29,30). The most efficacious siRNAs will be determined to be implemented in the chimera construct using *in vitro* and *in vivo* studies similar to what is described by Gu et al. (30).

4.3. Examining the aptamer/siRNA chimeric structure efficiency:

Aptamer/siRNA chimeric structures will be prepared using the method described by Zhou et al. (2009) (34). To evaluate the hypothesis and address the potential challenges in the application of the proposed

treatment, *in vitro* and *in vivo* experimental studies will be conducted.

4.3.1 *In vitro* studies

Vero/hSLAM cell lines will be maintained in Earle's Minimum Essential Medium (EMEM) and subsequently infected with SARS-CoV-2. SARS-CoV-2-infected cell lines will be treated with the following agents within four groups: chimera, aptamer alone, siRNA alone, and the control. Afterward, viral loads will be quantified by real-time PCR and will be compared with the control group (35). Besides, target gene expression levels will be determined by q RT-PCR, and their cleavage will be determined by the 5'-RACE PCR assay (16).

4.3.2 *In vivo* studies

First, humanized COVID-19 mouse models possessing human ACE2 will be prepared by exogenous delivery with a replication-deficient adenovirus. After infecting the mice with SARS-CoV-2 through the intranasal route, virus titers in the sera will be determined (36). Infected mice will receive the following agents within four groups: chimera, aptamer alone, siRNA alone, and the control. Afterward, the target gene expression level will be determined by q RT-PCR. Moreover, target RNA cleavage will be determined by a 5'-RACE PCR assay (16). To determine the agents' therapeutic efficiency, virus titers, pulmonary function, and weight loss will be assessed (36,37).

5. Discussion

Here, we have proposed a chimeric aptamer-siRNA structure that will be a dual anti-SARS-CoV-2 treatment strategy. Aptamers can neutralize the virus spike protein and halt its binding to the host cell surface, and the released siRNAs within host cells will cleave the virus genome. The chimeric aptamer-siRNA and SARS-CoV-2 will enter the host cells simultaneously, which offers targeted delivery of the proposed structure.

Aptamers are capable of highly specific binding and distinguishing between proteins sharing similar structures(38). Moreover, aptamers have shown easier, cheaper, and comparable functionalities to antibodies in terms of various research, diagnostics, and therapeutic approaches (39–42).

However, according to the novelty of SARS-CoV-2 emergence, there are still several unidentified factors related to virus pathogenesis and genetic variations. Thus, these factors might affect the treatment efficiency of the hypothesized structure. Furthermore, it has been shown that several functional SARS-CoV-2 S protein variants that confer resistance to commonly elicited neutralizing antibodies are now present at low frequencies in circulating SARS-CoV-2 populations (43). To overcome this resistance, it is possible to design and administer a cocktail of chimeric aptamer-siRNA structures to target various parts of the S protein, which will be lower in production cost and simpler in regulatory approval than using a cocktail of neutralizing antibodies.

Besides, after evaluating the efficiency of the hypothesized treatment strategy by *in vitro* and *in vivo* studies, several additional studies will need to be conducted to determine the pharmacokinetics and dynamics of the chimeric aptamer-siRNA treatment. Additionally, it is of the highest implication to discover specific targets for aptamers using SELEX technology.

In summary, we have proposed an anti-S-aptamer/siRNA chimera that will be a rational drug to employ both viral neutralizing and RNA interfering strategies in one structure. There is currently no effective cure for COVID-19. However, the treatment strategy proposed here is theoretically expected to be effective in this regard.

6. Abbreviations

COVID-19: Coronavirus Disease 2019

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2

siRNA: Small Interfering RNA

RBD: Receptor Binding Domain

ACE2: Angiotensin Converting Enzyme 2

mAB: Monoclonal Antibodies

ScFv: Single Chain Variable Fragment

RISC: RNA-induced Silencing Complex

gp-120: Glycoprotein 120

HIV: Human Immunodeficiency Virus

SELEX: Systematic Evolution of Ligands by Exponential Enrichment

NCBI: National Center for Biotechnology Information

PCR: Polymerase Chain Reaction

RT-PCR: Real-time Polymerase Chain Reaction

EMEM: Earle's Minimum Essential Medium

RACE: Rapid Amplification of cDNA Ends

7. Declarations

Ethics Approval and Consent to Participate:

Not applicable

Consent for Publication:

Not applicable

Data Availability:

Not applicable

Competing interests:

The authors declare that they have no competing interests.

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Author's contributions:

J Kh, M A-K, Y A and M J hypothesized the idea and wrote the manuscript. J Ki read the manuscript and revised it.

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Figures

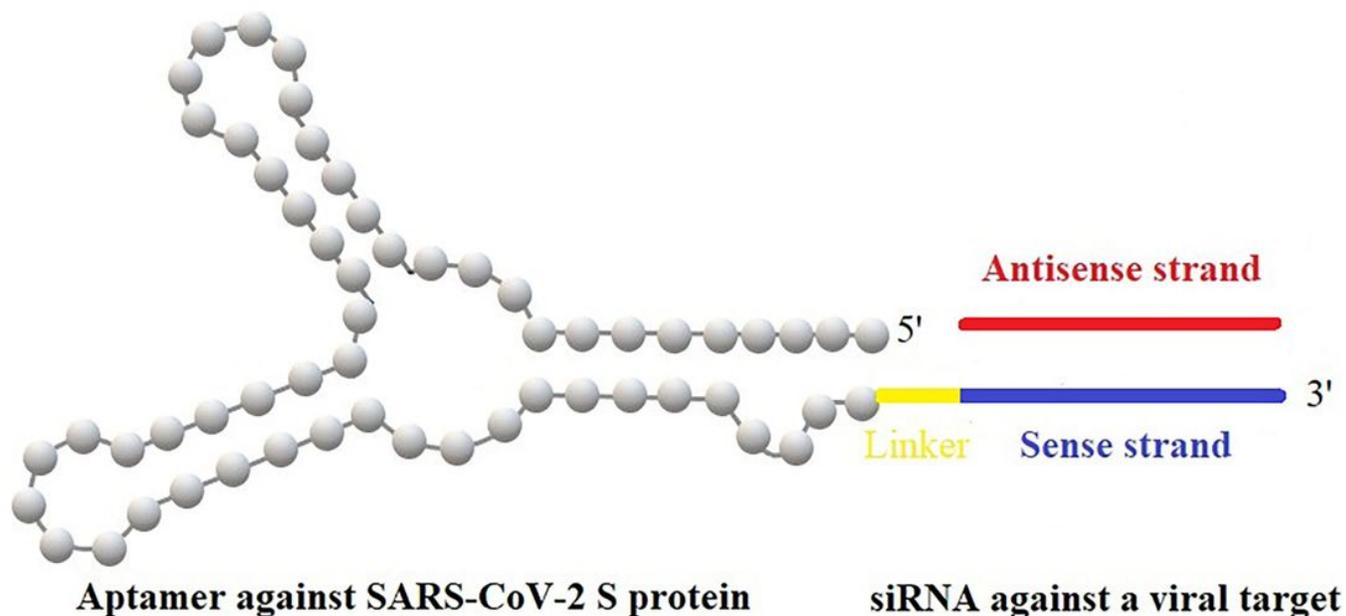


Figure 1

Schematic of Anti-S aptamer/ siRNA Chimera. Nuclease-resistant 2'-fluoro uridine triphosphate and 2'-fluoro cytidine triphosphate are implemented to shield the aptamer and sense strand segment of the siRNAs from nucleases (16). A UU linker is used to link the aptamer and siRNA portion.

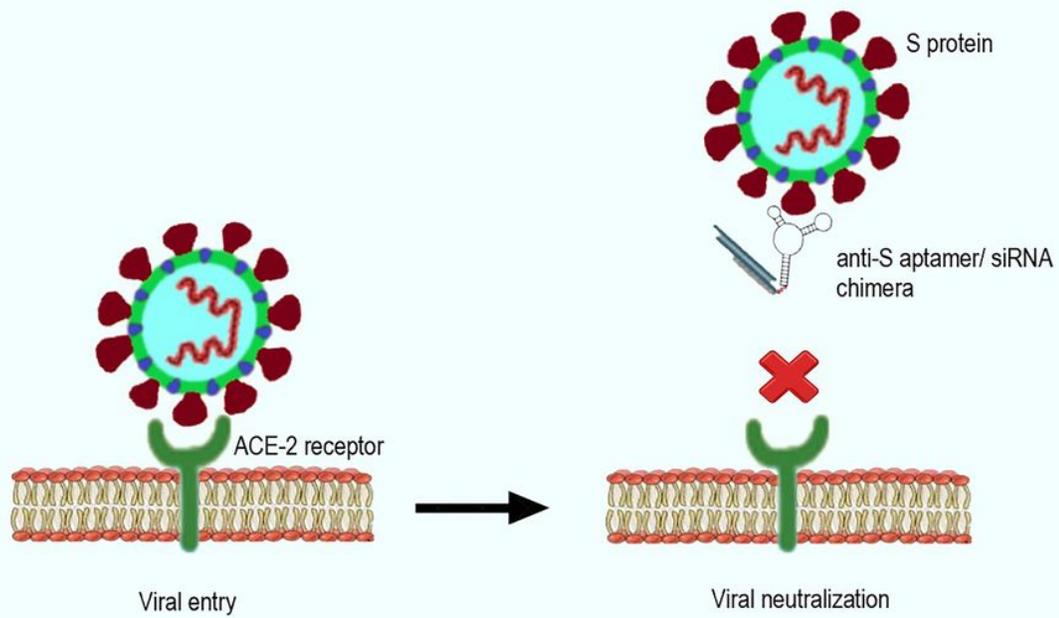


Figure 2

Virus Neutralization. The aptamer part of the chimera hinders the viral S protein-ACE2 receptor interaction (34).

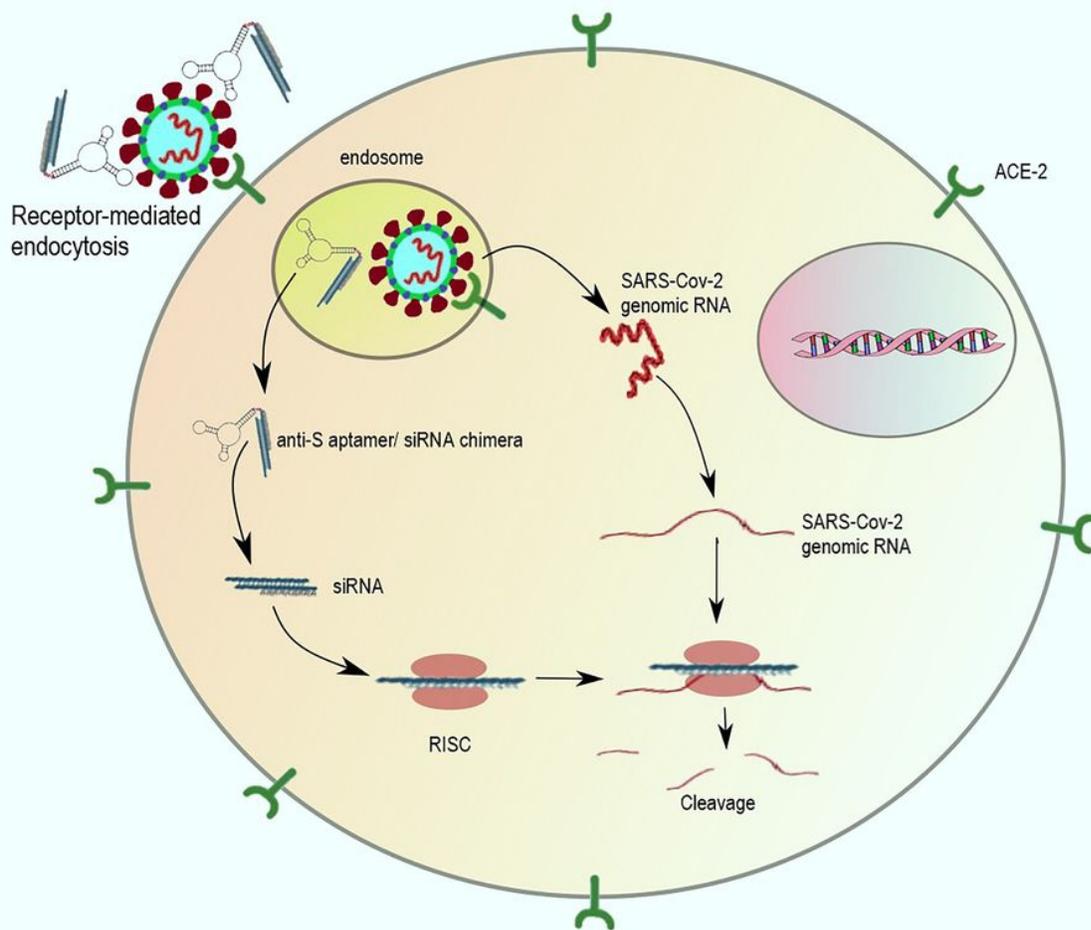


Figure 3

RNA Interference. The chimera will escape endosomes and release its siRNA part in the cytosol. Then, the antisense strand of the siRNA will guide the RISC complex to the SARS-CoV-2 (+ sense) RNA.