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Aptamers, the bivalent agents as probes and therapies for coronavirus infections: A systematic review

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ABSTRACT

The recently known coronavirus, SARS-CoV-2, has turn into the greatest global health challenge, affecting a large number of societies. The lack of specific treatment and gold-standard diagnostic system has made the situation more complicated. Efforts have led to production of several diagnostic kits that are associated with limitations such as inadequate sensitivity and accuracy. Aptamers as multipotent biological probes could be promising candidates to design sensitive and specific biosensors. Although few studies have introduced specific aptamer types of coronavirus, they may help us select the best approach to obtain specific aptamers for this virus. On the other hand, some of already-introduced aptamers have shown the inhibitory effects on coronavirus that could be applied as therapeutics. The present study has provided a systematic overview on use of aptamer-based biosensors and drugs to diagnose and treat coronavirus.

1. Introduction

1.1. Coronavirus and importance of the study

Coronaviruses (CoVs) are respiratory viruses that can cause infections (e.g. common cold and pneumonia) in mammals and birds. There are six types of CoVs (HCoVs) that may affect the humans: HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV and MERS-CoV. The causes of two major outbreaks in the first two decades of the contemporary century belong to the beta CoVs including Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) that was an epidemic in 2002–2003, and the Middle East Respiratory Syndrome Coronavirus (MERS-CoV), that has been epidemic since 2012. The latest onslaught of beta CoVs subfamily has been conducted by SARS-CoV-2 since December 12, 2019. COVID-19, the disease of this fighter virus, is now a vigorous threat to global health security [1].

World Health Organization (WHO) is concerned about the global public health and high-risk health systems since there are currently no specific vaccines or drugs to prevent or treat COVID-19 [2]. However, many efforts are being made to tackle this spiked virus.

The important bottlenecks for the management of COVID-19 are diagnostics and therapeutics. The common diagnostic test for COVID-19 is real-time reverse transcription polymerase chain reaction (RT-PCR) [3]. Regarding some reports the one-step rRT-PCR assays based on specific TaqMan probes have had a detection limit as few as 4 to 10 copies of RNA template per reaction [4,5]. However most of these tests show low sensitivity and specificity, so they do not work well enough to be routinely used. Thus, it is critical to invent new potent diagnostic tools.

The other traditional laboratory-based assays like virus culture, enzyme-linked immunosorbent assay (ELISA), western blotting, and serological antibody detection methods require virus isolation or DNA/RNA extraction that make them time-consuming and increasing the probability of virus spreading. These techniques also require expensive laboratory equipment and expert operators. These constrictions raise the response time and expenses which impact the quality of patient care [6].

Unlike the common laboratory assays, biosensors can produce quantitative signals proportional to the analyte concentration by the interaction of chemical or biological receptors with targets. Antibodies are applied as detector elements in the most viral biosensors. However, nucleic acids are recently recruited as the receptors which transduce signals to detect targets. DNA biosensors could detect a great variety of molecules with high affinity and specificity [7].

1.2. Diagnostic aptamers for viral infections

Aptamer, one of the novel DNA receptors, is a single-stranded folded RNA or ssDNA that can bind and detect various nucleic and non-nucleic acid molecules with high affinity and specificity. The application of aptamer probes for virus detection has increased in recent years. Aptamers could detect any viral infection markers, including viral genes, proteins, and antibodies. By applying some approaches, aptasensors can discriminate infected host cells from uninfected ones or active from inactive viral forms [8].

1.3. Therapeutic aptamers for viral infections

The highly mutated characteristic of viral genomes can lead to variability, and escaping from the host immune response makes most of drugs inefficient. Aptamers are novel potent candidates, which can treat viral infections by modulating the immune response, inhibiting the viruses penetrating the cells, or disrupting the replication enzymes. Furthermore, a variety types of modifiers, drugs and dyes could easily conjugate the aptamers without altering their primary properties to increase their *in vivo* stability and bioactivity [8].

1.4. Aptamer strengths and weaknesses

Despite the eligibility and the effects of aptamer as a diagnostic candidate for COVID-19, as well as their considerable advantages over similar molecules such as siRNA and monoclonal antibodies [8], there are some challenges toward the widespread use of aptamers for therapeutic purposes. The first challenge is the renal clearance which limits the circulation time of aptamers due to its low molecular weight that could be solved by conjugating with high weigh molecules. Also, the toxicological information regarding aptamers in humans is very limited. However aptamers have several advantages over antibodies, such as higher stability, simple synthesis, and modification.

Among the isolated aptamers, Pegaptanib (Macugen) is the only aptameric drug used for the treatment of neovascular (wet) age-related macular degeneration (AMD) that has been able to gain clinical and marketing approvals. Therefore, it is critical to continue the studies to find applicable therapeutic antiviral aptamers [9].

The present systematic review aimed to describe diagnostic and therapeutic approaches found by aptamer as a versatile tool for rapid diagnostics and therapeutics for coronaviruses.

2. Methods

2.1. Protocol

All stages of this review followed the published protocol by Liberati et al. [10]. The meta-analysis protocol was not applied due to the very limited available evidence on the topic and due to the urgency of the matter.

2.2. Eligibility criteria

In this review, these designs were considered for inclusion.

- Introduction of DNA and RNA aptamers for coronavirus
- Application of DNA and RNA aptamers for the diagnosis or therapy of coronavirus

No publication date, language, or publication status restrictions were imposed.

2.3. Sources of information

Studies were identified by searching through electronic databases

and scanning the reference lists of articles. The sources of the literature review encompassed Scopus, Pubmed, and Google scholars. The last search was run on June 21, 2020.

2.4. Search

The following terms were used to search all the trials, registers, and databases: SARS AND Aptamer, Corona AND Aptamer, COVID-19 AND Aptamer, SARS-CoV-2 AND Aptamer, SARS-CoV AND Aptamer, Coronaviruses AND Aptamer, MERS-CoV AND Aptamer, MERS AND Aptamer, (SARS OR MERS OR MERS-CoV OR Corona OR Coronavirus OR SARS-CoV OR SARS-CoV-2 OR COVID-19) AND Aptamer.

2.5. Study selection and data collection procedure

We included the entire original and review articles that had been designed or applied aptamers for coronaviruses. Then, in an unblended and standardized manner, we evaluated the eligibility of the articles by looking at the titles, abstracts, and full-text reports to determine inclusion and exclusion decisions and extract data from all eligible studies. To ascertain the validity of studies, pairs of reviewers worked independently and with adequate reliability determined the validity, efficiency, and application of aptamers. Finally, we used the MFold program to predict secondary structures of coronaviruses aptamers based on the Zuker algorithm [11].

3. Results

3.1. Study selection

After searching in Scopus, Pubmed, and Google scholar databases, a total of 41 studies involving aptamer and coronavirus were identified in this review. Then, after adjusting for duplicates and discarding articles that did not meet the criteria, three main articles on diagnostic aptamers against coronaviruses and two other studies on therapeutic aptamers remained. No reprinted relevant studies were obtained (Fig. 1).

3.2. Diagnostic aptamers

As we explored, there were only three published original research articles about diagnostic aptamers for coronaviruses (Table 1).

SARS-CoV nucleocapsid (N) protein forms a helical core in the viral envelope that plays a critical role during the viral life cycle. It can improve the efficiency of subgenomic viral RNA transcription [12]. According to the studies, N protein can be tracked in serum for the early detection of SARS-CoV [13].

Ahn et al. used a SELEX procedure to isolate two RNA aptamers (aptamer-1 and aptamer-2) binding specifically to the C-terminal region of SARS-CoV N protein with low dissociation constants (0.81 nM, and 3.35 nM, respectively). The result showed that aptamer-1 binds to N protein with a higher affinity than aptamer-2. They also applied SPR to confirm this result which appeared to be better than the antibody against N protein (KD = 1.65 nM for aptamer-1 *vs* KD = 2.1 μM for antibody).

To fabricate a detection system for N protein, Ahn et al. used a streptavidin-coated 96-well plate for immobilization of aptamer. After incubating N protein, a polyclonal antibody was applied to the captured agent and then a labeled monoclonal antibody to signal production. The detection limit of this method was 20 pg/ml (420 fM) of N protein [14] which was similar to that of ELISA with polyclonal and monoclonal antibodies [15].

Moreover, they used a polyclonal antibody and the FITC-conjugated secondary antibody to establish a nanoarray chip assay with a detection limit of 42 fM which was ten times more sensitive than previous chemiluminescence assay [14].

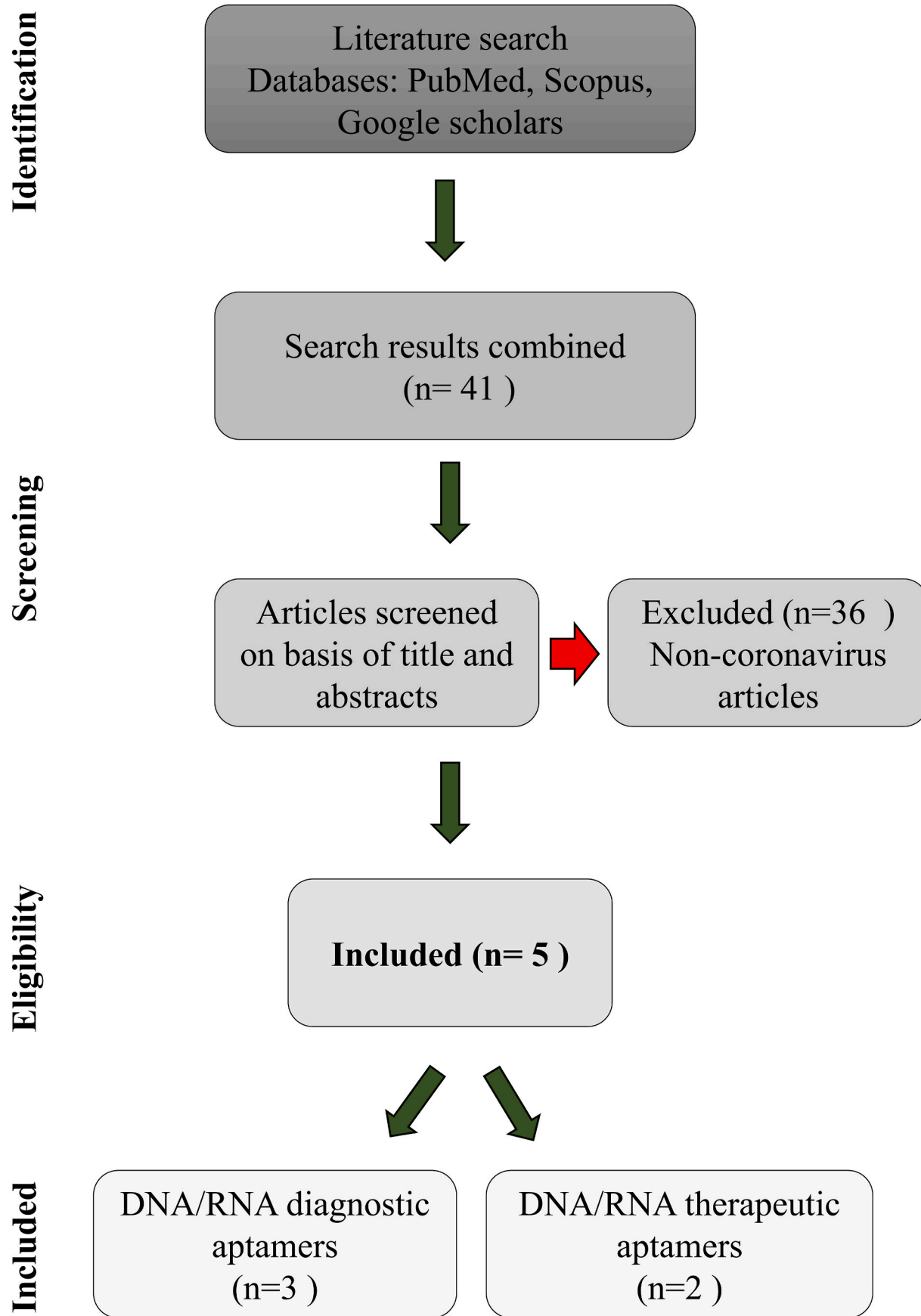In the second study, Cho et al., isolated a single-stranded DNA

Identification

Literature search
Databases: PubMed, Scopus,
Google scholars

Screening

Search results combined
(n= 41)

Articles screened
on basis of title and
abstracts

Excluded (n=36)
Non-coronavirus
articles

Eligibility

Included (n= 5)

Included

DNA/RNA diagnostic
aptamers
(n=3)

DNA/RNA therapeutic
aptamers
(n=2)

Fig. 1. Flow diagram of study selection.

Table 1

A brief overview of the aptamers probes against coronavirus.

No	Aptamer name	Type	Molecular Target	Length	Sequence	Kd (nM)	Viral target	SELEX method	Detection method	Ref
1	RNA aptamer-1-based sensitive detection of SARS-CoV nucleocapsid protein	RNA	SARS-CoV Nucleocapsid (N) protein	83	GGGAGAGCGGAAGCGUGCUGGGCCUGUCGUUCGCUGUGUCUUGCUACGUUACGUUAC ACGGUUGGCAUAACCCAGAGGUCGAUGG	1.65	SARS-CoV	Conventional SELEX	Aptamer-antibody hybrid immunoassays LD: 20 pg/ml (420 fM) Nanoarray aptamer chip LD: 2 pg/ml	[14]
2	RNA aptamer-2-based sensitive detection of SARS-CoV nucleocapsid protein	RNA	SARS-CoV Nucleocapsid (N) protein	83	GGGAGAGCGGAAGCGUGCUGGGCCUCAUUACACACAUCUCACGGGAGACAUAGCUGAC GAUAUCCAUAACCCAGAGGUCGAUGG-	3.35		Conventional SELEX	–	[14]
3	DNA aptamer specific to SARS-CoV nucleocapsid protein	ssDNA	SARS-CoV Nucleocapsid (N) protein	88	GCAATGGTACGGTACTTCCGGATGCGGGAAACTGGCTAATTGGTGAGGCGTGGGGCGGT CGTGCAGCAAAAGTGCACGCTACTTTGCTAA-	4.93		His-tagged N proteins immobilized on Ni–NTA sepharose beads	Western blot analysis (0–18.4 µg)	[16]
4	CoV2-RBD-1C	ssDNA	Receptor-Binding Domain of Spike Glycoprotein	51	CAGCACCGACCTTGTGCTTTGGGAGTGCTGGTCCAAGGGCGTTAATGGACA	5.5	SARS-CoV-2	Ni-Beads-SELEX	Flow cytometric analysis	[18]
5	CoV2-RBD-4C	ssDNA	Receptor-Binding Domain of Spike Glycoprotein	67	ATCCAGAGTGACGCAGCATTTCATCGGGTCCAAAAGGGGCTGCTCGGGATTGCGGATA TGGACACGT	19.9		Ni-Beads-SELEX	Flow cytometric analysis	[18]

(ssDNA) aptamer with specific binding to N protein of SARS-CoV with a high affinity (Kd = 4.93 ± 0.30 nM). They applied the selected aptamer in Western blot analysis, showing that this aptamer could be a worthy recognizer element instead of the monoclonal antibodies [16].

Since, SARS-CoV N protein is more than 90% homologous with protein sequence of SARS-CoV-2, a research group decided to investigate the affinity of the previously reported SARS-CoV N protein aptamers by Cho et al. [16] and modified variants against SARS-CoV-2 N protein by Enzyme-Linked Aptamer Binding Assay (ELAA). They immobilized SARS-CoV-2 N protein into the 96-well-plate and then added 5-bio-tinylated aptamers. Subsequently, the avidin-HRP was applied to recognize the biotin signal by measuring the absorbance using tetra-methylbenzidine as substrate. Their results showed that all aptamers could specifically bind to SARS-CoV-2 N protein [17].

It has been identified that SARSCoV-2 infects the human respiratory epithelial cells via interaction of its receptor-binding domain (RBD) of spike glycoprotein (S) with angiotensin-converting enzyme II (ACE2) on the host cells. Recently, Song et al. has isolated novel anti-RBD-SARS-CoV-2 aptamers using ACE2 competition-based aptamer selection strategy and a machine learning screening algorithm. They used His-tag RBD-modified Ni-beads (RBD-Ni-beads) to monitor the enrichment of interacted aptamers with SARS-CoV-2-RBD using flow cytometry. This research group found two high-binding-affinity aptamers with kd values of 5.8 and 19.9 nM. These aptamers could provide new hopes for detection of SARS-CoV-2 [18]. (Fig. 2).

3.3. Aptamers with therapeutic purposes

Despite of all efforts, scientists have not yet discovered the first line drug against COVID-19. However more than 500 complex clinical trials have been established for COVID-19 encompassing (a) direct-acting antiviral drugs such as Favipiravir, Arbidol, Interferon, Corticoste-roids, Remdesivir and lopinivir–ritonavir (LPV/r), (b) antimalarial medicines like hydroxychloroquine and chloroquine, (c) convalescent plasma therapy and stem-cell transfusion, (d) anti-inflammatory treatments and even (e) traditional medicine [19].

From a molecular biology point of view, viral helicase is a potential target for viral therapy because it is important in viral genome replication. The SARS-CoV helicase can be involved in a variety of biological pathways, such as replication, recombination, DNA repair, chromatin remodeling, catalytic process of conformational changes in the nucleic acids, movement of Holliday junctions, and numerous features of RNA metabolism. Thus, SARS-CoV NTPase/Helicase could be considered as a potent target to develop agents against SARS-CoV [20,21]. These drugs can bind to the helicase and inhibit its activity through blocking the binding to nucleic acids [22].

Nonstructural Protein 10 (nsP10) is an enzyme with NTPase/Heli-case activity which can threaten the life of SARS-CoV as its Achilles heel. In this line, we found two published original research articles about the therapeutic aptamers against coronaviruses (Table 2).

In the first research, Shum et al. isolated two different DNA aptamers in structure, G-quadruplex, and non-G-quadruplex, via Ni-NTA magnetic beads SELEX. Both of these aptamers had a positive effect on ATPase activity with low Km values. However, only the non-G-quadruplex one inhibits the SARS-CoV helicase activity to unwind nucleic acids. The aptamers could bind to the binding sites of helicase and change their conformation [23]. The MFold program was applied to predict the secondary structures of these DNA aptamers [11] (Fig. 3).

In the second research, Jang et al., presented specific RNA aptamers against nsP10. They used the conventional SELEX to find RNA aptamers against NTPase/Helicase in SARS-CoV from an RNA library, which resulted in an enriched RNA aptamer pool (ES15 RNA). This aptameric pool could decrease the unwinding of nucleic acid and ATPase activity of the helicase (IC50 = 1.2 nM) [24]. They pointed out that the inhibitory effect of these RNA aptamers was derived from AG-rich conserved sequence which could be considered as anti-SARS-CoV agents (Fig. 3).

4. Discussion

Currently, the major approaches to detect SARS-CoV-2 are nucleic

a — Ahn *et al.* [14] Reproduced by permission of The Royal Society of Chemistry

Aptamer 1 ΔG = -18.98 kcal/mol
Aptamer 2 ΔG = -14.95 kcal/mol

b — Cho *et al.* [16] License No: 4811860329657 License date: Apr 18, 2020 Licensed Content Publisher: Elsevier

DNA Aptamer ΔG = -11.11 kcal/mol

c — Song *et al.* [18] PERMISSION/LICENSE IS GRANTED FOR YOUR ORDER AT NO CHARGE License date: Jun 1, 2020 Publication: Analytical Chemistry Publisher: American Chemical Society

Cov-2-RBD-1C ΔG = -7.05 kcal/mol
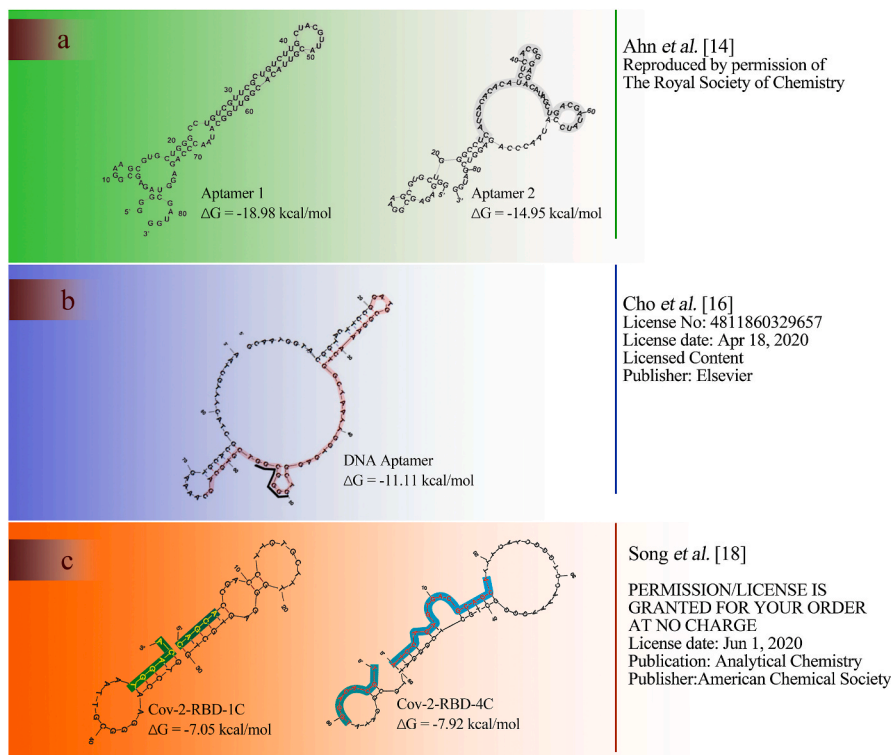Cov-2-RBD-4C ΔG = -7.92 kcal/mol

Fig. 2. Secondary structures of diagnostic Coronaviruses aptamers. a) The secondary structures of different RNA (14), b and c) DNA [16,18] were predicted using the MFold program or permitted previous studies. The variable regions are marked with different colors.
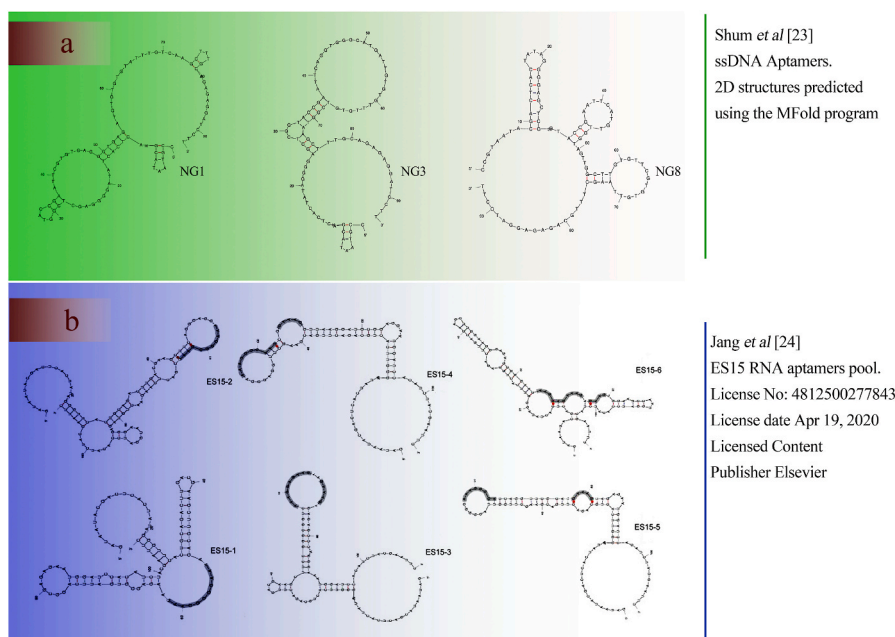

Fig. 3. Secondary structures of therapeutic Coronaviruses aptamers. a) The secondary structures of different ssDNA aptamer [23] were predicted using the MFold program. b) The AG-rich conserved sequences (Highlighted Regions) at the loop region in RNA aptamer pools, isolated by Jang et al., are assumed as binding motif structure to SARS-CoV RNA helicase [24].

Table 3
Advantages and disadvantages of nucleic acid amplification-based diagnostic methods vs Aptamer based diagnostic methods.

Method	Advantages	Disadvantages
Nucleic acid amplification-based diagnostic methods	Ability to differentiate among strains and species	Limited targets to nucleic acid
	High sensitivity and specificity	High-cost
	No need for viable organism	Expensive labeled reagents (probes)
	Simplicity and universal procedure	Pre-analytical sample preparation
	Availability in markets	Need for additional detection step after amplification
	Easy getting approved for clinical diagnostic usage	Need for expensive equipment and reagents
		Low stability
Aptamer based diagnostic methods	Recognize wide range of targets	Difficulties for generating for some molecules such as small molecules
	High sensitivity and specificity	Problems toward commercialization
	High stability	Sensitivity to nuclease existed in serum and real samples
	Ease of use	Changes in affinity and specificity in real situations
	Ease of production	Low chemical diversity of natural oligonucleotide
	Low-cost	
	Simple modification and labeling	
	Compatibility with different diagnostic approaches	

natural oligonucleotides. Some studies have attempted to solve this problem by using modified nucleotides. The other most important challenge in diagnosis field is nuclease sensitivity of unmodified aptamers. The nucleases in biological fluids such as serum are able to degrade the aptamer molecules. Fortunately, some chemicals could

increase their stability and half-life, but these modifications may decrease sensitivity and impose cost. The other issue is derived from *in vitro* SELEX procedures that may ignore some *in vivo* conditions. This ignorance dramatically impacts the structural arrangement of aptamers which may influence the affinity and specificity of aptamers in real samples. These challenges provide some restrictions for commercialization of aptamer-based methods. However, some studies have tried to resolve them by novel high-throughput technologies in automating SLEX procedure and fabrication of bisensors [36,37]. In spite of these challenges, there is a promising prospect toward aptamer-based diagnostic methods to fabricate ultrasensitive recognition tools for crucial targets such as SARS-CoV-2.

Therapeutic aptamers have more problems. In addition to nuclease sensitivity, low half-life and cost of modifications, therapeutic aptamers encounter new challenges such as high renal clearance, safety and drug delivery to enter therapeutic protocols. As mentioned before only one aptamer has been commercialized over the past quarter century. The few numbers of aptamers have entered in pre-clinical and clinical phases of treatment which are most related to cancers, but the scientists are attempting to develop new generation of aptamer-based drugs by combination of therapeutic aptamers with other novel technologies such as nanobiotechnolgy [38].

5. Conclusion

COVID-19 is a dangerous threat to public health, global disciples, and economics. The first step to treat this viral disease is a rapid and accurate diagnosis that can prevent its contagion. Recently, Nucleic acid aptamers have been confirmed to be useful as medications and diagnostic probes [39]. According to the previous studies and from a general point of view, it seems that the aptamer molecules could be effective anti-coronavirus agents in both diagnosis and treatment.

Although no aptamers have been isolated for SARS-Cov-2 until now, it seems that modeling of other coronavirus aptamers can be helpful to find an appropriate shortcut to achieve the best targets and subsequently the best antidote or diagnostic assays.

The Helicase enzyme has an important role in viral replication and proliferation. Therefore, it could be considered as a potent target to

develop the coronavirus therapeutic aptamers [23,24]. N protein could be also a potent target to detect and inhibit COVID-19 due to its crucial role in the synthesis of viral RNA and SARS detection. By applying aptamers as sensitive diagnostic elements, we will be able to fabricate rapid, sensitive, low-cost, and user-friendly diagnostic tools of small volume clinical samples.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mcp.2020.101636.

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