Chapter 6

Application of Nucleic Acid Aptamers to Viral Detection and Inhibition

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Additional information is available at the end of the chapter

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Abstract

Nucleic acid aptamers are small oligonucleotides that specifically bind to other molecules through noncovalent interactions that rely on complex tridimensional structural arrangements. Aptamers are generated through the iterative *in vitro* selection method called SELEX, resulting in specific binding against a wide variety of molecular targets including viruses. Because aptamers are obtained *in vitro* and can be synthetically produced, they have been envisioned as future diagnostic and therapeutic tools for human diseases including virus-borne pathologies. Aptamers have been isolated against a number of viruses including pandemic influenza virus, human papillomavirus and hepatitis C virus. Although aptamers have proven themselves as extremely sensitive detection tools triggering the development of affordable and highly diagnostic methods, their use as therapeutic moieties has been hampered by biostability, delivery and pharmacodynamical issues. Nevertheless, a new generation of chemically modified aptamers shows promise for the coming of age of protein-targeted noncatalytic oligonucleotides for the therapy of viral disease. The present review focuses on the most successful antiviral aptamers reported and includes a description of some of the novel methods developed for their use as diagnostic and therapeutic tools

Keywords: Aptamer, Oligonucleotides, Nucleic acids, RNA, DNA

1. Introduction

Nucleic acid aptamers are small single-stranded oligonucleotides capable of adopting complex tertiary structures that allow noncovalent interactions with other molecules. Because aptamers closely interact with their targets, their structural features are essential for highly specific binding. The term *aptamer* was coined in 1990 from the Latin "aptus" meaning "fitting" and

the Greek "meros" meaning "particle" [1]. Aptamers are generated through the iterative *in vitro* selection method called SELEX (systematic evolution of ligands by exponential enrichment) and are raised against a wide variety of molecular targets ranging from ions and macromolecules to whole organisms, including viruses, bacteria, yeast and mammalian cells [2]. Although many modifications to the SELEX method have been established to include new technologies and improve selection [3], the basic steps of SELEX remain immutable.

The SELEX method involves three well-defined steps [4]: the start point is the production of a synthetic oligonucleotide combinatorial library or oligonucleotide pool containing a central randomized region (15–70 nt) flanked by anchor sequences to allow polymerase chain reaction (PCR) amplification. The aleatory nature of the central region results in the production of an enormous pool of diverse oligonucleotides with diverse structures, thus providing the conformational variability necessary to produce moieties with binding capabilities for a desired target. The oligonucleotide pool can be directly used for SELEX to generate single-stranded DNA (ssDNA) aptamers, or as *in vitro* transcription template to produce an RNA pool to isolate RNA aptamers. Next, a selection procedure is performed based on the interaction properties of the library with the intended target. Only a very small fraction of the oligonucleotide pool tends to interact with the target, satisfying the selection criteria. Oligonucleotides that bind the target (aptamers) are recovered while the nonbound are removed through different strategies according to the nature of the aptamer–ligand complex (size, affinity, electric charge, hydrophobicity, etc.). In the final step, the recovered aptamers are amplified by PCR in order to regenerate a library with less variability but more affinity to the target that will be used in the next selection cycle. RNA pools are amplified by reverse transcription-coupled PCR (RT-PCR) and subsequent *in vitro* transcription before starting the next cycle.

The iterative selection cycles produce aptamers with high binding affinity to the target. Usually, a few cycles are required to isolate aptamers (4–20 cycles), but the precise number of cycles necessary for the isolation of highly specific aptamers depends on the selection criteria, the nature of the target and the type of library used. After the last selection cycle, aptamers are cloned and sequenced to obtain information on the individual oligonucleotides, which can be further characterized based on its ability to bind the target. It is common to observe conserved sequences or structures among the selected aptamers; these are indicative of efficient selection and may represent domains required for interaction.

Aptamer specificity is based on three-dimensional arrangements of a small number of contact points between the aptamer and its target, so the aptamer can achieve high selectivity to discriminate between two highly related molecules (i.e. enantiomers), or minimal structural differences such as the presence or absence of methyl or hydroxyl groups. The molecular recognition specificity and affinity level achieved by aptamers is comparable or even better than those of antibodies. These features place aptamers as an emerging class of molecules on their own with a huge range of diagnostic and therapeutic applications plus several advantages over antibodies including:

- Isolation by an *in vitro* process not dependent on animal cells or *in vivo* conditions. Therefore, the properties of aptamers can change on demand, and isolation can be manipulated to

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obtain aptamers with desirable properties for diagnosis. In addition, it allows aptamer isolation against toxins or poorly immunogenic molecules.

- Production by chemical synthesis with accuracy and reproducibility, thus insuring mass production with high quality control standards.
- Aptamers can be reversibly denatured allowing conditional binding through simple temperature control.

Since the development of SELEX, aptamers have been isolated against a wide diversity of targets such as amino acids [5, 6], antibiotics [7], nucleotides [8], enzymes [9], growth factors [10], mammalian cells [11], bacteria [12] and parasites [13]. Nowadays, some aptamers have even reached therapeutic applications in the clinic [14]. Furthermore, the first RNA aptamer for therapeutic purposes in humans (pegaptanib sodium or Macugen®) was approved by United States Food and Drug Administration (FDA) in 2004, as treatment for age-related macular degeneration (AMD) [15].

2. Aptamers against viruses

Many aptamers have been isolated against whole viruses or viral proteins to detect or inhibit infection. Viruses such as human papillomavirus (HPV), human immunodeficiency virus-1 (HIV-1), hepatitis C virus (HCV), hepatitis B virus (HBV), severe acute respiratory syndrome coronavirus (SCoV), influenza virus, herpes simplex virus (HSV), Ebola virus, Rift Valley fever virus, dengue virus, human T cell leukemia virus type-1 (HTLV-1), Epstein–Barr virus and human cytomegalovirus (HCMV) have all been targeted with aptamers [16].

Aptamer isolation to inhibit viral infection can be performed by using purified molecules from the viral surface through canonical SELEX approaches, or by modified SELEX methods with the use of attenuated whole viral particles. The advantage of this last variant is the isolation of aptamers through binding to the native viral conformation. Moreover, a deep knowledge of the viral infection mechanisms or potential surface target molecules is not required to obtain antiviral or neutralizing aptamers that tightly bind infectious particles. On the other hand, this method does not disclose the sites that directly interact with the aptamers, so further studies are required to determine specific interactions useful for potential aptamer improvement.

Many efforts have been focused on the isolation of aptamers to detect and treat viral diseases relevant to public health such as AIDS, hepatitis, influenza and some cancers. Here, we summarize the successful application of aptamers selected against HIV-1, HPV, HCV, and influenza.

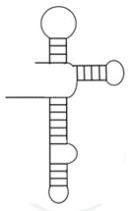
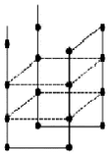
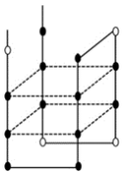
2.1. Aptamers against Rous Sarcoma Virus (RSV)

The first approach using whole viruses to isolate RNA aptamers without previous knowledge of the virion structural features was performed against Rous sarcoma virus (RSV), an avian retrovirus. Nineteen RNA aptamers were isolated from a canonical SELEX procedure and five

of them were able to neutralize the virus infection [17]. These results immediately revealed the potential of aptamers isolated against viral surface epitopes leading to the development of nucleic acid aptamers as novel diagnostic or therapeutic tools, especially on human viral diseases requiring fast diagnostics (i.e. pandemic influenza or Ebola) or asymptomatic chronic viral-induced conditions such as acquired immunodeficiency syndrome (AIDS), hepatitis C or cervical cancer [18].

2.2. Aptamers against Human Immunodeficiency Virus type I (HIV-1)

HIV-1 is the etiologic agent of AIDS [19, 20]. Most anti-HIV-1 aptamers are directed to HIV-1 reverse transcriptase (RT), RNaseH, integrase, Tat, Gag, nucleocapsid, gp120 and the TAR-element RNA (Table 1). The HIV-1 RT is the enzyme responsible for transforming the viral genomic RNA into dsDNA and contains a domain with RNaseH activity. HIV-1 RT is also the main target of several therapies against AIDS. So far, about a dozen of ssDNA and RNA aptamers have been reported to inhibit the RT activity in cell cultures showing K_D in the range of 25 pM to 30 nM [18, 21].

Aptamer	Nature	Sequence	Randomized region	Target	Action	Structure	Ref
P5	RNA	GGGAGCUCAGAAUAAACG CUCAACGGCACAGGGGUU GUAUCCUCCGGGACGAAU UCGACAUGAGGCCCGGAU CCGGC	30 nt	Integrase	Inhibit interaction between integrase and viral DNA		Allen P, *et al.* 1995
A54	RNA	GGGAGCUCAGAAUAAACG CUCAAGUCAAUCAUCGAU GUCCUGUGCCCUAGGGCU UCGACAUGAGGCCCGGAU CCGGC	30 nt	Integrase	Inhibit interaction between integrase and viral DNA		Allen P, *et al.* 1995
93del	DNA	GGGGTGGGAGGAGGGT	80 nt	Integrase	Block integrase actvity in vitro		Phan AT, *et al.* 2004, De Soultrait VR. Et. al. 2002
112del	DNA	CGGGTGGGTGGGTGGT	80 nt	Integrase	Inhibton of HV-1 integrase		, De Soultrait VR. Et. al. 2002

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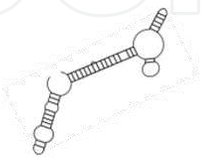
Aptamer	Nature	Sequence	Randomized region	Target	Action	Structure	Ref
RNA tat	RNA	ACGAAGCUUGAUCCCGUU UGCCGGUCGAUCGCUUCG A	120 nt	TAR	Inhibition of Tat dependent trasns-activation transcription. Biosensor		Yamamoto R, *et al.* 2000
B40	RNA	TAATACGACTCACTATAGG GAGACAAGACTAGACGCT CAaTGTGGGCCACGCCCGA TTTTACGCTTTTACCCGCAC GCGATTGGTTTGTTTTCGA CATGGACTCACAACAGTTC CCTTTAGTGAGGGTTAATT	40 nt	Gp120	Neutralizaton of HIV-1 infectivity		Khati M, *et al.* 2003. Dey AK, *et al.* 2005.
B40t77	RNA	TAATACGACTCACTATAGG GAGACAAGACTAGACGCT CAATGTGGCCACGCCCGAT TTTACGCTTTTACCGCACG CGATTGGTTTGTTTCCC	40 nt	Gp120	Neutralizaton of HIV-1 infectivity		Dey AK, *et al.* 2005. Cohen C, *et al*, 2008.

Table 1. Aptamers isolated against HIV proteins.

The HIV-1 integrase incorporates the viral DNA in the host genome [22]. RNA aptamers were isolated targeting HIV-1 integrase and classified into three groups according to their K_D (10 nM, 80 nM and 800 nM) [23]. Furthermore, DNA aptamers were also isolated targeting HIV-1 integrase by two different research groups, all with G-quadruplex structures and showing *in vitro* inhibitory activity [24, 25]. The characteristic structure of aptamers binding the HIV-1 integrase interacts within a channel of the tetrameric protein blocking catalytic amino acid residues essential for integrase function *in vitro* [26].

HIV-1 Tat protein regulates viral gene expression by interaction with the trans-activation responsive (TAR) elements within the long-terminal repeats (LTRs) [27]. Unlike the natural target of Tat (TAR-1 RNA), the isolated RNA aptamer (RNA^{Tat}) was highly specific to Tat and did not interact with other cellular factors. Moreover, RNA^{Tat} binds Tat protein over 100-fold higher than TAR-1 RNA and inhibited Tat function *in vitro* and *in vivo* [28, 29]. Based on these results, RNA^{Tat} was used in a preliminary study to develop a molecular beacon by flanking the 5′ and 3′ ends of the native aptamer stem-loop structure with a fluorophore and a quencher. In the absence of Tat, the quencher and fluorophore remain close to each other by the formation of the stem producing no signal. When the loop interacts with Tat, the complexed structure becomes more stable resulting in strand separation, thus holding apart the fluorophore and quencher allowing fluorescence [30].

Two other biosensors have been developed using RNA aptamers specific to HIV-1 Tat. These biosensors were created by immobilizing a biotinylated aptamer on a streptavidin layer over

quartz crystals included in surface plasmon resonance (SPR) chips [31]. Another approach used a diamond field-effect transistor (FET) technique to detect Tat protein by RNA aptamers. Aptamer-FET is based on a gate potential shift generated by the presence of HIV-1 Tat bound to the RNA aptamer on a solid diamond surface. Efficient detection showed a potential use for aptamer-FET in clinical applications [32].

HIV-1 Rev is essential to regulate the splicing and shuttle the viral mRNA through their nuclear and export localization signals. Also, Rev interacts with viral mRNA through a cis-acting Rev-binding element (RBE) within a Rev-responsive element (RRE). RNA aptamers against Rev have been isolated using random libraries or by randomizing the RRE minimal binding sequence [33–35]. Randomized RRE produced aptamers with up to 16-fold tighter binding than the minimal wild-type RBE (wtRBE). The RBE was then substituted by these RNA aptamers and tested *in vivo* using a reporter system [35]. The aptamer substitutions showed a better response than wtRBE [36]. Another technique used to isolate RNA aptamers against Rev protein was the cross-linking SELEX consisting of a 5′-iodo uracil (5-IU)–modified RNA library, which is reactive under long-wavelength UV irradiation producing cross-links between 5-IU oligonucleotides and the protein target. This method resulted in the selection of highly specific aptamers capable of forming covalent bonds with HIV-1 Rev [37]. Some other efforts have focused on the inhibition of HIV-1 replication in human T cells using RNA aptamers as decoys to sequester Rev [38]. Rev decoys and ribozymes have been combined to increase the anti-HIV effect relative to independent ribozyme or decoy effects [39–41].

HIV-1 gp120 is a surface glycoprotein involved in the early stages of HIV-1 infection. The gp120 protein interacts with the human surface receptor CD4 producing conformational changes and further receptor interactions to allow HIV-1 entry into the host cell. Due to its importance on the onset of the viral infection, gp120 represents a potential target for the isolation of aptamers to block HIV-1 entry. Several RNA aptamers have been isolated to block gp120 and CD4 interaction neutralizing diverse subtypes of the virus [42]. Characterization of aptamer B40 showed high specificity to HIV-1 R5 strain and neutralization in human peripheral blood mononuclear cells [43, 44]. Additional analyses produced a shorter synthetic B40 derivative (UCLA1) able to inhibit entry of HIV-1 at the nanomolar range. Moreover, the aptamer showed synergistic effects with a gp41 fusion inhibitor (T20) and anti-CD4 binding site monoclonal antibody (IgG1b12), suggesting a potential use as adjuvant [45].

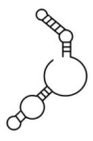
2′-Fluoride (2′-F) modified RNA aptamers selected to bind HIV-1_{Bal} gp120 and specifically internalized by cells expressing HIV-1_{Bal} gp120 were used to deliver anti-HIV siRNA into HIV-1–infected cells [46]. Two aptamer-siRNA chimeras were used: one covalent chimera presented a 2′-F-modified gp120 aptamer covalently attached to the sense strand of *tat*/*rev* siRNA and reduced the plasma viral load in a RAG-hu mouse model by suppressing HIV-1 replication and preventing CD4+ T cell decline. This effect was extended by several weeks beyond the last dose [47]. The second chimera consisted of a single aptamer with three different siRNAs targeting viral and cellular transcripts. The siRNA was linked to the aptamer by a bridge sequence of 16 nt that allowed complementary base pairing of one of the two siRNA strands to the aptamer. The aptamer–siRNA chimera showed a potent suppression of HIV-1 and protection from viral CD4+ T-cell depletion *in vivo*. In addition, the inhibitory effects were

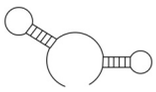
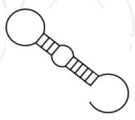
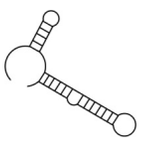
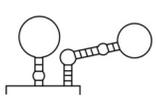
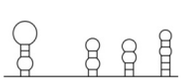
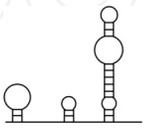
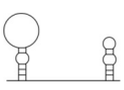
also extended several weeks after the last injection, providing an attractive therapeutic approach to HIV-1 therapy [48].

2.2.1. Aptamers against HPV

HPVs are small DNA viruses that infect squamous epithelia inducing proliferative lesions ranging from benign warts to cancer. High-grade papillomavirus, especially types 16 and 18 (HPV-16 and HPV-18) are associated with cervical carcinoma, the second most common cancer affecting women worldwide. HPVs have a circular double-stranded DNA genome of approximately 8 kb that is organized into three regions: the upstream regulatory region (URR), the early region (E) and the late region (L). The URR contains several transcription factor binding sites to control gene expression, the early region encodes six genes (E1, E2, E4, E5, E6 and E7) involved in viral replication, transcription and cell transformation and the late region encodes the L1 and L2 capsid proteins which self-assemble to produce the virion [49].

Because preventive vaccines for HPV infection are only protective for *naive* individuals [50], several research groups have been developing nucleic acid–based aptamers targeting HPV proteins in order to inhibit the oncoproteins activity, block viral infection or identify the absence/presence of viral proteins as biomarkers to determine cell transformation or cancer progression (Table 2).

Aptamer	Nature	Sequence	Randomized region	Target	Function	Structure	Ref
F2	RNA	GGGAAUGGAUCCA CAUACUACGAAUA UUCAACAUUCGAG GUGGAUGCUACGA AUCAACUUCACUG CAGACUUGACGAA GCUU	30 nt	E6	Inhbition of E6-PDZ (Magil1) interaction and Induction of apoptosis in SiHa cells	ND	Belyaeva TA, *et al.* 2014
F4	RNA	GGGAAUGGAUCCA CAUACUACGAAAA CUCGUUUCGAGGU UCGAAACGUUGUA AAGCCGUUUCACU GCAGACUUGACGA AGCUU	30 nt	E6	Inhbition of E6-PDZ (Magil1) interaction and Induction of apoptosis in SiHa cells	ND	Belyaeva TA, *et al.* 2014
A2	RNA	GGGAAUGGAUCCA CAUCUACGAAUCC CUUCAUCAUUAAC CCGUCCACGCGCU UCACUGCAGACUU GACGAAGCUU	30 nt	E7	Inhibition of E7-pRb interaction and Induction of apoptosis in SiHA cells		Nicol C. *et al*, 2013

Aptamer	Nature	Sequence	Randomized region	Target	Function	Structure	Ref
G5a3N.4	RNA	GGGAGACCCAAGC CGAUUUAUUUUGU GCAGCUUUUGUUC CCUUUAGUGAGGG UUAAUU	15 nt	E7	E7 high affinity binding on HPV-positive cervical carcinoma cells		Toscano-Garibay JD, *et al*. 2011
Sc5-c3	RNA	GGGAACAAAAGCU GCACAGGUUACCC CCGCUUGGGUCUC CCUAUAGUGAGUC GUAUUA	15 nt	L1	High affinity binding of HPV VLPs in murire biofluids		Leija-Montoya AG, *et al*. 2014
C5	RNA	GGGAGGACGAUGC GGAAGCATCAAGG GTGATCGTTTGACC CTCCCCAGACGAC UCGCCCGA	30 nt	HPV-16 E6/E7-HTECs	Internalization in HPV-16 E6/E7 HTEC as mechanism to deliver therapeutc agents		Gourronc FA, *et al*. 2013
13	DNA	ATACCAGCTTATTC AATTGGGCACAGA CGGAAGATGAGAA TTGTGGGGCTTAGT ATAGTGAGGTGCGT GTAGATAGTAAGTG CAATCT	52 nt	HF cell line	Detection of biomarkers lost in HPV-mediated cell transformation		Graham JC, *et al*. 2012
14	DNA	ATACCAGCTTATTC AATTGGGCGGGGA GTAGGGAGAGGGG TTTCCATCGGCGAC AGAGGAGTTATGTG TGTAGATAGTAAGT GCAATCT	52 nt	HF cell line	Detection of biomarkers lost in HPV-mediated cell transformation		Graham JC, *et al*. 2012
20	DNA	ATACCAGCTTATTC AATTGGGGAGGGA GACACAGTCATGG AGCAGTTATTAGGG TGTACCGGGTGTAG TAGATAGTAAGTGC AATCT	52 nt	HF cell line	Detection of biomarkers lost in HPV-mediated cell transformation		Graham JC, *et al*. 2012
28	DNA	ATACCAGCTTATTC AATTGGGGGACAC GGAGGTGGTGGAA	52 nt	HF cell line	Detection of biomarkers lost in HPV-mediated cell transformation		Graham JC, *et al*. 2012

Aptamer	Nature	Sequence	Randomized region	Target	Function	Structure	Ref
		AGGCTAAGATTTGA TGATGAGTAGTGTG GTAGATAGTAAGTG CAATCT					

Table 2. Aptamers isolated against HPV proteins.

The oncoproteins E6 and E7 are involved in cell immortalization and malignant transformation. E6 promotes the degradation of the tumor suppressor p53 [51], and E7 binds and destabilizes the cell cycle control protein pRb [52]. E6 and E7 have an important role in cancer progression, situating these oncoproteins as the principal potential targets to bind aptamers to block their oncogenic activity and cancer progression.

Several RNA aptamers were isolated against the PDZ-binding motif of the HPV-16 E6 oncoprotein, two of them were able to inhibit the interaction between E6 and proteins with PDZ domain (Magi 1) resulting in apoptosis. The aptamer interaction with PDZ domain was very specific and the interaction between E6 and p53 was not affected [53]. The same research group also isolated RNA aptamers against E7 oncoprotein that were able to disturb the E7–pRb interaction by targeting E7 for degradation and showed that one of them (A2) was able to inhibit cellular proliferation by inducing apoptosis in SiHa cervical carcinoma cells [54]. This effect was specific to HPV-16 transformed cells because it was not observed in HPV-free or HPV-18 cell lines [55]. Specific apoptosis induction of RNA aptamers targeting E6 and E7 oncoproteins suggests that these aptamers could have further applications in the future as therapeutic moieties.

A deeply characterized RNA aptamer targeting HPV-16 E7 oncoprotein named G5α3N.4 interacts with E7 through two stem-loop motifs in a clamp-like manner, suggesting a change in aptamer structure due to protein contact. The complex formation was observed exclusively in HPV-positive cervical carcinoma cells, suggesting that G5α3N.4 could be used to detect HPV infection and cervical cancer [56, 57].

The L1 protein is the main component of the HPV capsid. It is arranged in 72 capsomers, each consisting of five 55-kDa L1 monomers and a single 74-kDa L2 unit (theoretical 5:1 ratio). The L1 protein can self-assemble, forming virus-like particles (VLPs) that are structurally and immunologically similar to the infectious virions. HPV-16 L1 VLPs have been broadly used in HPV virology research, as delivery agents for epitopes or genes and to successfully produce prophylactic vaccines against HPV infection. The first RNA aptamer, targeting the L1 protein (Sc5-c3), was obtained using HPV-16 VLPs as targets [58]. Sc5-c3 structure consists of a hairpin structure with a 16-nt loop that directly binds VLPs with very low K_D (0.05 pM). This aptamer was able to specifically bind VLPs in complex protein mixtures (murine cervical washes), suggesting that Sc5-c3 may provide a potential diagnostic tool for active HPV infections and, with further refinement, could be used as a potential tool to inhibit viral infection [58].

Nucleic acid–based aptamers have been also isolated against whole HPV-infected cells. A cell-based SELEX protocol (cell-SELEX), was used to isolate RNA aptamers able to internalize into HPV-16 E6/E7 transformed human tonsillar epithelial cells (HTEC). This was the first report of aptamers that specifically internalize into HPV-16–transformed cells, providing a plausible mechanism to specifically deliver therapeutic agents into HPV-16–associated tumors [59]. Moreover, DNA aptamers have been isolated by a cell-SELEX modification for use with adherent cells (AC-SELEX). These aptamers recognize cell surface differences between HPV-transformed and nontumorigenic cell lines and one of them (Aptamer 14) was able to enter the cells independent of cell surface protein binding. These selected aptamers have potential to elucidate biomarkers for cellular changes associated to nontumorigenic phenotype in HPV-infected cells [60].

2.3. Aptamers against influenza virus

Influenza viruses are associated with most flu pandemics. They are enveloped RNA viruses of 80 to 120 nm diameter that infect the upper respiratory tract. The disease severity depends on the virus type: A, B or C. Influenza A virus infects birds and mammals, influenza B targets mainly humans and influenza C is less common than A or B but it also causes disease. Although the three virus types infect different hosts, it has been reported that all of them can infect humans and thus they have been the subject of several SELEX protocols.

2.3.1. Influenza A Virus (IAV)

The IAV genome comprises eight segments of linear RNA and two surface glycoproteins: hemagglutinin (HA) and neuraminidase (NA). These proteins are used to classify the IAV subtypes. Seventeen HA (H1–H17) and nine NA (N1–N9) variants have been identified and implicated on viral attachment, membrane fusion and viral entry to the host cell. Many aptamers have been isolated to bind HA and NA in order to inhibit and detect the viral infection, mainly H5N1, H9N2, H1N1 and H3N2 subtypes (Table 3).

Aptamer	Nature	Sequence	Randomized region	Target	Function	Structure	Ref
H3N2							
A22	DNA	AATTAACCCTCACTAA AGGGCTGAGTCTCAAA ACCGCAATACACTGGT TGTATGGTCGAATAAG TTAA	30 nt	HA (91-161)	Inhibition of viral infection		Jeon SH, *et al.* 2004
A21	DNA	AATTAACCCTCACTAA AGGGCGCTTATTTGTTC AGGTTGGGTCTTCCTAT TATGGTCGAATAAGTT AA	30 nt	HA (91-161)	Inhibition of viral infection		Jeon SH, *et al.* 2000

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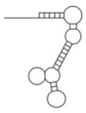
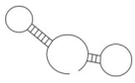
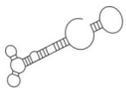
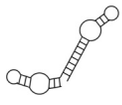
Aptamer	Nature	Sequence	Randomized region	Target	Function	Structure	Ref
P-30-10-16	RNA	GGGAGAAUUCCGACCAGAAGGGUUAGCAGUCGGCAUGCGGUACAGACAGACCUUUCCUCUCUCCUUCCUCUUCU	30 nt	A/Panama/2007/1999	Inhibition of viral infection. Discriminate between related H3N2		Gopinath SC, *et al*. 2006
H5N1							
10	DNA	GATTCAGTCGGACAGCGGGGTTCCCATGCGGATGTTATAAAGCAGTCGCTTATAAGGGATGGACGAATATCGTCTCCC	40 nt	HA	In vitro Inhibition of viral infection		Cheng C, et al.2008
2	DNA	GTGTGCATGGATAGCACGTAACGGTGTAGTAGATACGTGCGGGTAGGAAGAAAGGGAAATAGTTGTCCTGTTG	74 nt	HA and whole H5N1	H5N1 detection (QCM aptasensor)		Wang R,and Li Y. 2013
H9N2							
A9	DNA	GCTGCAATACTCATGGACAGCCTCCTGGGGTCAGGCTCAGACATTGATAAAGCGACATCGGTCTGGAGTACGACCCTGAA	40 nt	HA	Inhibition of viral infection		YueweiZhang, *et al*. 2015
B4	DNA	GCTGCAATACTCATGGACAGGGGCCGCGCCTGGTCGGTTGGGTGGGTGGCGCCCGGGACGGTCTGGAGTACGACCCTGAA	40 nt	HA	Inhibition of viral infection		YueweiZhang, *et al* 2015
H5N1 AND H7N7							
8-3S	RNA	GGGCAACCGCUGGAACUUGAAGUCGGUAAUGCGAGCGGAAAGCCC	70 nt	HA	Discriminate IVA subtypes, inhibition of receptor binding		Suenaga E and Kumar PK, 2014.
H5N1, HIN1 AND H3N2							
RHA0006	DNA	GGGTTTGGGTTGGGTTGGGTTTTTGGGTTTGGGTTGGGTTGGGAAAAA	30 nt	rHA	IVA detection (ELAA)		Shiratori I, *et al*. 2014
RHA0385	DNA	TTGGGGTTATTTTGGGAGGGCGGGGGTT	30 nt	rHA	IVA detection (ELAA)		Shiratori I, *et al*. 2014

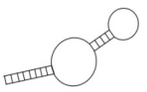
Aptamer	Nature	Sequence	Randomized region	Target	Function	Structure	Ref
RHA1635	DNA	GGGGCCCACCCTCTCG CTGGCGGCTCTGTTCTG TTCTCGTCTCCTTGATT TCTGTGGGCCCC	30 nt	rHA	IVA detection (ELAA)		Shiratori I, *et al.* 2014

Table 3. Aptamers against human IAV proteins.

Two DNA aptamers, A21 and A22, were isolated against an HA peptide containing amino acid positions 91–261. A22 was the most efficient aptamer to inhibit viral infection *in vivo* and *in vitro* by blocking the cellular receptor from binding HA. Moreover, A22 showed high binding activity against different IAV strains (H3N2 and H2N2) and reduced virus burden by 90%–99% in mice [61]. Further studies using the whole virus demonstrated the ability of RNA aptamers to distinguish between related strains within the H3N2 subtype of influenza type A viruses [62]. The selected aptamer P30-10-16 was able to discriminate between A/Panama/2007/1999 and A/Aichi/2/1968 H3N2 subtypes and its binding affinity to HA was even 15-fold higher compared with a monoclonal antibody specific to HA. A consensus aptamer sequence (5′-GUCGNCNU$(N)_{23}$GUA-3′) was selected by surface plasmon resonance (SPR) using an RNA pool based on randomized P30-10-16 (doped RNA pool). The GNCNU sequence was identified as the minimal element required to bind HA [63], suggesting a potential use as tools for influenza virus genotyping.

An aptamer selected against H5N1 HA (A10), showed inhibition of receptor binding producing *in vitro* inhibition of viral infection [64]. To increase the specificity, some aptamers were isolated using recombinant HA in the initial selection cycles and then the whole inactivated H5N1 virus for further selection cycles. The selected aptamers were able to discriminate among H5N2, H5N3, H5N9, H9N2 and H7N2, showing better specificity than anti-H5N1 monoclonal antibodies [65]. These aptamers were used on quartz crystal microbalance (QCM) biosensors coated with hydrogel. The hydrogel consisted of cross-linked hybridized ssDNA and aptamer. In the presence of H5N1 the hybridization is disturbed producing hydrogel swelling which is detected by a QCM sensor [66].

Although some aptamers have been isolated to bind a specific IVA subtype, some others identify more than one virus subtype. A 113-nt-long RNA aptamer (8-3) was isolated against HAs from H5-N1 and H7N7. The full 8-3 and shortened version called 8-3S aptamer were able to bind HA with high affinity and interfere with the cell surface HA–glycan interaction, suggesting a potential application in diagnosis and interference of virus–host interactions [67]. Furthermore, DNA aptamers were selected against recombinant hemagglutinin (rHa) to detect different subtypes of IVA such as H5N1, H1N1 and H3N2. The selected DNA aptamers: RHA0006, RH0385 and RHA1635 were able to successfully bind the three mentioned IVA subtypes. RHA0006 and RH0385 were also used in a sandwich enzyme-linked aptamer assay (ELAA), developing a novel, rapid and cost-effective diagnostic tool to identify various IVA subtypes [68].

2.3.2. Influenza B Virus (IVB)

Some aptamers have been isolated against whole virus or purified proteins in order to discriminate IVB from IVA (Table 4). An RNA aptamer against HA B/Johannesburg/05/1999 virus was able to discriminate between the HA from different strains and prevented viral infection by membrane fusion inhibition [62]. Two aptamers have been selected against intact HA of influenza strains B/Tokyo/S3/99 and Jilin/20/2003. The sensitivity of Tokyo aptamer was approximately 250-fold higher than a commercial antibody, demonstrating its potential to detect influenza viruses [69].

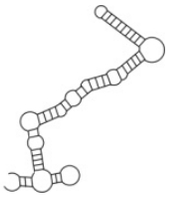
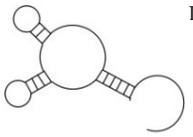
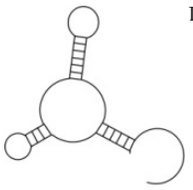
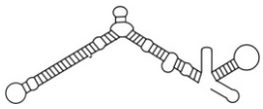
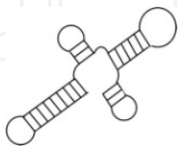
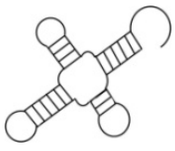
Aptamer	Nature	Sequence	Randomized region	Target	Function	Structure	Ref
Class A-20	RNA	GGGAGCUCAGCCUUCAC UGCACUCCGGCUGGUGG ACGCGGUACGAGCAAUU UGUACCGGAUGGAUGU UCGGGCAGCGGUGUGGC AGGGAUGAGCGGCACCA CGGUCGGAUCCAC	74 nt	HA	Discriminate between stran A and B. Membrane fusion inhibition		Gopinath JC, *et al*. 2006
Tokio virus aptamer (clone D)	RNA	GGGAGAAUUCCGACCAG AAGUUUUUGUUUAUAU UGUUGUUUUAUUCCUU UCCUCUCCUUCCUCUUC U	25 nt	Whole virus (Tokio virus)	Dscriminaton of influenza viruses and detection		Lakshmipriya T, *et al*. 2013
Jilin-HA aptamer	RNA	GGGAGAAUUCCGACCAG AAGGGUCUACGCCCGAA GGGUUGCCGUGCCUUUC CUCUCUCCUUCCUCUUC U	25 nt	HA (Jilin HA)	Dscriminaton of influenza viruses and detection		Lakshmipriya T, *et al*. 2013

Table 4. Aptamers against IBV.

2.4. Aptamers against HCV

HCV is one of the causes of chronic liver disease associated with end-stage cirrhosis and hepatocellular carcinoma. HCV are small enveloped viruses with a linear single-stranded RNA + genome containing a single ORF encoding a polyprotein flanked by untranslated regions (UTR) and processed into three structural proteins (C, E1 and E2) and seven nonstructural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B). The 5′-UTR contains an internal ribosomal entry site (IRES) important for mediated translation by association with the host cell small ribosomal unit (40S). Due to its importance in viral infection, replication and proliferation, HCV aptamers have been mainly isolated against NS3, NS5 proteins and some IRES domains (Table 5).

Aptamer	Nature	Sequence	Randomized region	Target	Function	Structure	Ref
10-G1	RNA	GGGAACUCGAUGAAGCGA AUUCUGUUGGCGAACUGU ACGCAAGUACACUGGAUG ACAGCCUAUCUAUCUAUC GGAUCCACG	10-18 nt	NS3	Inhibition of in vitro activity		Urvil PT, *et al*. 1997
G6-16	RNA	GGGAGAAUUCCGACCAGA AGGCUUGCUGUUGUUUCC CUGUUGUUUUGUCUCUCA ACUUUAUUGUGGUAAAGA UCACUGGGUUGAUAAGGG CUAACUCUAAUUUGACUA CAUGGUCGGACCAAUCAG UUCUUAUGGGAGAUGCAU AUGUGCGUCUACAUGGAU CCUCA	120 nt	NS3	Inhibition of proteolytic activity		Kumar PK, *et al*. 1997
G6-19	RNA	GGGAGAAUUCCGACCAGA AGCUCUUAUACUAUUAAC GCUACCGUGUCAUUGUAC UUGGUAGUGUUGAUGGUU UGGGUCGCAUUUGGCUUG GCUUAUGGUUUUUUCACC CUACCUCUCAUUGACGCA GUAGGCUCUCAUAUGUGC GUCUACAUGGAUCCUCA	120 nt	NS3	Inhibition of proteolytic activity		Kumar PK, *et al*. 1997
G9-I	RNA	GGGAGAAUUCCGACCAGA AGCUUCGGGAUUUGAGGG UAGAAUGGGACUACCUUU CCUCUCUCCUUCCUCUUC U	30 nt	ΔNS3	Inhibition of proteolytic activity		Fukuda K, *et al*. 2000
G9-II	RNA	GGGAGAAUUCCGACCAGA AGUGCUCUUAGAAUGGGA CUAAGACACGGGACCCUU UCCUCUCUCCUUCCUCUU CU	30 nt	ΔNS3	Inhibition of proteolytic activity		Fukuda K, *et al*. 2000
G9-III	RNA	GGGAGAAUUCCGACCAGA AGUACGACACGAUUGGGA CGUGUCUAUGGGACCCUU UCCUCUCUCCUUCCUCUU CU	30 nt	ΔNS3	Inhibition of proteolytic activity		Fukuda K, *et al*. 2000

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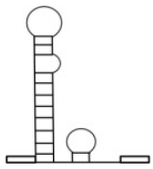
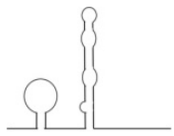
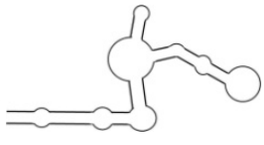
Aptamer	Nature	Sequence	Randomized region	Target	Function	Structure	Ref
B-2	RNA	GGGAUGCUUCGGCAUCCC CGAAGCCGCUAUGGACCA GUGGCGCGGCUUCGGCCC GACGGAGUGGUACCGCUU CGGCGGUACGUAAGCUUG GG	25 nt - 10 nt	NS5B	Inhibition of RNA polimerase activity in vitro		Biroccio A, *et al*. 2000
r10/43	DNA	GGGAGACAAGAATAAACG CTCAAGGGCGTGGTGGGTG GGGTACTAATAATGTGCGT TTGTTCGACAGGAGGCTCA CAACAGGC	36 nt	NS5B	Inhibition of specific subtype 3a polymerase activity		Jones LA, *et al*. 2006
r10/47	DNA	GGGAGACAAGAATAAACG CTCAATTGGGGTCTGCTCG GGATTGCGGAGAACGTGA ATCTTTCGACAGGAGGCTC ACAACAGGC	36 nt	NS5B	Inhibition of specific subtype 3a polymerase activity		Jones LA, *et al*. 2006
NS2-2	DNA	CAGGTACCACCTTCATGGG CGCGGAAGACGATGGTGTA CTA	40 nt	NS2	Distrup Ns2 - Ns5b interaction. Inhibition of NS2 activity	ND	Gao Y, *et al*. 2014
NS2-3	DNA	ACGGGGCAGGATTGTCCCC GCGCCTGGTTGAAGGTAGT CGC	40 nt	NS2	Inhibithion of NS2 activity	ND	Gao Y, *et al*. 2014
ZE2	DNA	GCGGAATTCTAATACGACT CACTATAGGGAACAGTCCG AGCCGAATGAGGAATAATC TAGCTCCTTCGCTGAGGGT CAATGCGTCATAGGATCCC GC	30 nt	E2	Competitive inhibithion of E2 - CD81 binding . Block HCV infection.		Chen F, *et al*. 2009
E1E2-6	DNA	ACGCTCGGATGCCACTACA G(N40)CTCATGGACGTGCTG GTGAC	40 nt	E1E2	Inhibition of aptamer binding to the host cell	ND	Yang D, *et al*. 2013

Table 5. Aptamers isolated against HCV proteins.

NS3 has a trypsin-like serine protease and NTPase/helicase activity [70, 71]. NS3 is required for proteolytic processing of nonstructural proteins [72]. The HCV protease domain disrupts the interferon (IFN) and toll-like receptor-3 (TLR3) signaling pathways by cleaving the caspase recruitment domain of mitochondrial antiviral signaling protein (MAVS) and the TIR domain containing an adapter-inducing interferon-β sequence (TRIF) [73]. As NS3 activity is crucial for viral replication, many aptamers have been isolated against NS3.

The 10G-1 RNA aptamer was selected against NS3 protease domain using a 12–18 nt randomized library and can reduce protease activity by 20% compared with serine protease inhibitors [74]. However, a new SELEX protocol was used to select anti-NS3 aptamers with improved binding and inhibition activities increasing the structural pool complexity by using larger randomized domains (120 nt) and competition against 10G-1. As a result, two new RNA aptamers were selected (G6–16 and G6–19) showing efficient NS3 binding. The G6–16 concentration needed to inhibit 50% of the NS3 activity was 3 μM, and although both aptamers inhibited the protease and helicase activity, they showed lower efficacy compared with known serine protease inhibitors [75]. To further improve aptamer efficacy and inhibit the NS3 RNA binding helicase, a truncated form (ΔNS3) only including the protease domain was used as a target, and the random sequence of the RNA pool was reduced to 30 nt to ease the SELEX process. Three highly specific aptamers (G9-I, G9-II and G9-III) were obtained against ΔNS3 containing the conserved sequence GA(A/U)UGGGAC that was present inside an identical loop in all aptamer structures. These aptamers showed K_D values of 11.6 nM, 6.3 nM and 8.9 nm, respectively. The G9 aptamers produced 90% of NS3 protease activity inhibition alone and 70% in the presence of NS4A used to simulate physiological conditions [76]. Structure analyses suggested that interaction of stem I and stem-loop II is essential to G9-I aptamer NS3 binding. To achieve *in vivo* applications, the G9-II aptamer was conjugated with cis-acting genomic human hepatitis delta virus (HDV) ribozymes. The aptamer was inserted into the nonfunctional stem IV region of the HDV ribozyme promoting *in vivo* stable structure that lasted up to 4 days after transfection. The HDV ribozyme–G9-II aptamer (HA) was attached to nuclear export signal CTEM45 (HAC) and ligated in tandem to increase the aptamers dosage in cells. These new constructs showed efficient NS3 protease inhibition *in vivo* and *in vitro* [77]. To also inhibit NS3 helicase activity, a poly U tail (14U) was added to the minimum functional sequence of the G9-I aptamer (ΔNEOIII) to mask and inhibit the helicase substrate-binding region [78]. NEOIII-14U displayed dual functions by inhibiting NS3 protease activity *in vivo* and *in vitro* and inhibiting the NS3 unwinding helicase reaction (IC_{50} 1 μM) [79].

More RNA aptamers were selected against NS3 helicase domain, including the conserved sequence GGA(U/C)GGAGCC at stem-loop regions. Further deletion and mutagenesis analyses demonstrated that the whole structure of the conserved stem-loop is needed for helicase inhibition. Aptamer #5 presented the best inhibition of helicase *in vitro* activity with an IC_{50} of 50 nM [77]. Bifunctional aptamers constructed conjugating RNA aptamers ΔNEOIII and G9-II with aptamer #5 through an oligo U spacer. The spacer length was optimized by protease and helicase inhibition assays [80]. The resulting advanced dual-functional (ADD) aptamers (NEO-34-s41 and G925-s50) showed superior inhibitory activities of NS3 [81].

NS5B is an RNA-dependent RNA polymerase that synthesizes the HCV-negative strand RNA using genomic positive RNA strand as a template. NS5B has an essential role in the HCV's life cycle and its variability has been associated with worse disease prognosis [82]. The highly specific B.2 RNA aptamer selected against a truncated NS5B target (NS5BΔC55) presented a conserved sequence that was folded on stem loop structure associated with a tight interaction to NS5B (K_D = 1.5 ± 0.2 nM). Also, B.2 demonstrated inhibition of NS5B activity by a noncompetitive mechanism [9].

Two DNA aptamers selected against NS5B (27v and 127v) showed inhibition of polymerase activity *in vitro*. Although both aptamers were isolated from the same SELEX procedure and presented an 11-nt conserved sequence, they displayed different mechanisms to inhibit NS5B. The 27v aptamer competed with RNA template and inhibited both initiation and elongation of RNA synthesis, while 127v competed poorly and just inhibited initiation. Also, 27v was able to inhibit RNA synthesis and HCV particles production on Huh7 cells [83, 84]. The RNA aptamers r10/43 and r10/47 were isolated against NSB5 of HVC subtype 3a and resulted in the inhibition of polymerase activity with an estimated K_D = 1.4 and 6.0 nM, respectively [85].

In a different approach, chemically modified RNA aptamers (2′-hydroxyl or 2′-fluoropyrimidine) were isolated against NS5B. The 2′-hydroxyl aptamer inhibited HCV replication on human liver cells without producing off-target effects or generation of escape mutants. The 2′-fluoropyrimidine aptamer showed increased affinity to NS5B and efficient inhibition of HCV replication in cultured cells. This last aptamer was further conjugated with cholesterol or galactose-polyethylene glycol ligand to increase its availability and specificity for the liver inhibiting replication of HCV genotype 1b and 2a [86].

Two other RNA aptamers targeting NS5A (NS5A-4 and NS5A-5) reduced the levels of intracellular infectious virions and viral RNAs by 3-fold and 1-fold, respectively, affecting virus assembly and release through prevention of the NS5A–core protein interaction. These NS5A aptamers were specific to HCV without affecting HBV replication and produced cytotoxicity in human hepatocytes [87].

NS2 contains a transmembrane segment in the N-terminal and a cytoplasmic region in the C-terminal domain. Although NS2 is essential for HCV RNA replication, its role in HCV's life cycle is still unknown. Aptamers NS2-2 and NS2-3 were isolated against NS2 and demonstrated reduced infectious virus production without *in vitro* cytotoxicity. These aptamers were specific to HCV and did not trigger innate immunity responses. NS2-2 aptamer produces its antiviral effects through binding the NS2 N-terminus thus disrupting NS2–NS5 interaction [88].

E2 is an enveloped glycoprotein implicated on initial steps of viral infection by the direct interaction with CD81. Through cell surface SELEX (CS-SELEX), specific DNA aptamers were isolated against E2 expressed on CT26 cells. Aptamer ZE2 showed the highest affinity and specificity to E2 and was able to detect HCV particles and block HCV infection on human cultured hepatocytes by CD81 binding inhibition [89]. A similar inhibition mechanism was observed on the DNA aptamer E1E2-6, which inhibited viral infection by blocking host cell binding [90]. A new system developed to quantify immobilized infectious HCV particles in microplates (so-called enzyme linked apto-sorbent assay or ELASA) used aptamers against E2 instead of antibodies and resulted in an effective and easy-to-use tool to quantify infectious units of HCV and to monitor anti-HCV drug efficacies [91].

3. Aptamer structures

Nucleic acid aptamers have a diverse range of secondary structures such as stems, loops, symmetric or asymmetric internal loops, bulge, single-base bulges and junctions. Aptamer

internal loops and bulges generally present different conformations in solution and adopt defined secondary and tertiary structures on ligand–aptamer complex [92]. This effect was observed on aptamer Sc5-c3 selected against HPV-16 VLPs. Sc5-c3 showed a hairpin structure with an internal loop, where the main loop (ML) presented two different structures in the absence of a target (Table 2). Sc5-c3 transition structure was demonstrated by ribonuclease mapping. Further experiments using Sc5-c3 mutants generated both stable stem and stable loop conformations, demonstrating that the loop structure binds better to the VLPs [58]. Thus, as observed in several aptamers, the binding region remains as a flexible single strand as bulges or loops stabilize conformation arrangements in the presence of a target, producing a very specific binding.

Although bulges and loops are quite common target-binding motifs in aptamer RNAs, they are not the only structures present in aptamer–target complexes. Pseudoknots and G-quadruplexes have also been reported as functional components of aptamers [93]. For example, some of the aptamers isolated against HIV integrase (93 del and 112 del) presented a G-rich nucleic acid sequence that was stabilized in the presence of K+ as G-tetrad, increasing their inhibitory effect [25]. Later reports showed that 93 del adopts an unusually stable dimeric quadruplex structure [94].

The binding properties of an aptamer are dictated by its sequence and subsequent folding into secondary and tertiary structures. Recently, functional RNA structures were classified as critical, connecting, neutral and forbidden structures regarding their particular roles within a structure [95]. This classification is also applicable to nucleic acid aptamers and is an important clue to design novel and functional variants for viral detection or therapy.

4. Challenges for aptamer technology

According to their molecular characteristics, RNA or DNA aptamers have some limitations in their use in animal models and humans. They have limited stability in biological fluids and are readily degraded by nucleases, unmodified aptamers in the bloodstream possess a half-life time of less than two minutes. However, many post-SELEX modifications have been developed to avoid nuclease attack and improve stability in biological fluids. Some modification examples include nucleotide substitutions by 2′-modified variants such as 2′-fluoro (2′-F), 2′-amino (2′-NH2) or 2′-O-alkyl. Because the most abundant nucleases in biological fluids are specific to pyrimidines, substitutions in pyrimidine positions appear to be sufficient to prevent degradation. Another method to stabilize RNA aptamers is the substitution of D-ribose by L-ribose. As a first step, the aptamers bind the mirror image of the target molecule to obtain a D-aptamer, then the selected aptamer sequence is synthesized in L-conformation. As a result of molecular symmetry, the L-ribose–containing aptamer can bind to the target molecule avoiding degradation by D-ribose–specific nucleases. Moreover, to efficiently overcome binding issues produced by the introduction of modified nucleotides on the aptamer sequence, the SELEX procedure can be carried out in the presence of modified libraries.

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Therapeutic aptamers selected against intracellular or nuclear proteins represent bigger challenges as they need to go across physiological barriers (i.e. cell membrane) before they reach their targets. DNA and RNA aptamers are characterized by rapid renal clearance leading to short half-lives in the bloodstream. To address this issue, aptamers can be conjugated to synthetic polymers such as polyethylene glycol (PEG) to increase their *in vivo* half-life and pharmacodynamics [96]. Additionally, PEG-conjugated aptamers show higher cellular uptake than the unconjugated form [96]. Alternatively, delivery systems such as viral and nonviral vectors may have improved aptamer cell uptake and nuclear distribution [97]. Vectors or aptamers alone can be delivered either *ex vivo* or *in vivo*. *In vivo* approaches include intravenous injection or local implantation and *ex vivo* refer to the removal of cells followed by *in vitro* genetic manipulation and the reintroduction of modified cells. These therapies are still under evaluation and further studies are necessary to demonstrate their clinical safety. Aptamers against extracellular or surface viral targets have obvious advantages over aptamers targeting viral proteins intracellularly expressed, as they can reach exposed areas of infection, such as the respiratory tract or reproductive organs. This availability makes it possible to develop new antiviral drugs administrated by noninvasive methods, such as aerosols in case of respiratory tract infection or topical creams/lotions in case of reproductive organ infections. Many aptamers are undergoing clinical trials, some of them administrated by noninvasive methods but, so far, no antiviral aptamer has been approved for human use [18, 98].

5. Conclusion

In the last few years, aptamers have become successful tools for specific viral diagnosis and genotyping, resulting in the development of many methods based on aptamer–target detection with very high sensibility and accuracy. On the other hand, aptamer's role as an antiviral drug has demonstrated the inhibition of viral infection through *in vitro* assays and *in vivo* experiments using cell lines or animal models. Nevertheless, most aptamers failed to produce results in clinical trials mostly due to nuclease-associated degradation. Therefore, further development of aptamer's stability in biofluids and improved pharmacodynamics and delivery methods are required to overcome clinical issues that would allow its successful therapeutic application.

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