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REVIEW ARTICLE

A review on the therapeutic applications of aptamers and aptamer-conjugated nanoparticles in cancer, inflammatory and viral diseases

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Abstract The advancement in early diagnosis and precise treatments options result in more predictable and powerful health care modalities. Aptamers are known as nucleic acid structures with three-dimensional conformation to selectively bind a target site. Physicochemical properties of aptamers, their conjugation with nanoparticles (NPs) in theranostics applications and their internalization have been found to be of interest in development of aptamer-based drug delivery systems. Therefore, we aimed to present an overview on the structure and generation of aptamers followed by advantages of aptamers-conjugated NPs and their theranostics applications in various diseases such as oncology, inflammatory diseases and viral diseases. Afterward, we discussed several reports on the internalization approaches of aptamers, efficiency of aptamers *vs*. their analogous, and implications of aptamers in clinical trials. Finally, we discussed the current challenges and future perspectives of actively targeted aptamers for clinical application. In conclusion, this review may hold a great promise for development of aptamer-based therapeutic platforms in clinical trials.

Contents

1. Introduction

The development of accurate diagnosis with successful therapy is important for favorable clinical application. So, the effort to develop small molecules capable of modulating the target's activities was increased. In the recent year, aptamer was introduced to fulfill desired goals related to clinical applications. Aptamers are the unique synthetic fold up structure of single-stranded DNA or RNA molecules with the capability to form secondary and tertiary structures. These unique characteristics make them potential candidates to bind several target molecules such as peptides, proteins, metal ions, bacteria, viruses, and other cellular targets. Aptamers research field is extending because of their remarkable potential for instance as potent anti-tumor activity, excellent circulation stability, biocompatibility, multimodal diagnostic functionalities, and high loading efficiency (Ni et al. 2011, Wan et al. 2019). Most conventional aptamer therapies stop cell transfer machines by dysregulation of transcription activator. In this regard, aptamers modulate cellular function through interfere with the DNA binding of the transcription activator (Zhao et al. 2006, Shi et al. 2007). Recently, the use of oligonucleotide-based drug has grown, providing new ways for the treatment of cancer, autoimmunity and inflammatory diseases. The treatment of wet form of age-related macular degeneration (wet AMD) was possible by Pegaptanib sodium, *i.e*. Macugen® (first FDA approved commercialized oligonucleotide-based drug) (Röthlisberger and Hollenstein 2018). Also, aptamers used in clinical phases include, Edifoligide (E2F Decoy), Metastatic Renal Cell Carcinoma (AS1411) and von Willebrand (ARC1779) (Nimjee and Sullenger 2020). Aptamers have also gained wide attention in the treatment of neurodegenerative, autoimmune, and bacterial or viral infections. Although aptamers are going to find their own niche of theranostic applica-

tions, their limitations still remained as challenges such as aptamer degradation, metabolic clearance, renal filtration, control of the duration of action, cross-reactivity, irreversible tissue uptake and generation by automated synthetic methods (Röthlisberger and Hollenstein 2018).

This review presents the applications and progress of aptamers in various diseases. First, we consider aptamer discovery, generation, possible modification to tackle aptamer degradation and aptamer internalization. Subsequently, recent progress in the use of aptamer-conjugated nanoparticles (NPs) and their theragnostic applications in various diseases such as oncology, inflammatory, and viral diseases were discussed. Afterwards, internalization approaches of aptamers, efficiency of aptamers *vs*. their analogous, and implications of aptamers in clinical trials were surveyed (Ni et al. 2011).

2. Aptamers

Aptamers are oligonucleotide compounds in the range of 15–100 nucleotides that exhibit a complex tertiary or quaternary structure. Aptamer detects targets in the micro- to picomolar range, which is comparable to antibodies (Morita et al. 2018). The first step in the aptamer coin, accompanied with identifying a chemical composition of the cell which is called nucleic acids (deoxyribonucleic acid or DNA) in 1869 by Friedrich Miesche (Dahm 2008). Likewise, studies continued in order to determine DNA structures, DNA functions, its effect on regulation of cellular pathways and ultimately its chemical synthesis and preparation *in vitro* (Alshaer et al. 2018). In 1990, aptamer was introduced by Tuerk and Gold in order to bind the T4 DNA polymerase (Tuerk and Gold 1990). High-affinity aptamer ligands were isolated by a procedure known as Systematic Evolution of Ligands by Exponential Enrichment (SELEX): process relies on ligand selection with alternate cycles from pools of different sequences and amplification of the bound species. In fact, they selected and enriched two variant types of RNA ligands from one eight-base random region library (65,536 species) which can interact with the T4 DNA polymerase. In the same year, the word of aptamer was coined by Ellington and Szostak to name RNA molecules with the ability to bind organic dyes. They isolated the aptamer that binds to organic dyes from 10^{13} different sequences of pools (Ellington and Szostak 1990).

Compared to antibodies (their proteinaceous counterparts), commercial success of aptamers still proceeds at a weak pace. However their unique features such as higher sensitivity and selectivity, small size, rapid penetration into target tissues, ease of chemical production on large scale, facile chemical modification, low cost, low immunogenicity, limited batch to batch variation, high thermal stability, simple storage, and resistance to denaturation reflect this fact that they can find their own niche of bioanalytical and pharmaceutical applications or even are expected to become an alternative platform for antibody applications (Dehghani et al. 2018, Röthlisberger and Hollenstein 2018, Ahmadyousefi et al. 2019). However, despite these favorable functions, aptamers suffer from two major shortcomings that often thwart their applications as therapeutic and diagnostic agents: limited stability and insufficient binding affinity and specificity (Röthlisberger and Hollenstein 2018, Nimjee and Sullenger 2020).

2.1. Structure

Aptamers are usually willing to form complementary base pairs based on their tendency to have certain structures. They can fold into different secondary structures such as internal loops, stems, pseudoknots, bugles, kissing complexes, tetra loops, hairpins, and G-quadruplexes. Followed by the specific and unique complex, three-dimensional structures can be formed from these secondary structures that are capable of specific molecular recognition of their cognate targets (Reinemann and Strehlitz 2014). Mixtures of interaction containing base stacking of aromatic rings, hydrogen bonding, van der Waals forces, complementarity in the geometrical shape, and electrostatic interactions, result in binding affinity and specificity of the aptamer (Zhou and Rossi 2017). In addition, enabling them to distinguish between conformational isomers, recognizing a distinct epitope of a target molecule and amino acid mutation, differentiate various functional groups or even closely similar targets such as theophylline and caffeine (Reinemann and Strehlitz 2014). Many of the selected aptamers show potential affinities comparable to those observed for monoclonal antibodies.

3. The generation of aptamers (SELEX Process)

Systematic evolution of ligands by exponential enrichment (SELEX) is a conventional aptamer engineering method that is used for *in vitro* selecting target-specific aptamers. Although there are numerous types of the SELEX process in new selection protocols, the main principles remain the same. In fact, the operation mimics a Darwinian type process, driving the selection towards relatively few (but optimized) structural motifs, which show the highest specificities and affinities to the selected target. Generally, the basic process can be divided into two repetitive stages of selection and enzymatic amplification. At the first level, the pool of original oligonucleotides with optimum concentration (around 10^{13}-10^{15} sequences) is chemically synthesized. Before starting the RNA SELEX process, the DNA library must be turned into the RNA library (Fig. 1A) (Röthlisberger and Hollenstein 2018). Initial library qualities play a crucial role in successful SELEX experiments. After incubating the oligonucleotide pools with the target and washing steps, the small amounts of the target bound oligonucleotides are amplified via a reverse transcription PCR (RT-PCR) for RNA and polymerase chain reaction (PCR) for DNA. Subsequently, this new enriched pool of selected oligonucleotides is re-exposed to the target in the next SELEX round. Iterative rounds up to the saturation concentration of target-interacting sequences dominate the population (Fig. 1A) (Röthlisberger and Hollenstein 2018). The selected aptamer pool is cloned to obtain individual aptamers and corresponding sequencing, which further analyzed to select representative aptamers in binding assays to characterize their binding characterization, including the affinities and specificities (Proske et al. 2005, Reinemann and Strehlitz 2014, Zhou and Rossi 2017, Röthlisberger and Hollenstein 2018).

Over the last few years, considerable efforts have focused on automating *in vitro* selection procedures. Since the advent of SELEX, the original method has evolved and improved in terms of time–cost optimization and efficiency. Despite consid-

erable success of aptamers, they contain some complications that prevent their widespread utilization in various applications particularly in biomedical fields. Aptamer degradation in biological media by nucleases is the first drawback. To tackle this issue, modified nucleotides before or after SELEX round, mirror image aptamers, and aptamer displacement screening are generally used. For instance, modification of 2′ sugar position (2′-amino pyrimidine nucleosides [20, 21], 2′-fluoropyrimidine nucleosides [22, 23], 2′-O-methyl purine, and 2′-O-methyl pyrimidine nucleosides [24, 25]) or 3′- and 5′-nucleotides, located L-ribose or L-deoxyribose in oligonucleotide backbone and displace aptamer with low-molecular-weight compound from the binding site of a target molecule, improve pharmacokinetics of the aptamer in blood. In the terms of the second problem, renal filtration of aptamer, conjugation with polyethylene glycol (PEG) and thus increasing aptamer size is a good way to increase the bloodstream circulation time. Third problem related to control action duration of aptamer, the use of polycationic biopolymers like porphyrin and conversion of an inactive aptamer to an active form are the most common solutions to this problem. Furthermore, Cell-SELEX and *in vivo* SELEX (Fig. 1B, (Zhou and Rossi 2017)), SELEX negative selection, automated SELEX and CE –SELEX were used to avoid aptamer generation with purified target molecules, cross-reactivity of aptamer, and automation of aptamer generation limitations, respectively (Lakhin et al. 2013).

4. Advantages of aptamer-conjugated nanoparticles (NPs) and their theranostic applications in various diseases

In recent years, combinations of aptamer and NPs are extensively used in the development of theranostic platforms because of their unique potential in targeted drug delivery systems, diagnosis and monitoring response to treatment (Khan et al. 2021b). In fact, Warner coined the new term 'theranostics' in order to implement simultaneous diagnosis and treatment into a single system. This is a useful concept when designing nanotechnology-based imaging contrasting agents and imaging-guided therapeutics (Ahmed et al. 2012, Morshed et al. 2020).

4.1. Oncology

Although significant advances in cancer treatment such as molecular biology, surgical procedures, radiotherapy, and chemotherapy have been achieved recently, cancer remains the most common cause of death worldwide. Various factors comprising microenvironment, genetics, and epigenetics affect tumor cells that ultimately, can lead to enhance the risk of therapeutic failure and thereby tumor relapse. In principle, the ultimate goal of cancer therapy is the development of targeted drug delivery systems (Liu et al. 2014). Rapid development of nanotechnology with the essential needs of selective inhibition of cancer cell proliferation at the initial phases of growth, making hybrid nanostructures as potential and powerful active targeting materials (Khan et al. 2021a, Khan et al. 2021c). Interaction of aptamers with nanomaterial has made this aim possible by increasing the efficacy of antitumor drugs on their target (Sharifi et al. 2019). Actually, the ability of aptamers to identify specific epitopes on cell surfaces can result in improved drug accumulation inside cancer cells (Reinemann and Strehlitz 2014, Alshaer et al. 2018). Furthermore, we summarized recent progress in the development of aptamer-NPs structures (Fig. 2A) that can deliver anticancer drugs to the specific tumor site in Table 1 (Grabowska-Jadach et al. 2019).

Although the use of aptamers can significantly reduce tumor activity according to Table 1, Kang et al. (2015) exposed that the use of aptamer E-selectin reduces the activity of breast cancer metastasis by inhibiting the adhesion of CD44 + breast cancer cells to blood vessels. They showed that intravenous injection of aptamer E-selectin reduced metastasis in syngeneic or xenogeneic breast cancer models without relocating the metastasis site (Kang et al. 2015). Moreover, Heo et al. (2016) in an animal model using an aptamer-antibody complex (oligobody) were able to prohibit the angiogenesis of xenograft A549 tumor (human adenocarcinoma cells) similar to the growth of tumor compared to control group with cotinine-specific antibody. Also, the oligobody increased the half-life in serum to 8.2 h, which can be very promising in drug stability. Meanwhile, in an animal model, it was determined that aptamer PD-L1 could induce lymphocyte proliferation and tumor growth by mimicking antibody functions, with a chemical nature without immunogenicity or inducing hepatotoxicity (Lai et al. 2016). The results of this study not only reduce angiogenesis compared to the previous study, but also significantly increase T cells with the markers CD4 + and CD8+, Interlukin-2, TNF-α, interferon-γ and the chemokines CXCL9 and CXCL10. These chemokines can further adsorb T cells to tumor tissues (Lai et al. 2016). In the following, Jain et al. (2018) revealed that the function of doxorubicin in the treatment of diffuse B-cell lymphoma (DLBCL) and other blood cancers depends on nucleolin silencing and non-binding to topoisomerase-II-α. Amplification of nucleolin quenching by aptamer AS1411 or Nocant N6L increases the activity of doxorubicin in DLBCL cells, which ultimately induces apoptosis significantly by DNA fragmentation. This finding is of potential clinical importance due to the high accuracy of aptamer in regulating the expression of nucleolin with low concentration.

4.2. Inflammatory diseases

Inflammation, which is primarily caused by immune molecules, acts an important role in promotion of a state of low-grade diseases due to the increased immune response to infections or injuries. Therefore, there is evidence that early detection of inflammatory molecules as well as their reduction by aptamers can accelerate therapeutic activity earlier than the clinical onset of the disease (Table 2). For example, cerebral inflammations, which generally have few clinical manifestations, can be detected and controlled by aptamers (Shahdadi Sardou et al. 2020). In this regard, Giorgi-Coll et al. (2020) in an animal model using an optical nanobiosensors based on the aggregation of AuNPs coated with two anti-murine interleukin-6 aptamers (ATW0082 and ATW0077) were able to detect interleukin-6 with a detection limit of 1.95 μg/mL (linear range of 3.3 to 125 μg/mL) as an indicator of acute inflammation. Interleukin-6 concentration, which is considered as an indicator of rheumatoid arthritis, has been reported in plasma samples from mice with different health conditions between concentrations of 1 and 1500 pg/mL (Nukina et al. 2001). In this line, Hekmatimoghaddam et al. (2019) to reduce

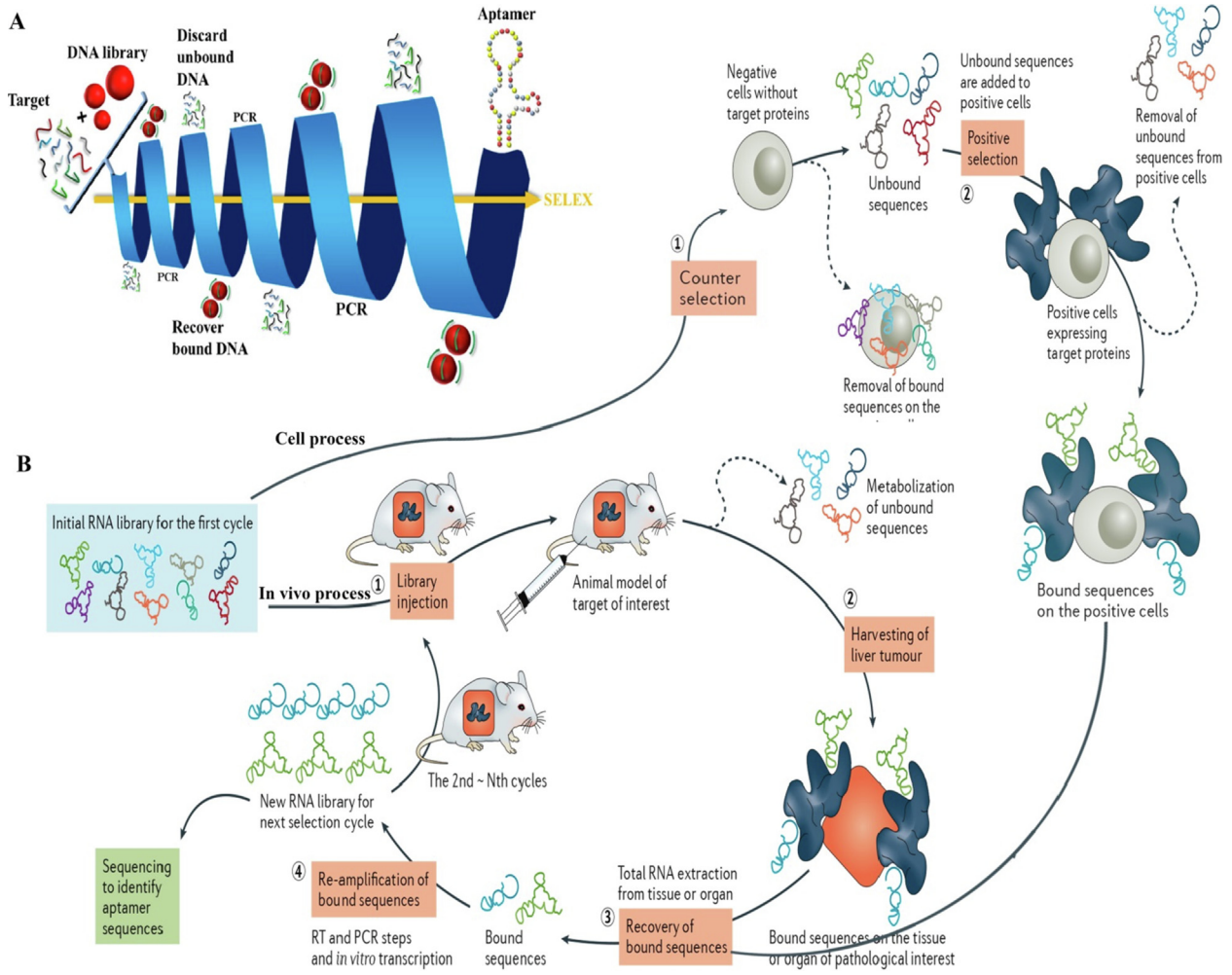

Fig. 1 **A)** Schematic representation of the SELEXmethod. An initial DNA library (of typically 10^{14} molecules) is incubated with the solid support-bound target. Unbound DNA molecules are discardedwhile the active species are recovered, amplified by PCR, and injected into subsequent rounds of selection. The stringency of the selection protocol can bemodulated by altering physicochemical parameters such as concentration, pH, temperature, or buffer composition. At the end of the protocol, the enriched population is sequenced and the individual aptameric sequences evaluated for their capacity at binding to the target (Röthlisberger and Hollenstein 2018). **B)** Cell-process: Step 1 involves counter selection by incubating the RNA library with negative cells that do not express the target protein. Step 2 involves positive selection by incubating recovered unbound sequences with positive cells expressing the target protein. Step 3 involves recovery of target-bound sequences. Step 4 involves re-amplification of recovered species and generation of a new RNA pool for the next selection round. *In vivo* process: After intravenous administration and circulation of an RNA library in the animal model (step 1), the tissue or organ of pathological interest is harvested (step 2) and the bound sequences are extracted (step 3). Subsequently, the recovered RNA sequences are re–amplified to make a new RNA library for the next selection cycle (step 4) (Zhou and Rossi 2017). Copyright 2018, reprinted with permission from Elsevier, (Röthlisberger and Hollenstein 2018) and Copyright 2017, Macmillan Publishers Limited, part of Springer Nature (Zhou and Rossi 2017).

brain inflammation induced by proteolipid protein and parathion showed that the use of gelatin hydrogel containing cerium oxide NPs coated with interleukin-17 aptamers could decrease the level of brain inflammation significantly by reducing the expression of interleukin-17, −10 and −6 genes as well as their serum concentrations.

4.3. Viral diseases

Aptamers are oligonucleotides that can be easily used as targeted agents in drug delivery and even in the development of biosensors to detect infectious agents (Eilers et al. 2020). Also, aptamers can target viral proteins involved in various stages of viral infection (Table 2) (Zou et al. 2019b). In this regard, Shiang et al. (2013) by designing an AuNPs (13 nm in diameter) containing two aptamers including RT1t49 and ODN 93 as a very effective suppressors for human immuno-deficiency virus type 1, showed that in the early stages of the HIV-lentiviral replication, the inhibitory effect of the loaded aptamers on AuNPs in the presense of the virus increases by 40.2% (Fig. 2B). Likewise, it was found that magnetic NPs containing E1E2 glycoprotein-aptamers reduce the amount of hepatitis C virus in human plasma samples with more than 91% capturing efficiency (Delaviz et al. 2015).

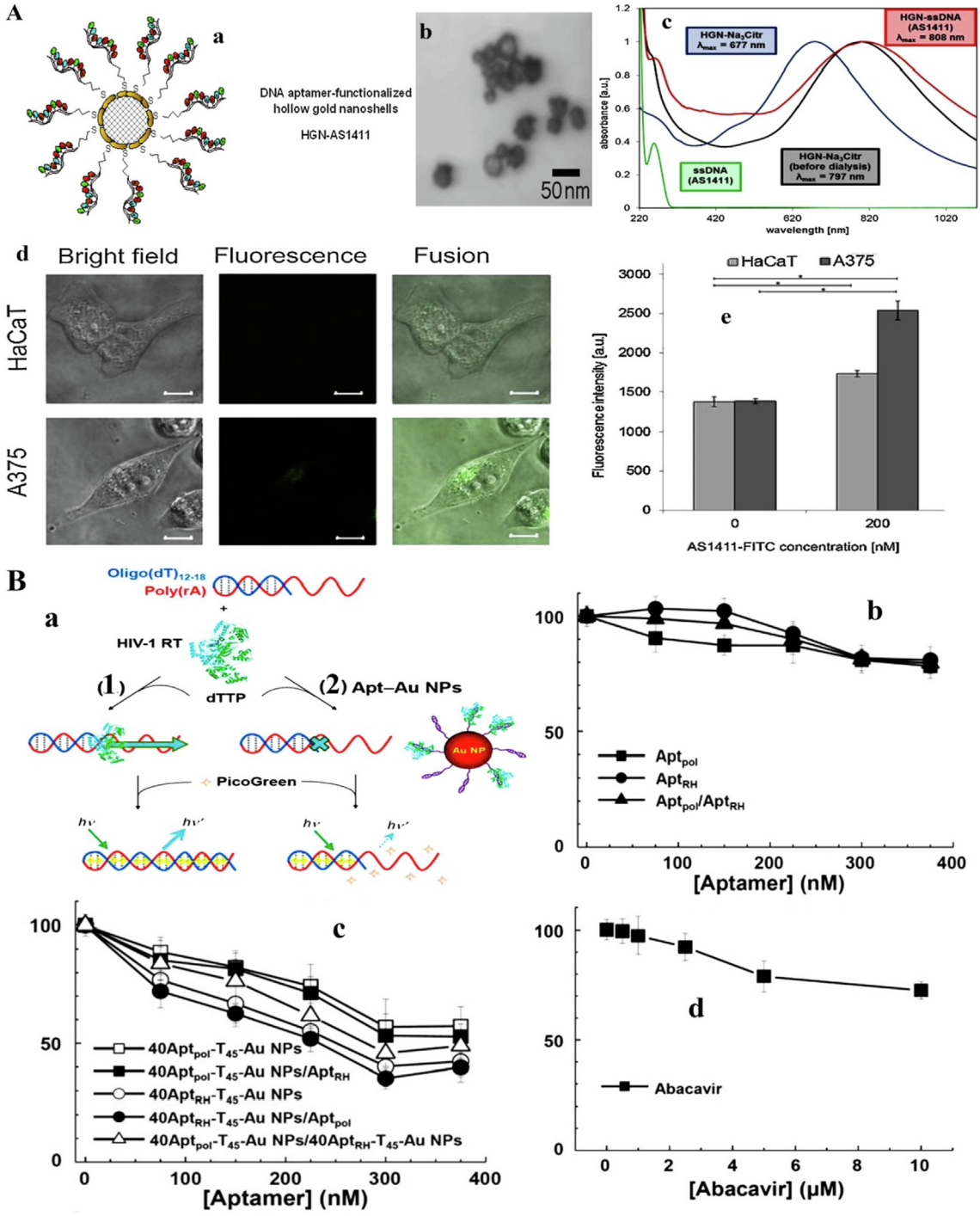

Fig. 2 **A)** a; Schematic illustration of HGN surface modification with thiolated ssDNA aptamer. b; TEM image of HGNs before ssDNA attachment. c; Absorption spectra of HGN samples at various stages of modification with ssDNA (AS1411 aptamer). Normalized spectra represent NPs: citrate estabilized before dialysis (black line), citrate-stabilized after dialysis (blue line) and DNA aptamer (red line). Green line represents spectrum of free ssDNA (AS1411) in solution. AS1411-FITC uptake by HaCaT and A375 cells after 24 h of incubation with cell cultures: d; confocal microscope images (scale bar 10 μm), e; measurement of fluorescence intensity (Grabowska-Jadach et al. 2019). **B)** a; Schematic representation of the binding and enzymatic activity of HIV-1 RT toward poly(rA): oligo (dT)12–18 in the absence (A) and presence (B) of Apt–Au NPs. Inhibition of viral infectivity to HepG2 cells by (b) aptamers, (c) Apt_Au NPs or (d) nucleoside RT inhibitor (Abacavir). The green fluorescent protein signal was measured after infection for 4 days. Results are expressed as a percentage of infectivity (Shiang et al. 2013). Copyright 2019, reprinted with permission from Elsevier, Under the terms of the Creative Commons CC BY license (Grabowska-Jadach et al. 2019) and Copyright 2013, Royal Society of Chemistry publishing group (Shiang et al. 2013).

Table 1 Summary of aptamers used in nano-carriers for the treatment of cancers.

Nanomaterials	drug	Aptamer	Target cell lines	Cancer type	Therapy method	Imaging	ref
Organic							
$Ru(bpy)_3^{2+}$- SiO_2 NPs	miRNA-21	AS1411	MCF-7	Breast cancer	Chemotherapy	Fluorescence	(Li et al. 2014)
Poly (ethylene glycol)-poly (caprolactone) NPs	Docetaxel	GMT8	U87 cells	Drain Glioblastoma	Chemotherapy	Fluorescence	(Gao et al. 2012b)
Poly (ethylene glycol)-poly (caprolactone) NPs	Docetaxel	AS1411	C6 cells	Brain glioma	Chemotherapy	Fluorescence	(Gao et al. 2012a)
CA-PLGA-*b*-TPGS NPs	Docetaxel	AS1411	MCF-7	Breast Cancer	Chemotherapy	Fluorescence	(Tao et al. 2016)
Exosome	Doxorubicin	sgc8	Ramos cells and CEM cells	Lymphoblast	Chemotherapy	Fluorescence	(Zou et al. 2019a)
Inorganic							
Gold (Au)	–	AS1411	A375	Skin cancer	Photothermal therapy (PTT)	Fluorescence	(Grabowska-Jadach et al. 2019)
Silica (SiO_2) NPs		YQ26	HEK293 BNL-CL2 H22 B16			Fluorescence	(Tan et al. 2017)
Mesoporous SiO_2 NPs	Doxorubicin	MUC1	MDA-MB-231 cells	Breast cancer	Chemotherapy	SPECT	(Pascual et al. 2017)
Au nanocluster	Doxorubicin	AS1411	U87MG cells	Brain glioblastoma	Chemotherapy	Fluorescence	(Chen et al. 2016)
Silver (Ag) nanocluster	miR-34a	MUC1	MCF-7	breast cancer		Fluorescence	(Chen et al. 2017)
Fe_3O_4	Epirubicin	5TR1	C26 cells	Colon carcinoma	Chemotherapy	MRI	(Jalalian et al. 2013)
Fe_3O_4	Doxorubicin	PSMA	LNCaP cells	Prostate-cancer	Chemotherapy	MRI	(Yu et al. 2011)
CdTe/CdS quantum dots	Doxorubicin	MUC1	MCF-7	Breast cancer	Chemotherapy	Fluorescence	(Du et al. 2015)
Au@ γ-Fe_2O_3 NPs	–	MUC-1aptamer	L929 CHO HT-29	Colon cancer	PTT	MRI	(Azhdarzadeh et al. 2016)
Au@Ag/Au	–	S6 aptamer	A549	Lung cancer	PTT	Fluorescence	(Shi et al. 2014)
Fe_3O_4@carbon	Doxorubicin	*sgc8c* aptamers	A549	Lung cancer	Chemo– PTT	MRI imaging	(Zhao et al. 2019)
Fe_3O_4–Au nanocomposite	Epirubicin	MUC-1 aptamer	MCF-7 and HT-29	Breast and colorectal cancer	Chemotherapy	Fluorescent imaging	(Binaymotlagh et al. 2019)
Au nanocage/ SiO_2	–	AS1411	MCF-7	Breast cancer	PTT	SERS Imaging	(Wen et al. 2019)
Fe_3O_4 co-loaded (PEG-PLGA) NPs	Doxorubicin	AS1411	C26 cells	Colon carcinoma cancer	Chemotherapy	MRI	(Mosafer et al. 2017)

5. Internalization approaches of aptamers

The cellular internalization of aptamer is an important factor for development of *in vivo* targeted drug delivery systems to reach the targeted site without harmful side effects to off-targeted cells (Wan et al. 2019). The cellular internalization depends on some factor such as charge, size, and even the stability of aptamers. The negatively charged aptamer and cell surface lead to electrostatic repulsion. In addition, cellular internalization is usually reduced in the case of aptamers with oligonucleotides longer than 25 bases due to self-hybridized conformations (Patil et al. 2005)

Since the advent of aptamers, exploring different signaling pathways mediating their cellular internalization have received a great interest in the development of aptamer-based diagnostic and therapeutic platforms. Endocytosis is a main pathway that is used by cells to internalize different types of aptamers. Generally, four pathways comprise of phagocytosis, micropinocytosis, clathrin-mediated endocytosis (CME) and caveolae mediated-endocytosis are introduced for endocytic mechanisms to internalized aptamer from the cell surface membranes. Besides, the mechanism of cellular internalization depends on aptamer targeting the specific receptor. The analysis of the localization of fluorescently labeled transferrin is used for determining CME of aptamers.

6. Efficiency of aptamers *vs*. Their analogous

Although, a variety of biological agents such as peptides and antibodies are used in biochemical assays, aptamers are able to detect very specific molecules by creating a variety of secondary structures like a loop, bugle, pseudoknot, and G-quadruplex, and even three-dimensional structures. This structural diversity and other advantages listed below make the use

Table 2 Summary of aptamers used in inflammatory and viral disease (Kaur and Roy 2008, Kanwar et al. 2010, Boshtam et al. 2017, Mor-Vaknin et al. 2017, Zhu and Chen 2018).

Nr.	Aptamer	Target	Action
1	DEK-binding	Nuclear chromatin protein DEK	Juvenile idiopathic arthritis
2	Aptamer M.G (RNA)	Acetylcholine receptors	Control of myasthenia gravis
3	ADR58 (RNA)	gp130 receptor	Control of rheumatoid arthritis
4	CD4-specific aptamer 14 (RNA)	Antigen-presenting cells	Immunosuppressant
5	DD7, ED1 (RNA)	hNE-specific ligand	Anti-inflammatory
6	IGEL1.2 and D17.4 (DNA)	Human IgE	Antiallergic response
7	LD201t1 (DNA)	L-selectin	Anti-inflammatory action
8	2′–NH2 −30-ligand (RNA)	IFN-a	Immunoregulatory
9	Spiegelmers NOX 2149	ORL-1R	Decrease in pain and stress
10	SE RNA	Hepatitis C virus NS3	Viral proliferation in chronic hepatitis
11	LIGAND 1.1	HIV-1 RT	Anti-HIV

of aptamers much more desirable than others. One of the most important advantages of using aptamers compared to other cases is the shorter production time of aptamers (1–3 months) compared to antibodies (4–6 months). Also, unlike antibodies and other proteins that require immunogenicity tests to be used in diagnostic and therapeutic processes, the use of aptamers show significantly lower immunogenicity (Dhar et al. 2020, Ni et al. 2020b). Also, unlike antibodies and other proteins that require immunogenicity tests to be used in diagnostic and therapeutic processes, the use of aptamers could have significantly lower immunogenicity (Jayasena 1999, Avci-Adali et al. 2013). On the other hand, the small size of aptamers along with flexible structures not only increases the concentration of aptamers compared to other compounds (Chen and Yang 2015), which can be more effective (5–10 times) in medical activities such as treatment (Ni et al. 2020b), but also their small size leads to more accurate tracking of biomaterials with sizes less than 60 Daltons (Zhang et al. 2014), which ultimately increases the accuracy of detection. Thus, their small size can reduce the restrictions on the penetration of compounds into tumors and the blood–brain barrier, although their small size accelerates the filtration rate of aptamers from the kidney (Chen et al. 2020). Furthermore, modification of aptamers to increase the detection accuracy *in vitro* through chemical synthesis is much simpler than other compounds, especially antibodies obtained through *in vivo* methods. Meanwhile, the production cost along with levels of impurities or contaminants of aptamers are much lower than those of other compounds. Finally, the results of the reports show that the shelf life of aptamers is much higher (∼up to 1 month) compared to other compounds and is very resistant to adverse environmental conditions such as changes of pH and temperature (Sun et al. 2014). In contrast, their level of stability in the body or tissues due to kidney filtration and nuclease activity are much lower than antibodies and other biological compounds that can be very effective in reducing aptamers toxicity. However, various results indicate that pharmacological modifications can improve the stability of aptamers for imaging and drug delivery activities (Han et al. 2019, Odeh et al. 2020).

7. Implications of aptamers in clinical trials

Despite extensive efforts to use aptamers in medical practice, their use faces numerous challenges and drawbacks. However, the first commercial and therapeutic example of the use of aptamers is Pegaptanib, which has been used to treat age-related macular degeneration (Ng et al. 2006). This aptamer acts as a vascular endothelial growth factor antagonist. However, clinical models have shown that this aptamer has no drastic effect on oncology applications. Nevertheless, successful aptamers have been developed in the treatment of cancer, such as AS1411 (a nucleolin-targeting DNA aptamer) and NOX-A12, which have shown good clinical activity (Ireson and Kelland 2006). The first aptamer has a desirable half-life due to its unique structure and using PEG (Ireson and Kelland 2006). The NOX-A12 aptamer with L-form also has a good half-life due to its high nuclear resistance (Ludwig et al. 2017). Aptamer AS1411 has shown approximately 47% success in the treatment of kidney cancer and myeloid leukemia after 4 to 6 months treatment (Soundararajan et al. 2009). However, phase II trials of this aptamer show that it needs other auxiliary biomarkers that can more effectively detect kidney tumors in all patients. Clinical activity in the field of hematologic malignancies of aptamer NOX-A12 shows that it not only reduced bone marrow niche microenvironment receptivity to multiple myeloma cells, but also effectively forbade chemotaxis of tumor cells towards CXCL12 along with reducing drug resistance by mediating adhesion in cancer cells (Ludwig et al. 2017, Waldschmidt et al. 2017). After treatment with NOX-A12 aptamer, approximately 86% of patients recovered within 16 months. However, the activity of this aptamer in the treatment of metastatic colorectal and pancreatic cancer with pembrolizumab is still under study. Summary of approved aptamers in clinical activities are presented in Table 3.

8. Challenges and future perspective

Despite the widespread attention in the field of using aptamers, they show several drawbacks in pre-clinical and clinical applications. In order to optimally use aptamers and reduce their side effects, it is necessary to pay special attention to these limitations as follows:

a) The rapid clearance of aptamers in the blood and other biological environments due to the activity of endonucleases has challenged the diagnostic activity or drug delivery based on aptamers. Reports indicate that changes in the conformational structure of aptamers

Table 3 Current aptamers in clinical and preclinical trials.

Aptamers	Nucleotide	target	Disease	Ref.
REG1	RNA	Coagulation Factor IX	Coronary Artery	(Vavalle and Cohen 2012)
E10030	DNA	PDGF	Age-Related Macular	(Jaffe et al. 2016)
NU172	DNA	Thromin	Heart	(Troisi et al. 2018)
ARC1779	DNA	vWF	Thrombotic thrombocytopenic purpura	(Mayr et al. 2010)
NOX-E36	RNA	CCL2	Type 2 Diabetes mellitus	(Oberthür et al. 2015)
BX499	RNA	Tissue Factor Pathway Inhibitor	Hemophilia	(Chang et al. 2012)
gp120	RNA	gp120	HIV therapy (pre-clinical)	(Shrivastava et al. 2020)
A10-3.2	RNA	PSMA	Prostate cancer (pre-clinical)	(Singh 2019)
Zimura	RNA	Anti-c5	Age-Related Macular (pre-clinical)	(Petrukhin 2020)
Rn-DsDsDs-44	DNA	Von Willebrand Factor	(pre-clinical)	(Matsunaga et al. 2017)
Kall1-T4	RNA	Kallikrein	(pre-clinical)	(Steen Burrell et al. 2017)

can be very effective in increasing their shelf life (Roxo et al. 2019, Odeh et al. 2020). However, time is still considered as a limiting factor, and this reduction in retention time in medical practice is not desirable. The use of SELEX methods (Kovacevic et al. 2018) with oligonucleotides containing modified nucleotides and NPs (Mignani et al. 2020, Thevendran et al. 2020) can have a significant effect on the stability of aptamers. However, it should be noted that the use of the above methods should not adversely affect their binding to target tissues or cells.

b) One of the challenges of using aptamers to load drug compounds within the nucleus is the interaction of aptamers with cell surface receptors, which can reduce access to intracellular target molecules. To reduce this problem, it is recommended to use compounds as inducers of cell surface receptor-dependent endocytosis on the drug carrier (Engelberg et al. 2018). After intracellular penetration of drug compounds, targeted access to intracellular organs can be provided by using protected aptamers via harpins in regions the 3′- and 5′- termini (Futami et al. 2019).

c) One of the most important pathways for the removal of aptamer in the body is filtration through the kidneys due to a molecular weight of less than 50 Da (Wang et al. 2019). Therefore, therapeutic applications of aptamers without preservatives are problematic. In this regard, the use of polymers, proteins and fats to increase their molecular weight can increase the shelf life of aptamers in the blood (Jeevanandam et al. 2020).

d) Another major challenge in using aptamers for medical activities is the complex and lengthy process of their production. Also, purification of produced aptamers is a difficult process and requires complicated processes. In addition, sometimes aptamers produced by microbial methods have no interaction with human cells due to detectable epitope changes. In this regard, it is possible to produce and purify aptamers using methods with negative selection approaches through normal cells and positive selection with modified cells (Sefah et al. 2010, Catuogno and Esposito 2017). Also, it is possible to use an organism to isolate and amplify aptamers by injecting them into the bloodstream and isolating the target tissue to extract aptamers.

e) Aptamers simply interact with the target molecule, so they can easily off-interact with structures similar to the target molecule (Liu et al. 2020), which can seriously challenge aptamer-based therapeutic activities. Although negative selection with molecules similar to the target molecule can drastically reduce these drawbacks, the results of some reports suggest that cross-activity based on the type of treatment is possible.

f) The use of unnatural nucleotides can increase chemical toxicity or immune responses. For example, an experiment has shown that nucleic acids as an aptamer increase the toxicity against liver cells (Burdick et al. 2014). Therefore, it is necessary to investigate the toxicity of the aptamers used and, for example, reduce their toxicity by their modification. On the other hand, the response of immune system against aptamer and additives such as polymers have raised concerns about the use of these compounds in therapeutic activities (Lincoff et al. 2016).

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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