REVIEW ARTICLE

Aptamer Insights in Targeting and Designing of Therapeutic Drugs

Mishra A*, Abdullah SM, Koley S, Modak S, Das A

Jharkhand Rai University, Rajaulatu Campus, Namkum, Ranchi, Jharkhand, India

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ABSTRACT

In the therapy of chronic and dangerous conditions like as cancer, heart disease, neurological illnesses, or retinal angiopathy, we frequently require most effective dosage schedule with adequate precision and sufficient therapeutic potential. Among these entities with the capacity to behave in a target-specific way are aptamers. Usually, they are produced by an iterative screening procedure named "Systemic Evolution of Ligands by Exponential Enrichment (SELEX)" which is used to complicate nucleic acid libraries. For several biological purposes, including targeted treatment, aptamers are potential and could be easily molded into desirable needs and also be tested in a manner that is not very extravagant or pricey. Chemical modification can be employed to enhance their pharmacological actions or prevent enzymatic degradation, hence guaranteeing their chemical integrity and bioavailability in a physiological setting. The recent advancements made in drug targeting and design with the use of aptamers are the main topic of this review. This review will highlight current developments and go over prospects and problems in the fields of aptamer-based targeted therapy, including aptamer therapeutics.

Keywords: Aptamer, SELEX, Targeted therapy, Drug discovery, Chronic disease.

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INTRODUCTION

Initially, RNA enzymes were employed in intricate processes and gained international attention in the 1980s. They were regarded as the initial step forward in the development of aptamers. In accordance to Kruger K. *et al*., (1982), a few researchers team developed the RNA sequence identification technique, which is further understood as "SELEX" in 1990; Tuerk and Gold named it after themselves. Later, the first dye-bound aptamers and thrombin-binding aptamers were found in 1992. However, the first SELEX-discovered aptamers were linked to the *Trypanosoma brucei* parasite. Macular degeneration, an ophthalmic disease, was the first medical condition in which aptamer was used to treat and was FDA-approved.

The entities resulting from natural selection have been named as Aptamers by Eelington and Sztosak, and it originated from the Latin term APTUS, signifying fitting.[1] Generally, short genetic entity pieces are called aptamers which can assemble into three dimensions with particular target molecules, including small molecules, peptides, and proteins.[2] These may be DNA or single-stranded RNA oligonucleotides with excellent selectivity and affinity for binding to certain species.[2] "Chemical antibodies" is another name for them because of their capacity to thwart the pathogenic activities of bacteria that cause illness.[3] Comparatively, aptamers are can be considered better option than antibodies since they can be produced chemically, are inexpensive, and don't vary from batch to batch. Nonetheless, despite its many benefits, the production of aptamers is always halted because of certain issues, such as the difficult and time-consuming discovery process, early patent protection, and more.[4]

In this review article, an attempt has been made to convey the importance of aptamers to readers and to explain how they might be used for better drug design and drug targeting.[5]

Detailed process to formulate the aptamers: SELEX

Aptamers are highly stable and don't lose their activity very easily, thus, they may be produced in bigger quantities. The phrase "SELEX," which is frequently used in the context of aptamer technology, stands for "Systemic Evolution of Ligands by Exponential Enrichment," which refers to a highly sophisticated *in-vitro* technique for aptamer synthesis. In 2005, Stoltenberg R. *et al.,* Aptamer production is undoubtedly a labor-intensive procedure. An oligonucleotide library typically comprises of 50 to 90 random single-stranded sequences that are fringed at the ends with random nucleotide sequences.[6]

To generate the aptamers by using the SELEX procedure following steps must be followed (Figure 1):

- Create a library of 1014–1016 single-stranded nucleotides.
- Incubate the oligonucleotide with its target.
- Separate bound nucleotides from unbound ones.

**Author for Correspondence:* anjalimishra674@gmail.com

- Choose and amplify the oligonucleotide using polymerase chain reaction (PCR) for DNA templates and real-time reverse transcriptase-PCR (RT-PCR) for RNA templates.
- Characterization through sequencing.[7]

Understanding of Core Complex and Steps Involved in SELEX

Single Stranded Nucleotide (nt) Selection

Aptamers, which enable PCR amplification using complementary primers, normally comprise a random region surrounded by two consistent sections.[8] The length of the random region, which is theoretically unique to each aptamer in a starting library, determines the potential number of sequence candidates.[9] For instance, a full library with a 40-nt random section would weigh about 50 kg overall and include 440 or about 1024 distinct sequences. The usage of strict in scope helps to minimize the occurrence of errors in the selection of long distinct sequences, which typically possess 1014 to 1015 distinct sequences.[10] SELEX performance can be influenced by the length of the random region and aptamer. Aptamers have been found in libraries in random areas as short as 22 nanometers and as long as 22 nm. The 50-nt and 70-nt choices had the target motif most frequently, according to Legiewicz M *et al.,* a study in 2005, which investigated how target-binding sequence abundance was affected by six distinct random area lengths (16, 22, 26, 50, 70, and 90). The most often occurring random area in recent papers has been found to be 36–52 nt long, or roughly 70 to 90 nt in total aptamer length.[11] The cost of synthesizing DNA also matters; lengthier syntheses are less efficient and more expensive. If the efficiency of adding a nucleotide base is 99.5%, for instance, an 80-mer aptamer has a yield of 69% and a 160-mer aptamer has a yield of 45%. Even though early research on aptamers primarily focused on RNA aptamers, the majority of aptamers that have been discovered and utilized are DNA-based. Despite the RNA backbone's greater flexibility, both the DNA and RNA backbones are capable of forming secondary and tertiary structures. This is because DNA is more stable than RNA and does not require the reverse transcription step during amplification, making selection easier.[12]

According to Elskens J.P. *et al*. (2020), synthetic or chemically modified nucleic acids also increase the diversity of aptamer libraries and make protein binding epitopes more accessible. In 2021, McCloskey *et al.,* employed "alpha-L-threofuranosyl nucleic acid (TNA)" in a recent study to create base- and backbone-altered aptamers that could bind target proteins and go through *in-vitro* selection.[13] These TNA aptamers, or "threomers," contain modified bases such as planar aromatic amino acids like tryptophan and phenylalanine, which are frequently found at the antibody-antigen interface. Following optimization, numerous threomers exhibited nanomolar to sub-nanomolar affinities. McCloskey *et al*. selected TNA libraries containing approximately 1014 distinct instances of the 40-nt random region for threomers that bind "SARS-CoV-2 S1 protein or TNF".

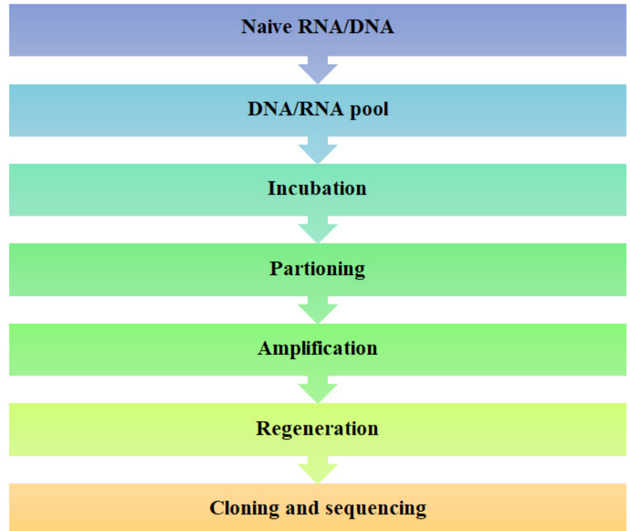

Figure 1: Sequential steps of SELEX cycle involved in aptamer technology

Nevertheless, utilizing threomers has several disadvantages, such as the requirement for extensive chemical synthesis (14–29 steps for each TNA nucleoside triphosphate) and TNA polymerase optimization.[14] Alkyne-modified uridine, to be more precise, 5-ethynyl uridine (EdU), which an azide-modified group operates, is also utilized in the "click-SELEX" approach. Since EdU is commercially available, unlike TNAs, it may be substituted for dT in DNA amplicons by employing standard DNA polymerases and PCR techniques. To improve restricting probability, Snap SELEX can be utilized with an azide that has a gathering present on the objective's normal fasteners too.[15]

Targets Selection

Numerous cell types, such as bacteria, T cells, and cancer cells, have been employed as selection targets.[16] Cell-SELEX is an easy-to-follow methodology that doesn't require any specialist tools to execute. Centrifugation makes it simple to divide cells, releasing unbound aptamers into suspension. After being eluted by high-temperature incubation, the cell-bound aptamers are separated by centrifugation. Cell-SELEX is beneficial due to its range of targets and genuine target presentation on the cell surface, in addition to its straightforward methodology. Receptor identification and binding reaction temperature are two issues with cell-SELEX, though. We choose candidates at 4°C in our procedure to prevent aptamer internalization, which would eliminate them from consideration. As a result, the discovered aptamers function well at 4°C but might need to be optimized for binding at 37°C or room temperature. However, "cell-SELEX" can also be performed at 37°C due to the fact that cell lysis permits the recovery of both internalized and surface-bound aptamers. Internalized RNA aptamers can be retrieved with the help of TRIzol and other chemical extraction methods. The other major obstacle is locating the aptamer's target, which is typically a membrane protein that can be identified using pull-down and mass spectrometry techniques.

However, because there are several possible targets and non-specific pull-downs, target identification is not simple. In order to quantify aptamer-specific pull-down, "stable-isotope labelling by amino acids in cell culture (SILAC)", a more recent method, compares the pull-down of "light" unlabeled cells to that of cells cultivated with heavy lysine and arginine. After optimizing the binding, cross-linking, and lysis conditions of each aptamer, the "SILAC-based approach" was able to identify two targets of aptamers with previously unknown receptors. All things considered, a considerable number of cell-targeting aptamers still have unknown targets and require novel tactics in order to identify their receptors.

Recombinant proteins are another common target of selection. Protein-SELEX does not involve a receptor characterization phase and may be performed at any temperature, in contrast to cell-based selection. In actuality, more research can pinpoint the binding epitope accurately using techniques like cryo-EM and protein binding experiments. "Aptamers that function well in protein binding tests but not in practical applications may result from the protein's presentation, which might not be consistent to its native shape. Hybrid cell- and protein-SELEX has proven to be an effective way to get around this. Hicke *et al*., who invented this method, employed tumor cells followed by recombinant protein to choose aptamers against tenascin-C, a protein linked to tumor development.[17] Combining SELEX techniques allowed for the identification of several aptamers. Targeting human programmed cell death protein 1, C42-aptamer was discovered following 13 rounds of protein and three rounds of cell SELEX. Transferrin receptor-targeting RNA aptamers were found by Wilner *et al*. following four rounds of selection against recombinant transferrin receptor protein and a fifth-round using Jurkat cells". After four rounds of protein SELEX and six rounds of selection using HEK293 that was stable in expressing spike protein, the "SARS-CoV-2 spike protein-binding aptamer SNAP4" was discovered. It is also possible to produce high-affinity aptamers by using different protein targets inside the same SELEX. After completing 13 selection rounds using SARS-CoV-2 S1 wild-type, Zhang *et al*. carried out five further selection rounds with other SARS-CoV-2 S protein variations.[18]

Bound and Unbound Aptamers Partitioning

A significant piece of SELEX is isolating restricting from non-restricting aptamers during choice. Centrifugation is an easy way to partition using cell-SELEX. Libraries are normally divided into one of two primary groups for protein-SELEX.[19] One technique is capillary electrophoresis SELEX (CE-SELEX), which uses different electrophoretic mobility constants to distinguish proteins, unbound aptamers, and aptamer–protein complexes from one another.[20] "Since the first development of CE-SELEX in 2004, other iterations have been created to enhance specific components of the procedure.: a single action CE-SELEX (ssCE-SELEX) saves time and materials by streamlining the selection in a single run; fraction collection CE-SELEX (FCE-SELEX) minimizes contamination by directly loading PCR reagents into oil-sealed mixtures"; "ideal-filter capillary electrophoresis (IFCE)" modifies the ionic potency of the running buffer to push "non-binding nucleotides" in the contrasting approach of nucleoprotein complexes. This significantly improves separation efficiency by preventing the carry-over of non-binding aptamers.[21] In general, CE-SELEX provides quantitative and customizable partitioning; nonetheless, it has significant limitations. The volume that CE-SELEX can process is limited, which affects the size and diversity of the sampled library. For each element to be separated, CE-SELEX additionally needs variations in charge and size between the aptamer-target complex and individual components.[22]

Amplification and strand separation

To prepare for additional selection or binding analysis, the pool must be amplified and the sense strand isolated after aptamers and bind the target have been gathered. The most familiar technique for strand split is PCR, which pull-downs a biotin-labeled backward strand. The ideal section is eluted by gentle denaturation and cushion trade after the PCR items are gathered on streptavidin agarose.[23] The most popular strand separation technique is the fast, easy-to-use, and high yield biotinylated PCR product pull-down method. "Lambda nuclease", which selectively digests "DNA with 5′ phosphorylation", is used in another technique. Through the use of a 5′ phosphorylated reversed primer, the antisense strand can be specifically digested during PCR. Asymmetric PCR is a third technique for library preparation.[24] To amplify each sense strand preferentially, different molar ratios of primers with forward and reverse orientations are employed. Next, in a time-consuming, low-yield procedure, ssDNA is eluted from the gel after the PCR result has been separated by gel electrophoresis.[25]

After gathering aptamers that tie to the substrate, it is essential to expand the reservoir and separate the explanation strand for additional picking via crucial analysis.[26] The considerably familiar technique for strand partition is PCR, which pulls down a "biotin-labeled reverse strand". After the PCR consequences are collected on "streptavidin agarose", the preferred strands are eluted by mild denaturation and "buffer exchange"*.[27] The most popular strand separation technique is the fast, easy to use, and high yield biotinylated PCR product pull-down method. Another method makes use of lambda nuclease, which selectively digests DNA through "5′ phosphorylation". During PCR, the antisense strand can be specifically digested with a "5' phosphorylated reverse primer". Asymmetric PCR is a third library preparation technique. Selectively amplifying the sense strand involves the employment of forward and reverse primers at differing molar ratios. After the PCR outcome has been divided by "gel electrophoresis", ssDNA is eluted from the gel in a time-consuming, low-yield procedure.[28]

Recent trends of aptamers in drug designing and drug targeting

In Breast Cancer Treatment

As one of the most predominant, muddled, and significant reasons for mortality on the planet as well as one of the best in mankind's set of experiences, malignant growth actually guarantees many lives.[29] In 2021, breast cancer will overtake lung cancer as the considerable typical cancer among women, according to GLOBOCAN 2020, resulting in two million new cases and 680,000 deaths worldwide. A multitude of factors can impact an individual's risk of breast cancer, such as race and ethnicity. Over time, our comprehension of selective therapy for breast carcinoma research has progressed in tandem with our expanding comprehension of breast cancer pathophysiology.[30] The best course of treatment for breast cancer can currently be determined by factors such as tumour type, volume, stage, and genesis location. Chemotherapy, endocrinology treatment, radiation treatment, medical procedures, and immunotherapy are the most frequently utilized methods of therapy. In an attempt to get around the challenges that come with traditional anticancer treatment approaches the research and development of nanoscale systems for delivering medicines have received a lot of attention throughout recent decades.[31] Some examples of these nanocarriers include "lipidic nanoparticles, polymeric micelles, carbon nanotubes, graphene, and metallic nanoparticles". Particles with a diameter of 1–100 nm are generally referred to as nanocarriers and are utilized in a range of biological elements. Liposomes, which are lipid-based nanocarriers, are frequently used as promising drug delivery systems among the many other nanocarriers that have been investigated and unexplored. They are circular vesicles with an aqueous center that hold a couple of lipid bilayers within. In 1965, Professor Alec D. Bangham made the initial discovery of banghosomes, a type of liposome. These vesicles, very closely resembling the membranes of living things, can spontaneously form when amphiphilic lipids, such as phospholipids, are present. Since the early 1980s, the potential of monoclonal antibodies to alter the function of target proteins has been thoroughly investigated.[32] However, with commercial antibody reagents, repeatability is a key source of worry. As a result, commercial antibodies are being replaced with antibodies derived from traditional sources[33.] It has been shown that aptamers based on cancer biomarkers increase the concentration and effectiveness of cancer remedies. Aptamers have a newly achieved rage as a tool for treating and interpreting exhaustive cancers due to their multiple benefits, such as their "low immunogenicity, long-term stability, and ease of synthesis". [34] Nowadays, trastuzumab and pertuzumab are used to treat breast cancers that express the HER2 gene. Eyetech Pharmaceuticals, Inc.'s Macugen is the only aptamer that was approved by the US FDA in 2004 and is being sold by "Bausch Health Companies". Because of their many advantages, like their low immunogenicity, long haul solidness, and Just four liposomal prescriptions have gotten promoting approval at this point for the therapy of bosom malignant growth. In each instance, liposomes were utilized to provide the "PEGylation effect" and transport shipment using the EPR effect. Only a small number of liposome-based formulations, including liposomal cisplatin (LiPlaCis®), "MM-310, MM-302, ONT-10, and AS1411", have been included in clinical trials.[35]

In Retinal Angiogenesis Prevention

Deregulation of retinal homeostasis, which is a subset of neovascular retinal illnesses, may be the source of pathological angiogenesis in the eye.[36] Retinal artery or venous blockage, "age-related macular degeneration (AMD), retinopathy of prematurity (ROP), and diabetic retinopathy (DR)" are a few of the main conditions that cause this pathological angiogenesis.[37] In these illnesses, the rupture of the "blood-retinal barrier (BRB)" in conjunction with the angiogenic process may result in irreversible blindness. Even though hypoxia episodes are normally the beginning of retinal angiogenesis, other neurotic cycles such as irritation, neurodegeneration, vascular injury, and oxidative pressure can likewise cause it.[38] The main molecule in this process is "vascular endothelial growth factor (VEGF)", but complement molecules like C5 and C3, "transforming growth factor beta (TGF-), platelet-derived growth factor (PDGF), interleukin-1 (IL-1) and IL-6" also play important roles. In relation to this overexpression of inflammatory cytokines, it has been demonstrated that the use of monoclonal antibodies against VEGF is a beneficial option to more conventional remedies like "surgery and laser photocoagulation."[39] Faricimab, aflibercept, bevacizumab, and ranibizumab are the most often used "anti-VEGF antibodies". However, there are many disadvantages to using these antibodies therapeutically, such as their high cost, short half-life, potential for adverse effects (such as inflammation, retinal detachment, or elevated ocular pressure.[40] or the requirement for Additionally, some people do not respond well to anti-VEGF, in which case corticosteroids like dexamethasone should be used instead. Aptamers have shown promise as a substitute for antibodies in blocking the retina's angiogenic process.[41]

Like antibodies, aptamers are short groupings of nucleic acids that collaborate with a solitary objective.[42] Aptamers, however, have a number of benefits over antibodies, such as being less expensive, immunogenicity-free, having a longer half-life, and being simpler to make and alter. Because of these benefits, aptamers—like antibodies—have been employed to target VEGF. The first aptamer to be approved for VEGF targeting was Macugen®. Despite the conceivable concessions of aptamers, Macugen® only targets one isoform of "VEGF—VEGF-165"—and its restorative effectiveness is lower than anticipated.[43] A number of other aptamers, including Fovista® and Zimura® (Clinical trial data), which target the molecules C5 and PDGF, respectively, have already been put through clinical studies. Another significant protein that may be involved in these diseases is "nucleolin (NCL)", an omnipresent protein that is a co-receptor of maturation characteristics and has significant regulatory functions in the nucleus.[44] NCL is phosphorylated upon overexpression of VEGF, which causes it to translocate into the cell membrane and serve as a co-receptor for a number of growth factors. Numerous contemplations have demonstrated that NCL plays a crucial role in "angiogenic neovascular retinal disorders" because its suppression upgrades an "antiangiogenic effect".[45]

Another aptamer under intense investigation for NCL targeting is AS1411.[46] One of the considerable mesmerizing elements of this aptamer is its use of a "G-quadruplex (G4) structure", whose stability is affected by the portion of salt in the circumstances. The specific interactions that aptamers have with their targets are based on their tri-dimensional structures, and the G4 design is significant for NCL to target Iturriaga.[47] The suppression of NCL on retinal angiogenesis has already been investigated in relation to the AS1411 aptamer. It is unknown, yet, whether conformation is the most physiologically significant because of its considerable polymorphism.[48] To address this problem, derivatives of AS1411, including AT11, have been developed. The bulge/linker size of AT11 and its derivatives varies slightly, ranging from zero to two thymines. Therefore, in order to lessen pathological angiogenesis, we assessed the G4 stability of AT11-L0 in this work. AT11-L0 was manufactured as a liposome containing either an acridine orange derivative called C8 or the anti-inflammatory drug dexamethasone.[49]

In targeted protein degradation

Samarasinghe KT *et al*. (2021) say that targeted protein degradation is a new treatment method that works by breaking down a "protein of interest (POI)" that is linked to the onset or progression of serious illnesses like cancer or neurological disorders.[50] "The highly conserved ubiquitin-proteasome system" "(UPS) and autophagy/lysosome degradation systems in eukaryotes" inspired several TPD methods, including PROTACs.[51] Through an event-driven pharmacology, PROTACs destroy disease-causing proteins and are reusable following the breakdown of the POI. Due to this catalytic mechanism, PROTACs can conduct virtually even at sub-stoichiometric dosage regimens.[52] On the other hand, the "non-specific biodistribution of PROTACs" in normal tissues and cells could lead to unanticipated "on-target degradation," which raises the risk of unpredictable issues with "on-target toxicity." Because the "event-driven mechanism" of action in PROTACs only demands intermediate binding movement from ligands, it has the potential to target undruggable proteins, in contrast to typical small molecules, which require long-term occupation of the active pocket.[53] In any case, finding fitting ligands for most POIs is as yet, a troublesome endeavor, particularly for framework proteins and record aspects. LYTACs can greatly increase the range of degradable proteins by eliminating extracellular and cell surface proteins.[54] Like "PROTACs, LYTACs are mainly composed of two components: one is used to recruit POI, typically with the help of antibodies, and the other is used to recruit an endosomal shuttling receptor" r. The POI is transported to the lysosome for destruction when LYTACs, the shuttling receptor, and the POI form a ternary complex.[55]

Summary

Due to vital and multiple properties, pharmaceutical medications find aptamers, which are nucleic acid ligands, to be appealing. Aptamers have a strong binding affinity and specificity, which makes them valuable in large-scale chemical reactions. The synthesizing of aptamers has been made easier by the adaptable technique of aptamer selection. By binding to buried receptors on the cell surface, aptamers have been used to carry a variety of payloads inside cells. Therefore, aptamers can be used to target cells that exhibit aptamer targets to supply molecules like siRNAs that would not otherwise be readily brought up by cells or to restrict the dispersion of molecules like the majority of chemotherapeutic medicines that are naturally assumed by organisms.

Suppose a target has high expression on all targeted cells, rapid internalization, and no expression in the outermost layers that make up non-target cells. In that case, it is ideal for aptamer-mediated delivery. A number of aptamers against appropriate targets have been found, notwithstanding the possibility that a perfect target presents issues. In trials employing these aptamers, aptamers have shown "proof of concept" by facilitating cell type-specific transport. Enzymes, poisons, chemotherapeutic drugs, imaging agents, and siRNAs are among the products that are shipped. The current hurdles include finding more superior objectives and aptamers to bind them, as well as optimizing the package's contents. With respect to the former, the swift progress being made in RNA interference and nanotechnology will lead to the development of increasingly complex aptamer structures. Aptamer technology has shown to be a very successful method for producing targeted protein inhibitors and detectors in recent years. It is a perfect fit for genomic and proteomic high-throughput target validation. Automation guarantees that aptamers are not intrinsically poisonous, reduces development times, simplifies the creation and modification of aptamers, and eases laboratory handling.

Another exciting perspective in clinical research is the benefit of aptamers for in vivo administration, protein visualization, arousal, or inhibition. A number of further advantageous and detectable aptameric compounds, including pegaptanib, commonly known as Macugen, a licensed therapeutic aptamer for age-related macular degeneration, are undergoing various phases of clinical preparations. Aptamers are artificial biological elements with a lot of potential applications, particularly in protein research.

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