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Mini Review

The Potential Use of Aptamers in The Process of Drug Development

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Abstract

Single-stranded nucleic acids can fold and create unique 3-dimensional structures when interacting with other molecules. The unique structure can achieve high specificity and affinity for the particular target. Synthetic oligonucleotide binding agents, also known as aptamers, are generated through the rational process of Systematic Evolution of Ligands by Exponential Enrichment (SELEX). As this technology matures, it shows increasing promise for use in the field of therapeutic drugs, drug discovery, development, and delivery, and this report seeks to detail how this technology may be applied.

Introduction

The continuous development of new medications to meet rising medical demand is subject to a tightly regulated system to ensure that emergent drugs are rendered safe and effective. Some drugs might take several years to be developed and an investment of millions of dollars is required.[1] The efficiency and efficacy of drugs could be improved while reducing the development time and investment costs.[1] The implementation of synthetic oligonucleotides, or aptamers, offers new methods for advancing the identification, production, and delivery of drugs with high efficiency and efficacy.

Aptamers are composed of single-stranded ribose nucleic acid (RNA) or deoxyribonucleic acid (DNA), which fold into target-specific unique 3D structures.[2] where some of the nucleotide bases interact directly with the target while the remainder of the nucleotide bases associate with each other to stabilize the structure. Aptamers can bind to a target with high affinity and specificity, making them comparable in use to antibodies.[3] This short communication endeavors to review how aptamers have been used as agents for drug discovery and drug development, as drug carriers, and as therapeutic drugs. We make emphasis on new approaches and novel technologies that use aptamers in therapeutics against cancer.

Evolution of Aptamers

Aptamer selection was first demonstrated in 1990 by Tuerk and Gold, and Ellington and Szostak, in two separate publications.[4,5] The word aptamer was chosen as a combination of the Latin term 'aptus', meaning 'to fit', and the Greek term 'meros', meaning 'part'.[4,5] These initial publications also described the *in vitro* process of generating aptamers, now commonly known as Systematic Evolution of Ligands by Exponential enrichment or SELEX.[4,5] SELEX begins with the target molecule being introduced to a synthetic custom-designed and randomized oligonucleotide library. Following an incubation period, oligonucleotides with no affinity to the target are removed from the reaction solution and the bound oligonucleotides are enriched through PCR. This cycle is repeated with the addition of various selection pressures in order to promote variants within the oligonucleotide library to attain improved affinity and specificity to the target molecule.[6] Provided sufficient evolution and selection pressures are subjected, the resulting aptamers evolved can discriminate between similar molecules such as caffeine and theophylline which differ in only by a single methyl group at nitrogen atom N-7, or between enantiomers of the same molecule such as L-arginine and D-arginine,[7,8] with the capability of binding the target in complex biological matrices.[9] Since the SELEX technique was first published, it has been modified into several subtypes to develop aptamers that bind to a diversity of targets including small molecules such as toxins and drugs, proteins, and contaminants. Also, the modification and improvement of SELEX techniques allows the enhancement of the natural properties of the aptamers upon binding such as changes in their 3D structure, to implement aptamers in several

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detection and quantification sensor platforms.[10-13] The use of synthetic affinity reagents such as aptamers offer multiple advantages over biologically generated antibodies.[9,14,15] (Table 1).

Chemical and Biological Properties

The oligonucleotide structure of aptamers has substantial impact on their properties and can be advantageous. Since their synthesis is carried out using well-established DNA synthesis laboratories, aptamers synthesis quality is maintained without the batch to batch variations expected from antibody synthesis. The synthetic nature of aptamers also allows for greater ease in chemical modifications, allowing them to mimic amino acid side-chains increasing their chemical diversity and affinity to targets that unmodified aptamers show low-affinity.[16] For example, Slow Off-rate Modified Aptamers (SOMAmers)[17] are aptamers where the 5' position of the uridine bases has been replaced with naphthyl, tryptophan, benzyl, or isobutyl groups, adding to the potential functional groups that can bind to the target, and thus increasing both the dissociation time and the binding affinity for proteins.[17,18] The next-generation aptamers also known as X-aptamers are another technology where various functional groups are added to the bases of the oligonucleotide to improve binding and specificity.[19] Aptamers with chemical modifications can be used in different detection platforms which enables the improvement of target-detection levels in complex matrices and permitting the measurement of target concentration.[11]

The nucleic acid composition of aptamers makes them non-immunogenic, unlike antibodies.[20] The non-immunogenic property contributes to their high rate of clearance rate by the kidneys and susceptible to degradation by exonucleases in the bloodstream.[3,21] The pharmacokinetics of aptamers or how aptamers move into, through, and out of the body, can be improved by modifying the natural oligonucleotide with un-natural forms. The use of spiegelmers, which are constructed from D-ribose instead of the L-ribose enantiomer recognised by exonucleases, can greatly extend the half-life of administered aptamers.[22-24] Spiegelmers are synthetized by creating a 'mirror image' of an existing aptamer out of D-ribose.[25,26] The use of non-natural bases can also decrease the degradation and renal clearance of administered aptamers.[27] A common method is to substitute the 2'-hydroxyl group of the pyrimidine bases with fluoro or amino groups, reducing the ability of exonucleases to recognize the nucleobase and thus slowing degradation.[14,28] Thioaptamers, which substitute sulfur with one or both of the non-bridging phosphoryl oxygens in the phosphate backbone of the aptamer, have a higher resistance to nucleases and can be processed by DNA and RNA polymerases. This gives rise to a higher binding affinity to proteins than unmodified oligonucleotides.[29,30] These chemical alterations can significantly increase the half-life of aptamer drugs in the body. However, it should be noted that conjugation of aptamers to carrier molecules can increase their bulkiness and may reduce their binding ability, especially to small molecules.[31,32] Table 2 identifies some of the companies that have reported work on developing aptamer-based therapeutic drugs.

The nucleic acid structure of aptamers allows for the rational design of antidotes.[27] Antibodies and conventional drugs have no systematic method of antidote design, but aptamers can be disabled by the introduction of the antisense strand to the original nucleic acid sequence.[33,34] The antisense oligonucleotide performs Watson-Crick base pairing and disables the aptamer's shape, so the aptamer is no longer capable of binding to the target. This has been a key advantage of aptamers in the design of

Table 1. The functional and chemical differences between antibodies and aptamers are comparable due to their ability to bind target molecules ranging from ions to proteins, all the way up to whole cells, and do so with high specificity and affinity. As shown here, aptamers have several key advantages over antibodies.

Aptamers	Antibodies
Synthetic origin	Biological origin
Size is ~12 – 30 kDa	Size is~150 – 170 kDa
Binding affinity down to pM	Binding affinity down to pM
In vitro and in vivo generation	In vivo generation
Adaptable to a variety of conditions	Restricted to function in biological conditions
Wide range of targets	Limited to targets that can be altered to provoke immune response
Enantiomer specific	Not enantiomer specific
Uniform batch performance	Non-uniform batch performance
Can be modified to improve pharmacokinetics, or how it moves into, through and out of the body.	Limited modification to improve pharmacokinetics
Selection time variable	Selection time long and affects specificity
Can regain function after denaturation	Loss of function after denaturation
Possibility of rational antidote design	No rational method of antidote design
Unlimited shelf life	Limited shelf life
Usually not immunogenic	Frequently immunogenic
Binding site can be modified to change specificity	Binding to target only
Targeted by exonucleases	Not targeted by exonucleases
High renal filtration	Low renal filtration

Table 2. The list of companies that are currently developing aptamers as drugs, their year of founding, and the field they work in.

Company	Established	Field
Antisoma Plc	2001	Aptamer-based cancer therapeutics
Apta Biosciences	2013	Aptamer-based therapeutics, diagnostics
AptaMatrix	2003	Aptamer discovery
Aptamer Sciences Inc	2011	Aptamer discovery
AptaTargets	2014	Aptamer-based therapeutics
Apterna	2011	Aptamers to assist drug delivery
Aptitude Medical Systems Inc	2011	Aptamer-based therapeutics, diagnostics
Archemix	2001	Thrombin-inhibiting aptamers
Centauri Therapeutics	2014	Aptamer-based immunogenic therapeutics
NOXXON Pharma	1997	Aptamer-based therapeutics
IVERIC Bio (formerly Ophthotech)	2007	Aptamer-based eye therapeutics
Ribomic	2003	Aptamer-based therapeutics
Somalogic	2000	Aptamer optimisation, biosensors
Veraptus	2011	Aptamer-based therapeutics for bacteria and viruses

anticoagulation drugs.[35,36] An aptamer can be introduced before or during surgery to stop blood clotting, and the antidote can be introduced as part of the recovery process, resulting in a faster resumption of the clotting process than a conventional drug and a better health outcome for the patient.[37] Anti-coagulation aptamers pegnivacogin and NU172 are currently undergoing drug trialing.[15,38]

Context of Aptamer Drugs

The potential use of aptamers as drugs was first discussed in 1995 due to the similarities between aptamers and antibodies, and the prevalence of antibody-based therapeutics.[3,39,40] It was postulated that, if aptamers could be adapted to be functional in the body, their ability to change the properties or envelop their targets could be beneficial to inhibit diseases.[41] The most popular targets for aptamer drugs were therefore diseases with a singular causative protein that could be inhibited by a suitably designed aptamer. Most notably, age-related macular degeneration, which is caused by the angiogenic VEGF, blood clotting disorders, and cancers were targeted by aptamer drug developers.[42] Aged related macular degeneration was also selected as a popular target due to the immediacy of intravitreal injections, which allows them to bypass issues such as renal clearance that affect aptamer pharmacokinetics.[43] The discovery of aptamers introduced the field of riboswitches and ribozymes, where folded pieces of RNA are capable of catalyzing reactions or altering gene expression.[44] Aptamers can interact with a target to change its structure or enable or disable processes.[45] This is the mechanism of Macugen, the first approved aptamer drug on the market, which targets VEGF and was approved in 2004. It was commonly used until recent years when antibody-based drugs superseded it in effectiveness.[15,38] Additional aptamer-based drugs that are currently in development can be found in Table 3.

Table 3. List of aptamer drugs currently in clinical trials, their developers, their structure, their target, and their progress. Macugen is the only aptamer drug that has been released onto the market.

Name	Developed by	Form	Target	Disease	Progress
Macugen (pegaptanib)	NeXstar Pharmaceuticals	27-nt RNA	VEGF	Age-related macular degeneration	On market
REG-1 (pegnivacogin)	Regado Biosciences Inc	37-nt RNA	Coagulation Factor IXa	Arterial thrombosis	Phase III (Suspended)
Zimura (avacincaptad pegol)	IVERIC Bio	38-nt RNA	Complement Factor 5	Age-related macular degeneration/Stargardt disease	Phase IIb
AS1411	Antisoma Research	26-nt DNA	Nucleolin	Acute myeloid leukemia	Phase II
NOX-E36 (emapticap pegol)	NOXXON Pharma	40-nt RNA	Chemokine CCL2	Type 2 Diabetes Mellitus/ Albuminuria/Liver Cancer	Phase II
NOX-A12 (olaptesed pegol)	NOXXON Pharma	45-nt RNA	CXCL12	Pancreatic, colorectal, brain cancer/Multiple myeloma	Phase II
NU172	Archemix Corp, Nuvelo	26-nt DNA	Thrombin	Heart disease	Phase II
NOX-H94 (lexaptepid pegol)	Pharma	44-nt RNA	Hepcidin	Anemia/renal disease	Phase II
RBM-007	Ribomic	37-nt RNA	Fibroblast Growth Factor 2	Age-related macular degeneration	Phase IIa

Development of Aptamer-Based Drugs

Drug development most commonly begins with target identification. Once a disease or condition has been identified as a possible target for treatment, there are multiple methods that can be used to discover a candidate drug. High-throughput screening is used to test large libraries of potentially therapeutic molecules for efficacy in a disease model.[46,47] Targeted methods use pharmacological principles to identify a potential drug target for the disease and develop an appropriate drug. Aptamers are best suited for targeted drug discovery since the oligonucleotides are developed against a specific molecule. The SELEX process can be modified to target different types of molecules; for example, cell-SELEX can screen libraries of oligonucleotides against a diseased cell, utilizing a healthy cell as a negative control in order to identify novel drug targets.[48] After candidate aptamers have been identified, they are subject to refinement (Figure 1) in order to alter their properties for *in vivo* pharmacology. As previously discussed, the properties of aptamers in the body can be substantially modified in order to increase their effectiveness as clinical drugs. The difficulty of transition from *in vitro* to *in vivo* has historically been one of the primary causes for aptamer drugs to fail during drug trials. Aptamer drugs are significantly less likely than traditional drugs to have issues with toxicity or immune reaction.[49] In contrast, aptamer drugs are instead more likely than comparable antibodies to have issues with efficacy. Future research is likely to focus on chemical alterations of aptamer drugs during the refinement stage in order to carry over *in vitro* efficacy in a biological platform. It will also be essential to improve the pharmacokinetic optimization of aptamer drugs so that the administration of these drugs will not be a limiting factor in clinical success. Macugen (pegaptanib), the aptamer drug that has been brought to market, was successful in administration as it is delivered via an injection directly into the eye, bypassing many pharmacokinetic issues that will need to be addressed as a wider range of diseases are targeted.[50] For example, the aptamer used for Macugen or pegaptanib is conjugated to polyethykene glycol or PEG to increase the intravitreal residence time and inhibiting the activity of the Vascular Endothelial Growth Factor (VEGF) for longer periods.[39]

Aptamers in the Drug Screening Process

The selective binding ability of aptamers makes them suitable for assays and screening applications,[51] and in this capacity, they can be exceedingly useful in the development of non-aptamer drugs. For the initial stage of target identification, aptamer microarrays and SOMAscans can be used to measure gene and protein expression and provide comparative information on diseased and non-diseased expression profiles.[52,53] Aptamers can also be modified with fluorochromes and quenchers in which the binding of the aptamer to its target protein triggers the uncoupling of the fluorochrome and a quencher allowing to track the aptamer activity *in vivo*.[54,55] Most notably, an RNA aptamer (spinach aptamer), was developed to bind the green fluorescence protein (GFP) fluorophore 4-hydroxybenzlidene imidazolinone (HBI), and activate its fluorescence upon binding. The spinach aptamer can be introduced to the cell via vectors or plasmids and expressed for fluorescent visualisation inside the cell.[56] The widely-used enzyme-linked immunosorbent assay or ELISA, which utilises antibodies, can be adapted into an ELONA (enzyme-linked oligonucleotide assay) which allows a greater range of targets and cheaper scaling due to the low synthesis and production costs of aptamers compared to antibodies.[57] The chemical structure of aptamers can also be used to produce aptabeacons, which use the structural change upon target binding to effect a measurable change such as activation of an attached fluorescent molecule.[58] These methods provide a useful toolkit for the identification of potentially novel candidates to be used as drugs.

Another use of aptamers for assays is their incorporation in microarrays. Microarrays are commonly used to identify molecules of interest in a mixed solution. Aptamer-based microarrays could bind to a variety of target molecules such as other oligonucleotides, organic and inorganic compounds, and peptides and proteins while antibody-based arrays are limited to capture larger molecules such as proteins.[59] Upon binding, the capture agent releases some kind of signal, such as a fluorescence, which is read by a high-resolution camera and used to quantify how much binding has occurred. Complementary DNA (cDNA) used in DNA microarrays and antibodies are currently the most popular capture agents, but aptamers are equally suitable for this purpose and offer higher shelf stability, and small molecule recognition.[60] Multiple aptamers can be used in concert

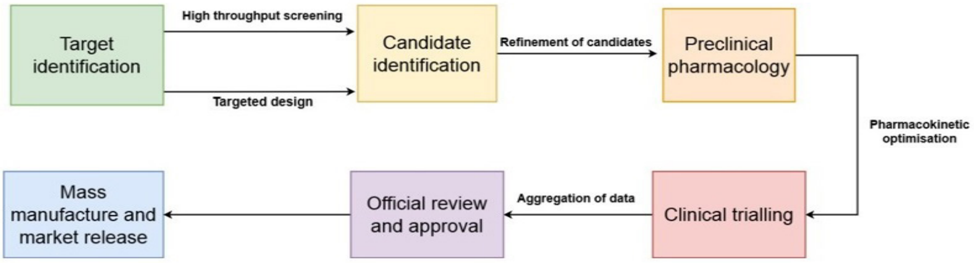

Figure 1. The generic drug development pathway.

with each other to test for multiple molecules whereas an antibody array would suffer from severe cross-reactivity. However, aptamer-based microarrays require significant optimization as the microarray format can interfere with the folding and structure adoption of aptamers when bound to their molecules.[29] Aptamer-based microarrays could be one of the most robust aptamer biosensor platforms and can be of use in all stages of drug development.

Aptamers in Drug Purification

Due to their relative cost-effective synthesis, high-affinity binding to specific targets, and ability to withstand repeated denaturation, aptamers can be utilized in the purification of other drugs. Aptamers have previously been utilized in the purification of the antibody-drug, Avastin, for the purification of the age-based macular degeneration target VEGF, and for the purification of a medley of human proteins from serum using chromatographic methods.[61] Their ability to selectively discriminate between enantiomers of a molecule and reach binding constants as low as the femtomolar are also strongly beneficial features when using aptamers for drug purification.[62] An aptamer produced for the drug of choice can reach yields that approach 100% recovery of the drug when utilized in affinity chromatography through the methods detailed in this research, and it is likely that aptamers will see greater use in the field of drug purification after this success.[63]

Drug Delivery

Site-specific drug delivery has increasingly become an area of focus in pharmacology as treatment methods are refined. For localized diseases such as cancerous tumors, or for drugs with a high level of off-target effects, it is essential to develop methods to ensure that the drugs are delivered to the correct part of the body in order to maximize efficacy and produce the best health outcome for the patients. The most common use of aptamers in the clinical context is in the delivery of drugs, toxins, liposomes, or siRNAs, using their high specificity to locate the target site and reduce off-target effects.[64] Aptamers are well-suited for this purpose as they are simple to manufacture, highly specific for a given target, easily modified, and generally have little to no immunogenicity.

The most notable example of a drug delivered by aptamer is doxorubicin, an anti-cancer drug that is only delivered to cancerous cells due to the aptamer's specific binding to PMSA-positive cells.[15] The versatility of the aptamer structure means that they can be conjugated to a given drug in a variety of different ways in order to reduce the impact that aptamers could have on the efficacy and sterics of the drug. Aptamers are most frequently used for the delivery of anticancer drugs since cancerous cells typically display a unique set of antigens that allows them to be distinguished from healthy cells by aptamers.[65] Small-interfering RNAs (siRNAs), which are a part of the RNA interference pathway, can be delivered to cells using aptamers as a targeting method, and since siRNAs and RNA oligonucleotides are both composed of RNA, the two are easily conjugated together.[38,66] The use of aptamers for drug delivery is likely to increase as the need for targeted drug delivery increases in the future.

Conclusion

Aptamers are currently being utilized in different capacities at all stages of drug development. However, they have yet to be adopted universally into this process and will require additional research in order to reach maximum effectiveness. Aptamers have shown some effectiveness as drugs and have strengths in their low toxicity and easy manufacture. It is clear that the promise of aptamers in the drug development field has yet to be utilized, which paves the way for future discoveries that may have significant impacts on the field.

Author Contributions

All authors contributed equally to this work. (acquisition and interpretation of data and drafting). Dr. Kumar as the correspondence author designed and revised the manuscript. They have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors report no conflicts of interest.

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