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Synthetic Antibodies: *The Emerging Field of Aptamers*

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Introduction

The current global market for aptamers is approximately $99 million annually and is anticipated to increase at an astonishing compound annual growth rate (CAGR) of 106.3% for the next five years resulting in an estimated value of $3.7 billion by the year 2017.[1]

Sometimes referred to as a "synthetic antibody," an aptamer is a nucleic acid or peptide molecule that binds to a target or antigen with high affinity and specificity. Aptamers have a wide range of applications including diagnostics, therapeutics, forensics, and biodefense. To date, hundreds of aptamer sequences have been identified and can now be chemically synthesized in the lab on demand, faster and less expensively, without the traditional issues associated with producing recombinant antibodies. This article will review aptamer technology, its advantages and limitations, as well as highlight a few of its many applications in the life sciences.

About Aptamers

Aptamers are single-stranded DNA or RNA oligonucleotides (short strands of nucleic acids) or peptides that have been engineered through a selection process to exhibit exceptional binding affinity and specificity to their target or antigen. Figure 1 shows the schematic of aptamer production and some of the shapes aptamers possess. Typical aptamer targets include heavy metals, small organics, peptides, proteins, tissues, and organs.

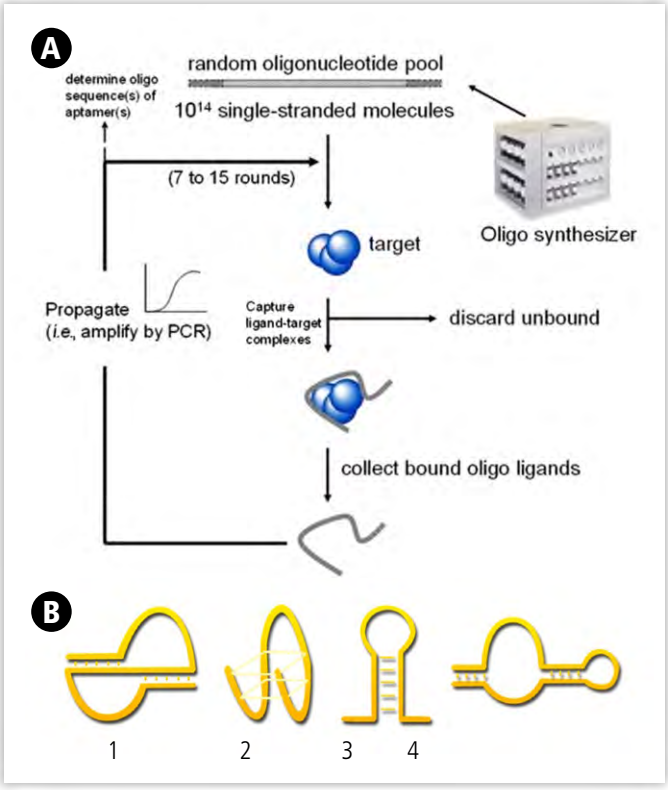

FIGURE 1. Illustration of aptamer production and various aptamer shapes. **(A)** A random library of 10^{13-14} oligonucleotides synthetized and used for selection against a target molecule (*e.g.*, protein). The bound oligonucleotides are collected and amplified using PCR. This selection step is repeated many times, followed by identification of the candidates with a DNA sequencer. **(B)** The conformational shapes of aptamers contribute to their specificity. The following are structural conformations of various aptamers: (1) pseudoknot (ligand for HIV-1 reverse transcriptase); (2) G-quartet (ligand for thrombin); (3) hairpin (ligand for bacteriophage for T4 polymerase); and (4) stem loop/bulge (ligand for adenosine-5′-triphosphate [ATP]).[2]

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In most cases, the aptamer identification process starts with a large, random pool of DNA or RNA oligonucleotides that are systemically subjected to negative and positive rounds of selection. Low-affinity or unspecific binders are filtered out and the remaining aptamers are amplified and used in subsequent rounds of selection. This process was described in 1990 by Tuerk and Gold in their paper, "Systematic Evolution of Ligands by Exponential Enrichment."[3] Their method, universally known as "SELEX", is currently the lab standard for selecting and identifying highly-targeted aptamers.

Once the aptamer sequence has been identified, the aptamer can be chemically synthesized in nanomole to micromole (*i.e.*, mg) quantities using an automated synthesizer. Larger g/kg scales are also possible for mass production of pharmaceuticals or diagnostic kits.[4]

Benefits to Using Aptamers Over Traditional Antibodies

Aptamers offer a wide range of clear-cut advantages over traditional antibodies, at all stages of development, production, and commercial applications.

No Damage to Surrounding Cells

With the ability to bind to a precise target, a wide range of diagnostic and therapeutic applications may soon be enabled without fear of damaging the surrounding cells, similar to the "magic bullet" scenario Paul Ehrlich first described over 100 years ago.[5] Numerous biomedical aptamers are now being studied with applications ranging from diagnostic biomarkers of breast cancer metastasis[6] to therapeutic drug delivery systems for targeting vascular disease.[7]

Higher Binding Affinity

While a variety of ligands may bind to the same receptor site, in many cases, aptamers have been observed to offer a much higher binding affinity to the target than either monoclonal antibodies or lead compounds obtained from random peptide libraries, small molecule libraries, or natural product extracts.[8, 9] In general, the higher the affinity, the less quantity of an antibody or aptamer is needed, which in turn reduces costs and increases profits. The dissociation constants (K_d) of aptamers have been reported to span from the μM to the nM range against various targets, and in some case pM[10] (one trillionth of a mole). Aptamers are also a fraction of the size of antibodies and therefore have greater capability to enter cellular compartments to bind to their target.[11]

Target Specificity

Aptamers are capable of excellent target discrimination. Aptamers have been generated that exhibit greater than 10,000-fold binding affinity for theophylline over caffeine, which differ from one another in structure by only a single methyl group. Antibodies have cross-reactivity or "false positive" issues.[12]

Discovery of Unknown Biomarkers

One of the perks of using aptamers is that it is unnecessary to actually know where the aptamer binds. Often, researchers spend several years studying a disease and how it functions before they can proceed to drug discovery. Aptamers, on the other hand, allow researchers to discover drugs without knowing the precise biomarkers or epitopes themselves. Various SELEX techniques can be used to identify unknown biomarkers. Disease discovery and therapeutics can therefore be developed quickly, potentially shaving years and millions of dollars off the development cost. Cell-SELEX can be performed to find unknown and yet-to-be-discovered targets on the surface or inside the cell of interest, or for discovering unknown targets specific to pathogenic organisms. *In vivo* SELEX has also been developed to identify unique biomarkers by performing selection in the chosen animal model.[13] Complex target SELEX can be performed on protein mixtures without requiring specific knowledge about the target.[14]

Additional Discovery Time and Cost Savings

Once the aptamer sequence has been established, automated synthesizers enable on-demand production of chemically-manufactured aptamers at a fraction of the cost of purchasing their biologically-produced antibody equivalent. For example, Aptagen, one of a handful of manufacturers worldwide offering custom aptamers, offers an ever-growing catalog of over 300 aptamers with prices ranging from less than $1–4 per μg, depending upon the design and purification level. Price/g at commercial scale decreases—typically less than $300/g (in mg volumes), and under $50/g (in g quantities). Manufacturing can be completed in as little as two days to two weeks as compared to three months for human monoclonal antibodies at an average cost of about $300/g.[15, 16]

After an aptamer sequence has been identified for a particular target, modifications can easily be made to improve the biological half-life or add extra functionality

through the addition of an inverted thymidine, polyethylene glycol (PEG), amino group, 2′-O-methyl, biotin, or fluorescent tags.

Minimized Batch-to-Batch Variation

Because aptamer sequences can be chemically synthesized, batch-to-batch variation is minimized. Furthermore, the chemical nature of the process eliminates many of the purification and toxicity issues typically associated with production of biologically-manufactured recombinant antibodies. The aptamer production process is also less prone to bacterial and viral contamination, which can be a concern during antibody production.[17]

In Vitro vs. In Vivo Testing Advantages

It is commonly understood that the failure rate for biologically-produced recombinant antibodies is high. For every one antibody-based drug that makes it to market, hundreds more fail during animal ADMET studies (absorption, distribution, metabolism, excretion, and toxicity), despite promising *in vitro* results. On average, antibody production takes seven to fifteen years from the lab bench to pharmacy shelves, and costs $4 billion in research and development, with the average failure rate as high as 80%. Half of this time and approximately 70% of associated biopharmaceutical R&D costs are spent on *in vitro* development.

By comparison, aptamers follow an *in vivo* testing model (directly testing the aptamer in the animal), thus avoiding the majority of the bench-testing process, saving several years and approximately 35% of the R&D cost. Some characteristics of aptamers, which make them suitable for clinical application, include the lack of immunogenicity, low toxicity, and high target specificity, avoiding off-target effects.[18]

Reduced Side Effects

While many aptamers have high specificity to their target, some have been discovered with affinities within the pM range. The high degree of specificity decreases the chance of adverse side effects and complications that often arise with the use of traditional pharmaceuticals. The non-immunogenic materials and minimal batch-to-batch variation further contribute to a reduced chance of inducing side effects.

Stable Product, Easy Storage

Aptamers have significantly better storage properties than antibodies. While antibodies require storage in a frozen state and have a limited 3–6 month shelf life once thawed, aptamers require no such special handling. Once dried down, aptamers can be stored at ambient temperature indefinitely. When resuspended within a buffer solution, they can safely be stored in a −20 °C freezer. Heat is not a problem when working with aptamers, and they may be denatured and renatured multiple times, unlike antibodies.

Limitations

Some of the limitations aptamers may face include the rapid clearance rate from circulation due to their small size and degradation by nucleases for the unmodified aptamers. However, these limitations can be easily resolved by chemically modifying the aptamers. Polymers such as PEG or lipids such as cholesterol can be conjugated to the aptamers to enhance aptamer circulation time. Modified nucleotides containing altered base, sugar, and internucleotide linkage groups can be used in the aptamer synthesis to increase resistance against nucleases. The strategies for producing chemically-modified aptamers are well-established and can be scaled-up for commercial manufacturing.[17]

Aptamers in Commercial Use

Therapeutics

In December 2004, less than 15 years after the conception of aptamers, Macugen®[19] became the first therapeutic aptamer to make it to market. This FDA-approved drug owned by EyeTech Inc. treats age-related macular degradation — an eye disease that affects the center of the visual field. Macugen is an anti-VEGF (vascular endothelial growth factor) aptamer conjugated to a PEG polymer for enhanced circulation time. While its rapid development and approval represented a huge step forward for the aptamer industry, Macugen remains the only aptamer-based therapeutic currently in use. However, there are many aptamers being investigated for clinical use. In 2012, there were nine DNA and RNA aptamers in the clinical pipeline.[18]

Diagnostics

Aptamers have enormous applications in diagnostics, especially in biomedical diagnostics for detection of disease biomarkers. Diagnostic-based aptamer kits have been developed for detecting mycotoxins and aflatoxins.[20] One very interesting technology developed by Aptagen is the aptamer-beacon (*i.e.,* Apta-beacon™) technology which can be used for rapid biomarker detection. This method of detecting analytes eliminates the need for multiple washes and lengthy incubation periods while also

increasing detection sensitivity. Apta-beacons have great potential as a point-of-care system for enhanced patient treatment.[2] Due to the rapid pace of advancements in aptamer research, many aptamer-based diagnostics will undoubtedly enter the market in the near future.

Other Applications Being Studied

Hundreds of aptamer-related experiments are now underway and/or have been recently reported in literature, covering a wide variety of fields. To give you a feel for some of the wide-ranging applications for aptamers, consider:

- Probes designed to detect trace amounts of mercury in water.[21]
- Rapid detection systems for *E. coli.*[22]
- Visualization of latent fingerprints in forensics.[23]
- Chemotherapy drug delivery systems for targeting pancreatic cancer cells.[24]

Wrap-Up

Aptamers are emerging as a key player in numerous applications such as drug discovery and therapeutics development, in addition to biodefense, food science, and more.

In biomedical applications, the unique chemistry of synthesized aptamers permits the selection of drug candidates in whole animal models, bypassing the test tube entirely. By using an animal model with the disease state of interest, one need not possess specific knowledge of the pathology or disease condition in question. As an added benefit, because this approach reduces the false starts, there are actually fewer animals needed for drug evaluation prior to the human clinical trial phase. The promise for these tools is great, and with the market expected to grow exponentially over the next few years, if you're not yet using aptamers in your studies, perhaps now is the time to start.

NOTE

Aptagen is a biotechnology company offering aptamer products and services as research reagents, diagnostic and biomarker discovery tools, as well as for use in drug discovery and targeted delivery for therapeutics, and bioindustrial applications.

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