Perspective: Aptamers as Drugs

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ABSTRACT Aptamers are nucleic acid molecules, either single-stranded RNA or DNA, that form secondary structures capable of eliciting affinities to a variety of targets ranging from small organics to whole proteins. They can be quickly developed and are relatively inexpensive to manufacture. Aptamers have the potential to be used as novel drug products, but lacks general acceptance within the pharmaceutical industry. Aptamers do not fall into the familiar classification of small organics and historical limitations of aptamer technology have placed the continued development of this field on the sidelines. However, new advances in aptamer chemistry and development promise to set aside doubts as to whether aptamers are a viable drug technology platform.

One of the most common reagents in biological research laboratories, considered an indispensable tool for research, is the oligonucleotide, which is a short, single-stranded nucleic acid molecule (e.g., DNA or RNA). Typically, oligonucleotides are used for antisense research to block gene expression[1], as primers for PCR to amplify minute quantities of DNA[2], and as components of microarray chips to analyze complex genetic profiles[3,4]. Most recently, small interfering double-stranded RNAs (siRNAs) are gaining in popularity as a therapeutic means to silence gene expression[5]. Familiarity with these oligonucleotide uses has stereotyped these biopolymers as being limited to a certain class of applications, involving Watson-Crick base-pairing. However, oligonucleotides have also been observed to exhibit intriguing functionalities that are completely different from and unrelated to their abilities to hybridize. In particular, libraries of randomized oligonucleotides have been shown to contain oligonucleotide ligands that bind tightly to various target compounds other than nucleic acid molecules having complementary sequences, revealing that oligonucleotides can have binding activities beyond those specified by Watson-Crick base-pairing[6-9].

RNA and single-stranded DNA biopolymer molecules can form a great diversity of structures by exploiting secondary and tertiary interactions, including nonstandard base-pairs, hairpin loops, bulges, multistem junctions, pseudoknots, and four-stranded G-quartet structures. Such biopolymers have structural specificity based on their shapes, and can be engineered to bind to many different compounds, proteins, and molecules. The affinity is imparted by the interplay between structural interactions of the biopolymer with its target, and is similar to the binding of an antibody to a corresponding antigen. For the past fifteen years, a variety of these tight binding nucleic acid ligands, which are generally known as "aptamers," have been developed and touted as an attractive alternative to conventional antibodies. One reason for the tremendous interest generated by aptamers is the practical advantages of aptamers over antibodies. In cell culture experiments and animal studies, for example, it has been demonstrated that aptamers exhibit neither intrinsic toxicity nor immunogenicity[10,11]. Further, there is no need to "humanize" an aptamer. Moreover, unlike the time and costs associated with producing protein-based antibodies, aptamers can be synthesized quickly and inexpensively using automated oligonucleotide synthesizers, as most tight-binding aptamers normally range from 25-80 nucleotides in length, well within the capacities of oligonucleotide synthesizers.

Aptamer Affinity

A growing body of literature supports the fact that aptamers exhibit high affinity binding to their cognate targets, and reinforces the notion that an aptamer can potentially be developed against virtually any target. There are a variety of reports of aptamers that exhibit binding affinities from micromolar to nanomolar ranges against various targets[12]. In many cases, the binding affinities are observed to be much greater than those of monoclonal antibodies, lead compounds obtained from random peptide libraries, small molecule libraries, and natural product extracts[12,13]. The selective binding affinity towards targets arises from complex interactive forces characteristic of the nucleotides. Specific interactions such as hydrogen bonding and phosphate group associations dictate the sequence-specific, 3-dimensional structures of the aptamer oligonucleotide ligands, providing a rigid scaffold for the arrangement of aptamer chemical surfaces for target interaction, and lowering the entropic cost of binding as compared to an induced fit interaction[14,15].

Aptamer Targets

Aptamers have been generated without difficulty that bind to organic dyes, drugs, amino acids, nucleotides such as ATP, vitamins, pharmacologically important proteins such as substance P[16], the anticoagulant thrombin[17], growth factors, proteases, and several other small and large proteins and enzymes[7,13,18]. Synthetic combinatorial methods have yielded a DNA ligand that inhibits infection by HIV in vitro[15] and a high affinity RNA ligand that inhibits infection by the Human Rhinovirus 14 for the common cold[19]. Such methods have also been used to generate antagonists of blood clotting and angiogenesis in thrombus clot growth[13]. Some aptamers that have been isolated exhibit stereoselectivity[20]. In addition, 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[12,15]. Another in vitro selection experiment generated an aptamer that binds to D-tryptophan with 670-fold greater affinity than to its counterpart L-tryptophan[15]. The first aptamer tested in animals was a DNA aptamer that efficiently blocks the proteolytic activity of thrombin. In a canine cardiopulmonary bypass model, this aptamer led to rapid anticoagulation and successfully replaced heparin[21]. More recently, an RNA aptamer has been described that reversibly antagonizes the blood clotting activity of coagulation factor IXa[22].

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Aptamer Delivery Agents

With the wide variety of targeting capability of aptamers, it was soon realized that these ligands can be used as carriers for known drugs to a therapeutic site of interest. For example, cancer cells were targeted by conjugating an aptamer to doxorubicin[23]. The aptamer binds to a membrane-specific antigen and doxorubicin is a well-known cancer drug. Doxorubicin is an effective drug in destroying cancer cells; however, the drug is associated with dose-dependent cardiotoxicity. By combining the membrane-specific aptamer to the cancer destroying ability of doxorubicin, the conjugate becomes a much more effective therapeutic. Other studies have extended this concept to target tumors in vivo using aptamer conjugates of radioisotopes[24], cytotoxic agents, or nanoparticles[25]. Aptamers were also enlisted for siRNA delivery to down regulate gene expression[26,27].

Aptamer Drugs on the Market

To date, the only aptamer clinically approved as a drug and on the market is Pegaptanib (Macugen), an anti-VEGF aptamer for the treatment of age-related macular degeneration[28].

Limitations

Even with all the acclaim for aptamers, all nucleic acid biopolymers, including aptamers, are limited by the diversity of the four member genetic alphabet of which they are made: adenine, guanine, thymine/uracil, and cytosine. The binding interactions and chemical reactions of aptamers are constrained by these four functional groups. Thus, even considering the results achieved using traditional drug discovery methods, as well as improvements provided by aptamer technology, there remains a great need for new, effective, and safe drugs to treat a wide variety of medical diseases and conditions, as well as improved methods for identifying such drugs.

Advances

The ability to expand the functional groups available to aptamers through the incorporation of a variety of chemical moieties could potentially open up new and improved chemistry and binding interactions. For instance, Famulok and his colleagues found that certain DNA polymerases are able to tolerate chemically modified nucleotides[29]. They were able to enzymatically incorporate a variety of different functional groups, i.e. acidic, basic, or lipophilic, into a growing oligonucleotide chain. However, again, there exists a limitation on the number of unique dNTPs which can be incorporated into the growing oligonucleotide chain. The functional equivalent of up to a four member genetic alphabet can only be tested at any one time. Recently, in an effort to expand the genetic alphabet, Hirao, et al[30] have generated two unnatural bases which can be incorporated by DNA polymerase and transcribed by RNA polymerase. In effect, they have extended the genetic alphabet from just two base pairs to three. Another approach developed by NuAce, Inc. (Rehovot, Israel) uses the recently discovered ability of DNA polymerases to incorporate dinucleotides into a growing oligonucleotide chain[31]. By functionalizing the dinucleotides, i.e. AA, AG, AC, AT, GA, GG, GC, GT, CA, CG, CC, CT, TA, TG, TC, TT, each with a unique functional group the effective genetic alphabet increases to sixteen (4 X 4 =16).

The ability to expand even further the functional genetic alphabet available to aptamers has been recently introduced by Aptagen, LLC (Jacobus, PA). A new class of molecules, called aptabodies™, is postulated to have a genetic alphabet orders of magnitude greater than other chemical methods[32].

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