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Review

SELEX methods on the road to protein targeting with nucleic acid aptamers

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ABSTRACT

Systematic evolution of ligand by exponential enrichment (SELEX) is an efficient method used to isolate high-affinity single stranded oligonucleotides from a large random sequence pool. These SELEX-derived oligonucleotides named aptamer, can be selected against a broad spectrum of target molecules including proteins, cells, microorganisms and chemical compounds. Like antibodies, aptamers have a great potential in interacting with and binding to their targets through structural recognition and are therefore called “chemical antibodies”. However, aptamers offer advantages over antibodies including smaller size, better tissue penetration, higher thermal stability, lower immunogenicity, easier production, lower cost of synthesis and facilitated conjugation or modification with different functional moieties. Thus, aptamers represent an attractive substitution for protein antibodies in the fields of biomarker discovery, diagnosis, imaging and targeted therapy. Enormous interest in aptamer technology triggered the development of SELEX that has underwent numerous modifications since its introduction in 1990. This review will discuss the recent advances in SELEX methods and their advantages and limitations. Aptamer applications are also briefly outlined in this review.

Contents

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1. Introduction to SELEX techniques

Aptamers are short single-stranded DNA (ssDNA) or RNA sequences generated by an *in vitro* process called Systematic Evolution of Ligands by EXponential enrichment (SELEX). The term aptamer is derived from the Latin words *aptus* and *meros* (meaning to fit and particle, respectively). These molecules can bind to a broad spectrum of target molecules such as proteins [1], peptides [2], nucleotides [3] antibiotics [4], toxins [5] and small molecules [6] with high affinity and specificity. The SELEX process was first developed by three independent groups in 1990 to isolate RNA sequences that could specifically bind to target molecules [7–9]. The conventional SELEX contains the repetitive cycles of incubation, binding, partitioning and amplification steps. A random pool of oligonucleotide sequences (initiating library) containing 10^{14} to 10^{15} different sequences is incubated with target molecules. During incubation, some of the sequences are bound to the target molecules whereas a number of sequences are weakly bound or do not interact. Partitioning is a step wherein bound sequences are isolated from weakly bound or unbound ones. The eluted oligonucleotides are amplified by polymerase chain reaction (PCR) (in case of DNA sequences) or reverse transcription PCR (RT-PCR) in case of RNA sequences) to enrich the library [10,11]. There are several methods to generate ssDNA from the resulting double-stranded DNA (dsDNA) including asymmetric PCR [12], denaturing urea-polyacrylamide gel [13], lambda exonuclease and T7 Gene 6 exonuclease digestion [14,15], and magnetic separation with streptavidin-coated beads [16]. The obtained ssDNAs are then used for the next round of selection. In most cases, the isolated aptamers *via* SELEX possess high affinity and low dissociation constant (K_D), ranging from micromolar to nanomolar. Therefore, aptamers are known as chemical antibodies. In contrast to antibodies, the aptamers are non-immunogenic, a feature that makes them superior to antibodies and attractive alternatives for various applications [17]. A number of successful therapeutic aptamers commonly used in clinic or clinical trials has been listed in Table 1. Among them, Pegaptanib, as a vascular endothelial growth factor (VEGF)-binding RNA aptamer approved by Food and Drug Administration (FDA), is used in the treatment of age-related macular degeneration (AMD) [18]. In addition to therapeutic applications, aptamers are applicable for many other purposes including diagnosis of microbial infections [19], disease biomarkers discovery [20], protein detection in Western blot [21], surface plasmon resonance (SPR) assays [22], microarrays [23] and biosensors [24]. Since SELEX invention, many changes have been made in this process to improve the efficacy and shorten the duration of SELEX from several days to several hours. In this review, we describe several types of oligonucleotide pool used as SELEX library and modifications of nucleic acid aptamers to increase their resistance to nuclease degradation and renal filtration. Then, some SELEX methods from the early conventional methods to the most sophisticated techniques are briefly explained. At the end, we will provide the audience with aptamer applications in a wide variety of fields, ranging from biomarker discovery, diagnosis, imaging to therapy.

2. Oligonucleotide pool design

The emergence and development of SELEX technology, especially during its first decade, was characterized by a dominance of RNA aptamers [25,26]. The inclination to RNA aptamers was likely because of the common opinion that only RNA molecules could form functional spatial structures [27]. Before long, it was demonstrated that ssDNA molecules are capable of folding into target-binding structures as well [28]. From the beginning of SELEX era to 2007, about 70% of all studies were associated with RNA aptamers. Since then, DNA aptamers have occupied 70% of SELEX studies [26]. Nevertheless, the isolated DNA and RNA aptamers against a number of small-molecule targets indicated similar affinities [29]. Therefore, both ssDNA and RNA library are applied in SELEX experiments.

The design of a suitable oligonucleotide pool (library) is the first step of a successful SELEX process. Oligonucleotide library used for SELEX process mainly contains a central region with a random sequence flanked by two regions with fixed sequences. The fixed sequences act as primer binding sites (PBSs) during amplification steps. By standard methods of single-stranded oligonucleotide synthesis, the ssDNA library is obtained. Synthesizing the random region is achieved by adding the mixture of all four deoxyribonucleotide derivatives to the reaction mixture. In the case of RNA library, the promoter sequence for T7 RNA polymerase is introduced into the 5′-end of ssDNA library. Double-stranded DNA is generated by PCR and *in vitro* transcription is performed to obtain RNA library. Different types of library used for aptamer selection are as follows: 1) Classical library: this type of library usually contains sequences