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APTAMERS AND THEIR USE IN BIOLOGY AND MEDICINE APTAMERS AND THEIR APPLICATIONS IN NANOTECHNOLOGIES, VIROLOGY AND BIOLOGY

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ABSTRACT

Aptamers are single-stranded DNA or RNA molecules that have a specific spatial structure and, due to this, are able to recognize other molecules or even exhibit catalytic activity. Note that such spatial structures can only be formed by single-stranded DNA or RNA, since their double-stranded forms have a double helix structure regardless of sequence. The name aptamer comes from the Latin aptus, meaning suitable.

Keywords: aptamers, use, biology, medicine, applications, nanotechnologies, virology, biology.

SELEX Technology

To obtain aptamers with desired properties, the SELEX technology (systematic evolution of ligands by exponential enrichment) was proposed [1, 2]. This method uses a library of oligonucleotides as a starting substance, in which each oligonucleotide has common 3' and 5' flanks of 17–25 nucleotides in length, and the middle regions of 20–60 nucleotides in length are unique. Such a library can be synthesized as a single preparation of the oligonucleotide, in which each of the four nucleotides is included with equal probability in the middle part at each position. This preparation is incubated with the target molecule, which is usually fixed on some solid support. Oligonucleotides that do not bind to the target are removed, and those that bind are amplified by PCR

using the common 3' and 5' flanks. This cycle is repeated several times, resulting in enrichment with sequences that have affinity for the target molecule.

Finally, aptamer molecules are cloned as part of plasmids and individually tested for their properties. This technology makes it possible to obtain aptamers from two weeks to several months. The experience of using SELEX has shown that aptamers can be obtained for almost any target: proteins, polysaccharides, small organic molecules, viruses, and whole cells.

It should be noted that the oligonucleotide libraries used in SELEX do not contain the full range of DNA or RNA molecules. Typically, such libraries contain about 10–8–10–10 M oligonucleotides, or 1014–1016 molecules, which corresponds to the number of oligonucleotide variants with a length of 25 (425 ≈ 1015). Thus, libraries with a random district longer than 25 are severely underrepresented. This opens up the possibility of further improvement of aptamers by mutagenic SELEX. This procedure differs from conventional SELEX only in that in each cycle of aptamer propagation, not conventional, but mutagenic PCR is used. Thus, it is possible to “fine-tune” aptamers and increase their affinity or specificity.

Aptamers can be considered as analogues of monoclonal antibodies. However, they have a number of important advantages over antibodies. Their production is much easier, cheaper and faster than the production of monoclonal antibodies. They are much smaller and therefore more easily penetrate into tissues and cells, and may have higher affinity and specificity.

Aptamers can be used in the following research, diagnostic and therapeutic tasks:

1. For the detection of various target molecules, both in scientific and diagnostic tasks. They can replace antibodies in Western blotting, in situ fluorescence hybridization and in ELISA.
2. A promising format for diagnostics is the creation of chips with many aptamers and the possibility of simultaneous detection of many proteins.
3. For affinity purification of target molecules.
4. For effective and specific inhibition of target proteins. Such inhibition can be used both for research purposes and for the creation of new drugs. Some of these drugs are already in clinical trials.
5. A promising direction for the use of aptamers is directed drug transport. Aptamers in this case determine the targeting of delivery (targeting ligands).

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Regulated Aptamers and Aptazymes

In addition to simple binding to a target, aptamers can also have more complex functions, such as controllable or catalytic properties.

For many research purposes, as well as in therapeutic applications, it can be useful to have aptamers whose binding to the target is amenable to regulation. Such a regulator can be an additional molecule that binds to the aptamer and changes its affinity for the target. To obtain such aptamers, the SELEX procedure is modified as follows. If an additional molecule should disrupt the binding of the aptamer to the target, then in each cycle the binding is performed as in the standard procedure, and the aptamer is eluted by adding an effector molecule. If an additional molecule is a binding condition, then the binding is carried out in the presence of this molecule, and the elution is carried out with a buffer that does not contain it. For example, in the selection of aptamers to formamidepyrimidine glycosylase (a DNA repair enzyme), the bound aptamers were eluted with neomycin [3]. As a result, binding of the resulting aptamers to the target was terminated in the presence of neomycin.

The SELEX method also makes it possible to obtain aptamers with catalytic activity dependent on target binding. Such aptamers are called aptazimes [4, 5]. The oligonucleotide library used to obtain Aptazyme initially contains some characterized ribozyme. For example, the hammerhead ribozyme, which can cleave its own sequence. There is also a random sequence in the library. In the course of selection, those molecules are selected that do not have catalytic activity in the absence of a target, and acquire such activity in its presence. Some molecules from this library will undergo self-cleavage initially; therefore, only full-sized molecules are selected for the SELEX cycle. They are incubated in the presence of the target and the cleaved molecules are collected. For the next cycles of SELEX, a constant cleavage moiety is attached to these molecules.

Aptazymes can be used, for example, for gene regulation in vivo. Thus, the sequence of aptazim regulated by theophylline was placed in the 5'-untranslated part of the mRNA. When theophylline was added to cells expressing such mRNA, aptazim was activated, this mRNA was cleaved, and its expression was terminated [6, 7].

Aptamers in virology

Aptamers can be used as antiviral agents. A convenient target in this case is the interaction of the virus with the host, although aptamers can be used to inhibit other stages of the virus life cycle.

Aptamers to HIV glycoprotein 120 (gp120) that compete with the HIV co-receptor CCR5 have been selected to inhibit entry of the HIV virus into helper T cells. Their binding to a highly conserved region of the gp120 molecule makes it possible to neutralize many different HIV isolates, disrupting the gp120-CCR5 interaction [8, 9]. This aptamer was used as the basis for chimeric bipotent particles containing also small inhibitory RNA (siRNA), which was achieved by packaging in the shell of the phi29 phage. This siRNA inhibited expression of the gp120 target.

Viral RNA-dependent RNA polymerases are often chosen as targets for aptamers, since such transcription is uncharacteristic for host cells. In the case of the HIV virus, inhibition of viral RNA polymerase by aptamers is a very attractive alternative to the currently used nucleotide-modified drugs azidothymidine and dideoxyinosine. Unlike such nucleotides, aptamers do not produce noticeable harmful side effects.

The described effects make aptamers a very promising tool for combating viral infections.

Use of aptamers in nanotechnology

One of the most promising applications of aptamers is associated with the development of nanotechnological approaches to the diagnosis and treatment of diseases. The small size of aptamers makes them especially suitable for nanotechnological applications, since the ability of molecules and particles to penetrate tissues greatly increases with decreasing size. Since aptamers are synthesized chemically, unlike antibodies, this allows adding various reactive groups to their ends, which can be used for covalent attachment to nanoparticles. Such particles can carry drug molecules that require targeted delivery to specific cell types. Aptamers can effectively provide targeted delivery.

The first example of this kind was the A10 aptamer, which recognizes prostate-specific membrane antigen (PSMA), a transmembrane protein that is overexpressed in prostate cancer. This aptamer was conjugated with drug-containing nanoparticles and was able to effectively deliver them to cancer cells [10]. In a mouse model of prostate cancer, a single injection of this conjugate into the tumor significantly reduced its size over 109 days of observation [11]. Aptamer-toxin conjugates have been investigated as anti-cancer drugs. Aptamer A9 was conjugated to gelonin, a ribosomal toxin that causes cell death by breaking a specific glycosidic bond in ribosomal RNA and disrupting protein synthesis. The ability of these conjugates to induce cell death was 600 times higher in

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relation to cells expressing PSMA, and toxicity to normal (non-target) cells was significantly reduced [10].

The promise of RNAi-based therapy has already been shown in preclinical trials. However, the problem of delivery of small interfering miRNAs to target cells remains an obstacle [13]. Using aptamers to the PSMA cancer-associated antigen, it was possible to create aptamer-siRNA conjugates for efficient delivery of siRNA to cancer cells. Based on the A10 aptamer to PSMA, its hybrids with miRNAs were created. These miRNAs were directed against genes important for tumor proliferation. When these hybrids entered the target cells, the siRNAs were automatically cleaved from the hybrid by the action of the Dicer enzyme. In this way, in a mouse model of prostate cancer, it was possible to achieve inhibition of tumor growth and regression [11].

Aptamers to amyloidogenic proteins and amyloids

One of the possibilities offered by the SELEX method is the production of aptamers that recognize different structural states of the same protein. At the same time, aptamers that recognize amyloidogenic proteins and recognize amyloid fibrils differ both in specificity and potential applications. Thus, RNA and DNA aptamers to the soluble form of the PrP prion protein were obtained [12, 13]. It has been shown that in vitro the RNA aptamer to human PrP blocks its transition to the pathogenic PrPSc form [15], which opens up fundamental possibilities for developing approaches to the treatment of prion diseases. Aptamers to the soluble form of other amyloidogenic proteins can be used similarly.

Alternatively, aptamers may specifically recognize amyloid and prion fibrils, but not interact with the monomeric form of the protein constituting these fibrils. The presence of an additional condition, non-interaction with the monomeric form of the protein, somewhat complicates the SELEX cycle. Thus, Wang et al. [16] first performed “subtraction”, i.e., the removal of oligonucleotides interacting with the protein monomer, in this case, the normal cellular form of PrPC; and then linked the remaining oligonucleotides to the target, the PrPSc prion. In the work of Surina et al. [13] used a more original approach: oligonucleotides were bound to a mixture of Sup35 yeast prionogenic protein amyloids and Sup35 monomers, and then the mixture was separated by centrifugation. In this case, aptamers, together with amyloids, fell into the sediment and then into the next selection cycle. The aptamers obtained in these studies actually recognized only the fibrillar form of target proteins.

However, interestingly, many of the resulting aptamers were also able to bind to amyloids of other proteins. In particular, all aptamers to fibrils of the Sup35 protein also bound to PrP protein amyloids, but not to PrP monomers [14]. In addition, some aptamers to Sup35 also recognized fibrils of the Rnq1 protein and polyglutamine. The polyspecificity of aptamers to amyloids was also found in other works. Thus, RNA aptamers to the amyloid form of β2-microglobulin also bound to lysozyme amyloids, although they did not bind to some other amyloids [16]; and DNA aptamers to α-synuclein associated with Parkinson's disease also interacted with amyloid β associated with Alzheimer's disease [15]. Such polyspecificity indicates the presence of common structural features in different amyloids. The most obvious application for amyloid aptamers is in the diagnosis of prion and amyloid diseases.

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