

DNA and RNA oligonucleotides as a new class of drugs

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Abstract

Aptamers are short synthetic single-stranded DNA or RNA oligonucleotides forming unique 3-D conformation. They are able to bind and neutralize their target molecules with high specificity and affinity. Therefore, they may specifically interfere in vivo with some protein function and thus display drug-like properties. This paper provides a brief description of possible clinical application of aptamers arguing for their tremendous therapeutic possibilities.

Key words: aptamer; DNA, RNA, cancer therapy, ocular diseases, viral diseases, circulations disorders.

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Introduction

Aptamers are short synthetic single-stranded DNA or RNA oligonucleotides which owing to unique 3-D conformation display an ability to bind in a specific complementary manner some target molecules such as proteins, lipids, nucleotides, carbohydrates, viruses, drugs, including toxins or nonimmunogenic antigens [1].

In early 80s Gold and collaborators from Colorado University (Boulder) developed a method of aptamers synthesis called SELEX (Systematic Evolution of Ligands by Exponential Enrichment), based on combinatorial nucleic acid selection and amplification [2]. Formation of a large combinatorial library containing up to 10^{15} random ssDNA or RNA sequences is a starting point of this technology. Central region of these sequences consists of 40-60 random nucleotides and is flanked by known primer sites for subsequent transcription, reverse transcription and amplification. Using PCR method the start library is amplified and, in case of RNA aptamers, transcribed to RNA start pool. Library is then incubated with the target molecule, e.g. some protein. Weakly bound or unbound sequences are removed from the reaction mixtures. Only molecules which bind to the target molecule with high affinity are enzymatically amplified. Such enriched pool of sequences is a source for the next selection and ampli-

fication cycle. Usually 25 cycles are needed to isolate sequences with highest binding affinity. Selected ligands are then sequenced and evaluated for their affinity to the targeted protein and their ability to inhibit the activity of the targeted protein *in vitro*.

Aptamers properties

Aptamers are able to display a great variety of three-dimensional structures specific for a variety of target molecules [1, 3]. Apart from their high specificity, aptamers are characterized by high affinity to target molecule, with typical dissociation constant at pico- to nanomolar range [3]. The mode of biological action of aptamers is inhibition with a high degree of target molecule function. Therefore, aptamers are often regarded as oligonucleotide analogs of antibodies [4]. Moreover, they display many attributes that make them superior to antibodies for therapy and diagnosis use. Their production is much faster and does not require laboratory animal use. Inhibitory potential of aptamers is much higher than antibodies. They are non-immunogenic, non-toxic and have faster tissue penetration. On the contrary to antibodies, they can achieve even intracellular targets. Resistance to denaturation and easiness to copy are also the features speaking well for aptamers predominance comparing to antibodies.

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Aptamers structure can be chemically modified enhancing their pharmacokinetic properties and stability against nucleases attack. The resistance to nucleases is dramatically increased by substituting the 2'-hydroxy-group with 2'-amino-deoxy, 2'-fluorodeoxy or 2'-methoxy modification [1]. Additional group can be inserted also at 3' or 5' end as well as inside the structure of nucleic acid of aptamer. Fluorescence dyes (*e.g.* rhodamine), affinity tags (*e.g.* biotin), reactive groups (*e.g.* thiols, amine) are examples of possible chemical modifications. Such modifications do not lead to aptamer function deprivation. On the contrary, they qualify them as diagnostic reagents, affinity matrices, and therapeutics. Furthermore, aptamers can act as small molecule tools to validate the function of extracellular targets and can be used in drug discovery fields.

During last years a lot of studies were published to show a broad possibilities of using aptamers in therapy. The first drug-aptamer was introduced to a clinical practice at 2006 [5]. Several other aptamers are currently under clinical investigations.

Aptamers in age-related macular degeneration

Age-related macular degeneration is a chronic, progressive disease occurring mainly in patients of the age over 55 years and leading often to irreversible blindness. The number of new AMD patients permanently increase, especially in rich highly developed countries [5].

Etiopathogenesis of neovascular AMD includes anoxia of the deep eye layers which results in enhanced production of Vascular Endothelial Growth Factor (VEGF) by retinal epithelium. VEGF generates abnormal growth of blood vessels under the macula and increases their permeability [5, 6]. When untreated, bleeding, leaking and scarring from these blood vessels eventually cause irreversible damage to the photoreceptors and supporting cells.

Pegaptanib (Macugen, RNA aptamer) is the first drug that positively passed through preclinical and clinical studies and was approved for human use in all types of neovascular AMD therapy by European Medicines Agency (EMA) [7]. Pegaptanib has a chemically modified molecule structure by polyethylene glycol (PEG) linking that ensure a long half-time of the drug. Pegaptanib binds VEGF165, an isoform primarily responsible for promoting the blood vessels growth and leakage in AMD with high specificity and affinity (Kd 200 pmol) thus leading to specific inhibition and diminished release of this cytokine. The drug is applied every 6 weeks by intravitreal injections at the dose of 0.3 mg/eye. Significant improvements in visual acuity as compared with the control group were seen at the doses 0.3 and 1 mg. The number of severe vision loss in pegaptanib group was significantly diminished number [5, 7]. The drug was reported to be

nonimmunogenic, nontoxic and well tolerated. Studies enrolling almost 2000 of patients after 2 years of pegaptanib treatment showed no increasing evidence of adverse events including stroke, hypertension, serious hemorrhagic or thromboembolism.

Aptamers in circulation disorders

The treatment of circulation disorders *e.g.* by coronary artery bypass surgery requires application of anticoagulant heparin and his neutralising polypeptide, protamine. This therapy, however, has some limitations and may result in some side effects such as bleeding or heparin-induced thrombocytopenia. There are at least two aptamers mimicking heparin action [8]. One of them binds to and inhibits thrombin, serine protease taking a part in processes of coagulation and hemostasis. This molecule is ssDNA composed of 15 nucleotides and has a tetraplex structure. Formation of aptamer-thrombin complex depends on interaction between negative phosphate groups of aptamer and positive group on thrombin molecule [9].

The number of studies has revealed that thrombin aptamer has rapid and predictable anticoagulant effect and effectively reduce bleeding complications [8, 9]. Due to very short half-life (2 min) there is no need for using of an antidote. Since 2004 it has been studied in clinical trial in coronary artery bypass surgery.

Rusconi et al. [10] isolated an aptamer specifically binding IXa coagulation factor which may be promising as a novel anticoagulation system. This is RNA aptamer modified by polyethylene glycol that prolongs the circulating half-life of the molecule *in vivo*. Its functional properties may be effectively attenuated by complementary RNA antidotes. Administration of antidote quickly (1 to 5 min) restored factor IX activity level. Bleeding or other adverse effects were comparable to placebo [11].

Aptamers in viral diseases

Aptamer production technique also opened the possibility to find new drugs effective in anti-viral therapy such as in HIV infection. There exist several aptamers that may be useful in HIV therapy. One example is tetraplex aptamer with specific d[TTGGGGTT] structure which is able to bind V3 loop of the gp 120 protein and thus prevent its interaction with CD4 molecule and subsequent T cell infection [12]. Second aptamer with deoxythymidine and deoxyguanine sequences forming tetraplex structure may inhibit HIV integrase activity responsible for its integration into host DNA [13]. This effect depends on the presence of dimeric quadruplex structure folding topology in K⁺. The presence of these ions increased the inhibitory efficiency of these agents dramatically. Yet another example is aptamer named RNA_{at} which is able to inhibit HIV replication via inhibition of TAT protein binding viral trans activation response region.

This aptamer has been reported to inhibit HIV replication *in vitro* with efficiency of 70% [14].

Aptamers may be also useful in treatment of other viral infections such as influenza. This may be exemplified e.g. by aptamers specific for viral surface hemagglutinin (HA) that may prevent infection by multiple viral strains [15].

Anticancer aptamers

The best recognized aptamer used in anticancer therapy is AS1411 (AGRO 100) [16]. This aptamer specifically binds nucleolin, a protein playing a role in control of cell proliferation and apoptosis and promoting cell survival. In normal cells nucleolin is found mainly in nucleus, whereas in cancer cells it is also present in big amount on the cell surface. AS1411 specifically binds surface nucleolin on cancer cells, then the nucleolin-aptamer complex is internalized leading to inhibition of DNA proliferation and cancer cell death [17, 18]. AGRO 100 has been studied in phase I clinical trial in patients with advanced solid tumours (renal and non-small cell lung cancer). Application of this agent resulted in clinical improvement and no visible toxicity effects were observed [16, 19]. AS1411 is now being tested in multicenter phase II trials in acute myelogenous leukemia and renal cell carcinoma.

Another promising aptamers recently applied in anticancer therapy are:

1. RNA aptamer specific to mutated receptor tyrosine kinase (RET) associated with multiple endocrine neoplasia (MEN) syndrome and familial medullary thyroid carcinoma which prevents RET dimer formation and blocks signal transduction [20].
2. Aptamer targeted against integrin $\alpha_v\beta_3$ that inhibits angiogenesis via integrin activated signaling pathways [21].
3. Aptamer to PDGF-B which was shown to inhibit pro-angiogenic activity of PDGF in experimental rat colon carcinoma [22, 23].
4. RNA aptamer which antagonizes activity of ced 9 – programmed cell death inhibitor, a homolog of human Bcl-2 [24].

Other possibilities of aptamers

Besides their therapeutic applications aptamers are also used in laboratory diagnostics and research such as well as were found to be useful for target validation in a variety of disease models [3, 24]. Aptamers can target both extracellular and intracellular molecules and there exist many examples for their usefulness in studies e.g. on HIV proteins as well as proteins involved in signaling pathways and regulation of cell proliferation (E2F, RAS/RAF1). For example, studies with specific aptamers have helped to revealed the role of cytohesin-2 in activation of transcription in addition to its previously described function in regulating cytoskeleton rebuilding and vesicles transport in the cell [25].

In conclusion, aptamers appear to be potential drugs in a variety of diseases and may be helpful in understanding the etiopathological mechanisms of various yet poorly recognized disorders.

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