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Invited Mini Review

Therapeutic aptamers: developmental potential as anticancer drugs

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Aptamers, composed of single-stranded DNA or RNA oligonucleotides that interact with target molecules through a specific three-dimensional structure, are selected from pools of combinatorial oligonucleotide libraries. With their high specificity and affinity for target proteins, ease of synthesis and modification, and low immunogenicity and toxicity, aptamers are considered to be attractive molecules for development as anticancer therapeutics. Two aptamers - one targeting nucleolin and a second targeting CXCL12 - are currently undergoing clinical trials for treating cancer patients, and many more are under study. In this mini-review, we present the current clinical status of aptamers and aptamer-based cancer therapeutics. We also discuss advantages, limitations, and prospects for aptamers as cancer therapeutics. [BMB Reports 2015; 48(4): 234-237]

APTAMERS AS ATTRACTIVE CANDIDATES FOR TARGETED CANCER THERAPIES

While 'traditional' cytotoxic chemotherapies usually kill rapidly dividing cells in the body by interfering with cell division, targeted cancer therapies are designed to interfere with specific molecules needed for tumor growth and progression. Given their greater precision and potential for causing fewer side effects, targeted cancer therapies have become a major focus of cancer research. Typically, targeted cancer therapeutics are classified broadly as small chemicals, peptides, nucleic acids, and monoclonal antibodies. Of these, therapies based on monoclonal antibodies, which can bind to target molecules with high specificity and affinity, are among the most successful and important current strategies for treating cancer patients (1). More than 30 therapeutic antibodies have been used clinically, and hundreds more are undergoing clinical trials (2). Although monoclonal antibodies have many advantages, monoclonal antibody-based medications face a number of issues that have prevented their more widespread use. For example, the high cost of therapeutic monoclonal antibody development is beyond the easy reach of many researchers. The extremely high production costs reflect the requirements for very large cultures of mammalian cells and extensive purification steps under Good Manufacturing Practice (GMP) conditions, but they hamper the widespread use of these drugs (3). Another issue is the therapeutic efficacy of monoclonal antibodies: because monoclonal antibodies are large (~150 kDa), tumor penetration may be limited (3-5), especially in the case of solid tumors, where entry into tumor tissue from blood vessels is critical for drug efficacy (6). As a consequence of these limitations, whereas over 85% of human cancers are solid tumors (7), only eight monoclonal antibodies that have obtained US Food and Drug Administration (FDA) approval for cancer therapy are used routinely with solid tumors.

Aptamers, which are composed of short, single-stranded DNA or RNA oligonucleotides, are often compared to antibodies because of their shared high specificity and affinity for target molecules (8, 9). Since the development in 1990 of the 'SELEX' (systematic evolution of ligands by exponential enrichment) system-an aptamer screening method-aptamers have come to be regarded as powerful therapeutic, diagnostic, and basic research tools (Fig. 1) (10-12). Over the past two deca-

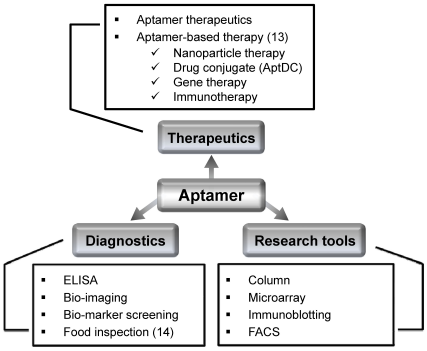

Fig. 1. Possible applications of aptamers. Aptamers bind to target molecules with high affinity and specificity. Because of these and other unique properties, aptamers are ideal tools for broad applications in therapeutics, diagnostics, and basic research.

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Table 1. Points to consider for successful cancer therapeutics

Properties	Requirements	Candidates
Target specificity & Binding affinity	Low nM∼pM	Antibodies, Aptamers, Peptides
Screening & Production Efforts	Screening: in vitro Fast, Low cost	Peptides, Aptamers
Immunogenicity	Low	Humanized antibodies, Aptamers
Modification	Easy to conjugation	Small molecules, Peptides, Aptamers
Stability	High pharmacokinetics & pharmacodynamics	Antibodies

des, aptamers have attracted increasing attention in the field of cancer therapeutics because they have several important advantages over other targeted therapeutics (Table 1). The fact that aptamers are obtained by chemical synthesis reduces their production costs, compared with monoclonal antibodies. It also means that chemical modifications can be easily and accurately introduced to fulfill different diagnostic and therapeutic purposes (15). Aptamers also show good thermostability and longterm stability as dry powders or in solution (16), and exhibit low immunogenicity and toxicity (17). Notably, aptamers are relatively small, compared with therapeutic monoclonal antibodies, and are thus expected to show greater penetration into tumor tissues (13).

APTAMERS IN CLINICAL TRIALS FOR CANCER TARGETS

AS1411. AS1411, a quadruplex-forming guanine-rich (G-rich) 26-mer DNA aptamer, is the most advanced aptamer and the first to enter clinical trials as a cancer therapeutic agent. AS1411 targets the protein nucleolin (18), which plays essential roles in cell growth and death through its involvement in rRNA transport and DNA transcription, replication, and recombination (19). Nucleolin, a nucleus- and cytoplasm-resident protein in normal cells, is overexpressed in the plasma membrane of many types of cancer, including lung cancer, breast cancer, prostate cancer, lymphocytic leukemia, and hepatocellular carcinoma (20). AS1411, developed by Antisoma, inhibits the proliferation of a wide range of cancer cell lines through a mechanism thought to involve disruption of the interaction of nucleolin with its binding partners. The steps involved in AS1411-induced cancer cell death have been proposed to include aptamer internalization via membrane nucleolin, interference with DNA replication, causing S-phase arrest, and stabilization of the mRNA for the anti-apoptotic protein, B-cell lymphoma protein 2 (BCL-2) (18, 20). AS1411, which exhibits minimal toxicity in patients with advanced solid tumors (21), is currently in Phase II clinical trials for acute myeloid leukemia (AML) and metastatic renal cell cancer (20).

NOX-A12. NOX-A12, which is developed by Noxxon Pharma, is a 45-mer long configuration (Spiegelmer) RNA aptamer that is linked to a 40-kDa polyethylene glycol (PEG). NOX-A12 targets CXCL12/SDF-1 (CXC chemokine ligand 12/stromal cell derived factor-1) (22), a chemokine that acts through binding to CXCR4 and CXCR7 chemokine receptors to play diverse roles in cancer biology, including regulation of leukemia stem cell migration to the bone marrow (23) and tumor growth and metastasis. CXCL12/SDF-1 expressed on leukemic cells also responds to the tissue microenvironment to play a role in the pathophysiology of chronic lymphocytic leukemia (CLL) (24). Neutralization of CXCL12/SDF-1 by NOX-A12 also has the potential to interfere with anchoring of leukemia stem cells in the bone marrow, allowing these cells to re-enter the cell cycle and become available for chemotherapeutic attack (25). The unique mirror-image configuration of NOXA12 makes the oligonucleotide resistant to hydrolysis and prevents hybridization with other nucleic acids (26). It has also recently been reported that NOX-A12 effectively inhibits cancer recurrence following irradiation in a glioblastoma multiforme model (27). NOX-A12 is currently in Phase II studies, designed to assess its therapeutic potential against CLL and multiple myeloma (26).

APTAMER-BASED TARGETED CANCER THERAPIES

One of the biggest advantages of aptamers, compared with antibodies, is the ease with which they can be modified chemically while retaining target specificity. Accordingly, there have been numerous efforts to combine the high target-specificity of aptamers with other anticancer modalities to provide targeted delivery of a variety of drug payloads. In these applications, aptamers that target cancer-specific membrane proteins mediate precise delivery of anti-cancer agents, such as nanoparticles, siRNA/miRNA, or cytotoxic drugs, to tumor cells (28). After binding target membrane proteins, aptamers are internalized into the cell together with their drug payload. Ultimately, the drugs are then released from the target molecules and exert their anticancer functions by damaging DNA or inhibiting microtubule polymerization (29). In one example of a nanoparticle designed for prostate cancer therapy, an RNA aptamer targeting prostate-specific membrane antigen (PSMA) was conjugated with a PLA (polylactide)-PEG or PLGA (polylactide-co-glycolide)-PEG nanoparticle encapsulating docetaxel (30, 31). In another example, paclitaxel-containing PLGA conjugated with an aptamer against mucin-1 (MUC1) was used to target MUC1-expressing cancer cells (32). siRNA/miRNA payloads have also been conjugated directly to aptamers. For ex-

ample, chimeric complexes of *Plk1* or *Bcl2* siRNA-PSMA aptamers and doxorubicin-PSMA aptamers have been developed for inhibiting PSMA-expressing prostate cancers (33). Aptamer-drug conjugates (ApDCs), which are conceptually similar to antibody-drug conjugates (ADCs), are also promising technologies for targeted cancer therapy because they can enhance therapeutic efficacy while reducing associated toxicities (34). Several potential problems with the ADCs approach remain to be resolved, such as undefined antibody-toxin ratios due to heterogeneous drug conjugation, a tendency to aggregate during synthesis, poor pharmacokinetics, and loss of immune reactivity (35). However, the beneficial properties of aptamers, such as accurate site conjugation and high solubility (> 150 mg/ml) (16), may ultimately surmount these potential issues.

LIMITATIONS OF APTAMERS AS CANCER THERAPEUTICS

When aptamers were first introduced, they garnered considerable attention as cancer therapeutics because of their advantages over monoclonal antibody therapeutics, highlighted above. However, even after 20 years, only two aptamers have reached clinical trials. Before aptamers can achieve widespread clinical application, they must clear several hurdles.

Degradation by nucleases. Because aptamers are composed of DNA or RNA oligonucleotides, they are rapidly degraded by exo- and endonucleases (36): the half-life of unmodified nucleotide aptamers in blood can be as short as 2 min (37). To increase the serum half-life of aptamers, researchers have introduced chemical modifications into the sugar moiety or phosphodiester linkages. "Capping" oligonucleotides by modification of 3′ and/or 5′ends of nucleic acid strands protects aptamers from attacks by exonucleases (36). One commonly used approach that achieves such a protective effect is incorporation of an inverted oligonucleotide at the 3′-terminus. The most widely used method for protecting against degradation by endonucleases is the incorporation of a fluoro or O-methyl group at the 2′position of the sugar moiety (38). Such modifications are typically combined to confer maximal protection. For example, pegaptanib sodium (Macugen), the first aptamer approved by the FDA in 2004, is 3′-capped, 5′-PEGylated, and internally modified with 2′-fluoro-pyrimidines and 2′-O-methyl-purines modifications that collectively extended the aptamer half-life to 131 h (39). Various modified nucleotides, including 2′amino pyrimidines, boranophosphate internucleotide linkages, 5-modified pyrimidines, and/or 4′thio pyrimidines, have also been used to increase the nuclease-resistant properties of aptamers (36).

Renal clearance. Aptamers usually range in size from 5 to 15 kDa (40). Thus, they are susceptible to rapid elimination from the blood by renal filtration. Target accessibility can be enhanced by increasing the size of an aptamer through conjugation to bulky molecules, such as high-molecular-weight PEG polymers, cholesterol, or certain peptides (41). Because the molecular mass cutoff for the renal glomerulus is 3050 kDa (42), 40-kDa PEGylation has been used extensively for extending the circulation half-life of aptamers. The circulation halflife of un-PEGylated aptamers is less than 20 min, but increases to as long as 1 day for 40-kDa PEGylated aptamers (43).

CONCLUSIONS AND PERSPECTIVES

Although aptamers have many properties that make them potentially advantageous for use as cancer therapeutics, their current market prospects are discouraging. Notable in this context is the failure of pegaptanib to make inroads in the marketplace dominated by therapeutic antibodies, such as bevacizumab (Avastin) or ranibizumab (Lucentis) (44, 45). Despite such setbacks, aptamers remain attractive molecules with the opportunity for development as cancer therapeutics. For aptamers to achieve success in the cancer therapeutic market, they will need to take full advantage of their unique features, rather than compete directly with antibody therapeutics. Our expectation is that efficacious aptamer-based anticancer agents will be developed in the near future.

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