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Applications of Aptamers in Cancer Therapy

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http://dx.doi.org/10.5772/intechopen.75603

1. Introduction

Aptamer is derived from one Latin and one Greek word combinations: “aptus,” which means “fit,” and “meros” meaning “particle” [1]. As a DNA or RNA oligonucleotide, aptamer
has low molecular weight (5–40 kDa) and three-dimensional (3D) structure with a high binding affinity against target protein. Synthesis of aptamers by selective evolution of ligands by exponential enrichment (SELEX) has been invented in 1990 by Larry Gold and Jack Szostak and the other laboratories concomitantly [2]. SELEX is a consecutive processes starting with binding of target with random sequence of oligonucleotide library, washing and elution of unbound oligonucleotides, amplification of 3D structure oligonucleotides against the target epitope via polymerase chain reaction (PCR), selection of aptamers with high binding affinity and specificity, and finally modifications of novel aptamers to increase stability and function [3]. Through SELEX, an aptamer against various targets can be synthesized such as amino acids, peptides, protein, phospholipids, carbohydrates, nucleic acids, antibiotics, metal ions, and whole cell (bacteria, viruses). Although aptamers are similar to antibodies due to their binding affinity to target molecule, they have a number of advantages such as small and low complexity with low immunogenic activity, high stability, high affinity and specificity for their targets, and easy to synthesize and modify in vitro [4].

1.1. SELEX method

The determination of oligonucleotides from a random ssDNA/RNA sequence pool is accomplished through in vitro selection referred as SELEX. SELEX method is composed of following various steps: (i) design and synthesis of oligonucleotide library (DNA or RNA), (ii) hybridization of target with oligonucleotide library, (iii) elimination of aptamers unbounded to target, (iv) selection of aptamers that are highly specific against target (4–20 rounds), (v) amplification of selected aptamers by PCR (ssDNA library) or RT-PCR (RNA library) in order to increase aptamer efficacy, (vi) cloning of selected and amplified aptamers in vector (TOPO cloning vector), (vii) sequence determination of cloned aptamers, and (viii) discriminate and identify the novel aptamer from aptamer database tools [5].

Oligonucleotide libraries are the major nucleic acid-based tool to generate aptamer via using SELEX method. Classical SELEX libraries are generally $10^{14}$–$10^{16}$ molecules with 20–80 nucleotides long and usually amount of $10^{-8}$–$10^{-10}$ M. They are arranged in order of sequences with three parts: 5′ sense primer sequence, random nucleotide, and 3′ antisense primer sequence parts, respectively. 5′- sense or 3′-antisense sequence part of the oligonucleotide libraries are 18–22 base long, and the random sequence between 5′ and 3′ sequences ranges from 20 to 40 nucleotides (Figure 1) [6].

Besides classical libraries, different featured libraries can be used for SELEX method such as structurally modified, based on a known sequence, free of fixed sequences or genomic sequences. In structurally modified libraries, between two fixed sequences, a random region constructed to form a secondary structure (G-quartets, hairpin, vs) in order to select more stable aptamer against target molecule [7]. A library used for SELEX is constructed with 4–6 nucleotide-fixed regions in both sides called a free of fixed sequence oligonucleotide library (tailored SELEX) [8].
In addition, to investigate for sequences such as transcription factors, translation regulators, and splicing sequences, genomic sequence-based libraries are used for genomic SELEX. In genomic SELEX, the oligonucleotide libraries are composed of 50–500 nucleotides long with fixed sequences on each region [9]. In order to protect aptamers from nucleases, chemical modifications such as L-form of nucleotides (L-ribose or L-deoxyribose); 5-iodo-, 4-thiouridine, 5-bromo oligonucleotides; or 5′ fluorescein isothiocyanate (FITC) dye and/or 3′- biotin labeling can be performed [10].

Since SELEX method is composed of generation of aptamer against target molecule by using a rich random nucleotide sequence of oligonucleotide libraries, there are modifications on SELEX method according to research aim such as nitrocellulose membrane filtration-based SELEX, affinity chromatography and magnetic bead-based SELEX, capillary electrophoresis-based SELEX, microfluidic-based SELEX, cell SELEX, and others [electromobility shift assay (EMSA), surface plasmon resonance (SPR)]. In nitrocellulose membrane filtration-based SELEX, following at least 12 SELEX selection rounds, aptamers are separated via the nitrocellulose membrane [11]. The strategy of affinity chromatography-based SELEX method is depending on the selection of aptamers generated against target protein that is tagged with glutathione-S-transferase (GST) or His-tag and immobilized on beads such as agarose [12]. By using affinity column containing target-immobilized beads to select the
SELEX-generated aptamers, however, the disadvantages of this technique because of untagged proteins or protein functional group unbeaded could not be accomplished. However, without bounding process for target protein, successful and specific aptamers can be selected by using magnetic bead technology [13]. In capillary electrophoresis-based SELEX, aptamer can be selected by electromobility difference of target, library, and target-library complex mixture under electroosmotic flow within few rounds such as 2–4. Theoretically, separation potential of aptamers depends on the speed, sample dilution, resolution, and ionic charge of each analyte under the influence of electric field within the capillary [14]. In order to decline the SELEX rounds, microfluidic-based SELEX is generated to increase the effective capacity of aptamer selection under the influence of microfluidic system principle on microchips. The most essential advantage of this method is rapid and automatically aptamer selection procedure [15]. Although SELEX is generally used for selection of aptamer against proteins, a whole cell can also be used as a target for SELEX method. In cell SELEX, extracellular proteins expressing on cell surface are generally regarded as a target for cell SELEX and, following round of SELEX method with whole cell and the oligonucleotide library, generation of aptamers against surface proteins on target cells. As the target cell expressing various extracellular receptors, ligands, etc. on cell surface, the aptamer selected from cell SELEX can be a mixture of aptamers targeting different surface proteins. However, aptamers generated by cell SELEX can be used for cell-specific diagnosis and therapy, targeting cell drug delivery (Figure 2) [16].

The investigation of potential chemotherapeutic effect of selected aptamers generated by SELEX is identified for their novelty in various bioinformatics tools given below [17]:

http://www.cas.org/SCIFINDER/scicover2.html
http://connection.ebscohost.com/c/articles/45243053/aptamer-database

By these different and multifunctional SELEX methods, a number of aptamers can be generated and selected. Subsequently, these aptamers can be used for aptamer-based sensors, new drugs, and drug delivery systems. Until now, various aptamers against target proteins are generated and investigated for preclinical studies, and some are under clinical trials such as Phases I and II (Table 1) [18]. Macugen (EYE001), FDA-approved modified RNA aptamer, phase II/III completed for the treatment of age-related macular degeneration (AMD). This RNA aptamer is referred as Pegaptanib (Pfizer, NY, USA), and it targets vascular endothelial growth factor (VEGF) [19]. Zimura (ARC1905) is an anti-C5 aptamer that targets essential inflammatory mediator, complement component. Phase I study of zimura and monoclonal antibody fragment for angiogenesis factor VEGF combination treatment was completed in AMD patients [20]. Another RNA aptamer against factor IXa is REG1 that acts as an anticoagulation system, and phase I and phase II trials are successfully accomplished to prevent thrombotic and ischemic complications [21]. Fovista is referred as E10030, an aptamer against platelet-derived growth factor (PDGF). The phase I and phase II studies of fovista in neovascular AMD have been accomplished. In addition, fovista with anti-VEGF therapy combined treatment is tried in
recruited participants [22]. AS1411 is a 26-nucleotide-long guanine-rich oligodeoxynucleotide aptamer that targets nucleolin-1, a phosphoprotein expression on various cancer cell surfaces. Phase II trials of AS1411 in treatment of myeloid leukemia and metastatic renal cell carcinoma have been accomplished. In addition, the treatment potential of AS1411 is inclined by combination of aptamer with chemotherapeutic drugs such as doxorubicin [23]. The AR19499 is a DNA aptamer with high binding affinity against tissue factors Xa and VIIa and leads to tissue factor pathway inhibition. Thus, AR19499 DNA aptamer is used in hemophilia which is a blood clotting deficiency due to improper clotting factor activity [24].
2. Aptamer-based drugs for cancer therapy

Drugs for the cancer treatment are generally focused on the inhibition of molecular signaling pathways, cell cycle inhibition, and induction of apoptosis. Chemotherapy is one of the major categories for the treatment of cancer; however, its success remains limited due to the ineffective accessibility of drugs to tumor cells. Furthermore, high doses of drugs are used to overcome this handicap causing intolerable toxicity and multidrug resistance. Drug resistance is the major obstacle in cancer therapy; thus the development of new technologies and agents needs to be investigated to overcome drug resistance and prevention of cancer invasion, or metastasis [25]. Because of their high binding affinity and specificity, aptamers become an essential target for cancer drug development. Aptamer-based drug design depends on targeting the cancer-specific biomarker proteins and increases the immunomodulatory functions by aptamers, using aptamers as a drug delivery system in cancer treatment [26].

2.1. Cell-specific targets for aptamer-based therapeutic strategies

New strategies for cancer treatment have been developed on the microarray analysis of cancer tissue samples compared to normal tissue’s expression profiles. According to microarray analysis, the significant upregulation of specific genes compared to normal tissue samples is shown with their biomarker potential. Therefore, the development of stable and selective inhibitors for these targets has been analyzed for cancer therapy [27]. One of the essential potential inhibitor for these target molecules are aptamers that are more sensitive and specific to their target via high affinity. Thus, aptamer-based drugs are designed and synthesized by SELEX method to investigate first for their binding affinity and potential chemotherapeutic potential in specific cancer types.

2.1.1. Pegaptanib

One of the essential molecules for induction of angiogenesis and to increase vascular permeability is vascular endothelial growth factor (VEGF). The isoforms of VEGF are 206, 189,
165, 145, or 121 amino acids long; one of these isoform VEGF165 overexpression in ovarian cancer cells has been demonstrated. In xenograft mice models, inclined levels of VEGF165 triggered ovarian cancer metastasis, and macrophage infiltration is also determined [28]. The successful designed aptamers against VEGF also put forward a clinical potential in the treatment of metastatic tumors [29]. 0.75–1.4 nm Pegaptanib, RNA aptamer against VEGF-165, inhibited VEGF-associated tumor vascularity, VEGF signaling via blocking phosphorylation of VEGFR2 receptor and phospholipase Cγ, and calcium mobilization [30]. Similar to Pegaptanib, SL(2)-B/RNV66, DNA aptamer is used to inhibit VEGF-mediated tumor neovascularization in hepatic and breast cancer cells [31].

2.1.2. A9g RNA aptamer

Prostate-specific membrane antigen (PSMA), as a prostate cancer marker when overexpressed, is a type II membrane-associated metallopeptidase. Many other solid tumors exhibited PSMA expression in their vasculature. Elevated expression of PSMA on the surface of prostate cancer cell membrane leads scientific focus on PSMA antigen to synthesize aptamer against it. A9g PSMA aptamer is used to target PSMA, which is poorly expressed in normal cells, and it is a transmembrane glycosylated protein (100 kDa), which exerts NAALADase/glutamate carboxypeptidase II activity on surface of the cells [32]. According to previous reports, block PSMA activity was obtained following A9g aptamer, a 2′-F modified RNA treatment. This modification increased the aptamer stability in serum. Although two different modifications were done during the experiments as 2′ F and 2′ O-Me adducts, 2′ O-Methylation did not increase stability. Treating animals with A9g by systemic administration prevented metastatic potential of prostate cells. The therapeutic efficiency of aptamer was high because only 10% mice models showed bone metastasis. When authors checked the safety of aptamer, animals did not exert any change for their weight, blood chemistry, or behavior within 4 weeks [33].

2.1.3. HPV16 E6 (F5) aptamer

Human papillomaviruses (HPVs) are DNA tumor viruses that infect epithelial cells, and they are known widely as causality factors of cervical, head, and neck cancers. Although there are more than 100 different types of HPV strains identified, one of the most common malignancy-associated HPV stain (HPV 16) causes the transformation of viral oncoprotein E6 and E7 in the cervical cells, which leads to inhibition of key tumor suppressors [34]. Postsynaptic density protein (PDZ) domain is discovered as Drosophila disk large tumor suppressor and is found in E6 targeting proteins (e.g., Magi, Dlg), which maintains interaction via short C-terminal PDZ-domain-binding motif (ETQV). The designed and selected RNA aptamer (F2) against HPV16 E6 was tested in cervical carcinoma cells, which express HPV16 E6 and E7 leading to induction of apoptosis through inhibiting interaction between E6 and the PDZ1 domains from Magi-1 in p53-independent manner [35].

2.1.4. ErbB targeting aptamers

The EGFR/ErbB family of receptor tyrosine kinases (RTK) comprises four members—EGFR (also known as HER1 or ErbB1), ErbB2 (Neu, HER2), ErbB3 (HER3), and ErbB4
containing an extracellular ligand binding region, a single membrane-spanning region, and an intracellular tyrosine kinase-containing domain [36]. Amplification and overexpression of ErbB2 have been also reported in numerous cancers, including breast, ovarian, stomach, bladder, salivary, and lung cancers. One of the promising RNA aptamers is developed against ErbB2, which overexpressed in majority of breast cancer cells. The designed RNA aptamer showed high affinity and specificity against extracellular domain of ErbB2 protein [37]. EGFR is a critical target, and FDA-approved two monoclonal antibodies (cetuximab, panitumumab) and three tyrosine kinase inhibitors (erlotinib, gefitinib, and lapatinib) are effective in the treatment of lung cancer patients [38]. However, the presence of increasing refractory cases is the major obstacle in the treatment of lung cancer. Thereby, more potent or synergizing therapeutically options gain researcher’s interest in cancer field. One of suggested therapeutic tools is CL4, a nuclease-resistant RNA aptamer. It is able to bind at high affinity to EGFR (ErbB1, Her1) on the surface of different cancer cells and to block EGFR downstream signaling via dimerization of receptors. The apoptotic effect of CL4 was shown in in vitro and in vivo experimental models. One of the other promising EGFR targeting RNA aptamers is E0727/CL428/KD11 30/TuTu2231, which blocks EGFR phosphorylation and EGFR-mediated PI3K/AKT and MAPK signaling, which led to increased apoptotic ratio in glioblastoma [39] and breast [40] and squamous cell carcinoma [41].

2.1.5. A30 aptamer

One of the members of receptor tyrosine kinase family member type I is human epidermal growth factor receptor-3 (HER3), and upregulation of HER3 expression has been demonstrated in various cancer types. Although the expression of HER3 is not high when compared to HER2 in cancer tissue samples and/or cancer cell lines, increase HER3 levels are evaluated to accelerate the drug resistance. RNA aptamers are selected for the extracellular domain of HER3 (82 kDa domain) by SELEX. One of the RNA aptamers with high binding affinity against HER3ECD is A30 aptamer, which has an inhibitive effect on heregulin-mediated HER2 phosphorylation and MAPK signaling in MCF-7 breast cancer cells [42].

2.1.6. OPN aptamer

Osteopontin (OPN) is a secreted phosphoprotein that induced tumorigenesis, local growth, and metastasis in a variety of cancers. It is a potential therapeutic target for the regulation of cancer metastasis. OPN is also referred to as cell attachment protein and cytokine that signals through two cell adhesion molecules: αvβ3-integrin and CD44 [43]. The selected RNA aptamer against OPN was effective to block cell migration and invasion in metastatic MDA-MB-231 breast cancer cells. The similar findings were confirmed in vivo through xenograft model of MDA-MB-231 cell-mediated tumor formation. OPN-R3 with 2′-O-methyl-substituted nucleotides, 5′-cholesterol modification, and 3′-inverted deoxy thymidine modifications were effective to decrease tumor volume. Therefore it was suggested that small RNA aptamers have potential in the treatment of cancers [44].
2.1.7. NAS-24 aptamer

Vimentin, one of the essential intermediate filamentous protein, play a role during cell adhesions play a role during cell adhesion, migration, and apoptotic cellular processes. Overexpression of vimentin in various cancer cells such as prostate cancer, malignant melanoma, lung cancer, breast cancer, and gastrointestinal tumor is demonstrated [45]. Increased vimentin expression is shown to be linked with cancer invasion and low prognosis potential. NAS-24, an 80-nucleotide-long DNA aptamer, targeting vimentin molecule has been investigated for its apoptotic potential in mouse ascites’ adenocarcinoma cells in vivo [46]. In order to increase the delivery of NAS-24, additional arabinogalactan (AG) and NAS-24 aptamer complex has been investigated in mice models with exposure of 1.6 μg/kg for 5 days, and treatment significantly triggered twofold apoptotic cell death compared to control mice group [47].

2.1.8. YJ-1 aptamer

According to cancer tissue microarray analysis, ample of tumor antigen is demonstrated in cancer progression, metastasis, and invasion. Overexpression of one of essential antigens, carcinoembryonic antigen (CEA), is associated with cell adhesion, cancer cell migration to the liver, and induction of hepatic metastasis of colon cancer cells. Aptamer against CEA is YJ1 aptamer, which is investigated for potential anticancer agent in colon cancer cells in mice models and revealed inhibition of metastasis of colon cancer to hepatocellular localization. However utilization of CEA aptamers is more potent to monitor disease progression [48].

2.1.9. A-P50 aptamer

A-P50 is a 31-nucleotide RNA aptamer against NF-κB molecule with a high binding affinity against DNA-binding domain of NF-κB p50. As a transcription factor, NF-κB activates various cytokine expressions and takes role in blocking the effect of radiotherapy and chemotherapy in cancer cells. Since NF-κB is a major key molecule in carcinogenesis or cancer drug resistance, potential therapeutic effect of aptamer against NF-κB has been investigated in hepatocellular carcinoma [49]. A-P50 aptamer targeting NF-κB p50 activation inhibition overcomes the doxorubicin-dependent drug resistance in lung cancer in vitro and in vivo mice models. Therapeutic effect of A-P50 aptamer is increased by Dox combined drug delivery, and this drug delivery system induced apoptotic cell death in A549 and H1299 in non-small cell lung cancer [50].

2.1.10. AP273 AFP aptamer

Hepatic cell proliferation, growth, and differentiation leading to development of hepatocellular carcinoma are associated with alpha fetoprotein (AFP). Active AFP signaling triggered oncogenic mRNA production, which results in investigation of the potential anti-carcinogenic effect of aptamer against AFP [51]. AP273, a DNA aptamer selected by capillary SELEX method, inhibited invasion and migration of HepG2 and SMMC7721 hepatocellular carcinoma cells. Microfluidic chips with magnetic bead bound AFP aptamer are constructed to determine and measure the circulating cancer cells as a diagnosis [52].
As we discussed above, there are a number of DNA or RNA aptamers with increased stability and function which are promising drug candidates in future cancer therapy models. When we checked the patents displaying this potential, aptamers are the important subjects for intellectual property rights. One of pancreatic ductal carcinoma treatment options with aptamers was shown with different functions, as synergistic drug with current therapeutic modalities for pancreatic cancer, single agent, and labeling of cancer cells. The selectivity of designed and selected aptamers for targeted cells, not normal epithelial cells, is one of the most important successful parts of aptamer therapy. Studies on severe combined immunodeficiency (SCID) mice models increased the potential therapeutic advantage of proposed aptamers for the next-term clinical trials. In the treatment of prostate cancer, one of the other promising DNA aptamers is DML-7, which binds to DU145 prostate cancer cells with high affinity in temperature-dependent manner. It is well established that the selectivity of DML-7 against metastatic cell lines was high due to the lack of interaction affinity to LNCaP or 22Rv1 prostate cancer cells [53].

2.2. Immunomodulatory aptamers in the treatment of different solid tumors

Tumor immunity is one of the important subjects in the current cancer therapy understanding. The approval of the immunomodulatory monoclonal antibodies in the treatment of different cancer types highlighted the role of effector T cells in antitumor immunity [54]. The activation of T cells via antigen-presenting cells (APCs) is proceeded due to T-cell receptor-triggered antigen-specific signals or co-stimulatory (COS) molecules (such as 4-1BB, CD28, and OX40). Although a number of attempts were done with antibody-based molecules to alter these interactions for COS molecules, the immunogenic properties of antibody-based options are found with limited effectiveness [55]. The designed and selected aptamer against 4-1BB (a COS receptor that is responsible for the activation and expansion of CD8+ T cells) was the first agonistic immunomodulatory aptamer. It is an artificial ligand with high similarity to anti-CTLA that initiates COS signals to boost T-cell survival. The aptamers against tumor necrosis factor receptor family members, OX40 and CD28, potentiated T-cell-mediated tumor immunity in mice models [56]. OX40 RNA aptamer could exert therapeutic potential in melanoma patients. Antagonist effect of Del 60 aptamer (against CTL4) on T-cell proliferation has been demonstrated in vitro studies. This RNA aptamer was found effective in in vivo tumor growth in melanoma and bladder tumors. In addition, programmed death-1 (PD-1) and PDL1 axis against DNA aptamer (MP7) was reported to inhibit PD-L1-induced apoptosis of tumor-specific T cells and IL-2 secretion [57]. Due to Treg activation following PD-1/PDL1 axis, MP7 aptamer may mediate the Tregs in antitumor immunity [58]. In order to deregulate the immunological functions of tumor microenvironment, inhibition of IL-10 and IL-4Rα-STAT6 signaling with RNA aptamers 5A1 and CL-42 was found effective to decrease tumor growth of colorectal and breast cancer cells. Similar to these achievements, DNA-based aptamers against IL-6 and VR11 prevented IL-6 or TNF-α receptor activation in myeloma cases [59]. In addition, the angiogenesis and invasion inhibition potential of aptamer binding IL-4Rα are demonstrated in myeloid-derived suppressor cells, and also IL4Rα aptamer induced the migration of T lymphocytes in tumor microenvironment in order to decline the cancer volume and size [60]. According to Spiegelmer technology, 2′-Fluoro- or 2′-amino pyrimidines or 2′-O-methyl nucleotides modified L-RNA aptamer generated that are resistant
against nucleases. One of the Spiegelmer (L-RNA aptamer, Noxxon Pharma, Germany) forms of L-RNA aptamer is Nox-E36, which is normally used to inhibit monocyte chemoattractant protein-1 (MCP-1) (CCL2). It is suggested to treat glioblastoma multiform patients, which display high MCP-1 expression profile [61]. Potential inhibition effect of Nox-E36 DNA aptamer on MCP-1 molecule revealed the use of Nox-E36 as an immunomodulatory cancer therapy agent in different cancer types. Similar to Nox-E36, Nox-A12 is an anti-chemokine suggested in the treatment of different malignancies. 45-mer L-RNA and PEG-derived formula of Nox-A12 is effective on stromal cell-derived factor-1α (SDF-1α). SDF-1α attracts and activates immune and nonimmune cells that bind to chemokine receptors CXCR4 and CXCR7. The immunological vulnerability potential of cancer cells increased the experimental success of L-RNA aptamers in hematological and solid tumors [62].

2.3. Targeted drug delivery in cancer therapy via aptamers

Recently, new targeted drug delivery approaches have been explored such as nanosystems and bio-conjugates leading to successful therapy. Targeted drug delivery systems enable drug accumulation within a target zone and usually catalyze the interaction between a drug and its receptor via four key steps: retain, evade, target, and release. The system selection depends on the specific site of the body. New strategies include the use of aptamers with their high affinity and specificity for targets, easy synthesis, modification, and tissue penetration. In addition, they are even integrated with a number of nanomaterials including gold nanoparticles, carbon nanotubes, DNA micelles, and hydrogels. Such specific approaches are expected to enhance the effect of chemotherapeutics with greater selectivity. In this section of the chapter, a number of aptamers used as effective drug delivery systems were discussed. Aptamer-based drugs for cancer therapy were listed in Table 2.

2.3.1. Prostate-specific membrane antigen

Two specific nuclease-stabilized aptamers, xPSM-A9 and xPSM-A10, able to inhibit the enzymatic activity of PSMA, were designed by Lupold et al. in LNCaP cells [63]. The study was the first of its kind that RNA aptamers were evaluated for prostate cancer. The truncated version of xPSM-A10 was highly selective to PSMA, showing strong affinity to LNCaP cells (PSMA+), but not PC3 cells (PSMA-) [64]. The high specificity of the aptamer was further used for silencing RNA (siRNA) delivery to cells. siRNA-aptamer complex knockdown was comparable to the positive control where siRNA-liposomal transfection reagent protocol was used [65, 66]. The study showed the decreasing cell proliferation and apoptosis ratios as a consequence of silencing polo-like kinase (PLK1) and B-cell lymphoma 2 (Bcl-2) genes via PSMA aptamer-siRNA complexes in LNCaP cells [67]. In addition, authors also delivered the siRNA-aptamer complex to the LNCaP cell-bearing mice, obtained tumor size and volume reduction, whereas no effect was observed in PC3 (PSMA-) cells. More importantly, siRNA-aptamer delivery overcame the cytotoxicity and immune response problem in vitro and in vivo experiments associated with the liposomal agent-mediated delivery. Anti-PSMA aptamer-conjugated siRNA strategy was also evaluated for other targets such as eukaryotic elongation factor 2 (EEF2) [68]. Due to the success of anti-PSMA aptamer-siRNA delivery system,
<table>
<thead>
<tr>
<th>Aptamer name</th>
<th>Aptamer target</th>
<th>Function</th>
<th>Cancer</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>A30</td>
<td>HER3</td>
<td>Inhibition of HER2 and MAPK signaling</td>
<td>HER-3 overexpressing lung cancer, breast cancer, gastric cancer, pancreatic cancer</td>
<td>[42]</td>
</tr>
<tr>
<td>A9-g</td>
<td>PSMA</td>
<td>Suppress the enzymatic activity of PSMA</td>
<td>In vivo and in vitro blockage of prostate cancer metastasis</td>
<td>[32, 33]</td>
</tr>
<tr>
<td>AFP-apt</td>
<td>Alpha fetoprotein</td>
<td>Suppression of AFP-mediated cancer progression pathways</td>
<td>In vitro hepatocelelular carcinoma growth and proliferation inhibition</td>
<td>[51, 52]</td>
</tr>
<tr>
<td>A-P50</td>
<td>NF-KB</td>
<td>NF-κB phosphorylation inhibition</td>
<td>Overcoming resistance profile of lung cancer against doxorubicin in vivo and in vitro</td>
<td>[49, 50]</td>
</tr>
<tr>
<td>AS1411</td>
<td>Nucleolin</td>
<td>Nucleolin-dependent NF-κB or Bcl2 signaling</td>
<td>Acute myelocytic leukemia, lung cancer, renal cancer, breast cancer, pancreatic cancer</td>
<td>[23, 84–90]</td>
</tr>
<tr>
<td>E0727/CL428/KD1130/TuTu2231</td>
<td>Epidermal growth factor receptor (EGFR)</td>
<td>EGFR-mediated PI3K/Akt and MAPK signaling inhibition</td>
<td>Squamous cell carcinoma, breast cancer, glioblastoma multiform, lung cancer</td>
<td>[36–41]</td>
</tr>
<tr>
<td>NAS-24</td>
<td>Vimentin</td>
<td>Inhibit cell growth and proliferation</td>
<td>Apoptotic cell death induction in adenocarcinoma in vitro and in vivo</td>
<td>[45–47]</td>
</tr>
<tr>
<td>NOX-A12</td>
<td>CXCL12</td>
<td>Suppress the migration and angiogenesis triggered by CXCL12</td>
<td>Multiple myeloma, chronic lymphocytic leukemia, glioblastoma multiform (with radiotherapy)</td>
<td>[62]</td>
</tr>
<tr>
<td>NOX-E-36</td>
<td>CXCL12</td>
<td>CXCL12-induced cell migration and angiogenesis inhibition</td>
<td>Incline in vivo drug sensitivity in hematological cancer cells, chronic lymphocytic leukemia, and multiple myeloma and in vivo radiotherapy success in glioblastoma multiform</td>
<td>[62]</td>
</tr>
<tr>
<td>OPN-R3</td>
<td>Osteopontin (OPN)</td>
<td>Inhibition of OPN binding to OPN receptor</td>
<td>In vivo and in vitro blocking of breast cancer cell migration and invasion</td>
<td>[43, 44]</td>
</tr>
<tr>
<td>Pegaptanib</td>
<td>VEGF-165</td>
<td>Inhibition of angiogenesis via suppressing the VEGF</td>
<td>Solid cancer extends angiogenic potential therapy</td>
<td>[28–31]</td>
</tr>
<tr>
<td>Yj-1</td>
<td>Carcinoembryonic antigen (CEA)</td>
<td>Blocking the cross-talk between CEA and ribonucleoprotein M4</td>
<td>Colorectal cancer invasion-metastasis inhibition in vitro and in vivo colorectal cancer</td>
<td>[48]</td>
</tr>
</tbody>
</table>

Table 2. List of aptamer-based drugs for cancer therapy.
researches have also investigated its potential for drug delivery. The first drug used for this purpose was gelonin which is a ribosomal toxin and protein synthesis inhibitor [69]. Studies with gelonin suggested that the effective doses are highly toxic due to its low membrane permeability. Chu et al. exposed LNCaP cells to anti-PSMA aptamer conjugated with gelonin in order to induce cell death [69].

Aptamer direct conjugation with drugs has a major limitation since drug concentration has a direct proportion with aptamer size and binding capacity. To enhance the efficacy of the drug delivery using aptamers, recent approaches focused on the conjugation of aptamers with functional polymers or nanoparticles. Dhar et al. used anti-PSMA aptamer conjugated with poly D, L-lactic-co-glycolic acid (PLGA), and polyethylene glycol (PEG) nanoparticles to deliver cisplatin to LNCaP prostate cancer cells [70]. The success of the entry of the aptamer-nanoparticle-drug complex to the cells was confirmed by fluorescence microscopy through green fluorescent-labeled encapsulation of the nanoparticles. Finally, authors were able to diminish the effective dose of free cisplatin which has a dose-limiting toxicity and acquired resistance problems, with the increase of the drug bioavailability. The same approach was also tested for docetaxel on mice with LNCaP tumor cell [70]. Results were promising in terms of tumor size/volume and survival. xPSM-A10-doxorubicin complex was used for drug delivery studies. The results from these studies increased the efficiency of doxorubicin in LNCaP prostate cancer cells. The aptamer-doxorubicin complex was further conjugated to a fluorescent quantum dot (QD) to provide high targeting potential. The same study revealed that PMSA-mediated endocytosis caused the release of doxorubicin [71]. However, aptamer-QD-doxorubicin complex was as toxic as free doxorubicin. Other anti-PSMA aptamers are the polyethylenimine (PEI)- or polyethylene glycol (PEG)-conjugated ones which were co-delivered with small hairpin RNA (shRNA) against Bcl-xL, the anti-apoptotic gene. These combinations were also succeeded to selectively and potently kill LNCaP cells \textit{in vitro} [72]. Therefore, all these studies demonstrated that aptamer-conjugated nanoparticles with chemotherapeutic drugs can be a powerful approach for targeted drug delivery with minimal side effects.

2.3.2. Tenascin-C

Tenascin-C (TN-C) as an extracellular matrix (ECM) protein having role in tissue remodeling is expressed during embryonic development, tissue repair, and pathological conditions such as chronic inflammation and cancer. It is highly expressed in tumor stroma in glioma and breast cancer [73]. TN-C affects several signaling molecules, Dickkopf-1 (DKK1) and Wnt, e.g., involved in survival, proliferation, invasion, angiogenesis, and metastasis [74]. Researches have designed a single-stranded DNA aptamer \textit{via} SELEX technology to target TN-C in U251 glioblastoma cells and modified them with a metal chelator MAG2 for an ideal binding affinity [75]. While they injected the final radiolabeled MAG2 aptamer to mouse bearing glioblastoma and breast cancer xenografts, resulting positron emission tomography (PET) imaging studies showed that the aptamer was localized in tumors. Recently, it was shown that the modified TN-C aptamer was uptaken by TN-C-positive U87MG glioblastoma and H460 lung cancer cells compared to Sc aptamer, the negative control [76]. In addition, the same study also showed that the aptamer has a fast clearance from the blood stream and kidneys. Further evaluations were obtained in terms of engineering a multimodal nanoparticles based Simultaneously Multiple
Aptamers and RGD Targeting (SMART) probe that targets TN-C, integrin αvβ3, and nucleolin at the same time. The SMART probe had a better affinity and specificity to several tumors like glioma, prostate cancer, cervical cancer, and lung cancer cells [77]. The next generation of TN-C aptamer was GBI-10 and shown that it can bind several TN-C peptides in vitro [75, 78]. The most important part of the fact that GBI-10 can target several binding sites of TN-C is the ability to address multiple splice variants at the same time. GBI-10 aptamer was also suggested for the diagnosis of tumor during MRI when subjected to gadolinium; a contrast agent helps to determine tumor localization, in a liposome capsule [79]. Therefore, TN-C aptamers due to target specificity, small size, and rapid tissue penetration has potential clinical advantages and is still tested for targeted drug delivery to the ECM of tumors.

2.3.3. Nucleolin

Nucleolin is an abundant nucleolar protein found in eukaryotic organisms including yeast, plants, and mammals. It has several structural domains which help the interaction of nucleolin with various proteins and RNAs, playing role in rDNA transcription and maturation, ribosome biogenesis, and nucleocytoplasmic transport. Although it was first described in 1973 by Orrick et al., its genomic and proteomic organization was clarified by the end of the 1990s [80]. It was elucidated that nucleolin is not only a nucleus resident protein but also is expressed at the cell surface of dividing cells, especially in tumor cells and angiogenic blood vessels in correlation with cell proliferation [81, 82]. The membrane-associated nucleolin has been shown to function as a growth factor receptor [83]. Human gastric cell lines express cell surface nucleolin which functions as receptors for TNF-α-inducing protein (Tipα) of Helicobacter pylori. Tipα upon nucleolin binding induced tumor progression via the activation of TNFα and NF-κB in human gastric adenocarcinoma cell lines MKN-1 [83]. Nucleolin silencing, by siRNA, resulted in the reduction of cell proliferation and migration. Therefore, studies put forward the cell surface nucleolin as a potential target for anticancer therapies.

AS1411, the first designed anti-nucleolin aptamer, is a 26-nucleotide-long guanosine-rich DNA sequence having antiproliferative activity [84]. It forms a G-quartet-containing structure which is resistant to nuclease degradation and remarkably stable in serum. AS1411 treatment caused the inhibition of NF-κB signaling, DNA replication, cell cycle arrest, and apoptotic induction. In addition, it was shown that AS1411 can bind Bcl-2 mRNA resulting its destabilization and consequent downregulation in breast cancer cells [85]. In vivo preclinical studies demonstrated the antitumor effect of AS1411 in mice bearing tumor xenografts with breast and lung cancer cells [86]. AS1411 was also tested in Phase II clinical trials on five patients with renal carcinoma and leukemia. The response against the aptamer was very promising in one renal cell carcinoma patient with 84% tumor size reduction and 2-year tumor-free progression. The patient whole exome sequencing revealed that AS1411 caused missense mutation in mammalian target of rapamycin (mTOR) and fibroblast growth factor receptor 2 (FGFR2) genes [23].

Besides the direct anticancer strategy using aptamers acting on nucleolin, cargo delivery into cancer cells was tested due to the fact that nucleolin shuttles between the cell surface, cytoplasm, and nucleus in dividing cells. Before aptamer strategy, F3, a 34-amino acid peptide
able to recognize nucleolin on the cell surface of angiogenic cells, has been used to target cancer cells [81]. Radio-labeled F3 peptide was used to deliver α-particle resulting tumor volume reduction in mouse xenograft models [87]. Aptamers are potentially better over peptides due to their high serum stability and immune tolerance. Therefore, AS1411 has been used for imaging and drug delivery systems in conjunction with nanoparticles. AS1411-human serum albumin (HSA) nanoparticles was used as drug carrier, and the uptake of the complex is increased compared to only-aptamer condition in MCF-7 breast cancer cells, but not in MCF-10A normal breast epithelial cells. This simple complex by modified HSA nanoparticles has been used for paclitaxel delivery and suggested as a way to overcome the limitations of paclitaxel toxicity [88]. A similar strategy was followed in gastric cancer. Behrooz et al. designed a single-stranded AS1411 together with polyamidoamine (PAMAM)-polyethylene glycol (PEG) complex. PAMAM-PEG-AS1411 complex dramatically increased the uptake of 5-FU by MKN45 gastric cancer cells without toxic effects [89]. PEG conjugated to AS1411 complex was also used to deliver Bcl-xL-specific shRNA and doxorubicin in AGS gastric adenocarcinoma which inhibited the cell growth and enhanced tumoricidal effect of doxorubicin [89]. AS1411 aptamer was conjugated to multimodal nanoparticles also (MFR-AS1411). MFR-AS1411-injected mouse bearing C6 rat glioma cell line was observed for the nanoparticle uptake. Authors suggested that aptamer-nanoparticle complex is a candidate for cancer diagnosis with MR imaging [90]. All these findings concluded that AS1411 is a particularly promising agent for targeted delivery approaches.

2.3.4. Mucin-1

Mucin-1 is a glycoprotein modified by O-glycosylation expressed on epithelial cell surfaces. Its expression is increased tenfold in malignancies such as breast, lung, and colon cancer [91]. Altschuler et al. showed that glycosylated MUC-1 is subjected to clathrin-coated endocytosis as well [92]. Since MUC-1 is a membrane protein and overexpressed in cancer cells with relatively low expression in normal tissue, it is an attractive tumor marker for targeted therapy. Therefore, MUC-1 aptamer, MA3, was developed to selectively deliver anticancer agents to cancer cells such as doxorubicin in vitro. Eighty six base DNA aptamer MA3 was applied to MUC1-positive A549 lung cancer and MUC1-negative HepG2 hepatocellular carcinoma cells [93]. The study showed that MA3-doxorubicin complex selectively delivered doxorubicin to MUC1-positive cells with minimal cross reactivity to albumin. Unlike free doxorubicin which enters cells by passive diffusion, aptamer-doxorubicin chooses receptor-mediated endocytosis in A549 cells [94]. In addition, three more anti-MUC-1 DNA aptamers were selected by Ferreira et al. that can selectively bind breast and pancreatic cancer cells [95]. The efficacy of the aptamer was also tested on ovarian cancer cells in a quantum dot-MUC-1-doxorubicin (QD-MUC-1-Dox) combination. In vivo imaging studies revealed that QD-MUC-1-Dox conjugate in a lower concentration was highly cytotoxic than free doxorubicin even in multidrug-resistant ovarian cancer cells [96]. MUC-1 aptamer was also conjugated to other nanoparticle structures such as mesoporous silica nanoparticles (MSNs). Confocal microscopy studies showed that MDA-MB-231 breast cancer cells that overexpress MUC-1 and MDA-MB-231 tumor-bearing Balb/c mice showed increased MSN-MUC-1 aptamer, but not the same scenario for non-tumorigenic MCF-10A breast epithelial cells. The complex was also used for a successful delivery of doxorubicin
to MCF-7 cells [97]. Another MUC-1-targeted aptamer L3 was combined with doxorubicin and exhibited selective toxicity to MCF-7 breast cancer cells. Most importantly, L3 aptamer was able to evade macrophages [98]. All these results indicated that anti-MUC-1 aptamers have potential for targeted drug delivery, diagnosis, and staging of cancer.

2.3.5. Protein tyrosine kinase 7

Protein tyrosine kinase 7 (PTK7) is one of the members of receptor tyrosine kinase family, also known as colon carcinoma-4 (CCK-4). PTK7 is highly expressed not only in colon but in various human malignancies inducing cell proliferation and metastasis. The first DNA aptamer targeting PTK7 was developed in 2006 by Shangguan et al. as the consequence of a cell SELEX protocol used in cancer biomarker search for acute lymphoblastic leukemia [99]. Forty-one-nucleotide-long aptamer called sgc8c was found selectively bound to PTK7. Subsequent studies are performed by other research groups with sgc8c internalized into PTK7 overexpressing cells [100, 101]. Sgc8c was conjugated with viral capsid protein MS2 linked to AlexaFluor488 in Jurkat T leukemia cells, and its efficient binding was determined by confocal microscopy [102]. Liposomal nanoparticles were also used for PTK7 aptamer delivery into CEM-CCRF cells with low molecular weight dextran as a simulation of drug delivery [103]. Next, researchers have conjugated sgc8 to various chemotherapeutics such as anthracycline for drug delivery experiments. Huang et al. were able to design the aptamer suitable for internalization and transportation to endosome in order to drug release in CCRF-CEM cancer cells [100]. Other anthracyclines were also combined with sgc8 such as daunorubicin which was highly effective to kill PTK7-expressing cancer cells but not PTK7 negative cells [104, 105]. More recently Sgc8 was labeled with a radiochemical, F-18 (\(^{18}\text{F}\)-fluorobenzyl azide). Sgc8-F18 showed high affinity to PTK7 expressing HCT 116 colon cancer and U87MG glioblastoma cell lines. The aptamer was rapidly cleared from the blood through kidneys. The study suggested that this complex may be suitable for aptamer targeting and drug delivery tracking in cancer cells [106]. Consequently, PTK7 targeting aptamers were suggested an effective strategy for the specific uptake of chemotherapeutic drugs and minimize their toxic effects on normal cells.

2.3.6. Epidermal growth factor receptor

Epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase which belongs to the ErbB family of receptor tyrosine kinases. EGFR is also considered as the prototype for receptor-mediated endocytosis. Upon binding with growth factors of EGF family, EGFR dimerization and autophosphorylation occur. Overexpression or mutant receptor expression lead a mitogen signals in various cancer types [107]. The inhibition of EGFR by monoclonal antibodies or small molecules such as cetuximab or erlotinib, respectively, has been shown as an effective strategy in the combat against cancer; however, it is not the case for all tumor types. The ability of ligand binding and endocytosis makes EGFR an ideal target for aptamer-mediated drug delivery. First experiments performed by Li et al. evaluated the EGFR aptamer conjugation with gold nanoparticles targeting human epithelial carcinoma cells line, A431 [108]. EGFR-overexpressing A431 cells were able to internalize the aptamer,
however MDA-MB-453 cells with low level of EGFR expression. Li et al. also suggested the aptamer has high in vivo stability [109]. Although the strategy is promising, currently there is only limited information for EGFR aptamer-mediated targeting.

In conclusion, a number of SELEX method-based aptamers have clinical potential to treat different malignancies as single agents or combination with another classical chemotherapeutics. In addition, the novel drug delivery methods enhanced the target-specific therapeutic potential of aptamers. Although early versions of aptamers exerted stability problems, chemical modifications increased their physiological availability. However, limited number of clinical trials for the treatment of malignancies with specific aptamers is an obstacle for near-future clinical modalities based on aptamers. Due to high specificity with increased stability, aptamers are more potent than monoclonal antibody-based drugs with low cost in cancer therapy.

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