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Tumor-Targeted Drug Delivery with Aptamers

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Abstract

Cancer is one of the leading causes of death around the world. Tumor-targeted drug delivery is one of the major areas in cancer research. Aptamers exhibit many desirable properties for tumor-targeted drug delivery, such as ease of selection and synthesis, high binding affinity and specificity, low immunogenicity, and versatile synthetic accessibility. Over the last several years, aptamers have quickly become a new class of targeting ligands for drug delivery applications. In this review, we will discuss in detail about aptamer-based delivery of chemotherapy drugs (e.g. doxorubicin, docetaxel, daunorubicin, and cisplatin), toxins (e.g. gelonin and various photodynamic therapy agents), and a variety of small interfering RNAs. Although the results are promising which warrants enthusiasm for aptamer-based drug delivery, tumor homing of aptamer-based conjugates after systemic injection has only been achieved in one report. Much remains to be done before aptamer-based drug delivery can reach clinical trials and eventually the day-to-day management of cancer patients. Therefore, future directions and challenges in aptamer-based drug delivery are also discussed.

Keywords

Aptamers; drug delivery; cancer; nanomedicine; theranostics; RNA interference; siRNA

INTRODUCTION

Cancer is one of the leading causes of death around the world. It is estimated that there were about 12.7 million cancer cases and 7.6 million cancer deaths in 2008 [1]. Among these, about 56% of the cases and 64% of the deaths occurred in the economically developing world. In the United States, cancer is the second leading cause of death (<http://www.cdc.gov>). In 2010, a total of 1,529,560 new cancer cases and 569,490 deaths from cancer were estimated to occur [2]. Over the last four decades, tremendous investment and effort has been devoted towards the “War on Cancer”, which has become one of the top priorities in pharmaceutical industry and the National Institutes of Health. With the development of new anti-cancer drugs with better efficacy and fewer side effects, accurate and efficient delivery of these agents to the tumor sites in cancer patients is of utmost importance [3,4]. A recent search of “drug delivery AND cancer” in PubMed returned > 19,000 publications. For successful delivery of anti-cancer drugs, many aspects have to be optimized simultaneously which include encapsulation, targeting, delivery, controlled

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release, among many others. Ideally, a combination of specific targeting and controlled drug release should be able to deliver sufficient doses of cytotoxic drugs to cancer cells (as well as cancer stem cells [5,6]) over an extended period of time without significantly affecting the surrounding normal tissue. Although a large number of anti-cancer drugs have been approved by the Food and Drug Administration (FDA) [7], most of them are not molecularly targeted which can give rise to significant toxicity and side effects.

Aptamers, typically generated through Systematic Evolution of Ligands by EXponential enrichment (SELEX; Fig. (1)) [8,9], have quickly emerged as a novel and powerful class of ligands with excellent potential for diagnostic and therapeutic applications [10]. These single-stranded DNA/RNA oligonucleotides (with a molecular weight of 5–40 kDa) can fold into well-defined 3D structures and bind to their target molecules with high affinity and specificity. To date, aptamers have been selected against a wide range of targets such as proteins, phospholipids, sugars, nucleic acids, whole cells, among others. Since wild-type RNA and DNA molecules can be easily degraded by nucleases, various strategies have been adopted to synthesize aptamers with enhanced in vitro/in vivo stability, such as the use of chemically modified oligonucleotides [11–13], unnatural internucleotide linkages [14], polyethylene glycol (PEG) conjugation [15], Spiegelmers (where the sugars are enantiomers of wild-type nucleic acid sugars) [16,17], among many others [10].

Aptamers possess several advantages over other ligands typically used in drug delivery such as antibodies. First, production of aptamers does not rely on biological systems hence is much easier to scale up with low batch-to-batch variability; Second, aptamers are quite thermally stable and can be denatured and renatured multiple times without significant loss of activity [18]; Third, the smaller size of aptamers than intact antibodies (~150 kDa) can lead to better tissue penetration in solid tumors; Fourth, lack of immunogenicity is another favorable advantage of aptamers over antibodies; Lastly, conjugation chemistry for the attachment of various imaging labels or functional groups to aptamers are orthogonal to nucleic acid chemistry, hence they can be readily introduced during aptamer synthesis. On the other hand, the disadvantages of aptamers include faster excretion than antibodies due to smaller size, potentially weaker binding to targets than antibodies, unpredictable toxicity and other systemic properties, susceptibility to serum degradation when unmodified aptamers are used, and intellectual property-related issues [10]. During the last two decades since aptamers were first selected through SELEX [8,9], Pegaptanib (Pfizer/Eyetech), an aptamer that binds to human vascular endothelial growth factor (VEGF), has been approved by the FDA for clinical use in treating age-related macular degeneration (AMD). A variety of aptamers against other molecular targets are currently in clinical investigation [10].

In this review, we will focus on the use of aptamers for tumor-targeted drug delivery. Although aptamers themselves can be employed for therapeutic applications in various diseases such as cancer, this aspect is out of the scope of this review. Generally speaking, the therapeutic agents that have been delivered using aptamers as the targeting ligands can be categorized into three major classes: chemotherapy drugs, toxins, and small interfering RNAs (siRNAs).

DELIVERY OF CHEMOTHERAPY DRUGS

Metastases are the cause of 90% of human cancer deaths [19,20]. Chemotherapy of cancer metastasis, although effective in some patients, has significant toxicity because of non-specific distribution of the cytotoxic drugs which severely limits the maximum allowable dose. However, rapid elimination and widespread distribution into non-targeted organs/tissues requires the administration of large doses. This vicious cycle of large doses and the concurrent toxicity is a major limitation of current cancer therapy. Sometimes patients

succumb to the adverse effects of the drug far earlier than the tumor burden [21,22]. Therefore, the development of an effective drug delivery strategy can significantly improve metastatic cancer patient management.

Doxorubicin (Dox), an anthracycline antibiotic which can intercalate within the double-stranded CG sequences of DNA and RNA, has been used in treating many cancer types such as hematological malignancies, carcinomas, and soft tissue sarcomas [23–25]. In many cases, the use of Dox can lead to dose-dependent cardiotoxicity such as dilated congestive heart failure and cardiomyopathy [26], which mandates the development of efficient tumor-targeted delivery strategies for Dox. In one report, an aptamer-Dox (Apt-Dox) physical conjugate (via intercalation) was prepared and tested in prostate cancer cells [27]. A10, a 2'-fluoropyrimidine RNA aptamer that binds to the extracellular domain of the prostate specific membrane antigen (PSMA) with a K_d of 2.1 nM [28], was used in this study. Composed of 57 base pairs, the molecular weight of A10 is 18.5 kDa. The Apt-Dox conjugate exhibited good stability in cell culture medium and was shown to target PSMA-expressing cells with high efficiency [27]. It was suggested that this strategy may be employed to develop novel targeted therapeutic agents for more effective cancer chemotherapy in the future.

In another report, Dox was conjugated to a DNA aptamer (termed “sgc8c”) via a hydrazone linker for specific killing of target cells [29]. The sgc8c DNA aptamer, which was selected using human T-cell acute lymphoblastic leukemia cells, can recognize protein tyrosine kinase 7 (PTK7) with nM binding affinity [30]. The high specificity and well-characterized DNA structure of sgc8c makes it capable of distinguishing target leukemia cells from normal human bone marrow aspirate, as well as identifying cancer cells closely related to the target cell line in clinical specimens. It was demonstrated that the sgc8c-Dox conjugate specifically bound to target cancer cells with high affinity, which led to high efficiency cell killing [29]. In some cases, chemical modification may change the binding characteristics of aptamers, as well as the safety and efficacy profile of the drugs. Therefore, Daunorubicin (Dau), another chemotherapy drug of the anthracycline family commonly used in the treatment of leukemia, has been physically bound to the sgc8 aptamer through intercalation instead of chemical linkage [31]. It was found that sgc8, serving as an escort aptamer, could specifically deliver Dau into PTK7-positive cells with high potency.

DELIVERY OF CHEMOTHERAPY DRUGS WITH NANOTECHNOLOGY

Nanotechnology can have a revolutionary impact on imaging and therapy of cancer [32–36]. With sizes smaller than a few hundred nanometers [37,38], several orders of magnitude smaller than human cells, nanomaterials can exhibit properties distinct from both molecules and bulk solids. Thus, they can offer unprecedented interactions with biomolecules both on the surface of and inside the cells, which may result in cancer detection and treatment in ways unimaginable before. Because of the large surface area/loading capacity and versatile chemistry, nanomaterials are excellent carriers for targeted delivery of anti-cancer drugs. Many nano-systems have been developed for systemic cancer therapy, such as liposomes, albumin-based particles, PEGylated proteins, biodegradable polymer-drug composites, polymeric micelles, dendrimers, among others [39]. The combination of nanoparticles and aptamers can have broad applications ranging from diagnostics to targeted delivery of drugs [40–42]. Over the last decade, a variety of nanoparticle-aptamer (NP-Apt) conjugates have been constructed for drug delivery applications.

In one early study, the anti-cancer efficacy of a NP-Apt conjugate (where the A10 RNA aptamer was used as the targeting ligand) was shown both *in vitro* and *in vivo* [43]. Docetaxel (Dtxl; an anti-mitotic chemotherapy drug [44,45]) was encapsulated into a NP-Apt conjugate where the nanoparticle was composed of a biocompatible and biodegradable

copolymer, poly(D, L-lactic-co-glycolic acid)-block-poly(ethylene glycol) (i.e. PLGA-b-PEG). It was found that the NP-Apt-Dtxl conjugate bound to PSMA on the cancer cell surface. Subsequent endocytosis into the cells led to significantly enhanced cellular toxicity than the non-targeted nanoparticles (i.e. NP-Dtxl). In addition, therapeutic efficacy and reduced toxicity were observed in vivo, where a single intratumoral injection of NP-Apt-Dtxl led to significant anti-tumor effect. The enhanced efficacy of NP-Apt-Dtxl over NP-Dtxl was attributed to the intracellular Dtxl release upon PSMA-mediated endocytosis of the former, while NP-Dtxl may release the drug in the extracellular space, thereby causing systemic distribution and absorption, increased toxicity, and decreased efficacy. Subsequently, similar strategy was also used to deliver cisplatin (another chemotherapy drug that can bind to and cause cross-linking of DNA [46]) into cancer cells [47].

In another report, a smart Bi-FRET QD-Apt-Dox conjugate (FRET and QD denote fluorescence resonance energy transfer and quantum dot, respectively) was constructed as both an imaging agent and a drug delivery vehicle (Fig. (2)) [48]. QDs are inorganic fluorescent semiconductor nanoparticles with many desirable properties for imaging applications such as high quantum yields, high molar extinction coefficients, strong resistance to photobleaching and chemical degradation, narrow emission spectra, large effective Stokes shifts, among others [49,50]. The A10 RNA aptamer was attached covalently to the QD surface, while Dox was intercalated in the double-stranded stem region of the A10 aptamer [48]. Since the fluorescence of QD was quenched by the absorbance of Dox and the fluorescence of Dox was quenched by the A10 aptamer, this QD-Apt-Dox conjugate is initially in the fluorescence “OFF” state. After taken up by PSMA-positive cancer cells, Dox was gradually released from the conjugate which led to a recovery of the fluorescence signal from QD and Dox (i.e. the “ON” state). Therefore, this multifunctional QD-Apt-Dox conjugate can be used for not only drug delivery to targeted cells, but also monitoring the delivery/release of the drug and concurrently imaging the target cells. Cytotoxicity of QD-Apt-Dox was found to be significantly higher in PSMA-positive cells as compared to PSMA-negative cells. Subsequently, similar strategies have also been adopted for cancer imaging and therapy applications by several other groups, where the A10 aptamer was attached to various nanoparticles such as thermally cross-linked superparamagnetic iron oxide nanoparticles (TCL-SPION, which could act as both a magnetic resonance contrast agent and a carrier for Dox) and gold nanoparticles (which served as both a contrast agent for computed tomography and a carrier for Dox) [51,52].

Due to the relatively large size and surface area of nanoparticles, dual-targeting can be employed for drug delivery when more than one type of aptamers are attached to the nanoparticle surface. Recently, one such dual-aptamer conjugate was constructed for targeting both PSMA-positive and PSMA-negative prostate cancer cells (Fig. (3)) [53]. Both the A10 RNA aptamer and DUP-1 peptide aptamer (selected against PSMA-negative cells [54]) were conjugated to streptavidin to form a dual-aptamer complex which can enable targeted delivery of drugs to both PSMA-positive and PSMA-negative cells. Dox was loaded on the stem region of the A10 aptamer similar as mentioned above [48]. Furthermore, Dox-loaded A10 RNA aptamer and DUP-1 peptide aptamer were immobilized on the surface of TCL-SPION for targeted delivery of Dox in cell culture. This proof-of-principle study demonstrated that tumor-specific drug delivery to multiple targets can be achieved using this dual-aptamer conjugate, which may potentially also be applied for image-guided drug delivery, as well as monitoring the therapeutic response.

DELIVERY OF TOXINS

Besides targeted delivery of chemotherapy drugs, aptamers have also been used for directing toxins to cancer cells. Gelonin, a ribosome-inactivating toxin with a molecular weight of 28

kDa, can cause cell death by cleaving a specific glycosidic bond in rRNA thereby interrupting protein synthesis [55]. Gelonin has low intrinsic cytotoxicity since it cannot be efficiently internalized into cells by itself. Over the years, gelonin has been conjugated to various antibodies or other proteins for targeted delivery to tumor cells [56–59]. In one study, gelonin was conjugated to an RNA aptamer A9, which also binds to PSMA with nM affinity [60]. The A9-gelonin conjugate exhibited dramatically enhanced potency to cells that express PSMA. Interestingly, it was also found that the A9-gelonin conjugate had 3-fold lower toxicity in PSMA-negative cells than unconjugated gelonin, suggesting that non-specific uptake of gelonin or presentation of the toxin may be inhibited by the A9 aptamer. This study suggested that aptamers may also be used to reduce the side effects of certain toxins or chemotherapy drugs.

Phototoxic-agent conjugated aptamers that can selectively enter and destroy cancer cells were reported [61]. DNA aptamers were selected for specific binding of short O-glycan peptides expressed only on the surface of certain cancer cells, and were further modified at their 5'-ends with chlorin e_6 (a photodynamic therapy agent that can be activated by laser illumination to produce cytotoxic singlet oxygen species [62,63]) via amide bond formation. Upon light activation, the modified aptamers had significantly enhanced toxicity than the drug or aptamer alone in targeted cells, while no toxicity was observed in cells lacking the O-glycan peptides [61].

A similar strategy was adopted to link another photodynamic agent, 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin (TMPyP4), to an AS1411 aptamer which could recognize and bind to nucleolin with high affinity and specificity [64]. Upon crossing the plasma membrane into cancer cells via nucleolin-mediated internalization of AS1411-TMPyP4, significantly higher phototoxicity was observed in nucleolin-expressing cancer cells than the control cells after light exposure (i.e. photodynamic therapy). The fact that AS1411 is currently in Phase II clinical trials as a cancer therapeutic [65,66] may speed up the translation of future AS1411-based conjugates for clinical use.

DELIVERY OF siRNA

Since the initial discovery in 1998, RNA interference (RNAi) has emerged as a powerful tool for therapeutic gene silencing due to its unique specificity, broad applicability, and high efficiency [67]. siRNAs composed of 20–30 nucleotides can lead to the degradation of a specific, targeted mRNA through the RNA-induced silencing complex (RISC) [68,69]. Although the mechanisms and the advantages of RNAi have been elucidated and many clinical trials are ongoing [70–72], clinical use of RNAi still faces a number of challenges. There is an urgent need for more efficient and specific delivery methods to deliver siRNAs to target cells or organs [70,73]. The ultimate goal of achieving RNAi-based therapies cannot be attained without improving the safety, effectiveness, and reliability of the delivery systems for siRNA or small hairpin RNA (shRNA). The attractive characteristics of aptamers, such as small size, high binding affinity/specificity, similar chemical composition as siRNA/shRNA, and desirable stability, make them excellent candidates for targeted delivery of siRNA.

In general, there are two major strategies for attaching siRNA to aptamers: 1) chemical conjugation and 2) covalently linking a siRNA and a RNA aptamer to form an aptamer-siRNA chimera. In a proof-of-principle study, biotinylated A9 aptamer was linked to a biotinylated 27mer anti-lamin A/C siRNA via a modular streptavidin bridge [74]. The disulfide linker between the sense strand of siRNA and biotin in the construct could be cleaved, after entering the cells, to enhance siRNA release. Effective PSMA-mediated internalization and siRNA-mediated inhibition of target gene expression was achieved in

tumor cells with this multivalent construct. Since streptavidin is a tetramer with four binding sites for biotin, it is possible to have more than one kind of aptamers/siRNAs within the construct which can be mixed and matched. However, the immunogenicity of streptavidin may limit its use as a delivery vehicle for siRNA. Less immunogenic streptavidin variants or linkers other than streptavidin would be desirable for future studies.

A truncated form of the A10 RNA aptamer was covalently linked to a siRNA specific for eukaryotic elongation factor 2 (EEF2) mRNA, forming monovalent or bivalent A10-anti-EEF2-siRNA constructs [75]. These conjugates were processed by the enzyme Dicer for siRNA release, after they were internalized by the targeted cells. Both the monovalent and bivalent A10-anti-EEF2-siRNA conjugates induced a sequence-specific knock-down of EEF2 mRNA and protein expression in vitro. The bivalent conjugate, with higher affinity than and same specificity as the monovalent conjugate, was found to be internalized much more efficiently.

A 2'-fluoro-modified A10 RNA aptamer was appended covalently to a 21mer siRNA which could knock down the expression of two survival genes commonly overexpressed in human tumors, polo-like kinase 1 (PLK1) and B-cell lymphoma 2 (BCL2) [76]. The aptamer-siRNA chimeras were shown to selectively enter PSMA-positive cells, effectively silence target gene expression, and lead to depletion of target proteins and cell death both in vitro and in vivo (after intratumoral injection). Specificity of the aptamer-siRNA chimera was confirmed with a number of controls including the use of PSMA-negative cells, a non-silencing siRNA, and a mutated A10 aptamer. It is worth pointing out that although the aptamers contained significant duplex structures, the chimeras did not induce any interferon response, suggesting that this strategy may be able to avoid certain adverse symptoms commonly observed in siRNA-based therapies [77].

To date, most of the aptamer-based anti-cancer agents were only investigated in vitro, with very few conjugates showing any efficacy in vivo. Direct injection of aptamer-based conjugates intratumorally has been reported to be effective in a few studies [43,76]. However, for potential clinical applications, systemic administration will be needed in most scenarios. Generally speaking, much higher therapeutic doses are required for systemic administration than local injection, which can lead to greater risk for harmful side effects due to unwanted exposure of the conjugates to normal non-targeted tissues. Further improvement of aptamer-siRNA chimeras that can minimize the dose and reduce the cost for efficacious anti-cancer therapy is of great value for future clinical investigation.

The abovementioned A10-anti-PLK1 siRNA chimera has been further optimized to achieve enhanced inhibition of prostate cancer xenograft growth after systemic administration (Fig. (4)) [78]. The aptamer portion of the chimera was truncated from 71 to 39 nucleotides, which still exhibited nM binding affinity. Such modification also made the chimera amenable to chemical synthesis because of significantly shorter nucleotide sequence. Furthermore, to increase the gene silencing activity and specificity of the chimera, several additional structural modifications were made to the siRNA to facilitate Dicer recognition, increase silencing specificity, as well as improve loading of the guide strand into the RISC. The optimized aptamer-siRNA chimera exhibited potent efficacy in vivo upon systemic administration, where ~70% of tumors in the treated mice completely regressed by the end of the treatment with no observed toxicity to normal organs. Subsequently, appending a 20 kDa PEG increased both the in vivo circulation half-life (from < 35 min to > 30 h) and serum stability of the chimera, without affecting its targeting and gene silencing capability. As a result, the therapeutic dose of the second-generation aptamer-siRNA chimera was reduced from 10 nmol to 1.25 nmol in 10 days, which not only reduced the cost of treatment but also minimized the risk of harmful side effects. Although the results observed in this

study were very promising, the mechanism regarding how the siRNA escaped from endosomes still remains unclear.

The large surface areas of nanoparticles can allow for the loading of a combination of anti-cancer drugs, which may lead to improved therapeutic efficacy if the drug combination is chosen rationally. In one recent report, co-delivery of Dox and Bcl-xL-specific shRNA was accomplished with the A10 RNA aptamer [79]. Branched polyethyleneimine (PEI) was grafted to PEG and aptamers were conjugated to the surface of the PEI-PEG construct. Dox was intercalated onto the aptamers and Bcl-xL-specific shRNA was complexed with the Dox-intercalated construct (i.e. PEI-PEG-Apt-Dox) through electrostatic interaction to generate the final conjugate. This conjugate efficiently and selectively delivered both shRNA and Dox to PSMA-positive cells through aptamer-mediated binding, which led to synergistic tumoricidal effect.

CONCLUSIONS AND FUTURE PERSPECTIVES

One of the major areas in cancer research is targeted delivery of drugs to cancerous cells, which can not only increase the therapeutic efficacy but also reduce the adverse side effects of the drugs [4,33]. Significant progress has been made over the last two decades since the initial appearance of aptamers. As therapeutics, one aptamer (i.e. Pegaptanib) has been approved by the FDA and many others are in late phase clinical trials. As delivery agents, aptamers have only been investigated for a few years. To date, a variety of anti-cancer agents (e.g. chemotherapy drugs, toxins, and siRNAs) have been successfully delivered to cancer cells in vitro. However, to the best of our knowledge, successful drug delivery to the tumor tissue after systemic injection of aptamer-based conjugates has only been achieved in one report [78]. Much remains to be done before aptamer-based drug delivery can reach clinical trials and eventually the day-to-day management of cancer patients.

The development of more efficient selection methods and easier strategies for aptamer conjugation are needed for potential clinical translation. With more and more academic groups and companies getting involved in this area of research after the earliest aptamer intellectual property expires [80], the development of aptamer-based therapeutics will speed up and aptamers will find their own niches in cancer treatment and bring revolution into targeted drug delivery as well as cancer diagnosis. Besides the three classes of agents mentioned in this review, aptamers can also be used to deliver other anti-cancer agents such as therapeutic radioisotopes (e.g. α -particle emitters such as ^{211}At and ^{225}Ac , β -particle emitters such as ^{90}Y , ^{67}Cu , ^{131}I , ^{177}Lu , ^{186}Re , and ^{188}Re , and Auger electron emitters such as ^{111}In and ^{125}I). Another interesting avenue of future research is to use an aptamer to deliver other aptamers that were selected against intracellular targets (e.g. nuclear factor- κB [81], high mobility group A1 [82], among others).

To date, researchers have identified high-affinity aptamers that target a broad array of protein families including cytokines, proteases, kinases, cell-surface receptors, cell-adhesion molecules, among others [10,83–85]. Since aptamers can be selected against most protein targets, the possible therapeutic applications of aptamer-based drug delivery range far and wide. One disadvantage of aptamers is the hard-to-predict and poorly understood pharmacokinetics, toxicity, and other properties upon systemic delivery. Although aptamers are unlikely to illicit an immune response, at least at the antibody level, the safety profile of each individual aptamer (conjugate) must be examined carefully. Off-target effects due to inhibition/activation of other proteins with similar structural conformations as the target protein need to be investigated empirically since they will likely differ for each aptamer.

The most significant barrier to the widespread use of RNA interference in the clinic is efficient delivery. Solving this problem will require the development of clinically suitable, safe, and effective gene delivery systems. Aptamer-siRNA chimeras represent a platform technology that can be applied to many other aptamers/siRNAs in the future [76,78]. Nanotechnology will also play a key role in the delivery of siRNAs (as well as other anti-cancer agents such as chemotherapy drugs and toxins) in the future [39,86].

There are many challenges facing nanoparticle-based drug delivery, and the most important of all is the (tumor) targeting efficacy. Although passive targeting based on the enhanced permeability and retention effect can be therapeutically efficacious in some cases and many liposome-based drugs have already been approved by the FDA for clinical use [39,87], this is by no means optimal. Long circulation half-life of nanoparticles is a double-edged sword. Although it can lead to higher level of passive targeting to the tumor, it also causes prolonged exposure of normal organs to the drug which can give rise to undesired toxicity. Based on the literature data to date, tumor vasculature targeting is the best bet for nanoparticles since many of these nanoparticles are too large to extravasate [88–91]. A circulation half-life of several hours, rather than a few days or weeks for liposomes, may be sufficient to allow for efficient tumor (vasculature) targeting. Nanoparticles are usually large enough to enable multiple targeting aptamers on their surfaces to simultaneously bind to multiple targets, an aspect that has been virtually unexplored to date. Targeting multiple different, but closely-related, receptors by incorporating different targeting aptamers on the same nanoparticle, with spacers of suitable length, require robust chemistry to minimize batch-to-batch difference and improve the reproducibility. For nanoparticle-based drug delivery, aptamers should be selected against those targets that are expressed on the tumor vasculature, such as integrin $\alpha_v\beta_3$ [90,92,93], VEGF receptors [90,94], CD105/endoglin [95,96], Tie-2 [97,98], c-Met [99,100], etc.

The future of nanomedicine lies in multifunctional nanoplatfroms which combine both therapeutic components and multimodality imaging (Fig. (5)). The integration of diagnostic imaging capability with therapeutic interventions, sometimes termed “theranostics” [101,102], is critical to addressing the challenges of cancer heterogeneity and adaptation. The ultimate goal of nanomedicine is that nanoparticle-based agents can allow for efficient, specific in vivo delivery of therapeutic agents (drugs, genes, radioisotopes, etc.) without systemic toxicity, and the dose delivered as well as the therapeutic efficacy can be accurately measured non-invasively over time. Much remains to be done before this can be a clinical reality. Continuous multidisciplinary efforts on the use/optimization of such nanoplatfroms will shed new light on molecular diagnostics and molecular therapy. With the capacity to provide enormous sensitivity and flexibility, aptamer-conjugated nanoparticles have the potential to profoundly impact anti-cancer drug delivery and patient management in the near future.

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Fig. 1.

A schematic depiction of SELEX (systematic evolution of ligands by exponential enrichment). The target can be either proteins or cancer cells. For cell-based SELEX, typically the nucleic acid library is first incubated with non-target cells. Only unbound nucleic acids are used for selection against the target cells. Typically, aptamer selection can be completed after 10–20 rounds of selection process. Adapted from [42].

Fig. 2.

A QD-Apt-Dox conjugate. **A.** QD-Apt-Dox is initially "off" as the fluorescence of QD is transferred to Dox and the fluorescence of Dox is quenched by the aptamer, both by fluorescence resonance energy transfer. **B.** Once QD-Apt-Dox is inside cancer cells, Dox is gradually released from the conjugate and the fluorescence of QD is recovered. **C.** Microscopy images of PSMA-positive cells after incubation with QD-Apt-Dox. QD and Dox are shown in green and red, respectively. Scale bar: 20 μm . Adapted from [42,48].

Fig. 3. A dual-aptamer (A10 RNA and DUP-1 peptide) probe that can target both PSMA-positive and PSMA-negative cells. The red parts indicate the cells and blue dots represent the stained superparamagnetic iron oxide nanoparticles. Adapted from [53].

Fig. 4.

An improved second generation aptamer-siRNA chimera exhibited excellent anti-cancer efficacy in a xenograft model of prostate cancer. **A.** The first and second generation chimeras. **B.** Bioluminescence imaging of mice bearing luciferase-expressing PSMA-positive tumors showed significantly lower signal after being treated with the second generation aptamer-siRNA chimera as compared to saline control. **C.** H&E staining of tumor tissue after treatment showed readily detectable areas of necrosis (asterisks) in the second generation aptamer-siRNA chimera-treated tumors, but not frequently in saline-treated tumors. TUNEL staining was detected in scattered cells throughout the tumor section of each group. Adapted from [78].

Fig. 5.

A multifunctional nanoplatform incorporating multiple receptor targeting with different aptamers, multimodality imaging, and multiple therapeutic entities. Not all functional moieties will be necessary and only suitably selected components are needed for each individual application. The various functional moieties may be either on the surface of or encapsulated inside the nanoparticle (NP).

Table 1

A brief summary of aptamer-based drug delivery in vitro and in vivo.

Aptamer	Target	Anti-cancer drug	Drug linkage	Delivery vehicle	In vivo/In vitro	Ref.
A10 RNA	PSMA	Dox	Intercalation	A10	In vitro	[27]
Sgc8c DNA	PTK 7	Dox	Hydrazone linker	Sgc8c	In vitro	[29]
Sgc8 DNA	PTK 7	Dau	Intercalation	Sgc8	In vitro	[31]
A10 RNA	PSMA	Doxl	Encapsulation	PLGA-b-PEG NP	In vivo (intratumoral)	[43]
A10 RNA	PSMA	Cisplatin	Encapsulation	PLGA-b-PEG NP	In vitro	[47]
A10 RNA	PSMA	Dox	Intercalation	QD	In vitro	[48]
A10 RNA	PSMA	Dox	Intercalation	TCL-SPION	In vitro	[51]
A10 RNA	PSMA	Dox	Intercalation	Gold NP	In vitro	[52]
A9 RNA	PSMA	Dox	Intercalation	TCL-SPION	In vitro	[53]
A10 RNA & DUP1 Peptide	Prostate cancer	Dox	Intercalation	A9	In vitro	[60]
A9 RNA	PSMA	Gelonin	Covalent	DNA aptamer	In vitro	[61]
DNA aptamer	MUC1 glycoform	Chlorin e ₆	Covalent	DNA aptamer	In vitro	[61]
AS1411	Nucleolin	TMPyP4	Intercalation & outside binding	AS1411	In vitro	[64]
A9 RNA	PSMA	Anti-lamin A/C siRNA	Steptavidin-biotin	A9	In vitro	[74]
Truncated A10 RNA	PSMA	Anti-EEF2 siRNA	Covalent	Truncated A10	In vitro	[75]
A10 RNA	PSMA	Anti-PLK1/BCL2 siRNA	Covalent	A10 RNA	In vivo (Intratumoral)	[76]
Optimized A10 RNA	PSMA	Anti-PLK1 siRNA	Covalent	Optimized A10	In vivo (Systemic)	[78]
A10 RNA	PSMA	Dox and anti-Bcl-xL shRNA	Intercalation & electrostatic	PEI-PEG NP	In vitro	[79]