Review

Nanotechnology and aptamers: applications in drug delivery

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Nucleic acid ligands, also known as aptamers, are a class of macromolecules that are being used in several novel nanobiomedical applications. Aptamers are characterized by high affinity and specificity for their target, a versatile selection process, ease of chemical synthesis and a small physical size, which collectively make them attractive molecules for targeting diseases or as therapeutics. These properties will enable aptamers to facilitate innovative new nanotechnologies with applications in medicine. In this review, we will highlight recent developments in using aptamers in nanotechnology solutions for treating and diagnosing disease.

Introduction

An exciting trend in biotechnology has been the development of targeting ligands that bind to biologically active molecules or receptors that are expressed differentially in diseased cells or tissues. Such targeting ligands can aid in the diagnosis and treatment of disease by, for example, helping to improve the sensitivity and/or specificity of diagnostic assays through molecular imaging [1,2], inhibiting disease processes [3–5] or targeting the delivery of drugs to diseased tissues [6,7]. Aptamers are a class of nucleic acid-based ligands that are characterized by an easy and effective selection method, a relatively fast and cheap production process with minimal batch-to-batch variability, slow degradation kinetics, low toxicity and relatively small size compared with antibodies. Their potential clinical utility might be best appreciated by considering that, despite being discovered less than two decades ago, an aptamer-based therapeutic (known as pegaptanib or Macugen®, marketed by Pfizer) has already received US FDA approval for the treatment of age-related macular degeneration (AMD) [8]. More recently, and still in preclinical development, aptamers have begun to find applications at the interface of nanotechnology and medicine in the form of aptamer–nanoparticle conjugates. In this review, we will introduce aptamer technology and discuss some of the recent preclinical work that aims to unite the two promising fields of aptamers and nanotechnology to improve the management of disease.

Aptamers as targeting ligands

Aptamers are single-stranded DNA or RNA oligonucleotides that fold into well defined 3D structures, which are able to recognize molecules, such as proteins, phospholipids, sugars and nucleic acids, with high affinity and specificity [9]. They range in size from 20 to 80 bases (~6 to 26 kDa). Aptamers can be isolated against most targets (referred to as 'aptamerogenic' targets), even those that are toxic (because, for their selection, they do not have to be injected into an animal) or have a low immunogenicity. Identifying new aptamer sequences can take from weeks to months, depending on the complexity of the selection process. Some of the selection methods have been automated to increase the throughput while also shortening the duration of the selection [10]. Once their sequence is established from the preliminary screening exercises, aptamers can be manufactured to clinical scales. Aptamers are relatively stable under a wide range of buffer conditions and are resistant to physical or chemical degradation, although nuclease activity *in vivo* and hydrolysis at the 2′OH of RNA aptamers can make them susceptible to degradation if appropriate modifications, as described later, are not made. In addition, aptamers are more capable of penetrating tissues than antibodies because of their smaller size. Aptamer-binding sites include grooves and clefts that might be present on their target molecules, similar to the structures that act as binding sites for so-called small-molecule drugs. This gives them highly specific, drug-like capabilities, such as antagonist activity, which can facilitate their integration into target validation and drug-screening programs. Aptamers are also amenable to a wide variety of chemical modifications, such as radioscopic or fluorescent reporters, affinity tags for molecular recognition, 2′ (deoxy)ribose ring modifications, such as 2′F and 2′O-Methyl, or construction from unnatural *L*-nucleotides ('Spiegelmers' [11]) to make aptamers

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0167-7799/$ – see front matter doi:10.1016/j.tibtech.2008.04.006 Available online 19 June 2008

nuclease resistant. It is also possible to chemically modify aptamers to facilitate covalent conjugation to nanomaterials, for example, with 5′ or 3′ amino or thiol groups. Aptamers can be stored long term under appropriate conditions, transported at ambient temperature and can sustain reversible denaturation. From a commercialization perspective, however, aptamers are more complex and more expensive to produce in a modified form compared with other ligand classes, such as peptides, small molecules or sugar moieties. There is also a fairly centralized ownership of the early patents that protect aptamer selection, although more recent patents protect specific compositions of matter (i.e. exact chemical composition) or the uses of aptamers. With the imminent expiration of the early patents and those protecting the proprietary reagents and chemistry used in aptamer synthesis, one can foresee a prospective decrease in the cost of aptamers and a simultaneous decentralization of aptamer-technology commercialization.

SELEX: the aptamer-selection strategy

In 1990, the Szostak [12] and Gold [13] groups independently described a method for identifying aptamers that bind to specific targets. This method, called '*in vitro* selection' or 'systematic evolution of ligands by exponential enrichment' (SELEX), took the core concepts of natural evolution – diversification, selection and replication – and developed a procedure based on these concepts that would result in the relatively rapid isolation of RNA ligands. Szostak and Gold hypothesized that, if a very large ($>10^{10}$) number of random sequences of RNA with unique tertiary conformation were subjected to a selection pressure in parallel, some of those sequences would have binding properties. The result was the establishment of SELEX as a new tool for developing nucleic acids that bind highly specifically to target molecules.

Over the years, SELEX has undergone several generations of refinements and modifications [14]. Aptamers can now be selected as either DNA or RNA and under conditions that match the environment of the intended application more closely. More automated selection processes are also possible. In this section, we will demonstrate how the concept of SELEX can be applied creatively to design more effective aptamer-guided nanomaterials. Although many complex forms of SELEX exist, we will focus on the basic fundamentals and on two forms of SELEX, Cell-SELEX and Automated SELEX, which are particularly interesting for targeted drug delivery with nanoparticles owing to their direct relevance for developing disease-targeting agents.

The SELEX process

In a typical SELEX protocol, the initial oligonucleotide selection pool (for either DNA or RNA aptamers) consists of DNA sequences that contain a 30–60-nucleotide variable region flanked by fixed regions on the 5′ and 3′ ends, which consist of 15–30 nucleotides to enable PCR amplification. In the case of DNA aptamer isolation, single-stranded DNA (ssDNA) oligonucleotides are required for the initial round of incubation with the target. Separation of the two strands of DNA is typically achieved by biotinylating the 3′ primer so that it can be immobilized and separated on streptavidin beads, followed by chemical or thermal denaturation. The pool of ssDNA is then incubated with the target and bound ssDNA sequences are collected, purified and amplified by PCR. The partitioned DNA (i.e. all selected DNA sequences) is then used for the next round and the process is repeated iteratively over several rounds.

For RNA aptamer isolation, the 5′ primer, which typically contains a T7 RNA polymerase-promoter sequence, is annealed with the 3′ primer and a dsDNA template is usually generated by Klenow fragment extension or several PCR cycles. The dsDNA is then transcribed to single-stranded RNA (ssRNA), which is available to interact with the target. At the end of each round, the 3′ primer is annealed to RNA in the presence of reverse transcriptase (RT) to create a cDNA (cDNA), which will be amplified by PCR to generate dsDNA. Please see Fitzwater [15] for a more detailed description of these techniques.

Cell-SELEX

The objective of a Cell-SELEX process is to develop an aptamer that will bind specifically to a particular target cell population (Figure 1). Cell-SELEX, or the similar process of 'target expressed on the cell surface SELEX' (TECS-SELEX), can be used to develop aptamers that are able to target nanomaterials to diseased cells by yielding ligands that bind preferentially to diseased cells compared with normal cells. Cell-SELEX was used successfully to develop an aptamer against glioblastoma, one of the most common of the human brain cancers, which bound to the extracellular matrix glycoprotein tenascin-C [16]. More recently, Cell-SELEX was applied to isolate aptamers that were able to inhibit the rearranged during transfection (RET) receptor tyrosine kinase, an oncoprotein implicated in various neoplasias [17].

As mentioned earlier, Cell-SELEX could improve the targeting of nanoparticle–aptamer conjugates to diseased cells. In addition, this selection process also has the potential to yield aptamer ligands that might be able to improve intracellular delivery of nanoparticles. For example, one could hypothesize that a counter-selection step can be included, which would eliminate those aptamers that bind to targets that cannot escape the endosome, thereby making it possible to enrich the pool of aptamers that are able to escape endosomal degradation and hence result in increased cytosolic delivery. Delivery to the cytosol is a primary challenge for achieving therapeutic efficacy using short-interfering RNAs (siRNA) as drugs. These have become the center of recent interest from the medical community because of their potential therapeutic value. However, even if aptamers were found that could facilitate delivery into the cytosol, aptamer function in the context of a nanomaterial would have to be re-validated and confirmed. Developing feasible strategies for delivering aptamers across the cell membrane is an important area of research in which innovation and ingenuity might facilitate interesting and novel applications of nanotechnology.

A recent promising approach using Cell-SELEX was used by the Tan group for the generation of aptamer molecular probes for the detection, characterization and isolation of tumor cells [18,19]. The authors were able to

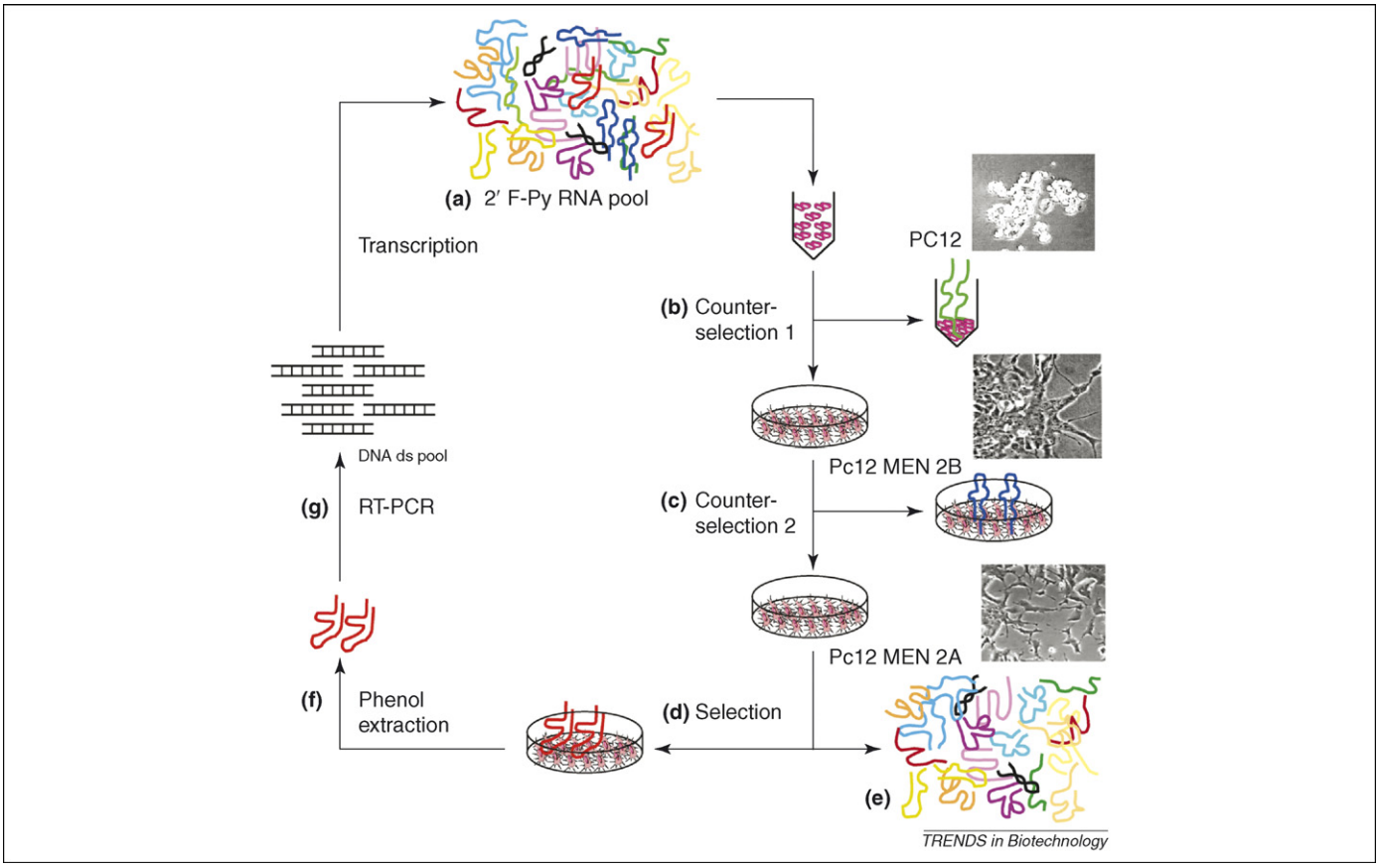

Figure 1. Example of a SELEX protocol used to develop aptamers against live cells. **(a)** A pool of 2′F-Py RNAs was incubated with suspended parental PC12 cells in a set of counter-selection steps **(b,c)**. The counter-selection steps served to eliminate oligonucleotides with affinity for motifs that are present on non-targeted cells, as well as those with affinity for other epitopes present in the tissue culture system, including the flasks and materials. (c) Unbound sequences yielded from the first counter-selection step are recovered by centrifugation and incubated with adherent PC12/MEN2B cells in a second counter-selection step. **(d)** Resulting unbound sequences in the supernatant were recovered and incubated with adherent PC12/MEN2A for the selection step. **(e)** Unbound sequences were discarded by several washing steps. **(f)** Bound sequences were recovered by phenol extraction. **(g)** Sequences enriched by the selection step were amplified by RT–PCR and *in vitro* transcription before a new cycle of selection was imitated. Adapted, with permission, from Ref. [17].

identify a panel of cell-specific aptamers against a T-cell acute lymphoblastic leukemia cell line, CCRF-CEM. Using fluorescently labeled aptamers and flow cytometry, target cells within biological samples, such as bone-marrow aspirates, could be identified. Subsequently, the internalization of these aptamers into leukemic T cells could be verified [20].

Automated SELEX

For nanotechnology applications for which a validated target protein already exists but no suitable aptamer has yet been selected, automated SELEX might be used to generate a novel aptamer-targeting ligand. Automated SELEX, as its name implies, is the automation of the multiple steps required to produce an aptamer from an appropriate starting material. The relative ease of this method as compared with the other more complex selection methods (for aptamers as well as other targeting ligand types: peptides, antibodies, etc.) makes it particularly interesting in the context of targeting nanomaterials. *In vitro* selection of aptamers can be a complex procedure that involves iterative rounds of preparation and purification of DNA or RNA, filtration of RNA–target complexes, reverse transcription (for RNA) or ssDNA separation (for DNA) and PCR amplification of selected species. Given that, typically, 5–20 rounds of selection are required to generate aptamers, most *in vitro* selection experiments proved to be extremely time consuming, taking months to complete. Furthermore, SELEX usually requires unique manipulations at each step that appear difficult to perform in an automated procedure. Despite these challenges, Ellington and colleagues [10] were the first to automate a typical *in vitro* selection protocol using a specially modified Beckman Biomek 2000 automated workstation and, subsequently, others have followed with their own procedures [21,22]. Using automated SELEX might contribute to a more rapid development of aptamers for targeted delivery applications by increasing the possibility of selecting aptamers in a high-throughput format.

Aptamers in nanotechnology

One of the most exciting applications for aptamers today is in developing nanotechnology approaches to diagnose or treat diseases. Aptamers are relatively small; therefore, they are especially useful in nanotechnology applications because the transport properties of molecules and particles through the microvasculature, as well as through tumor interstitium, depend strongly and inversely on size. As a solution, aptamers have a radius of gyration of only a few nanometers [23] and can therefore impart cell or tissue

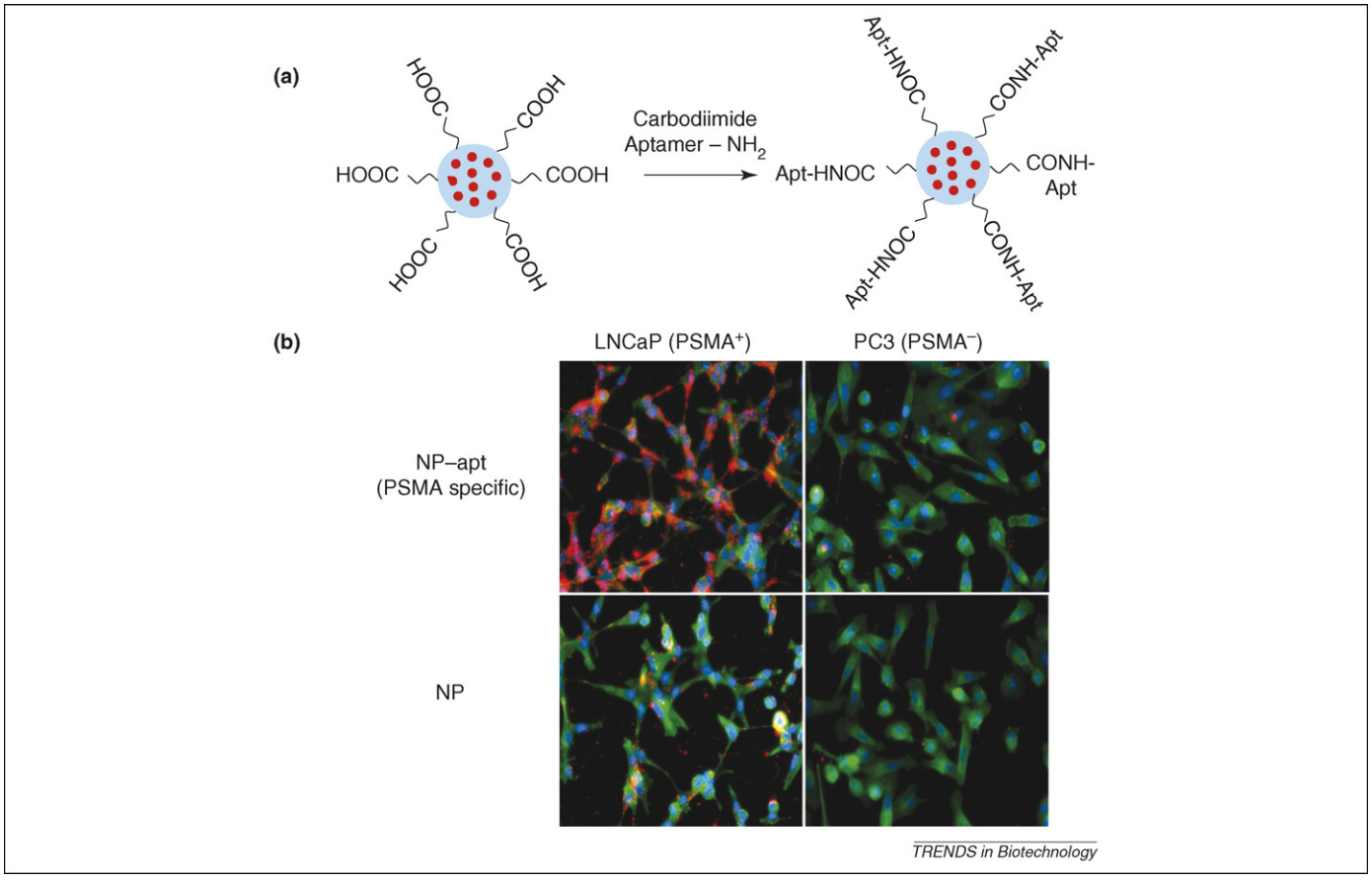

Figure 2. Nanoparticle–aptamer conjugate formation and cell-specific uptake. **(a)** Illustration of a nanoparticle that contains a fluorescent dye (in red) that is decorated on the surface with polymers containing terminal carboxyl groups. The amine-modification to the RNA aptamer molecule can be used to form nanoparticle–aptamer conjugates using carbodiimide chemistry. **(b)** Fluorescent nanoparticles (encapsulating rhodamine-labeled dextran) that are linked to RNA aptamers specific for the PSMA (NP–apt) were internalized into PSMA-expressing LNCaP cells ($PSMA^+$) but not PSMA-non-expressing PC3 cells ($PSMA^-$) after incubation for 16 h. The nucleus is stained in blue [4′,6-diamidino-2-phenylindole (DAPI)] and the cytoskeleton in green (adapted, with permission, from Ref. [35]). Abbreviations: Apt, aptamer; NP, nanoparticle.

selectivity while they contribute little to the overall size of a drug delivery device. In addition, aptamers can be synthesized chemically (unlike antibodies) and this makes it relatively simple to attach functional groups to their ends, which could then be used to link them covalently to nanomaterials, as shown in Figure 2a. In addition, aptamers are amenable to manipulation steps after binding. For example, the addition of an antidote that changes the conformation of an aptamer can result in release of the target. This technology was used in the context of finely controlling the degree of anticoagulation of patients who require anticoagulation therapy, as reported recently in Phase I trials [24]. However, it is worth mentioning that many of the advantages of aptamers discussed here are also common to other targeting ligands, such as peptides, small antibody fragments or small molecules. Indeed, large efforts of any targeting approach usually go into selecting the best targeting molecule for a particular application. From a scientific perspective, major factors that have to be considered are ligand size, charge, binding affinity and specificity, stability and any other relevant properties, such as antibody-dependent cell-mediated cytotoxicity and potential complement activation in the case of antibodies. From a practical perspective, other additional factors are important, such as the cost of production, ability to obtain intellectual property, ease of manufacture and scale-up, stability under manufacturing conditions (e.g. non-aqueous solvents, high temperatures) and ease of required modifications and conjugations (e.g. to nanomaterials).

Nanoparticle–aptamer conjugates

The variety of targets that can be bound by aptamers enables a wide array of drug delivery applications. Representative examples noted here include aptamers binding thrombin [9], hepatitis C virus proteins [25], HIV-1 [26,27], phospholipase A_2 ($sPLA_2$) [28], basic fibroblast growth factor (bFGF) [29], human thyroid-stimulating hormone (hTSH) and prions [30]. In addition, aptamers have been isolated to block autoimmune antibodies that bind to the human insulin receptor [31], which might have important implications for the treatment of insulin resistance. Each of these examples has the potential to open up new treatment methods for a variety of diseases, based on targeted nanotechnology, in which aptamers would be used to interrupt disease pathogenesis. However, the most important success of aptamers so far has been the development of aptamers that are able to bind vascular endothelial growth factor (VEGF) [32], a protein involved angiogenesis. This aptamer is now US FDA approved in the form of an intraocular injection for the treatment of AMD, a condition that can be slowed by reducing angiogenesis through

the inhibition of VEGF activity. This example also re-emphasizes an important potential of aptamers – that they can act as inhibitors in their own right (similar to antibodies) without the need for associated toxins. However, additional long-term data are needed to assess the potential toxicity and immunogenicity of aptamers that have been injected into the systemic circulation intravenously. Aptamers for medical applications, other than the US FDA-approved VEGF, are also making their way to the clinic and this has been reviewed recently elsewhere [33].

The development of nanoparticles that are loaded with drugs has been a novel and promising strategy for drug delivery, as demonstrated by recent preclinical studies [34]. Aptamers that were conjugated to nanoparticles resulted in increased targeting and more efficient therapeutics, as well as more selective diagnostics. For example, nanoparticle–aptamer (NP–Apt) conjugates of the A10 aptamer could be developed to target the prostate-specific membrane antigen (PSMA) [35] (Figure 2), a transmembrane protein that is upregulated in prostate cancer [36]. This was the first report of an aptamer that enabled the targeted delivery of drug-encapsulated nanoparticles. This formulation was further evaluated *in vivo* in a flank tumor model of LNCaP (PSMA-positive) prostate carcinoma and reduced tumor size effectively over a 109-day study, following a single intratumor injection [6]. Other reports that used NP–Apt conjugates include a colorimetric assay for the presence of platelet-derived growth factor (PDGF, which is over-expressed in some proliferative disorders) and its receptor using aptamer–gold nanoparticle conjugates [37], as well as aptamer-functionalized liposomes [38]. Aptamer–toxin conjugates have also been investigated as cancer therapeutics. In one study [23], the A9 aptamer was conjugated to Gelonin, a ribosomal toxin that causes cell death by cleaving a specific glycosidic bond in rRNA, thereby disrupting protein synthesis. The aptamer–toxin conjugates displayed more than 600-fold increased potency in inducing cell death compared with cells that do not express PSMA and, furthermore, the targeted toxin was considerably less toxic in non-targeted (PMSA-non-expressing) cells. In addition, aptamers have been used as vehicles to deliver anthracycline chemotherapeutics through their intercalation within the aptamer double-stranded regions [2]. This concept has enabled the engineering of polymeric nanoparticle systems to co-deliver anthracycline and taxane drugs in a temporally distinct manner [39]. Furthermore, by exploiting resonant-energy transfers occurring between anthracyclines and quantum dots, the production of quantum dot–aptamer bioconjugates that could not only detect cancer cells but could also detect whether the drug had been delivered was made possible [40]. Tumor imaging can be achieved with quantum dot–aptamer conjugates [41]. In this example, aptamers that bind to the PSMA were conjugated to luminescent CdSe and CdTe nanocrystals to be used as biosensors. This conjugate specifically targeted both fixed and live cells that over-expressed PSMA and were also able to label cells dispersed in a collagen-gel matrix, similar to live tissue. Aptamers have also been used in developing smart nanostructures that can detect the presence of analytes in simple dipstick-like devices that are reminiscent of home pregnancy tests [42]. Aptamers have also been immobilized on carbon-nanotube supports to capture and hence to detect the presence of analytes [43].

Aptamers in the molecular recognition of cancer

The molecular characterization of tumor cells is important not only for cancer diagnosis but it is also crucial for choosing the best possible therapy. Nanotechnology-based approaches for cancer imaging and therapy have demonstrated their potential in a variety of preclinical studies using aptamers and have already found broad application as therapeutic agents [44], markers for molecular recognition [19] and elements in nanotechnological assemblies [39].

A large number of aptamers have been raised against cancer-associated antigens (Table 1) and a representative list of such antigens includes pigpen [45] for targeting the tumor microvasculature, PSMA [36] for targeting prostate cancer and mucin 1 (MUC1) [46] for targeting various epithelial neoplasms that upregulate MUC1, whose expression has been associated with carcinomas. *In vivo* tumor targeting by fluorescently labeled aptamers could be demonstrated for glioblastoma and breast cancer xenografts, in which tenascin-C, an extracellular matrix protein that is up-regulated in tumor growth [1], was targeted. In addition, a recently developed protocol that enabled parental PC12 cells (cells that stop dividing and are

Table 1. Use of aptamers for binding cancer-related targets

Aptamer target protein or molecule	Application	Refs
PSMA	Prostate cancer diagnosis and therapy	[7,36,40,51,52]
WT1	Understanding Wilm's tumor pathogenesis	[53]
4,4′-methylenedianiline	Detecting DNA-damaging compounds	[54]
VEGF	Inhibiting angiogenesis	[8,32,38,55–57]
RET	Inhibition of pro-growth signaling	[17]
HER-3	Reducing drug resistance in HER-2$^+$ cancers	[58]
TCF-1	Colon cancer growth inhibition	[59]
Tenascin-C	Glioblastoma (brain cancer) detection	[1,16]
MUC1	Breast, pancreatic, ovarian cancers; targeting demonstrated	[46]
PDGF/PDGFR	Improving transport to tumors and targeting brain cancers	[37,60–64]
NF-κB	Targeting a transcription factor implicated in many diseases	[23]
Phosphatidylcholine:cholesterol liposomes	Triggering liposome degradation	[65]
Raf-1	Inhibiting pro-growth signaling	[66,67]
$\alpha_v\beta_3$ integrin	Targeting tumor-associated vasculature	[3]
Human keratinocyte growth factor	Inhibiting pro-growth signaling	[68]

Abbreviations: HER-3, human epidermal growth factor receptor 3; TCF-1, transcription factor 1; WT1, Wilm's tumor protein 1.

terminally differentiated) to be distinguished from cells that had undergone differentiation by using a subtractive SELEX technique sets the stage for being able to differentiate cancerous from normal cells [47].

Aptamers have helped in finding new approaches for the diagnosis of cancer. For example, TTA1, a modified RNA aptamer targeted against tenascin-C, was conjugated to the nuclear-imaging tracer isotope ^{99m}Tc for the purpose of performing single-photon emission-computed tomography (SPECT) [1], a 3D-imaging technique that can aid in visualizing tumors. Tenascin-C is known to be over-expressed in several tumors, including carcinoma of the lung, breast, prostate and colon, as well as lymphomas, sarcomas, glioblastomas and melanomas. Using murine xenograft models, Hicke and colleagues showed that TTA1-^{99m}Tc has rapid tumor penetration and blood clearance. Tumor retention was durable and there was high tumor-to-blood signal. Thus, by using TTA1-^{99m}Tc, Hicke and others imaged tumors in murine models of glioblastoma and breast cancer successfully.

To date, several aptamers for cancer therapy are in clinical trials. Toll-like receptor 9 (TLR9), which is expressed by certain immune cells, functions by detecting the presence of DNA (unmethylated CpG) in endosomes. The interaction of DNA with TLR9 in endosomes is thought to contribute to antitumor immunity. Two aptamers, CPG 7909 (Coley Pharmaceutical Group) and IMO 2055 (Idera Pharmaceuticals), that target TLR9 have been isolated. Phase II data for CPG 7909 showed stimulation of innate immune responses consistent with the proposed mode of action of the aptamer, with some patients showing a positive clinical response [48]. IMO 2055 is being evaluated currently in Phase I or II trials in combination with other drugs for non-small cell lung cancer and renal-cell carcinoma (www.iderapharma.com). Another example, nucleolin, an intracellular protein that is over-expressed on cell surfaces by tumor cells, has also been a target for aptamers in cancer therapy. AGRO100, also known as AS1411 (Antisoma), is an aptamer that is able to bind to nucleolin and it is in Phase II clinical trials currently as a cancer therapeutic [33]. AS1411 was proposed to function by inducing cell-cycle arrest, DNA-replication inhibition and the initiation of apoptosis. These effects appear to have been mediated by inhibition of the NF-κB signaling pathway [44].

Aptamer-targeted RNAi

The promise of RNA interference (RNAi)-based therapeutics has been demonstrated in several preclinical studies (for reviews, see [49,50]). However, one of the most important technical hurdles for successful RNAi therapy is the delivery of siRNA to target cells. Using anti-PSMA aptamers, two groups were able to form aptamer–siRNA conjugates for successful delivery of siRNA into tumor cells. In one study, the anti-PSMA aptamer (known as A9) was conjugated to an anti-lamin A/C siRNA using streptavidin as a modular bridge [51] and this resulted in aptamer-mediated siRNA delivery to the prostate tumor cells. The delivered siRNA was able to inhibit gene expression as efficiently as conventional lipid-based transfection reagents. In another study, aptamer–siRNA chimeras were generated for targeted delivery of siRNA [7]. The aptamer part of the chimera consisted of the A10 anti-PSMA aptamer and the siRNA component targeted the expression of tumor-survival genes. When these chimeras were internalized by the targeted cells, they were processed by the dsRNA-processing enzyme Dicer to release the siRNA portion of the chimera. Using murine xenograft models of prostate cancer, the authors were able to show that tumor-growth inhibition and regression were mediated specifically by the aptamer–siRNA chimeras. Although the results were positive, it is unclear how the siRNA was able to escape the endosome after internalization by means of the aptamer-targeting molecules. This issue remains a focus for future investigations.

Conclusion and future perspectives

The Gold [13] and Szostak [12] groups, 17 years ago, developed the SELEX method concurrently for isolating nucleic acids for molecular targeting. Since then, aptamers have been used in a wide variety of applications in medicine and nanotechnology, including US FDA approval for the AMD drug pegaptanib (Macugen). Recent advances in cancer nanotechnology have underscored the potential of molecular targeting in both diagnosing and treating disease. Their high binding sensitivity and specificity, their small size and their ease of identification by *in vitro* selection protocols (SELEX) make aptamers highly attractive for a variety of uses in molecular targeting. Aptamers are also particularly well suited for nanotechnology applications because they can be modified readily with functional groups that facilitate conjugation to nanomaterials. Innovative new selection protocols for aptamers, such as Cell-SELEX, or the rapid automated-SELEX selection protocols, provide the nanotechnologist with more versatile methods of selecting specific binding ligands. In addition, given today's widespread applications of antibodies, especially in cancer therapy, aptamers – as a similar but distinct technology – might further stimulate new diagnostic and therapeutic nanotechnologies in the near future.

In parallel to finding new aptamers, research being undertaken through the Human Proteome Organization (www.hupo.org) towards identifying target proteins for various disease states promises to develop a catalog of potential antigens for targeted therapy. The newly launched Swiss-Prot Current Science collaboration, called the Targeted Proteins Database (TPdb), has the similar aim of identifying all known proteins that have clear links to human disease (www.targetedproteinsdb.com). These efforts, which are focused on identifying relevant protein targets, could then be exploited for the development of relevant aptamer ligands for their use in nanotechnological assemblies directed against several diseases and conditions. These initiatives are therefore setting the stage for the emergence of exciting novel aptamer-enabled technologies.

Acknowledgements

This work is supported by USA National Institute of Health grant CA119349 (R.L. and O.C.F.) and EB003647 (O.C.F.). E.L-N. was supported by a postdoctoral fellowship from the USA Department of

Defense Prostate Cancer Research Program PtdCho 051156. A.F.R-M. would like to acknowledge support from the National Science Foundation Graduate Research Fellowship Program.

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