The future of aptamers in medicine

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ABSTRACT

Aptamers, simply described as chemical antibodies, are synthetic oligonucleotide ligands or peptides that can be isolated in vitro against diverse targets including toxins, bacterial and viral proteins, virus-infected cells, cancer cells and whole pathogenic microorganisms. Aptamers assume a defined three-dimensional structure and generally bind functional sites on their respective targets. They possess the molecular recognition properties of monoclonal antibodies in terms of their high affinity and specificity. The applications of aptamers range from diagnostics and biosensing, target validation, targeted drug delivery, therapeutics, templates for rational drug design to biochemical screening of small molecule leads compounds. This review describes recent progress made in the application of biomedically relevant aptamers and relates them to their future clinical prospects.

INTRODUCTION

Aptamers are nucleic acid ligands or peptides that can be isolated against a variety of targets (table 1) from massively complex combinatorial libraries by an iterative in vitro process called systematic evolution of ligands by exponential enrichment (SELEX). The SELEX process and its subsequent modifications have been extensively described in several primary research articles[1 2 22 38–47] and reviews,[34 48–54] hence I will not dwell on describing SELEX in this review. Since the seminal SELEX experiments, which were reported in 1990,[38–40] a large number of technical developments have been made that automated[42 55] and subsequently reduced SELEX to a single-step selection process called MonoLex.[46] The SELEX process and its subsequent technical innovations, which also include capillary electrophoresis[43] and microfluidic[44 45] SELEX, have increased the power and utility of the aptamer technology.[50]

Aptamers offer advantages over antibodies and other conventional molecules as they can be entirely produced in a test tube using enzymes, or can be readily produced by chemical synthesis within days rather than by tedious biological expression used to make antibodies. The in vitro process or chemical synthesis used to make aptamers represents a rapid, low cost and less batch-to-batch variation process than the in vivo process used for production of antibodies. Furthermore, toxins and molecules that do not elicit a good immune response and are not suitable targets for immunotherapy can be used as targets for the generation of high-affinity aptamers.

Another advantage of aptamers is that they elicit no immunogenicity,[56] are not toxic in therapeutic applications,[56–58] and can be modified to increase their stability[59 60] in biological environments. For instance, the stability in serum of the 2′-amino-modified anti-basic fibroblast growth factor aptamers was increased at least 1000-fold relative to unsubstituted RNA,[61] while modified anti-vascular endothelial growth factor (anti-VEGF) aptamer could survive for up to 17 h in urine.[62] Solid-phase synthesis of a specific aptamer also allows post-SELEX modification such as addition of 2′-oxymethyl groups that are not compatible with in vitro transcription. The resulting aptamers are typically stable in human plasma for 15–24 h at 37°C.[63] Conjugation of aptamers to either lipids or polymers such as polyethylene glycol improves their stability and distribution kinetics sufficient to produce therapeutic effects.[64–66]

The molecular recognition properties of aptamers are very similar to antibodies, which recognise a target with high affinity and specificity and in many cases effectively inhibit its function. Some of the best aptamers form complexes that have dissociation constants in the picomolar range,[61 62 67] while many have dissociation constants that are similar to the antigen-binding fragment of antibodies.[68] In terms of selectivity, aptamers can discriminate between very subtle structural differences, such as the presence or absence of a hydroxyl group or structural enantiomers (mirror images that have an identical chemical composition) of the target. Due to their relatively small size compared with antibodies, aptamers can fit into clefts where bulky molecules such as antibodies would otherwise be excluded. Their flexibility allows them to fold and assume the shape of relatively small binding pockets, thereby maximising surface contact with the target protein. These desirable properties of aptamers, combining the optimal characteristics of small molecules and antibodies, show great promise and have opened avenues for the development of therapeutic, antiviral, diagnostic and targeted drug delivery tools (table 1) in areas that have been hitherto refractory to other approaches.

THERAPEUTIC POTENTIAL OF APTAMERS

Aptamers achieved a major milestone in clinical therapy, on the 20 December 2004, when pegaptanib sodium—an anti-VEGF 165 ($VEGF_{165}$) aptamer developed by Pfizer and Eyetech[56 57] and marketed as Macugen—was approved by the US Food and Drug Administration for the treatment of age-related macular degeneration.[69] Other research groups are developing aptamers as therapeutics in a variety of indications: the treatment of cancer,[35 70] inhibiting proteins involved in Alzheimer disease,[71] against aberrantly folded pathological isoforms of prion proteins[72 73] that cause Creutzfeldt–Jakob disease, against *Mycobacterium tuberculosis*,[11] and against hepatitis C virus (HCV)[9 10 74 75] and several other viruses (reviewed by Gopinath[36]) including

Table 1 Recent examples and potential applications of aptamers isolated against various biomedically relevant targets

Target class	Target name*	Assayed activity of aptamers	Potential applications
Viral	HIV-1 gp120	(a) Neutralises HIV-1 infectivity[1] (b) Targeted delivery and dual inhibition of HIV-1[2 3] (c) High affinity to gp120[4]	(a) HIV entry inhibitor drugs[1] (b) Targeted delivery of siRNA into HIV-infected cells[2 3] (c) Microarray-based HIV-1 diagnostic[4]
	HIV-1 RT	(a) Inhibits drug-resistant RT[5–7] (b) Used to discover a small molecule that inhibits multidrug-resistant RT[8]	(a) HTS and identification of small molecule inhibitors of RT[8] (b) Second-generation RT inhibitor drugs[5–7]
	HCV	(a) Inhibits replication of HCV[9 10]	(a) HCV therapeutics
Bacterial	*M tuberculosis*	(a) Improves the survival of mice challenged with *M tuberculosis*[11] (b) High sensitivity and specificity to MPT64 protein of *M tuberculosis*[12]	(a) Antimycobacterial agent[11] (b) *M tuberculosis* diagnostic[12]
	Staphylococcus	(a) High specificity and affinity to *S aureus*[13]	(a) *S aureus* diagnostic[13]
	Botulinum neurotoxin	(a) Detects botulinum neurotoxin rapidly and with high sensitivity[14]	(a) Botulinum neurotoxin diagnostic[14]
Protozoan parasite	*Trypanosoma*	(a) Inhibits cell invasion[15]	(a) Prophylaxis for Chagas disease[15]
Human cytokines, chemokines, hormones and growth factors	VEGF	(a) High affinity to VEGF[16] (b) Inhibits angiogenesis[17]	(a) Cancer diagnostic[16] (b) Angiogenesis treatment[17]
	PDGF	Reduces retinal detachment in mice[18]	Treatment of ischaemic retinopathies[18]
	EGF	Inhibits tyrosine phosphorylation[19]	Lead compound for treatment of carcinomas[19]
	Insulin	Detects and measures insulin activity in solution[20]	Type 1 diabetes diagnostics
	Osteopontin	Decreased progression and metastases of breast cance.[21]	Anticancer drug[21]
Human cells, adhesion molecules, receptors and other cell surface proteins	Leukaemia cells	(a) High specificity to leukaemia cells in clinical specimens[22 23] (b) Targeted delivery of anticancer drug to leukaemia cells[24]	(a) Leukaemia diagnostic[22 23] (b) Targeted drug delivery to leukaemia[24]
	PSMA	(a) Targets delivery of anticancer drug inside nanoparticles to prostate cancer cells[25] (b) High sensitivity and specificity to PSMA[26]	(a) Targeted drug delivery to prostate cancer (b) Prostate cancer diagnostic[26]
	Nucleolin	Destabilises breast cancer cells[27]	Anticancer drug[27]
	O-glycan-peptide	Selectively kills epithelial cancer cells[28]	Epithelial cancer drug[28]
	EGFRVIII	Induces apoptosis of glioblastoma cells[29]	Targeted therapy for brain tumours[29]
Human coagulation components	APC	Selectively binds to APC with high affinity[30]	Potential therapeutic agent[30]
	VWF	Inhibits platelet aggregation[31 32]	Antithrombosis drug[31 32]
	Haem	Inhibits growth of malaria parasite[33]	Antimalaria drug[33]

*A comprehensive online database cataloguing a range of targets used to isolate aptamers is available (The Ellington Lab Aptamer Database: http://aptamer.icmb.utexas.edu/). In addition, reviews by Yan *et al*,[34] Cerchia *et al*,[35] Gopinath *et al*[36] and Held *et al*[37] contain comprehensive lists of aptamers isolated against various biomedically relevant targets published in the 1990s, various cancer targets identified in 1990–2002, and various viral and HIV-1 targets identified before 2007, respectively.
APC, activated protein C; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; HCV, hepatitis C virus; *M tuberculosis*, *Mycobacterium tuberculosis*; PDGF, platelet-derived growth factor; PSMA, prostate-specific membrane antigen; RT, reverse transcriptase; *S aureus*; *Staphylococcus aureus*; VEGF, vascular endothelial growth factor; VWF, von Willebrand factor.

various stages in the HIV-1 replication cycle (reviewed by Held *et al*[37]).

Within the HIV-1 life cycle, there are several key targets that are suitable for the generation of therapeutic aptamers. Aptamers that bind a variety of intracellular HIV-1 proteins, such as reverse transcriptase (RT),[5–7 76] Rev,[77 78] Tat[79] and integrase,[80] have been generated. Unlike antibodies, aptamers can fold properly and retain activity in the intracellular environment. However, the majority of aptamers with potential therapeutic utility selected to date target extracellular proteins.[19 21 56 57 62 81–89] Extracellular therapeutic targets such as HIV-1 gp120 have the advantage of ready access to aptamer intervention without the need for enabling access to cells. The aptamers against HIV-1 gp120 bind the protein with high affinity and high specificity, and neutralise a broad range of R5 HIV-1 clinical isolates.[1] In a recent review, these RNA aptamers were reported to have the most potent in vitro antiviral efficacy of all HIV-1 entry inhibitors described to date,[37] including antibodies. These aptamers prevented entry and suppressed viral replication in cultured human peripheral blood mononuclear cells by up to 10 000-fold.[1] While the emergence of HIV-1 escape mutants and drug-resistance variants appears to be inevitable, one extensively studied aptamer called B40 has recently been shown to penetrate the highly variable exterior surfaces of gp120 and bind the conserved core at the heart of the CCR5-binding site,[90 91] which the virus may be unable to mutate without compromising its fitness. While these aptamers have promising therapeutic potential, they remain to be evaluated in preclinical and clinical studies.

Some of the best examples of therapeutic aptamers that have progressed through preclinical to clinical development include antithrombin aptamer (ARC183),[92 93] anti-platelet-derived growth factor (PDGF) aptamer (ARC127)[18 94] and an anti-von Willebrand factor aptamer (ARC1779).[31 95] All these aptamers have been developed by Archemix (Cambridge, Massachusetts, USA) for use as an anticoagulant during coronary artery bypass graft surgery (ARC183), treatment of proliferative diseases such as intimal hyperplasia (ARC127), and thrombotic thrombocytopenic purpura and other thrombotic microangiopathies (ARC1779). These aptamers showed no acute toxicities, no evidence of genotoxicity, and no adverse effects in preclinical and clinical evaluations. Phase 1 clinical trials showed that administration of ARC183 resulted in a rapid onset of anticoagulation, demonstrated stable dose-related anticoagulation activity, and that the effects of the drug were rapidly reversed after administration of the drug infusion ceased.[96] The limitation with the therapeutic use of ARC183 is that the amount of drug needed to achieve the desired anticoagulation for use in coronary artery bypass graft surgery resulted in a suboptimal dosing.[96] However, ARC1779 was recently shown to be effective in treating an

otherwise refractory case of idiopathic thrombotic thrombocytopenic purpura in a 39-year-old patient.[32] These clinical and pharmacological data provide a rational basis for further trials with ARC1779 and related aptamers.

The most advanced aptamer in the potential treatment of cancer is AS1411,[97] which has been developed by Antisoma (London, UK). AS1411 aptamer binds nucleolin on the surface of cancer cells and induces apoptosis.[98] In a dose escalation (1 mg/kg/day to 10 mg/kg/day) clinical study, AS1411, then called AGR001, showed positive responses in patients with advanced solid tumours (including three renal and two pancreas cancer cases) without any adverse effects.[99] Recently, in a randomised phase II clinical trials, a 10 mg/kg/day or 40 mg/kg/day dose of AS1411 combined with high-dose cytarabine was well tolerated and showed promising signs of activity in patients with primary refractory or relapsed acute myeloid leukaemia.[100] AS1411 has also been shown to destabilise bcl-2 mRNA in vitro and is currently being evaluated for treatment of breast cancer.[27] An RNA aptamer, OPN-R3, isolated against osteopontin has been shown, in an in vivo xenograft model of breast cancer, to significantly decrease local progression and distant metastases.[21] Another aptamer, called SM20, isolated against plasminogen activator inhibitor-1, has demonstrated in vitro therapeutic potential as an antimetastatic agent and could possibly be used as an adjuvant to traditional chemotherapy for breast cancer.[101] There are several aptamers that have been recently isolated for potential treatment of other cancers such as glioblastoma,[29] T cell leukaemia,[22 24 102] and epithelial cancer cells in the breast, colon, lung, ovaries and pancreas.[28] Clearly, as aptamer research burgeons and more enter clinical trials, aptamers are likely to make a direct and significant contribution in the treatment of infectious and acute diseases, and chronic diseases such as cancer.

APTAMERS AS TEMPLATES FOR RATIONAL DRUG DESIGN AND SMALL MOLECULE LEAD COMPOUNDS

Other than using aptamers directly as therapeutics, they can also be used indirectly as templates for structure-based rational drug design and for biochemical screening of small molecule lead compounds. Generally, aptamers have a predilection of binding functional sites on target proteins in a manner similar to small molecule drugs. This property allows structural elucidation of the aptamer–protein complex to provide insights on the identity of the active site that could then be used for rational drug design. Co-crystal structures of aptamer–thrombin,[103 104] aptamer–bacteriophage MS2 coat protein,[105 106] aptamer–NFκB (nuclear factor κ-light-chain-enhancer of activated B cells),[107] aptamer–PreQ0 metabolite[108] and aptamer–HIV TAR[109] have provided valuable insights into the molecular recognition mechanisms adopted by aptamers to their respective targets. Typically, the intermolecular forces between aptamer and protein involve ionic interactions, hydrogen bonds and base stacking.[104 106 107 109] However, other architectural features, such as phosphate backbone flexibility and shape, complementarily contribute to aptamer affinity.[104] The structure–function relationship of gp120 binding and HIV-1 neutralising aptamer has shown that the aptamer sterically blocks the active site of the protein, and also interferes with protein activity by allosteric or non-competitive inhibition (Marisa Joubert, personal communication 2009). Taken together, these biophysical properties of aptamers make them desirable tools for structure-based rational drug design and for biochemical screening.

Aptamers can also be used in high-throughput screening of libraries of small molecules, where the displacement of target-bound aptamer by a small molecule in competition binding could be an effective method to identify hits (figure 1). This approach allows for small molecules acting at the same site as the parent aptamer to be screened directly. The seminal paper that described aptamer-based high-throughput screening of small molecule lead compounds used high-affinity aptamers that bind PDGF and wheat germ agglutinin.[110] In both cases, binding affinities of competing small molecules and aptamers were strongly correlated with their inhibitory potencies in cell-based functional assays. Labelled PDGF-binding aptamer was displaced by a small molecule antitumour agent called suramin from the PDGF-binding site.[110] In another study, an RNA aptamer that inhibits HIV-1 RT with high specificity[111] was used to biochemically screen and identify a small molecule competitive inhibitor of RT called SY-3E4.[8] SY-3E4 inhibits the replication of the HIV-1 NL4-3 wild-type strain and a multidrug-resistant mutant TN6-P5-5 in cell-based functional assays.[8] In addition to inhibiting a multidrug-resistant strain of HIV-1, SY-3E4 has been shown to bind at a different site from that of currently available anti-HIV-1 RT inhibitors.[8]

These data point to the value and prospect of aptamers in biochemical screening and identification of novel small molecule inhibitors with new mechanisms of action for validated targets and protein targets that have no known binding partners, such as orphan receptors. In recent studies, peptide aptamers have also been used for high-confidence validation of therapeutic targets and for guiding the discovery of small molecule drugs.[112 113]

DIAGNOSTIC AND BIOSENSING POTENTIAL OF APTAMERS

Another burgeoning area of aptamer research is their applications in diagnostics and biosensing. The high affinity and hence high sensitivity, high specificity, robustness and ease of modification, such as the attachment of fluorescence labels and differential dyes or colorimetric reporter molecules, are some of the defining properties that make aptamers desirable diagnostic or biosensing tools. For aptamer-based diagnostics or biosensors, binding of an aptamer to its target molecule must be reported by attaching a signal transduction mechanism to the aptamer sequence.

Allosteric aptamer-based florescence resonance energy transfer (FRET) for detection of molecular targets offers excellent choice because of the convenience of detection, sensitivity and availability of numerous flurophores and quenchers of nucleic acids. In the aptamer-based FRET, the aptamer acts as an affinity probe that specifically seeks a target analyte from a complex biological sample for binding, while the intermolecular and intramolecular FRET between the donor and acceptor through specific binding of fluorophore-labelled aptamer of the target molecule results in increased sensitivity. The limitations of using aptamer-based

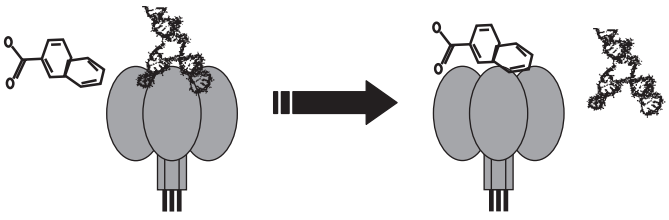

Figure 1 A hypothetical aptamer displacement assay for gp120-binding and HIV-1-neutralising aptamer. A gp120-binding aptamer (black) is displaced from the protein active site (grey) by a small molecule hit (white), which mimics and competes with the aptamer for binding. The aptamer displacement assay is used for high-throughput screening and discovery of small molecule leads with the same functional properties as the aptamer it displaces on the target.

FRET include the use of expensive fluorophores and the tedious process for labelling the aptamer. Another challenge of using FRET signalling is that it is difficult to apply directly in detecting analytes in their native environment or in complex biological fluids because of the interference of intense background signal, which can compromise assay sensitivity. Notwithstanding, there have been efforts to circumvent this problem such as the use of a light-switching excimer aptamer probe for rapid protein monitoring in biological fluids using steady-state and time-resolved fluorescence measurements.[114]

Important considerations in developing and evaluating any ideal diagnostic test are its sensitivity, specificity, rapidness, robustness, reliability, ease of performance and affordability by those at risk of infection. Because of their desirable properties, aptamers can be exploited and modified to fulfil the above criteria for developing ideal diagnostics. In proof-of-concept experiments, a protocol for aptamer-based colorimetric detection of a broad range of analytes has been developed.[115] In one experiment, DNA aptamers were attached to nanoparticles and used to construct a lateral flow colorimetric sensor with instantaneous colour response upon binding cocaine in undiluted human blood serum.[116] The beauty of this aptamer-based colorimetric sensor lies in its simplicity and user-friendliness because detection results can be observed with the naked eye without the need for sophisticated instruments or trained laboratory personnel.

Another simple proof-of-principle experiment used a sandwich assay based on single aptamer sequences for the direct detection of small molecule targets in blood serum and other complex matrices.[117] To demonstrate the utility and elegance of this approach, a single anticocaine aptamer and anti-ATP aptamers were used to fabricate electrochemical sensors directed against cocaine and ATP, respectively.[117] Both targets were detected at low micromolar concentrations, in seconds, and in a convenient, general, readily reusable, electrochemical format.[117] Both proof-of-principle diagnostics are selective enough to be adapted for various analytes of biomedical interest and can be deployed directly in blood, crude cellular lysates and other complex sample matrices. For example, a DNA aptamer isolated against surface proteins of *Campylobacter jejuni*, a bacterium that causes food poisoning in humans, has been used to develop a red quantum dot-based sandwich assay that rapidly (within 15–20 min) and specifically detects as little as 10–250 colony forming units of *Campylobacter jejuni* bacteria in various food matrices.[118] In another study, an aptamer-based electrochemical sensor has been developed to detect botulinum neurotoxin within 24 h and, with a high signal-to-noise ratio, allows a limit of detection of 40 pg/ml by two standard deviation cut-offs above the noise levels.[14] While this aptamer-based electrochemical detection of botulinum toxin compares favourably with monoclonal-antibody-based immunoassays in terms of sensitivity and specificity, it is superior in terms of rapidity and user-friendliness. To further demonstrate the simplicity and utility of aptamer-based diagnostics, the single-stranded DNA aptamer isolated against MPT64 protein from *M tuberculosis* has been used to construct a sandwich assay for diagnosis of *M tuberculosis*.[12] The sandwich assay scheme based on aptamer–protein complex has a high sensitivity (negative ratio, 24/27, 88.9%) and specificity (positive ratio, 46/52, 88.5%) in detecting MPT64 protein in the culture filtrates,[12] suggesting that this simple aptamer-based assay has potential for rapid and reliable diagnosis of *M tuberculosis*.

Aptamers may also be useful in detecting cells that express a particular protein, such as a tumour marker, on their surfaces. Using whole cell-SELEX, Sefah and colleagues[22] have isolated DNA aptamers that bind live acute myeloid leukaemia (AML) cells with high affinity and specificity. One aptamer, called KH1C12, shows selectivity to the target AML (HL60) cell line and recognises the target cells within a complex mixture of normal bone marrow aspirates, while two other aptamers, called KK1B10 and KK1D04, recognise targets with monocytic differentiation.[22] That study showed that the selected aptamers can be used as molecular probes for effective diagnosis and subcategorisation of AML. Aptamer-based cell-surface proximity ligation assay (PLA) has also been used to successfully detect and differentiate between cells that differentially express the prostate-specific membrane antigen (PSMA).[26] In PLA, two probes that bind adjacent to one another on a target analyte are ligated, yielding a unique amplicon that can be sensitively detected by real-time PCR.[26] This aptamer-based cell-surface PLA assay, which is specific for PSMA, has a great potential in the early diagnosis and typing of prostate cancer. Aptamers that recognise cell-surface biomarkers on cancer cells have also been used in a more novel assay configuration. For instance, a novel in situ tissue slide-based SELEX strategy targeting neoplastic tissues from breast cancer patients has recently been reported.[119] In situ tissue slide-based SELEX is a variation of the SELEX protocol, and it screens serial pathological tissue sections embedded on slides as targets for relevant aptamers, and evolves aptamers to all fractions of tissue in their natural positions.[119] Using in situ tissue slide-based SELEX, the Shao group identified a single-stranded DNA aptamer called BC15, which binds hnRNP A1—highly expressed in breast cancer tissues—and showed that it specifically recognises breast cancer cells within tissue sections or from culture medium.[119] The BC15 aptamer has also been used to probe tissues from several other pathological types of breast cancers, including lobular carcinoma, ductal carcinoma complicated with lobular carcinoma, comedo carcinoma, and lymph node metastasis of breast ductal carcinoma origin or breast lobular carcinoma origin.[119] These reports suggest that tissue slide-based SELEX has potential in the pathological diagnosis of cancer. An alternative novel assay configuration used an aptamer-modified microfluidic device to capture and enrich rare cancer cells from background healthy cells.[120] To accomplish this, sgc8 aptamer, which was isolated by cell-SELEX and specifically binds T cell acute lymphocytic leukaemia with K_d=0.8 nM,[23] was first immobilised on the surface of a poly (dimethylsiloxane) microchannel, followed by pumping a mixture of cells through the device.[120] This process allowed the use of optical microscopy to measure the cell-surface density from which the percentage of cells captured as a function of cell and aptamer concentration, flow velocity and incubation time were calculated. This aptamer-based microfluidic device captured rare cancer target cells, with >97% purity and >80% efficiency, within minutes.[120] This assay promises to play a key role in the early detection and diagnosis of cancer where rare diseased cells can first be enriched and then captured for detection.

Another approach to simplify the detection of analytes in aptamer-based diagnostics includes linking the aptamer to an enzyme that has activity that can be readily assayed. To illustrate the point, a biotin-labelled DNA aptamer was selected against insulin and used to construct an aptameric enzyme subunit, which allows detection and measurement of insulin activity in solution.[20] Detection and measurement of insulin activity is useful for the diagnosis of type 1 diabetes.

Another study has described linking an RNA aptamer isolated against C reactive protein, which is a biomarker for inflammation, sepsis and tissue necrosis, to a secondary antibody labelled

with a dye or enzyme that is easily measured in an immunoassay.[121] This aptamer-based sandwich immunoassay provides the unique potential of detecting C reactive protein in serum samples of low-risk patients (1–3 mg/l) as well as high-risk patients (>500 mg/l).[121] Through innovation and with the development of automated high-throughput isolation of aptamers, the aptamer-based sandwich immunoassays have evolved to high-throughput microarray-based diagnostics.[121–124]

Conjugation of aptamers to nanoparticles is another innovation that has opened further opportunities in the development of new generation rapid and reliable diagnostics.[125] In addition to the example described above for colorimetric detection of cocaine using aptamer–nanoparticle lateral flow technology, another study has reported highly sensitive and specific detection of PDGF via a 'sandwich' structure of two aptamer-binding sites and gold-nanoparticle-mediated amplification technique.[126] This aptamer–nanoparticle detection approach exhibits good stability and detects picomolar concentrations of PDGF in contaminated samples or undiluted human blood serum.[126] In nano-aptamer based diagnostics, aptamers have also been used to develop silicon-nanowire field effect transistor (SiNW-FET) biosensors for real-time detection of analytes.[16 127] In one study, anti-VEGF aptamer-modified SiNW-FET was used for real time, label-free and electrical detection of VEGF at concentrations as low as 104 pM.[16] VEGF drives angiogenesis in various tumours and hence its detection using the anti-VEGF aptamer-modified SiNW-FET can be used as a surrogate marker for cancer diagnosis. In another study, the antithrombin aptamer immobilised on SiNW-FET biosensor was applied for real-time detection of picomolar amounts of thrombin in blood samples.[127] A solid-state electrochemiluminescence (ECL) biosensing switch system based on special ferrocene-labelled molecular beacon aptamer (Fc-MBA) has also been developed successfully for sensitive and specific detection of thrombin.[128] The switch system includes an ECL intensity switch and an ECL substrate, which was made by special modification of gold nanoparticles.[128]

POTENTIAL OF APTAMERS IN TARGETED DELIVERY OF DRUGS

Nanoparticle-aptamer technology is dynamic and its application extends beyond diagnostics to targeted delivery of drugs. One of the common uses of nanoparticle–aptamer bioconjugates is for targeted delivery of drugs to cancer cells (figure 2). Using prostate cancer as a model, Farokhzad *et al*[39 47] encapsulated docetaxel (Dtxl) into nanoparticles formulated with biocompatible and biodegradable copolymer, and functionalised their surfaces with the RNA aptamers that recognise the extracellular domain of PSMA. These Dtxl-encapsulated nanoparticle–aptamer bioconjugates (Dtxl-NP-Apt) bound to PSMA expressed on the surface of LNCaP prostate epithelial cells and were taken up by these cells, resulting in significantly enhanced in vitro cellular toxicity as compared with non-targeted nanoparticles that lack the PSMA aptamer (Dxtl-NP).[25] The Dtxl-NP-Apt bioconjugates also exhibited remarkable in vivo efficacy and reduced toxicity as measured by complete tumour reduction, nearly 60% less mean body weight loss and 100% survival compared with 57% survival for Dxtl-NP and 14% for Dxtl alone in the 109-day study in LNCaP xenograft nude mice.[25] In other aptamer-based targeted drug delivery systems, the anticancer drug doxorubicin was covalently linked to the DNA aptamer sgc8c to specifically target T cell acute lymphoblastic leukaemia cells.[24] Another study showed that DNA aptamers selected against unique short *O*-glycan-peptide signatures on the surface of breast, colon, lung, ovarian and pancreatic cancer cells can be used for targeted delivery of phototoxic cancer therapy agent.[28] When modified at their 5′ end with the photodynamic therapy agent chlorine(6) and delivered to epithelial cancer cells, these phototoxic aptamers exhibited a remarkable enhancement (>500-fold increase) in toxicity upon light activation compared with the drug alone, and they were not cytotoxic towards normal cell types, which lack *O*-glycan-peptide markers.[28] Taken together, these results show that aptamer-based targeted delivery of anticancer agents is an intelligent, powerful and promising drug delivery technology that can increase the efficacy of chemotherapy yet at the same time mitigate the overall side-effect toxicity.

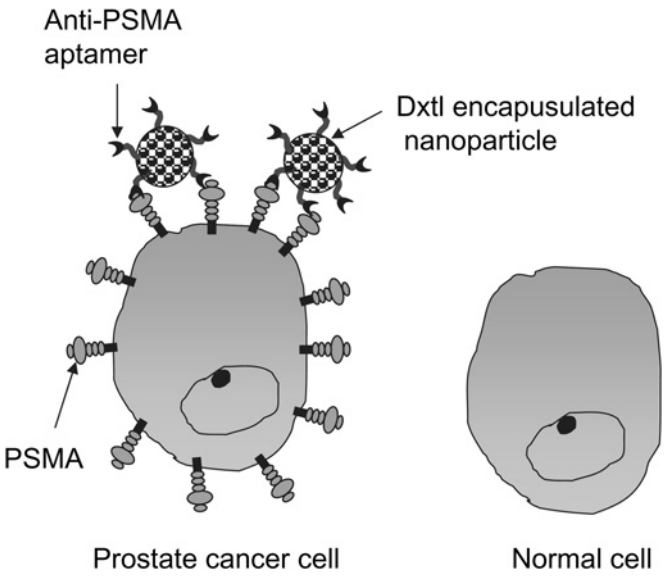

Figure 2 Targeted delivery of the anticancer drug docetaxel (Dxtl) encapsulated by the nanoparticle functionalised with an anti-prostate-specific membrane antigen (anti-PSMA) aptamer. The nanoparticle–aptamer bioconjugate selectively delivers the drug to prostate cancer cells expressing the PSMA on their surface and not to normal cells, which do not have the PSMA.

This aptamer-based targeted delivery of drugs can be adapted to selectively inactivate bacterial and viral pathogens in infected cells. Indeed, Tang and colleagues have developed DNA aptamers for selective targeting of vaccinia-virus-infected cells using cell-SELEX.[47] These aptamers bind selectively to vaccinia-virus-infected cells with apparent equilibrium dissociation constants in the nanomolar range. In another innovative study, Zhou and colleagues used anti-gp120 RNA aptamers to deliver Dicer substrate small interfering (si)RNA into HIV-1 infected cells.[2 3] The Dicer-substrate siRNA delivered by the aptamers is functionally processed by Dicer and has dual inhibitory function. It specifically inhibits HIV-1 replication and infectivity in cultured CEM T-cells and primary blood mononuclear cells.[2] In another recent study, the Giangrande group demonstrated the dynamic utility of the aptamer–siRNA duo by successfully delivering anti-PSMA aptamer targeted siRNA in prostate cancer animal models.[129] The optimised aptamer–siRNA chimera resulted in significant regression of PSMA-expressing tumours in athymic mice after systemic administration.[129] Taken together, these studies bode well for the future advancement of aptamer-based targeted drug delivery.

CONCLUDING REMARKS: CHALLENGES AND FUTURE PROSPECTS OF APTAMERS

While early stages in any technology have caveats and sceptics, and are hardly heralded, aptamer technology has made significant

Take-home messages

- Aptamers are synthetic oligonucleotide ligands or peptides that can be isolated in vitro against diverse biological targets.
- Aptamers assume defined three-dimensional structures and generally bind functional sites on their respective targets.
- Aptamers possess the molecular recognition properties of monoclonal antibodies in terms of their high affinity and specificity.
- Aptamers have a wide range of applications from diagnostics, targeted drug delivery and therapeutics.

strides since it was first described just 20 years ago. The challenges and limitations of aptamers hinge on issues of therapeutics formulations, administration route, bioavailability and costs of synthesis. Notwithstanding, the advantages and future prospects of aptamers outweigh their limitations. With remarkable target specificity and sensitivity, versatile biophysical and pharmacokinetic properties, opportunities for alternative formulations and schedule of administration, improvements in process chemistry and manufacturing economics, including economies of scale, aptamers have found themselves a substantial niche and are becoming established as a promising new class of medicines. Proof-of-concepts experiments illustrating that aptamers can specifically bind and regulate the function of various biomedically relevant proteins augurs well for future aptamer-based drugs and diagnostics development. My prescient prediction is that within the next 20 years aptamers are bound to revolutionise the drug discovery and targeted delivery process as well as the way we diagnose, treat and prevent diseases.

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