Cell-SELEX-based aptamer-conjugated nanomaterials for cancer diagnosis and therapy

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ABSTRACT

Nucleic acid aptamers, which are generated by a novel technique called SELEX (systematic evolution of ligands by exponential enrichment), have recently attracted significant attention in the field of early detection and treatment of cancer based on their numerous merits, such as high affinity, high specificity, small size, little immunogenicity, stable structures, and ease of chemical modification. Furthermore, aptamers can gain more flexibility as cancer cell targeting tools when conjugated to nanomaterials, including metallic nanoparticles, carbon nanomaterials, DNA nanodevices, and polymeric nanoparticles. We discuss the progress achieved in cancer diagnosis and therapy through the conjugation of cell-SELEX-based aptamers with different nanomaterials.

Keywords: aptamer, cell-SELEX, nanomaterial, cancer cells, cancer diagnosis and therapy

INTRODUCTION

Despite prodigious advances in our understanding of the disease, cancer is the most pressing health concern today [1]. A treatment modality that would combine early clinical diagnosis with optimal therapeutic regimens would improve prognosis. This could be achieved by a platform that selectively delivers drugs to target cancer cells [2]. From this perspective, a novel class of nucleic acid ligands, also known as aptamers, has been isolated and identified for specific cancer cell recognition. Aptamers, which are isolated through SELEX (systematic evolution of ligands by exponential enrichment), are single-stranded DNA (ssDNA) or RNA that can bind to their target molecules with high affinity and specificity by folding into distinct secondary and tertiary structures [3,4]. These targets include biomarker proteins [4–11], small molecules [3,12,13], and even whole live cells that express a variety of surface proteins of interest [14–21]. Owing to their automated synthesis, long-term stability as dry powder or in solution, ability to sustain reversible denaturation, well-established selection process, easy and controllable modification to fulfill different diagnostic and therapeutic purposes, slow degradation kinetics, non-toxicity and lack of immunogenicity [22,23], and fast tissue penetration, aptamers have become one of the most promising tools able to introduce target specificity to nanomaterials for intracellular imaging, diagnosis, and therapy.

Particularly, cell-SELEX has been developed to use living cancer cells as targets during aptamer screening, with the long-term goal of producing molecular probes for cancer theranostics. Using cell-SELEX, aptamers have been identified against cells of a panel of cancers, including acute lymphocytic leukemia, T cell leukemia [21], liver cancer [24], acute myeloid leukemia [25], lung cancer [26,27], ovarian cancer [28], B cell lymphoma [29], colorectal cancer [30], and breast cancer [31]. These aptamers could then serve as excellent probes for early detection of cancer cells or cancer-related biomarkers, as well as targeted delivery of therapeutics into cancer cells. The advantage of aptamer selection against live cells with target proteins expressed on the cell surface is straightforward in that cell-based SELEX has enabled the identification of aptamers that bind cell-surface proteins in their proper environments, thus retaining their native conformations. The cell surface is complex and has many different molecules, especially proteins. Cell-SELEX can produce aptamers that specifically bind to a certain cell line based on these unique extracellular characteristics; thus, it is not necessary to know the number or
types of proteins on the cell membrane. Moreover, selection is performed against whole cells having many protein receptors on their membrane surfaces; therefore, several aptamer sequences can be generated in one selection. This means that cell-based SELEX can provide an effective method of discovering new disease-related potential markers for cancer diagnosis and treatment [24,32,33].

A typical cell-based selection process is shown schematically in Fig. 1. First, for positive selections, a library of up to $10^{16}$ DNA sequences is used to generate aptamers. The target cells are incubated with the ssDNA library for a desired time and at a specified temperature. The unbound DNA is washed away, and bound sequences are eluted. The eluted sequences are then amplified by PCR or used for negative selection. In negative selection, negative cells are incubated with the eluted sequences. The eluted sequences pool can be used to carry out negative selection to filter out sequences that may bind to the molecules existing on the surface of both the target and control cell lines. DNA that does not bind to the negative cells after negative selection is amplified. It is nearly impossible to remove all these sequences from the pool with negative selection, and the remaining sequences can increase in number after performing PCR [20]. This eluted ssDNA pool from the first round of selection is then used as the library for the next round of selection. Normally, enriched pools are achieved after about 20 rounds; these DNA molecules can then be cloned and sequenced. Flow cytometry can be applied to monitor the enrichment process.

Over the past decade, concepts and tools derived from nanotechnology have been applied for early detection and treatment of disease. In particular, advances in nanotechnology have created new paradigms for theranostics, which is defined as the combination of therapeutic and diagnostic agents within a single platform. As a consequence of predictable structures and easy site-specific chemical modification, the combination of aptamers with novel nanomaterials, including nanomaterial-based aptamer bioconjugates, has attracted considerable interest and has led to a wide variety of applications in disease diagnosis and therapy. Nanomaterials, such as metallic nanoparticles (NP), semiconductor nanocrystals (quantum dots, QDs), upconversion nanoparticles (UCNPs), DNA nanodevices, and polymeric NP, have undergone many advances in synthesis and characterization in the past few years. These nanomaterials normally possess large surface area-to-volume ratio, unique shape, as well as composition-dependent physical and chemical properties, including surface plasmon resonance (SPR), fluorescence, magnetism, and/or loading ability. These properties provide aptamers with more flexibility as cancer cell targeting tools. More attractively, molecular functionalities incorporated into nanomaterials enable the specific recognition of molecular targets, a property which is particularly significant for the development of bionanotechnology. These properties provide aptamers with more flexibility as cancer cell targeting tools. Moreover, compared with antibodies, aptamers possess small size, quick and reproducible synthesis, slow degradation kinetics, non-toxicity and lack of immunogenicity, as well as easy and controllable modification to satisfy different diagnostic and therapeutic purposes [22,34,35]. These excellent biochemical properties make aptamers ideal candidates as molecular probes, particularly for the detection, diagnosis, and treatment of cancer. For instance, most nanomaterials can protect nucleic acids from nuclease digestion, which permits nucleic acids to maintain their activity, thereby enabling aptamer–nanomaterials conjugates to be applied in the living system. In this review, we discuss the progress achieved in cancer diagnosis and treatment through the conjugation of cell-SELEX-based aptamers with different nanomaterials, including metallic NP, semiconductor nanocrystals (QDs), UCNPs, DNA nanodevices and polymeric NP.
APTAMER–METALLIC NANOMATERIALS CONJUGATE FOR TARGETED CANCER DIAGNOSIS AND THERAPY

It is well known that metal nanomaterials have unusual optical and electronic properties, high stability and biological compatibility, controllable morphology and size dispersion, and easy surface functionalization [36–40]. For example, as the most common and stable metallic nanomaterials, gold nanoparticles (AuNPs) have attracted much attention over the past decade for their many features and properties. First, AuNPs possess strong distance-dependent optical properties by the variation of SPR, which can usually be demonstrated by a color change of AuNPs caused by unbalanced interval distance. Second, AuNPs exhibit good biocompatibility, high intracellular stability, high DNA-loading capacity, and easy surface modification. Third, the strong interaction between thiol and gold provides an easy-to-handle and low-cost approach for AuNP modification. In addition, rapid progress in the development of size- and shape-controlled metallic NP has led to their application in cancer cell targeting and therapy.

Many aptamer–metallic nanomaterials conjugate targeted to assemble on the surface of a specific type of cancer cell through the recognition of the aptamer to its target on the cell membrane surface have been reported. For example, by using cancer cell aptamer-conjugated AuNPs, Jon and co-workers reported prostate-specific membrane antigen (PSMA)-specific, aptamer-conjugated multifunctional AuNPs for combined computed tomography (CT) and cancer therapy [41]. In this study, AuNPs were first functionalized with an anti-PSMA aptamer and then loaded with doxorubicin (Dox). The in vitro cell assays were performed using targeted LNCaP cells and non-targeted PC3 cells. The result showed that PSMA aptamer-conjugated AuNPs had more than 4-fold greater CT intensity for an LNCaP cell than that of a PC3 cell. Furthermore, after loading of Dox, the PSMA aptamer-conjugated AuNPs killed 50% of LNCaP cells and 29% of PC3 cells.

Huang and co-workers also developed aptamer-functionalized AuNPs to co-deliver two different anticancer drugs to improve drug efficacy (Fig. 2) [42]. The surface of AuNPs (13 nm in diameter) was assembled with AS1411 aptamer, which is highly specific for overexpressed nucleolin receptors on the plasma membrane of both cancer cells and endothelial cells present in the angiogenic blood vessels; then, both the photosensitizer 5,10,15,20-tetrakis-(1-methyl-4-pyridyl)-21H, 23H-porphine (TMPyP4) and Dox were physically attached to the AS1411-conjugated AuNPs and delivered to target tumor cells, such as HeLa, and Dox-resistant MCF-7R cell lines. When exposed to visible light, the photodynamic action induced by the photoactivated sensitizers produced abundant ROS, followed by cell damage. In addition, triggered release of the complementary drugs also occurred simultaneously during the photodynamic reaction. In the presence of Dox molecules, the toxicity toward the target cells was superior to individual drug treatment. The combination of photodynamic therapy (PDT) and chemotherapy led to an improvement in the therapeutic inhibition of tumor cell growth over that of individual treatment. Moreover, the improvement of the photodynamic-stimulated triggered release was enhanced, thus proving immensely advantageous in combating tumor drug resistance with effective intracellular transport, as well as optimal antitumor efficacy.

In addition, AuNPs of different sizes and shapes, with optical properties tunable in the near-infrared (NIR) region, have been exploited for the hyperthermic destruction of cancer cells and have been used as drugs in photothermal nanotherapy [43,44]. By changing the aspect ratio, AuNPs may become nanorods, which present strong absorption in the NIR region. By their attractive optical properties, gold nanorods (AuNRs) have served as highly efficient energy quenchers [45] and hyperthermia agents for photothermal therapy (PTT) [46,47]. Taking advantage of this phenomenon, we have reported an aptamer switch probe (ASP) linking chlorin e6 (Ce6), a photosensitizer molecule, to the surface of AuNRs for PDT and PTT in multimodal cancer therapy [48]. As shown in Fig. 3, in the presence of target cancer cells, the ASP changes conformation to drive Ce6 away from the gold surface, thereby producing singlet oxygen for PDT upon light irradiation. AuNRs, which can convert light to heat,
enable further cell destruction by the photothermal effect. Consequently, this multimodal AuNR-ASP-Ce6 conjugate offers a remarkably improved and synergistic therapeutic effect compared to non-specific methods using either PTT or PDT alone. This strategy promises to be an efficient therapeutic regimen against cancer cells.

Recently, another AuNP, gold nanopopcorn, with optical properties tunable in the NIR region, has been exploited for destruction of cancer cells. For example, Ray and co-workers have reported a multifunctional, nanotechnology-driven, gold nanopopcorn-based surface-enhanced Raman scattering (SERS) assay for targeted sensing, nanotherapy treatment, and in situ monitoring of photothermal nanotherapy response during the therapy process (Fig. 4) [49]. Gold nanopopcorns have a unique shape that provides a sufficient field of enhancement since the central sphere acts as an electron reservoir, while the tips are capable of focusing the field at their apexes. Therefore, with popcorn-shaped AuNPs, low cross-section Raman signals can be amplified by several orders of magnitude, especially in narrow, nanoscale corners and edges. In their SERS-based assay, the authors used a well-characterized human prostate cancer cell line, LNCaP, which expresses a high level of PSMA, as a concept target cell line. In the presence of LNCaP human prostate cancer cells, multifunctional popcorn-shaped AuNPs could reach sufficient proximity to form several hotspots, thus providing a significant enhancement of Raman signal intensity by several orders of magnitude ($2.5 \times 10^9$). As a result, human prostate cancer cells could be recognized at the 50-cell level. After exposure to NIR light irradiation, the conjugate-bound cancer cells could be irreparably damaged by localized heating. Moreover, by real-time monitoring of SERS intensity changes, these authors had, for the first time, provided a tool for monitoring photothermal nanotherapy response throughout the therapy process.

QDs, or semiconductor nanocrystals, have been increasingly utilized as biological imaging and labeling probes. QDs have a number of attractive optical properties, including greater photostability, high quantum yields, size-tunable photoluminescence spectra, and continuous absorption spectra covering the ultraviolet to NIR ranges, low photo-bleaching, and resistance to chemical degradation.
The surface modification of QDs with aptamers that could bind to antigens present on the target cells has resulted in the development of sensitive and specific targeted imaging and diagnostic modalities for cancer. Bagalkot et al. have used smart aptamer-QDs conjugated with an anti-PSMA aptamer to functionalize QDs and superparamagnetic iron oxide NP for prostate cancer imaging, therapy, and monitoring of drug delivery (Fig. 5) [51]. The NP products have three major components: an aptamer used to recognize cell receptors, an NP used for optical or magnetic resonance imaging (MRI), and Dox used for anticancer therapy. In this system, QD fluorescence was quenched by Dox, and the Dox fluorescence was quenched by the aptamer, both by energy transfer. Because of high affinity and specificity, the aptamer can mediate cell-specific endocytosis of the NP upon molecular recognition between the aptamer and its receptor. After delivery into the cancer cell, Dox is gradually released from the system, which induces the recovery of QD and Dox fluorescence, providing a means of imaging target cells and monitoring drug delivery at the same time. The cytotoxicity assay showed that NP-mediated Dox delivery and delivery of free Dox were equally potent against PSMA-positive LNCaP cells [52]. More importantly, treatment with the aptamer-functionalized NP killed 47.5% of the PSMA-positive LNCaP cells versus 22.8% of the PSMA-negative cells.

Minko and co-workers also developed a tumor-targeted, pH-responsive quantum dot-mucin1 aptamer-doxorubicin (QD-MUC1-DOX) conjugate for the chemotherapy of ovarian cancer [53]. In their design, QD was conjugated with a DNA aptamer specific for mutated MUC1 mucin overexpressed in many cancer cells, including ovarian carcinoma. Dox was attached to QD via a pH-sensitive hydrazone bond in order to provide stability of the complex for systemic circulation and drug release in the acidic environment inside cancer cells. Results show that this bond was stable at neutral and slightly basic pH and underwent rapid hydrolysis in mildly acidic pH. The QD-MUC1-DOX conjugate was successfully applied for in vivo imaging and treatment and showed a higher cytotoxicity than free Dox in multidrug-resistant cancer cells, preferentially accumulating in ovarian tumor.

Another important type of multifunctional nanomaterial is superparamagnetic iron oxide nanoparticles (SPIONs). SPIONs have demonstrated their suitability for use as theranostic agents, particularly because their intrinsic properties endow them with diagnostic capabilities in MRI applications and because their surface can be easily modified by conjugation with various targeting ligands, dyes, and drugs to provide multimodal functionality. Jon and co-workers have reported a CG-rich duplex containing PSMA aptamer conjugated thermally cross-linked superparamagnetic iron oxide nanoparticles (TCL-SPIONs) as prostate cancer-specific theranostic agents. In this study, they conjugated PSMA aptamers to TCL-SPIONs in a non-covalent manner through hybridization between the aptamer and existing oligonucleotide (ONT) on the NP surface, followed by loading Dox to the conjugate via intercalation. Then, the targeting and therapeutic abilities of the resulting complex were evaluated in in vitro and in vivo human prostate cancer models by T2-weighted MRI using PSMA-overexpressing LNCaP cancer cells and PSMA-negative PC3 cancer cells. Their results suggest that these agents are capable of prostate tumor detection in vivo by MRI and selective delivery of drugs to the tumor tissue, simultaneously [54]. Pilapong group also
developed a smart magnetic NP-aptamer probe or theranostic nanoprobe for targeted imaging and as a drug carrier for hepatocellular carcinoma treatment. The theranostic nanoprobe combines the delivery potential of a non-toxic cellulose derivative polymer, specific capability of cancer-specific molecule (DNA-based EpCAM aptamer), and the imaging capability of magnetic iron oxide NP. The nanoprobe shows great potential as the next generation of cancer diagnosis and treatment [55].

UCNPs, in particular, lanthanide-doped rare-earth nanocrystals, are able to emit shorter wavelength photons under excitation by NIR light, thus making them good visible light generators with the ability to be remotely controlled by NIR light [56–58]. We have reported a specific aptamer-guided G-quadruplex DNA nanoplatform for targeted bioimaging and PDT [59]. In our design, a guanine-rich DNA segment is linked to an aptamer to form a bifunctional DNA sequence, termed G4-aptamer. TMPyP4, a porphyrin derivative broadly used in PDT, is then loaded in the G-quadruplex DNA sequence. Therefore, the G4-aptamer not only loads the photosensitizer but also specifically recognizes target cells. The G4-aptamer is bioconjugated to an UCNP, which brings the photosensitizer TMPyP4 and UCNP into close proximity, thereby causing energy transfer between the UCNP and TMPyP4. When the nanoplatform is delivered into cancer cells, the UCNPs are excited by NIR light to emit visible light to image cancer cells and, in turn, activate TMPyP4, which, finally, generates sufficient ROS to efficiently kill cancer cells. The nanoplatform is capable of selective recognition and imaging of cancer cells, controllable and effective activation of the photosensitizer, selective cytotoxicity to target cancer cells and improvement of the therapeutic effect. We anticipate that aptamer–metallic nanomaterials conjugate system could be applied to the design of similar multifunctional NPs through the use of other disease-specific aptamers and may be utilized to develop novel effective cancer theranostics methodologies.

APTAMER–CARBON NANOMATERIALS CONJUGATE FOR TARGETED CANCER DIAGNOSIS AND THERAPY

Carbon nanomaterials, including carbon nanotubes and graphene/graphene oxide, have been the focus of interest in electronics, chemistry, biosensing, and drug delivery research projects based on their unique structures and remarkable electrical, optoelectronic, chemical, and biocompatible properties. Because the ssDNA strands can either be adsorbed non-covalently onto the side walls or surface of carbon nanomaterials by virtue of $\pi-\pi$ stacking [60,61], or covalently bound to their carboxyl groups [62,63], aptamer-conjugated SWNTs have been found useful in both bioanalysis and imaging applications.

Abnous and co-workers have reported sgc8 aptamer-wrapped SWNTs for reversible targeting and controlled release delivery of daunorubicin (Dau) to cancer cells [64]. Sgc8 can bind to target protein PTK7, which is overexpressed on the target CEM cell membrane, but not on non-target Ramos cells. Dau was released from Dau-aptamer-SWNTs tertiary complex in a pH-dependent manner, and the rate of drug release increased about 6-fold at pH 5.5. Flow cytometric analysis showed that the tertiary complex was internalized effectively to Molt-4 (target) cells, but not to U266 (non-target) cells. Cytotoxicity of Dau-aptamer-SWNTs tertiary complex also confirmed the internalization result. Dau-aptamer-SWNTs tertiary complex was less cytotoxic in U266 cells when compared to Dau alone. No significant change in viability between Dau- and complex-treated Molt-4 cells was observed. Moreover, application of antisense oligos against the aptamer could almost prevent delivery of Dau to Molt-4 cells. Therefore, this system could reverse targeting and controlled release of Dau to cancer cells, while, at the same time, reducing the drug’s cytotoxic effects.
APTAMER–DNA NANOMATERIALS CONJUGATE FOR TARGETED CANCER DIAGNOSIS AND THERAPY

As a naturally occurring biomacromolecule, DNA exhibits excellent biodegradability, affords a large vocabulary of available sequences though programmable design, and is easily synthesized by automated instruments in large quantities. These unique properties make DNA a particularly promising candidate to serve as a building block material for a wide variety of nanostructures with potential applications in biomedicine and biotechnology [65–67]. So far, various DNA nanodevices have been developed, such as DNA origami, tetrahedral, nanotrains, and nanoflowers (NFs). The combination of aptamers and DNA nanostructures can thus merge the specific recognition capability of aptamers with the ability of DNA nanodevices for sensitive bioanalysis, disease-related biomarker detection, as well as targeted delivery of therapeutics. By molecular and nanoengineering, scientists have developed various aptamer-integrated DNA nanodevices to serve as drug carriers for targeted delivery of therapeutics and the subsequent regulation of biological activities in target cells. Herein, we mainly discuss aptamer-incorporated 2D DNA nanotrains and spherical DNA NP for targeted delivery of imaging agents, chemotherapeutics, as well as immunotherapeutics for disease theranostics.

Our group has reported aptamer-tethered DNA nanotrains (aptNTrs) as carriers for targeted drug transport in cancer theranostics (Fig. 6) [68]. An aptNTr is a long linear DNA nanostructure self-assembled very simply from two short DNAs upon initiation of aptamer-tethered trigger probes, through a hybridization chain reaction, such that each nanotrain is tethered with an aptamer moiety on one end for molecular targeting of cognate cancer cells and operating like locomotives to guide a

Figure 6. Schematic illustration of the self-assembly of aptNTrs for transport of molecular drugs in theranostic applications. Reproduced with permission from [68]. Copyright 2013 National Academy of Sciences.
series of tandem dsDNA ‘boxcars’ towards target cells. Two hairpin monomers (M1 and M2) were designed such that the stored energy in the loops is protected by the corresponding stems, preventing their polymerization in the absence of an initiation probe. To construct aptNTrs, aptamer sgce8 was chosen as a model. Importantly, the periodically aligned boxcars provide a large number of spatially addressable sites for high-capacity loading of therapeutics. Several widely used anthracycline anticancer drugs, including Dox, Daunorubicin and Epirubicin, were utilized as drug cargo models, and these drugs were able to preferentially intercalate into double-stranded 5′-GC-3′ or 5′-CG-3′. By flow cytometry, these nanodevices were demonstrated to selectively recognize target cancer cells, but not non-target cells. Using confocal microscopy, aptNTrs were shown to selectively deliver drugs into target cancer cells, as further verified by an in vitro MTS cell viability assay which indicated that sgce8-NTr-Dox induced cytotoxicity comparable to that induced by free drugs in target cells, but not in non-target cells. We next evaluated the in vivo therapeutic efficacy, both anticancer potency and side effects, of Dox delivered by this nanodevice using a CEM subcutaneous xenograft mouse tumor model. Mice were divided into three groups for comparative efficacy studies, in which the following regimens were administered by intravenous injections every other day: (i) sgce8-NTrs, (ii) free Dox, and (iii) sgce8-NTr-Dox. Compared to blank drug carriers (sgce8-NTrs), both sgce8-NTr-Dox and free Dox caused significant inhibition of tumor growth, with slightly stronger potency of sgce8-NTr-Dox than that of free Dox, most likely from the specific targeting ability and larger molecular weight of aptNTrs that endowed them with relatively long drug clearance time from blood, relatively high concentration of accumulated drugs, and long drug retention time in tumor. Consistently, both sgce8-NTr-Dox and free Dox led to longer mouse survival time than sgce8-NTrs. These results demonstrated the potent anticancer efficacy of drugs delivered via aptNTrs. Moreover, mice treated with free Dox lost significantly more weight than those treated with sgce8-NTr-Dox, while those treated with sgce8-NTrs showed a slight increase in body weight, indicating the reduction of drug side effects using aptNTrs, as well as the biocompatibility of aptNTrs. Overall, these data demonstrated the potent antitumor efficacy and the reduced side effects of drugs delivered via aptNTrs as the drug nanocarriers.

We also constructed a multifunctional aptamer-based DNA nanoassembly (AptNA) for targeted cancer therapy (Fig. 7) [69]. We first designed various Y-shaped functional DNA domains through predesigned base pair hybridization. These functional DNA domains include targeting aptamers, intercalated anticancer drugs, and therapeutic antisense oligonucleotides. Then, through complementary sequences, these functional DNA domains are linked to an X-shaped DNA core connector. Finally, hundreds (~100–200) of these basic building units with 5′-modification of acrydite groups are further photo-cross-linked into a programmable and multifunctional aptamer-based nanoassembly structure. AptNAs possess many advantages, such as high programmability, facile modular design and assembly, selective recognition and transportation, as well as excellent biostability and biocompatibility. With these properties, AptNAs were demonstrated to have specific cytotoxic effect against target cancer cells. Moreover, the incorporation of therapeutic antisense oligonucleotides resulted in the inhibition of P-glycoprotein (P-gp) expression, a drug efflux pump that increases the excretion of anticancer drugs and decreases drug resistance.

DNA has emerged as a building block material for the construction of DNA nanodevices for which conventional approaches typically rely on bottom-up assembly through Watson–Crick base pairing between short DNA building blocks. However, these approaches have intrinsic drawbacks. For example, limited compaction results from steric hindrance of DNA strands, yet highly compact DNA is typically favored for applications in nanotherapeutics and bioimaging nanoassemblies. The many different DNA strands needed to assemble relatively large and sophisticated nanodevices complicates design strategies. In addition, the bulky preparation of a large amount of DNA, the extensive intrinsic nicks of phosphodiester bonds in DNA building blocks,
which provide potential cleavage sites of many exonucleases [70–72], and dissociation that accompanies denaturation or extremely low concentrations all make bottom-up assembly challenging. Therefore, it would be desirable to assemble densely compacted multifunctional DNA nanostructures using elongated, less-nicked building blocks made from only a few DNA strands, without relying on Watson–Crick base pairing. Towards this end, our group has developed a non-canonical self-assembly of hierarchical DNA nanodevices, termed NFs, with densely packed DNA and built-in multiple functionalities (Fig. 8) [73]. Using only two DNA strands, one designer template and one primer, long DNA building blocks were generated through rolling cycle replication (RCR), an isothermal enzymatic reaction catalyzed by Φ29 DNA polymerase. Since the templates for RCR can be tailor-designed, various structural and functional moieties can be incorporated into templates and subsequently built into RCR products. The RCR template was designed such that the resultant RCR products consist of a series of aptamers, with sgc8 as a model, and drug-loading sites for Dox. The concatemer aptamers in elongated ssDNA are expected to enhance the binding affinity of the resultant NFs to target cells through multivalent binding. The tremendous drug-association sequences in NFs are expected to endow NFs with high drug payload capacity. The above-generated DNA then served as building blocks to assemble NFs. NFs were also demonstrated to self-assemble in a non-canonical manner through liquid crystallization of DNA, rather than through conventional DNA hybridization. These NFs have increased resistance to nuclease degradation, denaturation, and dissociation at low concentrations, as well as exceptional biostability. All these features make NFs suitable for versatile future applications, especially in biomedical situations. In our study, NFs were incorporated with functionalities, including aptamers, fluorophores, and drug-loading sites. The resultant NFs were then capable of selective cancer cell recognition, cell bioimaging, and targeted anticancer drug delivery. Using flow cytometry, NFs incorporated with FITC, drug-loading sites, and sgc8 were demonstrated to selectively recognize target HeLa cells and CEM cells, but not non-target Ramos cells, thus providing the basis for potential applications in cancer imaging and active cancer therapy. Using confocal microscopy, the FITC-incorporated NFs were able to elucidate the capacity of NFs for internalization into target cancer cells. NF-drug complexes were then evaluated for targeted drug delivery in vitro using an MTS assay, and these DNA nanodevices were found to be capable of targeted cancer theranostics.
Subsequently, we used a similar approach to make aptamer-conjugated FRET-NFs [74]. In this design, fluorescein (FAM), cyanine 3 (Cy3), and 6-carboxyl-X-rhodamine (ROX) were simultaneously incorporated into NFs via chemically modified deoxynucleotides, including FAM-dUTP, Cy3-dUTP, and ROX-dUTP, during RCR reaction. When the ratio of these fluorophores was varied in this enzymatic reaction, FRET-mediated emission signatures could be tuned such that NFs would exhibit colors under a single-wavelength excitation. As a result, these DNA NFs not only exhibited multiple and extremely bright fluorescence signals under a single-wavelength excitation but they also represented a target-specific and biocompatible drug delivery platform. FRET NFs also allow for a large Stokes shift, which benefits applications in complex biological milieu. As such, aptamer–DNA nanomaterials conjugate are promising for versatile biomedical applications.

APTAMER-CONJUGATED POLYMERIC NP FOR TARGETED CANCER DIAGNOSIS AND THERAPY

Another promising carrier system involves polymeric micelles, which were originally composed of an amphiphilic block, have become an increasingly exciting field in academic and industrial research due to its significant therapeutic potential. The use of amphiphilic polymers results in the formation of hydrophobic core–hydrophilic shell-structured NPs, which can encapsulate a variety of therapeutic compounds or imaging agents. More recently, a micelle constructed as a hybrid from hydrophilic oligonucleotide and hydrophobic polymer [75,76] has drawn close attention. In aqueous solutions, this type of amphiphilic block copolymer can self-assemble into a 3D spherical micelle structure or a nanorod-like micelle structure. This type of micelle has been shown to efficiently carry a variety of cargos to cells. To perform efficient targeted delivery, DNA micelles with a modified recognition molecule comprise a novel delivery system. We investigated the construction of well-defined oligonucleotide–micelle structures using a DNA–diacyllipid conjugate in 2010 (Fig. 9) [77]. In this work, we reported the design of a self-assembled aptamer–micelle nanostructure (Apt–micelles) able to enhance the binding ability of the aptamer moiety at physiological temperature (37°C), even though the corresponding free aptamer had lost its binding ability under the same conditions. The merits of Apt–micelles also include greatly improved binding affinity, low off rate once on the cell membrane, rapid targeting ability, high sensitivity, and effective drug delivery. To prove the potential detection/delivery application of this aptamer–micelle in biological living systems, we mimicked a tumor site in the blood stream by immobilizing tumor cells onto the surface of a flow channel device. Flushing the aptamer–micelles through the channel demonstrated their selective recognition ability under the condition of flow circulation in human whole-blood sample. The aptamer–micelles showed great dynamic specificity in flow channel systems that mimic drug delivery in the blood system. Therefore, our DNA aptamer–micelle assembly has shown high potential for cancer cell recognition and for in vivo drug delivery applications, thus paving the way for the construction of aptamer–micelles with applications in diagnosis and targeted therapy.

Polymeric NP are formed by self-assembly of biodegradable polymers, such as poly (D, L-lactic acid), poly (D, L-glycolic acid) and their copolymers, like poly (D, L-lactic-co-glycolic acid) (PLGA), which consist of two or more polymer blocks with different hydrophobicities [78,79]. The use of amphiphilic polymers results in the formation of hydrophobic core–hydrophilic shell-structured NPs, which can encapsulate a variety of therapeutic compounds or imaging agents [80]. Guo et al. have developed an antinucleolin aptamer (AS1411 aptamer) conjugated with PEG-PLGA NP for site-specific delivery [81]. AS1411 aptamer, as the targeting ligand that facilitates antigloma
delivery of paclitaxel (PTX), was conjugated to the surface of PEG-PLGA NP via an EDC/NHS technique. AS1411 aptamer–nucleolin interaction significantly enhanced cellular association of NP in C6 glioma cells and increased the cytotoxicity of its payload. Prolonged circulation and enhanced PTX accumulation at the tumor site was achieved for aptamer-conjugated, drug-loaded particles, which eventually obtained significantly higher tumor inhibition in mice-bearing C6 glioma xenograft and prolonged animal survival in rats bearing intracranial C6 gliomas when compared with PTX-NPs and a commercial formulation called Taxol®.

Another molecular engineering strategy, which combined aptamers and polymers, was developed by using the targeting property of aptamers and the intrinsic toxicity of polymers (Fig. 10) [82]. The hybrid polymer was synthesized by copolymerization of acrylamide, acrydite-modified, cell-based aptamer (Sgc8), and a high ratio of acrydite/FITC-labeled short DNA monomer. The resulting polymeric aptamers exhibited high specificity and internalization ability based on the coupling of the Sgc8 aptamer. Importantly, the polymer backbone built into the conjugate induced selective cytotoxicity in target cell lines, while having little effect on non-target cells. Furthermore, when the aptamer–polymer conjugate was tested with the drug-resistant K562/D cell line, significant cytotoxicity, similar to that of the corresponding non-drug-resistant K562 cell line, was observed. The results indicated that the aptamer–polymer conjugate could bypass P-gp on the cell membrane of the drug-resistant K562/D cells and interrupt cellular metabolism. Control assays have confirmed the non-toxicity of the aptamer itself, but they have also shown that the physical properties of the polymer backbone contribute to target cell cytotoxicity. It should, however, be noted that the exact mechanism of therapeutic effect remains unclear. Therefore, aptamer-conjugated polymeric NP have shown high potential for cancer cell recognition and for in vivo drug delivery applications and may shed new light on drug design and drug delivery.

CONCLUSIONS

Cell-SELEX provides an effective approach to generate a large number of aptamer probes that specifically target a variety of cancer cells. Aptamer–nanomaterials conjugates have become increasingly important molecular tools for diagnosis and therapeutics. The major advantages of these bioconjugates include (1) the ease of aptamer synthesis and modification, which facilitates the translation of aptamer functionality into clinical practice, (2) high binding sensitivity and specificity of aptamers, which guarantees targeted detection and binding, the first and most vital step in all kinds of detection and therapy, and (3) excellent optical, electrochemical, magnetic, and mechanical properties, as well as biocompatible loading ability, dramatically enhancing the intensity of the analytical signal and leading to highly efficient target cell recognition and delivery. The studies described in this review have demonstrated the potential of combining aptamers and nanomaterials to accomplish the goals of theranostic nanomedicine.

Although aptamer–nanomaterials conjugates are encouraging for targeted cancer diagnosis and therapeutics, recent studies have also shown that the development of such conjugate-based targeting theranostic tools faces several challenges. First, the toxicity and long-term health effects of nanomaterials need to be addressed for in vivo sensing and imaging. Therefore, surface modifications, such as immobilization with biocompatible compounds must be engineered. Second, the successful realization of aptamer-conjugated nanomaterials strategies requires proper attention to non-specific adsorption issues that commonly control not only the detectability of biosensor development, but also the toxicity to non-target cells in the drug delivery system. Therefore, surface physicochemical characteristics of nanomaterials must be engineered when coupling aptamers for the purpose of reducing non-specific binding and maintaining the aptamers’
active structures. Furthermore, novel nanomaterials with unique properties and little, or no, toxicity need to be designed and synthesized. For example, to lower the autofluorescence and shallow light penetration interference of cells or tissues, NIR fluorescent nanomaterials, or long-life luminescence nanomaterials, need to be synthesized and used.

In the future, for the development of aptamer-conjugated nanomaterials, much more attention could be directed toward creating novel nanomaterials and strengthening the performance of the aptamers. For example, the excitation of two-photon nanomaterials is founded on the notion that each of two photons carries one half the energy needed to excite the molecule and thus possesses a longer wavelength and lower frequency than that required in one-photon microscopy. As a consequence, photobleaching and autofluorescence in cells and tissues, as well as the shallow light penetration that occurs in one-photon excitation, are diminished, or eliminated, providing better 3D spatial localization, reduced photoinduced damage, and increased imaging depth. This new generation of powerful aptamer-conjugated nanomaterials could produce more outstanding features.

ACKNOWLEDGEMENTS

The authors would like to thank members of Molecular Science and Biomedicine Laboratory and the Collaborative Innovation Center for Molecular Engineering for Theranostics for helpful discussions related to this work.

FUNDING

This work was supported by the National Key Scientific Program of China (2011CB911000), the National Natural Science Foundation of China (212221003), and the China National Instrumentation Program (2011YQ03012412).

CONFLICT OF INTEREST

The authors declare no competing financial interests.

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