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Molecular aptamers for drug delivery

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Abstract

The active targeting of drugs in a cell-, tissue-, or disease-specific manner represents a potentially powerful technology with widespread applications in medicine, including the treatment of cancers. Aptamers, with properties such as high affinity and specificity to their targets, easy chemical synthesis and modification, as well as rapid tissue penetration, have become attractive molecules in diagnostics and therapeutics. They rival and, in some cases, surpass other molecular probes, such as antibodies. In this review, we highlight the recent progress in aptamer-mediated delivery for therapeutics and disease-targeting based on aptamer integration with a variety of nanomaterials, such as gold nanorods, DNA-micelles, DNA-hydrogels and carbon nanotubes.

Introduction

Aptamers are single-stranded oligonucleotides that can bind to their target molecules with high affinity and selectivity by folding into distinct secondary and tertiary structures. They are identified from an initial library containing 10^{13} -10^{16} random ssDNA or ssRNA sequences through an *in vitro* selection process termed SELEX (systematic evolution of ligands by exponential enrichment) (recently reviewed in [1, 2]). Aptamers are different from antibodies, yet they mimic properties of antibodies in a variety of diagnostic formats. Antibodies have made substantial contributions toward the advancement of diagnostic assays and have become indispensable in most diagnostic tests that are used routinely in clinics today. As an emerging class of molecules that rivals antibodies in both therapeutic and diagnostic application, aptamers are receiving attention for their novel properties, such as highly selective and specific target recognition and binding. Synthetic aptamers possess several advantages over natural antibodies [3-6], including economical and reproducible synthesis, as well as excellent molecular recognition ability, with K_d values in the nanomolar range. Examples include the DNA aptamers to thrombin (K_d = 25–200 nM [7]),

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MUC1 (K_d = 47.3 nM [8]), PSA-H protein (K_d =8.0 nM[9]) and CEM cells (K_d = 0.8 nM[10]). These values are all superior to those of antibodies, whose K_d's are in the micromolar to millimolar range, depending on concentration. Compared with antibodies, aptamers have additional advantages, such as biocompatibility and flexible modification, easy and controllable modification to fulfill different diagnostic and therapeutic purposes, long-term stability as dry powders or in solution, ability to sustain reversible denaturation, lack of toxicity and immunogenicity, and rapid tissue penetration. These physical and chemical properties make aptamers ideal candidates as probes for use in molecular medicine to elucidate the molecular foundations of diseases, particularly cancer and infectious diseases.

After more than a decade of development, aptamers have attracted interest for use as therapeutic agents and diagnostic tools by, for example, helping to improve the sensitivity and specificity of diagnostic assays through molecular imaging [10, 11], inhibiting disease processes [12, 13] or targeting the delivery of drugs to diseased tissues [14-16]. This review will focus on aptamer-mediated delivery for therapeutics and disease-targeting based on aptamer integration with a variety of nanomaterials, such as gold nanorods, DNA-micelles, DNA-hydrogels and carbon nanotubes.

Aptamers as therapeutic agents

In current research, aptamers have been effectively used for therapeutic applications, such as cancer cell detection and diagnostics [17-19] and targeted therapy [20-22], as well as sorting and enrichment [23, 24]. The generation of a pool of DNA aptamers for various types of cancer cells has been reported, including small-cell lung, non-small-cell lung (NSCLC), acute myelogenous leukemia (AML), liver and colon cancer, as well as virus-infected cells [25-28]. Using a similar strategy, DNA aptamers for mesenchymal stem cells [29], porcine endothelial precursor cells [30] and live bacterial cells [31] have also been developed by other research groups during the past two years. With their superior targeting performance, incorporation of aptamers with a defined therapeutic function and recognition capability for cancer therapy has raised considerable interest. Thus far, aptamers and aptamer assemblies have been validated as essential molecular tools in the areas of anti-infectives[32], anticoagulation [33], anti-inflammation [34], anti-angiogenesis [35], antiproliferation [36], and immune therapy [37].

In addition to their ability to recognize a target molecule with high specificity, certain aptamers can also modulate the activities of proteins implicated in pathological conditions, making aptamers potentially useful as pharmaceutical agents. For instance, Pfizer's Macugen® (pegaptanib), an aptamer-based anti-VEGF treatment for age-related macular degeneration, was approved by the United States FDA in 2004 based on findings from two clinical trials involving 1200 patients and all subtypes of neovascular AMD. as Another example is AS1411, an aptamer which specifically targets nucleolin, a bcl-2 mRNA binding protein involved in cell proliferation, which is found on the surface of many cancer cells. Once bound, the AS1411 aptamer is taken into the cancer cell, where it causes death by apoptosis (programmed cell death). This work is also in clinical trials [38, 39].

Aptamers as delivery agents

Aptamers can be designed as targeting ligands, particularly when generated by cell-based SELEX, and can differentiate diseased cells from healthy cells, thus enabling the selective delivery of therapeutic compounds to target cells (Table 1).

The emerging integration of aptamers with nanotechnology and chemical biology is envisioned to produce more versatile target-specific molecules, stimulate further new

diagnostic and therapeutic nanotechnologies, and provide significant potential for many research and clinical applications in the near future. Compared to applications of antibodies, aptamer research is still in its infancy, but progressing at a rapid pace.

SELEX: aptamer selection process

The technologies involved in the SELEX process can vary from traditional capillary electrophoresis or flow cytometry to, most recently, microfluidic systems [40]. SELEX has undergone several refinements and modifications. Most advances in aptamer isolation have aimed at improving selection efficiency, aptamer biostability and bioavailability. However, the overall goal of the various selection approaches has remained the same--to increase selection speed and efficiency, while identifying ligands that bind to targets with high specificity and affinity--and progress in this area has been recently reviewed [1, 2, 41]. Although many complex forms of SELEX exist, in this section we will focus on the fundamental ”cell-SELEX” strategy, which is of particular interest for targeted drug delivery where aptamers are integrated with such nanomaterials as liposomes [42] or DNA-micelles [14].

Cell-SELEX: a promising tool to generate clinically useful aptamers

Cell-SELEX is the process whereby live cells are used to select aptamers for target recognition (Figure 1). By yielding ligands that bind preferentially to diseased cells, compared with normal cells, cell-SELEX is a particularly promising selection strategy for the development of aptamers able to transport a payload of nanomaterials to diseased cells (Table 1). Live cells of different cancers have been used in this process, and as a result, cell-SELEX was successfully used to develop an aptamer against hepatocellular carcinoma (HCC), one of the most common and highly malignant cancers in the world, found in a human T cell acute lymphoblastic leukemia cell line CEM (used cell-SELEX target) [43]. More recently, cell-SELEX was applied to isolate aptamers that recognized acute myeloid leukemia (AML) cells with dissociation constants (K_d) in the nanomolar range [25].

The cell-SELEX process is easily managed, rapid and reproducible. Generation of aptamers using this technology has become an effective tool for molecular medicine and biomarker discovery. Specifically, when bound with cell membrane receptors, aptamers provide an effective approach for the discovery of biomarkers as disease signals. Since aptamer probes recognize molecular signatures on the cancer cell surface with high specificity, tumor cell profiles can be defined, perhaps leading to more “personalized” cancer treatment. In addition, with their ability to distinguish normal from cancer cells, aptamers allow a comparative strategy to identify differences at the molecular level and promote the discovery of molecular features of cancer cells.

A recent promising approach using cell-SELEX was developed for the generation of aptamer molecular probes that specifically recognize Burkitt's lymphoma cells, an acute blood cell cancer [44]. Early diagnosis and targeted therapy are crucial for patient treatment. However, the lack of molecular probes able to recognize cell-surface biomarkers prevents early diagnosis of such cancers as Burkitt's lymphoma and makes the study of their developing mechanism(s) difficult, if not impossible. The authors were able to identify a panel of cell-specific aptamers, called TDO5, against Ramos cells, a B-cell lymphoma cell line, leading to increased insight into the molecular activity of Ramos cells. As an effective molecular tool for identification of target cells in real biological samples for clinically meaningful biomedical studies and biomarker discovery, TD05 is advancing the potential for improved cancer diagnostics and treatment.

Aptamers in nanotechnology

The ability of DNA or RNA aptamers to act as targeting agents enables these molecules to be conjugated with therapeutic agents for use in targeted drug delivery. Early diagnosis of disease relies not only on the specificity of the molecular probes, but also on the detection sensitivity. Because of their predictable structures and functional groups for chemical modification, aptamers can readily be linked to advanced signaling mechanisms in the development of diagnostics or disease treatments. Some cancer cells, especially those in the early stages of disease development, may have a very low density of target on the cell surface available for detection. With their relatively small size, aptamers have shown promise in specifically targeting tumor cells and transporting small molecules, such as proteins, drugs or siRNA, through the microvasculature or the tumor interstitium [45]. In addition, by taking advantage of straightforward synthesis and chemical modification, aptamers can be conjugated to functional groups with relative ease, enabling their use as effective nanomaterials. Because each type of nanomaterial has different optical, electrochemical, and mechanical properties, medical diagnostic and drug delivery agents with diverse characteristics can be used for different applications, moving aptamer-based nanomedicine closer to reality. In this section, we will mainly focus on the applications of aptamers in nanotechnology for diagnostics and therapeutics.

Aptamers meet nanomaterials: nanoparticle conjugates

Applications for aptamers at the interface of nanotechnology and medicine in the form of aptamer-nanoparticle conjugates are actively being investigated. The large surface areas of nanoparticles offer excellent platforms for conjugating multiple aptamers. In addition, the interior volumes of nanoparticles can be used to store large quantities of drug molecules, thereby enhancing loading capacity.

Huang *et al.* synthesized Au-Ag nanorods (NRs) to serve as a platform for binding several aptamer molecules (Figure 2) [46]. In their work, an NR-scg8 aptamer conjugate combined the high absorption efficiency of Au-Ag NRs with the target specificity of molecular aptamers. When investigated with cell mixtures, the aptamer-NRs were demonstrated to have excellent hyperthermia efficiency on exposure to near-infrared (NIR) laser radiation, as well as selectivity for the target CEM cells. This technique can be used in clinical detection to enhance both binding affinity and signal strength when the concentration of target cells is relatively low. By functionalizing the surface of Au nanoparticles (NPs) with an RNA aptamer that binds to prostate-specific membrane antigen (PSMA), NP-aptamer conjugates were used for targeted molecular computed tomography (CT) imaging and treatment of prostate cancer [47]. Other than metallic NPs, aptamer-conjugated polymer particles are also attractive agents for drug-encapsulation and controlled-release in a cell- or tissue-specific manner. A bioconjugate composed of controlled-release polymeric aptamer-NPs has been used for targeted delivery to a prostate cancer cell line, LNCaP, which expresses PSMA [48]. These bioconjugates efficiently targeted and were taken up by LNCaPs. Compared to solid, metallic NPs, polymeric nanomaterials offer a more promising solution for encapsulation of chemotherapeutics and have been shown to reduce toxicity by providing a protective housing for the drug that limits its interaction with healthy cells [49].

Advanced aptamer–nanomaterial conjugates

Hydrogels are networks of polymer chains that are water-insoluble and superabsorbent, and they possess a degree of flexibility very similar to natural tissue. Target-responsive hydrogels that cross-link DNA aptamers with linear polyacrylamide chains have been fabricated (Figure 3) [50]. Competitive binding of the target to the aptamer causes a decrease in cross-linking density and, hence, dissolution of the hydrogel. Therapeutic

applications can therefore be devised using small molecules and proteins as the targets. To this end, an *in situ* injectable hydrogel has been functionalized with nucleic acid aptamers to control the release of proteins for human disease treatment [51]. The results showed that the protein release rate can be controlled by adjusting the affinity of the aptamers. Both of these studies [48,49] demonstrate that aptamer-based hydrogels provide a highly selective and controllable system, whereby efficient release of therapeutic agents can occur in the specific environment where the target biomarker is found.

In another report, a diacyl lipid tail was incorporated at the 5' end of oligonucleotides by solid-phase DNA synthesis. When dispersed in an aqueous solution, these amphiphilic DNA molecules spontaneously self-assembled into monodispersed micelle structures [52]. In one application of this technology, a self-assembling aptamer–micelle nanostructure was formed of hydrophilic aptamers linked to hydrophobic lipids by poly(ethylene glycol) (PEG) [14]. In aqueous solution, these conjugates self-assembled into3D spherical micelle structure with a hydrophilic aptamer targeting Ramos cells (from human Burkitt's lymphoma cell line) on the outside, and the lipid core on the inside (Figure 3a). The presence of more than one aptamer on the micelle surface provides an approximately 750-fold increase in target binding affinity. The aptamer-micelle assembly is also able to be internalized [37], indicating it is a promising strategy for clinical applications by increasing therapeutic effectiveness. Furthermore, after two days of incubation with the aptamer-micelle assembly, normal cells maintained over 80% viability.

Another application of aptamers is in photodynamic therapy (PDT). Singlet oxygen ($^1O_2$), one of the most important cytotoxic agents generated during PDT, is gaining wide acceptance as an alternative noninvasive treatment of cancers. The photosensitizer used in PDT, generally a chemical, transfers the light energy to tissue oxygen to generate highly reactive $^1O_2$, which can react aggressively with molecules in the cell, leading to cell damage and ultimately cell death. A novel molecular complex consisting of photosensitizer, a ssDNA aptamer, and single-walled carbon nanotubes (SWNTs) has been engineered for controllable $^1O_2$ generation (Figure 4) [53]. In the absence of a target, the close proximity of the photosensitizer to the SWNT surface causes efficient quenching of $^1O_2$. In the presence of its target, the binding between the aptamer and target molecule disturbs the DNA-SWNT interaction and causes the DNA to detach from the SWNT surface, resulting in a restoration of $^1O_2$ for PDT applications.

Because most aptamers cannot directly pass through the cell membrane, finding ways to increase membrane permeation has been an active area of research. Aptamer-conjugated, multifunctional liposomes have been fabricated to encapsulate and deliver cisplatin to breast cancer cells (MCF-7). [42]. The cell membrane is basically a dynamic lipid bilayer; therefore, this liposome nanostructure can efficiently increase cell permeability and enhance drug delivery. A cell-permeable sgc8-PEG-liposome nanostructure has also been reported for drug delivery [54]. With approximately 250 aptamers on each liposome, this design leads to multiple aptamer-receptor interactions, provides better binding to the target cells, and facilitates translocation through the plasmic membrane. Although liposomes are immobilized on the surface membrane of the CEM cells via aptamer recognition, no binding to the non-target NB4 cells is observed, again demonstrating aptamer specificity. Another advantage of liposomes is their ability to increase the plasma residence time of aptamers from several minutes to 23 h [55]. Here, polymeric nanocarriers have shown the benefit of being able to carry multiple drugs in the same vehicle. This, combined with aptamer-mediated targeting, can be used to selectively deliver dual-drug payloads to cancerous cells with high efficiency and specificity.

The lack of tumor-cell specificity often results in severe toxic effects for cancer patients undergoing traditional chemotherpay. To overcome this problem and to improve the selectivity of cancer therapy, cytotoxic drugs conjugated to aptamers have been designed for targeted delivery to tumor-specific sites. An antitumor agent doxorubicin (Dox) was covalently linked to the DNA aptamer sgc8c to specifically kill CEM cells, with minimal toxicity towards non-target cells [56]. The results demonstrated that the sgc8c-Dox conjugate possesses many of the properties of the sgc8c aptamer, including high binding affinity (K_d = 2.0 ± 0.2 nM) and efficient internalization by CEM cells. Moreover, the acid-labile linkage between sgc8c and Dox could be cleaved inside acidic endosomal environment. The delivered drug displayed potency similar to that of independent Dox, but with the target specificity lacking in most current drug delivery strategies. Furthermore, the nonspecific uptake of membrane-permeable Dox to non-target cell lines could also be inhibited by linking the drug with the aptamer; thus, it makes the conjugates selective to the cells which express higher amounts of target proteins. Compared to other reported Dox-antibody conjugates[57], these sgc8c-Dox conjugates make targeted chemotherapy more feasible with drugs having various potencies. When combined with the large number of recently created DNA aptamers that specifically target a wide variety of cancer cells, this drug-aptamer conjugation method has broad implications for targeted drug delivery.

Aptamer-mediated cell-type-specific siRNA delivery

Also known as chemical antibodies, aptamers are poised to surpass natural antibodies in therapeutics, diagnostics, and drug development. The pharmacologic properties of aptamers include wide therapeutic margins, stability, adjustable pharmacokinetics, and very low immunogenicity and toxicity – advantages that are drawing attention from major pharmaceutical companies. It has also been demonstrated that exogenous siRNAs can silence gene expression via the RNAi pathway in mammalian cells [58]. The challenge is learning how to direct those RNA molecules to the target cells and then deliver them through the membrane. With their specific recognition ability, aptamers are ideal candidates for this purpose. For the first time, cell-type–specific delivery of anti-human immunodeficiency virus (anti-HIV) siRNAs through fusion to an anti-gp120 aptamer has been demonstrated[59]. The envelope glycoprotein is expressed on the surface of HIV-1-infected cells, allowing binding and internalization of the aptamer–siRNA chimeric molecules. The chimera is specifically taken up by cells expressing HIV-1 gp120, and the appended siRNA is processed by Dicer; this releases an anti-*tat/rev* siRNA, which, in turn, inhibits HIV replication.

In a further study of this method [60], an anti-PSMA RNA aptamer (A10) was appended to a 21-mer siRNA portion, resulting in a chimera that targets polo-like kinase 1 (PLK1) and BCL2, two survival genes overexpressed in most human tumors [61] The aptamer portion of the chimeras selectively binds to PSMA, while the therapeutic siRNA portion interferes and knocks down gene expression and inhibits the xenograft growth activity of the cancer cells both in cell culture and *in vivo.* Because this delivery system consists only of RNA components, it offers several potential advantages as a therapeutic agent, including lack of immunogenicity, the possibility for chemical synthesis, and stabilizing modifications for *in vivo* application.

Conclusion and future perspectives

Aptamers are rapidly maturing into therapeutic tools with commercial potential. Given that aptamers mimic and extend many features of monoclonal antibodies (mAbs), they have the potential to make an impact in molecular medicine. For instance, aptamers that are highly specific to cancer cells can be used as drug targeting agents, thereby reducing toxicity (mice

body weigh loss only around 7%) while improving upon the efficacy of current therapeutic drugs (tumor size reduction from 5th day of injection and all the mice suvived in 109 days treatment [62]. Compared to antibodies in the 1990s, aptamers are on an even more accelerated trajectory for commercialization. Using cell-SELEX, aptamer probes capable of recognizing vaccinia virus-infected lung cancer A549 cells have been created [28]. It is therefore possible that a range of antiviral aptamers can be generated easily and that these might show synergistic activity, opening new prospects for antiviral prophylaxis or therapy.

With the improvements in SELEX technology and aptamer functionalities, as illustrated above, we believe that aptamers are poised to successfully compete with mAbs in therapeutics and drug development within the next few decades.

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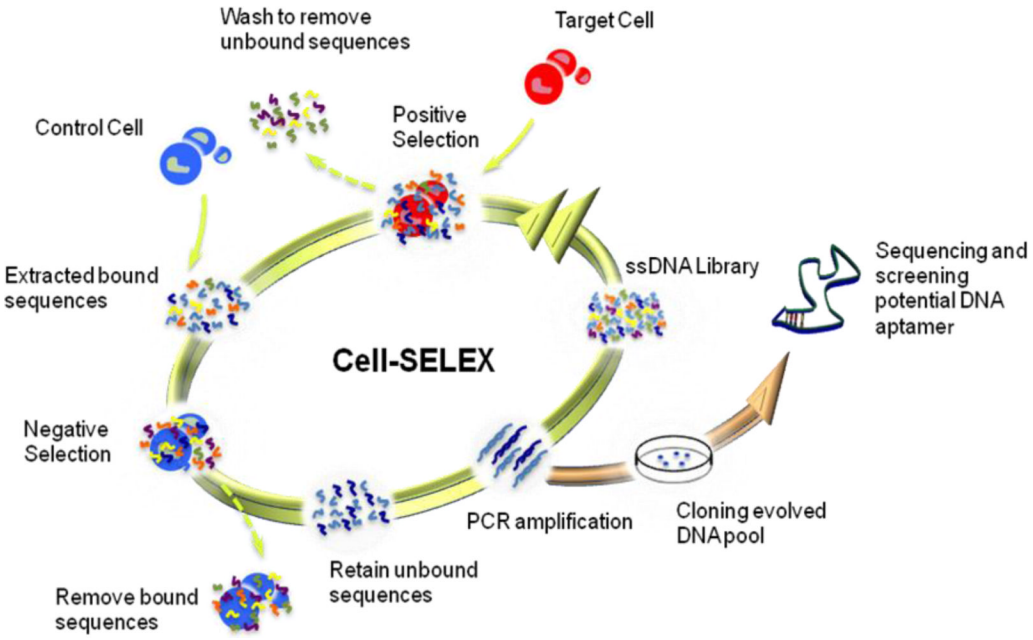

Figure 1.

Schematic representation of DNA aptamer selection using the cell-SELEX strategy. DNA sequences that have specific recognition to target cells are evolved to enrich the selection pools. The enriched pools are cloned, and the positive clones are sequenced to identify individual aptamers. The ssDNA pool is first incubated with target cells. After washing, the bound DNAs are eluted by thermal denaturation. The eluted DNAs are amplified by PCR. The double-stranded PCR products are then separated into ssDNAs and the sense strand DNAs collected for the next round of selection or tested by flow cytometry to monitor the SELEX progression. When the selected pool is sufficiently enriched, the PCR product of the evolved pool is cloned and sequenced for aptamer identification. Adapted from Ref. [70].

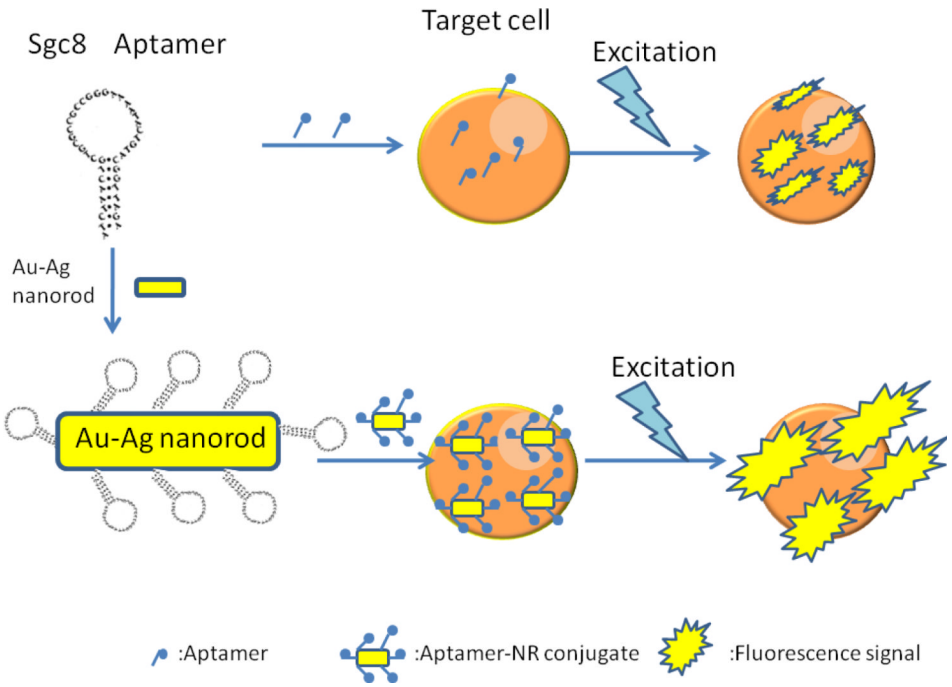

Figure 2.
Schematic representation of aptamer-nanorod signal enhancement.

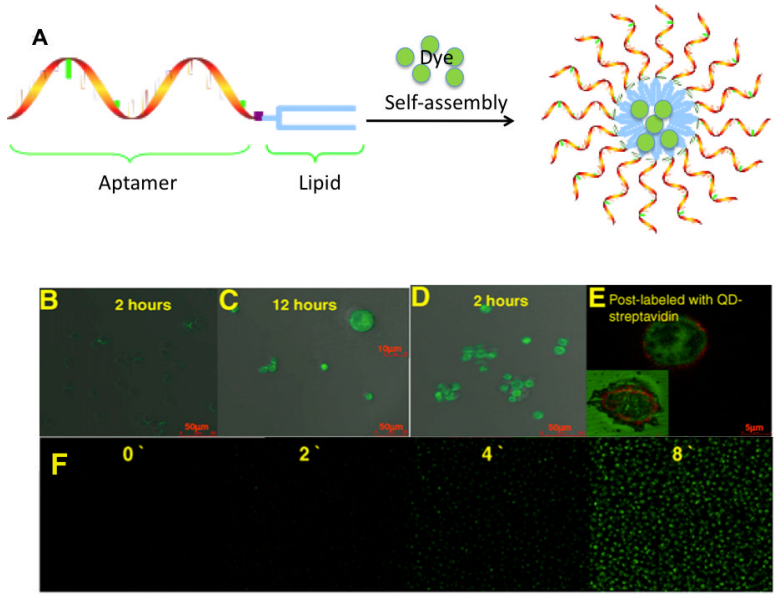

Figure 3.

(a) Design scheme of aptamer micelles containing dye. Fluorescent images of Ramos cells for (b) 2 h and (c) 12 h, or (d) incubation with biotin-TDO5-micelle for 2 h. (e) Enlarged fluorescent image after post-labeling the biotinylated TDO5 aptamer with QD705 streptavidin. The inset in image E is the fluorescent image of the dead cell. (F) Real-time monitoring of doped special dyes released from the core of the micelles and activated by intracellular enzymes.

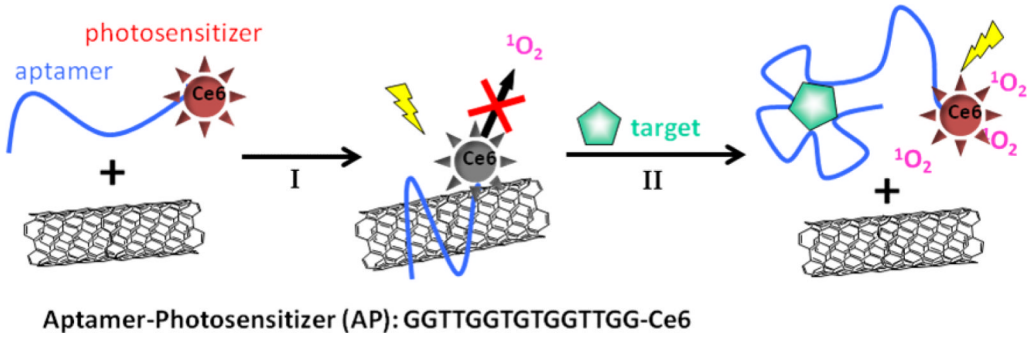

Figure 4.

Schematic representation of aptamer-photosensitizer-SWNT complex and the regulation of SOG upon target binding: (I) AP and SWNTs were mixed together to form an AP-SWNT complex. The ssDNA aptamer is wrapped onto the surface of SWNTs, which brings the photosensitizer close to the SWNTs to quench SOG. (II) Target binding with aptamers can disturb the interaction between AP and SWNTs, resulting in the restoration of SOG. Adapted from Ref. [53].

Table 1

Aptamers for drug delivery

Target name	Aptamer	Selection technique	Delivery application	Refs.
Epidermal growth factor receptor (EGFR)	RNA	Purified extracellular domain of EGFR	Nanoparticle delivery	[63]
Immunoglobin heavy mu chain (IGHM)	DNA	Cell-SELEX	Micelle nanoparticles for drug delivery	[14]
Mucin-1 (MUC-1)	DNA	Recombinant peptides	Photodynamic therapy (PDT) Radionuclide delivery	[64] [65]
Prostate-specific membrane antigen (PSMA)	RNA	Purified extracellular domain of PSMA	siRNA delivery Cytotoxin delivery Chemotherapeutic drug delivery	[66] [67] [68]
Protein tyrosine kinase- 7 (PTK7)	DNA	Cell-SELEX	Chemotherapeutic drug delivery	[69]