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Advances in the development of aptamer drug conjugates (ApDC) for targeted drug delivery

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Abstract

A key goal of modernmedicine is target-specific therapeutic intervention.However, most drugs lackselectivity, resulting in“off-target” side effects.To address the requirements of “targeted therapy,”aptamers, which are artificial oligonucleotides,have beenusedas novel targeting ligands to construct aptamer drug conjugates (ApDC)that can specifically bind to a broad spectrum of targets, including diseased cells. Accordingly, the applicationof aptamers in targeted drug delivery has attracted broad interest due to their impressive selectivity and affinity, low immunogenicity, easy synthesis with high reproducibility, facile modification, and relatively rapid tissue penetration with no toxicity. Functionally, aptamers themselves can be used as macromolecular drugs, and they are also commonly used in biomarker discovery and targeted drug delivery. In this review,we will highlight the most recent advances in the development of aptamers and aptamer conjugates, and discuss their potential in targeted therapy.

Graphical abstract

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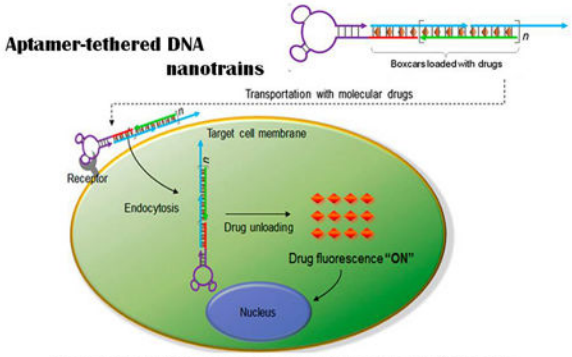

Aptamer-tethered DNA nanotrains used to transport drugs for theranostic applications as a freight train did for the goal of target-specific therapeutic intervention.

Keywords

aptamer; ApDC; cell-SELEX; conjugate; nanomaterials; targeted drug delivery

1. Introduction

Off-target drug delivery, especially in the cases of anticancer drugs, can cause serious side effects to normal tissues and organs. Moreover, the resulting suboptimal dosage in diseased cells may lead to inefficient therapeutic efficacy and even cause drug resistance. Targeted drug delivery, which can selectively and efficiently address chemotherapeutics to diseased sites,isexpected to significantly reduce systematic toxicity and improve therapeutic effect.[1]. Indeed, thus far, hundreds of approaches for targeted drug delivery have been developed, and some have been approved by the U.S. Food and Drug Administration (FDA) for cancer treatment[1, 2]. For example, OKT3 (muromonab-CD3), an IgG2a CD3-specific transplant rejection drug, was the first FDA-approved therapeutic antibody (Hooks et al., 1991)[3].Since then, antibodies have been widely used in targeted drug delivery. However, there are stilla number of limitations associated withantibody-based targeted therapy, includingtheirlarge size, high productioncost, high batch-to-batch variation, high immunogenicity, low thermal stability, and complicated modifications[4-7].

Aptamers were first reported in 1990[8]. They are artificialoligonucleotidesgenerated by an *in vitro*selection technique called Systematic Evolution of Ligands by Exponential Enrichment (SELEX). As demonstrated, their binding affinities arecomparable or superior tothose of most antibodies. In addition, aptamers have further advantages over antibodies, such as smallsize, low production cost, facile chemical modification, low immunogenicity, lowbatch-to-batch variation, high chemical stability, rapid tissue penetration, and no toxicity[9-17]. Because of these advantages, aptamers have attracted tremendous attention in the areas of biosensing, imaging and drug delivery.[10, 18-22] To date, numerous high-affinity aptamers have been selected for a broad range of target molecules, including metal ions, small molecules, peptides, proteins, and even whole cells or viruses[23-30]. Recently,theTan group used whole cells as targetsanddeveloped a simple and efficient cell-based aptamer selection strategy called cell-SELEX[31]. This process enables selection of aptamersagainst native molecular signatures present on the membrane surface of target cells. Taken together,

aptamers selected *via* cell-SELEX have greatpotential as highly specific ligandsfor targeted drug delivery. In this review, we will highlight the most recent advances in the development of aptamers and aptamer conjugates and discuss their potential in targetedtherapies.

2. Aptamerselectionand Cell-SELEX

2.1 Advantages of aptamers compared with antibodies

It has been nearly three decades since scientists first reported Systematic Evolution of Ligands by Exponential Enrichment (SELEX), a process for*in vitro* selection of aptamers against targets of interest. The term “aptamer” comes from the Latin *aptus,*orfit, and Greek*meros,* or part, which suggests the lock-and-key relationship between target and aptamer. More specifically, aptamers areRNA or DNA oligomers that spontaneously fold into specific three-dimensional conformations that can bind defined targets with high affinity and specificity. Compared to antibodies, these nucleic acid moleculesare easily engineered by a fully controlled synthesis process and, hence, are much more cost-effective to produce. As such, aptamers have been introduced as core elements in research disciplines ranging from materials science to biomedicine, particularly, targeted drug delivery[32-35].

2.1.1 Controllable and cost-effectivesynthesis—Because aptamers can be chemically produced*in vitro*, selection protocols can be controlled and conveniently adjusted in a test tube on demand.Because of their thermal and chemical stability, aptamers can be synthesized with no need to apply physiological temperature or buffers, whereas antibodies, as proteins, are prone to contamination and loss of biological activity under unstable experimental conditions. Moreover, antibodies often suffer from batch-to-batch variation and canbe denatured or easily degraded under poor storage or transport conditions. On the other hand, chemically synthesized aptamers are temperature-resistant and can tolerate transport with no restrictive requirements for cooling.This eliminates the need for a continuous cold chain and reduces the cost of long-term storage or transport of aptamers. In brief, aptamers can be readily synthesized in large quantities and atlow cost, whereas antibody production is relatively laborious and requires live cell screening.

2.1.2 Non-immunogenic and nontoxic withbroadtarget selection—Aptamers havelow immunogenicity and notoxicity*in vivo*. Compared with antibodies, aptamers with smaller size (~30 kDa) cansurpassantigen-antibody recognition, and they have been found to recognize the IgG Fc region in mouse[36]. In contrast to antibodies, aptamers can bind to hundreds of molecules of different sizes and structures without compromising immune response. To date, many aptamer targets have been identified, including cocaine[37, 38], growth factors[39-41], peptides[42, 43], toxins[44, 45], viral proteins[46, 47], even live cells and tissues[48-51], most of whichwereperformed successfully under controlled aptamer selection and translated into clinical practice.

2.1.3 Effective penetration—Based on their small size, aptamers can penetrate tissue barriers smoothly and be internalized, thus facilitating their use in biosensing and drug delivery. For example, as an alternative to epithelial cell adhesion molecule (EpCAM) antibody, Pu etal.[51]developed a time- and cost-saving molecular tool for the diagnosis

ofcancers of epithelial origin using a DNA-based EpCAM aptamer, SYL3C.Ristau et al.[52]demonstrated an EGFR aptamer that could recognize twice as many epitopes as its antibody counterpart, while aprostate-specific membrane antigen (PSMA) aptamer identifiedahundred-foldmore epitopes compared with the PSMA antibody.

2.2 SELEX and Cell-SELEX

Conventional SELEX is a well-controlled and efficient technology,involvingmultiple selection rounds of exponential amplification and enrichment for the screening of oligonucleotides with high affinities for their targets from random-sequence libraries. The total size of the library can range from 10^{14} to 10^{15}, providing for a wide range of 3-dimensional folding structures [53]. As shown in Figure 1, the SELEX procedure starts with the design of pools of nucleic acid sequences containing a randomized central core30-50nt long flanked by two conserved primer binding sites for enzymatic pool replication. Therandom sequences in the initial pool will fold into uniquesecondary and tertiary structures, some of whichcan form aptamer-target complexes. In the following step, the binding sequences are eluted and amplified by PCR (DNA aptamers) or RT-PCR (RNA aptamers), and reaction products are used as a new aptamer subpool for the next round of selection. These steps represent a single round of SELEX, but to obtain aptamers with high affinity and specificity, 7 to 20 iterative rounds are generally required.

Although the process has been developed over the course of many years, conventional SELEX still remainstime- and labor-intensive. In addition, some SELEX-selected aptamers fail to recognize their targets as expected. However,improvements have been made using classical techniques for aptamer selection, such as magnetic bead-based SELEX[54], capillary electrophoresis-SELEX[55,56], automated SELEX[57],and cell-SELEX[58, 59]. Suchtechniques have been able to immobilize SELEX targets, facilitate the capture of the targets, or even manipulate time-consuming repetitive cycles automatically during selection.It has been a challenge to select aptamers able to recognize a cognate target ligand in its native conformation. To address this, the Tan grouprecently developed a modified SELEX technology termed Cell-SELEX, which uses whole living cells, including myeloid leukemia, lymphocytic leukemia, liver cancer, small-cell and non-small cell lung cancer, as targets[60-62].This allows aptamer recognition of a target molecule in its native conformation with corresponding translational value and clinical application.

Moreover, cell-SELEX has brought the scientific community closer to realizing preferential binding to novel biomarkersand transport of drug payloads to disease cells. For instance,theTan group recently developed a truncated DNA aptamer termed XQ-2d[59], with high affinity and specificity for pancreatic ductal adenocarcinoma (PDAC), and applied it to *in vivo* imaging and clinical tissue recognition. Previously, they identifiedthe target of aptamer TOV6 to be a cell-surface membrane receptor, stress-induced phosphoprotein 1 (STIP1)[63],associated with poor survival outcome in epithelial ovarian cancer (EOC). They also found that aptamer TD05 targeted Immunoglobulin Heavy mu chain (IGHM) associated with Burkitt's lymphoma (American)(Ramos cells)[64]. In all, more and more aptamers selected through Cell-SELEX are advancing the potential for early diagnosis and imaging, as well as targeted therapy.

3. Aptamersasmacromoleculardrugs

The development of monoclonal antibodies is currently driving the targeted therapy revolution. However, aptamers have also been utilized as macromolecular drugs. Sullenger et al.[65]first found that nucleic acidscould prevent the activation of viral gene expression by overexpressinga trans-activation response decoy in host cells,resulting in the inhibition of viral replication. Since then, pegaptanib (Macugen, Pfizer)was approved by the FDA in 2004 as the first therapeutic aptamer for anti-VEGF treatment of neovascular age-related macular degeneration[66]. In addition to Macugen, abroad array of aptamers has beendesigned to inhibit or activate their targets in order to affect downstream signaling, thereby making them potentially useful as pharmaceutical or therapeutic agents in cancers. Notably, aptamer AS1411[67], which is currently in phase II clinical trials,can recognize a BCL-2 mRNA binding protein,nucleolin, associated with acute myelogenous leukemia (AML). Upon binding, AS1411 can beimmediately internalized,disruptingintracellular pathways andinhibiting cancer cell proliferation.Aptamersarealso considered good anticoagulant agents, as determined by Dobrovolsky et al. who developed DNA aptamers against thrombin to preventthrombin-induced clotting and platelet cell aggregation[67]. Anticoagulant aptamers are active against thrombin, prothrombin, coagulation factor VII, Factor IX, Factor X, and von Willebrand factor(vWF)[68-74].So far, many of the aptamers usedasanticoagulants are in the first phase of clinical trials.

Apart from aptamers in clinical trials or in clinical treatment, as noted above,developmental work is underwayto perfect more aptamer-based drugs. For instance, a DNA aptamer, termed RA10-6, efficiently blocks IL-17 binding to IL-17RA in a dose-dependent manner*in vitro*[75]. The injection of RA10-6 to an experimental mouse model of osteoarthritis resultedin thereduction ofIL-6 levels and synovial thickening, revealingRA10-6 as a potent adjunctive agent for the early treatment of osteoarthritis. Another study focused on advanced glycation end products (AGEs) and their receptor (RAGE), both of which play important roles in diabetic complications, such as nephropathy, retinopathy and neuropathy. According to the report, these diabetic complications result from inflammatory reactions activated by AGEs. Kaidaet al. established an animal model of type 2 diabetes with renal injury KKAy/Ta mice[76]. They detected increased urinary albumin and 8-hydroxy-2′-deoxy-guanosine levels inthese mice, as well as glomerular hypertrophy and enhanced extracellular matrix accumulation. However, the AGEs-aptamer was able to arrest experimental diabetic nephropathy in this mouse model.Thus, such aptamers with efficacy against cell proliferation, metabolic dysregulation,inflammation and coagulation are likely to make significant contributions to the treatment of various diseases in the near future.

4. Aptamer-drugconjugatesin targeted drug delivery

Aptamers linked toanticancer drugs have enabled the selective delivery of therapeutic compounds to diseased sites.In the section below, we cite representative examples of direct aptamer-drug conjugationbya chemical covalent linker or physical intercalation.

4.1 Covalent conjugation

Covalent conjugation between aptamers and chemotherapeutics dates back to an early experiment of Huang et al. using DNA aptamer sgc8, which specifically targetsprotein tyrosine kinase 7 (PTK7) overexpressed on human T-cell acute lymphoblastic leukemia (T-ALL) fromtheCCRF-CEM cell line[77]. Cleavage of a sgc8-Doxorubicin (Dox) conjugate was evoked in an acidic environment with pH 4.5–5.5 in order to control release of Dox. Tests of cell viability *in vitro* demonstrated that the sgc8c-Doxconjugate was potent in lowering toxicity towards nontarget cells compared with the unconjugated parent Dox(Figure 2). Nevertheless, this strategydid present some flaws,such as low copy number of drugs conjugated onto each aptamer. In response, Boyaciogul et al.[78] synthesized a novel dimeric aptamer complex (DAC) for high-capacity targeted drug delivery. More recently,Wang et al.[79]proposed and synthesized a more efficient strategy that not only enhanced the drug payload capacity of aptamer-drug conjugates, but also provided spatiotemporal controllability of intracellular drug release.A frequently prescribed anticancer drug, 5-fluorouracil (5-FU) for the treatment of colorectal cancer and pancreatic cancer, has been incorporated into anApDCsgc8-$(5\text{-FU})_5$conjugate, in which one sgc8 aptamer carries 5 copies of 5-FU, thereby increasing drug payload capacity and decreasing cost. On the other hand, a photocleavable (PC) linker has been used to link a drug moiety with the backbone of phosphoramidite, which served as a modular buildingblock.Under light irradiation, the cleavage of the PC linker released the tethered 5-FU molecules from the aptamer backbone, inhibiting cancer cell proliferation. Meanwhile, the recently recommended combinational chemotherapy has led to correspondingly increased side effects. To address this,Zhu et al.[80]utilized a simple biocompatible reaction to construct ApDCs with multiple drug copies, including anthracycline drugs (e.g., Doxorubincin, Daunorubicin, Epirubicin, and cisplatin), which could inhibit cell proliferation by disrupting cell division and inducing cell apoptosis. In this conjugate, a crosslinker, formaldehyde, was used to form a methylene linker with the 2-NH_2 on deoxyguanosine (dG) and the 3-NH_2 group of Dox on each side. The 2-NH_2 on the dG conjugate allowed one aptamer tocarrymultiple drugs in the ApDCs. Furthermore, temperature-dependent cleavage of the methylene linker offered gradual drug release at physiological temperature, thus enabling efficient production of ApDCs with high drug loading and drug release controllability.

4.2 Noncovalentaptamerconjugation through intercalation

In addition to stablecovalent bonding, noncovalent aptamer-drug conjugation, with the simplicity of programmable nucleic acid engineering, represents another attractive drug delivery strategy fortargeted therapy. In 2006, a PSMA-targeting RNA aptamer, A10, synthesized by Farokhzad et al. was considered as the first physical complex with drug molecules requiring no covalent modifications[81]and having an intrinsic intercalating site for Dox. According to their report, approximately 1.2 times dose Dox wasintercalated into the A10 aptamer, leading to selective cytotoxicity and relatively abundantdrug loading against PSMA-positive cells. Another aptamer-Dox complexwas then developed by Liu et al. for targeteddrug delivery to breast cancer. However, in retrospect, neither strategy was able to meet full drug-carrying capacity. To achieve this requirement for *in vivo* cancer treatment, aptamer-tethered DNA nanotrains (aptNTrs) wereexplored by the Tan group[82]. In this study, modified sgc8 aptamer acted as a locomotive for targeting, while the remaining dsDNA

nanoconstructs, which contained numerous Dox intercalation sites, acted as boxcars for drug delivery. The resultant sgc8c-NTrs displayed high cargo loading capacity with a Dox:sgc8-NTr molar ratio of 50:1. This special and efficient delivery platform holds promise for minimizing side effects and broadening applications for targeted drug delivery (Figure 3).

Another drawback involved the limited recognition of single aptamers given the heterogeneity of clinical samples from different patients.Zhu et al.[83] sought to solve this problem by developing a bi-specific aptamer-based drug delivery systemcontainingtwo aptamers, sgc8 and sgd5a, with drug-intercalating dsDNA as both linker and drug carrier, able to recognize two subtypes of a cancer with heterogeneous biomarkers, therebyovercomingmany diagnostic and therapeutic complications for future clinical applications.

5. Targeted delivery usingaptamer-functionalized nanomaterials

Combining size at the nanometer scale and unique structures, nanomaterials, such as nanoparticles, liposomes, and hydrogels,[84-93] offer many physical, chemical and biological properties conducive to aptamer functionalization and targeted drug delivery. For example, nanomaterials offer large surface area-to-volume ratios, the ability to travel through the blood stream withoutblockage of the microvasculature,and the ability to be taken up by cells through endocytosisand penetrate tissues.Broadening the scope of both aptamers and nanomaterials, many of the advantages of aptamers are compatible with nanomaterials, resulting in conjugates able to minimize the drawbacks of each technology when applied separately to address the same biological issues, e.g., targeted drug delivery. Below we discuss some of these innovative bioconjugates.

5.1 Aptamer-conjugated gold nanoparticles for targeted therapy

Gold nanoparticles (AuNPs)are the most common and stable metallic nanomaterialsclinically appliedover the last few decades. AuNPs can form thiolated complexes by stable Au:S bonds and introduce diverse molecules enabling multiple functionalities for drug delivery. Moreover, the inert nature of AuNPs ensures nontoxicity to living cells, as demonstrated by*in vitro* studies, making AuNPs ideal candidates for drug transport. The size and shape of AuNPs, e.g. nanorods, nanospheres, nanoshells, and nanocages, can be precisely adjusted for a variety of applications.

Among a wide array of cancer treatments, photothermal therapy (PTT) is relatively noninvasive and benign.PTT simplyexposes biological tissues to higher temperatures to promote the destruction of abnormal cells. Gold nanorods (AuNRs), providing a higher absorption cross section per unit volume than other AuNPs, are more feasible for future clinical PTT.Huang et al.[94]conjugated aptamer sgc8c to Au-Ag NRs for selective PTT *in vitro*. The binding affinity of this sgc8c-NR conjugate for targeted CCRF-CEM cells was foundto be 26-fold greater than that ofsgc8c used alone.Alongwith itshigh absorption efficiency and superior photothermal transfer, this bioconjugate is highly promising for specific recognition and targeted PTT.Following this work,aptamer CSC1 targeting DU145 prostate cancer cellsand aptamer CSC13 targetingasubpopulationof DU145cancer stem

cellswere linked to the surface of AuNRs. The bi-specific CSC13-AuNRcomplexes were able to kill both cancer cells and cancer stem cells using NIR laser irradiation[95].

For more effective drug loading,the hollow interior of AuNPs can be utilized, or their surface can be coated with mesoporous materials to enlarge pore volume and surface area. As an example of the first type, gold nanospheres (HAuNS) are composed of an Au shell with a hollow interior[96], which, compared to AuNPs, has similar size, surface charge, and equivalent Au concentration, but 3.5-fold greater drug loading capacity(Figure 4A).A delivery vehicle has also been explored using HAuNS together with a highly specific RNA aptamer to target CD30.TheresultantAptamer-HAuNS-Dox showed selective destruction of lymphoma tumor cells with minimal “off-target” effects. Coating AuNRs with mesoporous silica (AuMPs)has attracted attention as a potential drug delivery system owing to the high surface area for effective drug loading andmesoporous container for functional nucleic acids for controlled drug delivery and release[97, 98].Aptamer AS1411 has been utilized as a molecular gate grafted onto the surface of AuMPs forming a dimeric G-quadruplex structure to cap guest molecules. Whenirradiated by NIR light, the photothermal effect leads to a dehybridization of the linked DNA duplex that anchors the capping molecules, therebyallowing the specific release of the loaded cargo[99].Thus, by its functionalization as both targeting and capping agent, aptamer AS1411 makes this AuMP-based porous nanocarrier a good choice for remote-controlled targeted drug delivery, as its*in vivo*applicationis enabled by laser-induced thermal stimulus (Figure 4B).

5.2 Other aptamer-conjugated nanoparticles for targeted therapy

Softnanoparticles, such as liposomesand hydrogels, also exhibitunique advantages,including high water-solubility, enhancedaccumulation at the tumor cells, prolonged circulationtime in the blood andinherent biocompatibility.

Liposomes, which are artificially prepared vesicles composed of a lipid bilayer, are biocompatible and biodegradable. To form aptamer-liposome bioconjugates, cholesterol-modified aptamers can be spontaneously anchored on the outer shell during the formation of the NP conjugate.Thisadvantage has been utilized to engineer liposomesfor the delivery of toxic chemotherapeutic drugs, such as cisplatin and taxol. As reported, compared to nontargeting liposomes, the AS1411 aptamer-functionalized liposomes containing taxol have increased rates of cellular uptake and cytotoxicity for MCF-7 breast cancer cells[100]. Athymic nude mice bearing xenograft MCF-7 tumors treated with intratumoral injection of aptamer-functionalized liposomesexhibited earlier onset of tumor inhibition and improved anticancer efficacy.Kang et al. have successfully extended the application of aptamer-functionalized liposomes to the cellular level by simplification of the aptamer-modified liposome synthesis method[101]. Each liposome had approximately 250 aptamers tethered to its surface to facilitate target binding, and several thousand FITC-Dextran (FD) molecules (drug proxy)wereloaded inside. Within 30 minutes of incubation time, the aptamer-liposome conjugates could specifically bind with target cells and release the loaded model drug. Targeted drug delivery was guaranteed by sgc8 aptamer binding with its target CEM cells for breast cancer.

Hydrogels are crosslinked hydrophilic polymer structures that can hold large amounts of water or biological fluids in their pore spaces. As one of the newest classes of polymer-based systems, target-responsive hydrogels have found numerous biomedical and pharmaceutical applications[102-105].Li et al.[88]recently reported DNA nanohydrogels created through a self-assembly process. These DNA nanohydrogels consist of three elements, respectively termed Y-shaped monomer A with three sticky ends (YMA), Y-shaped monomer B with one sticky end (YMB), and DNA linker (LK) with two sticky ends. DNA nanohydrogels are size-controllable by varying the ratio of YMA to YMB. By incorporating different functional elements, such as aptamers, disulfide linkages, and therapeutic genes into different building blocks, the synthesized aptamer-based nanohydrogels (Y-gel-Apt) can be used for targeted and stimuli-responsive gene therapy. Y-gel-Apt strongly inhibited cell proliferation and migration in target A549 cells, but not in control cells. By taking advantage of both aptamers and nanohydrogels, this Y-gel-Apt with facile self-assembly, effective cellular uptake, and superior biocompatibility holdspromise as a candidate for targeted gene or drug delivery in cancer therapy.

6. Conclusion

Extensive genome sequencing and proteomic analysis in past decades have generated vast quantities of information for disease biology, changing, as a result,some inherent concepts in treating disease.The conjugation of less selective therapeutics withtarget-specific ligands has emerged as a promising alternativeto the use of antibodies.Based on their unique advantages and properties, compared to antibodies,aptamershaveattracted increasing attentionfor application in biomedicine to achieve targeted therapy. Cell-SELEX, whichselects aptamers againstwhole live cells without prior knowledge of molecular signatures onthe cell surface, is an ideal toolfor the selection of aptamers able to preferentially bind to diseased cells.Over past decades, this technologyhas enabled the generation ofbulk aptamers targetinga number of proteins,including PSMA, PTK7, IGHM, and nucleolin. Aptamerscan also act as macromolecular drugs in a myriad of human diseases, includingcancers andmetabolic diseases,as well as disorders resulting in inflammatory conditions and coagulation abnormality. Moreover, bioconjugation between nanomaterials and aptamers takes advantage of both technologies, making aptamer-based NP conjugates ideal vehicles for drug delivery applications, especially for the controlled release of drugs. Taken together, aptamershavethus farsuccessfully performedas drugs or guidance systems for targeted drug delivery and will certainly become even more widespread clinical tools in biomedical applications.

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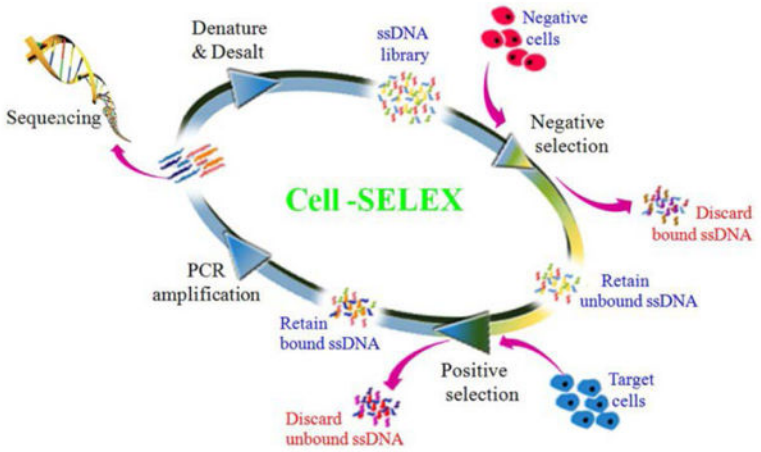

Figure 1.

Schematic illustration of the cell-SELEX process (Reproduced with permission from Reference59).

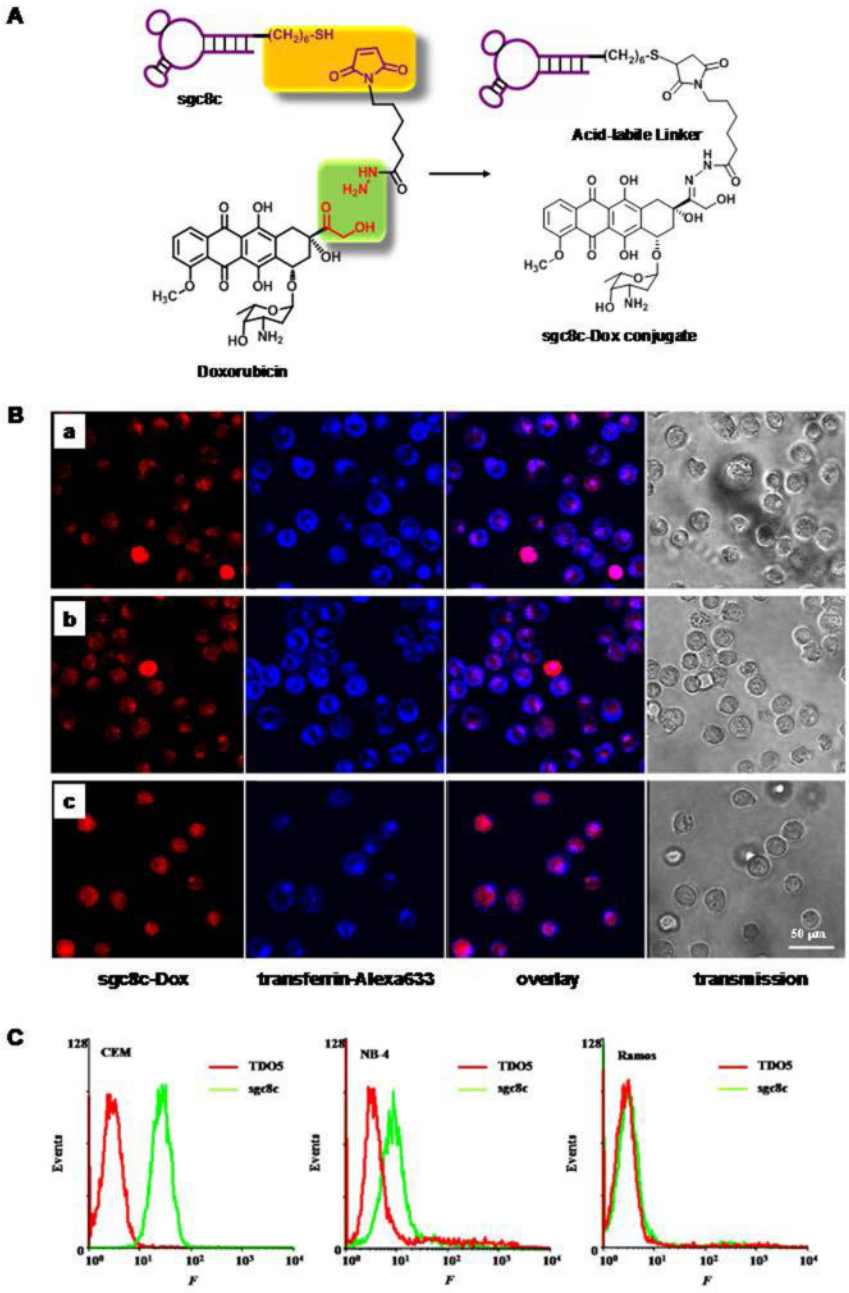

Figure 2.

Sgc8c-Dox conjugates for targeted drug delivery. (A) Schematic diagrams depicting sgc8c-Dox covalent conjugation via acid-labile linkages. (B) Distribution of sgc8c-Dox conjugates inside CCRF-CEM cells after incubation with cells for (a) 30 min, (b) 1 h, and (c) 2 h. From left to right, the fluorescence confocal images were monitored for sgc8c-Dox, transferrin-Alexa633, overlay of these two channels, and bright field channel, respectively. (C) Flow cytometry assay for the binding of biotin-labeled TDO5 and sgc8c with three different cell lines: CCRF-CEM, NB-4, and Ramos. Cells (10^5/mL) were incubated with biotin-labeled TDO5 and sgc8c at 37 °C for 20 min in 100 μL culture medium without FBS. After washing

twice, cells were mixed with streptavidin-(R-phycoerythrin) (20 min on ice), and the fluorescence was determined by flow cytometry(Reproduced with permission from Reference77).

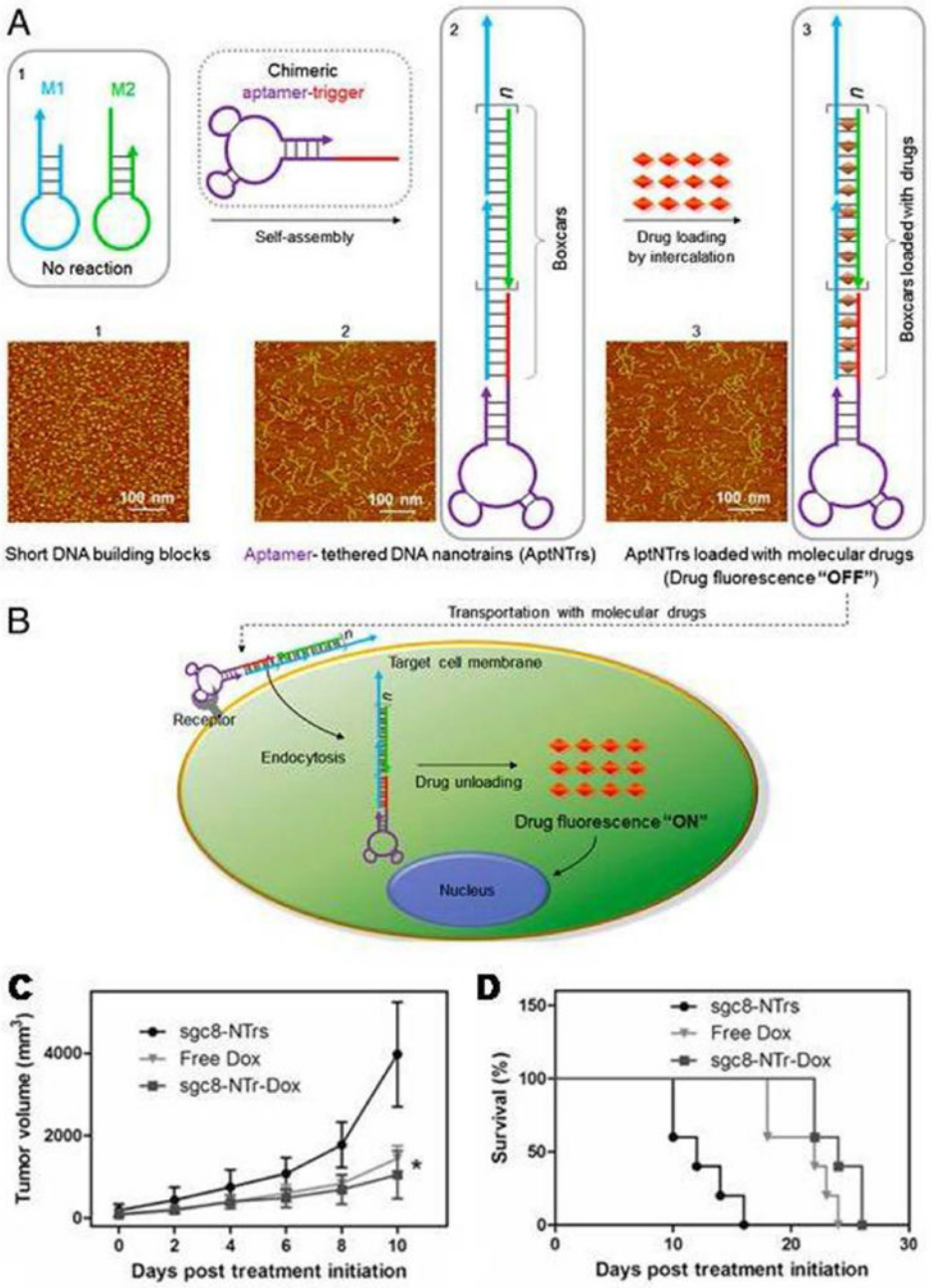

Figure 3.

Aptamer-tetheredDNAnanotrains (aptNTrs) used to transport drugsfortheranostic applications. (A) Schematic diagram depicting the self-assembly of aptNTrs from two partially complementary short hairpin monomers upon initiation by an aptamer-tethered probe through hybridization chain reaction (HCR). AFM images (1–3) show the morphologies of the corresponding nanostructures.(B) Drugs were specificallytransported to target cancer cells by aptNTrs and unloaded to induce cytotoxicity to target cells. (C) Potent antitumor efficacy and reduced side effects of drugs transported by aptNTrs. Tumor volumes of subcutaneousCEM xenograft mouse tumors were measured after drug administration up

to day 10 ($n = 5$). Asterisk on day 10 represents significant differences between tumor volumes of free Dox- and sgc8-NTr-Dox-treated mice (*$P < 0.05$, $n = 5$; Student's-t test). (D) Percentage of surviving mice after treatment initiation(Reproducedwith permission from Reference82).

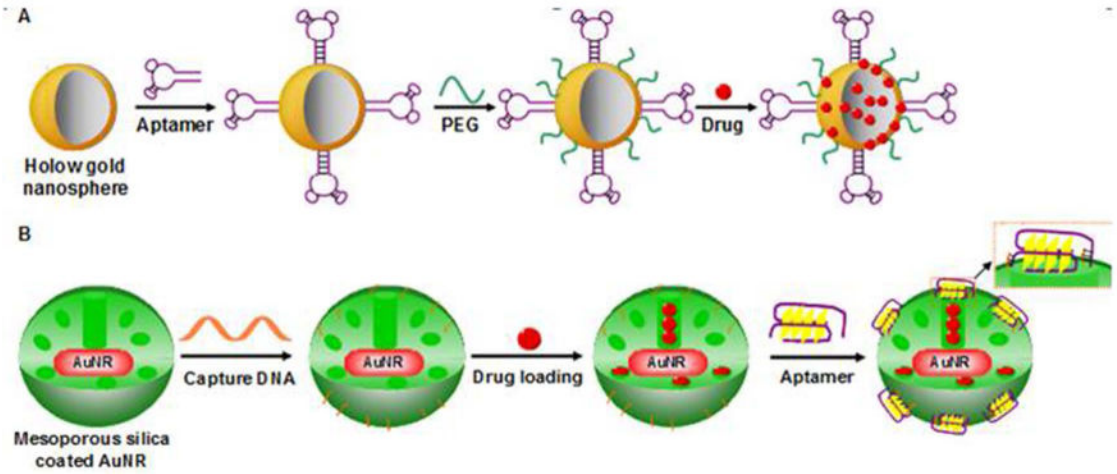

Figure 4.

Schematic diagrams of nanoplatforms based on aptamer-conjugated gold nanoparticles for targeted drugdelivery. (A) Apt-HAuNS-Dox nanoscale drug carrier. (B) AuNR-based mesoporous silica nanocarrier(Reproduced with permission from Reference97).

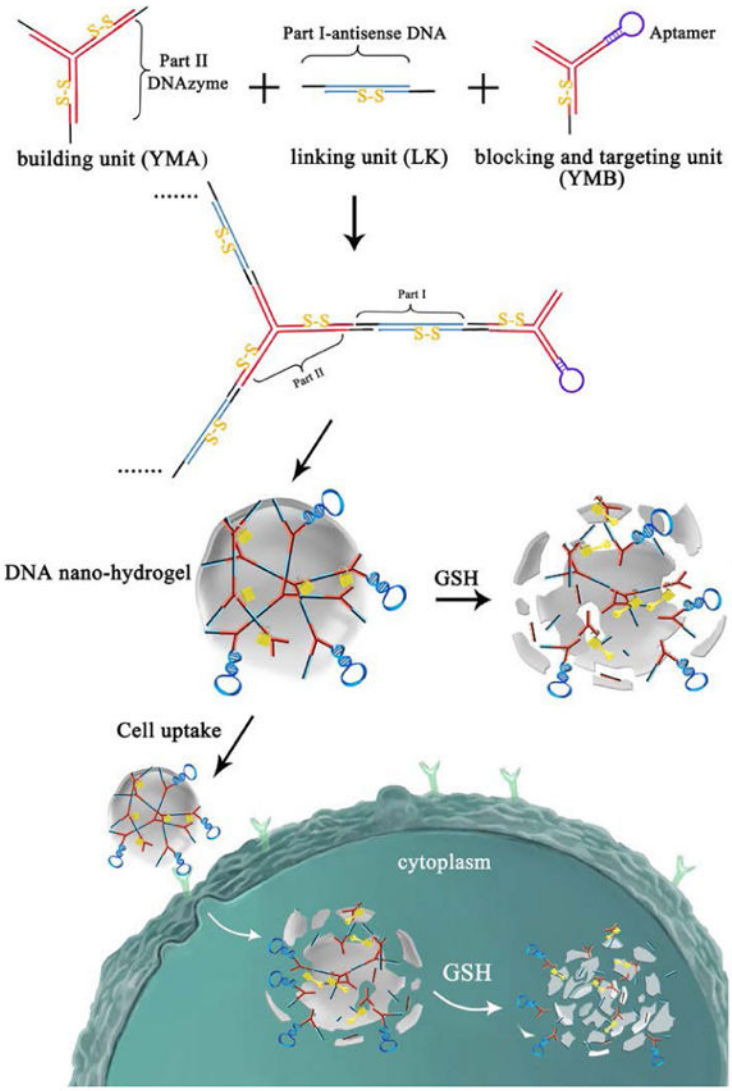

Figure 5.
Schematic illustration of Stimuli-Responsive DNA Nanohydrogel formation (Reproduced with permission from Reference88).