Title

Recent progress of aptamer–drug conjugates in cancer therapy.

Journal

Acta pharmaceutica Sinica. B, 13(4)

ISSN

2211-3835

Authors

He, Jiaxuan
Duan, Qiao
Ran, Chunyan
et al.

Publication Date

2023-04-01

DOI

10.1016/j.apsb.2023.01.017

Peer reviewed

Acta Pharmaceutica Sinica B 2023;13(4):1358−1370

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com

REVIEW

Recent progress of aptamer−drug conjugates in cancer therapy

Jiaxuan He[a], Qiao Duan[a,c], Chunyan Ran[a], Ting Fu[a,b], Yuan Liu[a,*], Weihong Tan[a,b,c,*]

[a]*The Cancer Hospital of the University of Chinese Academy of Sciences (Zhejiang Cancer Hospital), The Key Laboratory of Zhejiang Province for Aptamers and Theranostics, Hangzhou Institute of Medicine (HIM), Chinese Academy of Sciences, Hangzhou 310022, China*
[b]*Molecular Science and Biomedicine Laboratory (MBL), State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, College of Biology, Aptamer Engineering Center of Hunan Province, Hunan University, Changsha 410082, China*
[c]*Institute of Molecular Medicine (IMM), Renji Hospital, Shanghai Jiao Tong University School of Medicine, and College of Chemistry and Chemical Engineering, Shanghai Jiao Tong University, Shanghai 200240, China*

Received 20 September 2022; received in revised form 18 November 2022; accepted 15 December 2022

KEY WORDS

Aptamer;
Cancer therapy;
Aptamer−drug conjugate;
Drug delivery;
Small-molecule drug;
Therapeutic molecules;
Nucleic acid drugs;
Biotherapeutic drug

Abstract Aptamers are single-stranded DNA or RNA sequences that can specifically bind with the target protein or molecule *via* specific secondary structures. Compared to antibody-drug conjugates (ADC), aptamer−drug conjugate (ApDC) is also an efficient, targeted drug for cancer therapy with a smaller size, higher chemical stability, lower immunogenicity, faster tissue penetration, and facile engineering. Despite all these advantages, several key factors have delayed the clinical translation of ApDC, such as *in vivo* off-target effects and potential safety issues. In this review, we highlight the most recent progress in the development of ApDC and discuss solutions to the problems noted above.

*Corresponding authors.
E-mail addresses: yuanliu@ucas.ac.cn (Yuan Liu), tan@hnu.edu.cn (Weihong Tan).

Peer review under responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2023.01.017

1. Introduction

The severe acute respiratory syndrome coronavirus (SARS-CoV-2) caused an unprecedented crisis. mRNA vaccines have been recognized as one of the most effective ways to fight the pandemic[1,2]. Nucleic acid drugs, such as siRNA, miRNA, and aptamer, are playing a more and more important role not only in anti-virus but also in cancer therapy and other diseases[3]. Especially, nucleic acid drugs show prominent potential to treat diseases with undruggable targets for small molecules or protein drugs. Aptamers are a type of single-stranded DNA or RNA sequence that specifically binds to a target[4–6]. Aptamers are mainly screened from a technology known as Systematic Evolution of Ligands by Exponential Enrichment (SELEX). At present, a variety of aptamer screening methods have been developed[7–12], and aptamer targets include small molecules[13–15], ions[16,17], proteins[18–20], cells[21–23], and tissues[24,25]. Since Larry Gold[26] and Ellington A[27]. reported the aptamer in 1990, thousands of aptamers have been reported[28–33]. Aptamers are called chemical antibodies, but with specificity and affinity equal to, or better than, antibodies. Compared to antibodies, aptamers have the advantages of easy synthesis, facile chemical modification, high stability, and low cost (Table 1). Therefore, aptamers have shown promise in cancer therapy and diagnostics[34–38].

Antibody-drug conjugates (ADC) represent one of the fastest-growing anti-cancer drugs in the past decade, owing to their excellent anti-cancer effect compared to traditional chemotherapy[39,40]. The concept of ADC comes from the idea of "magic bullet" that Paul Ehrlich first presented over 100 years ago[41]. In his idea the "magic bullet" can specifically recognizes its target without harming the host organism. ADC research can be traced back to the 1980s; the U.S. Food and Drug Administration (FDA) did not approve the first ADC for marketing until 2000[42]. Public data show that 14 ADCs have been approved worldwide so far, and more than 150 ADCs are in clinical trials[43,44]. Besides ADCs, some peptide-drug conjugates (PDC) have shown promising anticancer effects and tumor penetration[45,46]. Last year, the FDA approved the first PDC, Pepaxto, to treat relapsed or refractory multiple myeloma (MM)[47]. ADCs and PDCs-alike consist of ligand, linker, and payload. However, the ligand in ADC and PDC is antibody and peptide, respectively. Huang et al.[48] first proposed aptamer–drug conjugate (ApDC) in 2009, a concept similar to ADC and PDC. Here, the aptamer can be easily conjugated with different drugs and act as the targeting ligand. In this review, we summarized the recent progress in developing and applying ApDC in cancer therapy (Scheme 1). We also defined the extended payload of ApDC beyond small-molecule drugs.

Table 1 Comparison of aptamer and antibody.

Character	Aptamer	Antibody
Molecular weight	6–30 kDa	150–180 kDa
Size	~2 nm	~15 nm
Affinity	High	High
Specificity	High	High
Stability	Very stable	Sensitive to temperature
Targets	Wide range of targets	Immunogenic molecules
Generation discovery time	Few hours to months	Several months
In vivo half-life	Short (~20 min)	Long (~one months)
Cost	Lower	Higher
Nuclease degradation	Sensitive	Resistant
Chemical modifications	Various modifications	Limited modifications
Secondary structure	Hairpin, stem, loop, G-quadruplex	A-helix and β-fold

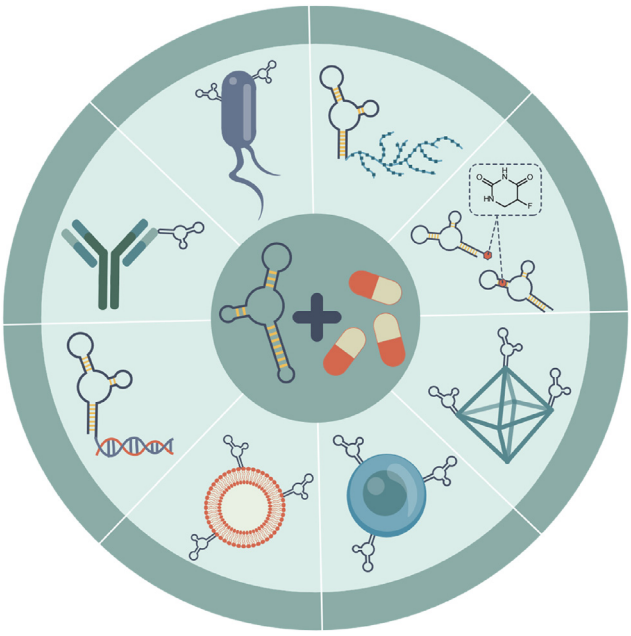
2. Small molecule drug-based ApDC

Despite the rapid development of biological drugs, small-molecule drugs still occupy a significant share of the pharmaceutical market. Small molecule drugs have the advantages of convenient storage and transportation, low immunogenicity, and oral administration. However, they have poor water solubility and relatively large toxic and side effects because of low specificity. Targeted delivery of small-molecule drugs *via* adding targeting ligands can effectively improve the water solubility, thus reducing the toxicity and side effects and enhancing drug efficacy. As an efficient recognition molecule, aptamer can couple with small-molecule drugs to achieve targeted drug delivery. The coupling of aptamers and small molecule drugs mainly adopts three forms: covalent conjugation, nucleic acid synthesis, and physical interaction.

2.1. *Covalent conjugation*

Aptamers have shown high efficacy as a delivery platform for small molecules in cancer therapy. Aptamers and drugs can form covalent coupling by modifying a reactive group, such as amino, thiol, or cyclooctyne (DBCO).

Since developing the first ApDC by covalent coupling in 2009 (Fig. 1A)[48], Tan group have reported multiple studies on covalent ApDC. In 2019, Zhou et al.[49] reported a cyclic bivalent ApDC (cb-ApDC), which can specifically recognize target cells, efficiently internalize into cells, and release drugs upon cleavage by esterase (Fig. 1B). At the same time, cb-ApDC has more stability than a single ApDC. In addition, cb-ApDC achieved precise control of the drug ratio *via* a simple chemical reaction. This cb-ApDC inspired the development of different drug combinations and proportions for combinatorial cancer therapy. For instance, in 2020, Yang et al[50]. Developed the aptamer-Mitomycin C conjugate (Fig. 1C). They found that the cytotoxicity of these ApDC was linker-dependent. The reductant-sensitive crosslinking strategy significantly enhanced the cytotoxicity of MMC for the treatment of target cancer cell lines. The cytotoxicity enhancement has several critical factors, such as specific binding, target recognition, and internalization. Furthermore, He et al.[51] developed a triptolide ApDC to treat Triple-negative breast cancer (TNBC) with high efficacy (Fig. 1D). The triptolide ApDC showed high specificity and cytotoxicity against the MDA-MB-231 cell line. It is noteworthy that the triptolide ApDC has excellent *in vivo* anti-tumor efficacy for TNBC and negligible side effects on healthy organs. In 2021, Li et al.[52] reported an aptamer-artesunate conjugate (Fig. 1E) which showed much higher cytotoxicity than artesunate alone because the aptamer caused an increased accumulation of artesunate in target cells. Meanwhile, *in vitro* experiments demonstrated that the aptamer-artesunate conjugate has the specific targeting capability and remains at the tumor site much longer than in control groups.



Scheme 1 Summary of aptamer-drug conjugate (ApDC).

In addition to the Tan group, other research groups have developed some effective ApDC *via* covalent coupling. Niu et al. collaborated with Prof. Tan[53] to develop the sgc8 aptamer and N-heterocyclic carbene (NHC)-Au(I) complexes to treat CCRF-CEM leukemia cells (Fig. 2A). This ApDC showed increased toxicity to CCRF-CEM cells compared to N-heterocyclic carbene (NHC)-Au(I) complexes alone. The IC_{50} decreased almost 30 times. They further tested other drug complexes and showed enhanced cytotoxicity in different cell lines[54]. Li et al.[55] reported a highly water-soluble aptamer AS1411-paclitaxel conjugate (AS1411-PTX), as shown in Fig. 2B, which can selectively deliver paclitaxel to tumor sites. Cathepsin B-cleaved dipeptide linker was used to connect AS1411 aptamer and the hydroxyl group at position 2 of paclitaxel. AS1411-PTX remains structurally stable in blood circulation. When AS1411-PTX enters tumor cells, the dipeptide linker is cleaved to release PTX to kill tumor cells. The selective enrichment of AS1411-PTX in ovarian tumor tissues significantly improves the *in vivo* antitumor activity of PTX and reduces systemic toxicity. The Gray group[56] screened aptamer E3, which has a high internalization ability. They used MMAE (monomethyl auristatin E) and MMAF (monomethyl auristatin F) to form ApDC E3-MMAE and E3-MMAF. These ApDCs can effectively kill prostate cancer cells *in vitro* with minimal toxicity on normal prostate epithelial cells. At the same time, aptamer E3 can specifically target tumor tissues *in vivo*, and the aptamer E3-MMAF conjugate has a significant growth inhibitory effect in the prostate cancer mouse model. In addition, using the complementary sequence of aptamer E3 as an antidote can attenuate the cytotoxicity of the ApDC.

Kratschmer et al.[57] used maleimide-MMAE and -MMAF conjugated with thiol-modified aptamers to form ApDC. These ApDCs showed high toxicity to the targeted cell lines. They could also specifically enter into and kill pancreatic cancer cells effectively. Pusuluri et al.[58] developed a modular aptamer−drug coupling model. It provided for selective drug combinations in a synergistic ratio for effective low-dose treatment through aptamers specifically targeting tumor cells (Fig. 2C). First, the peptide scaffold coupled doxorubicin (DOX) and camptothecin (CPT) with a precise ratio (Fig. 2D). Then, drug-peptide conjugate and aptamer were conjugated through an efficient chemical reaction to form an aptamer-cooperative drug conjugate. The ApDC showed a very low IC_{50} (31.9 nmol/L) in experiments using the triple-negative breast cancer cell line MDA-MB-231. Furthermore, *in vivo* antitumor study indicated that the effective dose was reduced 20−30 times compared with previously reported studies.

The ApDC formed by covalent conjugation is similar to ADC. Current studies have indicated that conjugation to small molecules has little effect on the aptamer binding affinity and specificity. However, the coupling strategy between aptamers and small molecules are restricted by traditional coupling methods such as thiol-maleimide, azide-alkyne, amino-carboxyl, etc. Therefore, developing new chemistry strategy for efficient conjugation is crucial for exploring highly efficient aptamer−drug conjugates.

2.2. *Nucleic acid synthesis*

Commercial nucleic acid is mainly synthesized through solid-phase and enzymatic synthesis. The small molecules can couple aptamers as base modules. The automated synthesis of ApDC, which uses inactive ingredients of drugs as a bonding moiety, usually does not affect the biological activity of conjugated drugs. When the ApDC enters cells, nucleases can release the drug.

Since the Tan group developed the first photoactivated ApDC *via* solid-phase synthesis (Fig. 3A) in 2014, they have developed multiple ApDC *via* automated DNA synthesis[59]. In 2019, Lv et al.[60] used 5-fluorouracil (5-FU) as a payload to form ApDC to investigate the internalization and subsequent transport of ApDC. Cellular uptake pathways of ApDC were found to be similar to that of aptamer, both being mainly directed by caveolin-mediated endocytosis.

In 2020, the Tan group reported an automated synthesis of Camptothecin (CPT) (Fig. 3B)[61], Combretastatin A4 (CA4) (Fig. 3C)[62], pyrochlorophyll A (PA) (Fig. 3D)[63], and an artificial prodrug base (Fig. 3E)[64]. The CPT ApDC demonstrated a general approach by converting traditional chinese medicine drug molecules into phosphoramidites to build ApDC through a DNA synthesizer[61]. The CA4 ApDC showed a more efficient and safer therapeutic effect than CA4 alone, including a systematic visualization of cellular events of CA4 ApDC upon entering the target cells[62]. Besides its use as a chemotherapeutic drug, PA ApDC also showed high efficacy for targeted photodynamic therapy. PA ApDC could take advantage of the hypoxia-associated tumor microenvironment for cancer treatment as a molecular domino reactor[63]. This ApDC provides a general strategy for creating functional ApDC to overcome the physiological barriers of the tumor microenvironment. Xuan et al.[64] designed a prodrug as an artificial base for the DNA synthesizer to synthesize an aptamer prodrug conjugate (ApPdC) for chemodynamic therapy. The ApPdC was combined with bioorthogonal chemistry to improve the chemodynamic therapeutic efficacy of the prodrug. The prodrug in ApPdC acts as a free radical generator for *in situ* generations of toxic free radicals in cancer cells. In 2021, Huang et al.[65] investigated the influence of linkers in ApDC (Fig. 3F). They developed three CA4 ApDC with disulfide bond, phosphodiester bond, or carbamate bond to decipher the drug release mechanism and anticancer efficacy. The phosphodiester bond could also be cleaved by a nucleophilic attack of glutathione. The repeated cleavage of the linker has a higher anticancer efficacy. This result

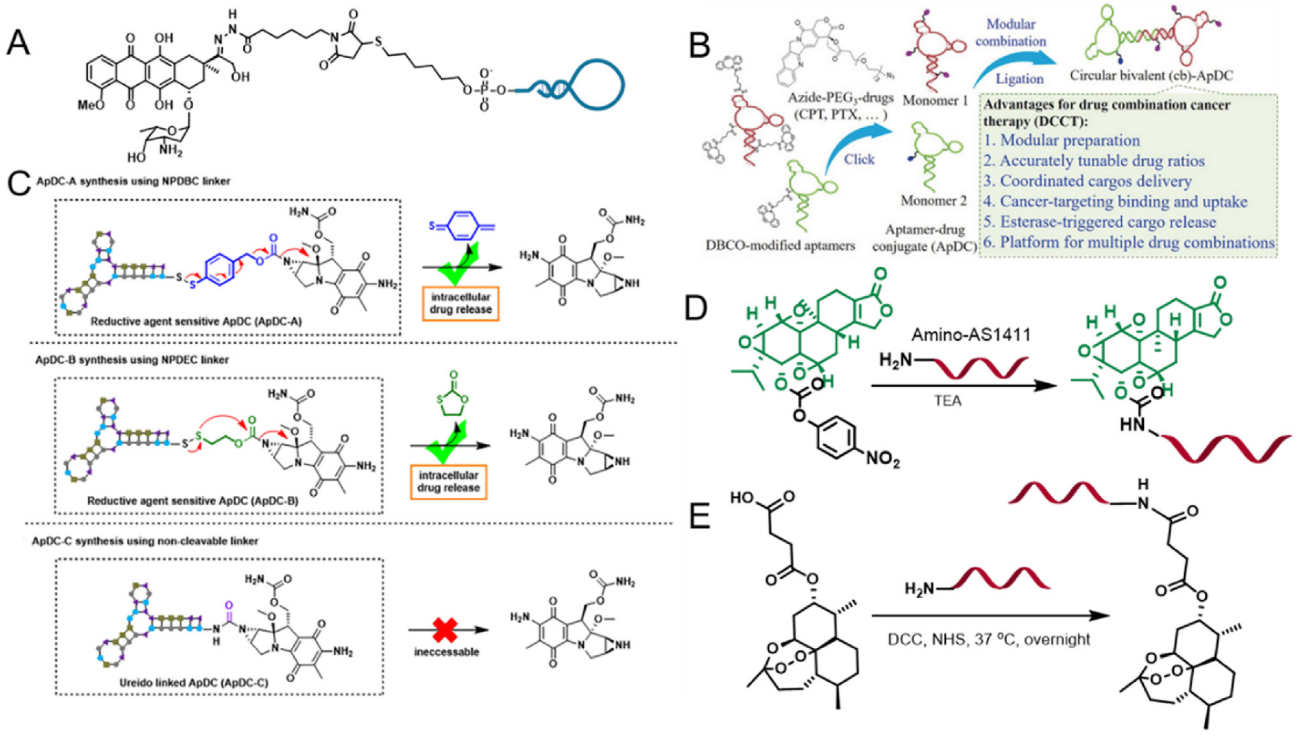


Figure 1 Examples of covalent-coupled ApDC developed by the Weihong Tan group. (A) Aptamer-DOX conjugate by covalent coupling. (B) Cyclic bivalent ApDC. Reprinted with permission from Ref. 49. Copyright © 2019 John Wiley and Sons. (C) Aptamer-Mitomycin C conjugate. Reprinted with permission from Ref. 50. Copyright © 2020, American Chemical Society. (D) AS1411-triptolide conjugate. Reprinted with permission from Ref. 51. Copyright © 2020, American Chemical Society. (E) Aptamer-artesunate conjugate.

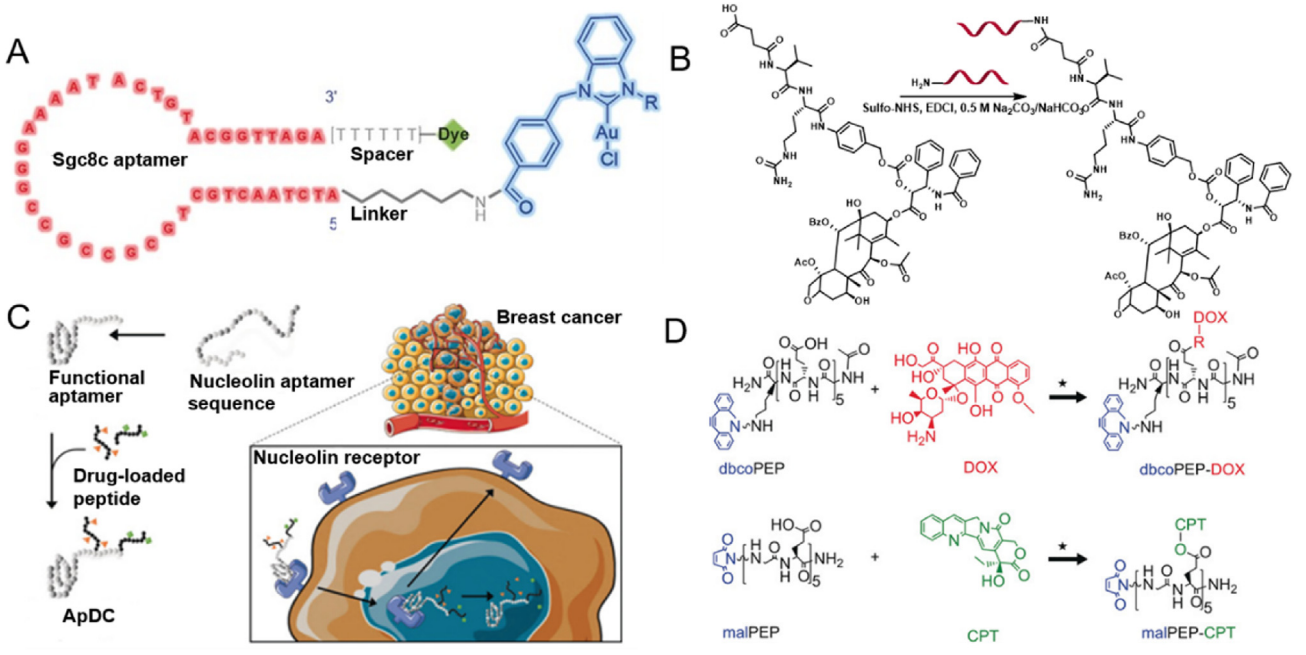


Figure 2 Examples of covalent-coupled ApDCs from other groups. (A) Sgc8 *N*-heterocyclic carbene (NHC)−Au(I) complex conjugate. Reprinted with permission from Ref. 53. Copyright © 2016 John Wiley and Sons. (B) AS1411-paclitaxel conjugate. (C) Modular ApDC. Reprinted with permission from Ref. 58. Copyright © 2019 John Wiley and Sons. (D) Peptide scaffold-coupled DOX and CPT. Reprinted with permission from Ref. 58. Copyright © 2019 John Wiley and Sons.

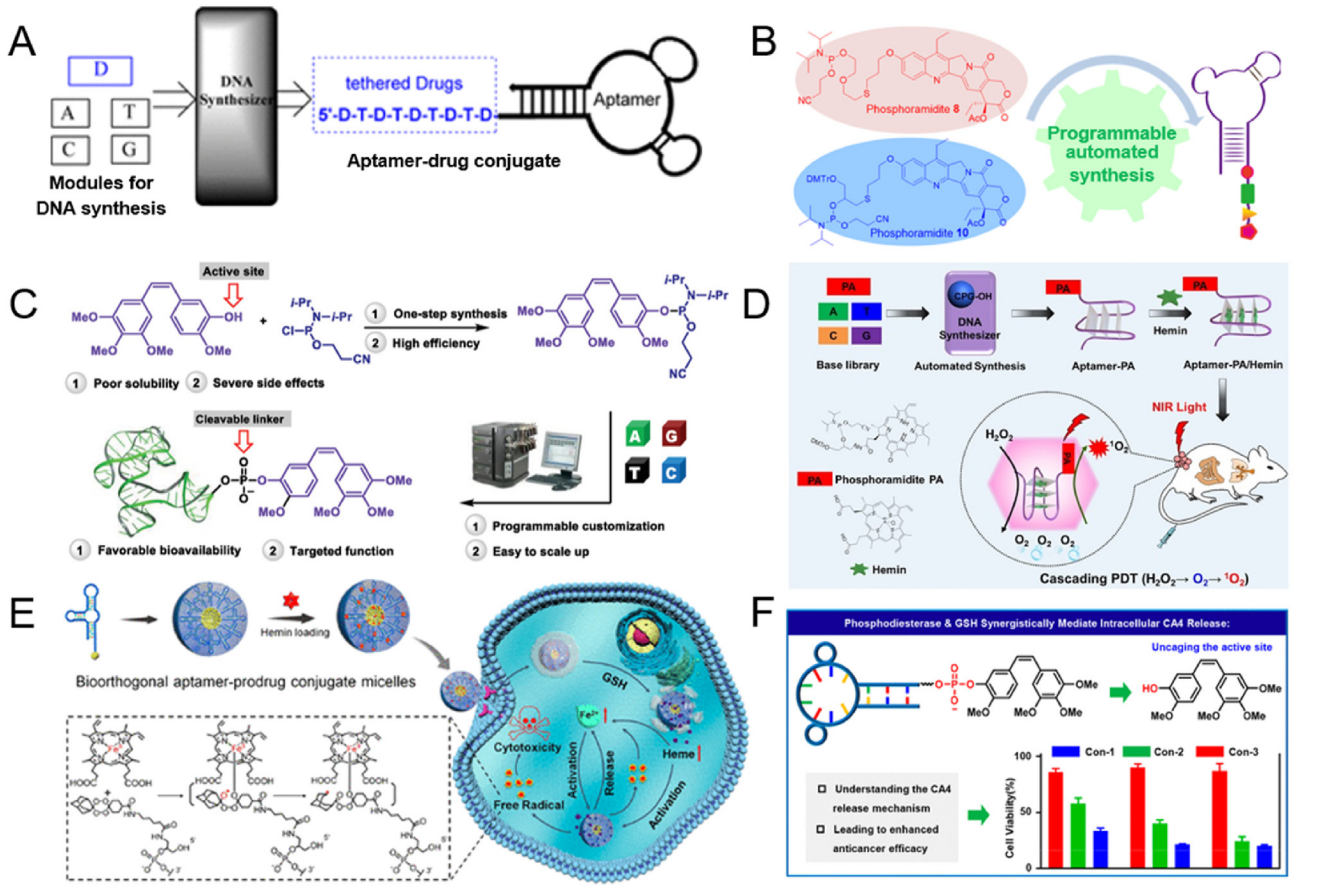


Figure 3 Examples of ApDCs from solid-phase synthesis. (A) The photo-activated ApDC across solid-phase synthesis. Reprinted with permission from Ref. 59. Copyright © 2014 American Chemical Society. (B) The camptothecin ApDC across solid-phase synthesis. Reprinted with permission from Ref. 61. Copyright © 2021 American Chemical Society. (C) The combretastatin A4 ApDC across solid-phase synthesis. Reprinted with permission from Ref. 62. Copyright © 2020 John Wiley and Sons. (D) The pyrochlorophyll A ApDC across solid-phase synthesis. Reprinted with permission from Ref. 63. Copyright © 2020 Ivyspring International Publisher. (E) Artificial prodrug ApDC across solid-phase synthesis. Reprinted with permission from Ref. 64. Copyright © 2020 American Chemical Society. (F) Combretastatin A4 ApDC with different linkers. Reprinted with permission from Ref. 65. Copyright © 2021 American Chemical Society.

is a reminder that the linker is also extremely important in the design of ApDC.

Nucleoside drugs are similar to bases and can form aptamers by enzymatic conjugation. Yoon et al.[66] developed a method for the enzymatic synthesis of 5-FU and gemcitabine ApDC. They used gemcitabine triphosphate (dFdCTP) and 5-fluorouracil (5-FU) triphosphate (5FdUTP) to enzymatically conjugate with P19 RNA aptamer. The ApDCs were found to significantly inhibit the proliferation of the targeted cell lines and induce the phosphorylation of histone H2AX on Ser139 (g-H2AX). Tran et al.[67] reported DNA nanoparticles of ApDC from enzyme-driven self-assembly. The ApDC nanoparticles showed highly efficient cellular uptake and enhanced serum stability. These properties led to significant *in vivo* suppression of tumor growth without apparent systemic toxicity. Enzymatic synthesis is the most effective method to get long-chain ApDC. However, only a small number of nucleoside drugs can be used for enzymatic synthesis, thus limiting the species of payload. Therefore, successful enzymatic synthesis of ApDC will ultimately depend on developing more versatile enzymes.

Nucleoside-based drugs have been widely used in clinics. The construction of ApDC by solid-phase synthesis or enzymatic coupling of various nucleoside drugs with aptamers is a clever strategy for the preparation of targeted nucleoside drugs. Despite many potential problems, the construction of ApDC based on nucleic acid synthesis is still an efficient and modular construction method for ApDC. At the same time, building ApDC based on nucleic acid synthesis is also conducive to the clinical translation of unnatural nucleic acids.

2.3. *Physical conjugation*

In addition, to achieve covalent crosslinking, it is convenient to couple drugs with aptamers using physical interactions. DOX is an anticancer drug widely used in the clinic[68,69]. Due to its flat aromatic ring structure, DOX can interact with double-stranded DNA[70]. Based on this, Bagalkot et al.[71] developed the first aptamer-DOX conjugate *via* non-covalent coupling in 2006 (Fig. 4A). Since then, DOX has become the most popular model drug in drug delivery, with nucleic acid as a carrier[72–74].

Zhu et al.[75] reported aptamer-tethered DNA nanotrains (aptNTrs) as a high-payload ApDC (Fig. 4B). The aptamer acted as the locomotive, guiding DNA "boxcars" to the cellular target. Li et al.[76] reported a multivalent ApDC called DNA nanocentipede. Compared to DNA nanotrains, the DNA nanocentipede has higher specificity owing to the multivalent aptamers. Wen

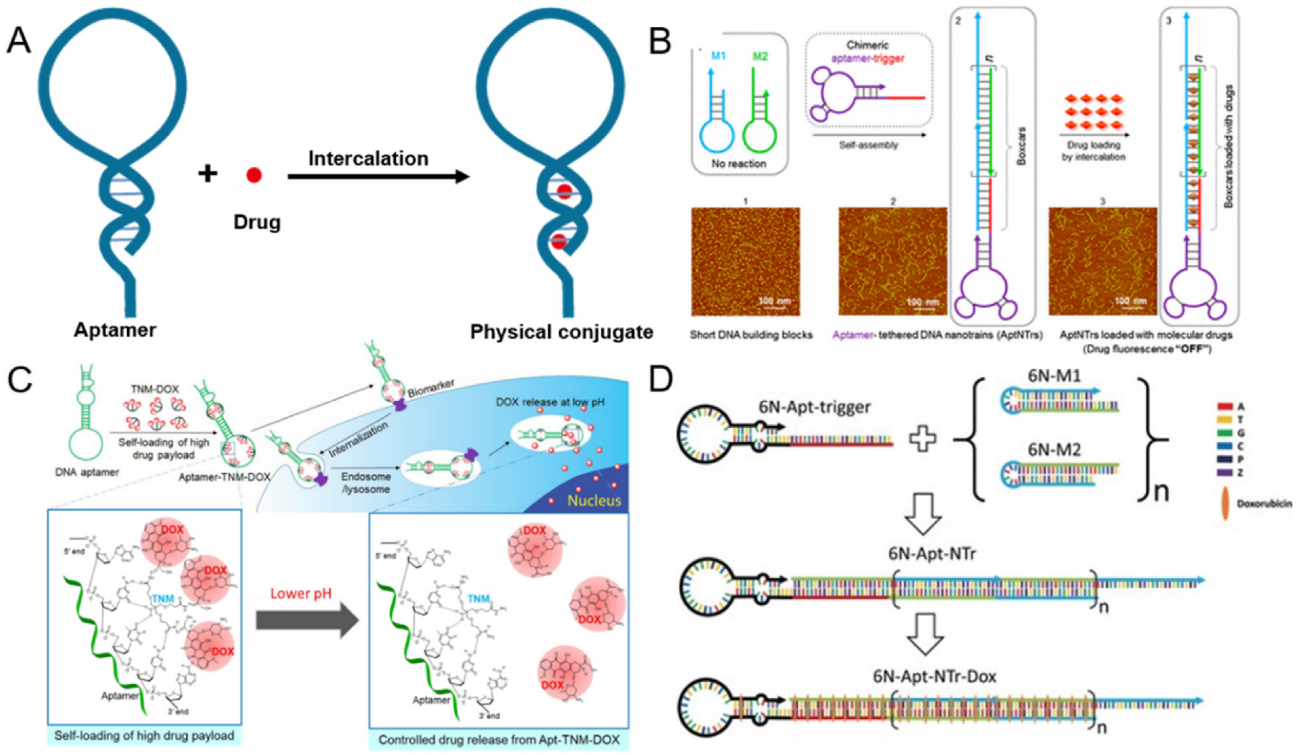


Figure 4 Examples of ApDC from physical conjugation. (A) The first aptamer-DOX conjugate *via* non-covalent coupling. (B) Aptamer-tethered DNA nanotrains. Reprinted with permission from Ref. 75. Copyright © 2013 National Academy of Sciences. (C) Aptamer−TNM−DOX conjugate. Reprinted with permission from Ref. 78. Copyright © 2021 MDPI. (D) Aptamer−DOX conjugates with artificial bases Z and P. Reprinted with permission from Ref. 79. Copyright © 2019 John Wiley and Sons.

et al.[77] reported a multiple myeloma cells-targeted ApDC. The aptamer was conjugated with DOX *via* hydrophobic interaction. The ApDC could specifically bind to CD38 protein on the cell membrane and internalize into the lysosome. Then, the ApDC released Dox in the acidic environment of the lysosome to kill tumor cells. The aptamer, combined with this active strategy, allowed this ApDC to specifically kill multiple myeloma cells with no significant off-target side effects. To improve the therapeutic efficacy of aptamer-DOX conjugate, Zeng et al.[78] used three DOX molecules to form a trifurcated Newkome-type monomer structure (TNM-DOX). TNM-DOX has pH-sensitive hydrazone bonds (Fig. 4C). The aptamer and TNM-DOX self-loaded together to generate an aptamer-TNM-DOX conjugate (Apt-TNM-DOX). Apt-TNM-DOX has a payload of 15 DOX molecules. DOX can be released from the Apt-TNM-DOX at pH 5.0. It can specifically target lymphoma cells without off-targeting to control cell lines. Apt-TNM-DOX is an example of a simple, high-payload aptamer-DOX conjugate. Zhang et al.[79] also reported an ApDC with artificial bases, such as Z and P (Fig. 4D), which can be conjugated with DOX *via* physical interactions like that of natural bases G and C. The displacement of standard nucleotide pairs by Z and P pairs may have improved the affinity between DOX and DNA. The ApDC formed by physical interactions has a promising application in cancer treatment. However, its *in vivo* safety and stability issues are still major challenges and remain to be studied.

Physical interaction is an early and widely used method for construction of aptamer−drug conjugates. Since the discovery that Dox can intercalate into nucleic acid GC bases, many studies regarding nucleic acid-Dox conjugation for tumor therapy have been conducted. Dox, an FDA-approved drug, is one of the most popular physical interaction-based ApDC drug candidates which can be released in acidic tumor environment. However, this method has a fatal flaw. The ApDC formed through physical interaction is extremely unstable and prone to drug leakage, thus inducing toxic side effects. Therefore, a more stable physical interaction-based ApDC system needs to be developed to enhance the therapeutic efficacy.

3. Biomacromolecule-based ApDC

Although ApDC shows excellent antitumor properties at the cellular level, some limitations remain for *in vivo* applications because of the low *in vivo* antitumor activity, short circulation time, and poor stability. Nonetheless, apart from small-molecule drugs, the ApDC concept has been expanded to proteins, nucleic acids, liposomes, viruses, bacteria, and even cells.

3.1. Aptamer-protein conjugate

Although the aptamer with small molecular weight confers good tumor penetration, it exhibits rapid renal clearance and limits *in vivo* efficacy. To enhance the targeting and therapeutic efficacy, conjugating aptamers to large biomacromolecules such as proteins is one of the means to increase the circulating half-life of aptamers.

Human serum albumin (HSA) has been a widely used drug delivery platform in the clinic because of the long circulating half-

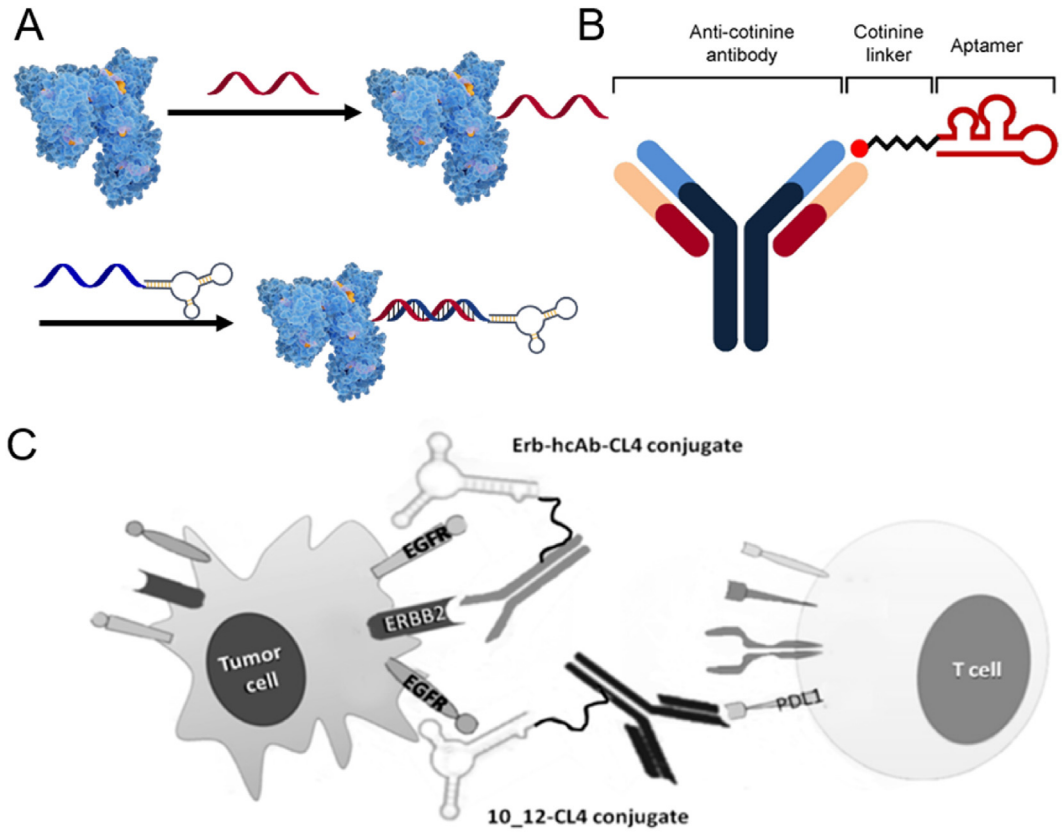


Figure 5 Examples of aptamer-protein conjugates. (A) Aptamer-HSA conjugate. (B) Aptamer-antibody conjugate. Reprinted with permission from Ref. 82. Copyright © 2016 Elsevier. (C) EGFR aptamer ErbB2 antibody conjugate and EGFR aptamer PD-L1 antibody conjugate. Reprinted with permission from Ref. 83. Copyright © 2019 MDPI.

life and engagement with the recycling neonatal Fc receptor (FcRn)[80]. The FDA has approved several albumin-based drug conjugates for clinical applications. The Kuhlmann group[81] reported a novel site-specific assembly method of designing an aptamer-albumin conjugate, as shown in Fig. 5A. The aptamer-albumin conjugate exhibits high serum stability and retains the specificity of the aptamer. This site-specific construction method has potential application prospects for combined drug delivery and half-life extension. An antibody is a kind of functional protein. In addition to increasing the half-life of the aptamer, the aptamer-antibody conjugate can also assume new functions. For instance, Heo et al.[82] reported an aptamer-antibody conjugate (Fig. 5B), called "oligobody" (oligonucleotide + antibody), which can overcome the limitation of short circulation time. Pegaptanib-targeted aptamer t44-OMe was coupled with cotinine to specifically bind with an anti-cotinine antibody to construct an aptamer-antibody conjugate. They found that the antibody extended the pharmacokinetics of the aptamer *in vivo* but had no effect on the binding affinity between aptamer and target. Compared to anti-VEGF antibodies, the oligobody can penetrate deep tumor tissues. Passariello et al.[83] developed an EGFR (epidermal growth factor receptor) aptamer ErbB2 (epidermal growth factor receptor 2) antibody conjugate and an EGFR aptamer PD-L1 antibody conjugate (Fig. 5C). These aptamer-antibody conjugates retained their inhibitory activity in cancer cells. The EGFR aptamer PD-L1 antibody conjugate efficiently activates T cells to kill cancer cells. The aptamer-antibody conjugate is an efficient way to form ApDC with better therapeutic activity by combining both advantages. The coupling of aptamer to protein significantly improves the circulating half-life of aptamers. However, there are still challenges in developing an efficient and general aptamer protein-specific coupling method to achieve precise coupling of aptamers and proteins.

3.2. *Aptamer-nucleic acid conjugate*

The FDA has approved many nucleic acid (NA) drugs since 1998[84,85], and more nucleic acid drugs are in the pipeline. The nucleic acid drug, such as mRNA, has shown prominent potential in therapy since the pandemic of COVID-19[86,87]. The targeted delivery of NA drugs is challenged by the intrinsic negative charge[88].

Aptamers can be conjugated with nucleic acid drugs *via* complementary hybridization. Using aptamers as an efficient carrier can precisely deliver NA drugs to target cells to improve efficiency[89]. MiRNA plays a significant role in messenger RNA expression regulation. Although no miRNA drug is available on the market so far. It is still considered as a promising new therapy candidate, especially in treating complex diseases such as diabetes and heart failure. However, targeted delivery of miRNA is crucial to effective miRNA-based therapy. Esposito et al.[90] designed an aptamer-microRNA (miRNA) conjugate to treat glioblastoma (Fig. 6A). They selected two aptamers that could respectively target and inhibit the receptor tyrosine kinases Axl and PDGFRβ as targeting ligands to deliver a variety of miRNAs into

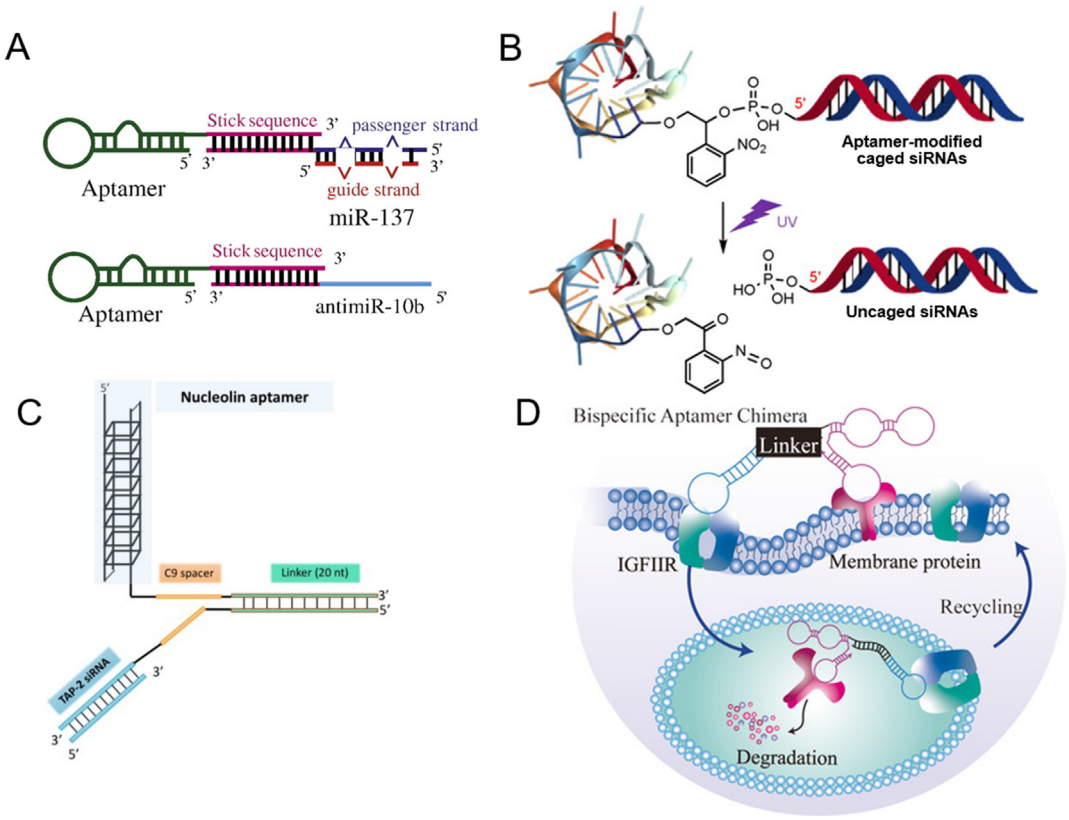


Figure 6 Examples of aptamer-nucleic acid drug conjugates. (A) Aptamer miRNA conjugate. Reprinted with permission from Ref. 90. Copyright © 2016 Elsevier. (B) Photoactivated aptamer-siRNA conjugate. Reprinted with permission from Ref. 92. Copyright. © 2018 John Wiley and Sons. (C) AS1411 TAP siRNA conjugate. Reprinted with permission from Ref. 94. Copyright © 2019 Springer Nature. (D) Aptamer conjugate as LYTACs. Reprinted with permission from Ref. 95. Copyright © 2019 John Wiley and Sons.

glioblastoma stem cells. The aptamer-miRNA conjugate could efficiently prevent the proliferation of glioblastoma stem cells. Russo et al.[91] reported an aptamer-tyrosine kinase receptor Axl conjugate GL21.T and miRNA-212. Aptamer-miRNA-212 could effectively deliver miRNA-212 into tumor cells while down-regulating PED protein expression and restoring TNF-related apoptosis-inducing ligand (TRAIL)-mediated cytotoxicity in cancer cells. They also developed an aptamer-GL21.T-miRNA-34c conjugate, which showed a dual inhibitory effect on Axl at the functional and transcriptional levels[72]. The aptamer-GL21.T-miRNA-34c conjugate could depress the proliferation of non-small cell lung cancer cells, thus overcoming the resistance to RTK inhibitors by targeting and inhibiting Axl.

Numerous clinical and preclinical data have demonstrated that siRNA is an efficient drug in gene-related diseases. With excellent targeting ability, aptamer also exhibits great potential in siRNA delivery. Zhang et al.[92] developed a photoactivated aptamer-small interfering RNA (siRNA) conjugate, as shown in Fig. 6B. The aptamer-siRNA conjugate could enter cells and cause gene silencing under light irradiation. Esposito et al.[93] reported a conjugation of tyrosine kinase PDGFRβ-binding aptamer Gint4.T- and STAT3- (signal transducer and activator of transcription 3) siRNA to treat glioblastoma. The conjugate effectively silenced STAT3 in glioblastoma cells, inhibiting the viability and migration of glioblastoma cells *in vitro* and depressing tumor growth and angiogenesis *in vivo*. Garrido et al.[94] reported the conjugation of AS1411 aptamer and TAP (transporter associated with antigen processing) siRNA, inhibiting the growth of various tumors without significant toxicity (Fig. 6C). This conjugate could transiently downregulate the expression of antigen processing-related transporters and induce the production of a set of universal neoantigens *in situ* in tumor cells.

Besides siRNA and miRNA, aptamers can also conjugate with aptamers to form multivalent aptamers or functional ApDC. Han et al.[95] developed an aptamer conjugate as lysosome-targeting chimeras (LYTACs) for targeted degradation of membrane proteins (Fig. 6D). They demonstrated that the ApDC could efficiently degrade membrane proteins, such as Met and PTK-7, *via* lysosomal protein degradation. This method provides a universal platform for LYTACs using aptamers.

The targeted delivery of nucleic acid drugs based on aptamers can significantly improve the efficiency of nucleic acid drugs in entering target cells or organs. The coupling of aptamer and nucleic acid drug can be achieved using molecular biological methods such as transcription. It's a unique advantage in targeted delivery of nucleic acid drug for aptamer compared with that of antibody. However, as a small-size targeting ligand, aptamer is facing challenges in the targeted delivery of large mRNA molecules. For example, aptamer-nucleic acid conjugates cannot avoid non-specific adsorption of proteins in serum caused by the electronegativity of nucleic acid molecules. Therefore, more exploration and research are still needed to develop aptamer-nucleic acid conjugates with high specific targeting ability.

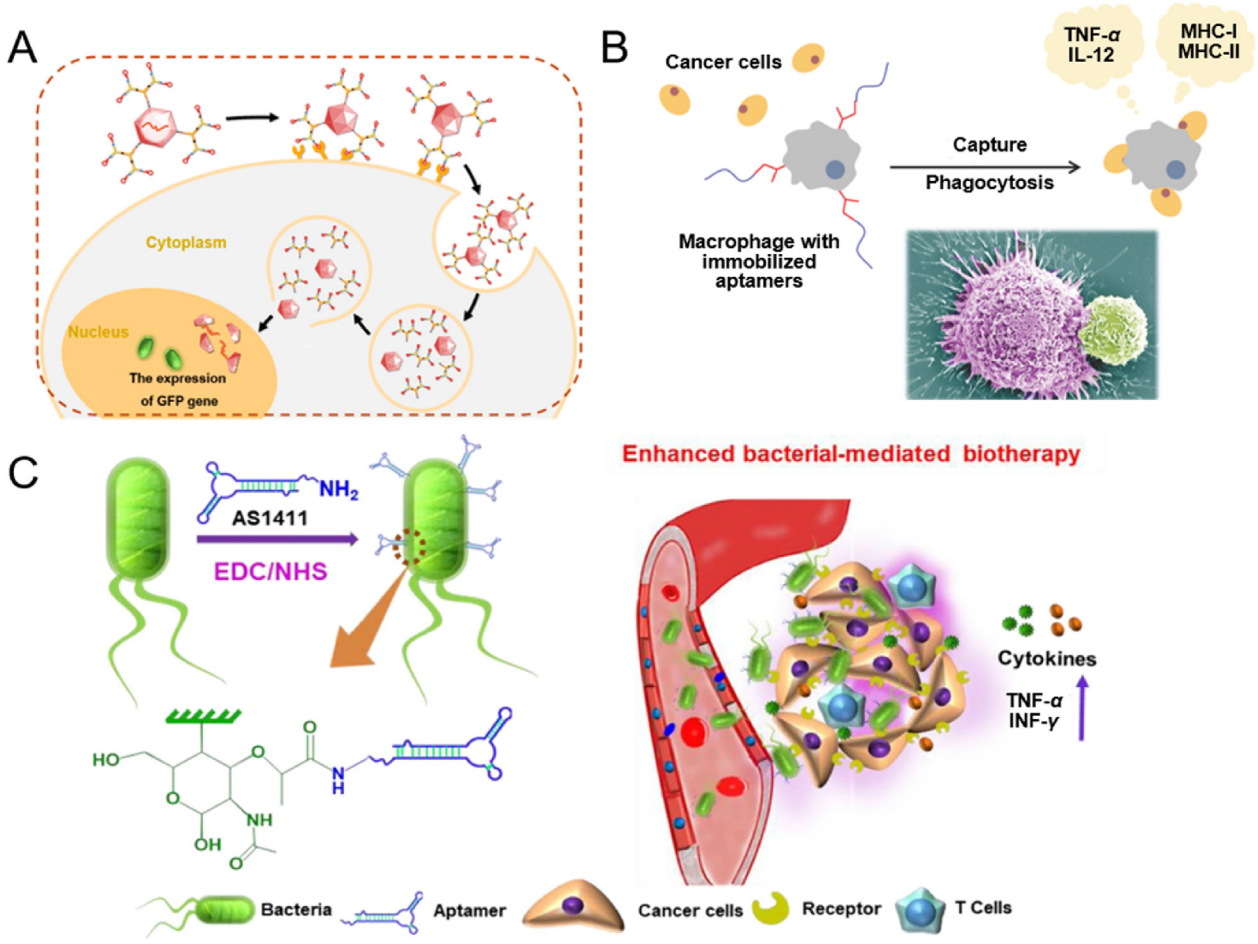


Figure 7 Examples of biotherapeutic drug conjugates. (A) Aptamer AAV2 conjugate. Reprinted with permission from Ref. 96. Copyright © 2017 American Chemical Society. (B) Aptamer-macrophage conjugate. Reprinted with permission from Ref. 97. Copyright © 2018 American Chemical Society. (C) Aptamer bacteria conjugate. Reprinted with permission from Ref. 98. Copyright © 2018 Springer Nature.

3.3. Aptamer-biotherapeutic drug conjugate

In addition to drugs, such as small molecules and proteins, biotherapeutic drugs, such as viruses, bacteria, and cells, can also be conjugated with aptamers for targeted delivery.

Wu et al.[96] reported multivalent aptamer AAV2 (adeno-associated virus vector) conjugate (G-sgc8-AAV2) for gene transfection (Fig. 7A). The G-sgc8-AAV2 conjugate has higher gene transfection efficiency owing to higher affinity and stability. Sugimoto et al.[97] developed an aptamer-macrophage conjugate for targeted tumor immunotherapy (Fig. 7B). The aptamer-macrophage conjugate showed stronger adhesion to tumor cells and more antigen presentation owing to the higher expression of major histocompatibility complex (MHC) class I and II molecules. Geng et al.[98] reported an aptamer-bacteria conjugate for the targeted localization of bacteria at the tumor site (Fig. 7C). They found that each bacterial conjugate with an average of 2.8×10^5 aptamers had the highest specificity to tumor cells *in vitro*. In different tumor-bearing mouse models, the aptamer-conjugated attenuated Salmonella showed enhanced antitumor efficacy and highly activated immune responses. These studies showed that aptamers could be an efficient drug delivery tool for biotherapeutic drugs in cancer therapy.

4. High-payload ApDC

Traditionally, each ApDC usually carries one or two drug molecules. High-payload ApDC with multi-drug molecules has great potential to enhance the efficacy of targeted therapy. It's convenient to couple with macromolecules such as polymers, framework nucleic acid (FNA), or liposomes to construct high-payload ApDC because of the intrinsic chemical conjugation superiority.

Deng et al.[99] conjugated an aptamer with a reduction-triggered prodrug and biocompatible brush-like backbone to develop aptamer−polyprodrug conjugates (ApPDCs) to improve drug loading and extend the *in vivo* circulation time of ApDC (Fig. 8A). ApDC with DNA octahedral wireframe was also developed (Fig. 8B)[100]. The ApDC have shown high serum stability, deep penetration capability, and significantly enhanced therapeutic efficacy. Luo et al.[101] also used cell-derived vesicles as carriers to form ApDC, thereby achieving a combined delivery of multiple drugs (Fig. 8C). The result showed that the drugs were efficiently encapsulated into the vesicles and entered the tumor cells through membrane fusion, resulting in a synergistic therapeutic effect. Geng et al.[102] reported a multivalent aptamer drug conjugate (ApMDC). ApMDC comprises hydrophilic aptamer and hydrophobic PAMAM monodendron (Fig. 8D). Each monodendron has

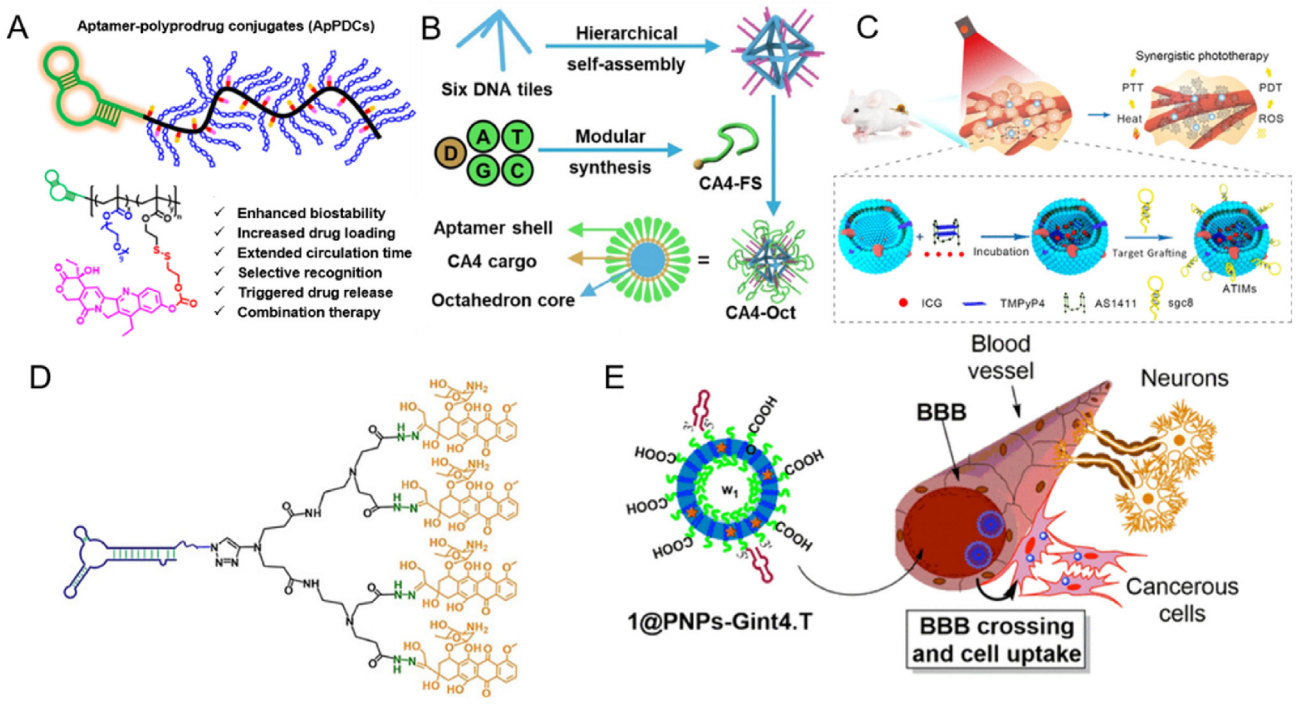


Figure 8 Examples of high-payload ApDC. (A) Aptamer–polyprodrug conjugates. Reprinted with permission from Ref. 99. Copyright © 2019 American Chemical Society. (B) ApDC with DNA octahedral wireframe. Reprinted with permission from Ref. 100. Copyright © 2020 American Chemical Society. (C) Aptamer-cell-derived vesicle conjugate. Reprinted with permission from Ref. 101. Copyright © 2019 American Chemical Society. (D) Multivalent aptamer-drug conjugate. Reprinted with permission from Ref. 102. Copyright © 2021 John Wiley and Sons. (E) Aptamer Gint4.T-polymer vesicle conjugate. Reprinted with permission from Ref. 103. Copyright © 2019 American Chemical Society.

four DOX molecules with an acylhydrazone linker. ApMDC can enhance antitumor immune responses and boost immunogenic tumor cell death *in vivo*. Monaco et al.[103] developed an aptamer Gint4.T-polymer vesicle conjugate (Fig. 8E). It could recognize platelet-derived growth factor receptor β and pass through the blood-brain barrier (BBB). The aptamer-polymer vesicle conjugate is characterized by higher uptake efficiency in glioblastoma U87MG cells and lower EC_{50} value (38 pmol/L) when loaded with PI3K-mTOR inhibitors. All these conjugates indicate that the ApDC, through transformation and optimization, can achieve high efficacy in tumor treatment.

5. Conclusions and future perspectives

Overall, the ApDC is very promising for clinical applications owing to its potential high efficacy in cancer treatment, as well as its intrinsic advantages. Although scientists have reported many ApDC, few of them have entered clinical trials. Some factors limiting the *in vivo* applications of ApDC are low *in vivo* specificity, low stability in serum, and rapid renal clearance.

To overcome the clinical limitations, several major strategies have been employed to obtain aptamers with excellent *in vivo* performance. The first strategy involves screening aptamers with a modified library. The introduction of unnatural nucleic acid enriches the aptamer library's diversity and improves the aptamer's affinity and specificity. However, there is currently a lack of nucleic acid amplification enzymes that can recognize unnatural nucleic acids to amplify unnatural nucleic acid libraries. The development of highly efficient nucleic acid amplification enzymes that recognize unnatural nucleic acids will significantly enhance the diversity of aptamer library and provide more excellent aptamers. The second strategy is to screen aptamer in a complex environment, such as *in vivo*-based SELEX. So far, most aptamers come from *in vitro* screening with high affinity and specificity *in vitro*. Only a few aptamers exhibit good binding specificity *in vivo*. Therefore, screening aptamers under the same target environment and same target conformation will have more opportunity to select aptamers with excellent *in vivo* performance. The critical factors for *in vivo* screening of aptamers are the efficiency of specific library enrichment and extraction and the amplification of the library from tissue. Thus, we suggest: (1) incubating the library with target tissue before *in vivo* screening to avoid loss of sequences because of circulation and metabolism; (2) increasing the *in vivo* circulation time to have more contact opportunities with target molecules; (3) Enhancing the extraction efficiency of the library with new technologies such as spatial omics. The third strategy involves post-SELEX of aptamer. Besides screening new aptamers, post-SELEX by mutation or truncation is also an efficient way to improve the *in vivo* efficacy of aptamers. However, the post-SELEX of aptamers lacks theoretical guidance and can only be obtained by trial and error. Future post-SELEX could be achieved through machine learning or artificial intelligence-assisted SELEX by building an extensive database of aptamer structures.

High-payload ApDC typically exhibits high anti-tumor activity. However, there is a trade-off between targeting efficiency and high payload. The targeting ability of aptamer may be lost as the payload increases since the aptamer size is getting smaller compared with excessive payload. The steric effect from the payload may cover the aptamer and resulting in the loss of

targeting ability. Too much loading of drug molecules may also induce conformational changes of the aptamer, while inadequate drug loading may lead to ineffective drug delivery. Therefore, it is necessary to develop precise, controllable and optimized ApDC with both high payload and high targeting ability to achieve satisfied tumor depression performance.

In addition to aptamer and payload, the linker is also critical to develop a high-efficacy ApDC. Covalent conjugation is the major strategy for the development of both ApDC and ADC. Although ApDC has more options in selecting a linker, the intrinsic properties of a linker such as stability, stimuli-responsive property need to be considered when constructing a new ApDC. New linkers which can hold high-payload and release the drug molecules upon reaching the target sites are eager to be developed for the next generation of high-efficacy ApDC system.

Owing to high-payload, facile conjugation, high stability, and readily modification, ApDC has shown more promising advantages over ADCs in some new therapeutic ways, such as proteolysis targeting chimeras (PROTACs), bifunctional molecules with two heads connected by one linker. From the labor work and time cost point of view, antibody screening is more time-consuming than aptamer SELEX, which may only take several weeks. Researchers need to find a drug delivery method for ApDC different from that for ADCs. For example, the administration of the first marketed aptamer drug Macugen was delivered through an ophthalmic intravitreal injection. Compared to the history of ADCs, the ApDC is still in its infancy and has a long way to go before clinical translation. We believe that ApDC represents a promising platform for targeted drug delivery and that further clinical translation of ApDC should be pushed forward for successful clinical translation.

Acknowledgments

This study was supported by the Zhejiang Provincial Natural Science Foundation of China (No. Y21C050001, China), Zhejiang Provincial Research Center for Diagnosis and Treatment of Major Diseases (No. JBZX-202003, China), the National Natural Science Foundation of China (Nos. 22104132 and 22204144), and the Zhejiang Province “Kunpeng” Program.

Author contributions

Jiaxuan He conceived the idea for this review and drafted the introduction, as well as the small-molecule aptamer−drug conjugate, aptamer-protein conjugate and aptamer-nucleic acid conjugate sections. Qiao Duan drafted the aptamer-biotherapeutic drug conjugate section. Chunyan Ran drafted the high-payload aptamer−drug conjugate section. Ting Fu, Yuan Liu, and Weihong Tan supervised the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Huang X, Kon E, Han X, Zhang X, Kong N, Mitchell MJ, et al. Nanotechnology-based strategies against SARS-CoV-2 variants. *Nat Nanotechnol* 2022. Availible from: https://doi.org/10.1038/s41565-022-01174-5.
2. Tang Z, Kong N, Zhang X, Liu Y, Hu P, Mou S, et al. A materials-science perspective on tackling COVID-19. *Nat Rev Mater* 2020;**5**:847−60.
3. Huang X, Liu C, Kong N, Xiao Yf Jr YA, Tabas I, et al. Synthesis of siRNA nanoparticles to silence plaque-destabilizing gene in atherosclerotic lesional macrophages. *Nat Protoc* 2022;**17**:748−80.
4. Fang XH, Tan WH. Aptamers generated from Cell-SELEX for molecular medicine: a chemical biology approach. *Acc Chem Res* 2010;**43**:48−57.
5. Keefe AD, Pai S, Ellington A. Aptamers as therapeutics. *Nat Rev Drug Discov* 2010;**9**:537−50.
6. Pastor F, Berraondo P, Etxeberria I, Frederick J, Sahin U, Gilboa E, et al. An RNA toolbox for cancer immunotherapy. *Nat Rev Drug Discov* 2018;**17**:751−67.
7. Bickle MBT, Dusserre E, Moncorge O, Bottin H, Colas P. Selection and characterization of large collections of peptide aptamers through optimized yeast two-hybrid procedures. *Nat Protoc* 2006;**1**:1066−91.
8. Hafner M, Vianini E, Albertoni B, Marchetti L, Grune I, Gloeckner C, et al. Displacement of protein-bound aptamers with small molecules screened by fluorescence polarization. *Nat Protoc* 2008;**3**:579−87.
9. Liu J, Lu Y. Preparation of aptamer-linked gold nanoparticle purple aggregates for colorimetric sensing of analytes. *Nat Protoc* 2006;**1**:246−52.
10. Mayer G, Ahmed MSL, Dolf A, Endl E, Knolle PA, Famulok M. Fluorescence-activated cell sorting for aptamer SELEX with cell mixtures. *Nat Protoc* 2010;**5**:1993−2004.
11. Wang Y, Tang LH, Li ZH, Lin YH, Li JH. *In situ* simultaneous monitoring of ATP and GTP using a graphene oxide nanosheet-based sensing platform in living cells. *Nat Protoc* 2014;**9**:1944−55.
12. Dunn MR, Jimenez RM, Chaput JC. Analysis of aptamer discovery and technology. *Nat Rev Chem* 2017;**1**:0076.
13. Lotz TS, Halbritter T, Kaiser C, Rudolph MM, Kraus L, Groher F, et al. A light-responsive RNA aptamer for an azobenzene derivative. *Nucleic Acids Res* 2019;**47**:2029−40.
14. Arroyo-Curras N, Somerson J, Vieira PA, Ploense KL, Kippin TE, Plaxco KW. Real-time measurement of small molecules directly in awake, ambulatory animals. *Proc Natl Acad Sci U S A* 2017;**114**:645−50.
15. Nakatsuka N, Yang KA, Abendroth JM, Cheung KM, Xu XB, Yang HY, et al. Aptamer-field-effect transistors overcome Debye length limitations for small-molecule sensing. *Science* 2018;**362**:319−24.
16. Yu TM, Zhou WH, Liu JW. Ultrasensitive DNAzyme-based Ca^{2+} detection boosted by ethanol and a solvent-compatible scaffold for aptazyme design. *Chembiochem* 2018;**19**:31−6.
17. Zhou WH, Ding JS, Liu JW. A selective Na^{+} aptamer dissected by sensitized Tb^{3+} luminescence. *Chembiochem* 2016;**17**:1563−70.
18. Lu YX, Liu YY, Zhang SG, Wang S, Zhang SC, Zhang XR. Aptamer-based plasmonic sensor array for discrimination of proteins and cells with the naked eye. *Anal Chem* 2013;**85**:6571−4.
19. Xue LY, Zhou XM, Xing D. Sensitive and homogeneous protein detection based on target-triggered aptamer hairpin switch and nicking enzyme assisted fluorescence signal amplification. *Anal Chem* 2012;**84**:3507−13.
20. Wu YW, Chen XL, Wang XF, Yang M, Xu FL, Hou CJ, et al. A fluorescent biosensor based on prismatic hollow Metal-polydopamine frameworks and 6-carboxyfluorescein (FAM)-labeled protein aptamer for CA15-3 detection. *Sensor Actuat B-Chem* 2021;**329**:129249.
21. Moon J, Kim G, Park S. Development of ssDNA aptamers for the capture and detection of Salmonella typhimurium. *Anal Methods-UK* 2014;**6**:7442−8.
22. Daniels DA, Chen H, Hicke BJ, Swiderek KM, Gold L. A tenascin-C aptamer identified by tumor cell SELEX: systematic evolution of ligands by exponential enrichment. *Proc Natl Acad Sci U S A* 2003;**100**:15416−21.

23. Moon J, Kim G, Park SB, Lim J, Mo C. Comparison of whole-Cell SELEX methods for the identification of *Staphylococcus aureus*-specific DNA Aptamers. *Sensors-Basel* 2015;**15**:8884−97.
24. Li L, Wan J, Wen XH, Guo QP, Jiang HS, Wang J, et al. Identification of a new DNA aptamer by tissue-selex for cancer recognition and imaging. *Anal Chem* 2021;**93**:7369−77.
25. Pu Y, Xiang J, Zhang XX, Deng YY, Liu HX, Tan WH. CD36 as a molecular target of functional DNA aptamer $NAFLD_{01}$ selected against NAFLD cells. *Anal Chem* 2021;**93**:3951−8.
26. Gold L, Tuerk C. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* 1990;**249**:505−10.
27. Ellington A, Szostak J. *In vitro* selection of RNA molecules that bind specific ligands. *Nature* 1990;**346**:818−22.
28. Lee J, Lee KH, Jeon J, Dragulescu-Andrasi A, Xiao F, Rao JH. Combining SELEX screening and rational design to develop light-up fluorophore-RNA aptamer pairs for RNA tagging. *ACS Chem Biol* 2010;**5**:1065−74.
29. Lavu PSR, Mondal B, Ramlal S, Murali HS, Batra HV. Selection and characterization of aptamers using a modified whole cell bacterium SELEX for the detection of salmonella enterica serovar typhimurium. *ACS Comb Sci* 2016;**18**:292−301.
30. Hanazato M, Nakato G, Nishikawa F, Hase K, Nishikawa S, Ohno H. Selection of an aptamer against mouse gp2 by SELEX. *Cell Struct Funct* 2014;**39**:23−9.
31. Meng HM, Fu T, Zhang XB, Tan WH. Cell-SELEX-based aptamer-conjugated nanomaterials for cancer diagnosis and therapy. *Natl Sci Rev* 2015;**2**:71−84.
32. Xu GH, Zhao JJ, Liu N, Yang MH, Zhao Q, Li CG, et al. Structure-guided post-SELEX optimization of an ochratoxin A aptamer. *Nucleic Acids Res* 2019;**47**:5963−72.
33. Chen ZJ, Liu H, Jain A, Zhang L, Liu C, Cheng K. Discovery of aptamer ligands for hepatic stellate cells using SELEX. *Theranostics* 2017;**7**:2982−95.
34. Cho HS, Yeh EC, Sinha R, Laurence TA, Bearinger JP, Lee LP. Single-step nanoplasmonic VEGF(165) aptasensor for early cancer diagnosis. *ACS Nano* 2012;**6**:7607−14.
35. Meng XD, Liu ZQ, Cao Y, Dai WH, Zhang K, Dong HF, et al. Fabricating aptamer-conjugated PEGylated-MoS_2/$Cu_{1.8}S$ theranostic nanoplatform for multiplexed imaging diagnosis and chemo-photothermal therapy of cancer. *Adv Funct Mater* 2017;**27**:1605592.
36. Wang D, Li SH, Zhao ZL, Zhang XB, Tan WH. Engineering a second-order DNA logic-gated nanorobot to sense and release on live cell membranes for multiplexed diagnosis and synergistic therapy. *Angew Chem Int Ed Engl* 2021;**60**:15816−20.
37. Wang T, Chen LM, Chikkanna A, Chen SX, Brusius I, Sbuh N, et al. Development of nucleic acid aptamer-based lateral flow assays: a robust platform for cost-effective point-of-care diagnosis. *Theranostics* 2021;**11**:5174−96.
38. Wu X, Chen J, Wu M, Zhao JXJ. Aptamers: active targeting ligands for cancer diagnosis and therapy. *Theranostics* 2015;**5**:322−44.
39. Tarantino P, Pestana RC, Corti C, Modi S, Bardia A, Tolaney SM, et al. Antibody-drug conjugates: smart chemotherapy delivery across tumor histologies. *CA Cancer J Clin* 2021;**72**:165−82.
40. Chau CH, Steeg PS, Figg WD. Antibody-drug conjugates for cancer. *Lancet* 2019;**394**:793−804.
41. Lambert JM. Antibody−drug conjugates (ADCs): magic bullets at last. *Mol Pharm* 2015;**12**:1701−2.
42. Tong JTW, Harris PWR, Brimble MA, Kavianinia I. An insight into fda approved antibody-drug conjugates for cancer therapy. *Molecules* 2021;**26**:5847.
43. Dean AQ, Luo S, Twomey JD, Zhang BL. Targeting cancer with antibody-drug conjugates: promises and challenges. *MABS-AUSTIN* 2021;**13**:1951427.
44. Joubert N, Beck A, Dumontet C, Denevault-Sabourin C. Antibody-drug conjugates: the last decade. *Pharmaceuti-Base* 2020;**13**:245.
45. Wang Y, Cheetham AG, Angacian G, Su H, Xie LS, Cui HG. Peptide-drug conjugates as effective prodrug strategies for targeted delivery. *Adv Drug Deliv Rev* 2017;**110**:112−26.
46. Zhu YS, Tang KX, Lv JY. Peptide-drug conjugate-based novel molecular drug delivery system in cancer. *Trends Pharmacol Sci* 2021;**42**:857−69.
47. Pepaxto Jessee JK. A new peptide-drug conjugate for heavily pretreated relapsed and refractory multiple myeloma. *Ann Pharmacother* 2021;**56**:951−7.
48. Huang YF, Shangguan DH, Liu HP, Phillips JA, Zhang XL, Chen Y, et al. Molecular assembly of an aptamer−drug conjugate for targeted drug delivery to tumor cells. *Chembiochem* 2009;**10**:862−8.
49. Zhou F, Wang P, Peng YB, Zhang PG, Huang Q, Sun WD, et al. Molecular engineering-based aptamer−drug conjugates with accurate tunability of drug ratios for drug combination targeted cancer therapy. *Angew Chem Int Ed Engl* 2019;**58**:11661−5.
50. Yang QX, Deng ZY, Wang D, He JX, Zhang DL, Tan Y, et al. Conjugating aptamer and mitomycin c with reductant-responsive linker leading to synergistically enhanced anticancer effect. *J Am Chem Soc* 2020;**142**:2532−40.
51. He JX, Peng TH, Peng YB, Ai LL, Deng ZY, Wang XQ, et al. Molecularly engineering triptolide with aptamers for high specificity and cytotoxicity for triple-negative breast cancer. *J Am Chem Soc* 2020;**142**:2699−703.
52. Li YY, Peng YB, Tan Y, Xuan WJ, Fu T, Wang XQ, et al. A new paradigm for artesunate anticancer function: considerably enhancing the cytotoxicity *via* conjugating artesunate with aptamer. *Signal Transduct Tar* 2021;**6**:327.
53. Niu WJ, Chen XG, Tan WH, Veige AS. *N*-Heterocyclic carbene-gold(I) complexes conjugated to a leukemia-specific DNA aptamer for targeted drug delivery. *Angew Chem Int Ed Engl* 2016;**55**:8889−93.
54. Niu WJ, Teng IT, Chen XG, Tan WH, Veige AS. Aptamer-mediated selective delivery of a cytotoxic cationic NHC-Au(I) complex to cancer cells. *Dalton Trans* 2018;**47**:120−6.
55. Li FF, Lu J, Liu J, Liang C, Wang ML, Wang LY, et al. A water-soluble nucleolin aptamer-paclitaxel conjugate for tumor-specific targeting in ovarian cancer. *Nat Commun* 2017;**8**:1390.
56. Gray BP, Kelly L, Ahrens DP, Barry AP, Kratschmer C, Levy M, et al. Tunable cytotoxic aptamer−drug conjugates for the treatment of prostate cancer. *Proc Natl Acad Sci U S A* 2018;**115**:4761−6.
57. Kratschmer C, Levy M. Targeted delivery of auristatin-modified toxins to pancreatic cancer using aptamers. *Mol Ther Nucleic Acids* 2018;**10**:227−36.
58. Pusuluri A, Krishnan V, Lensch V, Sarode A, Bunyan E, Vogus DR, et al. Treating tumors at low drug doses using an aptamer-peptide synergistic drug conjugate. *Angew Chem Int Ed Engl* 2019;**58**:1437−41.
59. Wang RW, Zhu GZ, Mei L, Xie Y, Ma HB, Ye M, et al. Automated modular synthesis of aptamer−drug conjugates for targeted drug delivery. *J Am Chem Soc* 2014;**136**:2731−4.
60. Lv C, Yang C, Ding D, Sun Y, Wang RW, Han D, et al. Endocytic pathways and intracellular transport of aptamer−drug conjugates in live cells monitored by single-particle tracking. *Anal Chem* 2019;**91**:13818−23.
61. Gao F, Zhou JM, Sun Y, Yang C, Zhang SY, Wang RW, et al. Programmable repurposing of existing drugs as pharmaceutical elements for the construction of aptamer−drug conjugates. *ACS Appl Mater Interfaces* 2021;**13**:9457−63.
62. Wang D, Peng YB, Deng ZY, Tan Y, Su YY, Kuai HL, et al. Modularly engineered solid-phase synthesis of aptamer-functionalized small molecule drugs for targeted cancer therapy. *Adv Ther* 2020;**3**:2000074.
63. Yang Y, He JX, Zhu WJ, Pan XS, Yazd HS, Cui C, et al. Molecular domino reactor built by automated modular synthesis for cancer treatment. *Theranostics* 2020;**10**:4030−41.

64. Xuan WJ, Xia YH, Li T, Wang LL, Liu YL, Tan WH. Molecular self-assembly of bioorthogonal aptamer-prodrug conjugate micelles for hydrogen peroxide and ph-independent cancer chemodynamic therapy. *J Am Chem Soc* 2020;**142**:937−44.
65. Huang ZY, Wang D, Long CY, Li SH, Wang XQ, Tan WH. Regulating the anticancer efficacy of Sgc8-combretastatin A4 conjugates: a case of recognizing the significance of linker chemistry for the design of aptamer-based targeted drug delivery strategies. *J Am Chem Soc* 2021;**143**:8559−64.
66. Yoon S, Huang KW, Reebye V, Spalding D, Przytycka TM, Wang YJ, et al. Aptamer−drug conjugates of active metabolites of nucleoside analogs and cytotoxic agents inhibit pancreatic tumor cell growth. *Mol Ther Nucleic Acids* 2017;**6**:80−8.
67. Tran BT, Kim J, Ahn DR. Systemic delivery of aptamer−drug conjugates for cancer therapy using enzymatically generated self-assembled DNA nanoparticles. *Nanoscale* 2020;**12**:22945−51.
68. Carvalho C, Santos RX, Cardoso S, Correia S, Oliveira PJ, Santos MS, et al. Doxorubicin: the good, the bad and the ugly effect. *Curr Med Chem* 2009;**16**:3267−85.
69. Cranmer LD, Hess LM, Sugihara T, Zhu YE. Impact of bolus versus continuous infusion of doxorubicin (DOX) on cardiotoxicity in patients with breast cancer (BC) and sarcomas: analysis of real-world data. *J Clin Oncol* 2020;**38**. e19123-e23.
70. Agudelo D, Bourassa P, Berube G, Tajmir-Riahi HA. Review on the binding of anticancer drug doxorubicin with DNA and tRNA: structural models and antitumor activity. *J Photochem Photobiol, B* 2016;**158**:274−9.
71. Bagalkot V, Farokhzad OC, Langer R, Jon S. An aptamer-doxorubicin physical conjugate as a novel targeted drug-delivery platform. *Angew Chem Int Ed Engl* 2006;**45**:8149−52.
72. Zang YD, Wei YC, Shi YJ, Chen Q, Xing D. Chemo/photoacoustic dual therapy with mRNA-triggered dox release and photoinduced shock-wave based on a DNA-gold nanoplatform. *Small* 2016;**12**:756−69.
73. Wu HP, Zhao M, Li J, Zhou XY, Yang TT, Zhao DZ, et al. Novel protease-free long-lasting chemiluminescence system based on the Dox-ABEI chimeric magnetic DNA hydrogel for ultrasensitive immunoassay. *ACS Appl Mater Interfaces* 2020;**12**:47270−7.
74. Xu Y, Huang SW, Ma YQ, Ding HM. Loading of DOX into a tetrahedral DNA nanostructure: the corner does matter. *Nanoscale Adv* 2021;**4**:754−60.
75. Zhu GZ, Zheng J, Song EQ, Donovan M, Zhang KJ, Liu C, et al. Self-assembled, aptamer-tethered DNA nanotrains for targeted transport of molecular drugs in cancer theranostics. *Proc Natl Acad Sci U S A* 2013;**110**:7998−8003.
76. Li WS, Yang XH, He LL, Wang KM, Wang Q, Huang J, et al. Self-assembled DNA nanocentipede as multivalent drug carrier for targeted delivery. *ACS Appl Mater Interfaces* 2016;**8**:25733−40.
77. Wen J, Tao W, Hao S, Iyer SP, Zu Y. A unique aptamer−drug conjugate for targeted therapy of multiple myeloma. *Leukemia* 2016;**30**:987−91.
78. Zeng ZH, Qi JJ, Wan QY, Zu YL. Aptamers with self-loading drug payload and pH-controlled drug release for targeted chemotherapy. *Pharmaceutics* 2021;**13**:1221.
79. Zhang LQ, Wang S, Yang ZY, Hoshika S, Xie ST, Li J, et al. An aptamer-nanotrain assembled from six-letter DNA delivers doxorubicin selectively to liver cancer cells. *Angew Chem Int Ed Engl* 2020;**59**:663−8.
80. Schmidt MM, Townson SA, Andreucci AJ, King BM, Schirmer EB, Murillo AJ, et al. Crystal structure of an HSA/FcRn complex reveals recycling by competitive mimicry of hsa ligands at a pH-dependent hydrophobic interface. *Structure* 2013;**21**:1966−78.
81. Kuhlmann M, Hamming JBR, Voldum A, Tsakiridou G, Larsen MT, Schmokel JS, et al. An albumin-oligonucleotide assembly for potential combinatorial drug delivery and half-life extension applications. *Mol Ther Nucleic Acids* 2017;**9**:284−93.
82. Heo K, Min SW, Sung HJ, Kim HG, Kim HJ, Kim YH, et al. An aptamer-antibody complex (oligobody) as a novel delivery platform for targeted cancer therapies. *J Control Release* 2016;**229**:1−9.
83. Passariello M, Camorani S, Vetrei C, Cerchia L, De Lorenzo C. Novel human bispecific aptamer-antibody conjugates for efficient cancer cell killing. *Cancers* 2019;**11**:1268.
84. Sridharan K, Gogtay NJ. Therapeutic nucleic acids: current clinical status. *Br J Clin Pharmacol* 2016;**82**:659−72.
85. Herkt M, Thum T. Pharmacokinetics and proceedings in clinical application of nucleic acid therapeutics. *Mol Ther* 2021;**29**:521−39.
86. Murata M, Ohno S, Kuroda M. Development of novel nucleic acid drug targeting KRAS to suppress lung cancer. *Cancer Sci* 2021;**112**:891.
87. Wong XK, Yeong KY. From nucleic acids to drugs discovery: nucleobases as emerging templates for drug candidates. *Curr Med Chem* 2021;**28**:7076−121.
88. Kulkarni JA, Witzigmann D, Thomson SB, Chen S, Leavitt BR, Cullis PR, et al. The current landscape of nucleic acid therapeutics. *Nat Nanotechnol* 2021;**16**:630−43.
89. Jain S, Kaur J, Prasad S, Roy I. Nucleic acid therapeutics: a focus on the development of aptamers. *Expet Opin Drug Discov* 2021;**16**:255−74.
90. Esposito CL, Nuzzo S, Kumar SA, Rienzo A, Lawrence CL, Pallini R, et al. A combined microRNA-based targeted therapeutic approach to eradicate glioblastoma stem-like cells. *J Control Release* 2016;**238**:43−57.
91. Russo V, Paciocco A, Affinito A, Roscigno G, Fiore D, Palma F, et al. Aptamer-miR-34c conjugate affects cell proliferation of non-small-cell lung cancer cells. *Mol Ther Nucleic Acids* 2018;**13**:334−46.
92. Zhang LL, Chen CM, Fan XL, Tang XJ. Photomodulating gene expression by using caged siRNAs with single-aptamer modification. *Chembiochem* 2018;**19**:1259−63.
93. Esposito CL, Nuzzo S, Catuogno S, Romano S, de Nigris F, de Franciscis V. STAT3 gene silencing by aptamer-siRNA chimera as selective therapeutic for glioblastoma. *Mol Ther Nucleic Acids* 2018;**10**:398−411.
94. Garrido G, Schrand B, Rabasa A, Levay A, D'Eramo F, Berezhnoy A, et al. Tumor-targeted silencing of the peptide transporter TAP induces potent antitumor immunity. *Nat Commun* 2019;**10**:3773.
95. Han D, Miao Y, Gao Q, Mao M, Zhang C, Yang L, et al. Bispecific aptamer chimeras enable targeted protein degradation on cell membranes. *Angew Chem Int Ed Engl* 2021;**133**:11267−71.
96. Wu Y, Zhang LQ, Cui C, Cansiz S, Liang H, Wu CC, et al. Enhanced targeted gene transduction: AAV2 vectors conjugated to multiple aptamers *via* reducible disulfide linkages. *J Am Chem Soc* 2018;**140**:2−5.
97. Sugimoto S, Iwasaki Y. Surface modification of macrophages with nucleic acid aptamers for enhancing the immune response against tumor cells. *Bioconjugate Chem* 2018;**29**:4160−7.
98. Geng ZM, Cao ZP, Liu R, Liu K, Liu JY, Tan WH. Aptamer-assisted tumor localization of bacteria for enhanced biotherapy. *Nat Commun* 2021;**12**:6584.
99. Deng ZY, Yang QX, Peng YB, He JX, Xu SJ, Wang D, et al. Polymeric engineering of aptamer−drug conjugates for targeted cancer therapy. *Bioconjugate Chem* 2020;**31**:37−42.
100. Wang D, Peng RZ, Peng YB, Deng ZY, Xu FY, Su YY, et al. Hierarchical fabrication of DNA wireframe nanoarchitectures for efficient cancer imaging and targeted therapy. *ACS Nano* 2020;**14**:17365−75.
101. Luo C, Hu XX, Peng RZ, Huang HD, Liu QL, Tan WH. Biomimetic carriers based on giant membrane vesicles for targeted drug delivery and photodynamic/photothermal synergistic therapy. *ACS Appl Mater Interfaces* 2019;**11**:43811−9.
102. Geng ZM, Wang L, Liu K, Liu JY, Tan WH. Enhancing anti-PD-1 immunotherapy by nanomicelles self-assembled from multivalent aptamer drug conjugates. *Angew Chem Int Ed* 2021;**60**:15459−65.
103. Monaco I, Camorani S, Colecchia D, Locatelli E, Calandro P, Oudin A, et al. Aptamer functionalization of nanosystems for glioblastoma targeting through the blood-brain barrier. *J Med Chem* 2017;**60**:4510−6.