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Recent Advancements in Aptamer-bioconjugates: Sharpening Stones for Breast and Prostate Cancers Targeting

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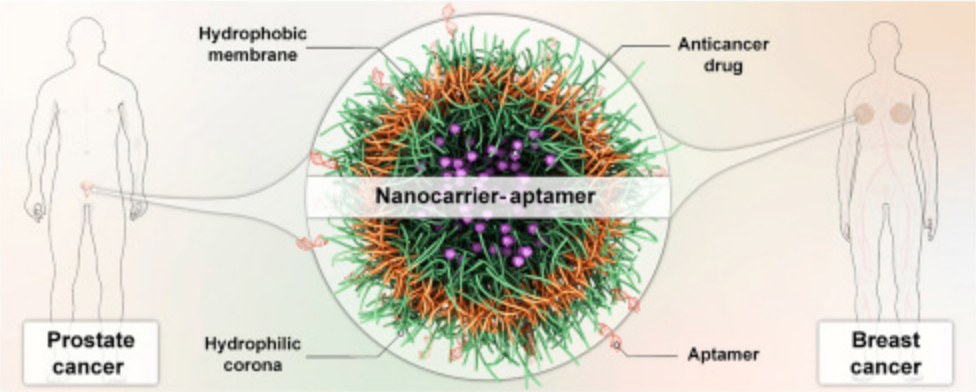
Abstract

Breast and prostate cancers are common types of cancers with various strategies, such as chemotherapy and radiotherapy, for their therapy. Since these methods have undesired side effects and poor target affinity, neoteric strategies—known as aptamer-based smart drug delivery systems (SDDSs)—have been developed in recent years to overcome the obstacles of current treatment, and investigated for a clinical trial. The high affinity and versatility of aptamers for binding to the corresponding targets make them highly noticeable agents in the drug delivery domains. In addition to their exceptional benefits, aptamers are able to overcome tumor resistance because of their high selectivity and low toxicity. Furthermore, aptamers can conjugate with various drugs, nanoparticles and antibodies and effectively deliver them to the specific breast and prostate cells. This review highlights the current researches in aptamer-conjugate developments for targeting breast and prostate cancers, with the special focus on the nanoparticle-aptamer bioconjugates, systematic evolution of ligands by exponential enrichment (SELEX) system and SDDS, especially cutting-edge articles from 2008 to present. Finally, the future prospects and challenges are described.

Graphical abstract

Breast and prostate cancers' mortality as the most important types of cancers have taken a heavy toll on women and men, respectively; hence there is a growing need for assessing diagnosis and treatment. Threfore, in this review, the recent advances in aptamer-bioconjugates treatments of breast and prostate cancers are highlited. Also, nanoparticle-aptamer bioconjugates, SELEX system and SDDS as the main approaches for these treatments are critically reviewed.



Keywords

Aptamer, Smart drug delivery system, Nanoparticle, Breast and prostate cancers, Tumor resistance, SELEX

1. Introduction

Breast and prostate cancers are the two most leading invasive human cancers, in terms of incidence, worldwide. For decades, therapy methods were confined to tumor cells or tissue morphologies instead of identifying the specifically targeted sites. Chemotherapy and radiation are primary therapies utilized for treating these cancers, but their major drawbacks forced researchers to discover neoteric treatment approaches [1]. Additionally, drugs that are commonly used for cancer treatment have some serious disadvantages, such as lack of solubility, bioavailability and fast blood clearance [2]. Therefore, concerns about diagnosis and therapeutic factors [3,4], along with recent developments in the pathophysiology of these cancers, open a new avenue leading to the novel treatment strategies [5].

Smart drug delivery systems (SDDS) have emerged as a cure-all for many useful chemotherapy drugs suffering from high toxicity. These systems are based on nanocarriers—such as dendrimers, liposomes, micelles and gold nanoparticles—in order to tackle the nonspecific and uncontrollable release [6] due to their low toxicity, half-life enhancement and cytotoxic protection from degradation [7]. These systems have benefits in comparison with traditional cancer therapies, such as reducing drug dosage and side effects [8]. Besides, targeted drug delivery systems exhibit dramatic results in identifying the malignant tumor, like improving the efficacy of selective distribution and controllable drug release at tumor locations [9]. Meanwhile, using nanocarriers-aptamers that are able to bind with various biological targets (i.e. peptides, antibodies and nucleic acids) are in the spotlight nowadays due to the shortage of immunogenicity and the higher proportion of target accumulation [10].

Aptamers are simple, small, single-stranded deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) that bind to target molecules with their high affinity and specificity by folding into a three-dimensional conformation similar to antibodies [11,12]. Aptamers can typically bind to various molecules, such as overexpressed receptors, through an *in vitro* iterative selection method termed SELEX (Systematic Evolution of Ligands by Exponential Enrichment) for diagnostic and therapeutic purposes [13]. In comparison with antibodies, aptamers are more beneficial, less toxic and easier to modify and synthesize in the lab. Additionally, aptamers have been chosen for their several benefits as a new family of cancer therapeutic compared to recent cancer therapies, such as monoclonal antibodies. These advantages consist of their promising affinity towards specific tumor cell lines, higher robustness than antibodies, fast *in vitro* selection, low immunogenicity and better penetration into solid tumor tissue [14,15]. Because of these unique advantages, scientists extensively have used aptamer-drug conjugates (ApDCs) with covalent or noncovalent conjugation in targeted cancer therapy [16,17]. Solid-phase

system aids researchers to produce sequence predesigned and automated DNA synthesis from particular phosphoramidite building blocks (A, T, C, G and D) (Fig. 1) [17].

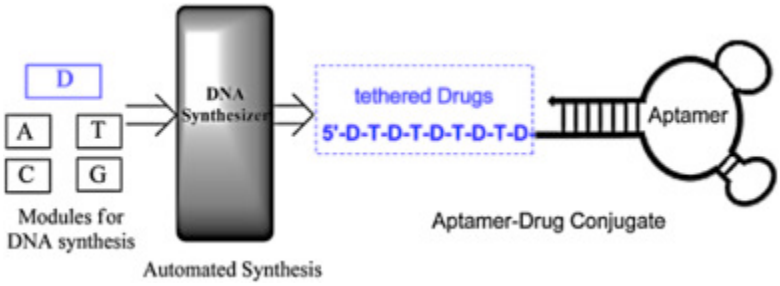


Fig. 1. Automated and molecular synthesis of ApDCs from phosphoramidite A, T, C, G and D [17]. Repreinted with permission.

Recently, aptamers have attracted remarkable interest for their competitive advantages compared with other biological materials [18]. Also, different aptamers have been extensively used to load drugs and nanoparticles (NP_S) for cancer therapy [19]. According to current studies, DNA aptamers were chosen for SK-BR-3 model breast cancer cell-line. By using the ionic-gelation procedure, NP_S-containing DNA aptamers for paclitaxel (PTX) targeted delivery systems comprising chitosan and Pluronic®F127 (PF) were synthesized [20].

Aptamer-drug-NP_S conjugates possess not only a wide range of potential benefits for clinical diagnostics—such as cancer bacteria, tissues and viruses—but also has outstanding ability in therapeutics [21]. Additionally, these platforms have succesfully appeared in biosensing. In recent work, Lu et al. performed a one-pot simultaneous detection of adenosine and cocaine using gold nanoparticles conjugated with aptamers and QDs (quantum dots). As a result, simultaneous colorimetric and fluorescent detection of mentioned molecules has been described [22,23]. This review represents the recent advances in aptamer-conjugates development for targeting breast and prostate cancers from 2008 until now. Also, the complementary discussion of the SELEX system, nanoparticle-aptamer bioconjugates and SDDS is presented.

2. SELEX technique

Aptamers can be identified through *in vitro* evolution called SELEX, which makes aptamer identification in a harmonic process [24]. SELEX is a process or principle for exploring high-affinity oligonucleotide ligand libraries by the combinational chemistry process [25,26]. In 1990, Tuerk and Gold selected two different RNA sequences that interact with the T4 DNA polymerase with high affinity and designed the SELEX method for the first time [27].

SELEX technique is repetitive rounds that allow the identification of unique RNA/DNA molecules from huge populations of random sequence oligomers that bind to the target, shown in Fig. 2 [28,29]. This process is performed in four steps:

(1) Selection step for introducing the chemical groups into DNA/RNA library

(2) Bound DNA section (selective to specific target)

(3) Wash to remove unbound DNA

(4) PCR (polymerase chain reaction) amplification

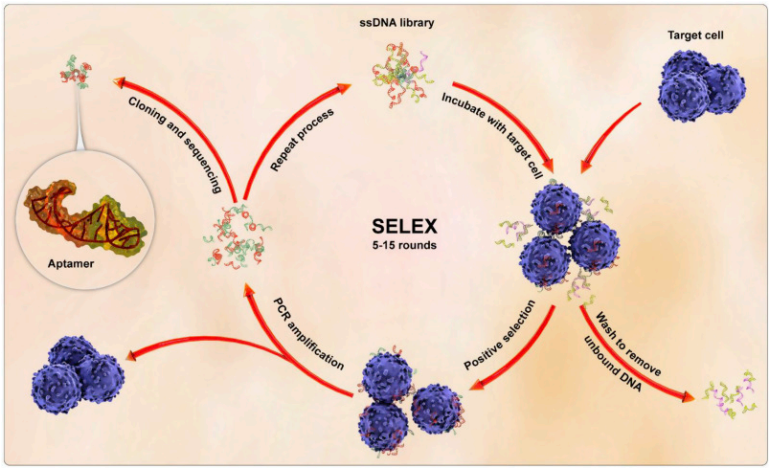


Fig. 2. *In vitro* selection starts with the generation of a diverse library of DNA or RNA molecules.

Generally, the library of ssDNA is reduced after 5 to 15 cycles to choose only aptamers with high affinity to targets [30,31]. In this repetitive process, some problems were observed that caused generation of modified aptamers. In order to fulfill this issue, Click-SELEX protocol has been introduced, which eliminates inappropriate enzymatic problems [32,33].

Click-SELEX is an attractive idea that allows the introduction of alkyne functional groups. Notably, the chemical modification of aptamers can be achieved by a combination of click-chemistry with nucleobase and *in vitro* selection processes.

3. Recent function of aptamers conjugates for cancer targeting

Design chemotherapy drugs has been really difficult, but this current research may affect the efficiency of antineoplastic remedy. Today, tumors become insensitive to several drugs in multi-drug resistance (MDR) [34,35]. Aptamers have been chosen for their several benefits as a new family of cancer therapeutic compared to recent cancer therapies, such as monoclonal antibodies. These advantages include their promising affinity towards specific tumor cell lines, higher robustness than antibodies, fast *in vitro* selection, low immunogenicity and better penetration into solid tumor tissue [14,15]. The aim of the review in this section is to focus on the published article over the four years in which aptamers-NPs-drug has been used for cancer targeting. Through alteration of NIR-absorbing nanocarriers with the ssDNA-caged sgc8 aptamer, Chen et al. demonstrated magnificent NIR light-activated particular cancer-cell binding with GNRs and SWNTs as a model system. This strategy has a dual-targeting capability in comparison with recently discovered cancer-targeting processes, such as reducing non-specific toxicity and increasing selectivity for treating tumor cells [36].

One of the most common drugs to the forefront of cancer therapy is 5-Fu (5-fluorouracil). In the recent study, Behrooz and his colleagues have developed new aptamer-drug conjugates in order to reduce side effects of 5-Fu in gastric cancer. They designed a complex consisting of nanocarrier PAMAM (Polyamidoamine), AS1411 aptamer and 5-Fu. The 5-FU-dendrimer- aptamer was capable of effectively delivering chemotherapy drug to cancer cells, reducing both quantity of cancer cells as well as the IC50 [37]. In a recent study, Martinez and his colleagues produce the first aptamer to target on tumor cell MRP1-expressing cells with a new combinatorial SELEX. They developed MRP1-CD28, which can identify the tumor and deliver the CD28 to tumor-infiltrating lymphocytes. Results have observed a noticeable delay in tumor growth (Fig. 3) [38].

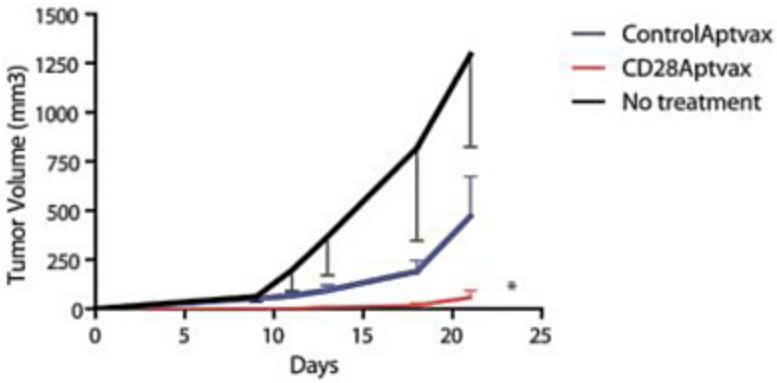


Fig. 3. The overall antitumor effect of the vaccine on mice vaccinated and challenge at day +15 was evaluated by tumor measurement (5 mice per group) [38]. Reprinted with permission.

4. Breast cancer targeting Aptamer

Breast cancer causes high female mortality, and the number of women who are in jeopardy has increased recently [39]. Usage of aptamers based on cell-SELEX is increasing for specific recognition of breast cancer cells [40].

In this section, we highlight some of the important achievements of aptamers, aptasensors and aptamers conjugated with [1] therapeutic agents [2], nanoparticles and [3] siRNA chimeras based on smart targeted delivery systems used for the diagnostics and therapeutics of breast cancer targeting in recent years.

4.1. AS1411 breast cancer aptamer

AS1411 is a significant antiproliferative G-rich phosphodiester oligonucleotide used as a remarkable anticancer agent in Phase II clinical tests [41]. AS1411 has an exceptional structure for binding to a particular cellular protein and resistance to nuclease degradation [42]. The DNA aptamer AS1411 largely demonstrates a strong binding affinity to nucleolin (K_d is in pM to low nM range) via its G-quadruplex structure [43]. AS1411 has been revealed to regulate Rac1 activation and antiproliferative properties that was measured by Bates et al. [44]. Recently, Ghahremani et al. demonstrated a new strategy for effective tumor targeting and megavoltage radiosensitizing by utilization of AS1411-aptamer conjugates with gold nanoclusters (GNCs) that are synthesized through BSA as the capping agent (Fig. 4). This method exhibited 39% of radiotherapy efficacy by taking advantage of flow cytometry and fluorescence microscopy. Importantly, the survival of the mice increased in 9 days [45].

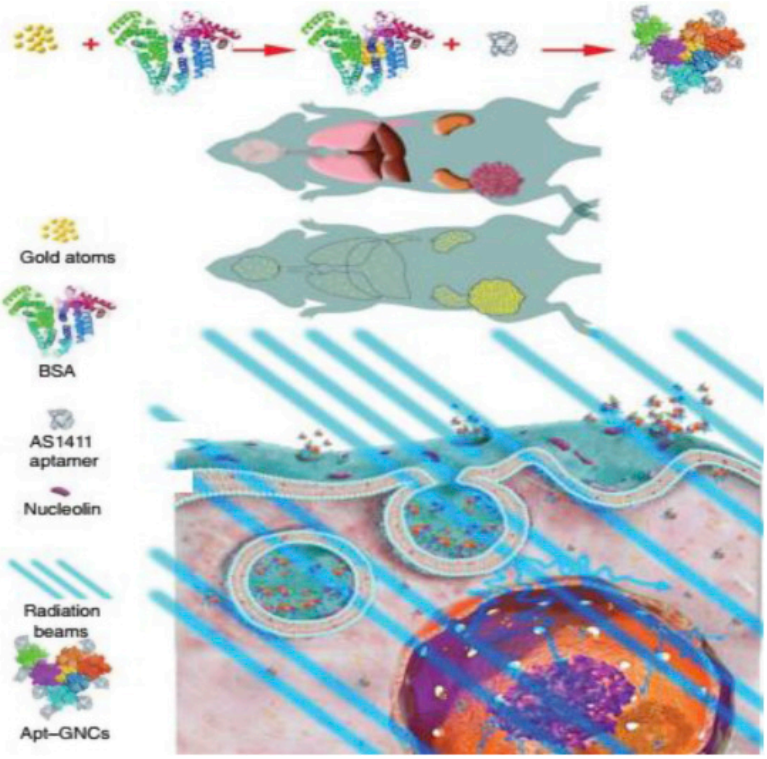


Fig. 4. AS1411 aptamer decorated GNCs for enhancement of radiation therapy effcacy.

Apt–GNCs: Aptamer-conjugated gold Nano-clusters; BSA: Bovine serum albumin [45]. Reprinted with permission.

In another report, Borghei et al. developed a colorimetric aptasensor based on aggregated nanocarriers in order to detect breast cancer cells. Fig. 5 illustrates the color change in the presence of the target (breast cancer) cell and normal cell. Due to the high affinity between the AS1411-aptamer and cancer cell, the removal of aptamers in the solution was triggered, and therefore, no aptamer remained to hybridize with ssDNA-AuNP, making the solution red. But, in the absence of breast cancer cell, aptamers assembled ssDNA-AuNP and remained in the purple solution [46]. Novel nanoparticle-AS1411 aptamer loaded docetaxel in a polydopamine (pD)-based surface (Apt-pD-DTX/NPs) demonstrated high affinity for both *in vivo* and *in vitro* cell studies, as described by Tao et al. This functionalized Apt-pD-DTX/NPs not only decreased adverse effects of Taxotere but also increased promising therapy and local drug concentration effects on breast cancer target [10].

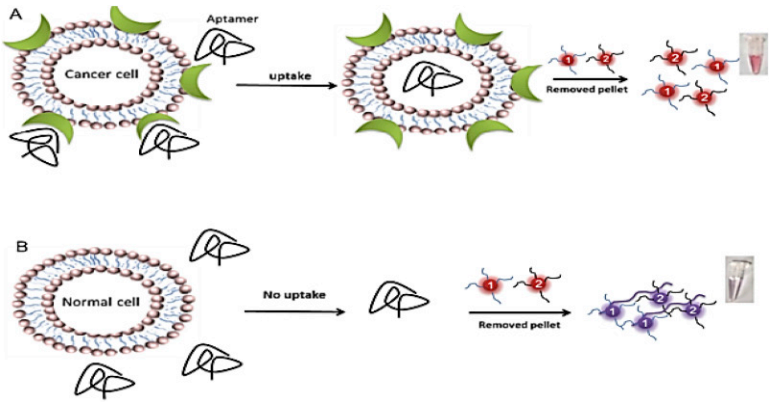


Fig. 5. Schematic representation of selective colorimetric method for detection of cancer cells by employing DNA probe 1,2 -functionalized gold nanoparticles and AS1411 aptamer [46]. Reprinted with permission.

A recent study reported the utilization of AS1411 aptamer conjugated with liposomes (containing DOX hydrochloride and ammonium bicarbonate) can effectively deliver and accumulate DOX to breast tumor cells (MCF-7/ADR). In this research, ammonium bicarbonate, a bubble-generating agent, helped to trigger the release of the DOX quickly into cancer cells simply by local heating (for generating CO_2 bubbles), and as a result, the cancer cells were destroyed (Fig. 6) [47]. In an interesting work in 2017, Zhang and coworkers designed a new process for targeted drug delivery to MCF-7 breast cancer cells. For this purpose, they offered polymeric micelles, including poly (ethylene glycol)-poly (β-amino esters) based on doxorubicin and AS1411 aptamer called PEG-PAEs NPs (PDANs) [48].

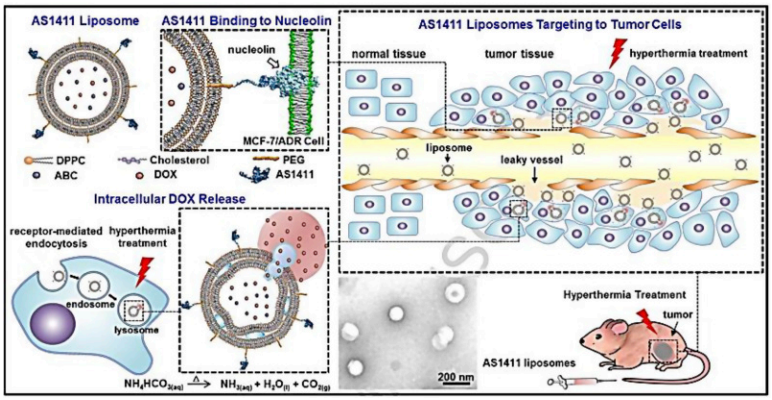


Fig. 6. Selective binding of the AS1411 lyposome to nucleolin on breast cell surface, effectively leading to accumulation of DOX by local heating and generating CO_2 bubbles and subsequent receptor-mediated endocytosis [47]. Reprinted with permission.

Malik et al. constructed a novel AS144-aptamer conjugated to 5 nm gold nanospheres (AS1411-GNS) and demonstrated their potential applicability in both *in vivo* and *in vitro* tests for breast cancer cells. Cells were incubated with nanospheres for 72 h and proliferation was measured by MTT assay. This conjugated aptamer exhibited significantly superior cellular uptake and high cytotoxic results. As illustrated in Fig. 7, two important breast cancer cells, including MCF-7 and MDA-MB-231, as well as non-malignant breast epithetical cells (MCF10A), were affected by AS1411-GNS. We can see how AS1411-GNS has the antiproliferative effects on MCF-7 and MDA-MB-231, but not major inhibitory impact on MCF10A. High selectivity, improved growth inhibitory and increased cytotoxicity are other advantages of this research, which make them prospective candidates for clinical translation [49].

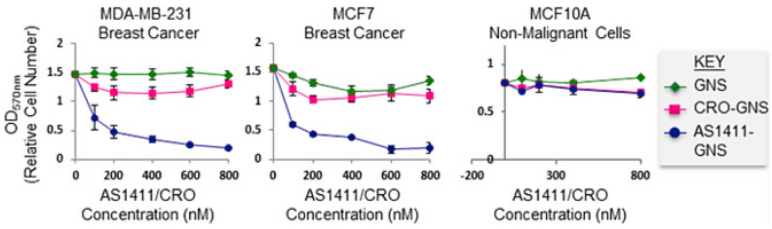


Fig. 7. Antiproliferative activity of AS1411-GNS on the growth of breast cancer cells toward MCF-7, MDA-MB-231 and MCF10A in different concentrations [49]. Reprinted with permission.

4.2. EpCAM breast cancer aptamer

Epithelial cell adhesion molecule (EpCAM also known as CD326 or ESA), a cell surface glycoprotein of approximately 40 kDa, is overexpressed in a variety of epithelial cancer cell lines such as breast, colon and ovarian cancer. Epithelial cell adhesion molecule is a well-known antigen that plays an important role human colon carcinoma tissue [50]. This molecule has gained enormous interest for novel cancer targeting, especially

for breast cancer cell surface [51] and efficiently applied in the case of developing new therapeutic approaches for targeted drug delivery [52].

To date, scientists have made several attempts to utilize more EpCAM-aptamer as a good way for cancer diagnosis and therapy, particularly for breast cancer. In this regard, Hadizadeh et al. developed an EpCAM-aptamer conjugated with PEG–PLGA copolymer for delivering doxorubicin (DOX) into a breast cancer cell *in vitro*. Fig. 8 demonstrates the procedure for synthesizing of doxorubicin-loaded aptamer conjugated nanopolymersomes, which were more cytotoxic ($P < 0.01$) toward MCF-7 than non-targeted nanopolymersomes. The results of this research were admirable due to the increasing the DOX release rate up to 8% over 5 days [53].

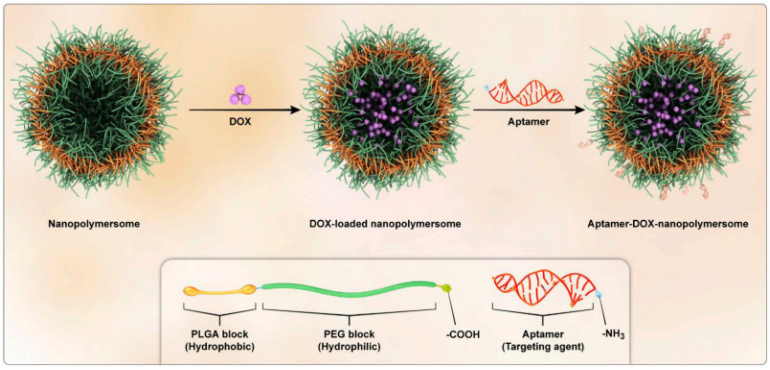


Fig. 8. Process of producing EpCAM-aptamer conjugated DOX-loaded nanopolymersomes.

In continuation, Shigdar et al. developed 19-nt RNA aptamer, which was isolated from 40-base RNA aptamer that binds selectively to EpCAM in the breast, colorectal and gastric cancers, followed by active internalization, for the first time. They evaluated the binding affinity of the EpCAM RNA aptamer to a specific target through flow cytometry and confocal microscopy and the affinity was approximately 55 nM. Importantly, this EpCAM RNA aptamer is efficiently internalized after binding to cell surface EpCAM [54]. In another report in 2017, Xiang and his group developed a novel EpCAM aptamer to tackle the problems with chemotherapy-resistant cancer stem cells and enhance the doxorubicin intercalation to colorectal, ovarian and breast cancer [55]. In an interesting effort, to address the Dox-resistance and deliver efficacy of the Dox to *in vivo* MCF-7 human breast cancer cells, a new strategy was reported by Wang et al. For this purpose, they used EpCAM aptamer-siRNA and Dicer Survivin, an important protein in drug resistance, which is highly expressed in cancer stem. As a result, this strategy demonstrated a high dose of the siRNA delivered to MCF-7 cell in xenograft tumors and silencing of survivin with EpCAM aptamer-siRNA chimera in cancer stem cell population [56].

Locked Nucleic Acid (LNA) modification is responsible for demonstrating increased hybridization affinity toward complementary of RNA and DNA [57], which was first synthesized by Wengel et al. [58]. The potential *in vivo* roles of LNA-modified EpCAM-aptamer was performed toward an experiment with the MDAMB453 breast cancer cells siRNA transfection as a positive control. Consequently, MDAMB453 was inhibited remarkably and showed high fluorescence intensity (MFI) [59].

4.3. MUC1 as a breast cancer aptamer

MUC1 is one of the important breast cancer targets that is overexpressed at the apical surface in various types of reproductive tract epithelia including lung, kidney, stomach, pancreas and breast [60]. It is also an effective tumor-associated antigen (TAA). Dai and his colleagues reported a valuable targeted delivery system for delivering DOX to breast cancer cells *in vitro*. In this project, MUC1 positive target was bound to DNA tetrahedron to make apt-td, which could deliver DOX to MUC1-positive breast cancer cells with high affinity, but

not to negative breast cancer targets. Compared to free MUC1-aptemer (one per aptamer), this material has a high capacity to load the DOX (25 per apt-td) [61]. In another work, based on the smart drug delivery approaches that aid scientists to develop cytotoxic drugs with reduced dosage, the novel 86-base DNA aptamer (MA3) was designed to selectively deliver the cytotoxic DOX only to the MUC1-positive breast cancer cells. However, an *in vivo* study is essential to assess the stability of MA3-apt in blood and tissue [62]. Mesoporous silica nanoparticles (MSNs) have attracted great attention as multifunctional smart nanocarriers for drug delivery system and cell imaging [63]. Following this approach, Hanafi-Bojd et al. designed a MUC1-aptamer conjugated MSNs in order to deliver Epirubicin (EPI) effectively to breast cancer cells. They demonstrated that MSN-MUC1-EPI has been a significant improvement in cytotoxicity versus MSN-EPI against MCF-7 cells [64]. In order to specifically release drugs in tumoral cells, MSNs loaded with safranin O conjugated MUC1 aptamer (S1-apMUC1) was developed by Pascual et al. The same procedure was used to load DOX instead of safranin O (S2-apMUC1). Both compounds were internalized significantly by MUC1 overexpressed breast cancer cells and delivered remarkable cargo (safranin O and DOX) to cancer cell line. S1-apMUC1-Tc (S1-apMUC1 with 99mTc radioisotope) was used as a radiolabeling tool (Fig. 9) [65].

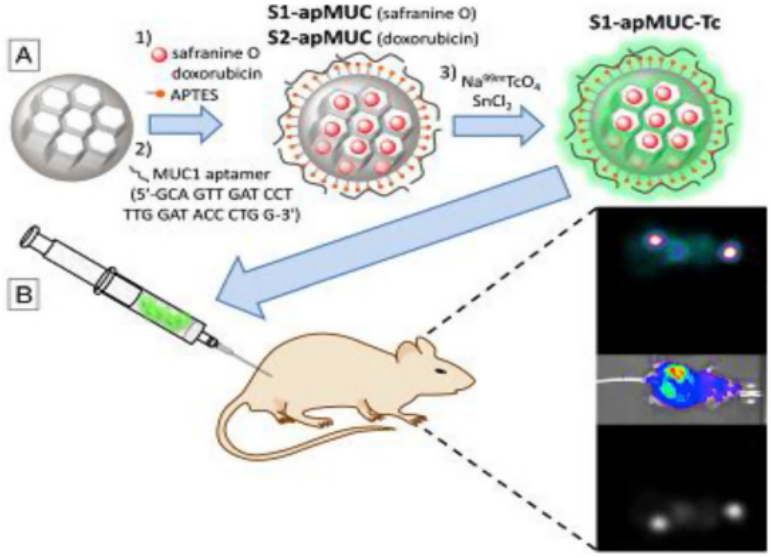


Fig. 9. (A) Schematic diagram for synthesis of S1-apMUC1, S2-apMUC1 and S1-apMUC1-Tc. (B) The role of S1-apMUC1-Tc [65]. Reprinted with permission.

Yu et al. exploited MUC1-aptamer for increasing drug delivery and cytotoxicity ($P < 0.01$) in comparison with paclitaxel to MCF-7 cancer cells loaded by liposomal formulations [8]. Also, Jo et al. developed a dual-aptamer modified silica nanoparticles (dye-doped) system with high affinity to both mucin 1 (MUC-1) $_{(+)}$ and epidermal growth factor receptor 2 (HER-2) $_{(+)}$ breast cancer cells. In comparison with the single aptamer, the mentioned system showed high selectivity and promising detection of breast cancer, as well as low cytotoxicity [66].

4.4. HER2 as a breast cancer aptamer

The human epidermal growth factor receptor 2 (HER2 or ErbB2) consists of HER1, HER3 and HER4 [67] and was discovered in 1985. HER2 overexpression plays an important role in different types of human malignancies [68]. HER2 is one of the important targets that has been examined in breast cancer therapy [69]. Having HER2 positive breast cancer in human increases mortality and causes high recurrence rates [70]. Determination of HER2 positive has been considered as an important method in cancer diagnosis [71]. Sett et al. made a point of developing a novel DNA aptamer against the extracellular domain (ECD apt1) of HER2. In this study, based on *in vitro* SELEX process, ECD apt1 demonstrated cytoplasmic staining in overexpression HER2 [72]. Likewise,

conjugation of ECD apt1 with biotin exhibited powerful cytoplasmic staining in SKBR3 than MDA-MB-231 and MCF-7 [73]. Nguyen et al. reported HER2 aptamer-micelle for delivering better of PTX and effective detection of human HER2 overexpressing SK-BR-3 breast cancer cell lines. The designed PTX-chitosan grafted to pluronic F127 copolymer micelles with DNA aptamer. The results showed a higher affinity and cytotoxicity of the designed platform in comparison with PTX and bare ap-micelles [74]. Recently, Yu et al. developed three-in-one aptamer-siRNA chimera that targeted EGFR/HER2/HER3 in one molecule, both *in vitro* and *in vivo*. They created a HER2 aptamer-EGFR siRNA-HER3 (H2EH3), shown in Fig. 10. H2EH3 exhibited high affinity and selectivity to breast cancer xenograft models, compared to both HER2 aptamer and HER3 aptamer. In addition, tumor growth was inhibited dramatically [75].

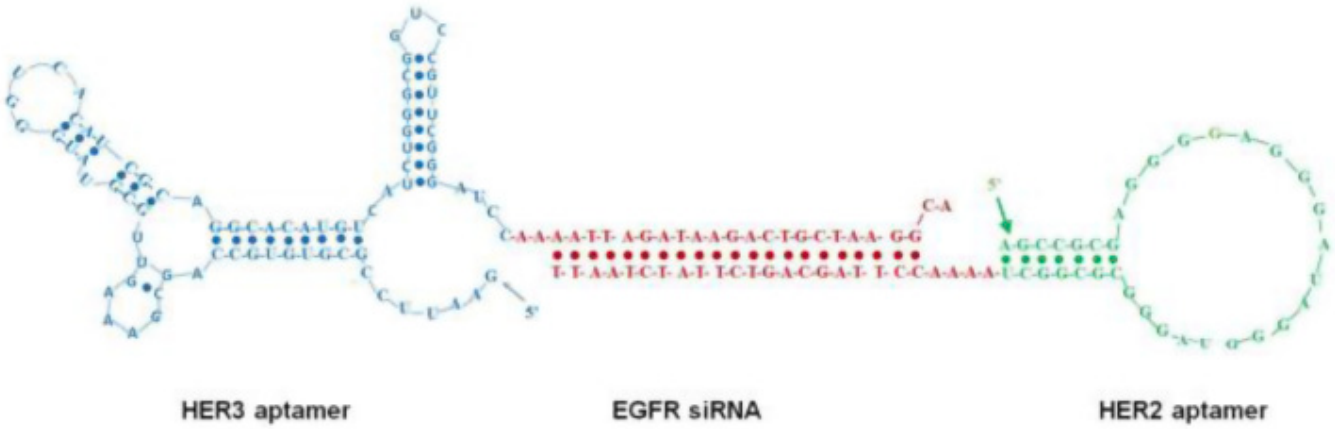


Fig. 10. Structure of H2EH3, which constitutes from conjugation of HER2 aptamer with HER3 aptamer through 21 bases of EGFR siRNA and 2–4 unpaired base linkers [75]. Reprinted with permission.

In 2018, Shen et al. developed pH-responsive micelle-like nanoparticles (MNPs) based-HER2 aptamer (HApt-MNPs). Compared to free HER2 aptamer, the HApt-MNPs was able to Ref. [1] deliver more effectively to a specific target and [2] enhance HER2 aptamer uptake and lysosomal transport in HER2 SKBR3 cells *in vitro* (Fig. 11) [76].

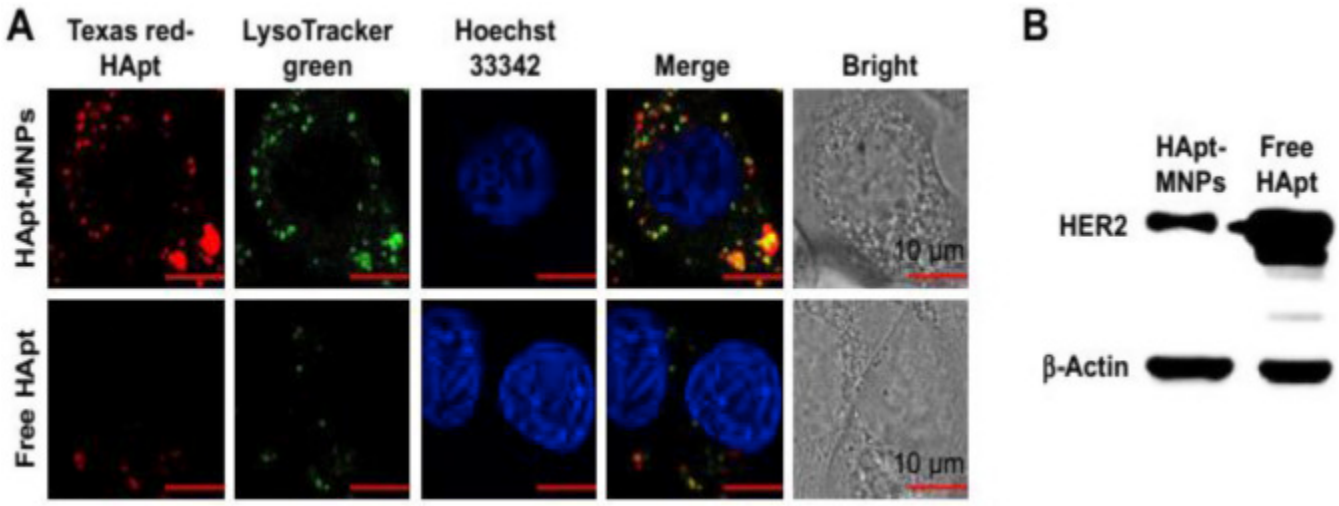


Fig. 11. sKBr3 cells were treated with hapt-MNPs or free hapt for 8 h at the same hapt concentration (125 nM), followed by fresh complete media for 16 h, then stained with lysosome tracker (green fluorescence) and Hoechst 33342 (blue fluorescence). (**A**) Confocal fluorescence microscopy images. Cellular signals were much stronger for hapt-MNPs (red, top panel) than free-hapt (red, bottom panel). scale bars = 10 μm. (**B**) Western blot of her2 protein expression. β-actin was used as a protein loading control. Mean (±sD) her2 band intensity was

24,454.43 (±1632.02) for hapt-MNPs and 79,276.08 (±2162.13) for free hapt (n = 3) [76]. Reprinted with permission.

4.5. The other nanocarriers-aptamer bioconjugates for breast cancer treatment

Since gold nanoparticle (AuNP) has a wide variety of applications on account of their color transition properties, aptamer-functionalized AuNP was developed for detecting the human estrogen receptor alpha (ERα), a crucial biomarker in breast cancer diagnosis, by Ahirwar and coworkers. This colorimetric aptasensor was tested on cellular extracts from MCF-7 and MDA-MB-231 breast cancer cells and subsequently changing the color of aptamer-protected nanoparticles became visible from red to the blue of aggregated AuNPs [77]. Notably, *in vitro* conjugation of aptamer 1(Apt1) and PEGylated liposomes demonstrated a high affinity to $CD44^{+}$ cancer cells line compared with $CD44^{-}$ 3T3 [78].

5. Prostate cancer targeting Aptamer

Prostate cancer (PC) is the second most common cancer among the men in the world [79] after lung cancer [80]. Current studies in prostate cancer demonstrate that localized prostate cancer can be cured significantly, do not have remarkable survival benefits and many men die annually for metastatic prostate disease [81,82]. Inhibiting the androgen receptor (AR), the main oncogenic driver in prostate cancer, is the ongoing projects that many drugs are attempting to achieve [83]. In this part, we demonstrate the most important studies of aptamers and aptamers conjugated with therapeutic agents and nanoparticles, based on smart targeted delivery system that have been used for diagnostics and therapeutics of prostate cancer targeting in recent years.

5.1. Prostate specific membrane antigen aptamer

Prostate-specific membrane antigen (PSMA, also known as A10) is the most famous cell surface antigen of prostate cancer that is highly expressed on the surface of human prostatic adenocarcinoma (LNCaP) [[84], [85], [86]]. It is assessed in primary and metastatic prostate tumor cells, which have been an important target for prostate cancer diagnosis and therapeutic [87]. Leach et al. synthesized a new RNA and DNA hybrid aptamer named A10-3-J1 based on A10-3 aptamer with high affinity to PSMA. In this study, A10-3-J1 is selectively conjugated with superparamagnetic iron oxide nanoparticles (SPIO-NP) in order to load DOX to $PSMA^{+}$ prostate cancer cells. This experiment brought some significant results, such as enhancing the cytotoxicity of targeted cells, mitigating collateral damage and increasing affinity of DOX [88]. Several studies have described gold nanoparticles as great candidates for prostate cancer treatment due to their positive effects on prostate cancer cell lines [89]. In this regard, Kim and coworkers developed a multifunctional gold nanoparticles conjugated with PSMA-specific A9 RNA aptamer for selective delivery of DOX to overexpressed PSMA of prostate cancer cells that enables combined prostate cancer imaging by computed tomography (CT). They demonstrated the effective capability of this aptamer for killing target prostate cancer cell more than non-target cells [90]. Efficient delivery of microRNA (miR-15a and miR-16-1) to *in vivo* and *in vitro* PSMA target was performed by Hao and his group. They used A10–3.2 aptamer (APT) conjugated with Atelocollagen (ATE), ATE-APT, which were loaded with miR-15a and miR-16-1 to PC3 ($PSMA^{-}$) and LNCaP ($PSMA^{+}$) targets. *In vivo* anticancer activity was investigated using the survival times of human PCa bone metastasis mice model. ATE-APT was able to deliver miR-15a and miR-16-1 to PSMA cells and, consequently, could kill the PCa cells in bone metastatic foci and improve cell viability (Fig. 12) [91].

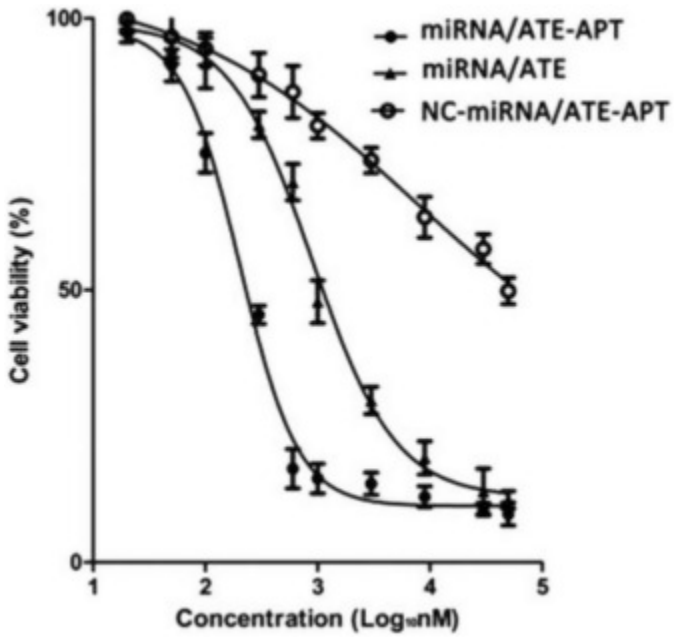


Fig. 12. Viability of LNCaP cells treated with miRNA/ATE–APT, NC-miRNA/ATE–APT and miRNA/ATE complexes (n = 3, error bars represent the standard deviation). APT, aptamer; ATE, atelocollagen; and NC, negative control [91]. Reprinted with permission.

Zhang et al. reported synthesis of a DNA nanoparticle containing PSMA-aptamer for targeted drug delivery of DOX using a pH-sensitive spacer composed of adenine repeats. Because of integration of pH-sensitive spacer, nanoparticles were able to break apart at acidic pH, and therefore caused fast release of the DOX. Moreover, a cell uptake study showed that DOX was bound to more $PSMA^+$ cells than PSMA-null cells for prostate cancer therapy [92,93]. Farokhzad et al. utilized docetaxel (DTX) encapsulated with PLG-PEG copolymer, which functioned with A10 2-fluoropyrimidine RNA aptamer (DTX-NP-Apt) that binds effectively to PSMA on the surface of LNCaP prostate cancer cell. *In vitro* ($P < 0.0004$) and *in vivo* cellular toxicity increased remarkably using a LNCaP xenograft nude mouse model of PCa that results in enhanced cytotoxicity and anticancer activity [94,95]. Antitumor activity of aptamer nanoparticle for acceptable loading of DTX (DTX-apt-NPs) was performed against both *in vivo* and *in vitro* prostate cancer. Sodium oleate and PLG-b-PEG were utilized through the solvent diffusion procedure. Gao et al. reported that DTX-apt-NPs could dramatically improve the delivery of DTX to $PSMA^+$ prostate cancer and increased the antitumor efficacy of DTX by aptamer-mediated intracellular delivery (Fig. 13) [96].

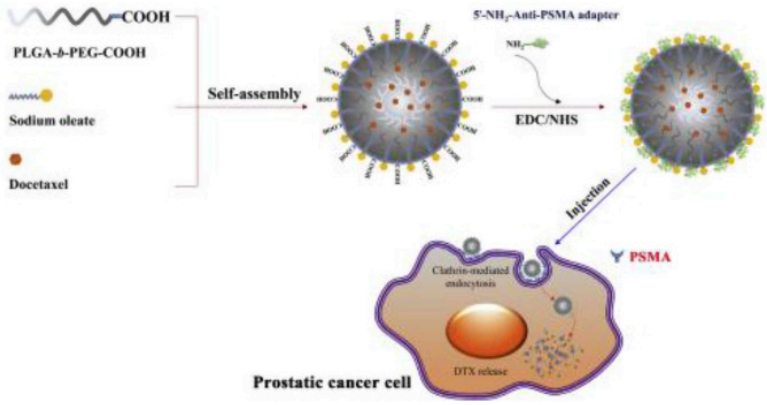


Fig. 13. Effective delivery of DTX through DTX-apt-NPs [96]. Reprinted with permission.

Cisplatin demonstrated high ability in a clinical trial to manage metastatic castration-resistance prostate cancer, however, its resistance and adverse effects make it a drug with limited use in cancer therapy [97,98]. In order to tackle these problems, Dhar and his group developed an applicable strategy by using Pt (IV)-encapsulated PSMA-aptamer of PLG-b-PEG nanoparticles. As a result, suitable dose of Cisplatin was loaded to target PSMA. Also, effective targeting against $PSMA^+$ LNCaP was described [99]. In addition, the combination of ApDCs with radiotherapy in $PSMA^+$ tumor and their noticeable therapeutic results has been described [100]. *In vitro* study evaluated the potential of targeted liposomes loaded with ^{225}Ac. In this study, A10 anti-PSMA-aptamer conjugated with PEGylated liposomes loaded with a-particle generator ^{225}Ac selectively killed prostate-specific membrane antigen. The results of this report confirmed the rapid internalizing, selective binding to specific target and $PSMA^+$ killing [101].

6. Conclusion and future prospects

Breast and prostate cancers' mortality as the most important types of cancers have taken a heavy toll on women and men, respectively; hence there is a growing need for assessing diagnosis and treatment. Several attempts have been made to address these cancers such as chemotherapy and radiation which most of them bring about several problems including low accumulation in tumor cells and low target selectivity. Therefore, targeted drug delivery may tackle these limitations and improve their disadvantages. In recent years, aptamers and synthetic oligonucleotide moleclules isolated *in vitro* demonstrated high target affinity as well as reduced toxicity. Aptamer-bioconjugates with a wide variety of benefits have demonstrated multifunctional capability for breast and prostate therapies and diagnostics. Nevertheless, remarkable challenges and issues remain to be tackled to use them for clinical use, such as low drug loading, weakly understood pharmacokinetics, toxicity and costly *in vivo* testing. More imporatantly, due to the small size and the rapid degradability of aptamers through different nucleases *in vivo*, they are more likely to be modified by different groups of agents resulted in increased cost and side effects. To solve this, scientists should carry out more animal models for the evaluation of safety and efficacy of aptamer-bioconjugates. Moreover, as finding potential targets needs boundless time and effort, the SELEX technology is quite time consuming and labor intensive even though a variety of methods are being developed for aptamer screening. Thus, different methods should be taken into consideration to decrease the cost of aptamer SELEX strategy *in vivo* in the future. In this sense, in order to reduce toxicity and improve target efficacy of aptamer-bioconjugates, some procedures can be investigated carefully such as surface charge, coating, biocompatibility and biodegradability of conjugates as well as optimization of the surface modification of conjugates. To the best of our knowledge, although aptamer-bioconjugates play an indispensable role in cancer therapy, to accurately assess therapeutic efficacy and ameliorate clinical practise of aptamer-bioconjugates, it is of paramount importance to evaluate more clinically and pathologically in relation to animal models. Furthermore, the SELEX strategy requires more experiments to choose appropriate aptamers from an oligonucleotide library. For the near future, we should be hopeful for the design of new generations of nanocarriers-aptamer bioconjugates to address all limitations, particularly, for clinical use. Some of the aptamer-drug conjugates systems are summarized in Table 1.

Table 1. Some other aptamer-drug conjugate systems for targeted drug delivery.

Target	**Aptamer(DNA/ RNA)**	**Therapeutic agent**	**Status**	**Ref**
PSMA(PC)	E3	MMAE and MMAF	In vivo and In vitro	[102]
PSMA(PC)	A10	Plumbagin	In vivo	[103]
PSMA(PC)	A10–3.2	Paclitaxel	In vivo and In vitro	[104]
PSMA(PC) nanobubbles	A10–3.2	siRNA-cationic	In vivo and In vitro	[105]

PSMA(PC)	A10	TFO	In vivo	[106]
MUC1(BC)	DNA aptamer	Doxorubicin	In vivo and In vitro	[107]
MUC1(BC)	DNA aptamer	Paclitaxel	In vitro	[108]
MUC1(BC)	DNA aptamer	miR-34a	In vivo and In vitro	[109]
Nucleolin FOXM1(BC)	AS1411 and	Doxorubicin	In vivo and In vitro	[110]
Nucleolin	AS1411(BC)	Doxorubicin	In vivo and In vitro	[111]
Nucleolin	AS1411(BC)	Paclitaxel	In vitro	[112]
Nucleolin	AS1411(BC)	Cisplatin	In vitro	[113]
Nucleolin	AS1411(BC)	Doxorubicin	In vivo and In vitro	[18]
Nucleolin	AS1411(BC)	Fluorescein	In vivo and In vitro	[114]
HER2+(BC)	HER2	Docetaxel	In vivo and In vitro	[115]
HER2+(BC)	A6	P-gp	In vitro	[116]
HER2+(BC)	HB5	Doxorubicin	In vitro	[117]
EpCAM(BC)	EpCAM	Nutlin-3a	In vitro	[118]
EpCAM(BC)	EpCAM	Neocarzinostatin	In vitro	[119]

PSMA: Prostate-specific membrane antigen, MMAE: Monomethyl auristatin E, MMAF: Monomethyl auristatin F, TFO: Triplex forming oligonucleotides, HER2: Human epidermal growth factor receptor 2, EpCAM: Epithelial cell adhesion molecule.

Conflicts of interest

The authors declare that they have no conflict of interest.

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