Review

Aptamer-Based Targeting Cancer: A Powerful Tool for Diagnostic and Therapeutic Aims

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Highlights

Cancer is the most important reason of death all over the world.
Timely diagnosis and treatment of cancer have a considerable role in
Aptamer are frequently used for designing the molecular diagnostic methods.
Molecular diagnostic methods include targeted delivery systems and biosensors.

Abstract: Cancer have been known as a most important global mortality reason, which in spite of novel therapeutic ways, the leading death is still considerable. Molecular targeted diagnosis and therapy using aptamers with high affinity have converted to a popular technique for pathological angiogenesis and cancer therapy scientists. In this paper several aptamer-based diagnostic and therapeutic techniques such as aptamer-nanomaterial conjugation, aptamer-drug conjugation (physically or covalently) and biosensors, which successfully designed for biomarkers, are critically reviewed. The results obviously demonstrated that aptamer have potentially incorporated with targeted delivery systems and biosensors for detection of biomarkers which expressed by cancer cells. Aptamer-based therapeutic and diagnostic methods, as the main field of medical sciences, possess high potential in cancer therapy, pathological angiogenesis and increase of community health. The clinical use of aptamer is so limited due to unreliability resulted from targets impurities, inaccuracy in SELEX stages process, and *in vitro* synthesis which led to the lower selectivity for *in vivo* targets. Moreover, size behavior, probable toxicity, low-distribution and unpredictable behavior of nanomaterials in *in vivo* media make critical their usage in clinical assays. This review can be helpful for implementation and designing the future studies which are effective and applicable for clinical use.

Keywords: aptamer; cancer; diagnosis; therapy; biosensor

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1. Introduction

Aptamer is known as a synthetic nucleic acid strand which can specifically bind to a wide range of targets such as proteins, cells, bacteria, toxins, DNA, miRNA, ions, drugs molecules with high affinity [1]. Aptamer was firstly introduced at 1990 by Larry Gold and his colleagues at Colorado University during process of systematic evolution of ligands by exponential enrichment (SELEX). Briefly this technique includes introduction of target into a library of the random sequences of ssRNA or ssDNA (10^{13}-10^{16}), cleaning up the unbounded strands, washing and reproduction of bounded strands with PCR (6 to 15 times) [2].

Although antibody and aptamer, which known as chemical antibody, possess the same role in the specific binding to the targets, but some advantages of aptamer over the antibodies make preference use of aptamer for designing the targeted delivery and biosensing platforms. The interesting features of aptamers are specificity to molecules in cells and also monoclonal antibodies, easy penetration to the tissue and cells, easy and inexpensive production, and lower size [3–6].

The folding of aptamer with secondary and tertiary conformation lead to high affinity to the targets [1,7]. This feature enables usage of aptamer as a targeting and nanocarrier agent for the delivery of therapeutic and imaging agents to the tumor cell through recognize of biomarkers. Moreover, aptamer can be used as a therapeutic agent. According to the food and drug administration (FDA) approval, age-related macular degeneration can be treated with an aptamer-based drug Macugen ® (pegaptanib) [8]. From the other side, aptamer can be used for detection of various biomarkers related to the various diseases such as cardiovascular, cancer, neurodegenerative, infections, organ harms. So, as shown in Scheme 1, aptamer can be introduced as a powerful therapeutic and diagnosis agent.

Based on the American Cancer Society, about 1,918,030 cancer cases and 609,360 cancer deaths were estimated in 2022 in united state. Moreover, about 350 deaths per day were recorded for lung cancer which was the most leading reason of death. But the statical studies of the National Center for Health Statistics (NCHS) of USA demonstrated that the mortality patterns contain stagnated progression for prostate and breast cancers and increase for lung cancer [9]. This statical estimation shows the importance of cancer survival, which can be achieved by timely treatment and diagnosis. Due to importance of this subject, in this paper we review application of aptamer in designing targeted delivery systems and biosensors and critically discuss methods, which can be helpful for future studies.

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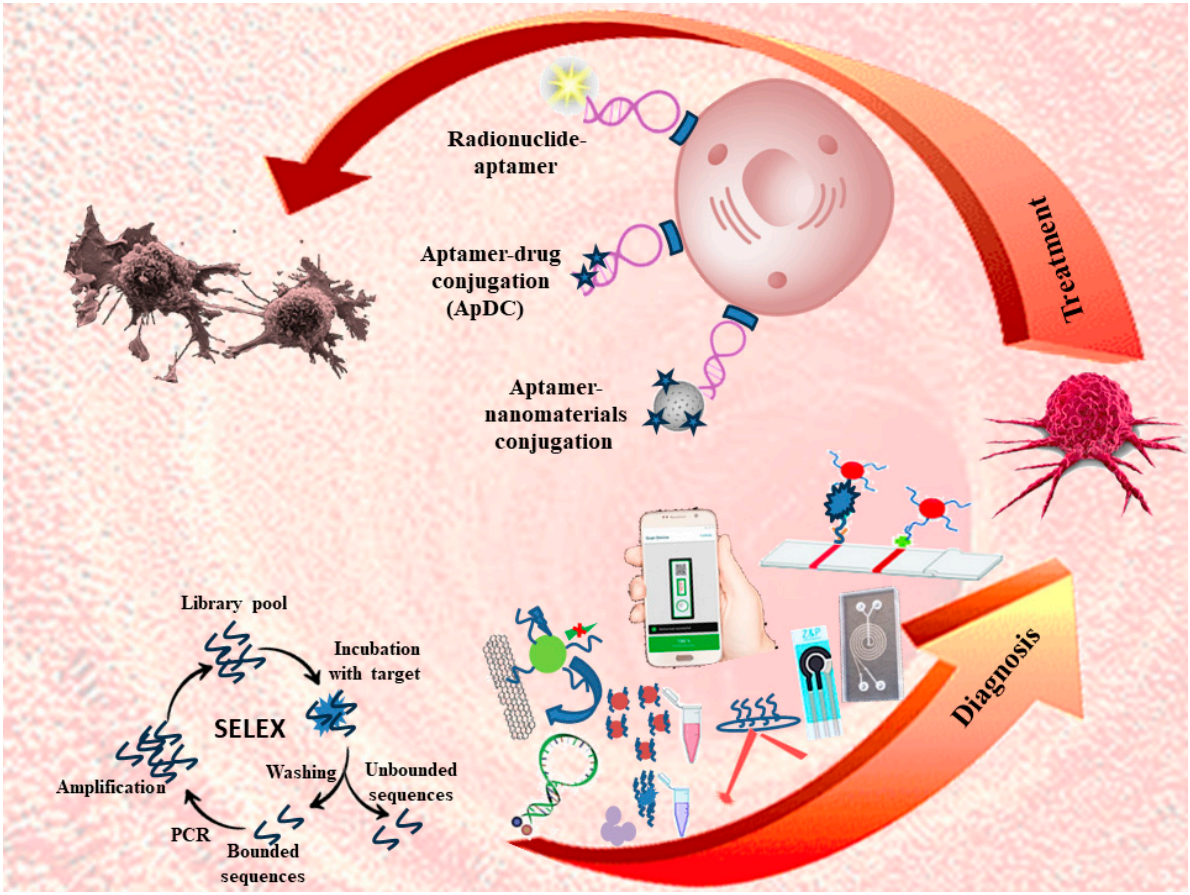

Scheme 1. Application of aptamer as a therapeutic and diagnosis agent for tumors.

2. Aptamer-Based Targeted Delivery Systems

Targeted-delivery as a main field of medical sciences, have highly applied for cancer therapy and pathological angiogenesis. This field can be performed in the both form of aptamer-nanomaterials conjugations and aptamer-drug conjugates (ApDC). The former is benefited from high surface area of nanomaterials for loading the different anticancer drugs and possible therapeutic effects of nanomaterials like thermal or ROS production. The later mainly used for loading the intercalating drugs such as doxorubicin (DOX) which reduce the toxicity on the normal cells, by targeting the specific cells [10,11]. The combination of aptamer with therapeutic agents can resolve some problems such as low *in vivo* sensitivity and selectivity, high toxicity of drugs, and tumor imaging difficulty, etc. These systems are able to cancer diagnosis or prognosis, and cancer signal pathways blocking [7]. In this section the studies which employed the aptamer in targeted-delivery systems are discussed and their information are summarized in Table 1.

Table 1. The summarized information aptamer-based targeted-delivery systems.

Method of delivery	Sequences of aptamer (5′ to 3′)	Cell or animal	Marker	Therapeutic agent	Ref.
Nanocomplex of ATP aptamer/QDs and MUC-1 aptamer/ AuNPs	ATP-aptamer: NH2-AACCTGGGGGAGTATTGCGGAGGAAGGT MUC1-aptamer: 5′-SH-GAAGTGAAAATGACAGAACACAACA-3′	MCF-7	Muc-1, ATP	-	[12]

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EpCAM aptamer-conjugated SWNT/piperazine–polyethylenimine	EpCAM aptamer: GCG ACU GGU UAC CCG GUC G SiRNA: GGAUGUUCAAGAUCCCAUGCAGCTC	MCF-7	EpCAM	siRNA for suppressing BCL9l	[13]
silica coated-Gd-Zn-Cu-In-S/ZnS QDs/PEG/EpCAM DNA	EpCAM aptamer: CAC TAC AGA GGT TGC GTC TGT CCC ACG TTG TCA TGG GGG GTT GGC CTG	4T1, MCF-7	EpCAM	DOX	[14]
Sgc8 aptamer-modified silica nanoparticles system	Sgc8 aptamer: ATCTAACTGCCGCCGCGGGAAAATGTAC GGTTA G(T)10-COOH	CCRF-CEM human acute T lymphocyte leukemia	protein tyrosine kinase-7 (PTK-7)	DOX	[15]
As141 aptamer conjugated-pluronic F127 /beta-cyclodextrin-linked poly (ethylene glycol)-b-polylactide block copolymers (β-CD-PELA)	As141 aptamer: TTGGTGGTGGTGGTTGTGGTGGTGGTGG	MCF-7, female BALB/c nude mice	Nucleolin	DOX	[16]
Encapsulation of aptamer-DOX in liposome	Aptamer AS1411: GGT GGT GGT GGT TGT GGT GGT GGT GGT T	Human breast tumor MCF-7/Adr cells	nucleolin	DOX	[17]
irradiation therapy using AgNPs functionalized with PEG and As141 aptamer	As1411 aptamer: (CH2)6-NH2-GGTGGTGGTGGTTGTGGTGGTG GTGG	C6 glioma, C6 glioma-bearing mice	nucleolin	-	[18]
Photodynamic therapy by aptamer-conjugated superparamagnetic iron oxide nanoparticles (SPION) loaded by daunomycin (DNM) and 5, 10, 15, 20-tetra (phenyl-4-N-methyl-4-pyridyl) (TMPyP)	As1411 and DNM aptamer: 5′-NH2-GGG GGG GGT TGT CCC CCC CCT TTT TTG GTG GTG GTG GTT GTG GTG GTG GTG G	C26, A549	nucleolin	DNM, TMPyP	[19]

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Fe_3O_4@ UiO-66-NH2 MOF/DOX/CDs/ AS1411 aptamer	As1411 aptamer: GGTGGTGGTGGTTGTGGTGGTG GTGG	MDA-MB-231	nucleolin	DOX	[20]
photosensitizer protoporphyrin IX/ AS1411/NaYF4:Yb, Er nanocluster (UCNP)	As1411 aptamer: NH2–GG TGGTGGTGG TTG TGG TGGTGG TGG	MCF7, Hella	nucleolin	photosensitizer protoporphyrin IX produced ROS	[21]
assembling five DNA strands to form five-point-star motif	strand I: ATAGTGAGTCGTATTAATTAACCCTCACT AAAAAGGATCCGGATCCTT strand II: TTTAGTGAGGGTTAATCATACGATTTAGGT GAAAGGATCCGGATCCTT strand III: TCACCTAAATCGTATGGGAGCTCTGCTTA TATAAGGATCCGGATCCTT strand IV: ATATAAGCAGAGCTCCTAGAAGGCACAG TCGAAAGGATCCGGATCCTT strand V: TCGACTGTGCCTTCTATAATACGACTCAC TATAAGGATCCGGATCCTT	MCF7	MUC1	DOX	[22]
Assembling two strands contains a DNA aptamer with G-quadruplex and double-stranded DNA	CCCCCCCCCCTGTTGGGGGGGGGGTTTTT TTTTGGTGGTGGTGGTTGTGGTGGTGGTG G	MCF7	MUC1	DOX	[23]
Conjugation of aptamer P19 to gemcitabine, 5-fluorouracil (5-FU), monomethyl auristatin E (MMAE) and derivative of maytansine 1 (DM1)	GGGAGACAAGAAUAAACGCUCAAUGGC GAAUGCCCGCCUAAUAGGGCGUUAUGA CUUGUUGAGUUCGACAGGAGGCUCACA ACAGGC	PANC-1 AsPC-1	cells	Gemcitabine 5-FU MMAE DM1	[24]
Covalently binding sgc8ca aptamer-doxorubicin	ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GA	Human T-cell ALL (CCRF-CEM), human B-cell	kinase 7 (PTK7)	DOX	[25]

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		Burkitt's lymphoma (Ramos)			
self-assembly of biotinylated hairpin DNAs followed by streptavidin-aptamers (Zy1) conjugation	H1: Biotin-CGT CGT GCA GCA GCA GCA GCA GCA ACG GCT TGC TGC TGC TGC TGC TGC H2: Biotin-TGC TGC TGC TGC TGC TGC ACG ACG GCA GCA GCA GCA GCA GCA AGC CGT Trigger: TGC TGC TGC TGC TGC TGC ACG ACG Zy1: ACG CGC GCG CGC ATA GCG CGC TGA GCT GAA GAT CGT ACC GTG AGC GCG $T(T)_{10}$- streptavidin	SMMC-7721	cell	DOX	[26]
self-assembly of 8 sequences of S1−S4 DNA and linker L1−L4 DNA to form nanohydrogels: -unmethylated cytosine-phosphate-guanine oligonucleotides (CpG ODNs) - DNA nanohydrogels (CpG-MUC1-hydrogel) - I-motif cytosine (C)-rich single-stranded DNA	S1(I-motif): TCAACACTAATCCCCAATCCCAATCCCA ATCCCAAACG A S2: TCAACACTAATCCGTTTGGGATTGGGAC AAAACGACGAA S3(CpG-MUC1): TCAACACTAATCCCCAATCGTCGTTTTGT CGTTTTGTCGTT-S-S GCAGTTGATCCTTTGGATACCCTGG S4: TCAACACTAATCAAAACGACAAAACGAT TGGGATTGGGA L1: GATTAGTGTTGAAACGACA-S-S-AAACG L2: ACAAAA-S-S-CGACGAGCCCTCCCCC L3: GATTAGTGTTGAGGGGGAGGGC L4 (CpG): TCGTCGTTTTGTCGTTTTGTCGTT	MCF-7, A549, HepG-2, Female BALB/c (nu/nu) athymic nude mice (5−6 wk)	MUC1	DOX	[27]
antimiR-21 DNAzyme linked to the aptamer of low-density lipoprotein receptor (LDL-R)	TCA ACA GGC TAG CTA CAA CGA CAG TCT GAT AAG CTA TTTTA GGA CAG GAC CAC ACC CAG CGC GGT CGG CGG GTG GGC GGG GGG AGA ACG AGG TAG GG	Huh-7, MDA-MB-231	LDL-R	antimiR-21 DNAzyme	[28]
Covalently bonding paclitaxel (PTX) to the nucleolin AS1411 aptamer (NucA) through dipeptide bond	TTGGTGGTGGTGGTTGTGGTGGTGGTGG	SKOV3 (ATCC HTB-77), OVCAR3 (ATCC HTB-161)	Nucleolin and cathepsin	PTX	[29]
HER2- aptamer - conjugated to the mertansine (DM1)	AGC CGCGAG GGG AGG GAU AGG GUA GGG CGC GGC U	BT-474, MDA-MB-231 MCF-7, A549,	HER2	DM1	[30]

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		mouse xenografts with BT-474 breast cancer			
conjugation of HER2 aptamer to siRNAs targeting Bcl-2	GGGAGGACGAUGCGGCGAUGCUUACGU GCACGCGCCAGACGACUCGCCCGAGCU GUCACAGAGGGGCUACUU	N202.1A	HER2	siRNAs targeting Bcl-2	[31]
^{18}F-fluoride-HER2 aptamer	TCCTGGCATGTTCGATGGAGGCCTTTGAT TACAGCCCAGA	tumor-bearing mice	HER2	^{18}F-fluoride-HER2 aptamer	[32]

2.1. Aptamer-Nanomaterials Conjugation

Nanomaterials as an inseparable part of targeted delivery systems can be incorporated with aptamer for aims of therapeutic and imaging systems. Nanomaterials are known as a favorable candidate for functionalization with aptamer due to properties of biodegradability, high loading capacity, easy bioconjugation, high permeability, and easy release of drugs. Targeted delivery of drugs to cells and tissues, can considerably reduce the cytotoxicity and enhance drug efficacy [33]. Moreover, controllable release of drug can be happened because of easy and fast response of nanoparticle carriers to the environmental condition or stimuli in cells [34]. This kind of targeted delivery synergically benefits from advantages of both parts including high surface area of nanomaterials for binding of aptamers, protected situation of aptamer from nuclease degradation, high sensitivity and selectivity [35,36]. Here we will discuss more on the nanoparticles which have frequently used for designing the drug delivery systems such as gold nanoparticles (AuNPs) [37], carbon-based nanoparticles [38], quantum dots (QDs) [39,40], silica nanoparticles [41], polymeric nanomaterials [42], liposomes [43], magnetic nanoparticles [44], metal organic frameworks (MOFs) and upconversion nanoparticles (UCNPs) [45].

Gold nanoparticles with advantages of unique optical and electrochemical properties, surface plasmonic effect, considerable biosafety, high easy bioconjugation with Au-S chemistry have been widely applied for designing the targeted delivery platforms [37]. The AuNPs can be used as a carrier for delivery of therapeutic or imaging agents to the cancer cells or tissues. Mohammadinejad et al. introduced a nanocomplex for specific diagnosis, and imaging of MCF-7 based on detection of ATP molecules in cancer cells [12]. Nanocomplex was designed based on conjugation of AuNPs with MUC-1 aptamer (MUC1 apt – AuNPs) and conjugation of QDs with ATP aptamer (ATP apt-QDs). As shown in Figure 1A after adsorption of ATP apt-QDs on the MUC1 apt – AuNPs, the intensity of the QDs was quenched due to fluorescence resonance energy transfer, resonance energy transfer (FRET) phenomena. In the following internalization of complex in the MCF-7 cells through binding to the MUC 1 biomarkers on the MCF-7 cells, ATP apt-QDs can bind to ATP molecules leading to separation from MUC1 apt – AuNPs and ends up recovery of the intensity.

Carbon-based nanoparticles which mainly includes graphene oxide (GO), carbon nanotubes (CNTs), and carbon dots (CDs) possess features of easy functionalization, low toxicity, great surface area which is suitable for drug loading, and considerable *in vivo* stability. This feature makes possible frequently use of carbon-based nanoparticles incorporated with aptamer as a biorecognition element. This platform can be a powerful platform for aims of therapeutic and delivery systems. Benefiting from these materials Mohammadi et al. designed a nanocarrier for siRNA based on conjugation of RNA aptamer of EpCAM with single-walled carbon nanotube (SWNT)/ piperazine–

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polyethylenimine non-viral vector. This platform was able to successfully suppress BCL9l in positive EpCAM cell line MCF-7 [13].

QDs are assigned to the semiconductor nanoparticles containing periodic elements II/VI or III/V groups with interesting features such as tunable size and emission (UV to near-infrared), high intensity fluoresces intensity with narrow width, and considerable photostability [46]. Akbarzadeh et al. introduced a drug delivery and imaging platform based on loading the DOX on the silica coated-Gd-Zn-Cu-In-S/ZnS QDs/PEG/EpCAM DNA (Figure 1B) toward 4T1 and MCF-7 cell lines [14]. *In vitro* studies demonstrated high toxicity of platform for cell lines and high efficiency of proposed platform. Moreover *in vivo* studies on the 4T1 tumor-bearing Balb/c mice proved applicability of designed platform by prevention of tumor growth.

Recently silica nanoparticles have received huge consideration of scientists for designing of targeted delivery due to high porous structure for loading the therapeutic agents, easy modification and high biocompatibility. Yang et al. synthesized a silica-based platform for targeted delivery of DOX to CCRF-CEM human acute T lymphocyte leukemia cells [15]. In this design they used Sgc8 aptamer-conjugated mesoporous silica nanoparticles and results demonstrated successful designing with good releasing and cell killing.

The polymeric nanomaterials are assigned to a wide range of particles such as micelles, hydrogels, and polymeric nanoparticles. The micelles are structured from self-assembling the copolymers with amphiphilicity feature which can be produced in a critical micelle concentration (CMC) [16]. Hydrogels with high hydrophilicity are designed from cross-linking structure of polymers which possess the high elasticity with considerable diffusivity of bifunctional molecules [47].

This nanocarriers with advantages of great drug loading and easy release, biocompatibility, high biodegradability is a potent candidate for incorporation with aptamer for therapeutic and imaging aims. Polymeric nanoparticles are a wide range of hydrophilic polymers such as polyethylenimine (PEI), polyethylene glycol (PEG), polyethyleneimine (PEI), polycaprolactone (PCL), etc., which can be formed as nanoparticles [48] or a coating for other nanoparticles [13,18].

Benefiting from conjugation of the aptamers with polymeric nanoparticles, Zhao et al, introduced a irradiation therapy platform using AgNPs functionalized with PEG and aptamer As141 [18]. The proposed platform with synergically advantages, showed an interesting radiosensitizing effect with high rate of apoptotic cell death on the C6 glioma cells tumor cells. Moreover *in vivo* studies showed more accumulation of synthesized nanoparticles in tumor tissues.

Liposomes are known as a phospholipid bag with a core made of aquas media which allows loading the drug in different parts of core, bilayer, or interface layer. This nanocarriers have interesting features super biocompatibility, hydrophilic drugs delivery, and negligible toxicity [49]. Taking advantages of this nanocarriers, Li et al, introduced a liposomes-based delivery system for loading the aptamer-DOX conjugation and delivery to the reversing drug resistance of MCF-7/Adr cells [17]. The nanoparticles successfully were applicable to up taking with cells, entrance and release of the Ap-Dox complex. Afterwards the interaction between aptamer AS1411 and nucleolin lead to the entrance of Dox into the nucleus and prevention of drug efflux.

Recently incorporation of aptamer with magnetit nanoparticles has widely applied for targeted delivery systems due to super and para magnetism features which enables the drug delivery, magnetic resonance imaging (MRI), and hyperthermia therapy for cancer [50]. Sun et al. designed a targeted delivery chemo-photodynamic platform using conjugation of aptamer strand to the superparamagnetic iron oxide nanoparticles (SPION) [19]. The sequences of aptamer contained the AS1411 sequence with G-quadruplex folding and stem loop folding. The nanocarrier sequences loaded 5, 10, 15, 20-tetra (phenyl-4-N-methyl-4-pyridyl) porphyrin (TMPyP) as a photosensitizer molecule and anticancer drug of daunomycin (DNM), respectively. The nanocarrier (TMPyP&DNM&Apt-S8@SPION) can show high cytotoxicity to nucleolin-overexpressed C26 and A549 cells with producing cytotoxic reactive oxygen species (ROS).

Metal-organic frameworks (MOFs) with advantages of high porosity, crystalline-like structure structures, and synthetic adjustability can be used in the different field of biosensor [51], separation

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[52], energy saving [53], catalytic effect [54], thermal stability, and loading protection against enzymatic degradation [55]. Alijani et al. designed core-shell Fe_3O_4@MOF nanocarrier (Figure 1C) using zirconium 2-amino-1,4-benzenedicarboxylate (UiO-66-NH_2) MOF loaded DOX, CDs and then conjugated to AS1411 [20]. The proposed nanocarrier successfully applied for selective delivery to triple-negative MDA-MB-231 human breast cancer cells resulted in pH-depend release, inhibition of cell proliferation and induction of apoptosis.

Due to some problems for *in vivo* imaging of photodynamic therapy, and poor penetration of UV or visible wavelength into deeper tissues for excitation of photosensitizer (PS), in the recent years NIR-excitable up conversion nanoparticles (UCNP) have gained considerable attention of science due to excitation with NIR and emission of UV-vis wavelength. Also, they have some superiors such as auto-fluorescent background free, deep penetration accessibility for excitation, and photochemical stability [56]. In this regard, Lin et al., introduced a NaYF4:Yb (20%), Er (2%) nanocluster (UCNP) for photodynamic therapy [21]. For this purpose, they used AS1411 for targeting the MCF-7 and Hella cells. In this design a photosensitizer protoporphyrin IX was loaded on the aptamer which can be excited by UV-Vis which emitted from UCNP to produce the ROS.

Recently in order to application of aptamer for evaluation of molecular and metabolic changes in living cells, radiolabeled approach was proposed using radionuclide agent bound to the strand. Radionuclide agents can be gamma-emitting probe including indium-111 (^{111}In), iodine-123 (^{123}I), and ^{99m}Tc which can be used in single-photon emission computed tomography tomography (SPECT) system. Also, other agents are gallium-68 (^{68} Ga) or fluorine-18 (^{18} F) which can be used in positron emission tomography (PET). In this way Kim et al. proposed ^{18}F-fluoride- HER2 aptamer (Figure 1D) for *in vivo* molecular imaging [32].

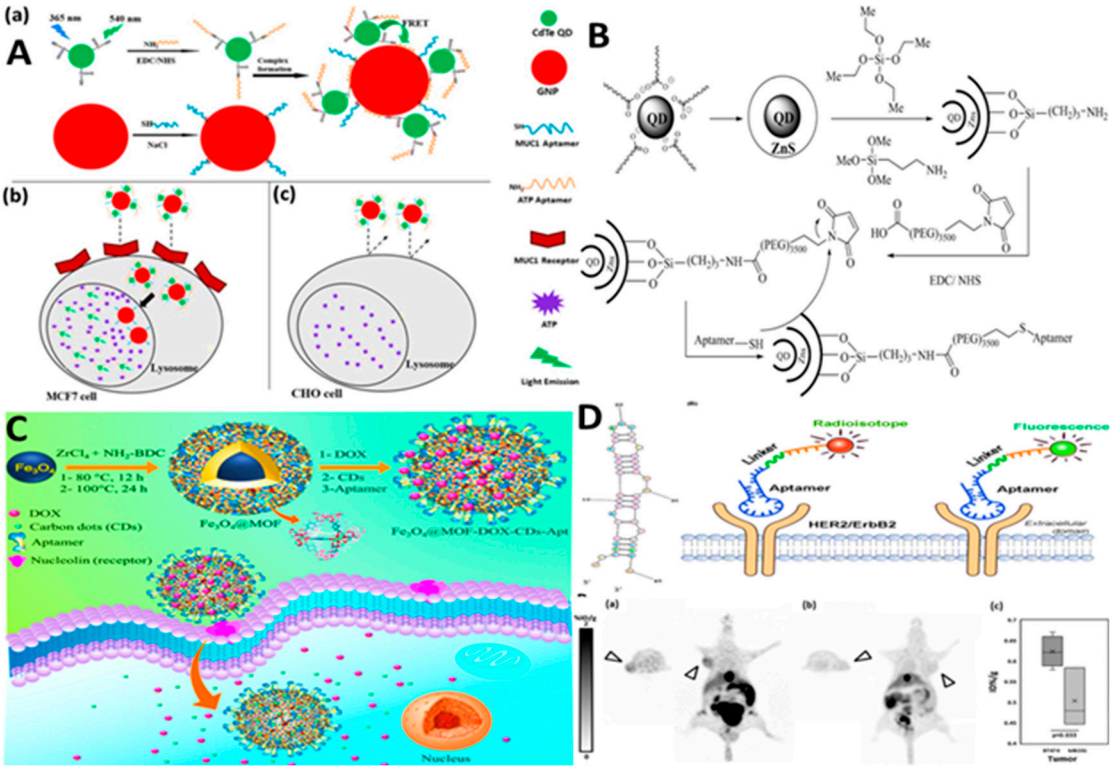

Figure 1. (A) Application of AuNPs and QDs in MCF-7 cells imaging: **(a)** preparation of MUC1 apt – AuNPs/ ATP apt-QDs complex; **(b)** Selective internalization of complex into MCF7 cell by interaction between MUC1 aptamer and MUC1 biomarker overexpressed on the MCF7 cell surface. **(c)** Failure to entrance of complex into CHO cell due to absence of MUC1 biomarker. (AuNP: GNP). "Reprinted from with [12] permission from Elsevier." **(B)** Synthesis of Gd-Zn-Cu-In-S/ZnS QDs/PEG/EpCAM DNA and conjugation of thiolated-apatamer with maleimide group. "Reprinted from [14] with permission from Elsevier". **(C)** Synthesis of Fe_3O_4@ UiO-66-NH_2 MOF-DOX-CDs-Aps aptamer nanocarrier and selective entrance to MDA-MB-231 human breast cancer cells through

interaction of AS1411 apatmer with nucleolin biomarker. "Reprinted from [20] with permission from Elsevier". **(D)** Application of ^{18}F-fluoride- HER2 aptamer: Up section**:** mechanism of entrance to tumor cells; Down section: *in vivo* application for radiography: (a) HER2-positive BT474 tumor; (b) HER2-negative MB-MDA231 tumor; (c) %ID/g of tumor estimated for 18F-labeled HER2 aptamer. "Adapted from [32] in accordance with the Creative Commons Attribution 4.0 International (CC BY 4.0) Creative Commons Attribution License".

2.2. Critical Note

The most important disadvantages of the nanoparticles-based targeted delivery systems are lack of nanoparticles clearance studies in the *in vivo* systems. So, it is proposed to add more details about biodistribution and clearance pathways in the future. Although most studies have used core-shell nanoparticles for targeted delivery, but administration of some nanoparticles such as QDs for *in vivo* studies may have some toxic effect in the bio-environmental due to some inevitable heterogeneous distribution and leakages phenomena. So, it is better in the future the long-term toxicity is done for designed platforms which use nanoparticles specially QDs in the formulation. Moreover, another problem can be related to the carbon-based nanoparticle which benefited from a wide range of advantages but poor solubility and low dispersion in liquid media can limit the delivery systems. Also, another problem of some of nanoparticles as AuNPs is the agglomeration in the culture media which make critical the condition of targeted delivery system. From the other side, quenching and low intensity of fluorescent nanoparticles due to FRET or inner filter effect in the culture and biologic media can limit the activation of scientists [57]. Another limitation of use of fluorescent nanoparticles like QDs for *in vivo* imaging is poor penetration of UV-Vis into deep tissues. Moreover, the wide range of UV-Vis may have the toxicity effect on the normal cells and tissues. So, near-infrared (NIR) wavelength with the range of 700 nm to 1000 nm light which possess low phototoxicity can be used [58–60]. For this purpose, UCNPs can be applied as a valuable luminescent alternative due to unique advantages with high contrast for *in vivo* imaging. But some disadvantages may limit usage of UCNPs for clinical or preclinical procedures. One of the challenges is suitable excitation power to reach the enough conversion luminescence and brightness, due to tissue damage. This may be resolved by modification of cross section of absorption and increase of quantum yield. Moreover, the use of excitation wavelength more than 980 nm can resolve the probability of tissue damage [61]. Another challenge can be providing a microscope which is equipped with an external 980-nm laser which can increase the price and accessibility.

Another alternative for imaging can be radio-labeled aptamer, which can use lower energy for excitation of radioactive material. Although this technology can be used for killing, slowing the cancer cells growth and imaging but use of this material has some inevitable problems for human healthy. Some of this side effects are feeling exhausted, memory problems and distracting, hair loss, skin defects, and vomiting [62].

Although the proposed platforms for targeted-delivery show high sensitivity toward considered cell, but it may be better some other studies are performed in the future such as scalability for entrance to cells, and comparison with other clinical methods for assessment of applicability and evaluation of entrance of nanocomplex to the tissues and release of therapeutic or imaging agent. The most important problem of the studies [14] with pH-sensitive release claim, is low release (e.g. 15%) after 24 hours at acidic condition which can be critical for drug delivery systems. Also, *in vivo* stability is another problem of studies [15] which must be more conducted in the future. Although the covalent binding of aptamer to nanoparticles possesses advantages of strongness, and stability in different conditions of pH and temperature, but the problem of probable denaturation during chemical reaction for covalently binding, may limit its applicability. This phenomena lead to the poor functionality of aptamer and less efficiency of the targeted delivery system [63]. In this way incorporation of aptamer with liposomes can be performed in the both form of noncovalently and covalently. Former can be created electrostatic interaction with cationic liposomes, and later can be trough chemical conjugation, which both of them lead to placing the aptamer in core or on the surface

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of liposomes. Moreover, stability of the encapsulation with liposome in various physiological conditions must be evaluated which the study of Li et al. [17] lacks it.

Although the application of magnetite nanoparticles for targeted-delivery possess extraordinary features, but some disadvantages such as difficulty to use outer magnetic field for delivery of magnetite nanocarriers to organs, limitations of frequency, exposure time, and intensity of magnetic field for patients [64].

Application of MOF possess some deficiencies which may limit its applications includes weak stability of some of them which may lead to prompt release and leakage of drugs or high stability in aqueous media which can reduce the precent of drug release and efficiency of method. Moreover, due to lack of metal-carboxylate sites which can be necessary for modifications, some pretreatments must be done for MOF which may lead to structural failures and material amorphization [65].

2.3. Aptamer-Drug Conjugation (ApDC)

Aptamers with advantages of high stability, reversible conformation, powerful targeting and easy conjugation and modification, and programable conformation can be used as a carrier for ApDC technique. This technique possesses three main members includes aptamer as a ligand for targeting and delivery to sites or biomarker, warhead which is therapeutic agent (drug), and linker with function of loading the therapeutic agent [66,67]. According to the covalently or non-covalently of linker, two modes of physical conjugation or intercalation and chemical linker can be described.

2.3.1. Physical Conjugation (Intercalation)

The DNA nanostructures are known as the biodegradable carriers with high biocompatibility which donot need any elements for internalization into the cells and possess more bio-friendly features in comparison with inorganic and polymeric nanomaterials. Due to folding ability of aptamer the anticancer drugs, mainly anthraquinone family specially doxorubicin (DOX), are able to intercalate into between bases of DNA double helix. The intercalation can be through opening the deoxyribose-phosphate and laminated interaction with plane aromatic base. The intercalation process lead to unzipping two strands of dsDNA and after internalizing the intercalated agent can be released. This process can be reversible, if the DNA structure is not destroyed. This method is easy to performance, without any chemical synthesis process, and inexpensive [68,69].

In this way, icosahedral DNA complexes was formed and used as nanoparticles for carrying doxorubicin toward MCF7 cells [22]. The 3D DNA nanostructures was formed through assembling five DNA strands to form five-point-star motif. The internalization to cells was happened through recognize of MUC1 on the surface of cells. DOX was able to release from DNA nanostructures in the acidic medium of lysosomes resulting in prevention of side effect to considerable extent and increasing effectiveness.

Taking advantages of DNA complexes Liu et al. designed a similar nanostructure includes a DNA aptamer with G-quadruplex structure for interaction with MUC1 biomarker on the MCF 7 cell surface and double-stranded DNA, for loading DOX [23].

In another study by Lopes et al., a delivery system was designed based on the conjugation of the pancreatic cancer RNA aptamer P19 to therapeutic agents such as gemcitabine, 5-fluorouracil (5-FU), monomethyl auristatin E (MMAE) and derivative of maytansine 1 (DM1) [24]. The results demonstrated that delivery systems were able to inhibit the proliferation of PANC-1 and AsPC-1 cell lines with mechanism of phosphorylation of histone H2AX protein on Ser139 (γ-H2AX). Moreover, mitotic G2/M phase arrest was mechanism of action of delivery systems of MMAE and DM1.

Li et al. introduced an innovative design based on hybridization chain reaction (HCR) with scaffold formation for delivery of DOX to SMMC-7721 cell [26]. In this design a short DNA trigged the self-assembly process of biotinylated hairpin DNAs (Figure 2A). After formation of scaffold hybridization, streptavidin-aptamers (Zy1) were able to conjugate to hybridization trough streptavidin-biotin binding. The DOX was loaded on the scaffold hybridization body and aptamers Zy1 as legs was used for targeting.

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Benefiting from one-step self-assembly method, a cross hybridization formation of DNA (C-DNA) nanohydrogels was designed using eight ssDNA [27]. As shown in Figure 2B this nanostructure formed by S1−S4 DNA sequences and linker L1−L4 DNA sequences which mainly includes unmethylated cytosine-phosphate-guanine oligonucleotides (CpG ODNs), I-motif cytosine (C)-rich single-stranded DNA with quadruplex formation ability in acidic conditions, and MUC1 aptamer. After internalization in cells, in the acidic condition, I-motif desire to form quadruplex leading to dissembling C-DNA and release of DOX.

2.3.2. Covalent Conjugation

Covalent and chemical bonding of drugs to the aptamers have been widely used for designing the drug delivery systems due to advantages of simple modifications of aptamer and easy cleavage of drugs. The linkers can be non-cleavable or cleavable. The cleavage of linkers can be depended to the enzymatic reaction, pH, and temperature. But non-cleavable are applied when therapeutic agent is usually a sequence such as small interfering RNAs (siRNAs) or nucleotide analogs and nucleobases which is conjugated to targeting aptamer through a sequence [31].

There are three kinds of chemical linkers which usually applied for conjugation of drugs such as hydrazone, thiol-to-thiol and dipeptide bonds. Hydrazone is formed by reaction of hydrazine on aldehydes and ketones with hydroxyl group. This linker can be dissociated by hydrolysis in acidic condition of endosomes and lysosome [70]. The thiol-to-thiol linker can be used for bonding thiolated drugs with thiolated aptamers. Sometimes in order to increase of stability, the thiolated maleimide or PEG can be used between drug and aptamer. The disulfides can be decomposed in acidic conditions of endosomes. The dipeptide bonds which are created between amino acids, such as valine-citruline (Val-Cit) and phenylalanine-lysine (Phe-Lys), can specifically decomposed by Cathepsin B as a lysosome protease which is overexpressed in cancer cells.

Nucleotide analogs and nucleobases with different functionalities of antiviral and immunosuppressive, anticancer, etc. [71,72] can be inserted as a sequence in aptamer strands. In this design both functionality of targeting of aptamer and therapeutic effect of nucleotide analogs strand can be retained to a high extent with more safety in comparison with chemical linkers.

Huang et al. designed a targeted drug delivery using sgc8ca apt-DOX conjugate to tumor cells [25]. In this study the DOX was conjugated to the aptamer through hydrazone binding while the applicability and naturality of the aptamer was maintained (Figure 2C). The results of several assays such as selective internalization, and toxicity confirmed successful targeted drug delivery to tumor cells and specific killing the Human T-cell ALL (CCRF-CEM) and human B-cell Burkitt's lymphoma (Ramos) cell lines. The evaluation of fluorescence intensity, obviously proved the successful uptake of conjugation to cells. After recognition of proteins on the cells, the drug can be released from the acid-labile linkages inside the endosomes to transport to nuclei.

Benefiting from linker-based therapeutic agent binding, Wang et al. introduced a therapeutic conformation to low-density lipoprotein receptor (LDL-R) as a biomarker target on the surface of cancer cells such as Huh-7 liver cancer cells and MDA-MB-231 breast cancer cells [28]. In this design antimiR-21 DNAzyme was linked to the aptamer through a bridge containing 5 sequential deoxythymidine (d-TTTTT-) to retain the applicability of both aptamer and DNAzyme. The results showed that expression of miR-21 decreased up to 56%.

In this way, paclitaxel (PTX) with hydroxyl group at 2′ position of PTX was covalently bound to the nucleolin aptamer (NucA) through dipeptide (S-S) bond [29]. After entrance of NucA- PTX to tumor cells, the linker was cleaved through cathepsin as intracellular lysosomal protease leading to release of PTX.

Also Theil et al. introduced a delivery system based on conjugation of HER2 aptamer to siRNAs targeting the anti-apoptotic gene, Bcl-2. This system was able to potentially internalize the HER2 positive cells (N202.1A cells) to silence and prevent Bcl-2 gene expression [31].

Benefiting from covalently binding, HER2-specific aptamer was conjugated to the mertansine (DM1), through disulfide bonding between DM1 and 3′ end of aptamer which can be cleaved in early and late endosomes [30]. This cleavage can increase anticancer activity of drug through

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facilitation of DM1 release from the endocytic pathways. Moreover, in this design PEG molecule was bound to the 5′ end of aptamer to increase of biocompatibility and circulation ability *in vivo*. In this study aptamer-DM1 conjugation was used for *in vivo* studies mouse xenografts with BT-474 breast cancer.

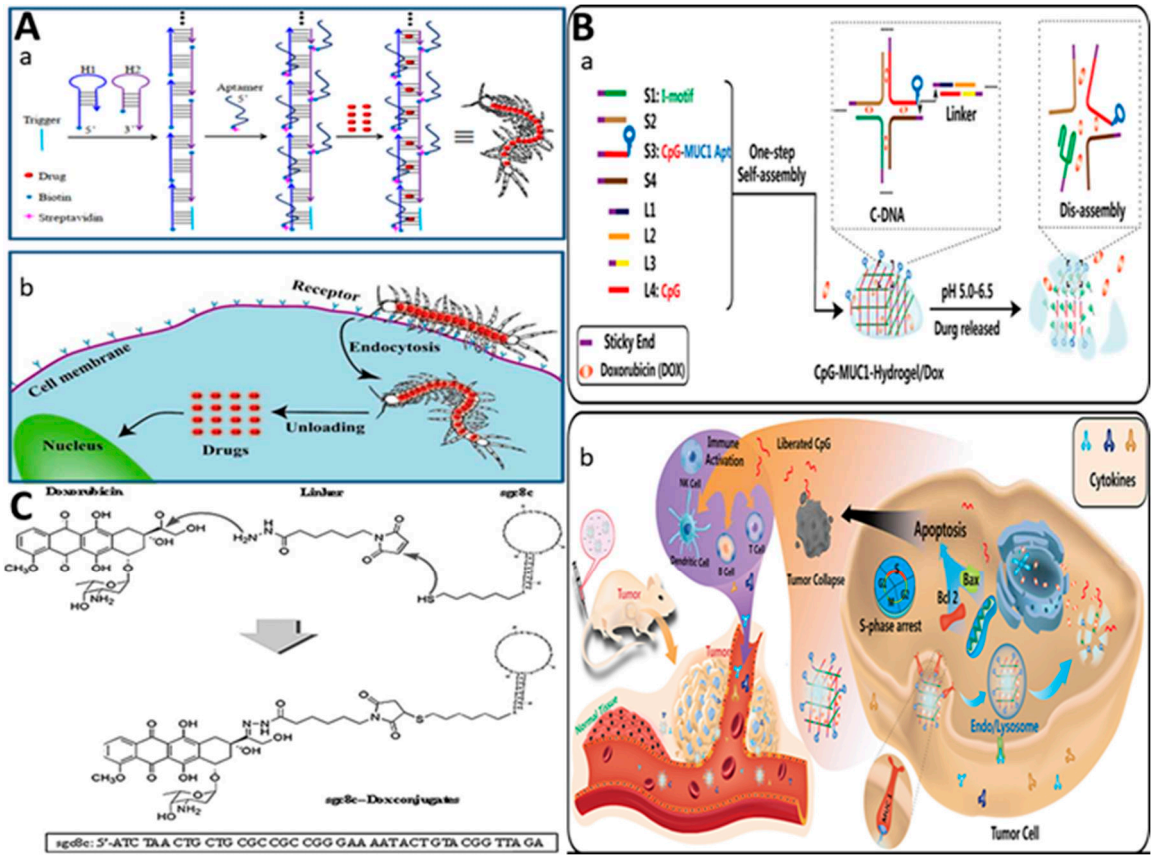

Figure 2. (A) Application of HCR in drug delivery: **(a)** Formation of nanocentipede and loading of DOX; **(b)** Eternalization of nanocentipede-DOX system into SMMC-7721 cell. " Reprinted from [26] with permission from ACS."; **(B)** Application of cross hybridization formation of DNA (C-DNA) **(a)** Formation of CpG-MUC1-hydrogel for **(b)** targeted drug delivery to cells with MUC1 biomarkers. "Reprinted from [27] with permission from ACS."; **(C)** Covalently binding of sgc8ca to DOX through hydrazone. " Reprinted from [25] with permission from John Wiley and Sons." .

2.3.3. Critical Note

The studies demonstrated that incorporation of aptamer and drugs have been formed as well for targeting delivery to the cells. This promising technique is potentially more applicable in the human treatment domain provided that a lot of completed procedures and assays are done. Although these studies have been so valuable but may be limited with some disadvantages. In most studies it is not well detailed in description of characterization and synthesis of aptamer-drug conjugation. Although studies reported successful conjugation for targeted drug delivery but some disadvantages such as the *in vivo* applicability and cancer treatment effects including immune system reactions could be tested. Most of papers deals with *in vitro* evaluation to validate the applicability of delivery systems. Also, it was better to discuss more on the off-target, toxicity and side effects of the designed ApDC on the normal cells or tissues [11]. Moreover, covalently bonding required the chemical process and reagents which may reduce the nature of aptamer and increase the toxicity of delivery system. Besides, other challenges can be the low amount of drug loading, expensive reagents requirements, and several boring steps requirement for covalently conjugation. On the other hand, physical conjugation of drugs may face to the loading challenge, especially in the *in vivo* environment, which contains a lot of macromolecules and interreferences, while lead to release of drug, low loading and

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low efficiency of drug delivery. Moreover, probable changes of formation and folding during processes, which contain different media and conditions, can be inevitable leading to the low selectivity and failed efficiency. In order to evaluation of therapeutic efficacy of results, the designed delivery system can be replicated in other animal models or even human clinical assays.

3. Biosensors

The timely diagnosis of cancers is an inseparable part of fast therapeutic aims, has a highlighted role in the increase of community health. In order to diagnosis of cancers and biomarkers a lot of traditional methods such as enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (RT-PCR), flow cytometry, mass spectrometry [73] have been applied. Unfortunately, aforementioned methods suffer from some disadvantages such as expensiveness, highly expert persons requirement, and time-consuming. So, development of simple, sensitive, easy to operate techniques is urgent. Nowadays biosensors can be a potent alternative for traditional methods. Biosensors are known as a potent diagnosis technique for detection of a wide range of molecules including proteins, toxins, cells, bacteria, DNAs, miRNAs, etc, which are important in different fields of medical, food and the environment. Biosensors are able to record and transmutation the biological response to readable signal through transduction system during processes of collection, amplification of signal. In term of manner of traducing, different biosensing systems can be introduced such as optical, electrochemical, piezoelectric thermometrical and magnetical [63]. Biosensor should be designed so that they can diagnosis the biomolecules with high selectivity among the interferences of biological system. Recently several biosensors have been designed for diagnosis of cancers through detection of biomarkers in medical sciences. In this way various sensors have been fabricated using bioreceptors such as antibody, enzyme, aptamer, etc. Among them aptamer have received high attention due to some advantages such as small size (12-30 KDa), wide range of targets, simple synthesis, high stability, easy modification, long term storage, inexpensive, low immunogenicity [74]. In the recent years point-of-care (POC) technique has been pervasive for the clinical aims. The usual medical assays which are centralized in clinical or hospital labs, need large amount of sample and long time for interpretation of results. POC devices enable person-centered assays in the short time which required small amount of sample, and enable cheaper test in emergency condition [75]. Due to unique features of aptasensors, they can be accomplished for development of POC devices. Concerning the kind of the platforms, aptasensors which mainly designed for cancer cell lines, can be classified into optical, electrochemical, paper based, microfluidic and smartphones. The summarized data of studies are shown in the Table 2.

Table 2. Summarized data of aptasensors for detection of biomarkers.

Method	Sequence	Biomarker	Linear range	LOD	Ref.
Exosomes on the molecularly imprinted polymer (MIP)-coated Fe_3O_4 release the aptamer-FAM from GO	CD63: FAM-CACCCCACCTCGCTCCCGTGACACTAATGCTA MUC1: FAM-CAGCCTGCACTCTAACGCAGTTGATCCTTTGGATAGCCTGGGTTAGA	CD63, MUC1	1.19×10^{-6} - 4.76×10^{-5} mol/L	2.27×10^{-6} mol/L	[76]
tetrahedral DNA (L1–L4) hybridized with MAB	L1: ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGA GCCGCCATAGTA L2: TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTA	telomerase	50 - 2000 HeLa cells	35 HeLa cells	[77]

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	TGGCGGCTCTTCTTTTTAATCCGTCGAGCAGAGTT L3: TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCG AGGGTCCAATACTTTTT/iBHQ2dT/TGCACCCTAACCCTAACCCT L4: TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTG GACCCTCGCAT MB: Cy3-AACGATTAGGGTTAGGGTCGTT-Cy5				
Prevention of AuNPs aggregation by aptamer	CCGATCTCTCCCACTCTCTCCAACTCACAGGCTACGGCACGTAGAGCATCACCATGATCCTGTGGGTGTGTTGTTGATGGATCGGATCATCATGGTGAT	platelet-derived growth factor (PDGF)	0.01–10 μg/ml	0.01 μg/ml	[78]
Gold chip modified with CA125 aptamer through streptavidin–biotin	CTC ACT ATA GGG AGA CAA GAA TAA ACG CTC AA-biotin	Mucin 16 (MUC16) or cancer antigen 125 (CA125)	10–100 U/mL	0.01 U/mL	[79]
Magnetic glass carbon electrode (MGCE)/α-Fe_2O_3/Fe_3O_4/Au/complementary strand/aptamer	Complementary strand: SH-TTTTTTTTTTTTTTTTTTTTCCCTATAGTGAG Aptamer: CTCACTATAGGGAGACAAGAATAAACGCTCAA	cancer antigen 125 (CA125)	5–125 U/mL	2.99 U/mL	[80]
CRISPR/Cas12a + RPA + electrochemistry (Modified electrode with complementary 1 (CP1) and MB /Fe_3O_4@COF/PdAu modified with complementary 2 (CP2))	crRNA template: AAGTACCCAGCAGTTTGGCCCGCCATCTACACTTAGTAGAAATTCCtatagtgagtcgtattag CP1: SH-ACACTTGAAGTGTATTTCCTAAATA CP2: AATTGCAAGTATGTAGAAGTTCACA-SH Synthetic target: ATGTCAAGATCACAGATTTTGGGCTGGCCAAACTGCTGGGTGCG	ctDNA EGFR L858R	10 aM – 100 pM	3.3 aM	[81]
Microfluidic system incorporated with screen-printed gold electrode was	GCCTGTTGTGAGCCTCCTAACCGTGCGTATTCGTACTGGAACTGATATCGATGTCCCCATGCTTATTCTTGTCTCCC–SH	α6β4 integrin on A549 cells	50–5×10^5 cells/mL	14 cells/mL	[82]

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modified with integrin α6β4-specific aptamer (IDA)					
Lateral flow assay (LFA) with streptavidin (SA)-biotin-CD63 aptamer on T-line	CD63 aptamer: 5′-GTGGGGTGGACGAGGGCACGTGATTACGTA-3′ complement aptamer: CACCCCACCTCGCTCCCGTGACACTAATGCTA-Biotin	CD63 on the non-small cell lung cancer (NSCLC)	-	6.4×10^9 particles/mL	[83]
Smartphone control of emission from gold nanoclusters (GNCs)-aptamer as emitter and polyurethane (PU) - coated with GO as quencher	SH-CCCCCCGATCCTTTGGATA	Mucin 1 (MUC1)	250–20,000 cells /mL	221 cells /mL	[84]

Optical platforms contain three main members such as the light source, the sensor of target – light, and a detector which collect the light from sensor and evaluate it. Optical biosensors with advantages of high sensitivity, simplicity and easy miniaturization have been highly desired to incorporation with aptamer.

In this way florescence resonance energy fluorescence resonance energy transfer (FRET)-based aptasenors frequently have designed for detection of cancer cells biomarkers. FRET as a nonradiative phenomenon usually happens through transmission of energy of fluorophore as donor to an acceptor which may able to emit the energy to longer wavelength [85]. Benefiting from this phenomenon several biosensors have designed FRET biosensor based on energy transfer between fluorophore-conjugated aptamer and GO as a quencher. As an example, in this filed Feng et al. designed a FRET system based on the selective adsorption of exosome on the molecularly imprinted polymer (MIP)-coated Fe_3O_4 nanoparticles followed by competition with aptamer in the GO/aptamer-FAM system (Figure 3A) [76]. This competition led to release of aptamer-FAM and recovery of fluorescence intensity.

Taking advantage of aptamer-incorporated FRET, molecular aptamer beacon (MAB), which possesses both fluorophore and quencher moieties at two ends of strand have been introduced with aim of imaging and quantification. The design of molecular is based on switching the folding of stem-loop structure to recognize the target by on/off yielding [86]. Yue et al. designed an interesting targeted delivery system combining tetrahedral DNA nanoprobe (TDNp) and MAB which contains fluorophores Cy3 as donor and Cy5 as acceptor [77]. As shown in Figure 3B in this system TDNp prepared from L1–L4 which includes primer (TP) at strand L2 end and hybridization section for MAB at L3. The delivery was able to internalize to cell trough pathway of caveolin-medicated endocytosis. In absence of telomerase, after hybridization and folding change, the Cy3 and Cy5 can stay at far distance leading to the FRET inhibition. But presence of telomerase lead to duplication of TP at 3◎end of L2 which extended to L3 with consecutive TTAGGG sequences. The hybridization with L3 resulted in the release of MAB and FRET phenomena.

Nowadays, colorimetric biosensors with advantages of easy to use, simple interpretation, inexpensive, visibility, etc, have been frequently incorporated with biomarker detection. In this way

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AuNPs-based colorimetry have been widely applied for designing this kind of sensors through color change from red to purple which happens through aggregation of AuNPs and variation of wavelength at 520 nm. AuNPs with dispersion form possess inter-nanoparticles distance more than the particle diameter which can seem red, but in aggregation form this distance becomes lower leading to seem purple [87]. The most interesting design of AuNPs-aptamer colorimetric biosensor is aggregation-resulted by adding the salt. In this design the aptamer remained among AuNPs preventing aggregation in presence of NaCl salt. By adding the target, it can be bind to the aptamer leading to the conformation changes and poor defense from AuNPs against aggregation. Hasan et al designed this kind of biosensor for detection of platelet-derived growth factor (PDGF) (Figure 3C) [78].

Surface plasmon resonance (SPR) can be happened through nanoplasmonic phenomena using noble metal nanoparticles which are able to focus the light into nanoscale regions. This incidence of light lead to the increase of collective oscillation which is produced by surface free electrons. This phenomenon results in the scattering or absorption of light leading to the plasmon resonance frequency change. This technique includes the advantages of label-free possibility, inexpensive, easy sample preparation [88]. Taking advantages of SPR, Shahbazlou et al. introduced a SPR platform (Figure 3D) designed by immunization of CA125 aptamer, through streptavidin–biotin, on a bare gold chip [79]. This design was used for detection of CA125 in human serum samples.

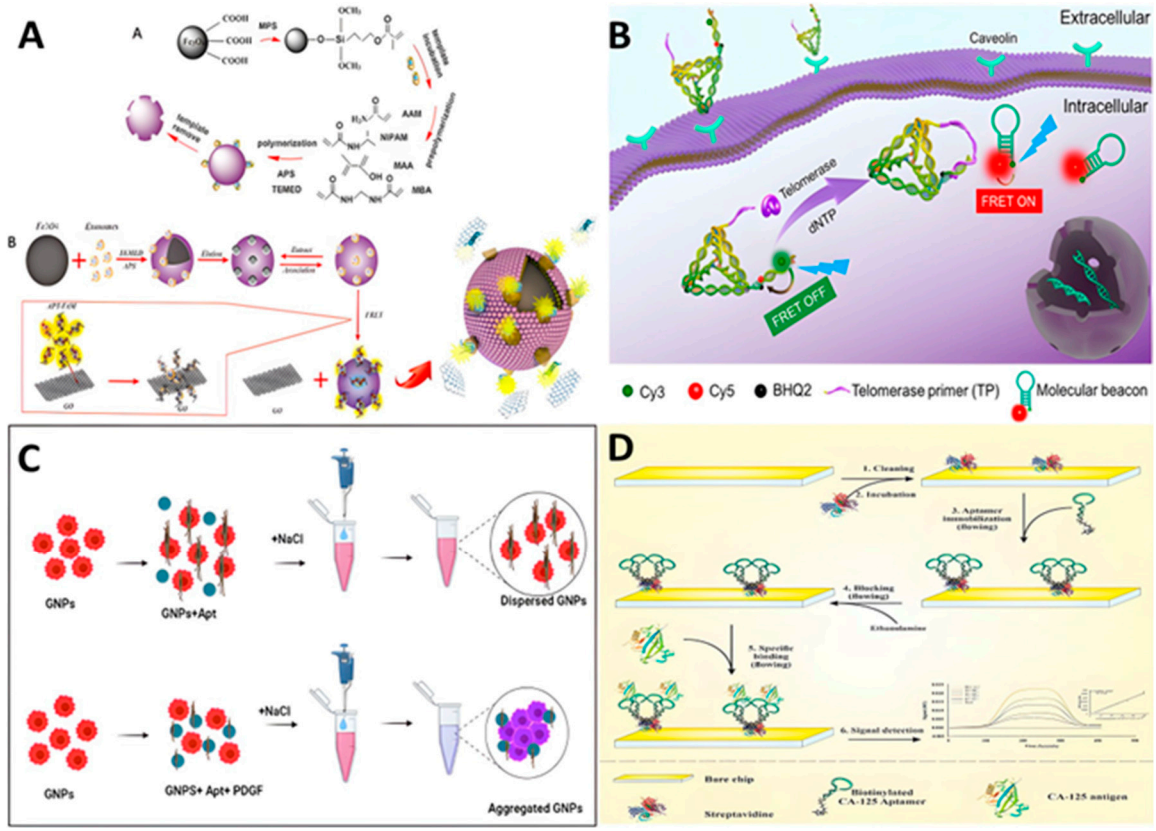

Figure 3. Optical aptasensors: **(A)** and **(B)** FRET-based; **(C)** colorimetric; **(D)** SPR. "Reprinted from [76–79] with permission from Elsevier".

Electrochemical biosensors which synergically contains sensitivity of electrochemical transducer and specificity of bioreceptors, can be classified to biocatalytic and affinity sensors according to the kind of biorecognition element. The biocatalytic sensors usually use enzymes, tissue slices, etc, and affinity sensors use antibody, aptamer, DNA [63]. Among different kind of electrochemical sensors, aptasensor have frequently applied due to simple design, sensitivity and low price. According to the labeling with electrocatalytic agent, aptasensors classified to the label-free and labeled-based. Ni el al. designed a label-free aptasensor for cancer antigen 125 (CA125) by modification of electrode using

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magnetic α-Fe_2O_3/Fe_3O_4/Au nanocomposite followed by immobilization of the partial complementary strand on the electrode. As shown in Figure 4A, in presence of CA125, aptamer is released from complementary strand, and current increased [80]. Moreover, recently a new generation of electrochemical sensors was developed based on the CRISPR-Cas systems. Cas12a nuclease is known as a powerful tool for detection of nucleic acids strands and known as an RNA-guided DNases which possesses advantages of considerable sensitivity and specificity, and efficient cutting ability. Cas12a which directed by CRISPR RNA (crRNA), contains T-rich protospacer adjacent motif (PAM), create a Cas12a/crRNA complex to specifically detect DNA with *trans*-cleavage and *cis*-cleavage activities to indiscriminate ssDNA or detect DNA. Taking advantages of this technique, Liu et al. designed an electrochemical biosensor for detection of EGFR L858R as a circulating tumor DNA (ctDNA) in lung cancer [81]. In this design MB /Fe_3O_4@COF/PdAu bound to a complementary strand and another complementary strand was immobilized on the electrode. As shown in Figure 4B, the resulted ssDNA from Cas12a *trans*-cleavage activity, can be hybridized by complementary strands leading to the increase of MB signal.

In order to improvement of the sensitivity of biosensors, incorporation with microfluidic systems always have been a good idea for scientists due to enhancement of mass transfer to the surface of biosensor. Microfluidic technique is able to donate valuable features to the biosensors such as low sample requirement, high-throughput and fast detection, miniaturization and portability [89]. Taking advantages of microfluidic assay, Khaksari et al. introduced a platform for electrochemical detection of A549 cells. In this design screen-printed gold electrode was modified by integrin α6β4-specific DNA aptamer (IDA) and inserted in on-chip gas-actuated microvalves microfluidic platform to provide the flows (Figure 4C) [82].

Lateral flow assay (LFA) as a paper-based technique, which possess valuable advantages including low cost, simple design, visual results interpretation, can be used as POC device for diagnosis of cancer[90]. It can be used for detection of a wide range of targets such as bacteria, toxins, hormones, biomarkers, etc. Recently application of aptamers instead of antibodies have been pervasive due to superior advantages of aptamer over antibodies which was discussed in aforementioned sections. The LFA strips contains the sample pad for sample loading, the conjugated pad for loading the labeled-bioreceptors, the test pad which is a nitrocellulose (NC) membrane for capillary migration of sample and separation, the absorbent pad for absorption of buffer and sample, and the support back for retaining the components of LFA strips. Moreover, the NC normally coated with two lines of test line (T-line) and control line (C-line). T-line usually can be dispensed target bioreceptor and C-line also is bioreceptor for labeled-bioreceptor [91]. As shown in Figure 4D, Yu et al. fabricated a lateral flow test strip for diagnosis of identification of CD63 on the non-small cell lung cancer (NSCLC) exosomes [83]. In this design, they dispensed streptavidin (SA)-biotin-CD63 aptamer complementary on the T-line, and AuNPs-CD63 aptamer was used as probe.

Smartphone is known as a new generation of POC devices which possess high integrability with different type of biosensors, simple design and portability. This kind of biosensors enable control of reaction process of biosensor with a simple software on the smartphone [92]. Sanati et al. designed a lab-in-a-tube technique for detection of MUC1 on the circulating tumor cells (CTCs) using gold nanoclusters (GNCs)-aptamer as emitter and polyurethane (PU) - coated with GO as quencher [84]. As shown in Figure 4E they designed the dark chamber equipped with UV-LED emitters, aluminum heat sink for exhaust of heat, and a cylindrical chamber for reaction tube which can be controlled through a smartphone with imgeJ software from top of chamber.

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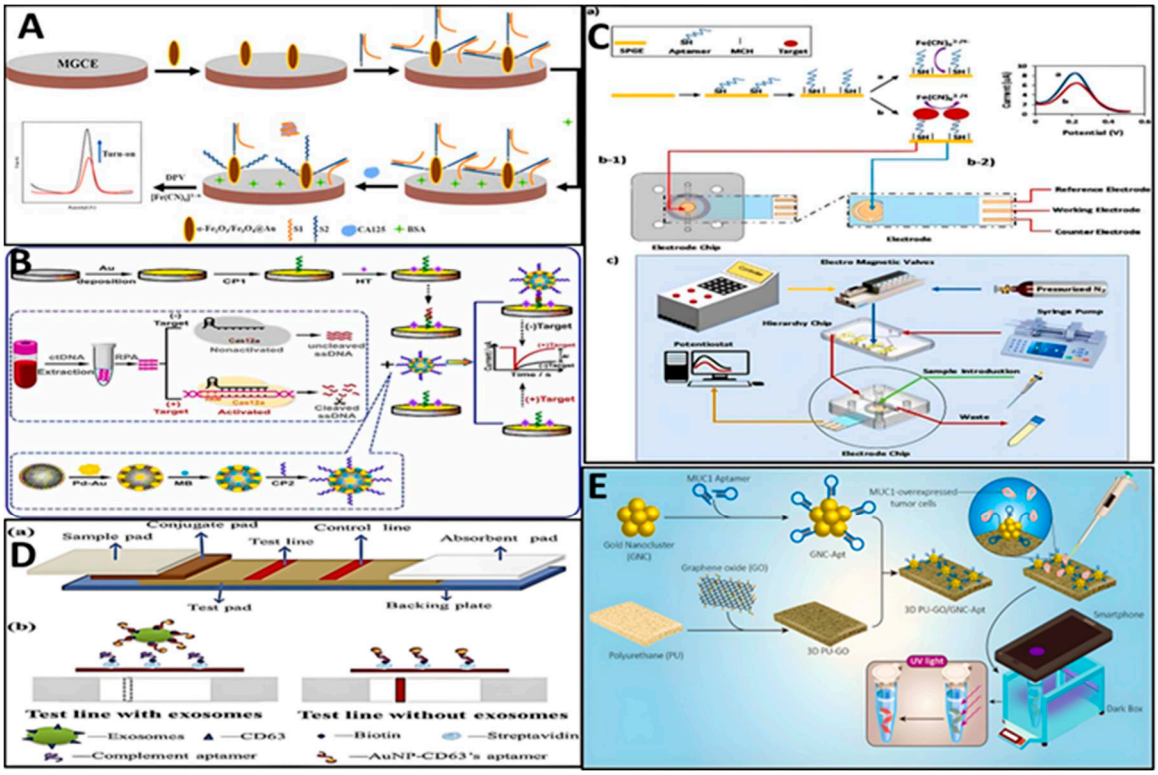

Figure 4. Designing of aptasensor for cancers: **(A)** Label-free electrochemical sensor; **(B)** Cas12a/crRNA based electrochemical sensor ; **(C)** microfluidic; **(D)** LFA; **(E)** smartphone." Reprinted from [80–84] with permission from Elsevier.".

4. Critical Note

Although designed biosensors can be introduced as the most potent technique for early diagnosis of cancer, but some disadvantages and failures such as inaccessibility of some methods for public and non-expert persons may prevent development of these methods as POC devices. For example, microfluidic device which is designed with Khaksari et al., [82] although resulted acceptable sensitivity, but design of this platform requited large and expensive equipment which can be used with highly expert persons. Also, FRET based techniques which are based on the transmission of energy, may suffer from autofluorescence interferences or FRET between fluorophore and macromolecules in biological samples leading to the negative or positive errors. Colorimetric methods based on AuNPs, seriously exposed to aggravation in biological samples. On the other hand, sensitivity of some technique like MAB, which are highly depended to the aptamer folding, may be changed by conformation and 3D folding change in different conditions such as PH, solutions, and temperature. Moreover, LFA and smartphones, although provided some conditions such as simplicity and accessibility for development of POC techniques, but low sensitivity and lack of validity of results may affect on development of them as potent POC technique. Some enzymatic methods such as Cas12a may suffer from instability for long term, which lead to poor accuracy of biosensors. The electrochemical and SPR sensors are most important, reliable, and accurate techniques which have potential of miniaturization for accessibility and label-free ability for use of bioreceptors. The hand-held platforms with easy interpretation of results and low price can be designed using these techniques which possess heighted role in healthcare and treatment.

5. Conclusion and Future Perspective

This review potentially demonstrates that aptamer have been successfully introduced as a therapeutic and diagnostic tool for designing the imaging, drug delivery and biosensors due to unique features of aptamers including the high selectivity, affinity, and compatibility with different methods. The targeted-delivery technology significantly benefited from aptamer advantages for

efficacy cancer therapy and decrease of toxicity. Studies obviously demonstrated that aptamers potentially played role of targeting and nanocarriers for drug delivery systems with drug intercalation, or chemical linkers, and also nucleotide analogs. There are still some challenges in aptamer-based targeted-delivery systems such as fast clearance from body and kidneys excretion, nucleus degradation, and in vivo thermal instability [93]. Moreover drug-aptamer conjugation mostly limited to DOX use which can easily intercalate into the nucleic acid strands. So, developing the delivery systems with different drugs can be the most important challenge which must be evaluated in the future. This review demonstrated that incorporation of aptamer with biosensors platforms was implemented as well to produce the accurate, selective and sensitive diagnosis tools for biomarkers. But the most important shortcoming of these biosensing platforms is being inaccessibility and most of them remain limited to articles and studies. So, it is highly required to development of POC devices for cancers which are accessible for public, like glucometers or pregnancy checkers. Although it is mentioned that aptamer possess superior features in comparison with antibodies for therapeutic aims, but actually application of aptamers is limited which must be more assessment in the future.

This review demonstrated aptamer well-incorporated with *in-vitro* and *in-vivo* assays for diagnosis and therapy of different kinds of cancers in preclinical stages. Although a lot of commercialized aptamer-based drugs have been introduced in the recent years but there has been also a considerable desire for biomarker diagnosis and therapeutic aims using antibodies in clinical assays. The main reason for distrust of implementation of aptamer in clinical assays can be some defects in SELEX stages due to some inaccuracy in process, impurity of targets, and mostly *in vitro* synthesis which lead to the lower selectivity and interaction with other *in vivo* targets. One of the powerful and effective technique which can be applied for resolving the poor tissue permeability problem is *in vivo* SELEX which can be directly performed in a live media [94]. In this technique nucleic acid strands which are nuclease-resistant, injected intravenously to the tumor-bearing mice followed by separation of tissue, isolated of nucleic acid strands, and *in vitro* amplification. This strategy assumed to create an effective targeted delivery system which enable the diagnostic and therapeutic aims in clinics. Unfortunately, this technique may suffer from some disadvantages, which may limit clinical applications, such as differences in specificity, polymerases inability in amplification, costly extraction and unreliability for *in vivo* screening. So, future developments in this area must be focused in the low cost, fast, and improved identification after screening. In the next few years some technologies such as single molecule or cell screening, identification and sequencing can be so promising to resolve the problems. Moreover, artificial nucleotide bases can be developed to increase the biostability of screened aptamers for clinical assays. The detailed information clearly demonstrated that nanoparticles possess the undeniable role for the diagnosis and therapeutic aims. But some conditions such as size, low-distribution and unpredictable behavior of materials in *in vivo* media make critical their production and usage in clinical assays. Nanomaterials mainly assigned to the unbound particles with size distribution of < 100 nm, where at least 50% of particles must meet that condition without agglomeration. So, it is required to performance of safe-by-design assays for clinical development due to changes of physicochemical and magnetic properties at range size of < 10 nm and also biological properties at range size of > 200 nm [95]. Moreover, another anxiety in this field is toxicity of nanoparticles in body which may be remained unresolved in the future. Specially the aptamer-nanoparticles conjugation required chemical agents which increase the possibility of toxicity for body. The reported information by articles and studies contains some characterizations which cannot be used as a citable reference for companies in order to production of diagnostic and therapeutic products. Some efforts in this filed have been performed for evaluation of materials toxicity which more limited to the cosmetics without any nano-toxicology evaluations. So, in future it is expected more efforts are done in *in vivo* studies including computational biology, biostability, size effects, nano- toxicity, coating, biodegradability, biocompatibility, dose of therapeutic agent-incorporated with size, evaluation of penetration and diffusion, etc. The biotechnology companies must focus on the evaluation and reduce of toxicological effects of nanoparticles to produce the medicinal products. Moreover, drug delivery studies should describe

and explain predictive effects more than simple report of results. Recently aptamer-based CRISPR-Cas, as a new generation of biosensors, have gained attention of scientists for diagnosis of biomarkers and toxins. This new design of sensors is a promising field which can be helpfully and powerfully applied in different fields such as bioanalytical sensors, molecular biology, and enzymatic engineering. Although some shortcomings such as complicated processes, optimization of acting conditions of Cas proteins, and highly expert requirement may limit its application and accessibility, but due to high accuracy, this method must be more developed and incorporated with electrochemical methods for designing handheld and smartphone platforms which can be simply used in clinics.

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Abbreviations

AuNPs	Gold nanoparticles
ApDC	Aptamer-drug conjugates
β-CD-PELA	Beta-cyclodextrin-linked poly (ethylene glycol)-b-polylactide block copolymers
ctDNA	Circulating tumor DNA
COFs	Covalent organic frameworks
CNTs	Carbon nanotubes
CDs	Carbon dots
C-DNA	Cross hybridization formation of DNA
CCRF-CEM	Human lymphoblasts T-cell ALL
CTCs	Circulating tumor cells
CpG ODNs	Cytosine-phosphate-guanine oligonucleotides
crRNA	CRISPR RNA
CA125	Cancer antigen 125
DOX	Doxorubicin
DNM	Daunomycin
DM1	Maytansine 1
EGFR L858R	Exon 21 mutations in L858R substitution
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and drug administration
5-FU	5-fluorouracil
FRET	Fluorescence resonance energy transfer, resonance energy transfer
GNCs	Gold nanoclusters
GO	Graphene oxide
HCR	Hybridization chain reaction
IDA	Integrin $\alpha 6\beta 4$-specific DNA aptamer
LDL-R	Low-density lipoprotein receptor
LFA	Lateral flow assay
MMAE	Monomethyl auristatin E
MAB	Molecular aptamer beacon
MOFs	Metal organic frameworks

MB	Methylene Blue
NSCLC	Non-small cell lung cancer
NCHS	National Center for Health Statistics
PAM	Protospacer adjacent motif
PDGF	Platelet-derived growth factor
PTX	Paclitaxel
QDs	Quantum dots
Ramos	Human B-cell Burkitt's lymphoma
RT-PCR	Real time polymerase chain reaction
ROS	Reactive oxygen species
SELEX	Systematic evolution of ligands by exponential enrichment
SPION	Superparamagnetic iron oxide nanoparticles
TMPyP	5, 10, 15, 20-tetra (phenyl-4-N-methyl-4-pyridyl)
TDNp	Tetrahedral DNA nanoprobe
UCNP	Up conversion nanoparticles

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