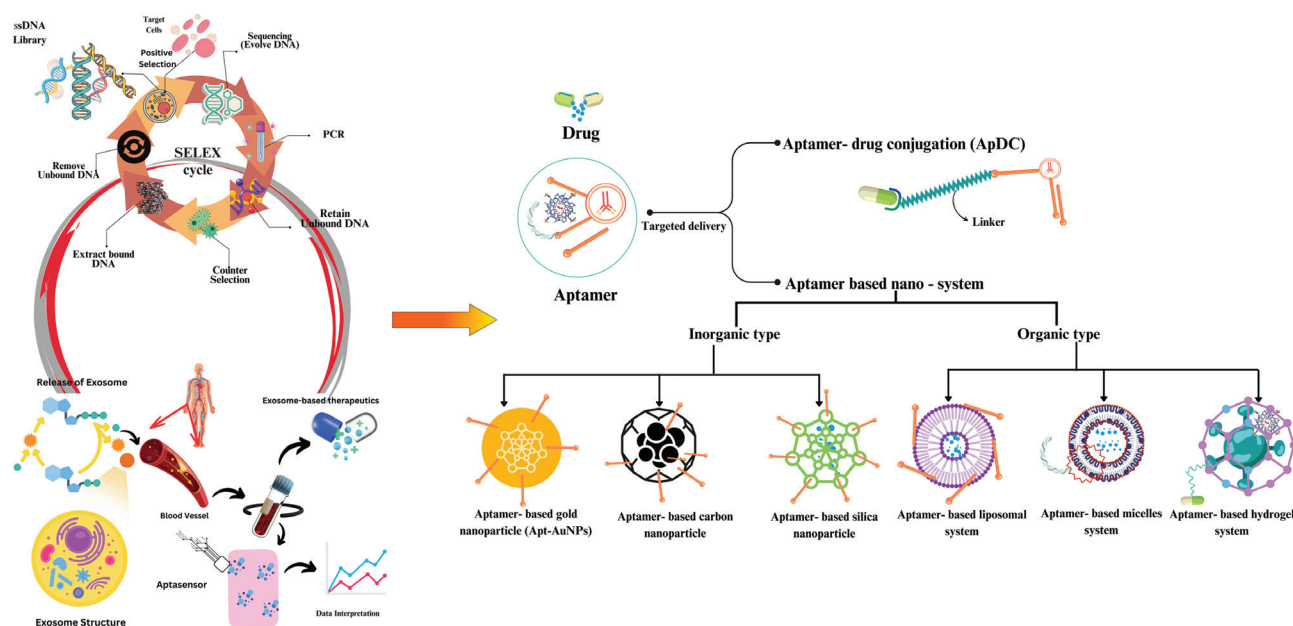


Aptamer-based Theranostics in Oncology: Design Strategies and Limitations

Jyoti Trivedi¹, Mohammad Yasir^{1,*}, Rahul K. Maurya¹ and Alok Shiomurti Tripathi²

Graphical abstract



Oncology related design, strategy, and implementation of aptamer based nano-system in theranostics.

Aptamer-based Theranostics in Oncology: Design Strategies and Limitations

Jyoti Trivedi¹, Mohammad Yasir^{1,*}, Rahul K. Maurya¹ and Alok Shiomurti Tripathi²

Abstract

Cancer remains a major global health burden, necessitating innovative approaches for improved diagnosis and treatment. Aptamer-based theranostics have gained attention in the field of cancer research and treatment. Aptamers can be used as targeting ligands for the delivery of therapeutic agents to cancer cells, as well as for the detection and imaging of cancer, due to high binding affinity and selectivity. Aptamers are also being investigated as anticancer drugs. Specifically, aptamers serve as a tool for controlling protein activity via protein-protein and protein-ligand interactions. The aptamer-exosome technology improves aptamer targeting. To gather relevant data, we searched scientific databases, including PubMed/Medline, Google Scholar, Wiley, Web of Science, and Springer. Despite challenges, theranostics, environmental monitoring, biosensing, and other fields could benefit from the use of aptamer technology. This review discusses the standard methods for producing aptamers, including green aptamers, and potential applications in diagnostics. Aptamers are useful in biotherapy and as anticancer drugs, and this article gives a thorough overview of both with examples. We also covered aptamer-exosome technologies, aptasensors, and their diagnostic and therapeutic applications. We investigated recent systematic evolution of ligands by exponential enrichment (SELEX) methodologies with a focus on carrier materials and technical advances, and discuss the difficulties in creating aptamers that are more practical, highly efficient, and stable.

Keywords

Aptamer, exosome technology, SELEX, theranostics.

¹Amity Institute of Pharmacy, Lucknow, Amity University Uttar Pradesh, Noida, Uttar Pradesh, India

²ERA College of Pharmacy, ERA University, Lucknow, Uttar Pradesh, India

*Correspondence to:
Dr. Mohammad Yasir, Assistant Professor (G-III), Amity Institute of Pharmacy, Amity University Uttar Pradesh Lucknow Campus, Near Malhaur Railway Station, P.O. Chinhat, Lucknow 226028, Uttar Pradesh, India, Mobile: +919179417284. E-mail: yasirmohammad6@gmail.com; myasir@lko.amity.edu

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Introduction

Cancer causes death worldwide with an estimated 10 million new cases of cancer diagnosed every year. Because the prognosis for some types of cancer is still poor, ongoing research into the causes, prevention, and treatment is helping those who are affected [1]. Nanotechnology has emerged as a promising field in the development of novel cancer theranostics by combining diagnostic and therapeutic modalities [2]. Aptamer-based theranostics have gained attention in the field of cancer research and treatment.

Diseases that can penetrate or spread across various body parts because of aberrant cell proliferation are collectively referred to as cancer. There are many types of cancer, the most common forms of which are breast, prostate, bladder, and brain. The possible cancer treatments include surgery, radiation, chemotherapy, immune therapy, and targeted delivery systems. Prevention strategies involve lifestyle changes. Cancer is becoming more widely known and understood despite the difficulties it causes and

various advances in technology. Therapies change the outlook of patients more on the positive side and include a greater quality of life for those who are impacted [3]. Treatments strategies, like chemotherapy and radiotherapy, affect not only cancer cells but also healthy cells, leading to various adverse effects, such as fatigue, nausea, hair loss, and immune suppression [4].

Furthermore, the efficacy of these treatments are limited by cancer cell acquired resistance. However, aptamer-based theranostics have the potential to overcome these limitations. By specifically targeting cancer cells, aptamer-based theranostics minimize off-target effects and reduce toxicity to healthy cells. Moreover, aptamers can be engineered to overcome acquired resistance by targeting alternative pathways or biomarkers that are still present in resistant cancer cells. Furthermore, combining aptamer-based theranostics with other treatment modalities, such as chemotherapy or immunotherapy, can enhance the overall efficacy of cancer therapy [5].

Aptamers are short, target-specified, single-stranded oligonucleotides with

high selectivity and affinity for target molecules. Aptamers are akin to deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). Aptamers are made *in vitro* by applying the systematic evolution of ligands by exponential enrichment (SELEX) method. Aptamers can replace or substitute antibodies in some applications due to excellent affinity and selectivity. Aptamers are easily modifiable with functional groups to create novel sensing and targeted delivery platforms with numerous possible uses, including diagnostics, therapy, and biosensing [6].

Aptamers are used as biosensors to detect small molecules and proteins, for targeted drug delivery, in diagnostic assays to identify cancer markers, infectious diseases, and genetic disorders, and as a tool to change the activity of proteins by determining how proteins interact with other proteins and ligands [7].

This introduction provides a brief overview of aptamers and aptamer applications. Further, we discuss perspectives and related challenges in aptamer technology design for cancer and targeting cells in theranostics [8].

Aptamer design

A unique nucleotide sequence that has a binding to the target site is chosen from a library of randomly produced sequences to create aptamers. Various techniques are used in the selection process and the aptamer synthesized is stable under various environmental conditions [9].

Short single-stranded oligonucleotides, which are known as nucleic acid aptamers, have high selectivity and affinities towards binding target sites (low molar-picomolar range) that are comparable to antibodies [10]. Nucleic acid aptamers combine with a variety of targets, including whole cells [7, 11], proteins [7, 11], small molecules [12], metal ions [13], and viruses and bacteria [14, 15]. Aptamers are preferred compared to antibodies owing to their smaller size, faster selection in *in vitro*, production of chemicals without the use of cells, improved tissue perforation, and lower immunogenicity [7].

Aptamers having high affinity and specificity towards various targets and have great potential as anticancer drugs/agents, so an overview of aptamer roles according to target classification is discussed below.

Proteins: Aptamers can target specific proteins, such as growth factors or cell surface receptors, that are implicated in the progression of cancer. Aptamers can block signalling pathways essential for tumour growth and metastasis by attaching to these proteins.

A flexible approach to treatment is provided by aptamers, which target proteins implicated in the development of cancer. For example, vascular endothelial growth factor (VEGF) is an important angiogenesis mediator that is required for tumour growth and metastasis. Some aptamers, including pegaptanib, have been created to exclusively bind to VEGF, preventing VEGF from interacting with receptors and preventing angiogenesis [16]. Similarly, overexpression of the epidermal growth factor receptor (EGFR) is frequently observed in a variety of malignancies, in which EGFR

promotes cell survival and proliferation. Aptamers, such as E07, have demonstrated encouraging outcomes in EGFR signalling inhibition and cancer cell death induction [17]. Aptamer AS1411, for example, targets nucleolin, a protein that is overexpressed in cancer cells, causing apoptosis and inhibiting the growth of cancer cells [18].

Small molecules: Small molecules linked to the onset of cancer or resistance to treatment can be bound by aptamers through engineering. As inhibitors to prevent these molecules from functioning, these aptamers can also serve as drug delivery vehicles.

Innovative therapeutic options are provided by aptamers that target small molecules implicated in the development of cancer or resistance to treatment. For example, adenosine triphosphate (ATP) is frequently dysregulated in cancer cells and is essential for the metabolism of cellular energy. Aptamers that target ATP, like aptamer-ATP-Cy3, have been created to specifically transport drugs to cancer cells and increase the effectiveness of chemotherapy [19]. Furthermore, small molecule medications can be coupled with aptamers that target cell surface receptors, including nucleolin, for targeted distribution that reduces off-target effects and improves therapeutic outcomes [20].

Metal ions: Metal ions have a variety of functions in cancer biology. Aptamers can be tailored to bind to specific ions for theranostic purposes. Aptamer-based sensors (Aptasensor) have been developed for the purpose of detecting metal ions linked to cancer, such as lead or cadmium. Aptamer-based sensors have been created, which potentially provide tools for early diagnosis [21].

Aptamers that target ions have the potential to be used for both diagnosis and treatment. Metal ions have a variety of roles in cancer biology. Aptamer-based sensors that target metal ions linked to cancer, including gold, lead or cadmium, present opportunities for personalised treatment and early cancer diagnosis [22].

Viruses: Aptamers can be engineered to specifically target viral components that are essential for infection or reproduction in viral infections that have been linked to malignancies.

Aptamers that target viral components have special therapeutic prospects. For example, aptamers that target human papillomavirus (HPV) proteins, like E6 or E7, have been created for targeted therapy because HPV infection is a major risk factor for cervical cancer [23]. Furthermore, aptamers that target viral proteins necessary for viral replication, such as the HIV reverse transcriptase, offer promise for antiviral treatment as well as the prevention of malignancies linked to viruses. Aptamers have been created against HIV-1 envelope glycoprotein gp120 and may be used as antiviral medicines to prevent cancers linked to viruses [24].

Bacteria: Some cancers are related to persistent bacterial infections and aptamers can be used to target bacterial components or toxins connected to carcinogenesis.

Aptamers that target bacterial components or toxins present new therapeutic options. For example, *Helicobacter pylori* infection has been linked to gastric cancer and aptamers that target toxins or bacterial surface proteins, like VacA or CagA, have been created for targeted therapy [25]. Furthermore, by reducing chronic inflammation linked to bacterial infection, aptamers that target bacterial toxins,

such lipopolysaccharide (LPS), have the potential to prevent cancer [26].

The methodology for purifying and describing the aptamer should be incorporated into the aptamer design, as discussed below.

Traditional

SELEX methodology

Three distinct teams used a selection technique (SELEX) in the 1990s to isolate RNA aptamers [11, 27]. The four general selection steps are typically involved in technology, as shown in **Figure 1**.

- The target interest and a baseline library of DNA and RNA are combined, which marks the beginning of the selection cycle. A library typically contains up to 10^{15} chosen 20–60 nucleotide sequences that are flanked at the 5' and 3' ends by fixing primer regions.

- Target- and un-bound sequences are separated after incubation using a variety of partition methods.
- For the next selection cycle, a new library is created using the bound sequences that have been recovered and reamplified.
- Prior to utilizing polymerase chain reaction (PCR) procedures, such as transcription and amplification to form a new RNA library, retrieved RNA patterns need to be converted into cDNA using the reverse transcription technique, whereas new DNA libraries can be generated directly using the PCR technique [7].

After 2–15 iterations of the selection cycle, analysis of the RNA patterns utilized to recognise specific patterns from the collection is enhanced. Throughout the selection procedure, modifications are made in the library with respect to incubation time, target ratio, and buffer composition. Selection stringency improves the enhanced target-bound sequences. Various SELEX technologies targeted at select molecules are summarised in **Table 1**.

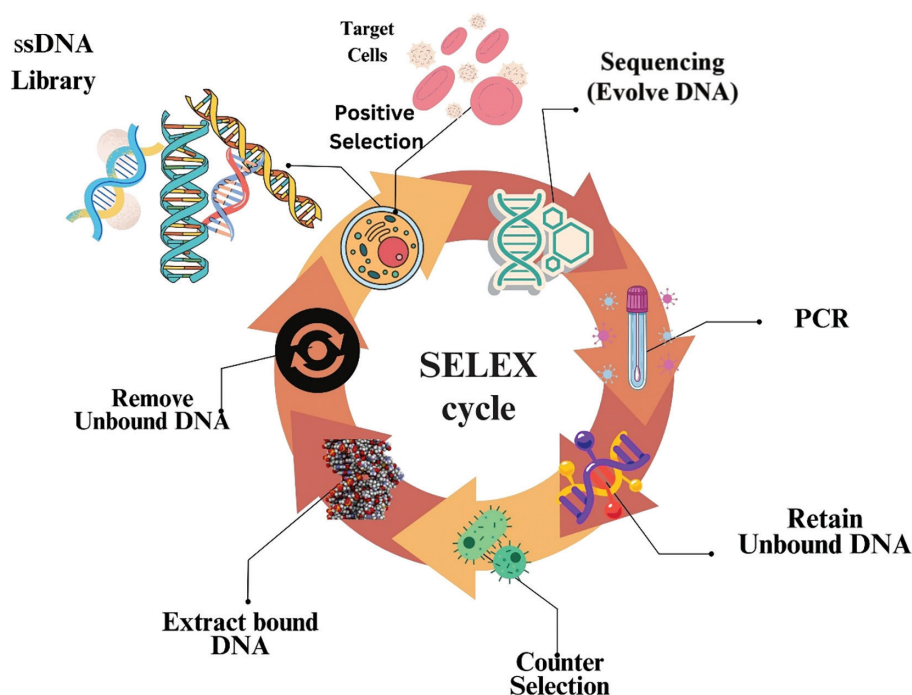


Figure 1 Flowchart representing the various steps of SELEX technology.

Table 1 Various SELEX Technology Targeted to Selected Molecules with Examples

SELEX Technology	Selected Molecule	Examples	References
DNA-SELEX	DNA-binding proteins, DNA aptamers	HMGB1 protein, TATA-binding protein, DNA aptamers for cocaine	[11, 131]
RNA-SELEX	RNA-binding proteins, RNA aptamers	HIV-1 Rev protein, nucleolin protein, RNA aptamers for ATP	[10, 14]
Chem-SELEX	Small molecules, peptides, proteins	ATP, streptavidin, tau protein	[132, 133]
Cell-SELEX	Whole cells, cell surface proteins	Leukaemia cells, breast cancer cells, T lymphocytes	[134, 135]
Protein-SELEX	Proteins, peptides	TGF- β 1 protein, PDGF-BB protein, peptides that bind to fibrinogen	[136, 137]
Spiegelmer-SELEX	Mirror-image oligonucleotides, mirror-image aptamers	Thrombin protein, VEGF protein, mirror-image RNA aptamers for IFN- γ	[10, 138]

Green aptamer

Cell imaging studies are crucial to understanding cellular physiology. Green fluorescent protein (GFP) and targeted cells (proteins) have long been studied for their interactions, positioning, claims, signals, and functions, which are key parts of cellular imaging applications. Yet, a large amount of RNA research has begun to broaden the function of RNAs, leading to the creation of cutting-edge intracellular imaging technologies, including fluorogenic aptamers, such as those found in spinach [28], broccoli [29], mangoes [30], and corn [31], which cause fluorescence to be activated when target fluorophores are bound. These green or fluorescent aptamers can be made into imaging tags to determine how RNA moves inside cells or as biosensors to track metabolites inside cells in a laboratory setting.

Spinach as a fluorogenic

Green fluorescence is caused when the small molecule, fluorophore 3,5-difluoro-4-hydroxy-benzylidene-imidazolinone (DFHBI) and its variations, including DFHBI 1T and DFHBI 2T, connect to the spinach fluorogenic RNA aptamer [28]. The spinach-DFHBI complex was determined using crystal structure analysis and this information was used to investigate the exact mechanism underlying fluorescence activation.

The spinach-DFHBI complex co-crystal structures were shown to have an unexpected quadruplex core coupled with a combination of a mixed base and two G-tetrads. The unpaired guanine residue (G31) is encased by a base-triple-inserted DFHBI. In the fluorophore-free state, in contrast, the base tier of the quadruplex is rearranged with the fluorophore collapsing binding site, in which the helical stack can grow to G31 [32].

Broccoli as a fluorogenic

The broccoli isolation technique was created to increase fluorogenic aptamer functionality and specificity. Following an initial round of traditional aptamer selection, fluorescent RNA aptamers were introduced into plasmids using experimental techniques. Fluorescence-activated cell sorting was utilized for isolation of fluorescent cells after cloned plasmids were transformed into *E. coli* for DFHBI treatment. The green aptamer, broccoli, which is fluorogenic, was successfully obtained using this method and displayed vigorous folding in low concentrations of salt, enhanced thermal stability, and higher fluorescence than spinach [29]. Broccoli was dimerized into tdBroccoli after being shortened to tBroccoli for any further *in vitro* imaging. The dimerized tdBroccoli fluoresced with twice the intensity of broccoli [29]. Without a tRNA scaffold, broccoli or dBroccoli display greater fluorescence intensity than a tRNA scaffold. In transfected HEK293T cells, microscopy was used to compare fluorescence activation [29].

Mango as fluorogenic

The mango RNA aptamer exhibits orange-coloured fluorescence in significant levels. Derivatives of the fluorophore thiazole orange (TO1) increase fluorescence by a factor of 1100. In an A-form duplex, two-tiered all-parallel G-quadruplexes were anticipated to fold the structure of mango. Three antiparallel quinines creating G-quadruples were revealed by the crystal structure investigation of the mango-TO1 complex [33]. The G-flat quadruplex face on which TO1 is bound, stabilizes the mango structure and turns on the orange fluorescence.

For imaging purposes, equimolar quantities of RNA mango were used to directly inject TO1 into the syncytial gonads of *Caenorhabditis elegans* (*C. elegans*). The gonad nuclei were shown to have a high fluorescence composition [30].

Corn as a fluorogenic

Yellow fluorescence is triggered by the corn RNA aptamer, which combines to form 3,5-difluoro-4-hydroxynenzylideneimidazolinone-2-oxime [i.e., DFHO] [31]. Corn exhibits much greater photostability than broccoli. Fluorescence is visible at 320 ms imaging periods but broccoli is only visible at 160 ms [31]. In contrast to the broccoli-DFHBI complex, which loses >50% of fluorescence past 200 ms, the corn-DFHO combination in the irradiation trials reveal very little fluorescence loss after 10 s. Corn is therefore regarded as a newly emerging aptamer that is fluorogenic and acts as a biosensor. The corn aptamer yields three primary subtypes (tRNA, 5S RNA, and U6 [Pol III promoters]). The tRNALys scaffold plasmid facilitates the assessment of viability as a fluorescent RNA tag that is genetically encoded [34].

Aptamer-exosome technology

All live cells secrete extracellular vehicles (EVs), which are membrane-bound bionanoparticles [35]. Based on the mechanism underlying synthesis and size, EVs are categorised as exosomes, oncosomes, exomers, apoptotic bodies, or microvesicles [36]. EVs have progressed from the early days as “platelet dust” [37] to becoming an important intermediary for *in vivo* interaction across some cellular entities, tissues, and cross-kingdom molecules [38]. Exosomes and microvesicles have been identified after long-lasting cell biology and biochemistry research. Exosomes have particle sizes ranging from 50–150 nm with surface biomarkers known as tetraspanins [CD63, CD81, and CD9] [39], while microvesicles have particle sizes ranging from 100–1000 nm and annexin A1 as a distinctive biomarker.

Exosomes carrying cargo acquired from the cells of origin have been discovered in blood and different body fluids, circulating in a variety of cell types. These developments have created the groundwork for exosomes to become a cutting-edge source for identifying new biomarkers. Tumour-susceptibility gene 101 (Tsg101), heat shock proteins, the endosomal sorting complex needed for transportation

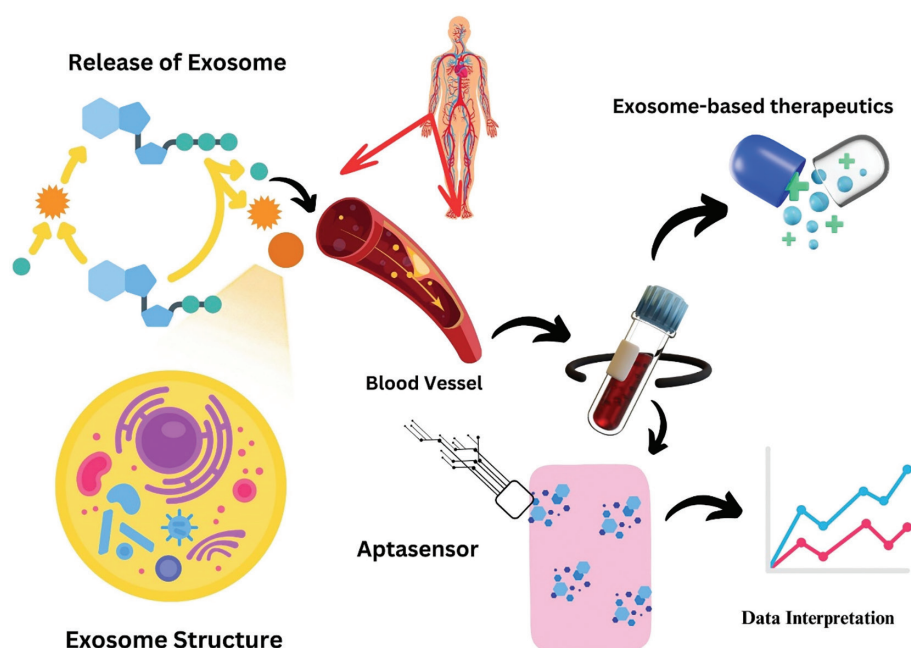


Figure 2 Extracellular vesicles as theranostics.

(ESCRT-3) binding protein, Alix, and the major histocompatibility complex, both class I and class II composites, are known to be carried by exosomes [40].

Exosome attachment and fusion with the plasma membrane of the recipient cell may be facilitated by the additional attachment of molecules to the surface, such as lymphocyte function-associated antigen-1 (LFA-1) integrin and intercellular attachment of molecule-1 [ICAM-1 {often called CD54}] [41]. In addition, the fact that exosomes contain a lot of transmembrane proteins, such as EGFRs and epithelial cell adhesion molecule (EpCAM), suggests that exosomes arise from living structures. These proteins are used as excellent biomarkers because the proteins are connected to the typical pathophysiology and pathogenesis of numerous diseases [42].

Exosomes are extremely durable in a variety of *in vivo* and *in vitro* settings because the membranes are significantly abundant in lipid rafts [43]. These proteins are used as excellent biomarkers because they are connected to the typical pathophysiology and pathogenesis of numerous diseases [44], as shown in **Figure 2**.

Capturization-aptamers to exosomes

Exosomes include information about the prognosis and development of a specific condition in addition to the parent cell, making exosomes useful diagnostic indicators. For example, exosomes produced by cancer cells may contain membrane proteins vital to the development of the disease.

Membrane proteins are involved in the development of cancer. Programmed death ligand 1 (PD-L1) has been shown to suppress CD8⁺ T lymphocytes and promote tumour growth, based on a recent study involving exosomal PD-L1 derived from metastatic melanoma cells [45]. Integrins, such

as $\alpha 6$, αv , and $\beta 1$, that are present in exosomes produced by cancerous cells can be used to forecast the tumour stage and differentiate between different types of cancer, including the breasts, kidneys, colon, and ovary [46] because more of these proteins were released by aggressive progenitor cancerous cells.

Research has demonstrated that exosomes can pass through the blood-brain barrier, at least in some cases. Therefore, the quantity and composition of exosomes in plasma and cerebral fluids can reveal important details about the pathogenesis and development of neurodegenerative disorders [47]. The biological properties of exosomes enable vesicles to efficiently transport therapeutic compounds. This homing effect of exosomes suggests that therapies using exosomes may have better results in preventing cancer in the cancer cells from which exosomes arise [48].

Technology in theranostics

Exosome diagnostics using aptamers

Aptamers can be chosen to precisely bind with biomarkers on exosome surfaces and confine the exosomes to enhance the enrichment efficiency of the exosome and facilitate exosome isolation. The following sections provide an overview of the development of aptamer-guided exosome diagnostic tools.

Exosomes can be easily captured using peptide/RNA/DNA aptamers

A diagnostic tool, the aptamer-guided exosome-capturing nanoplatform technique, is used by choosing an aptamer contrary to an overexpressed polypeptide on exosomes. An aptamer that connects to the extracellular domain of HSP70 was developed by Garrido et al. [49] with the aim of targeting the A8 peptide, which is increased in numerous

cancers. HSP70-positive exosomes were discovered using this aptamer in urine samples from patients with breast, lung, and ovarian cancer [49].

Exosome levels in the blood and urine can be measured. After multiple iterations of SELEX, Murakami et al. [50] chose two RNA aptamers with lengths of 55 and 30 nucleotides (nt) each. According to surface plasmon resonance (SPR) analysis, the 55- and 30-nt aptamers have similar levels of attraction for exosomes. Circular dichroism spectroscopy showed that the two aptamers are capable of forming G-quadruplex structures in between loop regions that are supported by K⁺ or potassium ions [50]. Sun et al. [51] devised ways to use DNA aptamers and DNA to separate exosomes of a specific size and identify the surface proteins at the same time in different studies. Stage II breast cancer patients with varying HER2 expression patterns and different breast cell lines could be distinguished using a machine learning algorithm implemented to assess exosomal size and marker signatures [51].

Aptasensor

Aptasensors have developed into a crucial diagnostic tool for identifying cancer-derived exosomes due to high sensitivity, quick response, accessibility, and low volume of sample [51].

Other diagnostic methods based on labs-on-a-chip technology

The rationale behind aptamer-guided exosome diagnostic tools is that aptamer-guided exosome diagnostic tools selectively attach to exosomes that carry disease-related information. Aptamers are mixed with polymers and other inorganic substances to make a platform that is built right into lab-on-a-chip exosomal diagnostic tools. The ExoAPP test, an aptamer nanoprobe-based exosome profiling technique, was used to characterise the surface proteins present in exosomes and detect cancer-derived exosomes [52].

Exosome therapeutics using aptamers

Exosomes with paclitaxel, an anticancer medication frequently used in chemotherapy regimens, were described by Wan et al. [53] as a novel platform for clinical applications in treating cancer.

The cholesterol-PEG-covalently-conjugated nucleolin targeting aptamer AS1411 was then fixed to murine dendritic cell membranes. Then, to produce aptamer-guided exosomes, these cells were physically extruded. This method used extrusion to make 3×10^{10} aptamer-guided exosomes with a diameter of 105 nm from approximately 1×10^7 cells/h. Because of amphiphilicity and corresponding stiffness, cholesterol-PEG2000 was chosen because cholesterol-PEG2000 stabilizes exosomes at the lipid bilayer membrane through hydrophobic contacts. The technique used to create cholesterol-PEG2000-aptamers could be potentially applied to the mass manufacturing of specific exosomes released through immune cells for the treatment of various malignancies. The cells needed to generate exosomes are collected from

the patients who require therapy, making this procedure relatively more secure than cell-based immunotherapy, such as CAR T-cell therapy.

Such derived exosomes can be used to be genetically engineered without drug loading [53]. An alternate strategy involving reprogramming of exosomes leads to effective targeted exosome delivery and tumour suppression, which can be achieved by controlling surface ligand presentation and siRNA/miRNA loading on exosomes with an RNA aptamer [54].

Aptasensor

Having high affinity, stability, ease of modifying kinetic parameters, relatively quick creation without the use of animals, and a wide range of targets from tiny molecules to entire cells, aptasensors are essentially antibody-based sensors [55]. To identify cancer, there are numerous optical, electrochemical, and colorimetric aptasensor techniques. This section will concentrate on current developments in aptasensors for the sensing and identification of cancer, with a focus on developments.

Electrochemically activated aptasensors

Sandwich-style aptasensor

The sandwich-style aptamer system basically consists of an electrode base and two aptamers (Figure 3). The analyte is captured and immobilised by a capturing aptamer coupled to an electrode surface, whereas another aptamer named secondary aptamer identifies and binds different parts of the analyte base and forms an aptamer-analyte-aptamer sandwich. The electrode [56] can be used to detect an electroactive label present in the secondary aptamer, such as cadmium sulphide quantum dots [57], glucose dehydrogenase [58], or gold nanoparticles [AuNPs] [59]. Numerous sandwich-based detection systems were designed due to the relative simplicity in cancer cell targeting.

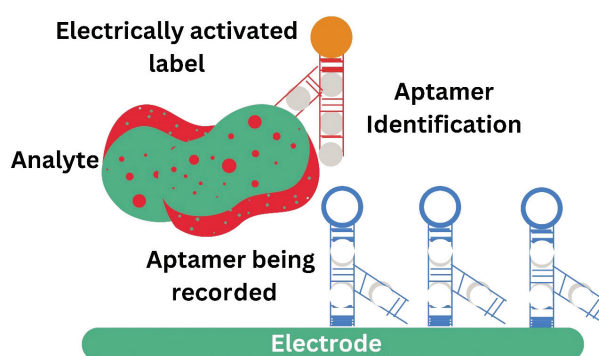


Figure 3 Representation of sandwich-style aptasensors activated electrochemically.

Zhang et al. created an aptasensor that is electrochemically activated by means of an aptamer against the surface glycoprotein, Mucin1 (MUC1), which is markedly overexpressed in many cancers. A similar MUC1 aptamer-conjugated magnetic bead was caught by an extra lectin-based nanoprobe functionalized on AuNPs, which then attracted cells that express MUC1 [60]. In this experiment the reduction of silver ions by gold caused voltage fluctuations. Utilizing electrochemical stripping analysis, these voltage differences were read to determine the amount of MUC1 expression, and potentially, a carcinogenic diagnosis.

Label-free aptasensors

The properties of aptamers, such as the distinctive attributes changing upon binding to a target, enhance resistance triggered by the creation of double-stranded DNA and decrease signalling when an electrode electroactive group is moved by aptamer binding, have been harnessed to create label-free, electrochemically activated aptasensors [56, 61]. Various electrochemically activated aptasensors, which are label-free, have been created against cancerous cell targets and aptasensors that benefit from the conformational shift that occurs as aptamers attach to their targets.

Wang et al. [62] recognized MCF-7 breast malignant cells using a polyadenine-modified aptamer system using the voltage drop caused by target binding that is detected by differential pulse voltammetry.

Aptasensor-activated fluorescence

The first aptamer-based biosensor was created using a fluorescent aptamer against human neutrophil elastase (HNE) in 1996. It was shown that the HNE fluorescent aptamer is equally efficient as an antibody in detecting HNE on beads. However, the HNE fluorescent aptamer also had additional benefits, such as the capacity to include functional groups faster, a smaller size that makes internalisation easier, the ability to identify intracellular targets, less off-target binding, and higher storage stability [63].

Herr et al. [64] created a fluorescent sandwich system for the identification of cancerous cells using aptamers coupled with fluorescent nanoparticles. Herr et al. [64] created a useful technique for therapeutic usage using magnetic nanoparticles to assist with taking these cells out of entire blood samples.

Chen et al. [65] developed a multiplexed detection technique in 2009 that uses aptamer-conjugated Förster resonance energy transfer (FRET) using silica nanoparticles to identify several cancer cell targets. Numerous fluorescent aptasensors have been created to detect cancer. Fluorescent aptasensors are used to detect cancer indicators in addition to malignant cells. VEGF is one of the markers that a fluorescent aptamer was initially intended to detect.

Freeman et al. [66] reviewed several optical aptasensor techniques that exploit the structural change of an anti-VEGF aptamer when an anti-VEGF aptamer interacts with its target. Specifically, some sensing approaches were

evaluated based on fluorescence resonance energy transfer, luminescence emission via chemical means, and luminescence resonance energy transfer methodologies that enable target detection [66].

Cho et al. [67] used the optical phenomenon known as nanoplasmonic sensing, in which fluorophore intensity is altered when the fluorophore encounters free electrons on a metal surface to create a one-step method for VEGF165 detection. Using this technique, cyanine (Cy3) was used to mark the surface of AuNPs with anti-VEGF aptamers. The aptamer conformation is modified when the aptamer is bound to VEGF, releasing the aptamer from the AuNPs and causing a substantial drop in fluorescence intensity [67].

Aptasensor-activated colorimetry

Several aptamer-based colorimetric assays facilitate the identification of cancer markers, allowing for easy and quick recognition of targets that range from tiny metal ions to complex structures, such as proteins.

Xu et al. [68] designed a colorimetric biosensing technology designed to identify the proto-oncogene (K-Ras) using a DNA molecular machine. The main part of the device is a hairpin detector that can find K-Ras and connect to a primer-linked polymerization unit to make an anti-hemin aptamer. This anti-hemin aptamer facilitates the conversion of the colourless substrate, 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) [ABTS], into a green-coloured form, allowing visual identification without any equipment. This process activates a DNazyme that mimics the activity of horseradish peroxidase [68].

Ahirwar and Nahar et al. [69] designed a nanoparticle-based colorimetric aptasensor system for the recognition of the human oestrogen receptor alpha (ER α), a usual marker in breast cancer.

Theranostics benefits of aptamer

Aptamers serve a purpose in theranostics because of a dual function (therapeutic and diagnostic purposes). Aptamers provide high affinity and specific selectivity for target molecules that have the capacity to bind to certain sites, which allows aptamers to be used as delivery systems for medicinal drugs, allowing for tailored or conjugation therapy, as well as for diagnostic purposes by identifying biomarkers. By optimizing therapeutic outcomes and offering real-time monitoring of treatment response, this dual functionality advances the theranostics use of medicine [70].

Chemotherapy

One of the limitations of traditional cancer treatments, such as chemotherapy and radiotherapy, is the potential for significant side effects. These treatments often affect not only

cancer cells but also healthy cells, leading to various adverse reactions, such as fatigue, nausea, hair loss, and immune suppression. Furthermore, the efficacy of these treatments can be limited by cancer cells acquired resistance. However, aptamer-based theranostics have the potential to overcome these limitations. By specifically targeting cancer cells, aptamer-based theranostics minimize off-target effects and reduce toxicity to healthy cells. Moreover, aptamers can be engineered to overcome acquired resistance by targeting alternative pathways or biomarkers that are still present in resistant cancer cells [71].

Various drugs that have been used in chemotherapy studies are below.

Doxorubicin (DOX)

DOX is often used in several cancer treatments. When small molecules are coupled to DOX, the resistance caused by cancer cells is decreased, which therefore enhances the efficiency of chemotherapy [72]. As a constituent of the anthracycline family, DOX has the Food and Drug Administration (FDA) approval. DOX inhibits DNA replication by intercalating DNA and subsequently causes adverse reactions, such as irreversible cardiomyopathy brought on by oxidative stress, myocardial fibre loss, apoptosis, and downregulation of genes that regulate contractile proteins [1].

The AS1411 aptamer is a G-quadruplex-structured anti-proliferative DNA aptamer that was created by targeting nucleolin, a proteinaceous structure, which is abundant in cancerous cells. This oligo-nucleotide, the first aptamer, provided the basis for clinical trials involving a variety of cancer treatments and was subsequently used in additional studies to improve the effectiveness of the treatment against cancerous cells [73]. Details of the clinical trials based on aptamer along with its NCT number are shown in **Table 2**.

Li et al. [74] worked on an advanced aptamer system (AS1411) that delivers DOX to patient malignant breast cells (MCF-7). The hearts of mice bearing the MCF7 tumour showed no indication of drug accumulation. Micelle technology significantly increased AS1411 affinity for MCF-7 receptors. The reduced accumulation of DOX in cardiac cells led to a reduction in cardiac tissue necrosis and damage, which provided a feasible avenue for aptamer-based therapy. Moreover, AS1411 exhibited anticancer activity and a decreased level of cardiotoxicity by DOX in MCF-7/ADR tumour-bearing mice, as well as decreased resistance to multiple drugs mediated by glycoprotein-P (P-gP) discharge [74], as shown in **Table 3**.

The same investigation used a bubble generating agent. When the microenvironment is warmed to 42°C, intracellular DOX content rises and potentially harmful drug release occurs, leading to permeability defects in the bilipid layers. The use of novel aptamers that bind and block the proteins necessary for the formation of P-gP is intended to remove restrictions on the therapeutic application of DOX. This maneuver will increase DOX release and decrease carcinoma cell survival without having a deleterious effect on cardiac muscle failure. Trinh et al. [75] also developed AS1411 and DOX into a human model of hepatocellular carcinoma.

No negative effects on the heart or endocrine system, as well as no weight loss were noted [75].

Atabi et al. reported that MUC-1 aptamers make DOX persist longer in human prostate cancer cell lines (LNCaP cells). Jing et al. concluded that the double aptamer system decreases cancerous growth in prostate cancer (LNCaP and PC3 cells). The combined effects of DOX and MUC-1 aptamers on MCF-7 malignant cells found in breast tissues were investigated by Jeong et al. Similarly, Sun et al. worked on a human colorectal adenocarcinoma cell line (HT-29) to observe the effect of the SL2B aptamer. These investigations provided a path for the development of aptamers, which resulted in chemotherapy that is administered at lower doses, reducing the afore-mentioned negative effects [76].

Docetaxel (DTX)

DTX is a taxane-family anticancer medication that promotes cell death by depolymerizing tubulin and causing microtubule aggregation. Intravenous DTX has been associated with toxicity in malignant brain tumours [77].

Gao et al. [78] utilized AS1411 as a booster for the DTX anti-glioma impact in the C6 glioma line. Treatment for gliomas is particularly challenging because the blood-brain barrier (BBB) and brain-glioma barrier inhibit the uptake of drugs into tumour cells. Double aptamer systems can be used to improve such investigations, both for BBB detection as well as cancer identification.

Paclitaxel (PTX)

PTX is a chemotherapy agent that treats malignant glioma cells, although reduced solubility in water reduces PTX efficacy. In the interest of promoting enhanced permeability and retention within tumours, aptamers may be used to increase the solubility of PTX. Increasing tumour penetration and retention of macromolecular medications can lessen toxicity to healthy cells [79].

Epirubicin (Epi)

Taghavi et al. [80] used the anti-MUC-1 aptamer, 5TR1, and the augmented Epi medication delivered to CHO cells, a hamster ovary cell line, and MCF-7 breast cancer cells. Epi is a medication from the anthracycline family that when used in high quantities can lead to cardiotoxicity. Novel aptamers may improve MUC-1 glycoprotein recognition to reduce myocardial cell cytotoxicity, improve Epi release to boost DNA intercalation, block topoisomerase II, and increase degradation of cancer cells.

Gemcitabine (Gem)

The RNA aptamer utilized by Ray et al. to increase the efficacy of the chemotherapeutic medication, Gem, targets EGFR-overexpressed pancreatic carcinoma cells. Gem is a

Table 2 Clinical Investigation Done on Aptamer in the Field of Oncology

S. No.	NCT Number ^a	Drug/Intervention	Cancer	Study Type	Volunteers/ Clinical Trial	Summary	Status	References
1.	NCT01830244	Nab-paclitaxel (Abraxane)	Breast	Interventional Primary purpose: Treatment	60 participants Phase 2	This project aims to assess tailored primary systemic therapy using sequential nab-paclitaxel, epirubicin, and cyclophosphamide for early breast cancer. It is an open-label phase II clinical trial hypothesizing that this regimen is feasible and yields high response rates. The study planned to enroll 60 patients, including 40 with chemotherapy-sensitive tumours and 20 with ER-positive tumours, potentially responsive to hormonal treatment. The target group was women with early breast cancer eligible for primary systemic therapy. The primary outcome was overall response rate in the breast. Secondary outcomes included response rates in axillary lymph nodes, safety, tolerability, and breast conservation rate. Participants underwent genotype testing to explore chemotherapy sensitivity prediction, with results not affecting therapy selection. Optionally, patients can contribute tumour tissues for laboratory studies on cancer-initiating cells and aptamer-based tumour targeting.	Unknown	[139, 140]
2.	NCT00056199	EYE001	von Hippel-Lindau disease	Interventional Primary purpose: Treatment	5 participants Phase 1	von Hippel-Lindau (VHL) disease is an inherited disorder causing various benign and malignant tumours across multiple organs. Retinal angioma, a common symptom, can lead to vision loss due to retinal edema. Traditional treatments, such as photocoagulation or cryotherapy, may not work for all lesions. The study aimed to test the effectiveness of anti-VEGF therapy (EYE001) on retinal angiomas associated with VHL in five patients. The patients received 6 injections over 30 weeks and were monitored for improvements in vision, retinal health, and any adverse effects.	Completed	[141]
3.	NCT00740441	AS1411	Metastatic renal cell carcinoma	Interventional Primary purpose: Treatment	30 participants Phase 2	The aim of this study was to evaluate the efficacy of AS1411 in patients with metastatic renal cell carcinoma.	Unknown	[142]
4.	NCT01486797	NOX-A12	Chronic lymphocytic leukaemia	Interventional Primary purpose: Treatment	28 participants Phase 2	The purpose of this study was to evaluate the safety and efficacy of NOX A12 in combination with a background therapy of bendamustine and rituximab chemotherapy in previously treated patients with chronic lymphocytic leukaemia.	Completed	[143, 144]
5.	NCT03385148	68Ga-Sgc8	Colorectal	Interventional Primary purpose: Diagnostic	70 participants Early Phase 1	The protein, tyrosine kinase-7 (PTK7), is overexpressed in various types of human cancers. As a specific imaging agent of PTK7, 68Ga-Sgc8 was investigated in this study to assess its safety, biodistribution, and dosimetric properties in healthy volunteers, and to preliminarily evaluate its application in colorectal patients.	Unknown	[145]
6.	NCT06258330	AM003	Solid tumour	Interventional Primary purpose: Treatment	15 participants Phase 1	This is a phase 1, first-in-human study to assess the safety and tolerability of AM003 in patients with locally advanced and metastatic solid tumours.	Recruiting	[146]

^aNCT Number- ClinicalTrials.gov Identifier

Table 3 Various Drugs with Aptamers Specifications with Cell Line used in Chemotherapy Studies

S. No.	Name of Drug	Aptamer	Cell Line	Cancer Type	References
1	Doxorubicin (DOX)	AS1411	MCF-7	Breast	[35, 94, 96]
		MUC-1	LNCaP	Prostate	
		SL2B	HT-29	Human colorectal adenocarcinoma	
2	Docetaxel (DTX)	AS1411	C6 Glioma	Brain	[37]
3	Epirubicin (Epi)	Anti-MUC-1	CHO	Ovarian	[36, 37]
			MCF-7	Breast	
4	Gemcitabine (Gem)	RNA	EGFR	Pancreatic	[1]

nucleoside molecule that has been linked to neutropenia-related illnesses in high concentrations because of normal cell toxicity. Aptamers that affect Gem phosphorylation status during internalisation can be exploited to circumvent the toxicity effect limits. A nucleoside, Gem, that phosphorylates intracellular cells to limit the function of ribonucleotide reductase stops growing cancer cells from lengthening DNA, as shown in **Table 3**.

These studies demonstrated that unexplored territories remain in aptamer-based chemotherapy. Aptamers can undoubtedly enhance cancer treatment by increasing therapeutic efficacy and/or decreasing adverse effects [1].

Biotherapy

Immuno-theranostic

Melanoma, and non-small cell lung, head, and neck cancer are just a few of the many cancer types that can be treated with immunotherapy, which is a successful and innovative treatment approach. Several biotherapy tools have been developed by integrating several aptamer features with other additional related molecules, such as proteins or reporter groups.

Zhao et al. [81] configured a bivalent RNA aptamer that prevents heat shock factor (HSF1) from interacting with associated DNA promoter regions, down-regulating expression of HSF1 and making carcinomas more susceptible to anti-cancerous drugs. By using a CD28 2'-fluoro RNA aptamer, Lozano et al. created an aptamer in place of Foxp3 [82]. The initial findings indicate that the P60 Foxp3 inhibitor peptide couples alongside a CD28-targeted aptamer to enable peptide delivery to CD28-articulating cells, thereby suppressing regulatory T-cell immune responses when the cells become trapped within the tumour microenvironment.

While formulating combination medicines, aptamers may also be crucial because aptamers may aid in locating the biomarkers responsible for unfavourable reactions. For example, the mesothelial surfaces that are present on lung pleura are home to drug-resistant malignant pleural mesothelioma (MPM), the biomarkers of which lack sensitivity and specificity [83]. The success rate of treatment could be considerably increased by the creation of precise biomarkers for MPM. One of the frequently used MPM markers is CD44, although there is no reliable measurement method for CD44. The creation of aptamers against this marker may aid in the earlier diagnosis of this condition, enabling a more structured course of therapy.

Various organizations are currently focusing on a category of biomarkers, specifically metabolic biomarkers. This category of markers is consistent amongst the four functional levels (genome, metabolome, proteome, and transcriptome) linked to cell function. This approach has been used in colorectal cancer (CRC) therapies. Accumulation of arginine, proline, glutamine, threonine, lactate, phenylalanine, and a few other amino acids have been identified as an important CRC biomarker [84–86]. The abovementioned metabolic biomarkers, in particular those that are released by cells, can all be detected using aptamers. Because the behaviour of tumour cells can be understood with the help of such metabolites and how the tumour cells react, if at all, to traditional remedies, implementing aptamers for quantification of these metabolites can be beneficial in cancer progression studies.

Drug delivery system: aptamers in anticancer

Drug delivery methods have been in use for some time and have indeed advanced medicinal modalities. Nanocarriers are colloidal nano-scale systems that can carry anticancer substances. Drugs and large molecules with small molecular weights are examples of nanocarriers that facilitate therapy by preventing pharmaceutical deterioration, lowering renal clearance, extending half-life, and helping to control kinetic release. Drugs and large molecules with small molecular weights assist with solubility [87]. Among tumours that grow quickly, cancerous cells are usually situated near an endothelium barrier. As a result, nanocarriers containing targeting molecules will adhere to the primary receptors encountered rather than trying to penetrate the entire tumour [88]. Cell-SELEX is best-suited in this scenario. The procedure selects aptamers that might be beneficial for the nanocarriers by attaching nanocarriers to cellular receptors that are stated above and blocking access to the remaining tumour through receptor saturation.

Targeted drug delivery: aptamer-drug conjugate (ApDC)

ApDCs have drawn interest as therapeutic agents as well as vehicles for delivering a variety of therapeutic agents, including toxins, small interfering RNAs (siRNAs), microRNAs (miRNAs), and chemotherapeutics [Figure 4] [89]. Conventional ApDCs comprise a combination of aptamers and linkers which are linked via various cleavable or

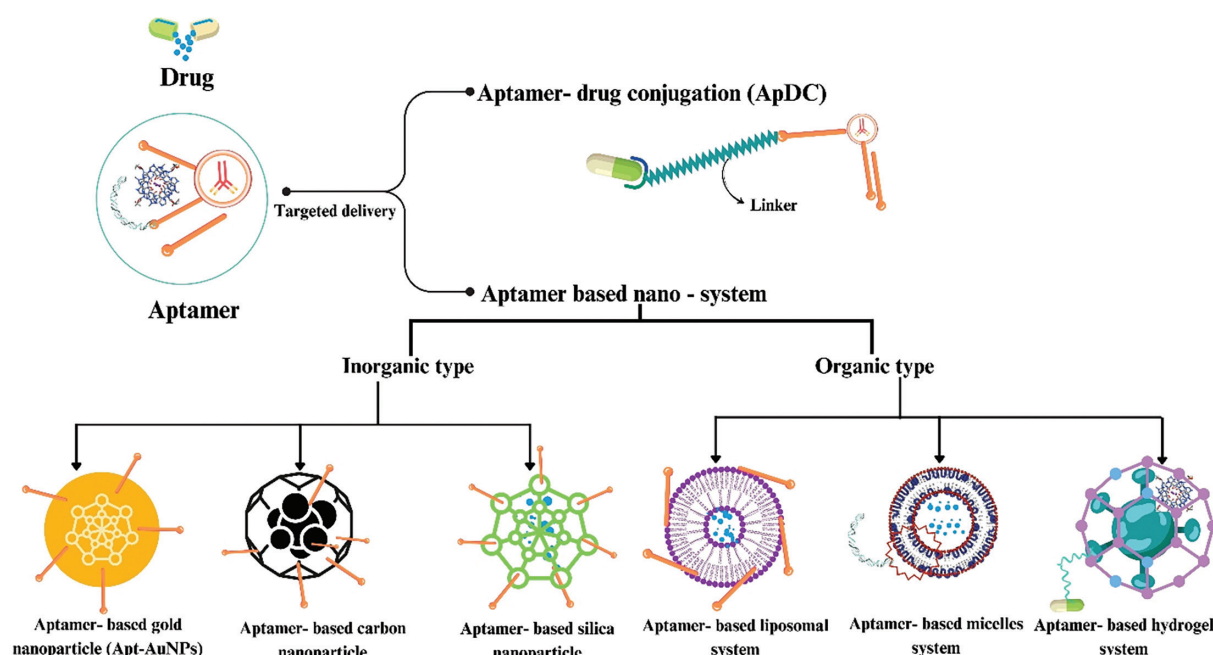


Figure 4 Schematics of drug delivery methods for aptamers explain aptamer-drug conjugation and aptamer-based nanosystems.

non-cleavable linkers. Compared to antibody-drug conjugates, which have been applied in clinical cancer treatment, ApDCs have several benefits, including quick tissue penetration, facile chemical modification, smaller size, and a cost-effective manufacturing procedure. ApDCs have been produced for a wide range of therapeutic techniques, including immunotherapy and chemotherapy, by targeting biomarkers linked with specific diseases [90].

Tan et al. [91] created and synthesized a sgc8-DOX conjugate to deliver DOX in a targeted manner. In these ApDCs, the anticancer drug, DOX, and the aptamer, sgc8, were conjugated at an equal ratio using an acid-labile hydrazone linker, which enabled DOX to be selectively delivered to acidic tumour environments [91].

Aptamers can be used to overcome these difficulties. Receptor-mediated endocytosis, for example, could account for concentration issues. This strategy could be implemented using aptamers that target cell-surface receptors associated with cellular uptake.

Aptamers may accumulate in tumour cells, possessing a major disadvantage to the system, the use of which could potentially be advantageous for drug delivery systems based on lipids. An aptamer against mTOR (Raptor) that performs in a similar manner was chosen by Berezhnoy et al. (4-1BB aptamer-siRNA chimera). When mechanistic target of rapamycin (mTOR) is expressed, mTOR hinders conversion of effector cells into T-memory cells when mTOR is expressed. Activated T cells are targeted by the 4-1BB aptamer, and after internalising 4-1BB, release Raptor siRNA into the cytoplasm of the cell (CD137). When memory T cells are being induced, mTOR is inhibited by the very existence of siRNA within the cell [92]. Dassie et al. proposed a specimen for an aptamer-siRNA chimera that alters gene articulation. Dassie et al. identified an aptamer that binds to siRNA and causes tumours that express prostate-specific membrane antigen (PMSA) to recede [93].

Aptamers have the ability to conjugate with chemotherapy agents as well as therapeutic RNA or DNA. This interesting hypothesis might be extended to tumours that secrete MMP and/or kallikrein (klk). Use of an iRNA chimera aptamer as a delivery vehicle has also been described by Dassie et al. Moreover, Dassie et al. selected an RNA aptamer that targets genes that help prostate cancer cells remain viable and sends cytotoxic siRNA directly to the cancer cells [94]. The ability of aptamers to deliver active RNA to cells *in vitro* as well as *in vivo* was demonstrated for the first time in this work.

Many organizations have begun to use similar delivery strategies. An aptamer was used to administer DOX to HER2-positive cancerous breast cells according to Liu et al. DOX must bind to DNA to form the aptamer-DOX complex (Apt-DOX). Due to the structure of HB5, DOX is able to reach HER2-positive breast cancer cells and inhibit HER2-negative cells from drug uptake *in vitro*, resulting in less cytotoxicity [95].

Thiel et al. used RNA aptamers to deliver chemotherapy-sensitizing siRNAs to HER2-positive breast cancer cells. The goal was to find RNA aptamers that are specific for a certain type of cell that has cell-internalising properties. Thiel et al. developed a unique cell-based selection method [96]. These aptamers (anti-apoptotic genes) have siRNAs that are coupled covalently and target the Bcl-2 gene. Application of the HER2 aptamer-Bcl-2 siRNA pair specifically targeted HER2-positive cells, lowered the expression of the Bcl-2 gene, and made the cells more vulnerable to cisplatin therapy. These projects hold great potential because the disease is so unique and the treatment is difficult because of the unusual properties of malignant cells.

Targeted drug delivery (aptamer-based nanosystem)

An aptamer-based nanosystem contains various nanomaterials that have an important role in the application of

biological medicine and analysis [97]. Nanomaterials have advantageous characteristics, such as ultra-micro size with large surface area and having high loading capacity over conventional theranostic strategies [Figure 4] [98].

Inorganic nanomedicine-based system

Silica-based nanoparticles: Silica nanoparticles are becoming an appropriate carrier in delivery of drugs in an inorganic system because silica nanoparticles provide the controlled drug release by changing pH, temperature, and redox and photochemical reactions [99]. When coupled with specific components, such as aptamers, silica nanoparticles improve the therapeutic effects of cancer treatment at a lower medication dose [100].

A new redox-responsive nanocontainer based on mesoporous silica nanoparticles (MSN) for targeted triplex cancer therapy was described by Cai et al. [101]. The AS1411 aptamer was customised for CytC-sealed MSNs [101]. Additionally, this mechanism may cause the S-S connections to dissolve, allowing DOX to be released specifically into the tumour cells. FRET-based two-photon MSNs were initially created by Tan et al. [102] in 2021 for targeted drug delivery and multiplexed intracellular imaging. By altering the doping ratio of the 3 dyes, the MSNs exhibit distinct two-photon multi-color fluorescence. Moreover, the anticancer medication, DOX, can be released from the DOX-loaded, aptamer-capped nanosystem and internalised into cancer cells with efficiency. An abundance of work has also been performed on gene-targeted delivery using aptamer-targeted MSNs, which can shield gene therapy molecules from nuclease degradation [103].

Carbon-based nanoparticles: Carbon nanomaterials functionalized with aptamers are perfect as nanoplateforms for cancer treatment and diagnostics [53]. Multi-functionality was recently produced by Wang et al. and demonstrated sustained release and heat-stimulating capabilities. This nanoparticle exhibits remarkable recognition ability for targeting MCF-7 breast cells due to the incorporation of MUC1 aptamers [104].

Gold-based nanoparticles: The biomedical field has widely accepted gold nanoparticles because of exceptional stability, low toxicity, high surface-to-volume ratio, and biological compatibility [105]. The use of aptamer-conjugated gold nanomaterials (Apt-AuNPs), which combine the unique benefits of aptamers with gold nanoparticles, has been extensively adopted in cancer treatment and diagnosis [106].

Yang et al. reported the use of apt-AuNPs in conjunction with graphene oxide for photothermal therapy of breast cancer [107]. Dou et al. [108] reported the use of aptamer-functionalized AuNPs-Fe₃O₄-GS capture probes for tracking circulating tumour cells in whole blood.

Organic-based system

Liposomal system: Liposomes are a promising drug delivery technology because of the high drug loading efficiency, low immunogenicity, low toxicologic profile, and superior biocompatibility [109]. For targeted cancer gene therapy, a recent attempt to package the CRISPR/Cas9 complex into liposomes functionalized with an aptamer [110]. Liang et al. created a lipopolymer based on aptamers to transport

CRISPR/Cas9 to specific tumours and control vascular endothelial growth factor (VEGFA) in osteosarcoma. The LC09 aptamer has the potential to enable the targeted delivery of CRISPR/Cas9 plasmids in this system, thereby reducing VEGFA expression and preventing lung metastasis and orthotopic osteosarcoma malignancy [111].

Micelle-based system: Micelle structure is another interesting class of organic nanomaterial based on aptamers. Because of the multivalent effect, this drug delivery method exhibits good aptamer binding to targets. A micelle-based system can therefore be developed for a wide range of bio-applications [112].

A cross-linked aptamer-lipid micelle system was created by Li et al. [113] in 2018 for target cell recognition that offers exceptional stability and specificity. In this simple method, an effective photoinduced polymerization process connected the aptamer and lipid segments to a methacrylamide branch [113].

Hydrogel-based system: Target-responsive DNA hydrogels are an aptamer-based organic nanomaterial system that has been applied extensively in pharmaceutical and biomedical fields. Target-responsive DNA hydrogels have outstanding mechanical properties and programmable features [114]. For possible drug release, the first adenosine-responsive hydrogel was created in 2008. This study used two oligonucleotide-incorporated polyacrylamides and carefully thought-out cross-linking oligonucleotides to create DNA nanohydrogels. The adenosine aptamer sequence was present in the DNA linker. The hydrogel will dissolve and the cross-links will break when the aptamer binds to target molecules that already contain adenosine. Accordingly, target-responsive drug release may be investigated using this method [115].

Challenges and overcoming methods in aptamer technology

Stability and nuclease resistance

Aptamers, especially aptamers based on RNA, are susceptible to degradation by nucleases. Enhancing stability and nuclease resistance is a significant challenge. Various modifications, such as chemical modifications or using modified nucleotides, can be used to increase the stability of aptamers. However, these alterations may impact aptamer binding properties and introduce additional complexities.

Identification of suitable aptamers from the aptamer library

As is well known, an aptamer sequence ultimately controls the 3-dimensional conformation, which in turn allows the aptamer to bind to its target. An aptamer library with highly varied sequences will include a wide variety of structurally unique aptamers. This diversity increases the likelihood of developing aptamers that bind tightly to desired targets

and contrasts with proteins that utilize 20 different amino acids to present diverse functional groups, while nucleic acids rely on only 4 bases (adenine, guanine, cytosine, and thymine/uracil) and have fewer functional group options. Consequently, aptamers have functional groups added that mimic the side chains of amino acids to broaden the chemical range and confer protein-like properties [116].

A pioneer in expanding the variety of aptamer sequences in a library is SomaLogic, a top business in aptamer creation. New functional groups, such as naphthyl, benzyl, tryptamino, and isobutyl hydrophobic side chains, were added at the 5' position of dUTP nucleotides. Slow off-rate changes in aptamers (SOMAmers), which were created utilizing these changed nucleotides. Therefore, aptamers modified with SOMAmer technology have been shown to improve aptamer selection compared to unmodified aptamers when targeting difficult proteins. The success rate rose from approximately 30% for standard aptamers to >90% for SOMAmers [117].

Target selection criteria for aptamers

Aptamers can be developed through an iterative process of selection and amplification from a large pool of randomized single-stranded DNA/RNA oligonucleotides (with $>10^{15}$ random sequences) called SELEX. This method allows for internalisation of therapeutic compounds into cells when aptamers are chemically conjugated [118] to compounds as well as aptamer binding to extracellular targets on the cell surface [119], from which therapeutic aptamers have been produced. Although the selection process for creating aptamers for either use is fundamentally the same, a higher level of success must be achieved with the cell-internalising aptamers. A crucial factor that needs to be considered for cargo efficient biological activity is endosomal escape and delivery to the cytosol.

Filtration process through the kidney

For effective therapeutic use, it is necessary to overcome the phenomenon of renal filtration of small molecule medications. Aptamers experience this difficulty because of the diminutive size. The size of an aptamer with a mass of 6–30 kDa is approximately 5 nm [120]. Even with stabilizing backbone changes, unmodified aptamers supplied intravenously are prone to fast renal filtration excretion, which reduces circulation time.

Aptamers can be chemically modified by the addition of large compounds, such as polyethylene glycol (PEG), liposomes, proteins, and organic or inorganic nanomaterials. Aptamers can also be multimerized. These modifications enable the aptamers to achieve an overall mass that exceeds the molecular weight cut-off for glomerular filtration (30–50 kDa).

To circumvent this problem we used further modifications as follows.

PEGylation

One of the most popular techniques for preventing renal filtration of aptamers is PEGylation. PEG is highly lipophobic (i.e., hydrophilic), a flexible, uncharged polymer that is frequently used with medicinal medicines to increase medication efficacy, prolong circulation time, and decrease reticulo-endothelial clearance [121].

The only aptamer-based drug that the FDA has currently approved is Macugen. Macugen utilizes PEGylation, a chemical strategy that grafts PEG modifications. PEG groups reduce clumping behaviour and heightens the solubility of conjugated parameters. For Macugen, PEGylation enables substantial enhancement of the plasma half-life (9.5–12.5 h) after intravenous delivery. Similarly, the vitreous humour half-life reached approximately 94 h upon subcutaneous administration owing to the PEG groups [122].

Chol-aptamer

An RNA aptamer with modifications to all pyrimidine nucleotides was previously shown to inhibit replication of hepatitis C virus (HCV). Researchers subsequently conjugated this aptamer to cholesterol, yielding a cholesterol-aptamer construct. When applied to cells, this cholesterol-linked aptamer exhibited efficient cellular uptake and halted HCV RNA amplification. Furthermore, systemic administration of the cholesterol-aptamer in a mouse model demonstrated good tolerability along with extended plasma circulation time [123].

Diacylglycerol (DAG) conjugation

DAG was conjugated at the 5' end of the VEGF aptamer by Willis et al. [124]. After being added to a liposome bilayer, aptamers having enhanced anti-VEGF activity were generated by this DAG-aptamer combination, both *in vitro* and *in vivo*. This DAG-aptamer-liposome combination showed a much longer plasma residency time than an unaltered aptamer [124].

Toxicity

Aptamers have a significant advantage over antibodies with respect to limited or non-immunogenicity. Following efficacy and stability assessments, a critical next stage in medication testing is the evaluation of immunogenic potential, toxicity, and adverse effects. The aptamer-based drug, pegaptanib sodium (Macugen), which is used to treat wet age-related macular degeneration, did not show any signs of being harmful in preclinical tests [120].

Although there is insufficient clinical evidence of the cytotoxicity of aptamers, research using different animal models exhibited minimal or no evidence of cardiac toxicity, liver damage, or renal toxicity. Non-specific absorption causes cardiotoxicity, which is a side effect of the widely used anti-cancer drug, DOX. PEGylated or liposomal DOX has not

been able to resolve this issue. In contrast to non-targeted administration, the aptamer-DOX conjugate is reported to minimize this cardiotoxicity and boost the efficacy of tumour suppression *in vivo* [125].

Targeting action of aptamer

Targeting the tumour-initiating cells that are developing and stem-like system

Most epithelia have an EpCAM, which is a glycosylated cell surface receptor that helps cells divide, migrate, and differentiate [126].

Most carcinomas, including the lung, breast, colon, pancreatic, and prostate, have high levels of EpCAM expression. This protein is also authorised as a biomarker for detecting circulating tumour cells and is implicated in the formation of stem-like tumour-initiating cells [38, 54, 127].

Focusing on intracranial brain cancer

The tight cerebrovascular endothelium that makes up the BBB, a physical barrier that poses a significant obstacle to the advancement of gene therapy drugs for brain tumours, the barrier hinders the flow of big macromolecules from the blood-to-the brain, including peptides and nucleic acids, which reduces the efficacy of treatments [128].

Jiang et al. recently explored transporting PTX, a highly potent chemotherapeutic for various cancers, including gliomas, across the BBB. Jiang et al. utilized AS1411, an aptamer targeting nucleolin receptors, as the guiding molecule [129]. Scientists made AS1411-PGG-PTX by connecting AS1411 and poly-(α -glutamyl-glutamine)-paclitaxel (PGG-PTX). This compound has two different functions. Incorporating PGG improved PTX solubility and diminished toxicity [130].

Addressing these challenges requires interdisciplinary collaboration and ongoing research efforts. Continued advances in aptamer technology have the potential to revolutionise various fields, ranging from theranostics to environmental monitoring and biosensing. By overcoming these challenges, aptamers can offer innovative solutions and contribute to the development of more efficient and targeted approaches in diverse applications.

Conclusion

Aptamer technology has developed tremendous potential as a tool centred on theranostics. Aptamer technology utilizes perspectives and various challenges in the design of aptamer technology for targeted cancer therapeutics. Aptamer technology is involved in the design of the aptamers and various fluorogenic aptamers, such as green aptamers, concluded the diagnostic approach. Various modifications, such as PEGylation and cholesterol-containing parameters, and many more to overcome challenges are particularly derived from the aptamer technology.

Various SELEX technologies targeted at selected molecules were presented in this article. SELEX technologies also involves various drugs that are used in chemotherapy studies, and we studied aptamers as biotherapy and chemotherapy agents and involved various types of aptamers.

We investigated the aptamer-exosome technology and how aptamer-exosome technology captures aptamers. We also investigated how exosomes diagnose aptamers and how exosomes can help with theranostic effects in specific systems.

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Authors' contributions

J.T. and M.Y. have made substantial contributions to the conception and design of the manuscript, acquisition, analysis,

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