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Review Article

Aptamer-based approaches for *in vitro* molecular detection of cancer

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Abstract

Cancer is typically associated with abnormal production of various tumor-specific molecules known as tumor markers. Probing these markers by utilizing efficient approaches could be beneficial for cancer diagnosis. The current widely-used biorecognition probes, antibodies, suffer from some undeniable shortcomings. Fortunately, novel oligonucleotide-based molecular probes named aptamers are being emerged as alternative detection tools with distinctive advantages compared to antibodies. All of the existing strategies in cancer diagnostics, including those of *in vitro* detection, can potentially implement aptamers as the detecting moiety. Several studies have been performed in the field of *in vitro* cancer detection over the last decade. In order to direct future studies, it is necessary to comprehensively summarize and review the current status of the field. Most previous studies involve only a few cancer diagnostic strategies. Here, we thoroughly review recent significant advances on the applications of aptamer in various *in vitro* detection strategies. Furthermore, we will discuss the status of diagnostic aptamers in clinical trials.

Keywords: Aptamer; Biosensor; Cancer detection; Tumor marker.

1. INTRODUCTION

Cancer, as one of the most important health problems, is mainly originated from some genetic or epigenetic alterations and typically represented as uncontrolled cell growth with abnormal production of various molecular products (1,2). Probing cancer-specific markers called tumor markers, which are mainly resulted by the alterations could be beneficial for cancer diagnosis and management. Therefore, utilization of efficient approaches for punctual and accurate tumor marker recognition is of great clinical significance (3).

The ability of the conventional tumor marker recognition strategies is restricted by the lack of proper detecting agents. Current widely-used biorecognition probes, antibodies, suffer from limited chemical modification, easy denaturation and degradation, animal-dependent production, poor penetration (due to large size), and immunogenicity (4,5). Fortunately, another class of molecular probes named aptamers, which can efficiently recognize a range of targets from small molecules to the whole intact cells, are being emerged as alternative detection tools with distinctive properties (5-7). Aptamers are typically obtained from an oligonucleotide (single-stranded DNA or RNA) library using a cyclic selection process known as the systematic evolution of ligands by exponential enrichment (SELEX) (8-11). The target binding occurs through their three-dimensional structure with reasonable affinity and specificity at low target concentrations.Their binding affinity is comparable to that of antibodies (9,12,13).

There is a growing interest in aptamer investigations. A search on Science Direct database (https://www.sciencedirect.com/) on January 10th, 2020, showed a progressive increase in the number of the review or research articles, in which their keywords, title or abstract include the word “aptamer” since the year 2000 (Fig. 1).

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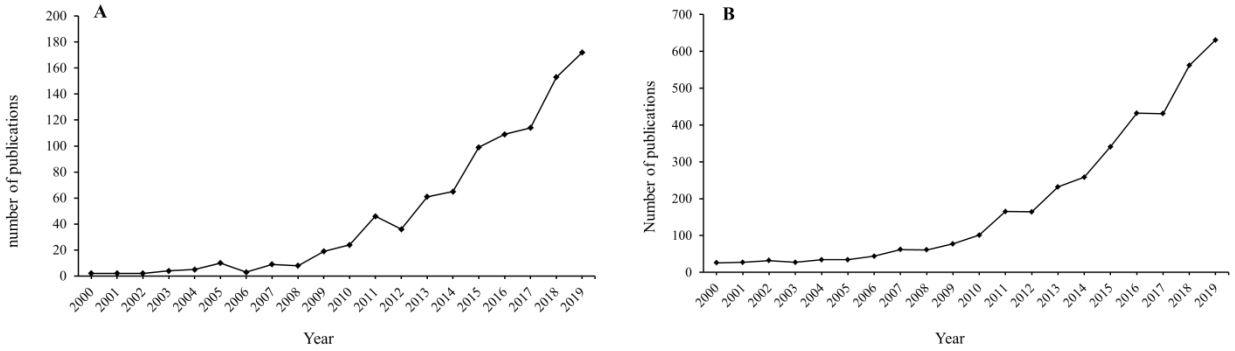

Fig. 1. Number of articles published each year in the field of aptamer since 2000. The result of a search on Science Direct database on 10 January 2020 presenting the number of the review or research articles, which their keywords, title or abstract include the word (A) "aptamer", and (B) "aptamer and biosensor", have progressively increased since 2000.

Several detection strategies have been introduced to date to detect oncological biomarkers both *in vitro* and *in vivo*. These strategies include tissue-related marker detection, enzyme-linked assays, flow cytometry, biosensing, and bioimaging. Aptamers could be used as the detection moiety in all of these approaches. However, the *in vitro* application of aptamers is more applicable since oligonucleotides would be degraded by various nucleases available in living systems. In some cases, the use of aptamer in place of traditional probes has certain advantages. For instance, because of the nucleic acid nature, immobilized aptamer probes in the plates of enzyme-linked assays could be simply reused for several experiments (14-16). In the case of biosensing, the conformational change of aptamers after binding to their intended targets makes these nucleic acid-based probes an appropriate tool for switchable systems.

In this study, we will comprehensively review a variety of aptamer-based detection techniques used to visualize and quantify tumors. However, our focus will be on recent advances in use of aptamer probes for *in vitro* detection of various cancers.

2. TUMOR-SPECIFIC APTAMERS FOR IN VITRO DETECTION OF CLINCAL SAMPLES

Aptamers have been extensively used *in vitro* to detect a wide variety of cancers. There are numerous reliable tumor-markers in cancer tissues, on circulating tumor cells (CTCs), and soluble in the bloodstream of patients, which can be detected utilizing appropriate probes for a dozen of purposes such as early cancer diagnosis, molecular monitoring of treatment progression, prognosis, detection of invasion and metastasis, and biochemical monitoring of recurrence.

Quantification of nucleotide aptamer can be easily achieved by quantitative real-time polymerase chain reaction (qRT-PCR). However, since aptamers could be easily chemically manipulated, various signaling tools including fluorescent agents, biotin/streptavidin- horseradish peroxidase (HRP) conjugates, and electro-chemicals could be implemented in aptamer selection techniques to give accurate and sensitive quantitative probes (17). In this regard, researchers developed a variety of aptamer-based technologies for *in vitro* cancer diagnosis. Traditional quantifying strategies that have been adjusted to implement nucleotide aptamers as the targeting moiety consists of aptahistochemistry, aptamer-based enzyme-linked immunosorbent assay (ELISA), qRT-PCR, and aptamer-based flow cytometry. More recently, researchers have offered various innovative platforms to enhance sensitivity and accuracy of target detection including a variety of aptamer-based molecular biosensing techniques (18,19).

3. APTAMER APPLICATION IN TRADITIONAL CANCER DETECTION METHODS

The use of aptamers as a traditional *in vitro* cancer diagnostic tool has been frequently

reported in numerous studies. These include the detection of tumor markers on histopathological tissue sections and those present in circulation.

3.1. Tissue-marker detection (aptahisto-chemistry analysis)

Along with the hematoxylin and eosin (H&E) staining of tumor tissue slides as the gold standard of cancer detection, histopathological immunostaining is also used to improve the sensitivity of recognition, predict prognosis, and determine subtypes of various cancers. In comparison with the application of antibodies in immunostaining of formalin-fixed paraffin-embedded tissue sections, aptamers are smaller, therefore penetrate more easily and efficiently (20). It has been also reported that the nonspecific signal of the necrotic area is efficiently omitted using aptamer probes (20). Visualization is usually carried out using 3′-diaminobenzidine tetrahydrochloride, as a horseradish peroxidase substrate, or fluorophores-based techniques (21,22). Fortunately, both of them are simply applicable in aptamer-based staining of tissue slides, because of the adaptable nature of nucleotide aptamers (4,5). As shown in Fig. 1, an increasing number of studies have reported the use of aptamer in histopathological staining of tumor sections.

In an investigation, Wang *et al.* generated a fluorescent dye-labeled aptamer named Wy-5a against prostate cancer, which could efficiently differentiate high-risk groups with metastasis from benign prostatic hyperplasia (22). In another study, Duan *et al.* introduced a fluorophore Cy5-labelled-aptamer having excellent selectivity for recognition of the metastatic prostate cancer (23). In 2017, Huang *et al.* produced a Cy5-labelled-aptamer against prostate cancer, capable of binding to its target in the microenvironment of tissue sections (24). In another work, Stuart *et al.* developed a biotin-labeled vitronectin binding aptamer to stain human breast cancer tissue slides. They also realized that in contrast to the monoclonal antibody which preferentially binds to the extracellular matrix and vessel walls, where vitronectin can become multimerized, the aptamer directly binds to tumor cells, with much lower binding to extracellular matrix (25). Shigdar and her colleagues reported their isolated aptamer against epithelial cell adhesion molecule, a type I glycosylated membrane protein, which is more sensitive and specific than existing antibodies for the detection in breast cancer tissue slides. This aptamer showed no non-specific signal with tissues negative for expression of epithelial cell adhesion molecule (25). Using biotinylated anti-estrogen receptor aptamers and HRP-streptavidin, Ahirwar *et al.* reported their successful attempt in estrogen-receptor (ER)-positive breast cancer tissues (26). The results demonstrated that their aptamer-based histopathological tissue staining can be used efficiently for proper grading of ERα expression (26). In another study by using 3′-diaminobenzidine tetrahydrochloride and HRP, Han *et al.* developed an aptamer against matrix metalloproteinase 2 and confirmed its potential in binding to the target on tissue sections (21). Yuan *et al.* selected and labeled (with fluorescent dye Cy5) an aptamer with the ability to distinguish lymph node tissue sections with colorectal cancer metastasis using cell-SELEX on metastatic colorectal cancer cell lines (27).

3.2. Detection of circulating markers

Detection of circulating tumor markers has great importance in management of the disease because cancer tissues are not always accessible. Sometimes, cancer cells are located in remote parts of the body making them unreachable (28). Also, the removal of tissue samples *via* a biopsy or surgical procedure is considered an invasive procedure with noticeable post-operational complications (29). In addition, repeated sampling is almost impractical; therefore, histopathological-based methods are not appropriate for monitoring of cancer progression or treatment. Unlike tissue-specific markers detected in histopathological immunostaining, some circulating tumor markers could be spotted at the early stages of cancer, allowing early detection and intervention (30,31).

Using appropriate aptamers, circulating tumor-markers including soluble proteins, analyst, and CTCs can be effectively spotted

through a dozen of methods converting detection to an assessable signal. These strategies include qRT-PCR, ELISA, flow cytometry, and molecular biosensing.

3.2.1. qRT-PCR

Quantification of nucleic acids is simply and reliably achievable using qRT-PCR (32,33). The exact amount of the initial nucleic acids is calculated using a standard curve drawn with known concentrations of the intended DNA (34). Consequently, by implementing this technique, it is possible to directly quantify a nucleotide aptamer which bind to the target of interest. In this way, the identified amount of each aptamer will be proportional to the amount of its specific target, excluding the need for expensive labeling of the detection aptamer. However, despite the use of this method in other areas (35-39), few studies on cancer have been performed in this regard (40,41).

In a study, Li *et al.* could simply detect serum biomarkers of patients with lung cancer using magnetic carboxyl agar beads as the aptamer selection method and qRT-PCR as the quantification strategy. They showed that their pioneering aptamer-based system led to a much more sensitive diagnosis than the conventional antibody-based diagnostic methods (40). In a recent attempt to select reliable DNA aptamer against serum of colorectal cancer patients, Li *et al.* implemented qRT-PCR in the selection procedure, the assessment of the affinity and selectivity, and bio-detection in human blood samples (41).

3.2.2. Aptamer-based enzyme-linked assay

ELISA has been considered as a reliable quantitative assay in traditional cancer diagnostics, which uses antibody probes as the tumor marker recognizing moiety. Introducing nucleic acid aptamers as novel molecular recognition agents with some superior features comparing to traditional antibodies led to development of an aptamer-based ELISA named enzyme-linked aptamer sorbent assay (ELASA). Like the original antibody-based assay, ELASA can be performed in various modalities including direct, indirect and sandwich assay (Fig. 2). In addition to its general advantages, the specific benefit of this method is that the immobilized capturing aptamer can be simply reused by heating and refolding after each experiment (42). Also, there are a variety of innovative ways other than heating to make the plates practically reusable, including the use of chaotropic reagents, surfactants, or chelating agents (14-16). To validate a DNA aptamer-based sandwich ELISA, Lee *et al.* succeed in recognizing a well-known tumor-marker named lipocalin-2 (LCN2) in the serum of patients with hepatocellular carcinoma. This assay platform benefits from a sandwich pair of aptamers including an immobilized NH_2-modified capture-aptamer and an HRP-labeled reporter-aptamer. The researchers claimed that the developed assay platform is capable of quantifying low-medium abundance tumor-markers presented in patient serum ranging from ng to µg/mL (43). Ahirwar *et al.* established an aptamer-based ELISA to show the potential of their proper selected aptamer in probing target of interest related to human breast cancer (26). In another study, Ferreira *et al*. successfully designed an aptamer-antibody sandwich ELISA to identify and quantify mucin 1 (MUC1) in solutions; therefore they could establish innovative diagnostic tools against this biomarker for detection of various epithelial tumors (44). Two innovative aptamer sandwich-based microfluidic ELISA assays for recognizing free prostate specific antigen (FPSA) in patients with prostate cancer were developed by Jolly *et al.*, where a DNA aptamer was used as the capturing probe and an antibody or a lectin was utilized as the detecting agent to quantify the target (FPSA) by chemiluminescence (45).

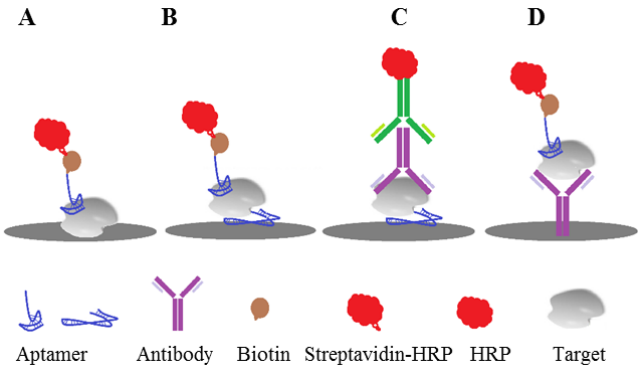

Fig. 2. Schematic picture of enzyme-linked aptamer sorbent assay. (A) Direct target-aptamer ELASA; (B) sandwich aptamer-target-aptamer ELASA; (C) sandwich aptamer-target-antibody ELASA; and (D) sandwich antibody-target-aptamer ELASA, Enzyme-linked aptamer sorbent assay; HRP, horseradish peroxidase.

In a recent study, Zhu *et al.* used an enzyme-labeled anti-MUC1 aptamer to establish an aptamer-based sandwich configuration of ELASA. In the proposed assay, the immobilized aptamer is fabricated on a gold electrode surface and the reporting aptamer is conjugated with HRP (46). In another study, Kavosi *el al.* utilized sandwich-type enzyme-linked aptamer incorporation with electrochemical biosensors and gold nanoparticles as a triple signal amplification method to PSA (47).

3.2.3. Aptamer-based flow cytometry

Fluorophore labeled aptamers that are designed against cell surface tumor markers can be simply measured using flow cytometry technique. By implementing the cell-SELEX technique, our group validated a fluorophore labeled ssDNA aptamer against the B-lymphocyte antigen (CD20). The expression of CD20 is various in different types of acute lymphoblastic leukemia (ALL), which helps to differentiate various types of the disease including B-cell precursor-ALL (30-50%), and mature B-cell ALL (80-90%). Using flow cytometry and fluorescein isothiocyanate-labeled probes, we could ultimately distinguish B-cell with different expression of CD20 in real patient specimens of bone marrow (48). In another work on isolating DNA aptamer probes by a novel pipeline approach, Yang *et al.* could successfully define the phenotype of normal hematopoietic cells and acute myelogenous leukemia (AML) in patient samples by flow cytometry (49). In order to distinguish different types of cells (T- and B-cells) in patients with blood cancer, Shangguan *et al.* could develop a set of aptamer probes based on molecular differences between cells using cell-SELEX technique. Assessed by flow cytometry, the isolated aptamers with fluorescein isothiocyanatelabel could specifically recognize various cells in bone marrow aspirates, proposing specific tools for cancer detection and therapy (50). In an attempt made to generate a multivalent aptamer probe specifically binding to the B-cell receptors in patients with leukemia and lymphoma, Mallikaratchy *et al.* employed flow cytometry on tumor cell lines and real clinical specimen to show the ability of their selected aptamer in bivalent staining of the target of interest. They reported that their obtained bivalent aptamers could distinguish between patients with non-B-cell malignancy, chronic myeloid leukemia, and those with B-cell malignancy, B-chronic lymphocytic leukemia, and hairy cell leukemia (51). In another study, Sefah *et al.* reported a properly isolated aptamer obtained using the cell-based selection technique with the ability to target AML cells in both cell culture and real clinical samples by flow cytometric assay. They also developed two other aptamers recognizing targets related to differentiation of monocytes. Their results showed the potential of cell-SELEX and flow cytometry in recognizing subclasses of AML, and introduced new potent cell-membrane markers (52). Tan *et al.* introduced an innovative method based on aptamer-modified fluorescent silica nanoparticles to specifically target leukemia cells. The principle of the strategy is the formation of amid links between amino groups of amino-labeled aptamers and the carboxyl group of the carboxyl-modified fluorescent silica nanoparticles. Their final assessment was through flow cytometry and fluorescence microscopy to show the sensitivity and specificity of the isolated probes (53). Using flow cytometry analysis, Zhang *et al*. showed the power of the Cy5-labeled RNA aptamer targeting CD30 in detecting anaplastic large cell lymphoma cells and Hodgkin's lymphoma cell lines both in cultured cells and mixed cell specimens (54).

3.2.4. Aptamer-based detection of CTCs

Tsai *et al.* proposed an integrated microfluidic system based on aptamer technology to establish an authentic way to spot ovarian CTCs which have extremely low concentration in peripheral blood circulation. The first step of the procedure is elimination of erythrocytes followed by depletion of white blood cells. Then the ovarian CTCs could be captured using appropriate aptamers. The authors claimed that their innovative approach yields a higher recovery rate for CTCs than the traditional methods using antibodies (55). Zheng *et al.* introduced a novel barcode particle technology using various dendrimer-amplified

aptamer probes in order to capture a wide variety of CTCs in the peripheral bloodstream, promising new perspectives in cancer detection using CTCs (56). Zamay *et al.* developed specific aptamer probes which were capable of detecting CTCs in peripheral blood of patients with metastatic lung cancer. They claimed that the aptamer could be rapidly and specifically generated for each individual patient, opening up the opportunity of personalized diagnostics (57).

In an investigation, Li *et al.* developed an interesting method taking advantage of aptamer-functionalized hydrogels to catch CTCs and restriction endonucleases to efficiently release them (approximately 99%) (58). In another work on patients with non-small cell lung cancer, a microfluidic assay was successfully set up based on a cocktail of synergistic aptamers. Implementing aptamer cocktails enhanced the performance of CTCs catch compared to solitary probes (59).

4. VARIOUS TYPES OF SENSING STRATEGIES IN APTAMER BIOSENSORS

Biosensing technology is emerging as an important strategy to visualize and quantify biochemical targets of interest. A biosensor, that calculates the concentration of a specific analyte by converting the molecular detection event into a computable signal, can be attached with various high-affinity bioprobes allowing efficient detection of intended biomolecules (18). Biosensors commonly composed of the following components: an analyte detecting part named bioreceptor, a signal transducer part converting detection of the analyte into a computable signal, electronic part for signal amplification, and a display part that visualize the outcomes (18).

Using antibodies as traditional detecting agents, several studies have been done on biosensors aimed at cancer detection, most of them based on the sandwich detection system (19,60). According to the general advantages of aptamer probes, recent studies have tried to replace antibodies with their aptamer counterparts. In addition, the conformational change of aptamers after binding to their intended targets makes these nucleic acid-based probes an appropriate tool for developing switchable biosensing systems. Various sensing technologies including electrochemical (using impedimetric, voltametric, potentiometric, amperometric, electrochemiluminescence, and *etc.*), and optical sensors (using fluorophores, quantum dots, surface plasmon resonance, intercalating dyes, bioluminescent, and *etc.*) along with a variety of nanomaterials like metallic nanoparticles, graphene, graphene oxide, carbon nanotubes, and nanowires of different agents have been sophisticatedly incorporated to set up authentic bio-recognition tools to date(18,19). Schematic views of aptamer-based biosensing are depicted in Figs. 3 and 4.

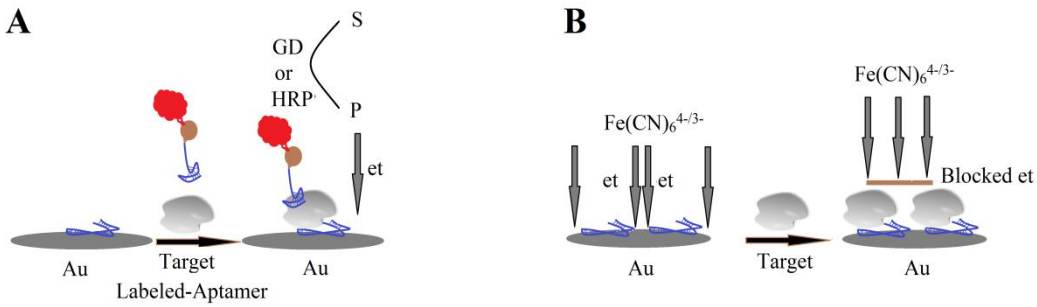

Fig. 3. Schematic picture of electrochemical biosensing. The interaction between aptamers and targets impact electron transfer to the gold nanoparticle coated electrode, making signals proportional to the detected targets. (A) Conjugated aptamer-based approach in electrochemical biosensing and (B) free aptamer-based approach in electrochemical biosensing. HRP, Horseradish peroxidase; GD, glucose dehydrogenase; et, electron transfer; Au, gold; $Fe(CN)_6^{4-/3-}$, Ferrocyanide.

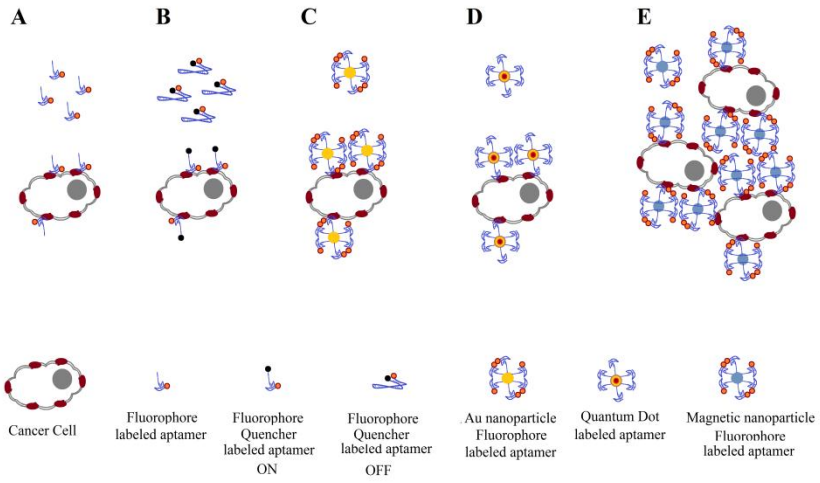

Fig. 4. Schematic picture of fluorescence biosensing. Various types of fluorescence biosensors are depicted as described in the picture. (A) Simple fluorophore-labeled aptasensors; (B) fluorophore/quenchere-based aptasensors; (C) gold nanoparticle/fluorophore-labeled aptasensors; (D) quantum dot aptasensors; and (E) magnetic nanoparticle/fluorophore-labeled aptasensors.

4.1. Electrochemical aptamer-based biosensors

In 2020, Zhou *et al.* introduced an electrochemical impedance sensor based on free aptamers, gold electrodes and ferrocyanide ($Fe(CN)_6^{4-}$) to detect serum CD44 biomarkers in the range of 0.1-1000 ng/mL with a detection limit of 0.087 ng/mL(61). In another study, Safavipour *et al.* implemented TiO_2 nanotubes-reduced graphene oxide (TiO_2 nanotube-rGO) to obtain MUC1 aptamer-based electrochemical biosensor to recognize breast cancer cells (MCF-7) (62). Presenting an electrochemical platform by gold electrodes and using a HER2-specific DNA aptamer, Sunil K. Arya *et al.* launched a powerful electrochemical aptasensor for breast cancer classification. Their developed aptasensor was able to detect human epidermal growth factor receptor 2(HER2) from 1 pM to 10 nM in unprocessed patient serum, promising future success in developing similar aptasensors for other possible protein tumor markers (63). In another study, a glassy carbon electrode (GCE) containing multiwall carbon nanotubes and poly glutamic acid was used by Yazdanparast *et al.* to set up a dual aptamer sandwich sensor detecting the breast cancer cell line, MCF7. The immobilizing probe was a MUC1 detecting aptamer and the recognizing part was an MCF7-binding aptamer with a silver nanoparticle label. In order to follow the electrochemical signal of the silver nanoparticles, the obtained sandwich electrochemical system used differential pulse anodic stripping voltammetry (64). Reporting a bipolar electrode system that benefits from electrochemiluminescence technology and using two aptamers detecting nucleolin, Motaghi *et al.* were able to sensitively detect cancer cells in the acceptable linear range of 10-700 cells and selection limit of 10 cells (65). In an attempt to develop an accurate and efficient platform capable of tumor-marker detection, Nie *et al.* implemented electrochemiluminescence assay along with a non-enzymatic amplification and aptamer-triggered emitter release strategy. Therefore, they were able to improve the analytical accuracy of breast cancer biomarker recognition (66). Amouzadeh Tabrizi *et al.* could effectively capture and release promyelocytic leukemia cells (HL-60) from real patient serum using a novel nanomotor containing manganese oxide nanosheets-polyethyleneimine with nickel/gold nanoparticles (MnO2-PEI/Ni/Au) and HL-60 specific aptamers. The suggested electrochemical platform was able to detect cancer cells in the acceptable linear range of 25-5×10^5 cells and the selection limit of 250 cells (67). Preparing the first complementary metal oxide semiconductor (CMOS) biosensor for electrochemical tumor detection *via* a peptide

aptamer-based microneedle exclusively binds to vascular endothelial growth factor (VEGF), Song *et al.* could directly spot the biomarker in unprocessed patient blood. The presented system, which benefits from a two-step capacitance-to-digital converter visualizing small changes in VEGF concentrations, could efficiently spot VEGF in the range of 0.1 to 1000 pM (68). In another study, an elaborate electrochemical sandwich-based aptasensor containing tetrahedral DNA nanostructures-aptamers on the outside of gold electrode as the capturing probe, the nanozyme Mn_3O_4 with the second aptamer as the first amplifying nanoprobe, and an HRP/complementary DNA (cDNA) nanoprobe to further intensify the signal was generated to specifically and sensitively detect the breast cancer HER2 biomarker. Results demonstrated that the proposed platform can operate in a wide linear concentration range (0.1-100 ng/mL) (69). Implementing gold nanoparticles, platelet-derived growth factor specific aptamer, and electrochemical measurement technology, Hasanzadeh *et al.* established an aptasensor assay capable of recognizing MCF7 breast cancer cell line. The researchers claimed that the developed electrochemical biosensor is applicable in straight patient plasma specimens (70). A complimentary list of similar works is presented in Table 1.

4.2. Fluorescent aptamer-based biosensors

A combination of cell-SELEX derived aptamer and gold nanoparticles were used to develop a strip biosensor against Ramos cancer cells in real human bloodstream by Liu *et al.* The proposed strip biosensor system provided a promising fast quantitative way to detect circulating tumor cells with a reasonable sensitivity (103). In an investigation, Bayat *et al.* constructed a fluorescent aptamer-based biosensor for detection of CD70-positive tumor cells and isolated a DNA aptamer against tumor marker CD70 (104). Gedi *et al.* designed an on-chip aptamer-antibody based cancer-detecting platform containing a CA125-specific DNA aptamer, an immobilized antibody against CA125 and a three-dimensional network of carbon nanotubes. The authors demonstrated that this on-chip platform is superior to other approaches like graphene oxide-based and ELISA assays (105). An aptamer gold-nanostructures immunochromatographic strip was introduced in a study aimed at quantifying a new tumor marker named N-glycolylneuraminic acid by Gong *et al.* in 2018. The platform provided rapid and sensitive detection of the target of interest with a quantitative detection limit of 5.38 ng/mL (106). An innovative method based on bifunctional aptamer and catalytic hairpin assembly was designed and successfully applied by Liu *et al.* to efficiently detect cancer cells in clinical specimens. Division of the fluorophores from their related quenchers in the presence of captured cancer cells leads to signal production. The measurement of targets in real patient samples did not need any additional process (107).Luo *et al.* established a switchable cytosensor using hairpin DNA, including MUC1 aptamer and initiation strand of the catalytic hairpin assembly-mediated Y-junction nicking enzyme assisted signal amplification circuits to identify MUC1 positive breast cancer cells (MCF-7) (108). Using photoactive knowledge and aptamer-based recognition technology, Mazhabi *et al.* set up a novel photoelectrochemical cytosensor identifying HeLa cervical cancer cell lines with a limit of detection of 5 cells/mL. In the proposed assay, g-C3N4-AgI nanocomposites were utilized as light-sensitive resources (109). In another work, molybdenum disulfide (MoS_2) nano sheets and carcinoembryonic antigen (CEA)-specific aptamers with fluorescence labels were used by Zhao *et al.*, to construct a fluorescent biosensor detecting CEA tumor marker in the patient specimens. Following probe-target interaction, a conformational change will be occurred leading separation of aptamer from the surface of the nanosheets and then the production of the fluorescence signal. The linear range of detection was reported to be 100 pg to 100 ng/mL with the limit of 34 pg/mL (110). In an observational case-control clinical trial started in June 2015, 100 patients with bladder cancer are being monitored using a colorimetric aptamer biosensing approach to follow bladder cancer biomarkers (111). A complimentary list of similar works is presented in Table 1.

Table 1. Complementary list of studies describing aptamer-based biosensors in cancer diagnostics.

Target	Transducer	Electrode/ Nanoparticle	Aptamer-label	Refs
Mucin 1 (MUC1)	Surface Plasmon Resonance	Au nanoparticles		(71, 72)
	Surface Plasmon Resonance	Magnetic nanoparticle		(73)
	Electrochemiluminescence	Au nanoparticles-deposited glassy carbon electrode (depAu/GCE)		(74)
	Differential pulse voltammetry (DPV)	negatively charged ITO electrode		(75)
VEGF	Surface Plasmon Resonance	Carboxyl-coated polystyrene microsphere		(76)
	Cyclic voltammograms (CVs) /amperometric	Glassy carbon electrode (GCE)	Ag/Pt bimetallic nanoclusters	(77)
	Electrochemiluminescence/electrochemical impedance spectroscopy (EIS)	Cys-CdS:Eunanocrystals (NCs) modified GCE		(78)
PDGF	Potentiometric a field-effect transistor (FET)	Carbon Nanofiber Carboxylatedpolypyrrole-coated hybrid carbon nanofibers (CPMCNFs)		(79)
	Linear sweep voltammetry (LSV)	Au nanoparticle		(80)
	Differential pulse voltammetry (DPV)	Molybdenum selenide-graphene composites		(81)
	differential pulse voltammetry (DPV)	Au nanoparticle		(82)
	Fluorescence resonance energy transfer (FRET)	poly-L-lysine (PLL)-coated Au nanocomposites	TAMRA	(83)
Carcinoembryonic (CEA)	cyclic voltammograms (CVs): terminal deoxynucleotidyltransferase (TdT) CA ,chronoamperometry	Au nanoparticle electrode Platinum electrode, and an Ag/AgCl electrode		(84)
	Cyclic voltammetry (CV)/SWV ,square wave voltammetry	Au nanoparticle (AuNPs)	rolling circle amplification (RCA)	(85)
	Photoelectrochemical (PEC)	CdS/TiO2/ITO PEC electrode		(86)
	Electrochemiluminescence	ZnS-CdS/MoS2/glass carbon electrode		(87)
	Fluorescence	AuNPs	AgNCs	(88)
HER2	Voltammetric electrochemical impedance spectroscopy (EIS)/ differential pulse voltammetry (DPV)	Graphene oxide (GO) reduced graphene oxide-chitosan (rGO-Chit)		(89)
	Differential pulse voltammetry (DPV)	Au nanoparticles	ferrocene-labeled DNA/Au nanospheres (FcNS)	(90)
	Non-Faradic impedance spectroscopy (nFIS)	Capacitor microelectrodes		(91)
HepG2	Impedimetric Electrochemical impedance spectroscopy (EIS)/ cyclic voltammograms (CVs)	Au nanoparticles gold nanoparticles (AuNPs) modified the glassy carbon electrode (GCE) surface	(Fe_3O_4/MnO_2/Au@Pd)	(92)
	Differential pulse voltammetry (DPV)	HRP and platinum nanoparticles (PtNPs)		(93)
	Differential pulse voltammetry (DPV)	HRP and MIL-101@AuNPs		(94)

Table 1.(continued)

Target	Transducer	Electrode/ Nanoparticle	Aptamer-label	Refs
MCF-7	Voltammetric Electrochemical impedance spectroscopy (EIS)/ cyclic voltammograms (CVs)	Au nanoparticles/graphene oxide		(95)
	Fluorescence	Graphene oxide (GO)	Quantum dots coated Silica nanoparticles	(96)
	Chronoamperometry (CA)	Au nanoparticles		(97)
K562 leukemia cells	Voltammetric Electrochemical impedance spectroscopy (EIS)/ differential pulse voltammetry (DPV)	Magnetic nanoparticles		(98)
	Voltammetric Electrochemical impedance spectroscopy (EIS)/ cyclic voltammograms (CVs)	Hemin/RGO/Au Nanoflower		(99)
	Fluorescence	Graphene oxide (GO)	Quantum dots coated Silica nanoparticles	(96)
HL-60 cells	Electrochemiluminescence	Au nanoparticles/ Graphene/ Cs ITO glass (Au NPs-GA-CS/ITO)		(100)
	Fluorescence	Graphene oxide (GO)	Quantum dots coated Silica nanoparticles	(96)
CD44	Fluorescence	GO/Au nanoparticles		(101)
hepatoma SMMC-7721 cell	Surface Plasmon Resonance	Au nanoparticles	Magnetic nanoparticles	(102)

4.3. Colorimetric aptamer-based biosensors

There are a few recent studies on developing colorimetric aptamer-based biosensors for cancer biomarker recognition. For instance, in 2020, Dong *et al.* developed a highly sensitive colorimetric aptasensor against the $VEGF_{165}$ in human serum (112). Also in another recent study, Heydari Shayesteh and Ghavami stablished a label-free colorimetric aptamer-based biosensor for highly sensitive determination of PSA using gold nanoparticles and a cationic polymer (113). Xu *et al.* developed a colorimetric aptasensor against K-Ras, which showed a wide linear range (0.01-150 nM) and the detection limit of 10 pM (114). In another study, Ahirwar *et al.* could successfully establish a nanoparticle-based colorimetric aptasensor recognizing the human estrogen receptor alpha in breast cancer (115).

5. CONCLUSION

Application of aptamers in cancer diagnostic has been studied for a long time and developed to meet the urgent need of an authentic probe in various detection approaches. Based on the distinctive advantages of nucleotide aptamers compared to antibodies including higher environmental stability (for all purposes), better tumor penetration properties (for aptahistochemistry), easier chemical modification (for all methods using labeled aptamers like fluorescent-aptamers), and the capability of conformational change for switchable systems (especially for biosensors), aptamers are now considered as promising tools for cancer diagnostics. Also unlike antibodies, label-free oligonucleotide aptamers could be simply quantified using qRT-PCR as an affordable quantifying approach. However, the *in vitro* application of aptamers is more applicable since oligonucleotides would be degraded by various nucleases available in living systems. Various types of oligonucleotide aptamers have been successfully isolated and employed in numerous cancer biorecognition strategies *in vitro*, including tissue-marker detection, enzyme-linked assay, flow cytometry, and biosensing. Various chemical and reporting labels like fluorophores, biotin, HRP, *etc*. leads toutilizing biomarker specific aptamers as detection moiety of various cancer diagnostic strategies. In particular, some multifunctional

nanoparticle-based labels are developed for enhancing detection capability. In this study, the progress of aptamer applications in the field of cancer detection is summarized over recent years. Aptamer-based strategies are becoming gradually more common. As shown in Fig. 1, the aptamer-based investigation is a high-speed growing field, indicating the special place of this method in future studies. Although, the number of aptamer-guided biosensing studies has suddenly elevated since 2012, but its commercial applications are limited. The investigations will lead to improved existing aptamer-based strategies for biosensing and bioimaging, and promising better performance in the future. Until quite recently, the practical properties of two promising aptamer-based methods in cancer diagnostics are being evaluated in clinical trials.

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7. CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest for this study.

8. AUTHORS' CONTRIBUTION

All authors contributed equally in this work.

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