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DNA nanotechnology-based strategies for gastric cancer diagnosis and therapy

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ABSTRACT

Gastric cancer (GC) is a formidable adversary in the field of oncology. The low early diagnosis rate of GC results in a low overall survival rate. Therefore, early accurate diagnosis and effective treatment are the key to reduce the mortality of GC. With the advent of nanotechnology, researchers continue to explore new possibilities for accurate diagnosis and effective treatment. One such breakthrough is the application of DNA nanotechnology. In this paper, the application of exciting DNA nanomaterials in the diagnosis and treatment of GC is discussed in depth. Firstly, the biomarkers related to GC and the diagnostic strategies related to DNA nanotechnology are summarized. Second, the latest research progress of DNA nanomaterials in the GC targeted therapy are summarized. Finally, the challenges and opportunities of DNA nanomaterials in the research and clinical application of GC are prospected.

1. Introduction

Currently, the incidence of gastric cancer (GC) ranks fifth among global malignancies and is continuously increasing [1]. Due to the non-specific early symptoms of GC and limited screening conditions, the majority of patients are diagnosed at an advanced stage, resulting in suboptimal treatment outcomes. Therefore, the development of appropriate early diagnostic methods and effective treatment strategies is crucial for reducing GC mortality. Biomarkers are measurable molecules within an organism that provide important information about individual physiological status and disease progression. The detection of biomarkers helps achieve early disease diagnosis and personalized treatment, holding significant clinical application prospects. In recent years, various biomarkers associated with GC have been discovered, and the detection of these markers provides valuable reference for the accurate diagnosis of GC.

Unlike most systemic cancers, GC is a localized tumor, and local metastasis is a significant factor that hinders curative treatment. Therefore, the diagnosis of GC not only involves whole-body imaging examinations but also requires local imaging examinations. Clinically, the main method for locating gastric tumors is endoscopy, followed by histological examination to confirm malignant transformation. However, limited efficiency and accuracy and complex operation of current clinical methods cause physical and psychological distress to patients. There is an urgent need for efficient, simple, and non-invasive methods for early diagnosis and treatment. Currently, various nanotechnologies with high sensitivity and multifunctional diagnostic and therapeutic capabilities have been developed for tumor marker detection and tumor treatment, offering a promising strategy for GC diagnosis and treatment. The limitations of current clinical applications urgently require the translation of basic research into relevant clinical applications.

Deoxyribonucleic acid (DNA), as the most important genetic material of life, encodes the genetic information of most organisms, including human beings. Due to its good biocompatibility and programmability, DNA has become a biopolymer for building biomedical materials. Over the past three decades, two - and three-dimensional DNA nanostructures, such as triangles [2], tiles [3], polyhedral [4], and nanospheres [5], have been constructed by hybridizing multiple cleverly designed DNA strands. These multi-dimensional DNA nanostructures with high biocompatibility, enhanced nuclease resistance and high drug loading ability have a good biomedical application prospect. Some nucleic acid amplification techniques, such as polymerase chain reaction (PCR) [6], hybridization chain reaction (HCR) [7], strand

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displacement amplification (SDA) [8], helicase-dependent amplification (HDA) [9] and rolling circle amplification (RCA) [10], have been introduced into DNA nanotechnology, and have been widely used for the sensitive detection of biomarkers [11], which is expected to be applied in the diagnosis of clinical disease indicators.

The Watson-Crick base pairing principle endows DNA molecules with properties such as recognition and construction of functional nanostructures, which enables a wide range of applications in targeted cancer diagnosis and therapy. The application of some functional nucleic acids (e.g., Aptamer; DNAzyme) provides a broader research direction for the development of DNA nanotechnology.

The design principles of DNA nanotechnology for cancer diagnosis mainly include three aspects: (1) Target recognition: Aptamer can be used for target detection by recognizing proteins or exosomes specifically expressed by cancer cells. The identification of nucleic acid biomarkers is based on the principle of complementary base pairing. (2) Signal amplification. A variety of nucleic acid amplification techniques or target enrichment techniques are mainly used to amplify the target signal. (3) Signal output. In cancer diagnosis, the optical, electrical or catalytic properties of nanomaterials are used to construct the signal output mode of various sensors.

Due to the complex environment *in vivo*, the design principles of DNA nanostructures for cancer therapy have higher requirements. (1) Good biocompatibility. Good biocompatibility can avoid the toxic side effects of the nanostructure itself on the body. Due to its high biocompatibility, DNA is widely used in the construction of drug delivery carriers. (2) High stability. The high stability can avoid drug release due to degradation of nanocarriers during blood circulation. The rational design and modification of DNA nanostructures significantly improve the stability of DNA nanostructures and enhance their potential applications in biological environments. In addition, the combined application of some non-nucleic acid nanomaterials also improves their biological stability. (3) Targeting. At present, the most common treatment for cancer is chemotherapy. Most chemotherapeutic drugs are small molecules, not targeted, and have obvious side effects. Cancer cell targeting receptors such as aptamer or folic acid have been functionalized on DNA nanostructures to enhance their targeting. (4) Ability of controlled drug release. Controlled drug release triggered by intracellular stimuli is also a current research hotspot. (5) Efficient treatment capacity. The combination of chemotherapy, gene therapy or phototherapy can improve the efficacy of cancer treatment.

This article mainly reviews the application of DNA nanotechnology in the diagnosis and treatment of GC (Fig. 1). The different designs and progress of nucleic acid nanoprobes targeting different GC biomarkers are first described in detail, and then the recent progress of nanomaterials in the treatment of GC is discussed. Finally, the challenges and limitations from basic research to clinical application of DNA nanotechnology in the diagnosis and treatment of GC are discussed, and it is envisaged that it can be applied to the clinic in the near future.

2. GC biomarker diagnosis

The detection of biomarkers can be quantified and can be used to reflect physiological function and pathological manifestations, which is an important indicator for early diagnosis and disease monitoring. In this chapter, we summarize the various types of nucleic acid detection techniques for different GC-related biomarkers.

2.1. CEA

Carcinoembryonic antigen (CEA) is a tumor-associated antigen that was first extracted from colon cancer and embryonic tissues by Gold and Freedman in 1965 [12]. It is an acidic glycoprotein with human embryonic antigen characteristics. CEA is a broad-spectrum tumor marker. Although the specificity is low, it has important clinical value in the differential diagnosis, disease monitoring, and efficacy evaluation of

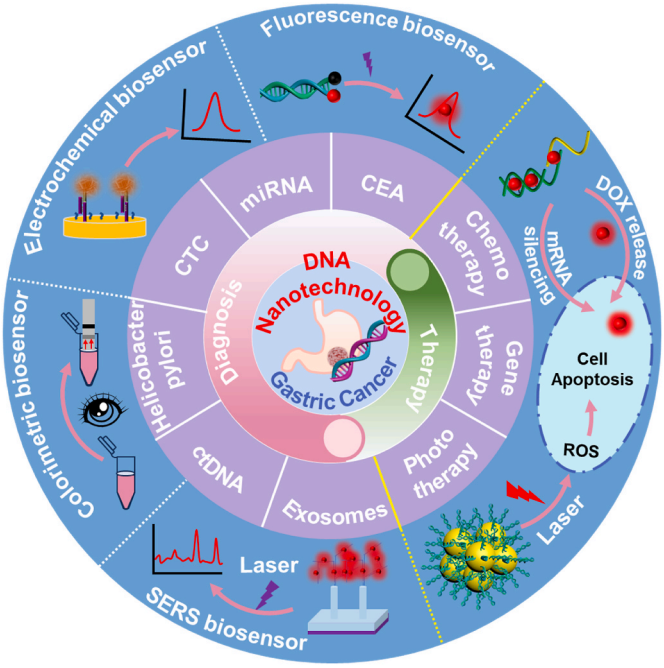

Fig. 1. DNA Nanotechnology-based strategies for GC diagnosis and therapy.

malignant tumors. In recent years, biosensors based on DNA nanotechnology have attracted extensive research from researchers due to their high sensitivity, high specificity, low cost and simple operation. According to the different ways of signal transduction, biosensors used to detect CEA mainly include optical sensors and electrochemical sensors.

2.1.1. Colorimetric-based DNA biosensor

In DNA-based optical biosensors, colorimetric method has attracted much attention due to its low cost, simple operation and fast analysis speed. Nanomaterials suitable for colorimetric detection mainly include Au nanoparticles (AuNPs) [13] and DNAzyme [14]. Because of its simple operation without any precision instrument, it can be used for on-site quick testing, and has the potential for commercial application.

AuNPs with clear physical and chemical properties have a wide range of applications in the fields of chemical and biological sensing, disease diagnosis and therapeutics. The morphology and color of AuNPs are closely related to their optical properties. The core size of AuNPs changes, and the relative absorption peak changes, which in turn affects the color of AuNPs. This feature is the basis for the development of colorimetric biological detection methods [15]. Based on the CEA responsive dissociation of AuNP-DNA aptamer complex, Wang et al. constructed a sensitive, accurate and simple colorimetric method for CEA detection [13]. As shown in Fig. 2A, the sulfhydryl group modified single-stranded (ss) CEA target binding aptamer is bound to AuNP surface by Au-S bond. The AuNP-DNA aptamer complex is uniformly dispersed in high saline solution and has high stability. In the presence of CEA, DNA aptamers target CEA, resulting in conformational changes and dissociation from AuNP. The free AuNP accumulates in a highly saline solution and changes in color from red to blue. Under optimal experimental conditions, the detection limit of CEA is 3 ng mL^{-1}. The detection method is simple, rapid and universal, and can meet different detection requirements.

Based on the Hg^{2+}-assisted peroxidase-like activity of AuNPs, Liu et al. designed a colorimetric method on the test strip for threshold-readout detection of CEA [16]. The colorimetric sensing platform includes a sandwich-type magnetic bead (MB) capture module based on the binding between CEA aptamer and CEA, a signal transduction module composed of CEA triggered HCR system with tunable Hg^{2+} combining capacity and a colorimetric readout module based on the free

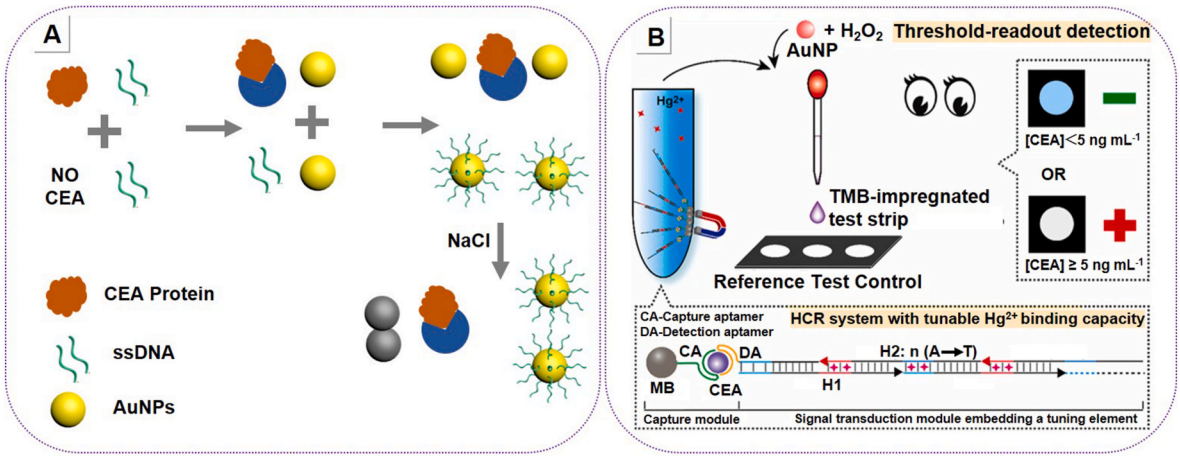

Fig. 2. Colorimetric-based DNA biosensor for CEA detection. Schematic illustration of unmodified AuNPs and CEA aptamer -based colorimetric method (A) and HCR-based colorimetric sensing platform includes a sandwich-type MB capture module and a colorimetric readout module based on the free Hg^{2+}-assisted peroxidase-like activity of AuNP (B). Reproduced with permission [16]. Copyright 2022, Elsevier.

Hg^{2+}-assisted peroxidase-like activity of AuNP (Fig. 2B). Only when CEA is combined with the capture aptamer fixed on the surface of MB and the detection aptamer at the same time, the sandwich-type MB capture module can play a role, ensuring the high specificity of detection. Then the initiator domain of detection aptamer triggers the HCR system capable of binding Hg^{2+}. The MB sample was removed by magnetic separation, and the remaining free Hg^{2+} in the solution was used to enhance peroxidase-like activity of AuNPs. The test strips impregnated by 3,3′, 5,5′-tetramethylbenzidine (TMB) as an indication of AuNP activity. The CEA concentration was inversely related to the residual free Hg^{2+} in the solution and AuNP activity. When the CEA concentration is below a certain threshold, the strip appears blue. When the CEA concentration was higher than the threshold, the blue color of the test strip disappeared. In addition, the threshold concentration can be adjusted by changing the number of T bases chelating Hg^{2+} in the HCR hairpin probe. The colorimetric method exhibits high sensitivity, low cost, convenient and fast detection, and reliable results, which further enhances the clinical application value. In addition, the possibility of detecting other disease markers can be realized by targeting changes in the aptamer.

2.1.2. *Fluorescence-based DNA biosensor for CEA detection*

Fluorescence-based biosensor is one of the most frequently used methods in the field of optical sensing because of its high sensitivity, good repeatability, strong versatility, simple operation and non-destructive [17]. Since some detected targets have no optical output signal, the biometric identification process is converted into optical signal by incorporating fluorescence technology into the biosensor [18]. DNA nanomaterials can be used as signal amplification probes, fluorescent signal generators, and signal conversion carriers. In this chapter, we discuss the progress of DNA fluorescence biosensing in the detection of CEA.

DNA nanotechnology can be used to construct a signal amplification probe for CEA detection. Wang et al. designed a smart DNA

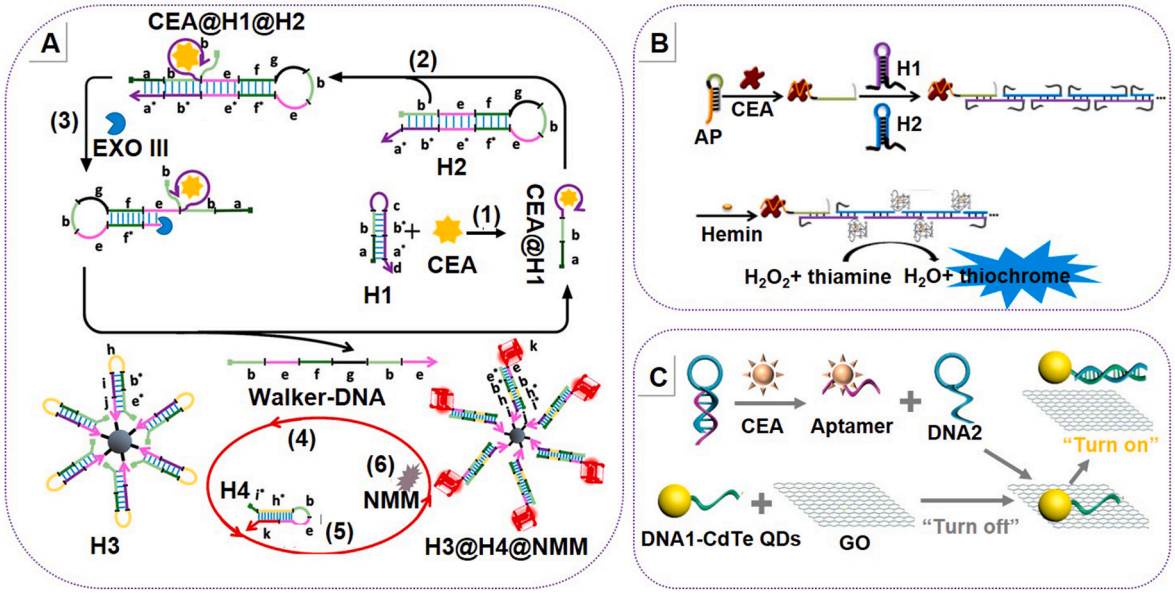

Fig. 3. Fluorescence-based DNA biosensor for CEA detection. Schematic illustration of the DNA nanomachine based on the ERA and DNA walker cascade amplification strategy for CEA detection (A), HCR and G-quadruplex DNAzyme based enzyme-free detection platform (B), and fluorescent biosensor based on the interaction of DNA modified with quantum dot (QD-DNA) and GO (C) for CEA detection. Reproduced with permission [19,20]. Copyright @ 2017 American Chemical Society; Copyright 2020, Elsevier.

nanomachine with high sensitivity and specificity for CEA detection by introducing exonuclease (Exo) III-assisted target recycling amplification (ERA) and DNA walker cascade amplification strategy [19]. The illustration for the principle of the smart DNA nanomachine for the CEA detection was shown in Fig. 3A. CEA aptamers were introduced into the ERA process for specific recognition of CEA. Once the CEA aptamer recognizes and binds CEA, ss walker-DNA strands are generated with the assistance of CEA technology. Subsequently, the walker-DNA strand moved spontaneously on silica microspheres modified with hairpin strand 3 (H3), facilitating the hybridization of hairpin strand 4 (H4) to H3 and formation of G-quadruplex, which produced an ultra-sensitive fluorescence signal with the help of N-methylmesoporphyrin IX (NMM). Interestingly, a single walker-DNA triggered autonomous hybridization of multiple H3 and H4, which in turn generate multiple signaling molecules, enabling sensitive detection of CEA. The specificity and practicability of the smart DNA nanomachine were confirmed by selective experiments and real samples, indicating that it has great potential for clinical application.

To further simplify the operation process, a variety of enzyme-free isothermal DNA amplification techniques, such as HCR and strand displacement amplification (SDA), have been developed. Feng et al. reported an enzyme-free CEA detection platform based on HCR and G-quadruplex DNAzyme [20]. The schematic is shown in Fig. 3B, once binding to CEA, the conformation of CEA aptamer changes and the initiator is released, which in turn triggers reciprocal hybridization of hairpin probe 1 and hairpin probe 2 to form a nicked linear DNA duplex. With the participation of hemin, the G-rich ends of hairpin probes 1 and 2 self-assemble to form a hemin/G-quadruplex DNAzyme that catalyzes the formation of fluorescent products from thiamine. The enzyme-free fluorescence detection platform has high sensitivity and high specificity and simple operation, which is especially suitable for clinical diagnosis. In addition, Yu et al. reported an SDA-based label-free, enzyme-free ratio fluorescent biosensor for CEA detection [21]. CEA can trigger a conformational change of hairpin fragment containing the CEA aptamer to release the G-quadruplex sequence, leading to enhanced Thioflavin T (THT) fluorescence. At the same time, the fuel DNA strands split silver nanoclusters (AgNCs) pair, resulting in a decrease in the fluorescence of AgNCs. The experimental results show that the detection ability of the ratio fluorescence sensor is better than that of the single fluorescence detection strategy.

In addition, some inorganic nanomaterials with luminescence quenching ability, biocompatibility and low toxicity, such as AuNPs and graphene oxide (GO), have been combined with DNA nanotechnology to construct fluorescent biosensors [22–25]. Wang et al. reported a fluorescent biosensor based on the interaction of DNA modified with quantum dot (QD-DNA) and GO [24]. As shown in Fig. 3C, CEA triggers the unwinding of the CEA aptamer-DNA2 duplex. And the released DNA2 from aptamee-DNA2 duplex binds to QD-DNA on GO, allowing the fluorescence signal to be restored. Alternatively, Wang et al. constructed a biosensor for CEA detection by taking advantage of the surface-enhanced fluorescence properties between AuNPs and Ag nanoclusters (AgNCs) [25]. Specifically, DNA-modified AuNPs and DNA-modified silver nanoclusters were linked by a third DNA strand, the CEA binding aptamer via base-complementary pairing. The proximity of AuNPs enhances the fluorescence signal of silver nanoclusters. However, once CEA is added to the system, the CEA aptamer competitively binds to CEA, making Au and AG inaccessible, resulting in a weakened fluorescence signal. Based on the detection principle, the system can be used for the detection of CEA. The experimental results show that the system has a high sensitivity for CEA detection, and has a high application prospect in the field of bioanalysis.

2.1.3. Electrochemical-based DNA biosensor

Electrochemical biosensor is a kind of biosensor based on electrochemical principle, which is used to detect and analyze biological molecules or biological processes. Compared with fluorescent biosensors, the key advantage of electrochemical transduction is that it does not require fluorescent labeling, simple operation, and low cost. In addition, electrochemical biosensors combine the advantages of high specificity, high sensitivity and low background signal in the biological measurement process, and become an important tool for research and application in the field of biological analysis [26,27].

In order to achieve high sensitivity detection of CEA, Zou et al. constructed an electrochemical biosensor based on 3D DNA nanotweezers composed of highly ordered self-assembly of five oligonucleotide strands (N1, N2, L1, L2, T1) [28]. As shown in Fig. 4A, in the absence of the target, the five DNA strands bind to each other to form a closed nanotweezers and block the recognition site of the target. Once the target CEA is added to the nanotweezers homogeneous solution, the binding of CEA to the linker segments leads to the opening of the nanotweezers and the release of the T1 strand. The released T1 strand was captured by the ferrocene (Fc) labeled signal probe E1 fixed on the gold electrode to form a E1/T1 duplex. Subsequently, the signal probe E1 was gradually digested by EXO III, which led to the decrease of electrochemical signal and the release of T1 strand. Subsequently, the released T1 strand is involved in the cyclic cutting of the next E1 strand, thereby achieving signal amplification and increased sensitivity. The target recognition process of the electrochemical biosensor is carried out in homogeneous solution, which simplifies the experimental operation and improves the reaction efficiency. At the same time, the recycling of the T1 strand greatly improves the detection sensitivity and reduces the detection cost. This sensing strategy achieves the convenient and sensitive detection of CEA, which can provide reference value for the design of detection strategies for other biomarkers.

To detect sensitively CEA in human blood accurately and specifically, Lin et al. constructed an enzyme-free electrochemical biosensor including DNA tetrahedrons (TDNs) and catalytic hairpin assembly (CHA) [29]. Specifically, TDN with CEA aptamer/trigger DNA duplex (TDN-duplex) and another TDN with hairpin strand 1 (TDN-H1) were all fixed on gold electrodes (Fig. 4B). In the initial state, the trigger strands were silenced by complementary CEA aptamer, such that there's no interaction between two TDNs. In the presence of CEA, CEA competitively binds the CEA aptamer, resulting in the release of CEA aptamer. The free trigger strands, still anchored to TDNs, were specific binding and opening H1 on TDN-H1, forming trigger/H1 duplex. Upon addition of methylene blue (MB) modified H2, MB-modified H2 competitively binds H1, releasing trigger strand. The released trigger strand can open the next H1 and initiate CHA cycle, allowing more and more MB to be immobilized on the gold electrode, which in turn enables signal amplification detection. The enzyme-free electrochemical biosensor has simple design, low cost and strong practicability. In addition, the experimental results show that the biosensor has high sensitivity, high specificity, good repeatability and high stability in the field of target detection, which has broad application prospects in the clinical diagnosis and prognosis of cancer.

Common enzymes are proteins or RNAs. However, DNAzyme was obtained by *in vitro* screening and is an enzyme that cleaves the target RNA. Compared to the protein or RNA enzyme, DNAzyme has the advantages of simple operation, high stability, good specificity and low cost, and has been widely used in the development of biosensors. Kraatz et al. developed a DNAzyme-mediated DNA walker on hollow carbon nanospheres (HCS) support as electrochemical immunosensor for the detection of CEA and α-fetoprotein (AFP) [30] (Fig. 4C). In the presence of CEA (or AFP), the Arm1-Ab1 can specifically bind to Arm2-Ab2 via antigen/antibody (Ag/Ab) interactions. With the addition of Mg^{2+} cofactor, the Mg^{2+}-dependent DNAzyme is activated and cyclically cleaves the substrate strand (SP) immobilized on the HCS surface, which in turn produces a large number of iDNA strands. The released iDNA strands and HP strands bind to CP strands immobilized on AuNP modified indium tin oxide electrode (Au/ITO), forming Y-junction DNA. Under the catalysis of Nt.*Bbv*CI, the 5′-CP was cleaved and separated from electrode surface, resulting in a decrease in the electrochemical

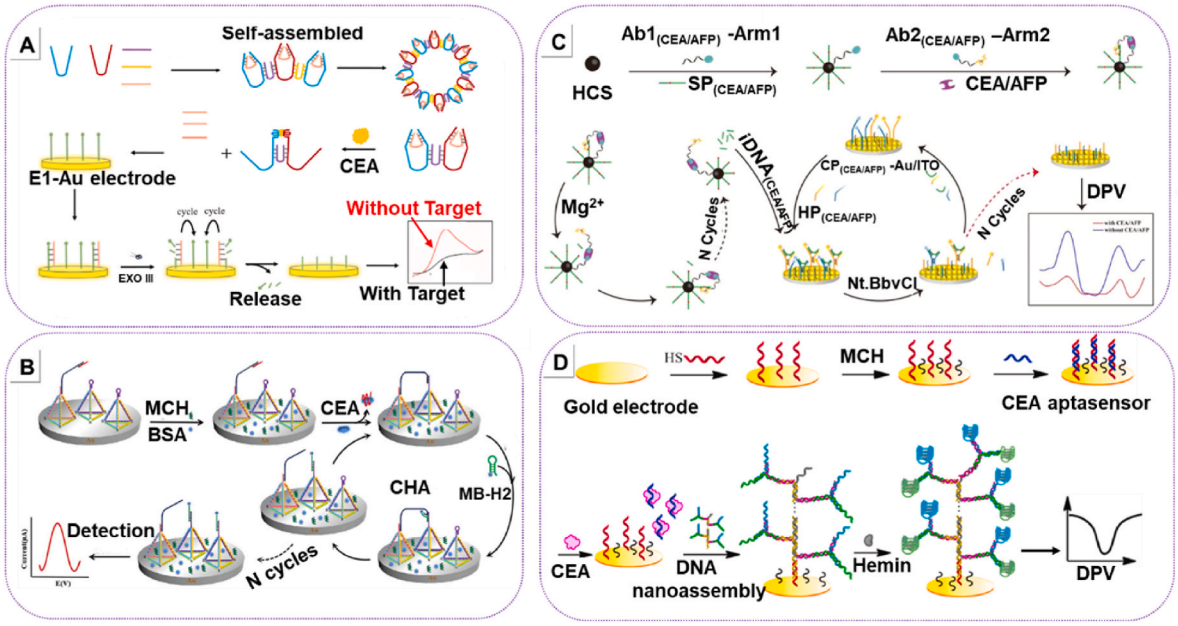

Fig. 4. Electrochemical-based DNA biosensor for CEA detection. Schematic illustration of (A) electrochemical biosensor based on 3D DNA nanotweezers composed of highly ordered self-assembly of five oligonucleotide strands; (B) enzyme-free electrochemical biosensor based on TDNs and CHA; (C) DNAzyme-mediated DNA walker on hollow carbon nanospheres (HCS) support as electrochemical immunosensor and (D) label-free G-quadruplex DNAzyme-catalyzed electrochemical aptasensor based on a multifunctional dendrimer-like DNA nanoassembly for CEA detection. Reproduced with permission [28–31]. Copyright @ 2022 Elsevier; Copyright @ 2021 Elsevier; Copyright @ 2020 Elsevier; Copyright @ 2021 Elsevier.

signal. Research results show that the electrochemical immunosensor has good selectivity, high sensitivity, enhanced stability, high accuracy, high reproducibility and good universality, and has great application potential in the early diagnosis and prognosis of clinical tumors.

Upon binding of the G-quadruplex to hemin, the catalytically functional G-quadruplex DNAzyme is obtained. To obtain a very prominent and applicable tool for clinical CEA detection and cancer diagnosis, Cui et al. reported a label-free G-quadruplex DNAzyme-catalyzed electrochemical aptasensor based on a multifunctional dendrimer-like DNA nanoassembly [31]. As shown in Fig. 4D, the DNA nanoassembly was self-assembled from a DNA concatemer containing the CEA aptamer and multiple Y-shaped DNA units with G-quadruplex DNAzyme through hybridization. The sulfhydryl modified cpDNA strands were captured on the gold electrode and hybridized with the CEA aptamer. In the presence of CEA, the CEA aptamer binds CEA and makes cpDNA exposed to capture DNA nanoassembly. Upon the addition of hemin, the high load of G-quadruplex shows strong peroxidase-like activity, resulting in an amplified electrochemical signal. The electrochemical aptasensor has the advantages of simple operation, high sensitivity, high accuracy, stable storage, good reproducibility and low cost, which greatly meets the requirements of early diagnosis of cancer. This experiment provides a more sensitive and simple method for the detection of malignant tumors, which can promote the development of tumor detection and has broad development prospects in the future clinical field.

The detection sensitivity of various biosensors mentioned above to CEA were listed in Table 1. The detection sensitivity of CEA in current clinical practice were listed in Table 2. At present, the Elecsys CEA Kit (Roche) is commonly used in clinical practice with the highest accuracy, and its detection limit is 0.6 ng/mL. Most of the biosensors mentioned in Table 1 are more sensitive than Elecsys CEA Kit (Roche).

Table 1
The detection sensitivity of various biosensors to CEA.

Method	Biosensor	Detection Range	Detection Limit	Reference
Colorimetric detection	AuNPs and CEA aptamer-based DNA biosensor	10–120 ng/mL	3 ng/mL	13
	HCR-based DNA biosensor	4–25 ng/mL	0.19 ng/mL	16
Fluorescence detection	ERA and DNA walker cascade amplification strategy-based DNA biosensor	10 pg/mL ~100 ng/mL	1.2 pg/mL	19
	HCR and G-quadruplex DNAzyme based enzyme-free detection platform	0.25–1.5 nM	0.2 nM	20
	QD-DNA and GO-based DNA biosensor	5–250 ng/mL	1.17 ng/mL	24
Electrochemical detection	3D DNA nanotweezers-based DNA biosensor	10 fg/mL ~50 ng/mL	4.88 fg/mL	28
	enzyme-free electrochemical biosensor based on TDNs and CHA	1–30000 pg/mL	0.04567 pg/mL	29
	DNAzyme-mediated DNA walker on HCS support	1 pg/mL ~1000 ng/mL	0.3 pg/mL	30
	label-free G-quadruplex DNAzyme-catalyzed electrochemical aptasensor	2–45 ng/mL	0.24 ng/mL	31

2.2. MiRNA

MicroRNA (miRNA) is a kind of endogenous non-coding RNA, which participates in a variety of biological processes, including cell proliferation, differentiation, and development. Aberrant expression of miRNA is closely related to the occurrence of GC. and it is a relevant tumor

Table 2
The detection sensitivity of clinical CEA detection kits.

Clinical Human CEA Kit	Method	Detection Range	Detection Limit
Human CEA (Carcinoembryonic Antigen) ELISA Kit (Finetest, EH0090)	Enzyme-linked immunosorbent assay (ELISA)	0.156–10 ng/mL	0.094 ng/mL
Elecsys CEA (Roche)	Electrochemiluminescence	0.3–1000 ng/mL	0.6 ng/mL
Human CEA ELISA Kit (abcam, ab264604)	ELISA	78.13–5000 pg/mL	24.68 pg/mL
Carcinoembryonic Antigen (CEA) Kit (Wondfo)	Immunochromatography	2.5–400 ng/mL	2.5 ng/mL
Carcinoembryonic Antigen (CEA) ELISA Kit (LMAI Bio)	ELISA	19.531–1250 pg/mL	8.0 pg/mL
VIDAS CEA (S)	Enzyme-linked fluorescent Assay (ELFA)	0.5–200 ng/mL	0.5 ng/mL

marker of GC. To date, a variety of miRNAs aberrated in GC, such as miRNA-21, miRNA-106a, miRNA-135b, etc., have been identified and applied in preclinical studies [32].

2.2.1. *Colorimetric-based DNA biosensor*

Studies have found that the expression of miRNA-135b and miRNA-21 is increased in gastric epithelial cells in the process of gastric inflammation and carcinogenesis, which can be used as a biomarker for the diagnosis of GC [33–35]. Lateral flow immunoassay (LFIA) is a kind of intuitive visual diagnosis strategy based on antigen-antibody reaction, which is usually applied to the detection of proteins such as antigens or antibodies. Recently, LFIA has also been applied to genetic detection [36]. However, the technology can't accurate detection target genes at low concentration. In order to achieve the ultrasensitive detection of target genes, LFIA was combined with molecular amplification technology to improve its detection sensitivity. Lim et al. designed and developed an isothermal amplification-based lateral flow biosensor (IA-LFB) as visual *in vitro* diagnostic platform to detect miRNA expression levels in blood [37]. The detection principle of IA-LFB responds to the miRNA is shown in Fig. 5A. In the presence of miRNA, the biotin modified capture probe was bind to miRNA, forming capture probe/miRNA duplex. The captured miRNA hybridizes to circular DNA and initiates RCA. The obtained RCA products was then bound to FAM labeled reported probes. Finally, LFB was incubated in complex buffer containing RCA products. Streptavidin on TL of test strip specifically binds biotin modified RCA products, while FAM-modified reported probes can be combined with AuNPs modified with anti-FAM antibodies, resulting in the appearance of TL band. In the absence of the target, the TL band could not be observed. IA-LFB has good sensitivity and selectivity, and can accurately distinguish the overexpression of miRNA-135b and miRNA-21 in the blood of healthy people and patients with different stages of GC, which is helpful for the early diagnosis and prognosis of GC.

2.2.2. *Fluorescence-based DNA biosensor*

Due to the low cost and high detection sensitivity and high

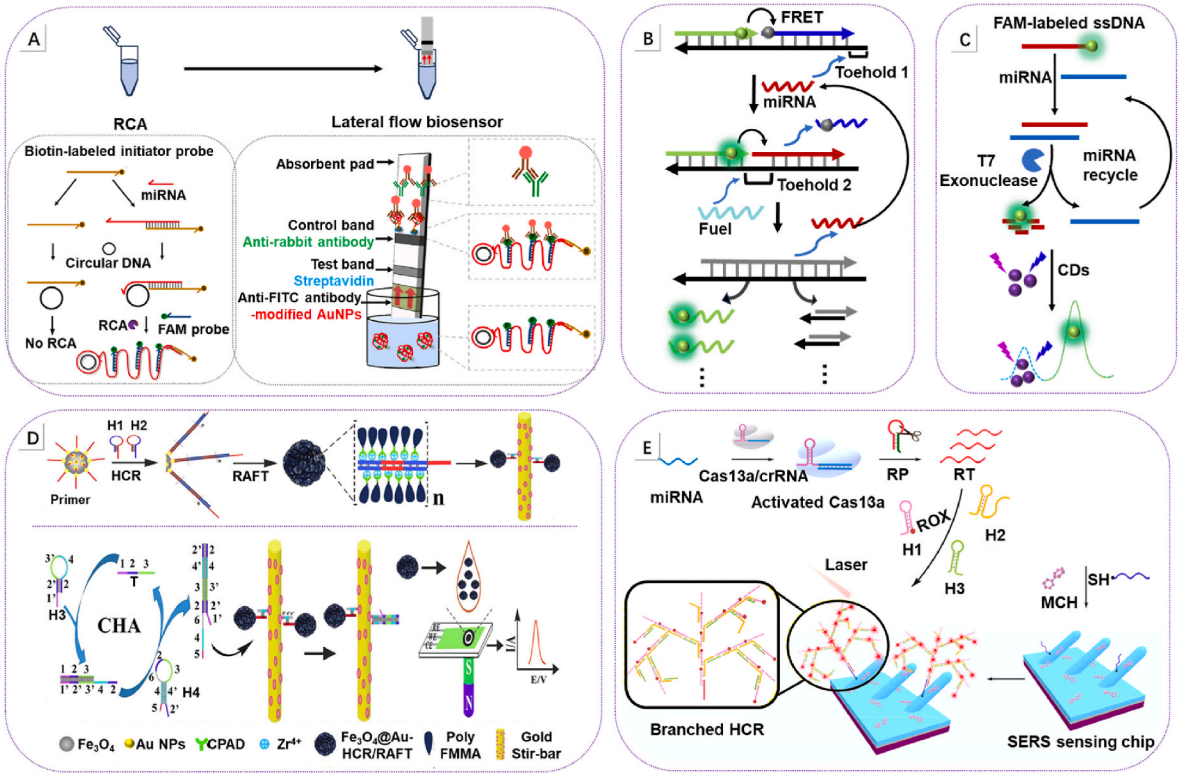

Fig. 5. DNA biosensor for miRNA detection. (A) The detection principle of IA-LFB responds to the miRNA. Reproduced with permission [37]. Copyright @ 2022 Elsevier. (B) Schematic illustration of fluorescence signal amplification technology using a combination of hydrogel and fuel-assisted DNA cascade amplification reaction for the detection of circulating miRNAs. Reproduced with permission [40]. Copyright @ 2022 Elsevier. (C) The ratiometric fluorescence strategy based on CDs and FAM fluorophore using T7 exonuclease mediated cyclic signal amplification technology for the detection of miRNA-21. Reproduced with permission [41]. Copyright @ 2020 Elsevier. (D) The detection principle of electrochemical sensing platform based on CHA, HCR, and reversible addition fragmentation transfer (RAFT) polymerization multiple signal amplification techniques for early detection of miRNA. Reproduced with permission [44]. Copyright @ 2022 Elsevier. (E) Schematic illustration of a signal amplification strategy (Cas13a-bHCR) based on CRISPR/Cas13a system and branched hybridization chain reaction on SERS-active silver nanorod sensor chip for ultrasensitive SERS detection of miRNA markers in GC. Reproduced with permission [50]. Copyright @ 2023 Elsevier.

specificity, hydrogels-based biosensor is expected to be promising biomedical diagnosis platform [38,39]. However, the sensitivity of these sensors cannot meet the needs of miRNA detection at low concentrations in body fluids. To address the clinical diagnostic challenge, Lim et al. developed a fluorescence signal amplification technology using a combination of hydrogel and fuel-assisted DNA cascade amplification reaction for the detection of circulating miRNAs in the serum of GC patient [40]. A shown in Fig. 5B, the fuel stimulant powered amplification (FSP) probes are attached on hydrogel via photo-crosslinking. In the initial state, the fluorescence of DNA1 is quenched by the adjacent quencher modified on the DNA2. Upon the addition of target miRNA, the quencher modified DNA2 was replaced by toehold 1-mediated strand replacement reaction, and the fluorescence signal was recovered. Under the action of fuel, miRNA can be competitively replaced, and then trigger the next cycle to realize the amplification and detection of fluorescence signal. The enzyme-free hydrogel-based fuel-assisted DNA cascade amplification reaction can specifically and sensitively detect the overexpression of miR-135b and miR-21 in blood samples of GC patients, which is expected to be applied to the diagnosis and prognosis of GC.

Fluorescence biosensor is widely used because of high sensitivity and high selectivity. However, the strategy based on single fluorescence emission may be disturbed by the external environment and produce false positive signals. To correct for possible interference factors from the environment, Weng et al. designed a ratiometric fluorescence sensor with carbon dots (CDs) and FAM dual-emission system for the quantitative detection of GC biomarker miRNA-21 [41]. The specific amplification mechanism mediated by T7 exonucleases is illustrated in Fig. 5C. In the absence of miRNA-21, FAM-modified ssDNA was adsorbed and quenched by CD. Once miRNA is added, miRNA-21 binds to FAM-modified ssDNA to form an RNA/DNA duplex that is cleaved by T7 exonuclease. The reduced binding affinity of the cleaved FAM-modified ssDNA and CDs results in the dissociation of FAM in solution. Finally, the ratio fluorescence of FAM and CD was used for miRNA quantification. The ratiometric fluorescence strategy with high accuracy, high selectivity and good repeatability, able to distinguish miRNA-21 expression level in clinical blood samples between healthy people and patients with gastrointestinal tumor.

CLDN18 are the main components of the tight junction chain of cells, which act as a physical barrier to prevent ions and water from freely passing through the paracellular space of the epithelium or endothelium. The CLDN18 gene has two spliceosomal forms: CLDN18.1, which is mainly expressed in the lung, and CLDN18.2, which is mainly expressed in the stomach [42]. The increased accessibility of CLDN18.2 in tumors makes CLDN18.2 a promising therapeutic target for gastrointestinal tumors. However, due to its high similarity to CLDN18.1, the preparation of CLDN18.2-specific antibodies has become an important challenge. Hence, based on the high similarity between CLDN18.1 and −18.2, CLDN18.2 RNA would be a better target for detection. Shen et al. reported a phosphorothioate and 2′-O-methyl modified molecular beacon (MB) probes for the detection of CLDN18.2 RNA [43]. The modifications of phosphorothioate and 2 ′-O-methyl on MB probe increase the stability, which avoid the generation of false positive signals. The fluorescein and quencher were modified at 5-end and 3-end of the MB, respectively. In the absence of target, the fluorescence signal was quenched by adjacent quencher. Once upon the CLDN18.2 RNA, the fluorescence signal increased with the opening of the MB probe. This MB probe was shown to discriminate CLDN18.2 in multiple model cells with high resolution and specificity. In addition, the MB probe successfully detected CLDN18.2 RNA in circulating tumor cells (CTC), which is expected to help more GC patients with high CLDN18.2 expression to receive timely treatment.

2.2.3. Electrochemical-based DNA biosensor

Electrochemical detection is also a common method for miRNA detection. Kheirollahi et al. designed a sensitive electrochemical miRNA sensing platform based on CHA, HCR, and reversible addition fragmentation transfer (RAFT) polymerization multiple signal amplification techniques for early detection of miRNA markers in GC [44]. As shown in Fig. 5D, firstly, blackberry-like magnetic DNA/ferrocene methyl methacrylate (FMMA) nanospheres were constructed using HCR and RAFT technologies. In this DNA nanosphere, each dsDNA fragment is labeled with a large amount of electroactive FMMA, which amplifies the electrochemical signal and improves the sensitivity of the detection. Then the blackberry-like DNA nanospheres modified on gold stir-bar were also replaced by DNA duplexes generated by the target-triggered CHA reaction. Finally, the nanosphere probe was magnetically enriched to amplify the signal for electrochemical sensitive detection. This electrochemical detection method is highly sensitive, reproducible, specific and simple to operate, especially suitable for the detection of miRNA in biological samples. It has a wide application prospect in the early detection and therapeutic evaluation of clinical GC.

Ghanbarian et al. reported an electrochemical biosensor based on gold/magnetic nanocomposites combined with bidirectional probe recognition for ultra-sensitive detection of miRNA-106a [45]. Firstly, Fe_3O_4 nanoparticles were sequentially coated with N-trimethylchitosan (TMC) and gold nanoparticles to form three-layer nanocomposites, and then coated with a layer of streptavidin by carbodiimide hydrochloride/N-hydroxysulfosuccinimide (EDC/NHS). The P1 probe was immobilized on the nanoparticles by streptavidin-biotin interaction. At the same time, the biotin-modified P2 probe was immobilized in the screen printed carbon electrode (SPCE) working region covered by streptavidin. In the presence of target miR-106a, the target would capture the gold magnetic nanoprobe and hybridize with P2 immobilized on the SPCE electrode, resulting in an electrochemical signal. The electrochemical sensor has good selectivity, specificity, short detection time, good repeatability and no need for sample pretreatment, which is an ideal method for clinical evaluation of miRNA.

A single miRNA may be related to a variety of diseases, which is not conducive to clinical diagnosis [46]. It has been reported that miR-106a is an oncogenic circulating miRNA that is up-regulated in GC [47]. In contrast, the expression level of let-7a was suppressed in GC [48]. Therefore, Omidfar et al. constructed a dual-signal nanobiosensor using magnetic nanocomposites of Au nanoparticles and CdSe@CdS quantum dots as electrochemical labels for simultaneous quantitative detection of two GC related miRNAs (miR-106a and let-7a) [49]. To prepare an electrochemical biosensor with two different signals, two well-characterized nanocomposites (Au/TMC/Fe3O4 and CdSe@CdS/TMC/Fe3O4) were used as electrochemical markers, resulting in the final detection signal. The magnetic core of these nanocomposites was captured on the SPCE surface modified with polythiophene (PTh) and reduced graphene oxide (rGO) using the same principle of streptavidin-biotin interaction and target double recognition, which enhanced the surface conductivity of the electrode. This dual-signal biosensor has good detection specificity, good selectivity, strong stability and significant sensitivity for both miRNAs, showing attractive application prospects in the clinical diagnosis of GC.

2.2.4. SERS-based DNA biosensor

Surface-enhanced Raman scattering (SERS) is an optical detection technique that can significantly enhance the intensity of molecular Raman scattering spectra on some special materials. Compared with the traditional detection techniques such as fluorescence and chemiluminescence, SERS has the advantages of high sensitivity, good repeatability and high specificity, and is more suitable for the detection of biological samples. Wang et al. constructed a signal amplification strategy (Cas13a-bHCR) based on CRISPR/Cas13a system and branched hybridization chain reaction (bHCR) on SERS-active silver nanorod sensor chip for ultrasensitive SERS detection of miRNA markers in GC (Fig. 5E) [50]. After addition of the target (miR-106a), the target was hybridized to the Cas13a/crRNA complex to activate Cas13a, which in turn cleaved a large number of recognition probes (RP). The cleaved RP

probe sequentially triggers the three hairpin probes H1, H2, and H3 for bHCR. The bHCR products were specifically captured on the SERS sensor chip. Due to a large number of ROX molecules in bHCR product and the good surface enhancement effect of the Ag nanorods array, the ROX modified on the hairpin probe produces an enhanced Raman signal under laser excitation and exhibits high sensitivity of detection. Meanwhile, the SERS sensor based on Cas13a-bHCR also has good specificity and repeatability, which has broad application prospects in the early diagnosis of diseases.

Vo-Dinh et al. also constructed a label-free SERS-based plasma-coupled interference (PCI) biosensor for multiplex detection of miRNAs [51]. Specifically, the complementary binding between reporter probe modified on a silver nanoparticle and capture probe modified on another silver nanoparticle results in the dimerization of the two silver nanoparticles, which in turn causes an enhancement of Raman scattering. The capture probe was designed to be fully complementary to the target miRNA. In the presence of the target, the capture probe can competitively bind and interfere with the formation of the nanonetwork, leading to the decreasing of SERS signal. The PCI nanoprobe is simple to operate and can detect multiple targets at the same time, which has great potential in medical applications.

Due to its low cost and good stability, lateral flow assay (LFA) has attracted widespread attention in the field of point-of-care testing for food detection and infectious disease diagnosis [52,53]. However, the detection method is limited by visual inspection and low sensitivity [54]. To overcome these limitations, Deng et al. integrated the SERS technique into the LFA technique and greatly improved its detection sensitivity [55]. Au rhombic dodecahedron (AuRD) surface was modified with Raman reporter molecule (4-MBA) and ssDNA as SERS tag. In the absence of the target, AuRD-SERS tags will bind to the T-line by base-complementary pairing between hpDNA and ssDNA, showing an enhanced SERS signal. When miR-96-5P was present in the sample, miR-96-5p competitively bound to the hpDNA probe immobilized on the T line, resulting in reduced AuRD-SERS tags binding on the T line, which in turn resulted in reduced SERS signal intensity. The SERS-LFA probe has good repeatability and specificity, and can quantitatively detect miR-96-5p in the GC serum, which has a wide application prospect in the clinical diagnosis of GC.

2.3. CTC

Circulating tumor cells (CTC) are living cancer cells that spread from solid tumors to the circulatory system, which is one of the main biomarkers of tumor liquid biopsy. It has important clinical significance in the early diagnosis, prognosis, efficacy evaluation, recurrence, metastasis and drug resistance mechanism of tumors. However, the clinical application of CTC detection is hindered by the rarity and heterogeneity of CTC.

Epithelial cell adhesion molecule (EpCAM), a transmembrane protein expressed on the surface of a variety of epithelial cells, is an ideal tumor antigen candidate on the surface of CTCs. Yang's group screened a set of DNA aptamers that specifically recognized EpCAM proteins [56]. At the same time, the length of the obtained specific aptamer was optimized to obtain the aptamer sequence SYL3C. Compared with antibodies, DNA aptamers are small, easy to synthesize, have high affinity, good selectivity and high stability, and have a bright application prospect in CTC diagnosis and tumor targeted therapy.

After enrichment of CTCS in blood, signal amplification is a major challenge for sensitive detection of CTCS. Yang's group reported an electrochemical DNA biosensor based on the RCA reaction for ultrasensitive detection of CTCs [57]. Firstly, CTCs were captured by EpCAM antibody-modified magnetic nanospheres. In addition, CTC surface can also bind to DNA Aptamer-primer strand, and then assemble a large number of primer-mediated RCA products onto the electrode surface to generate electrochemical signals. In this study, breast cancer was used as an example to confirm the good detection performance of the biosensor. However, its signal amplification strategy combined with magnetic nanospheres also has a broad application prospect in the diagnosis of CTCs in GC.

The single EpCAM specific antibody/aptamer strategy cannot recognize EpCAM-negative CTC, which limits its clinical application. Here, Yu's group proposed a simple, low-cost, double-aptamer (EpCAM and PTK7) modified immunomagnetic Fe_3O_4 particles (IMNs) for sensitive capture of rare CTCs with epithelial and mesenchymal phenotypes in GC patients and subsequent genetic analysis (Fig. 6A) [58]. This IMN-based immunomagnetic bead sorting strategy was further applied to the analysis of clinical GC patients, revealing that the number of captured CTCs was closely related to the chemosensitivity, diagnosis and clinical progress of patients. This study has greatly improved the sensitivity of CTC detection in clinical practice, and provided a powerful tool for the diagnosis and individualized treatment of CTC-related cancers, which has broad clinical application prospects in the future.

2.4. Exosomes

Exosomes are a kind of double phospholipid membrane vesicles, which contain a variety of components such as proteins, lipids, and nucleic acids, and play an important role in the connection between cells. Some studies have proved that exosomes secreted by GC cells may be involved in the pathological process of GC and are considered as potential diagnostic biomarkers for cancer [59,60].

2.4.1. Electrochemical-based DNA biosensor

By using the hemin/G-quadruplex system and isothermal RCA amplification technology, Li et al. constructed an electrochemical sensor for specific detection of GC exosomes [61]. Specifically, anti-CD63 antibody modified gold electrodes were used to capture exosomes, only GC exosomes could specifically recognize GC exosome-specific aptamers (Fig. 6B). This aptamer was complementary to a circular template containing G-Quadruplex complementary fragments and trigger the RCA reaction to generate multiple G-Quadruplex units. Once hemin is added, the hemin/G-quadruplex system can mimic the deoxyribozyme catalyzing the reduction of H_2O_2 and generate a sensitive electrochemical signal. Experiments show that the electrochemical sensor has high sensitivity and specificity for exosome detection. In addition, the electrochemical sensor is simple to operate and low cost, which is especially suitable for the detection of clinical samples and has great application potential in the diagnosis and prognosis of GC.

2.4.2. SERS-based DNA biosensor

At present, it is still difficult to accurately detect trace amounts of cancer-derived exosomes in complex blood.

Based on the high sensitivity of SERS, the high specificity of CRISPR/Cas13a and the signal amplification ability of nucleic acid amplification reactions, Song's group proposed a SERS biosensor based on CRISPR/Cas13a trans-cleavage and CHA signal cascade amplification reaction for ultrasensitive detection of GC-derived exosomes [62]. In the presence of GC-derived exosomes, MUC1 aptamer specifically binds to exosomes and exposes RNA fragments. The exposed RNA fragment activates CRISPR/Cas13a to cleave the uracil modified hairpin reporter, which in turn triggered a downstream CHA reaction to form a large number of duplexes that can capture the AuNP-based SERS tags onto the AgNRs sensing chip, resulting in a significantly enhanced Raman signal. This SERS biosensor has the advantages of rapid response, high sensitivity, good specificity and universality. It can also be detected specifically in human serum, indicating a bright application prospect in early cancer diagnosis.

The involvement of CRISPR and its associated proteins (Cas) greatly increases the cost and complicates the procedure. In order to simplify the operation steps and reduce the cost, Song et al. proposed an ultrasensitive SERS sensor based on trivalent MUC1 aptamer coupled DNA tetrahedrons (triApt-TDN) and trigger MUC1 aptamer (tgApt) driven

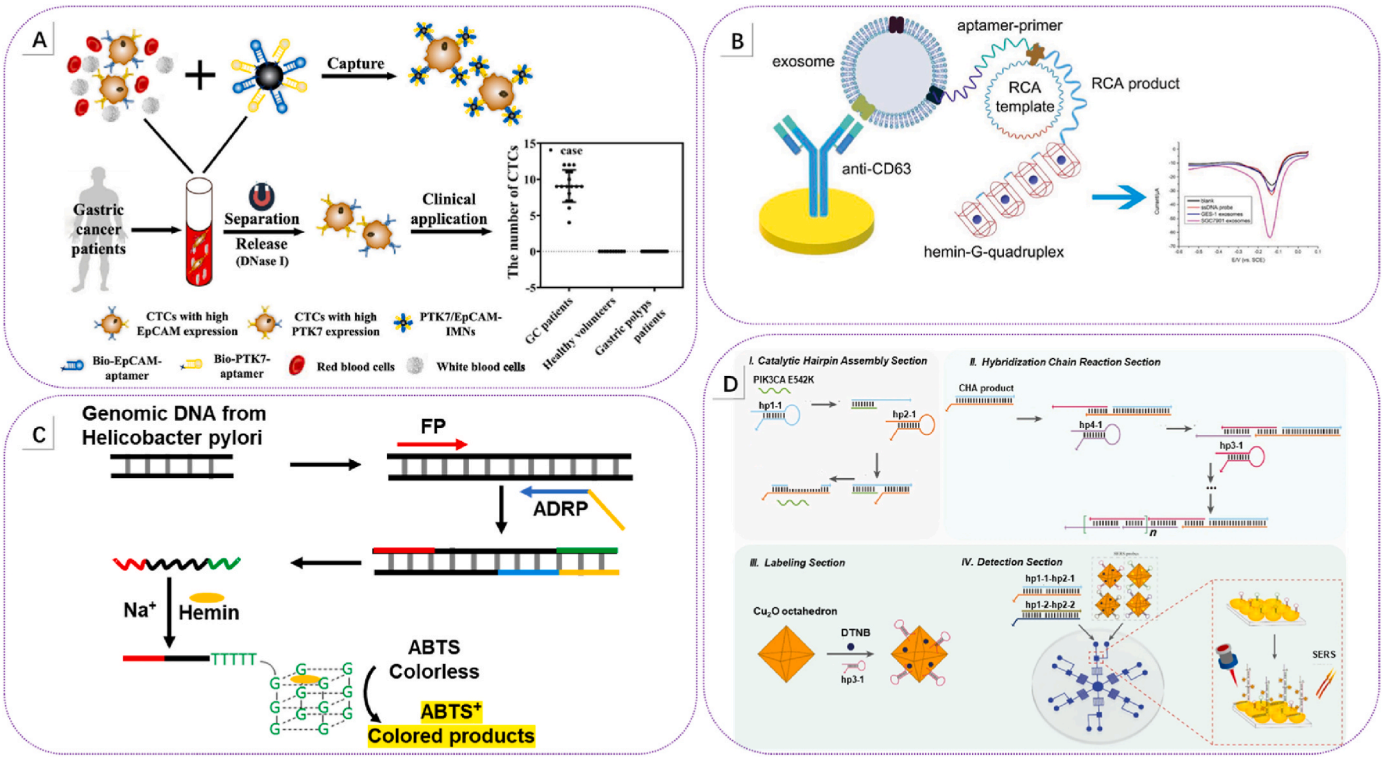

Fig. 6. DNA biosensor for CTC, exosomes or helicobacter pylori detection. (A) Schematic Illustrations of the double-aptamer (EpCAM and PTK7) modified IMNs for CTC detection in its clinical patient samples. Reproduced with permission [58]. Copyright @ 2022 American Chemical Society. (B) Schematic Illustrations of electrochemical sensor for specific detection of GC exosomes. Reproduced with permission [61]. Copyright @ 2019 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (C) Schematic Illustrations of a visual *H. pylori* detection method based on PCR technology and G-quadruplex DNAzyme catalyzed reaction. Reproduced with permission [67]. Copyright @ 2018 Elsevier. (D) Schematic Illustrations of the pump-free high-throughput microfluidic chip with CHA and HCR signal amplification strategies for SERS detection of PIK3CA E542K and TP53. Reproduced with permission [75]. Copyright @ 2022 Springer Nature.

branching hybridization chain reaction (bHCR) for accurate detection of GC cell-derived exosomes [63]. Specifically, triApt-TDN was immobilized on the surface of silver nanorods (AgNRs) arrays to construct a SERS-active sensing chip that could specifically capture exosomes. Subsequently, trigger aptamers (tgApts) specifically bound to exosomes triggered bHCR. At the same time, large amounts AuNP-based SERS tags were captured by bHCR product, triggering a strong SERS signal. It has been proved that the SERS sensor has excellent sensitivity, repeatability and specificity, and has good anti-interference ability in the clinical detection of human serum samples, which is expected to be applied to the early diagnosis of GC in clinic.

2.5. *Helicobacter pylori*

Helicobacter pylori (*H. pylori*) is a spiral-shaped gram-negative bacterium found in the stomach and duodenal bulb. It has a strong ability to survive and is the only bacteria found to survive in the stomach. *H. pylori* infection in gastric mucosa is the basis of chronic infection, inflammation and tumor formation, and the inflammation caused by it will activate effector cells and eventually lead to GC [64]. The diagnosis of *H. pylori* infection is of great significance for the prevention and treatment of GC. There are various methods for the detection of *H. pylori*, including non-invasive detection and invasive detection. Non-invasive tests, such as urea breath test, stool antigen test, and serological tests, are preferred because of their low cost of detection, ease of operation, and user friendliness [65]. However, these methods are limited by diet, sample storage, and quantitative detection [66].

In order to increase the sensitivity of detection, Liu et al. constructed a visual detection method for *H. pylori* based on PCR technology and G-quadruplex DNAzyme catalyzed reaction [67] (Fig. 6C). Firstly, PCR primer pairs targeting *H. pylori* genomic DNA were designed to ensure the production of DNA aptamer of DNAzyme at the 3-end of PCR products. After the addition of hemin, the G-quadruplex DNAzyme/hemin complex showed peroxidase-like activity to catalyze colorless 2, 2′-azo-bis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) to colored product $ABTS^{\bullet+}$, thereby enabling the amplification of the signal. This method can be read by the naked eye, has high sensitivity and specificity, and is simple to operate. However, this visual detection method requires expensive instrument assistance and has high temperature requirements. To simplify the experimental manipulation, Tang et al. constructed an enzyme-free isothermal detection method using the color and absorbance changes of AuNPs of different sizes [68]. In the presence of *H. pylori* in the samples, AuNPs modified by *H. pylori*-specific aptamer aggregated and the color changed from red to dark purple. The qualitative and quantitative detection of *H. pylori* can be achieved by naked eye observation and ultraviolet spectrum analysis, respectively. This method has high sensitivity, rapid detection rate, low detection cost, and unlimited test subjects, especially suitable for the detection of clinical samples.

2.6. *Circulating tumor DNA*

To achieve rapid, economical and non-invasive early detection of GC, liquid biopsy has become the most popular detection technique. The level of circulating tumor DNA (ctDNA) can reflect tumour burden, which is an important indicator of tumor initiation and progression [69]. Tumor-specific genes carried by ctDNA in blood can be used for precise diagnosis of specific cancers. Compared with imaging and histological examination, ctDNA can detect the possibility of cancer at an ultra-early stage.

Mutations in PIK3CA are also commonly found in GC [70,71]. To detect GC-associated PIK3CA genes of ctDNA, Cui et al. reported a

DNA-based electrochemical biosensor. Specifically, L-arginine and GO coated AuNPs were deposited on a glass electrode as a reaction platform. At the same time, a DNA probe specifically capturing the PIK3CA gene was immobilized on this reaction platform [72]. The experimental results show that the electrochemical sensor has high sensitivity, good stability and wide dynamic response range. In addition, the detection platform is simple to operate and low cost, which has great application potential in the clinical diagnosis of GC.

In addition, TP53 is the most studied gene in tumors and may be a potential biomarker for GC, playing an important role in regulating cell proliferation, apoptosis, and DNA repair [73,74]. Therefore, it is of great significance to design sensors for sensitive detection of PIK3CA and TP53 for early diagnosis of GC. To construct an ultrasensitive detection technique for PIK3CA E542K and TP53, the enzyme-free DNA signal amplification strategies, such as CHA and HCR, were introduced into a pump-free high-throughput microfluidic chip for SERS detection of PIK3CA E542K and TP53 [75]. In Fig. 6D, two different hairpin DNA strands (hp3 and hp4) modified octahedral Cu_2O were used as SERS probes, and HP3-modified AuNB arrays were used as capture substrates. In the presence of the target PIK3CA E542K (or TP53), the CHA response is initiated to open the corresponding hp1. Then, the hp2 competitively replaces the target, forming a large number of hp1-hp2 duplexes. The hp1-hp2 duplex and SERS probe were passed through a capillary pump and captured by HP3-modified Au nanobowl arrays, which subsequently initiated the HCR reaction between hp3 and hp4 to form long double-stranded DNA (dsDNA). This CHA-HCR dual amplification strategy resulted in an increasing number of SERS probes aggregated on the surface of the Au nanobowl array, significantly enhancing the signal intensity. Based on the CHA-HCR dual amplification strategy, the chip can detect PIK3CA E542K and TP53 in an ultra-sensitive manner, and the detection process was completed within 13 min. In addition, the chip can also analyze multiple samples at the same time with high precision, which is expected to be used for early diagnosis of GC.

3. GC therapy strategies

The therapeutic efficacy and safe delivery of anticancer drugs is a major challenge in cancer treatment. The treatment of cancer is still largely dependent on chemotherapy. However, while killing tumor cells, chemotherapy can also damage normal cells and produce serious side effects. In order to reduce the side effects of chemotherapy, scientists are urgently seeking safer targeted drug delivery strategies. Nucleic acid nanomaterials can not only achieve the accurate detection of specific markers of GC, but also meet the needs of precision treatment. The large specific surface area of DNA nanomaterials enables efficient loading of small molecule drugs. At the same time, the easy modification of DNA nanomaterials allows the targeted delivery of drugs to the tumor site, which improves the effectiveness of treatment and reduces the side effects of drugs. In addition, DNA nanomaterials are more suitable for *in vivo* therapeutic applications due to their high biocompatibility and biodegradability. The development of DNA nanotechnology provides a safe and effective drug delivery system for cancer treatment.

3.1. Chemotherapy

To overcome the systemic side effects of conventional chemotherapy, Ma et al. constructed an AS1411 aptamer mediated AuNPs (AS1411-AuNPs) as a targeted delivery carrier for chemotherapy drugs for efficient treatment of GC (Fig. 7A) [76]. Specifically, functional hairpin DNA was modified on the surface of AuNPs for loading doxorubicin (DOX). AS1411 aptamer-nucleolin specific interaction achieve precise tumor targeting of AS1411-AuNPs. More importantly, upon laser irradiation, AS1411-AuNPs released DOX at pH 5.0, showing enhanced anti-tumor effects. Based on the advantages of good stability, safety, specific targeting ability and enhanced anti-tumor effect, AS1411-AuNPs system has great clinical application potential in the targeted therapy of GC.

3.2. Gene therapy

The combined application of metal and DNA nanostructures shows great potential as functional nanomaterials, such as DNAzyme [77], which can play a powerful application in specific detection and targeted therapy with the assistance of metal ions. The catalytic activity of metal ions combined with the therapeutic function of nucleic acids can play a great advantage in the treatment of cancer. Zhang et al. constructed a Ca^{2+}-dependent aptamer-DNAzyme metal-nucleic acid framework (MNFs) carrying interferon regulatory factor-1 (IRF-1) for targeted regulation of glucose transporter (GLUT-1) expression in human epidermal growth factor receptor-2 (HER-2) positive GC cells [78]. Specifically, the HER-2 targeting aptamer was integrated into the Ca^{2+}-dependent GLUT-1 DNAzyme to extend the length of the oligonucleotide for promoting Ca^{2+}-assisted self-mineralization and target into GC cells for visualization and silencing of GLUT-1 mRNA (Fig. 7B). The results showed that the MNF nanodrug disrupted GSH/ROS homeostasis, depleted ATP, synergistically enhanced ROS-mediated DNA damage, and significantly inhibited tumor growth.

Therapeutic DNAzymes have great potential in cancer diagnosis and

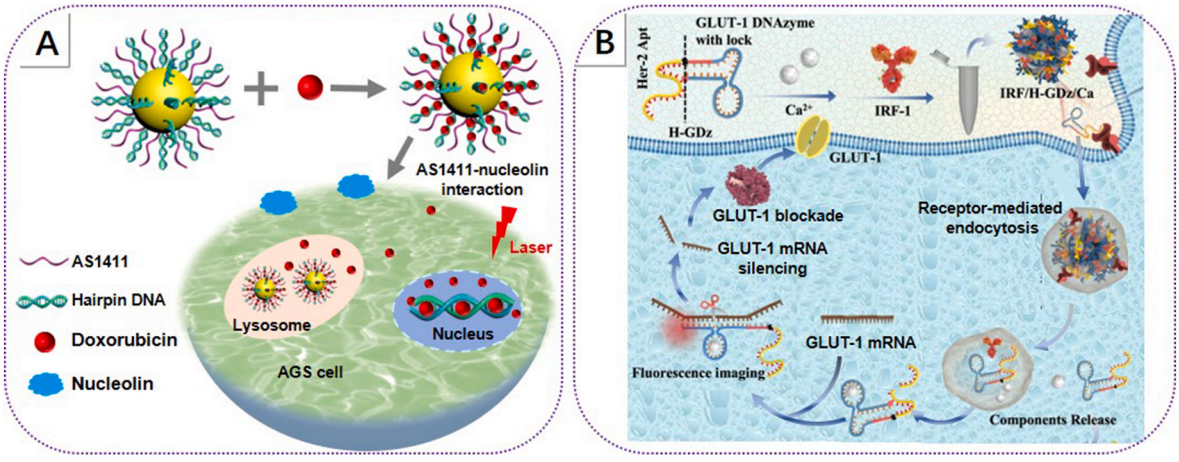

Fig. 7. Strategies of nanoparticles in the treatment of GC. (A) AS1411 aptamer mediated AuNPs (AS1411-AuNPs) carrying chemotherapy drugs for efficient treatment of GC. (B) Ca^{2+}-dependent aptamer-DNAzyme metal-nucleic acid framework (MNFs) carrying interferon regulatory factor-1 (IRF-1) for targeted regulation of glucose transporter (GLUT-1) expression in human epidermal growth factor receptor-2 (HER-2) positive GC cells.Reproduced with permission [78].Copyright @ 2024 Springer Nature.

treatment. However, a major obstacle that has limited the clinical use of DNAzymes is the lack of ability to specifically recognize diseased cells. Healthy cells can also be damaged to varying degrees during treatment. Therefore, multi-component deoxyribozymes (MNAzymes), which can derive additional DNA recognition fragments, have attracted much attention [79,80]. Shen's group proposed a Ca^{2+}-dependent MNAzyme with Her-2 targeting recognition ability to cleave GLUT1 mRNA specifically activated by endogenous mirRNA-21 for targeted therapy of GC cells overexpressing Her-2 [81]. Specifically, a human Her-2 targeting aptamer was integrated into the MNAzyme sequence to target GC cells overexpressing Her-2. Upon uptake by cancer cells, endogenous miRNA-21 specifically recognize and activate Ca^{2+}-dependent MNAzyme to cleave GLUT1 mRNA, which in turn inhibits GLUT1 and disrupts the pentose phosphate pathway (PPP) to induce DNA damage in cancer cells. In the study, the advantages of DNAzymes and MNFs are combined to overcome the limitations of therapeutic DNAzymes. Meanwhile, the carrier-free MNAzyme/Ca delivery system can encapsulate proteins efficiently under mild conditions, greatly expanding the medical application prospects of DNAzyme.

In order to enhance the effect of tumor treatment, researchers mostly use multiple combination therapies, such as gene therapy and chemotherapy. Ramezani et al. constructed a tumor-targeted Bcl-xL shRNA and chemotherapy drugs co-delivery system based on AS1411 aptamer-modified single-walled carbon nanotubes (SWCNT) [82]. A modified branched polyethylenimine (PEI) was conjugated to SWCNT via PEG as the delivery carrier of shRNA. Subsequently, the AS1411 aptamer was linked to the SWNT-PEG-PEI conjugate to achieve targeted delivery. At the same time, DOX was embedded into the AS1411-SWNT-PEG-PEI carrier to achieve efficient loading of chemotherapy drugs. It has been proved that this targeted co-delivery system can specifically recognize GC cells and is an efficient and safe anti-tumor method.

3.3. Phototherapy

Phototherapy mainly includes photodynamic therapy (PDT) and photothermal therapy (PTT). In recent years, PDT and PTT has attracted great attention because of its advantages of high selectivity, repeatable operation and non-invasive. Therefore, minimally invasive treatment of gastrointestinal tumors can be achieved by endo-laparoscopic PDT. By binding to aptamers, Na et al. developed a highly targeted photosensitizer (PS) for effective PDT of GC [83]. The AS1411 aptamer was coupled to PS (Chlorin e6, Ce6) using polyethylene glycol (PEG) during the synthesis process, resulting in Aptamer-PEG-Ce6. This Aptamer-PEG-Ce6 can effectively target tumor cells overexpressing nucleolin, and can produce ROS under laser irradiation, thereby effectively killing tumor cells. It has broad application prospects in the minimally invasive treatment of tumors.

PTT is used to treat tumors by converting near-infrared light into cytotoxic heat using photothermal agents. Sun et al. successfully constructed a dual-targeted gold nanoprism by modification of the AS1411 aptamer and Zn-tetraphenylethene (TPE@Zn) on the bare gold surface (Au-Apt-TPE@Zn) for dual-model imaging and precise PTT of cancer [84]. In this study, TPE functionalized with phenanthroline derivatives has aggregation induced emission (AIE) properties and TPE@Zn can specifically target the early apoptotic cells membrane. In addition, the AS1411 aptamer mediates specific targeting of Au-Apt-TPE@Zn to the nucleus. Under NIR irradiation, Au-Apt-TPE@Zn showed cancer-targeted fluorescence and light-up photoacoustic dual-model imaging properties and exhibited a strong photothermal therapeutic effect, resulting in excessive production of ROS, which in turn induces apoptosis of GC cells.

In summary, DNA nanotechnology has promising application prospects in targeted drug delivery, chemotherapy, gene therapy, phototherapy and other fields of GC. The programmability, biosafety and easy access of DNA nanotechnology ensure its wide application in biomedicine. Most of the current therapeutic strategies for GC involve the combined application of nucleic acids and non-nucleic acid nanomaterials, such as inorganic nanoparticles (graphene and gold nanoparticles) and cationic materials (polymers, proteins and peptides). Despite the significant progress of non-nucleic acid systems in the laboratory research phase, the transition to clinical applications has been hampered by the complex synthesis process, high cost, and uncertain cytotoxicity. In the future, the construction of pure nucleic acid nanomedicine system will be a safe and effective strategy for the treatment of GC.

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4. Integrated platform of diagnosis and treatment

In nanomedicine, a single diagnostic or therapeutic model can no longer meet the people's pursuit of high efficacy/specificity. Therefore, multimodal nanomedicine has been developed to co-encapsulate multiple diagnostic and therapeutic modalities in a single nanomedicine platform. The integration of diagnosis and treatment may provide a practical solution for cancer cure or early treatment in the near future. Diagnostic probes and therapeutic agents can be adsorbed, conjugated or encapsulated in DNA nanomaterials to achieve the construction of diagnosis and treatment integrated platform. The unique optical properties and thermal efficacy of some nanomedicines should show applications in diagnosis and therapy. The ideal diagnosis and treatment integration platform should target the lesion site, diagnose the morphological and biochemical changes of tissues/organs, and provide effective treatment.

Wang's group proposed a diagnosis and treatment integration platform based on a split aptamer for in *vivo* imaging and *in situ* drug release of cancer cell-specific activation [85]. The platform is assembled from fluorophore modified long DNA strand containing the aptamer and complementary quencher-modified short DNA strand. In its initial state, the platform showed a double-stranded structure that could be loaded with chemotherapeutic drugs, and the fluorescence signal was quenched. Once the split aptamer in this platform encounters the target cell, it triggers a conformational change, which results in the activation of a fluorescent signal and chemotherapy drugs release. The integrated diagnosis and treatment platform can not only specifically identify cancer cells, but also realize the imaging and treatment of cancer *in vitro* and *in vivo*. As a promising multi-function diagnosis and treatment platform, the platform has broad application prospects in future personalized medicine. In addition, as described above, the dual-targeted gold nanoprism (Au-Apt-TPE@Zn) constructed by Sun et al. [84]. The platform can be used for both fluorescence/photoacoustic dual-imaging and precise PTT of GC, which is an ideal platform for diagnosis and treatment integration.

The integration of diagnosis and treatment provides patients with continuous and comprehensive medical services through the organic integration of diagnosis and treatment, which is a promising way to achieve the best treatment effect of patients and is also the key research direction in the future.

5. Conclusion

GC is a global health problem with significantly reduced survival after metastasis. In the clinical diagnosis and treatment of gastrointestinal malignant tumor, accurate early diagnosis can make patients get the most effective treatment, and thus prolong patient survival time and improve the quality of life, therefore urgently needs to strengthen the early diagnosis and effective treatment method. However, the clinical diagnostics and treatment of GC effect is not satisfactory. The good biocompatibility and programmability of nucleic acid nanotechnology make it show great application potential in early screening and targeted therapy of GC. The application of nucleic acid nanotechnology improves the detection sensitivity of tumor biomarkers in biological samples, which has reference significance for the development of non-invasive and accurate clinical cancer screening and monitoring tools.

Although the nucleic acid nanoprobes have made some progress in the detection of biomarkers, there are still some challenges in the future clinical research. 1) Various nucleases in biological samples, such as blood, degrade nucleic acid nanomaterials and reduce their stability, making it difficult to achieve the purpose of long-term detection. Based on the characteristics of nucleic acids that are easy to be modified, covalent or non-covalent modification can improve the stability of DNA without affecting the detection ability of nucleic acid probes. 2) For the detection of nucleic acid targets, nucleic acid nanoprobes rely on the recognition method of base pairing, but the non-specific recognition of various non-target DNA/RNA in biological samples will lead to false positive signals. 3) In the process of disease progression, may be accompanied by a variety of changes in the biomarkers. A single biomarker cannot accurately reflect disease progression. Probes that can simultaneously detect multiple biomarkers need to be designed to achieve accurate disease diagnosis. 4) There are relatively few innovative studies based on the clinical specific characteristics and needs of GC. Most of the tumor biomarkers expressed in GC are also expressed in other types of cancer. The specificity of these biomarkers is limited, which limits the accuracy of diagnosing a particular type of disease. We need to find new biomarkers of GC, better understand the molecular mechanism, further promote the development of nucleic acid nanomedicine in the diagnosis of GC, and increase interdisciplinary cooperation and communication.

DNA nanotechnology is faced with greater challenges in clinical treatment, such as low drug targeting delivery efficiency, uncertain cell toxicity of ion, unclear mechanism of ion mechanism of action and escape. First of all, most of the current studies are preclinical or *in vitro* studies, and the safety of nucleic acid nanomaterials is not clear. Due to the high heterogeneity of tumors, there may be differences in biodistribution and targeting of nucleic acid nanomedicines. Secondly, due to the species differences between humans and experimental models, as well as the heterogeneity between diseases, there are many obstacles in the translation of basic research to human clinical research. Finally, the single treatment strategy for GC has inherent limitations. The construction of multifunctional nucleic acid probe and collaborative platform is the key point of further clinical application. Based on the rapid development of nucleic acid nanomaterials, scientists are expected to explore more effective clinical diagnosis and treatment methods for GC.

CRediT authorship contribution statement

Congcong Li: Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Conceptualization, Validation, Visualization. **Tongyang Xu:** Writing – original draft, Investigation. **Guopeng Hou:** Software, Investigation. **Yin Wang:** Writing – review & editing, Funding acquisition, Validation. **Qinrui Fu:** Writing – review & editing, Visualization, Validation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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