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Review Article

Application of Aptamer-based Biosensors in Early Diagnosis of Gastric Cancer: A Promising Method for Early Detection

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Abstract

Personalized medicine is a relatively new approach that addresses differences between patients based on unique features such as genetic make-up, environment, and physiology. Aptamers are synthetic sequences of single-stranded DNA or RNA with a particular three-dimensional conformation that binds to a target. Aptamer-based biosensors are promising tools to detect disease markers, especially in cancer. Gastric cancer is the third most common cause of cancer-related deaths worldwide and has very high prevalence in Asia. Currently, there is a lack of effective screening tools for the early detection of gastric cancer. Thus, identifying new methods to detect markers of gastric cancer is crucial. In this study, the role of aptamer-based biomarkers in early diagnosis of gastric cancer is reviewed.

Introduction

Precision medicine or personalized medicine provides a more comprehensive insight into why patients respond differently to medical treatments following the same diagnosis.[1,2] Different physiological conditions, genetic makeup, and unique molecular characteristics underlie the variation in responses and prognosis among patients.[1] Tissue biopsy has been the primary method for determining disease pathology and the underlying mechanisms and characteristics. Currently, liquid biopsy, which is a less invasive alternative method for conventional tissue biopsy, has been used in personalized medicine to detect disease biomarkers such as circulating tumor cells (CTC), microRNAs (miRNA), proteins, and extracellular vesicles.[3] Different techniques have been expanded to facilitate early disease diagnosis, especially life-threatening diseases such as cancer, by detecting biomarkers in bodily fluids or tissues. Antibodies have been widely used in these detection methods, but some alternative methods including aptamer-based biosensors have been developed. Aptamers are synthetic sequences of single-stranded DNA or RNA (ssDNA or ssRNA, respectively) with a particular three-dimensional conformation that binds to a target such as purified proteins, miRNA, metal ions, lipids, whole cells, virus, and bacteria.[4,5] Aptamers have some advantages over traditional antibody-based methods, including the following: 1) Aptamers are inexpensive, feasible, and their chemical synthesis can be scaled without using animal models; 2) Aptamers allow for better detection of low expression molecules compared to immunohistochemistry staining; and 3) Aptamers are more resilient under wide ranges of pH, temperature, and other harsh environmental conditions.[5,6]

In 1990, Tuerk *et al.* and Ellington *et al.* developed a technique termed systematic evolution of ligands by exponential enrichment (SELEX), which is an *in vitro* process of selecting aptamers for detecting specific targets.[7] SELEX is an efficient technology that can generate and select oligonucleotides with a prominent target affinity.[8] The SELEX procedure consists of the following steps: 1) preparing a library of 10^{14}–10^{16} single-stranded oligonucleotides (ssDNA or ssRNA) that are 20 to 50 bases in length; 2) incubation of the counter-selected protein with the library, selection of oligonucleotides under conditions such as temperature, ionic strength, and time, and elimination of unbound oligonucleotides and separation of bound oligonucleotides; 3) incubation of the target ligands with the library; 4) elimination of unbound oligonucleotides and separation of bound oligonucleotides, then amplification of the oligonucleotides using polymerase chain reaction (PCR) for the next round of selection; 5) repeating the selection rounds to achieve op-

Keywords: Gastric cancer; Aptamer; Antibody; Biosensor; Biomarker.

Abbreviations: BRCA, branched rolling circle amplification; CEA, carcinoembryonic antigen; CTC, circulating tumor cells; DNA, deoxyribonucleic acid; EpCAM, epithelial cell adhesion molecule; FMNS, fluorescent-magnetic nanospheres; GC, gastric cancer; GHRH, growth hormone releasing hormone; *H. pylori*, *Helicobacter pylori*; HER2, human epidermal growth factor receptor 2; miRNA, microRNAs; MMP2, matrix metalloproteinase 2; MUC1, mucin 1; PARD3, par-3 family cell polarity regulator; PCR, polymerase chain reaction; RNA, ribonucleic acid; SELEX, systematic evolution of ligands by exponential enrichment; ssDNA, single-stranded DNA; ssRNA, single-stranded RNA.

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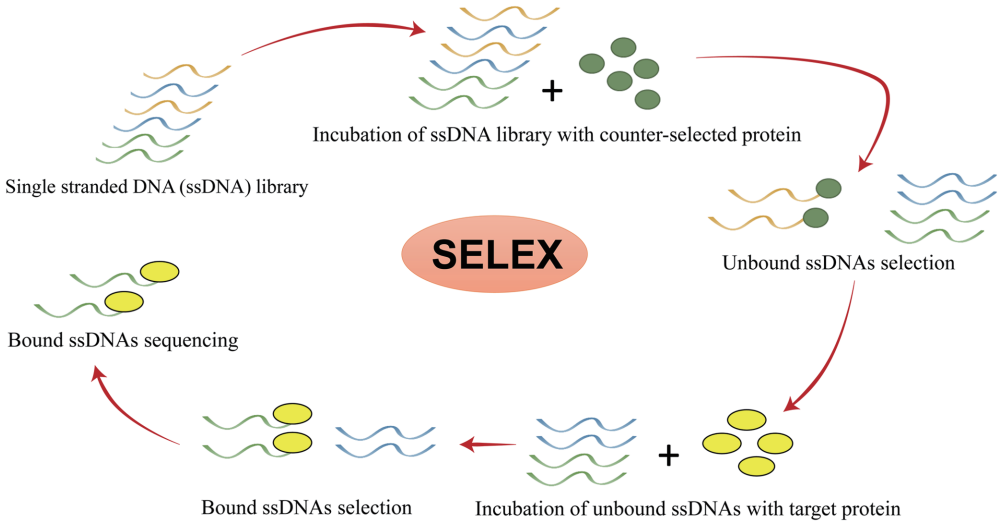

Fig. 1. The conventional SELEX method. First, the ssDNA library is incubated with a counter-selected protein. The unbound ssDNAs are selected to be incubated with the target protein. The bound ssDNAs are then entered into several rounds. The bound ssDNAs are then sequenced. SELEX, systematic evolution of ligands by exponential enrichment; ssDNA, single-stranded DNA.

timal specificity of aptamers for the target; and 6) sequencing the selected aptamers for further characterization.[9] The conventional SELEX method is illustrated in Figure 1.

Despite growing attention focused on the SELEX method, its time-consuming nature, the possibility of errors during PCR, and the possibility of obtaining non-specific aptamers during amplification present barriers to its use. Other detection methods that were developed based on the biases of the traditional SELEX method therefore also have their pros and cons. Since some sequences may be selectively over-amplified during PCR, high-throughput SELEX was developed to obtain more proper aptamers using emulsion and droplet digital PCR and cloning the sequences into *Escherichia coli* for further high-throughput sequencing and bioinformatics analysis.[10] As mentioned above, a unique purified protein is a target for traditional SELEX, but Cell-SELEX was also developed for use with whole cells as the target. By selecting aptamers using the Cell-SELEX method, it is possible to target unknown cell markers and proteins on the cell surface, in the cytoplasm and in the nucleus.[11] Microfluidic SELEX (M-SELEX) has been developed to prepare a system with a larger surface-to-volume ratio and smaller amount of reagents to enhance the target binding specificity.[5] Capillary Electrophoresis SELEX (CE-SELEX) was first applied for human immunoglobulin E (IgE) as the first M-SELEX, and this system can separate unbound sequences from bound ones in the mixture based on their mobility.[12] Today, magnetic bead-based SELEX is widely used as another M-SELEX technique, which uses a magnet in the form of nanoparticles or microparticles to catch and isolate the target in SELEX.[13]

In Asia, gastric cancer (GC) is one of the most fatal cancers; only 30% of patients with GC will survive after 5 years.[14] GC is the fifth diagnosed cancer (5.7% of all cancers) and the third among cancer-related deaths (8.2% of all deaths) worldwide.[15] According to reports, 75% of all GC diagnosed patients and deaths have occurred in Asia, and 80% of patients with GC are diagnosed in stage IV.[16] Therefore, it is crucial to apply an efficient screening method in the general population to detect and prevent GC in its early stages. Carcinoembryonic antigen (CEA) and CA125 are widely used biomarkers for early diagnosis and determining GC patient prognosis.[17,18] Although there is still a gap between the optimal use of aptamer-based biosensors for GC detection, some studies have successfully reported the application of aptamers in GC screening and treatment. Here, we review the current place of aptamers in GC screening and early detection.

Application of aptamers in GC

Some candidate markers are currently being considered in practice and research to screen for cancer in the general population. CEA, cancer antigen 19-9 (CA19-9), cancer antigen 50 (CA50), and cancer antigen 72-4 (CA72-4) are used as markers of gastrointestinal tumors.[19] Growth hormone releasing hormone (GHRH) is a 44 amino-acid neuropeptide secreted from the hypothalamus that induces the pituitary gland to produce and secrete growth hormone.[20] Given that GHRH antagonists can inhibit GC cell proliferation, GHRH could be a good novel target for aptamer synthesis to be used in the diagnosis and treatment of GC.[21] The x-aptamers against GHRH 1–44 and its first 29 amino-acids (GHRH 1-29) have been synthesized using magnetic bead selection.[22] Epithelial cell adhesion molecule (EpCAM) is another non-specific biomarker for GC that is related to worse prognosis, larger tumor size, and metastasis to the lymph nodes. EpCAM is a transmembrane protein that regulates cell adhesion and plays a role in epithelial-to-mesenchymal transition of carcinoma cells in various cancers.[23] Producing an aptamer against EpCAM could be useful to detect CTC in the blood of GC patients. A DNA aptamer against EpCAM has been introduced as a useful and non-expensive method compared to RNA aptamers. The DNA aptamers can be synthesized with an appropriate size and stability compared to anti-EpCAM antibodies to screen patients.[23] Since some CTCs are not EpCAM positive, other targets such as protein tyrosine kinase 7 (PTK7) can enhance the affinity of aptamers to detect CTCs in GC patients.[24] PTK7 is expressed in the cytoplasm of some GC cells and is associated with a well-differentiated GC type.[25] This is an example of a new concept termed "dual-aptamers", which was applied in conjugation with immunomagnetic Fe_3O_4 particles.

Matrix metalloproteinases (MMPs) are a family of enzymes that

digest extracellular proteins.[26] MMP2 specifically plays role in cell migration, making MMP2 a treatment target for various diseases such as atherosclerosis, arthritis, and cancers including GC, breast cancer, pancreatic cancer, among others.[27] A DNA aptamer conjugated with a magnetic fluorescence nanoprobe has been generated against MMP2, and this aptamer has been shown to be comparable to conventional immunohistochemistry staining of GC tissue.[28]

Human epidermal growth factor receptor 2 (HER2) and CEA are well-known non-specific tumor markers that have been widely studied and used in aptamer synthesis. Several studies have generated some aptamers against CEA and HER2, but the non-specificity of these markers for GC has led to new studies using a GC cell line to improve their specificity.[29–33] Using high-throughput sequencing SELEX, six aptamers were selected from a huge library against CEA, CA50, and CA72-4 in the GC adenocarcinoma AGS cell line.[34] The fluorescent signal of the aptamers was comparable to traditional antibody detection, suggesting that aptamers could be an effective alternative to antibody-based immunoassays.

Helicobacter pylori (*H. pylori*) is a micro-aerobic pathogen that is one of the major causes of gastroduodenal disorders such as gastritis, gastric and duodenal ulcers, and gastric malignancies, especially GC and gastric mucosa-associated lymphoid tissue lymphoma.[35,36] *H. pylori* pathogenesis is associated with socioeconomic status and hygiene conditions, and its prevalence is rising because of antibiotics resistance particularly in developing countries.[37] Currently, more than 4.4 billion people are infected by *H. pylori* worldwide.[38] Thus, *H. pylori* could be a substantial target for aptamer early detection of *H. pylori* infection and subsequent GC development. The existing tests for *H. pylori* detection, such as the rapid urease test (RUT) and urea breath test (UBT), are time consuming and require professional operators and instruments. *H. pylori* surface recombinant protein (HP-Ag) has been used as a target for making an HP4 DNA aptamer. This aptamer could be a promising cost-effective method to detect *H. pylori* infection in the general population, especially in high-incidence countries.[35] The HP4 DNA aptamer could be applied as a screening tool for *H. pylori* without the need for professional operators, making detection safer and easier.

The focus on miRNAs as biomarkers for cancers has been widespread. MiRNA let-7d is a member of the human lethal-7 (let-7) family and is considered a key suppressor of cell growth because let-7d can inhibit oncogenes in various pathways resulting in the prevention of cancer growth.[39] A study showed that the combination of let-7d and an anti-nucleolin aptamer suppressed the JAK2/STAT3 pathway in human GC MKN-45 cells.[40] Nucleolin is a protein located in the nucleolus, cytoplasm, and on the membrane of normal cells, and it is overexpressed on the surface of cancer cells. Some studies suggested that nucleolin could be a GC biomarker.[41–44] Thus, nucleolin could be a promising target for GC treatment and early diagnosis. The combination of miRNAs and aptamers could increase the efficacy and specificity of aptamers to detect or diagnose GC.

Imaging has been used for early diagnosis and follow-up of GC patients. Cell-SELEX is the best method to optimally identify aptamers to target cells when there is no known collection of biomarkers. Some studies have been conducted to show how the higher specificity of aptamers could improve the imaging, diagnosis, and follow-up of patients with GC. The poorly differentiated GC cell lines BGC-823 and MGC-803 have been used for selecting aptamers for GC using Cell-SELEX.[45] PDGC21-T and MGC-803 aptamers conjugated to quantum dots (QD605) were developed as specific probes for the targeted imaging of GC and to distinguish poorly differentiate cell lines from the moderately differentiated GC cell line SGC-790. Cell-SELEX was also implemented to select aptamers termed cy-apt 20 to target the GC adenocarcinoma AGS cell line. Cy-apt 20 can bind to GC cells with a 7-fold higher selectivity compared to hepatocellular carcinoma and colon carcinoma cell lines.[46] AGC03 is a newly identified aptamer that was determined using Cell-SELEX with the GC cell line HGC-27. AGC03 demonstrated an ability to enter GC cells,[47] and this internalization could be facilitated by a surface transporter like nucleolin, which could make HGC-27 a candidate aptamer to deliver medications into GC cells or even into the nucleus. However, while Cell-SELEX is a feasible and effective method *in vitro*, *in vivo* studies should be conducted to investigate its feasibility as a screening method in the general population. One advantage of using aptamers over antibodies is their ability to attach under temperature changes in the body. Another study showed that GC aptamers could be detected in the human gastric adenocarcinoma SGC7901 cell line using imaging following intravenous injection of fluorescent-magnetic nanospheres in mice.[45] The high fluorescent signal induced by GC specific aptamers is a highly sensitive method to detect GC cells in the tissue or blood.

A study conducted by Zheng Y *et al.* using whole serum of patients with GC, lung cancer, colorectal cancer, hepatocellular carcinoma, and normal patients used DNA specific aptamers synthesized with magnetic beads to identify apolipoprotein A-I (APOA-I), apolipoprotein A4 (APOA4), par-3 family cell polarity regulator (PARD3), and importin subunit alpha-1 as GC protein biomarkers.[18] APOA-I was previously shown to be a specific GC biomarker in a proteomics analysis.[48] PARD3 was also introduced as a hepatocellular carcinoma and esophageal squamous cell carcinoma marker to detect gastrointestinal cancers.[49,50]

Exosomes are nanovesicles that are formed by membranes. Exosomes contain functional molecules like miRNA, protein, nucleotides, and lipids in high concentration to facilitate cell to cell communication[51] and are often used for angiogenesis, metastasis, and tumor progression.[52,53] Considering the important role of exosomes in the pathogenesis of cancers, they could be substantial markers to be targeted by aptamers. Haung *et al.* demonstrated the role of mucin 1 (MUC1), an overexpressed glycoprotein on the surface of GC derived exosomes, as a specific biomarker for generating aptamers against GC. Subsequently, they showed that branched rolling circle amplification (BRCA) could be a more effective method to produce a MUC1 aptamer.[54] BRCA has some advantages over other amplification methods: it can be done at room temperature and produce high molecular weight DNAs with high sensitivity. Exosomes derived from the SGC7901 human GC line were entered into BRCA cycles and were labeled with SYBR Green I fluorescent dye. The high fluorescent signal from the aptamers against GC exosomes showed that exosomes could be promising markers to select aptamers in GC. CD63, a member of the Tetraspanins family, is an exosome marker that can promote cell differentiation, adhesion, and tumor invasion.[55] CD63 has been shown to be expressed in GC, and some studies have introduced it as a marker for GC.[56,57] A new generation of CD63 aptamers used to detect GC derived exosomes has evolved.[58] By only using one marker, aptamer selection against exosomes is non-specific. Combining several markers on GC derived exosomes make this selection more sensitive and specific. A study conducted by Chen *et al.* showed the effectiveness of a specific aptamer-coupled Au-decorated polymorphic carbon to detect early released exosomes in the urine of GC patients and healthy controls. This study extracted three metabolic biomarkers from these exosomes with more than 90% accuracy, providing a new perspective for using aptamers to screen GC in the early stage. A summary of the studies reviewed herein is presented in Table 1.[18,22,28,32–35,40,45–47,54,58–63]

Table 1. Aptamers used in the gastric cancer diagnosis and treatment.

Type	Description	Author
NCL-Apt-miRNA let-7d chimera	JAK2 expression and activity were significantly decreased in MKN-45 gastric cancer cells treated with a conjugate of NCL-APT and miRNA let-7d.	Ramezanpour M *et al.* 2019[40]
Aptamer-siRNA Chimera/PEI/5-FU/ Carbon Nanotube/ Collagen Membranes	Chimera/PEI/5-FU/CNT/collagen membrane nanoparticles were shown to have strong targeting capability within gastric cancer cells. Through the use of siRNA-aptamer chimeras, 5-FU molecules can be delivered to gastric cancer cells and drug-resistant genes can be silenced. Chim/PEI/5-FU/CNT nanoparticles significantly inhibit 5-FU-resistant gastric cancer cell invasion and proliferation.	Chen W *et al.* 2020[59]
Aptamer PDGC21-T-QD	The PDGC21 aptamer was truncated by removing nucleotides at its 5′ and 3′ ends in order to make it a shortened aptamer. Despite being truncated, the aptamers retained their binding specificity for BGC-823 and MGC-803 cells. However, they were not able to bind to SGC-7901 and MKN28 cells, indicating that the aptamer is most efficient against poorly differentiated gastric cancer cells.	Li W *et al.* 2019[45]
Aptamers conjugated with fluorescent-magnetic nano-spheres (FMNS)	A fluorescent-magnetic nanosphere (FMNS) added to selected aptamers enhanced their ability to capture and release cancer cells. Fluorescence is activated after an aptamer probe binds to a cancer cell, resulting in changes in its conformation. The aptamer probe strategy can be used to develop molecular probes that are highly sensitive and specific.	Ding F *et al.* 2015[60]
DNA aptamers using Cell-SELEX	DNA aptamers specific to gastric cancer serum were screened with subtractive SELEX using gastric cancer serum as the target serum, and normal serum as the negative serum. APOA1, APOA4, PARD3, and Importin subunit alpha-1 were relatively high-scoring potential biomarkers. Therefore, the four ssDNA aptamers generated here may prove to be effective molecular diagnostic tools for the recognition of gastric cancer.	Zheng Y *et al.* 2019[18]
RNA aptamers targeting biomarkers CEA, CA50 and CA72-4	Six novel RNA aptamers for three gastrointestinal cancer biomarkers were discovered, CEA, CA50, and CA72-4. SELEX has been enhanced by the use of high-throughput sequencing.	Pan Q *et al.* 2018[34]
Fluorescence aptasensor based on branched rolling circle amplification (BRCA)	BRCA was used to amplify the signal using the MUC1 specific aptamer. In order to prepare a circular probe, the aptamer was employed as a ligation probe. The detection procedure was greatly simplified, thus reducing time and costs.	Huang R *et al.* 2020[54]
AS1411 aptamer-functionalized/ PAMAM dendrimer nanocarriers	The number of cancer cells were reduced by the 5-FU-dendrimer-aptamer complex. An aptamer-5-FU complex on nanoparticles could efficiently administer chemotherapy to cancerous cells while reducing dysfunctional side effects in healthy cells.	Barzegar Behrooz A *et al.* 2016[61]
Aptasensor Based on a Hemin/G-Quadruplex-Assisted Signal Amplification Strategy	Evidence suggests that mucin 1 (MUC1) (particularly MUC_3) can be utilized as a biomarker for gastric cancer invasion. In the gastric cavity, most epithelial cells express mucin glycoproteins. This aptasensor uses MUC_3 as its detection probe. As a result, this aptasensor is simple, cheap, and it may be useful in diagnosing gastric cancer and predicting its prognosis.	Huang R *et al.* 2019[62]
Nucleic acid aptamers using H pylori surface recombinant antigens	HP-Ag was used as a target to screen aptamers. The affinity and specificity of aptamers were enhanced by trypsin, while non-specific sequences were reduced by using BSA as a negative target. In total, three aptamers showed high affinity for HP-Ag, including Hp1, Hp2, and Hp4. Hp4 showed excellent specificity for H pylori cells when incubated with E coli, S aureus, and V anguillarum.	Yan W *et al.* 2019[35]
Aptamer-based microchip	By using magnetic beads to induce strand circles, microchip electrophoresis assays were improved. By using the proposed MCE-LIF assay (microchip electrophoresis with laser-induced fluorescence), a proof-of-concept study showed CEA could be detected in both cancer and healthy serum samples rapidly and accurately.	Zhao S *et al.* 2019[32]
ECD_Apt1	HER2 expression can be detected by the aptamer ECD_Apt1. The use of DNA aptamers against HER2 is capable of evaluating therapeutic agents and assessing patient compliance with cancer treatments including gastric cancer.	A Sett *et al.* 2017[33]
ssDNA X-aptamers	Many cancers (including gastric cancer) are inhibited by GHRH (NH2 1–29) peptide antagonists. X-Aptamer technology was utilized to target GHRH NH2 (1–44) and NH2 (1–29). By using the SELEX method, X-Aptamer technology can synthesize up to 5 targets simultaneously, but it is limited to targeting molecules or targets that have a molecular size of 10 amino acids or above.	Ayhan-Sahin B *et al.* 2022[22]

(continued)

Table 1. *(continued)*

Type	Description	Author
Cy-apt 20	A number of experiments have demonstrated that the aptamer Cy-apt 20 binds more strongly to gastric cancer cells than to non-gastric cancer cells. For this case, Cy-apt 20 proved to be a highly specific and sensitive molecular probe for detecting gastric cancer.	Cao H *et al.* 2014[46]
AGC03	Cell lines isolated from gastric cancer, including HGC-27, BGC-823, MGC-803, and SGC-7901, are specifically bound by AGC03, and cannot bind to other cell lines. It has been shown that it can be absorbed by cancer cells on its own.	Zhang X *et al.* 2014[47]
SYL3C aptamer	Recombinant EpCAM protein can be binded with an aptamer. The length of this aptamer is reduced to 48 nt, and there is a small weight reduction. SYL3C aptamer recognition results showed that target cancer cells could be distinguished from mixed cells during flow cytometry analysis.	SongY *et al.* 2013[63]
Aptamer-conjugated fluorescent nanoprobe	This aptamer was designed to target the MMP2 protein. Using Aptamer, gastric cancer tissues were precipitated and detected. Magnetic fluorescent nanoprobes were created using EDC conjugation of the MMP2 aptamer.	Han M *et al.*2014[28]
ROX-Apt	In order to recognize CD63, the Aptamers are stacked on the MoS2-AuNS surface. It has been shown that ROX-APT can reduce SERS signals when it is released by binding specifically to exosomes in nanocomposites composed of exosomes.	Pan H *et al.* 2022[58]

BRCA, branched rolling circle amplification; CEA, carcinoembryonic antigen; EpCAM, epithelial cell adhesion molecule; FMNS, fluorescent-magnetic nanosphere; GHRH, growth hormone releasing hormone; HER2, human epidermal growth factor receptor 2; miRNA, microRNAs; MUC1, mucin 1.

Future direction

Endoscopy is the most commonly used screening method for GC, but more effective screen methods are needed. Aptamers are less expensive than antibodies and they bind to a variety of molecules, making them an alternative method for detecting and screening GC. *H. pylori* infection is the most prevalent cause of GC that could be screened by aptamers with high sensitivity and specificity as an alternative approach to conventional antibody-based methods. By replacing antibody-based methods with aptamer-based biosensors, the cost of laboratory tests and treating patients could be reduced.

Conclusions

Aptamers are easy to synthesize, have various target molecules, and are stable in harsh conditions, making them an optimal alternative to antibody-based detection systems for GC. As such, aptamers could enhance GC screening in its early stages in the general population. More studies need to be performed to evaluate the use of aptamers in GC diagnosis and treatment.

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Conflict of interest

We have no conflicts of interest to disclose

Author contributions

Contributed to study concept and design (RR, FA, and FM), writing and data collection (RR and FA), figure and table preparation (FM and RR), and critical revision of the manuscript (FA and FM).

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