



Review Article

Advances in CRISPR Based Biosensing Strategies for Cancer Diagnosis



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Abstract

With the development of CRISPR/Cas technology, its application in tumor biosensing and diagnosis is becoming increasingly widespread. CRISPR/Cas-based platforms can enable sensitive detection of tumor-related targets through programmable target recognition, cleavage, and signal amplification. However, challenges remain in translating these platforms into routine clinical diagnosis. This review focuses on CRISPR/Cas-based biosensing strategies for cancer diagnosis, including the detection of tumor-derived exosomes, circulating tumor DNA, circulating tumor cells, and tumor biomarkers. These approaches combine CRISPR/Cas systems with aptamers, nucleic acid amplification, electrochemical, fluorescent, and other sensing strategies to facilitate the detection of low-abundance tumor-associated targets. Related CRISPR applications for investigating the tumor microenvironment are also briefly discussed. Current studies demonstrate the versatility and analytical sensitivity of CRISPR/Cas-based biosensing; however, further improvement in assay performance and clinical validation is needed before these strategies can be routinely applied to cancer screening or diagnosis.

Introduction

Tumors are heterogeneous diseases that arise from the malignant transformation of somatic cells and can be classified as benign or malignant tumors.¹ Early and accurate diagnosis of tumors is crucial for improving treatment efficacy. The diagnosis of tumors primarily relies on pathological examination,² imaging examination,³ and cytological examination.⁴ For example, Balaur and colleagues created a microscope slide with an innovative optical design that ena-

bles various cells and tissues to display various colors without the need for additional processing. By observing these color variations, researchers successfully distinguished between healthy epithelial cells, precancerous tissue, and breast cancer tissue.⁵ Yuan *et al.*⁶ designed a strategy that relies on intracellular self-assembly controlled by proteinase to concurrently enhance magnetic resonance imaging signals. This approach not only enables the diagnosis and imaging of tumors but also facilitates the real-time monitoring of drug distribution for image-guided tumor therapy. Stephen *et al.*⁷ explored fine needle aspiration cytology (FNAC) for diagnosing orbital lymphoid tissue tumors. FNAC, being a minimally invasive and effective method, provides essential cytological diagnostic information for orbital lymphoid tissue tumors, particularly malignant tumors. In addition, recent advances in metal composite sensors,⁸ including a self-calibrating magnetic aptamer sensor for PD-L1-positive exosomes,⁹ contribute to innovation in oncological diagnostics. These studies not only broaden the spectrum of tumor diagnostic techniques but also enhance their reliability in clinical applications. Early and accurate tumor diagnosis holds great significance in improving cure rates and survival rates, guiding individualized treatments, predicting patient prognosis, formulating follow-up strategies, conducting high-risk population screening, and avoiding overtreatment. The treatment of smaller tumors and those with slower growth rates, while accurate determination of tumor types and characteristics, allows for the development of appropriate targeted therapy. Currently, the key focus for advancing tumor diagnosis and treatment is enhancing screening sensitivity and specificity, achieving non-invasive and minimally invasive detection, guiding precise treatments, and monitoring treat-

Keywords: CRISPR/Cas biosensing; CRISPR/Cas; Exosomes; Circulating tumor DNA; Circulating tumor cells; Tumor biomarkers; Tumor microenvironment.

Abbreviations: AFP, alpha-fetoprotein; Cas12a, CRISPR-associated protein 12a; Cas13a, CRISPR-associated protein 13a; CD, cluster of differentiation; CRISPR, clustered regularly interspaced short palindromic repeats; crRNA, CRISPR RNA; CTCs, circulating tumor cells; ctDNA, circulating tumor DNA; DL, deep learning; DPV, differential pulse voltammetry; dTTP, deoxythymidine triphosphate; EVs, extracellular vesicles; FNAC, fine needle aspiration cytology; FQ, fluorophore-quencher reporter; GCE, glassy carbon electrode; GR, graphene; gRNAs, guide RNAs; HCR, hybridization chain reaction; HDPC, high definition photoconductor; MMP, matrix metalloproteinase; MNPs, magnetic nanoparticles; NLS, nuclear localization signal; NUC, nuclease lobe; PAM, protospacer adjacent motif; PD-L1, programmed death-ligand 1; REC, recognition lobe; SDA, strand displacement amplification; sgRNA, single guide RNA; TALEN, transcription activator-like effector nucleases; TME, tumor microenvironment; ZFN, zinc-finger nucleases.

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ment responses. The exploration of new diagnostic strategies holds significant value in tumor treatment in the future.

CRISPR/Cas technology, with its programmable target recognition and cleavage capabilities, has demonstrated great potential in tumor biosensing and diagnosis.^{10,11} This technology refers to the recognition and modification of target genes via various tool enzymes, including in-cell gene editing and somatic cell gene editing techniques.¹² Three primary types of gene editing technologies are zinc-finger nucleases (ZFN),¹³ transcription activator-like effector nucleases (TALEN), and CRISPR/Cas.¹⁴ Among them, the CRISPR/Cas system is currently the most widely used and achieves specific cleavage of DNA or RNA through various Cas nucleases.¹⁵ The CRISPR/Cas system presents both advantages and challenges in tumor diagnosis. Compared to ZFN and TALENs, CRISPR systems have the advantage of being easier to design and customize, offering greater flexibility in laboratory studies. Additionally, they show greater efficiency in gene editing, which is crucial for accurately targeting diagnostic biomarkers. Despite potential challenges such as off-target effects, these risks can be mitigated through the design of more specific guide RNAs (gRNAs) selected with the aid of advanced algorithms and prediction tools. Compared to traditional diagnostic methods, CRISPR has the advantage of high specificity, allowing for more accurate identification and editing of tumor biomarkers, enabling real-time monitoring for accelerated results. However, it is important to note that CRISPR technology can create technical complexity for researchers with limited experience in gene editing, as well as challenges in delivery systems, especially when applied in vivo. Cas9 cleaves double-stranded DNA near the Protospacer Adjacent Motif (PAM) sequence after the hybridization between the single guide RNA (sgRNA) and target DNA.^{16,17} Cas9 systems, employed for precise gene editing, are strategically adapted for tumor marker detection. Design considerations involve selecting highly specific target sequences to ensure accurate detection of particular tumor markers. The adaptation strategy employs gRNA to guide Cas9, facilitating efficient recognition and precise cutting of target genes. This approach ensures a highly specific response during the detection of tumor markers.¹⁸ The mechanism of Cas12 is to initiate dual cleavage after the hybridization between the crRNA and DNA, and the activated Cas12 can cleave along the chain of crRNA and DNA (cis cleavage) as well as nonspecifically cleave the other chain (trans cleavage).¹⁹ For the Cas12 system, utilized for specific target gene identification, design considerations involve selecting highly specific target sequences suitable for Cas12 in the context of tumor markers. The adaptation strategy includes designing specific gRNAs to ensure precise cuts on target genes by Cas12. Furthermore, the system exhibits a notable signal amplification effect after cutting the target, contributing to enhanced detection sensitivity. Cas13, employed for RNA detection, recognizes RNA sequences and nonspecifically cleaves nearby RNA guided by designed crRNA.²⁰ For the Cas13 system, widely used in RNA detection, particularly for certain tumor markers involving RNA, design considerations encompass the suitability for RNA detection. The adaptation strategy involves optimizing the gRNA design for Cas13 to ensure high specificity for target RNA. Leveraging the “cleavage activation” mechanism of Cas13 enhances the effective detection of tumor markers. These design considerations and adaptive strategies collectively ensure optimal specificity and sensitivity for each CRISPR/Cas system in detecting diverse tumor markers. Compared to traditional methods, CRISPR/Cas-based biosensing has potential advantages in operational procedures, detection time, and analytical sensitivity. This not only enables precise tumor diagnosis but also predicts tumor occurrence and development, determines prognosis,

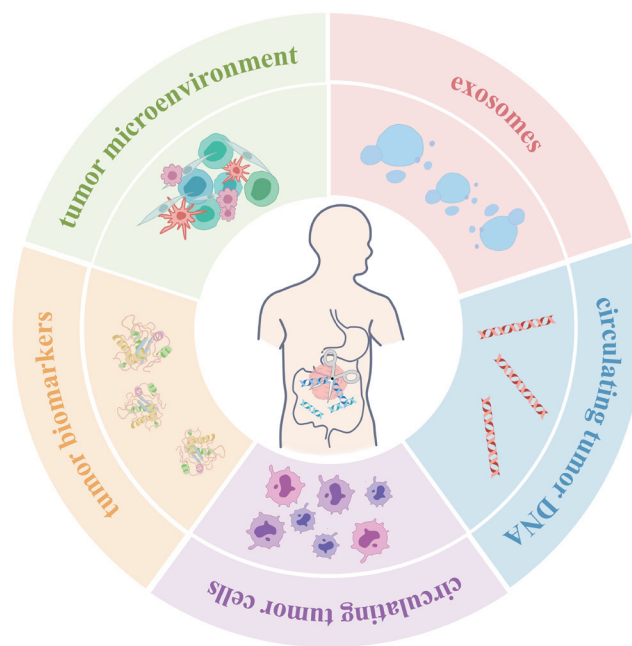


Fig. 1. Applications of CRISPR/Cas-based biosensing strategies in tumor diagnosis.

and guides the selection of treatment plans, thereby greatly improving the level of tumor diagnosis and treatment.^{21,22}

This paper discusses the applications of the CRISPR/Cas system in tumor diagnosis, including the detection of tumor exosomes, circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), biomarkers, and tumor microenvironment (TME) factors. A diagram of the study is shown in Figure 1. Firstly, the detection principles and advantages of these applications are introduced. Then, the effects of various CRISPR/Cas-based detection strategies in tumor diagnosis are described. Finally, the prospects of CRISPR/Cas-based biosensing for tumor diagnosis are discussed, aiming to provide references for early tumor detection and precision treatment.

Detection of exosomes

Exosomes, small vesicles secreted by cells, carry components such as proteins, lipids, mRNAs, and miRNAs. Their surface contains specific protein markers, such as CD9 and CD63. Exosomes play important roles in cell-to-cell signaling, tumor metastasis, immune regulation, and other processes.^{23,24} CRISPR/Cas-based exosome detection strategies can effectively improve detection sensitivity, expand the range of detectable tumors, and potentially achieve non-invasive early tumor diagnosis and non-invasive “liquid biopsy”. Researchers have achieved specific capture and identification of tumor exosomes by designing CRISPR-Cas systems targeting tumor-related proteins or RNA,^{25,26} and then introduced various signal amplification strategies to achieve highly sensitive detection.^{27,28} Xing *et al.* proposed an innovative approach, apta-HCR-CRISPR assay, for the direct and highly sensitive detection of tumor-derived extracellular vesicle (TEV) protein biomarkers.²⁹ The schematic diagram is illustrated in Figure 2a.^{29–32} Combining hybridization chain reaction (HCR) and CRISPR-Cas12a, the method achieved a low limit of detection (LOD) of 102 particles/μL, surpassing traditional assays like aptamer-ELISA and apta-HCR-ELISA. Application to

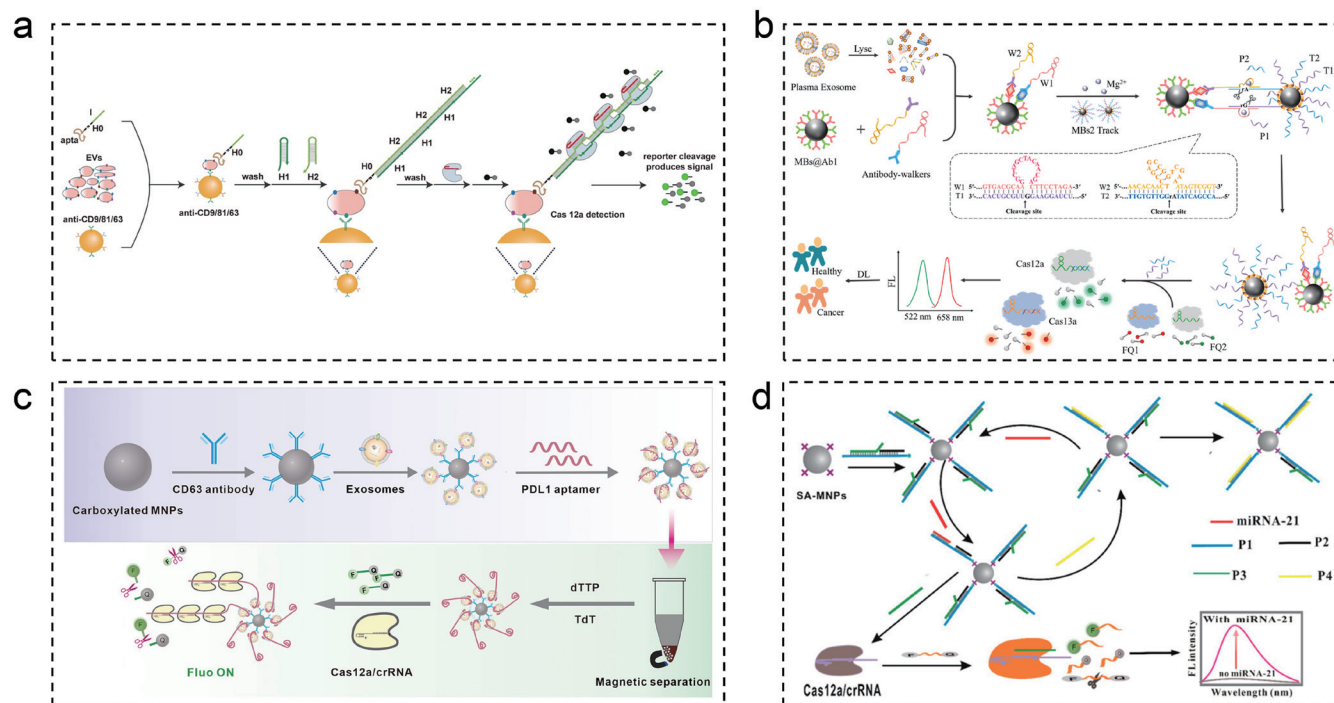


Fig. 2. The CRISPR-Cas system combined with multiple signal amplification strategies can detect tumor exosome components. (a) Utilizing HCR and CRISPR-Cas12a double amplification technique, a highly sensitive apta-HCR-CRISPR method was devised for the detection of TEV protein.²⁹ (b) A novel CRISPR-Cas12a/Cas13a approach, activated by DNAzyme walkers, is suggested for the concurrent identification of exosomal proteins such as SAA1 and clotting FV.³⁰ (c) A fluorescence aptasensor was created to detect NPC-derived exosomes by utilizing a combination of MNPs, TdT, and CRISPR/Cas12a.³¹ (d) An innovative biosensor, incorporating MNPs, CSDR, and CRISPR/Cas12a, was created to identify the exosome miR-21 in lung cancer.³² Cas12a, CRISPR-associated protein 12a; Cas13a, CRISPR-associated protein 13a; CD, Cluster of Differentiation; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; crRNA, CRISPR RNA; DL, Deep Learning; EVs, Extracellular Vesicles; FQ, fluorophore-quencher reporter; PD-L1, Programmed Death-Ligand 1. (a) Reproduced under the applicable open-access license. (b) Reproduced with permission from Elsevier. (c) Reproduced with permission from Springer Nature. (d) Reproduced under the applicable open-access license.

clinical samples revealed diagnostic potential for nucleolin+ TEVs in nasopharyngeal carcinoma (NPC) and therapeutic monitoring using PD-L1+ TEVs. The assay's simplicity suggests its utility for sensitive and versatile TEV protein quantification in clinical settings. Ding *et al.*³⁰ designed a CRISPR-Cas12a/Cas13a dual system driven by DNAzyme walkers. The schematic representation is shown in Figure 2b. They constructed two DNAzyme walker probes that specifically bind to two tumor-related exosome surface proteins. When probes bind to their targets, DNAzyme cleavage reactions are initiated, releasing DNA sequences that guide gene editing. The activated Cas enzymes then cleave fluorescence labeled short chains, generating a fluorescent signal. This system can sensitively detect exosome proteins in various body fluid samples and achieve intelligent diagnosis through the fluorescent ratio of the two Cas enzymes. The assay is highly sensitive (with limits of detection as low as 30.00 pg/mL for serum amyloid A-1 protein and 200.00 pg/mL for coagulation factor V), highly specific, and ideally accurate. Moreover, this approach enables the simultaneous detection of multiple targets in tumor exosomes and demonstrates the potential of liquid biopsy technology. Yi *et al.*³¹ reported a fluorescent aptamer sensor based on magnetic nanoparticles, CRISPR/Cas12a system, and terminal transferase for detecting exosomes from nasopharyngeal carcinoma. The illustrative diagram is presented in Figure 2c. They coupled the Cas12a enzyme with terminal deoxynucleotidyl transferase (TdT), and once the target gene sequence was recognized, the activated Cas12a guided the TdT catalytic reaction to synthesize repetitive

sequences and produce a fluorescent signal. Under the optimized conditions, the ability of the fabricated fluorescence aptasensor to detect nasopharyngeal carcinoma-derived exosomes was excellent, with a linear range between 500 to 5×10^4 particles mL^{-1} and a limit of detection of 100 particles mL^{-1} . Liu *et al.*³² proposed a method for detecting tumor exosome miR-21 via a gene editing system, magnetic nanoparticles, and cascade displacement reaction fluorescence. Figure 2d illustrates the conceptual diagram. Utilizing the chain displacement activity of the Cas12a enzyme activated by miR-21, they constructed a cascade signal amplification system, using magnetic nanoparticles for exosome extraction and enrichment from the samples. The CRISPR-Cas system can transform exosome detection into specific nucleic acid or protein recognition,^{33,34} with higher integration and simpler operation,^{35,36} enabling synchronous detection of multiple components such as miRNAs and proteins.^{37,38} These methods overcome the challenges of detecting exosomes with extremely low abundance, achieve high-throughput and precise analysis of trace tumor exosomes in body fluids, and broaden the possibilities for accurate diagnosis of exosomes.³⁹

Circulating tumor DNA testing

ctDNA refers to fragmented DNA released by tumor cells undergoing apoptosis or necrosis, which can be obtained from blood samples.⁴⁰ It represents the entire tumor genome and can be used as a real-time and dynamic monitor for tumor changes. It serves as a cru-

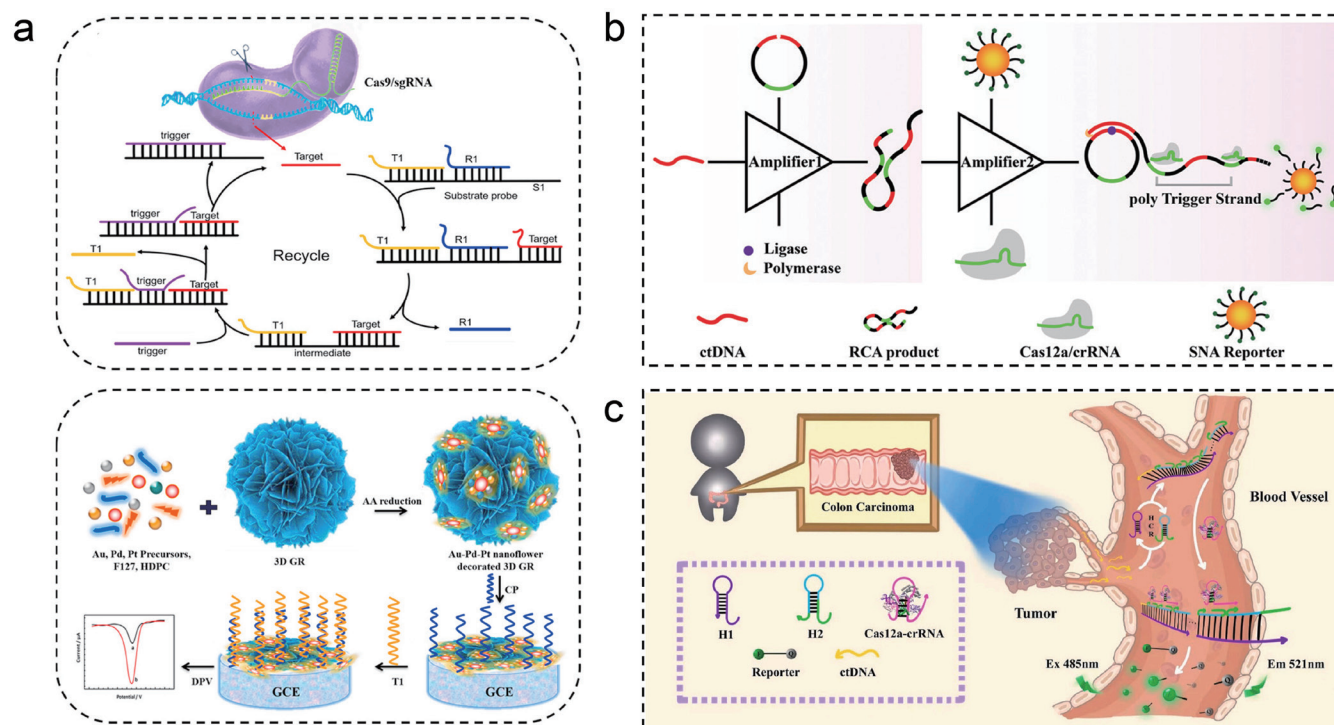


Fig. 3. The CRISPR-based recognition and cleavage model, in conjunction with signal amplification and detection techniques such as electrochemistry and fluorescence, was able to identify ctDNA. (a) A novel 3D GR/AuPtPd nanoflower sensing platform, based on CRISPR/Cas9 cleavage-triggered ESDR, has been developed for the effective detection of ctDNA.⁴² (b) An amplifier utilizing spherical cascade CRISPR/Cas12a and nucleic acid reporter was designed to enhance the accuracy and responsiveness of ctDNA detection.⁴³ (c) A method was devised for the precise identification of ctDNA through the utilization of HCR and adjacent hybridization regulated CRISPR/Cas12a, employing a dual-signal amplification strategy.⁴⁴ ctDNA, circulating tumor DNA; DPV, differential pulse voltammetry; ESDR, entropy-driven strand displacement reaction; GCE, glassy carbon electrode; GR, graphene; HCR, hybridization chain reaction; HDPC, high definition photoconductor. (a) Reproduced with permission from Elsevier; (b) Reproduced with permission from Royal Society of Chemistry. (c) Reproduced with permission from Elsevier.

cial indicator for liquid biopsy of tumors, although its concentration in samples is relatively low.⁴¹ Chen *et al.* utilized a complex of Cas9 protein and sgRNA to target and cleave ctDNA,⁴² exposing hydroxyl groups captured by 3D graphene/AuPtPd nanoflower materials. As depicted in Figure 3a,^{42–44} the diagram provides a visual representation. Subsequently, platinum ions are released and deposited on the working electrode surface, generating a strong electrochemical signal. With these methods, the content of ctDNA in the sample can be further calculated. Zhou *et al.*⁴³ devised a cascaded amplification system employing CRISPR/Cas12a. The schematic representation is shown in Figure 3b. Upon recognition of the target DNA by the first-level Cas12a, the second-level Cas12a is triggered to amplify the signal. Spherical nucleic acids serve as reporting molecules due to their high stability and exhibit a notable signal amplification effect, leading to enhanced stability and sensitivity (5 orders of magnitude). Li *et al.*⁴⁴ proposed a targeted amplification strategy by designing gRNAs that specifically target ctDNA sequences, identifying and cleaving them to achieve highly selective amplification. The diagram in Figure 3c serves as a visual representation. Subsequently, a protonated probe is introduced, which hybridizes with the adjacent cut end of the ctDNA, triggering a fluorescent signal and achieving dual signal amplification of the product, thus enabling precise detection of ctDNA with extremely low abundance in body fluid samples. This strategy exhibits high sensitivity for ctDNA detection with a low limit of 5.43 fM. Liu *et al.*⁴⁵ employed an electrochemical method to detect the EGFR gene in ctDNA from non-small cell

lung cancer (NSCLC) patients. They designed crRNAs targeting the EGFR gene's 19del and L858R mutation sites, which were mixed with Cas12a, substrate strands, and the target ctDNA. When the crRNA recognizes the mutation site, Cas12a cleaves the substrate strand, generating an electrochemical signal. Wang *et al.*⁴⁶ also proposed a method for detecting mutations in ctDNA from NSCLC patients. They designed a crRNA library targeting various gene mutation sites, such as those in EGFR, KRAS, and TP53. The ctDNA was pre-amplified using multiplex PCR to enrich the mutation sites, and then Cas12a was employed with the crRNA library. When the crRNA paired with the target mutation, Cas12a was activated, and the probes were cleaved, resulting in a fluorescent signal. This method achieved high-throughput, multi-site parallel detection of common driver gene mutations in NSCLC, offering broader applicability compared to single-target detection. Wu *et al.*⁴⁷ proposed a chain displacement amplification fluorescence resonance energy transfer technique. This technique utilized the non-specific cleavage activity of the Cas12a enzyme and introduced initiation probes and reporting probes to initiate a chain displacement reaction and amplify the signal.⁴⁸ These studies have designed targeted CRISPR/Cas-based recognition and cleavage models, combined with signal amplification and techniques such as electrochemistry and fluorescence.⁴⁹ Extremely low abundance ctDNA and mutations in bodily fluid samples have been successfully detected.⁵⁰ This approach has the potential to greatly improve the accuracy of early cancer diagnosis.⁵¹

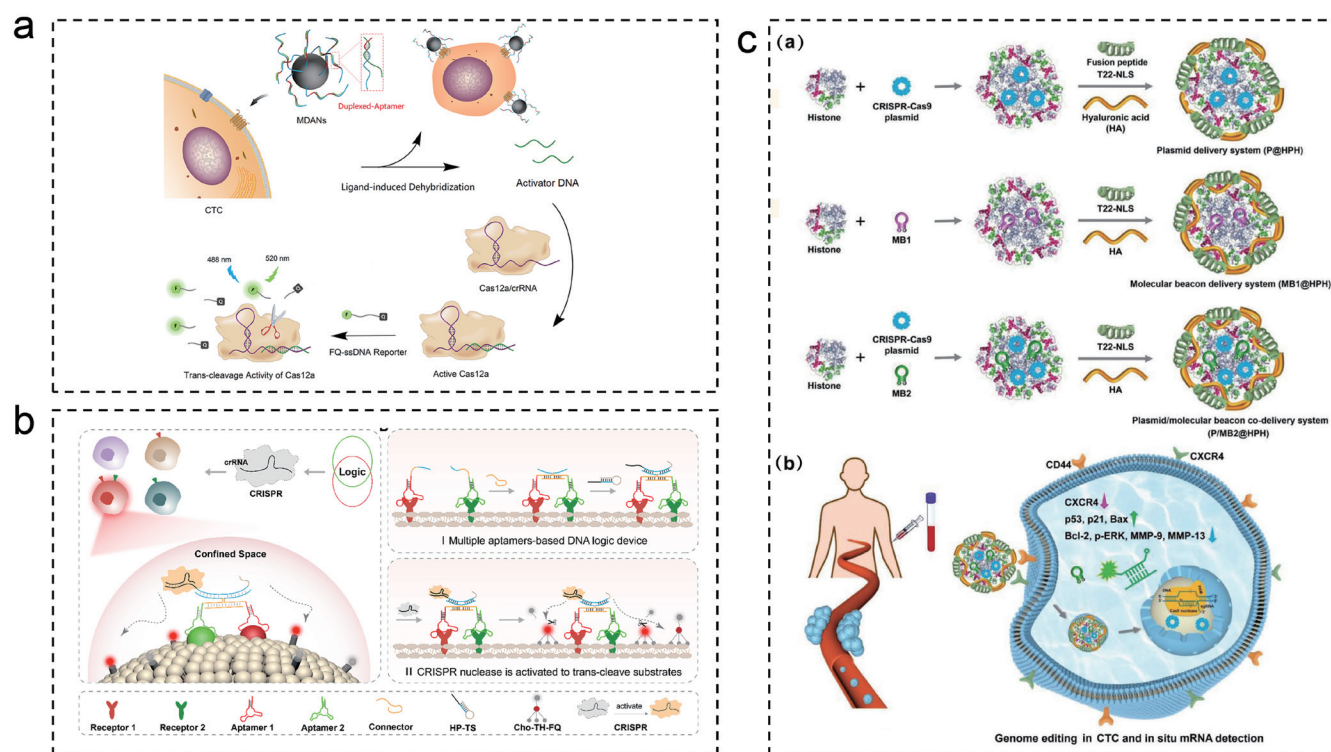


Fig. 4. An innovative CTC detection system, utilizing DNA aptamers and CRISPR-Cas effectors, was devised to attain precise capture and precise detection of CTC. (a) A sensor that detects CTCs using CRISPR-Cas12 MDANs has been reported.⁵⁶ (b) A FINDER was successfully developed on living cell membranes, enabling the rapid and sensitive identification of cancer cells in clinical blood samples.⁵⁸ (c) The reprogramming of CTCs with in situ detection of biomarkers at the single-cell level is achieved through the utilization of multi-functionalized nano-systems containing a delivery vector consisting of histone for plasmid loading, hyaluronic acid for tumor targeting, and a fusion peptide for CXCR4 targeting, along with nuclear localization.⁵⁹ CTC, circulating tumor cell; MDANs, multivalent duplexed-aptamer networks; FINDER, fluidly confined CRISPR-based DNA reporter; CXCR4, C-X-C motif chemokine receptor 4; MMP, Matrix Metalloproteinase. (a) Reproduced under the applicable open-access license. (b) Reproduced with permission from Wiley. (c) Reproduced under the applicable open-access license.

Detection of CTCs

CTCs are tumor cells that detach from primary tumors or metastatic lesions and enter the circulation.⁵² They play a crucial role in tumor progression and metastasis.⁵³ Various biosensing strategies have been developed for the detection and analysis of CTCs.^{54,55} CRISPR/Cas-based approaches have further shown potential for sensitive CTC detection.^{56,57} Lv *et al.*⁵⁶ designed and constructed a multivalent double-stranded DNA aptamer network that specifically recognizes tumor surface markers. Figure 4a displays the conceptual diagram.^{56,58,59} Upon binding the aptamer to the target, the connected Cas12a enzyme is activated to cleave the reporter gene, generating a fluorescent signal. The detection limit of this method can reach 26 cells/mL. Wang *et al.*⁵⁷ designed a dual-nucleic acid adapter DNA network to enhance the specific capture of CTCs. Using CRISPR/Cas12a as a signal amplifier, the DNA network can release captured CTCs for subsequent analysis through chemotaxis by inputting a release signal DNA strand. The specificity and sensitivity of the test were demonstrated in human blood samples. Yin *et al.*⁵⁸ developed a fluidly confined CRISPR-based DNA reporter (FINDER) on live cell membranes for the rapid and sensitive identification of cancer cells. The schematic illustration is shown in Figure 4b. FINDER consists of sgRNA and Cas proteins that target specific sequences of cancer-associated genes. When FINDER interacts with the target sequences, it triggers the enzymatic activity of Cas proteins, releasing a fluorescent signal for real-time detec-

tion of live cells. The FINDER rapidly identified target cells in only 20 min, and achieved over 80% recognition efficiency even with only 0.1% of the target cells being present in clinical blood samples. This approach holds great potential for precision medicine and biosensing applications. Ren *et al.*⁵⁹ designed a histone-composed nanodelivery system for in situ detection of biomarkers at the single-cell level. The schematic representation is presented in Figure 4c. They combined histones with CRISPR/Cas9 plasmids and surface-modified hyaluronic acid to target CD44, as well as a fusion peptide of T22 sequence to target CXCR4 and a nuclear localization sequence. This system selectively delivered the gene editing plasmids to CTCs in whole blood, especially those overexpressing CD44 and CXCR4, enabling efficient operation of gene editing. Molecular beacons for p53 and p21 were loaded and detected within the delivery system at a single-cell level. In addition, gene editing technology not only can be used to repair disease-causing genes for treatment,⁶⁰ but also to detect the expression and mutations of specific genes, evaluate the risk of drug resistance in tumors, and guide individualized drug treatment,⁶¹ laying the theoretical foundation for precision medicine.⁶²

Detection of tumor biomarkers

Tumor biomarkers are a series of biological indicators associated with the formation and progression of tumors, including

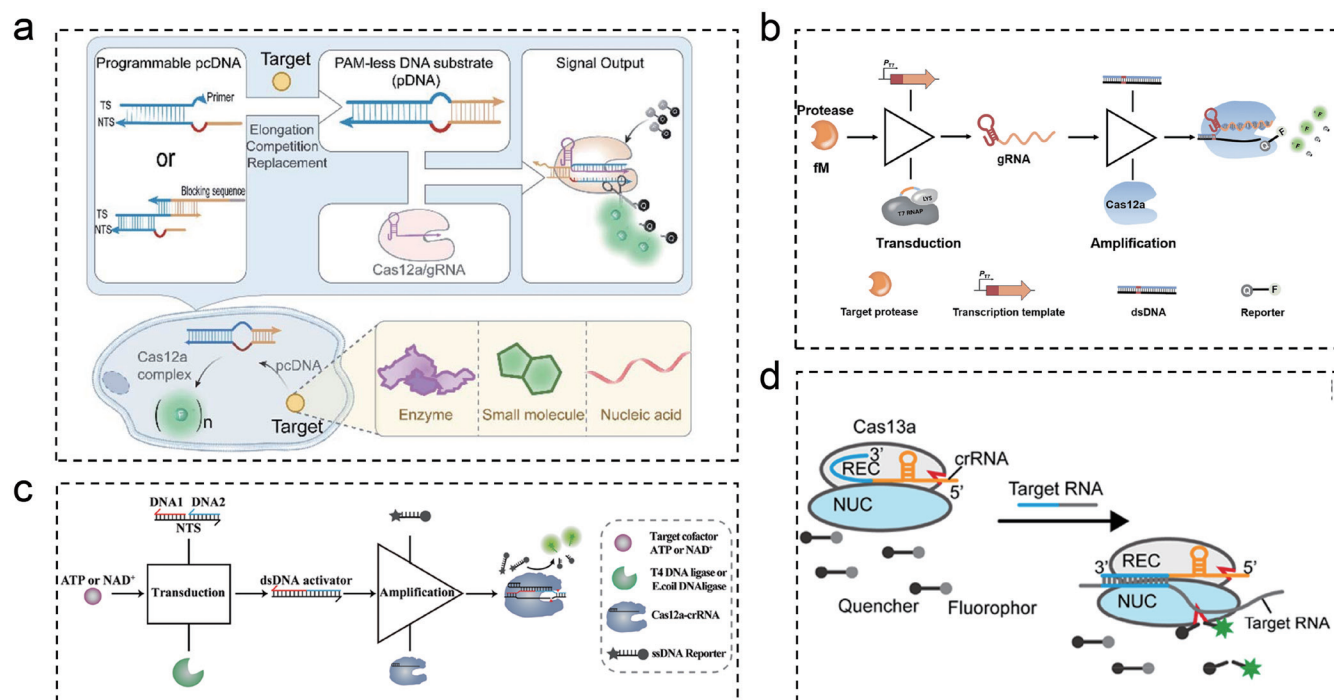


Fig. 5. The innovative combination of CRISPR/Cas technology with immunoassay, DNA nanotechnology, and multiple signal amplification technologies provides a fundamental platform for tumor marker monitoring. (a) A CRISPR-Cas12a system was designed that uses a generalized stimulus-response switching mechanism based on pcDNAs. DNA substrates that do not necessitate the PAM and bubble structure are used to construct stimulation-responsive pcDNAs. The CRISPR-Cas12a transcleavage capability was then employed for signal reporting, and a multifunctional CRISPR-based living cell biosensing system was set up to identify tumor markers.⁷⁰ (b) The utilization of both protease-induced transcriptional activation and CRISPR-Cas12a technology allowed for the identification of tumor marker protease.⁷³ (c) The purpose of this paper is to elucidate a CRISPR-Cas12a sensing platform that is activated by DNA linking reactions triggered by biomolecules, leading to the production of DNA double strands that activate Cas12a's nuclease activity. Consequently, this platform generates an amplified fluorescent signal, enabling the sensitive detection of non-nucleic acid targets, including NAD⁺. ATP and PNK are involved in the process.⁷⁴ (d) The mechanism utilizes a CRISPR/Cas13a-based signal output amplification strategy to double the output signal through T7 RNA polymerase transcription and CRISPR/Cas13a side cutting activity. Detection of inflammatory factor human IL-6 and tumor marker human VEGF.⁷⁵ NUC, nuclease lobe; REC, recognition lobe; pcDNAs, PAM-less conditional DNA substrates; PAM, protospacer-adjacent motif; PNK, polynucleotide kinase; VEGF, vascular endothelial growth factor. (a) Reproduced with permission from Royal Society of Chemistry. (b) Reproduced with permission from Springer Nature. (c) Reproduced with permission from Royal Society of Chemistry. (d) Reproduced with permission from American Chemical Society.

molecular markers reflecting genetic changes, related cytokines and receptors, tumor cell-specific metabolic products, enzymes with increased activity or expression, and antigens suggesting the occurrence of tumors.⁶³ These biomarkers serve multiple purposes, such as tumor detection, classification, prognosis assessment, and clinical observation.^{63,66} Combining CRISPR/Cas-based biosensing with immunodetection, DNA nanotechnology, and various signal amplification techniques can enable highly sensitive detection of key tumor biomarkers.^{64,65,67} For example, telomerase activity is suppressed in normal human tissues, but is reactivated in approximately 90% of cancer cells, allowing them to maintain telomere length during cell division and achieve immortality.⁶⁸ Therefore, aberrant telomerase activity is considered to be associated with various tumors.⁶⁹ Chen *et al.*⁷⁰ designed a special double-stranded DNA structure as a substrate, in which the guiding sequence and the reporting sequence bind complementarily to form a helical structure. Figure 5a shows a depiction of the conceptual diagram.^{70,73-75} When telomerase cleaves the guiding sequence, the DNA structure is unwound, releasing the guiding sequence to bind to Cas12a, activating Cas12a's cleavage activity to cut the fluorophore on reporting sequence, generating a fluorescent signal. Prostate-specific antigen (PSA) is secreted by prostate epithelial cells and belongs to the kallikrein

family of proteins. It is present in prostate tissue and semen, and its concentration in normal human serum is extremely low. PSA is the preferred biomarker for diagnosing prostate cancer. Liang *et al.*⁷¹ linked anti-PSA antibodies to Cas12a. When PSA is present, it binds to the antibody, inhibiting the endonuclease activity of Cas12a, thereby suppressing the cleavage of the reporting molecule, resulting in a decreased fluorescent signal. The optical density transducer created by platinum nanoparticles (OD-CRISPR) assay achieved a minimal detectable concentration of 0.01 ng/mL. Jia *et al.*⁷² utilized the CRISPR system along with a personal blood glucose meter for the quantitative detection of the liver cancer biomarker alpha-fetoprotein (AFP). The detection module comprises a gRNA to guide the CRISPR system, Cas12a enzyme linked to glucose oxidase, and a reporting module. They quantitatively detected the AFP biomarker in spiked human serum samples with a detection sensitivity down to 10 ng/mL. In the presence of AFP, it specifically binds to the gRNA, activating the enzyme activity of Cas12a. This, in turn, cleaves the sequence linking glucose oxidase, releasing the enzyme to catalyze glucose and generate glucuronic acid, which can be detected using a personal blood glucose meter. In another innovative approach, Hong *et al.*³⁹ combined the CRISPR/Cas13a system with liposome technology for direct detection of trace amounts of

miRNA in cellular and bodily fluid samples without nucleic acid extraction and amplification. They detected miR-21-5p in plasma samples from ovarian cancer patients. Additionally, Yang *et al.*⁷³ designed a CRISPR-Cas12a-based protease-inducible transcription activation system, where the protease cleaves the peptide chain to activate downstream gene expression and drive the CRISPR-Cas12a system, enabling highly sensitive detection of tumor-associated protease biomarkers. Figure 5b provides an illustration of the concept. In the subsequent study, they overcame the limitation of CRISPR-Cas system in detecting only nucleic acid by designing DNA linker reactions, enabling the detection of tumor-associated proteins and small molecules.⁷⁴ The diagram presented in Figure 5c provides a visual representation. Chen *et al.*⁷⁵ combined CRISPR/Cas13a with immunoassay technology to design a dual signal amplification mechanism, greatly improving the detection sensitivity of biomarker proteins. The conceptual diagram is visually represented in Figure 5d. Liu *et al.*⁷⁶ integrated the CRISPR/Cas13a system with gold nanoparticles technology, achieving direct detection of low-abundance RNA in cells without nucleic acid amplification or labeling. Zhao *et al.*⁷⁷ proposed a multifunctional biosensing platform integrating the CRISPR-Cas12a system with aptamers, which can detect a variety of target molecules. Chen *et al.*⁷⁸ integrated CRISPR-Cas12a with rolling circle amplification, significantly improving the detection sensitivity of circular non-coding RNA. Wu *et al.*⁷⁹ used the CRISPR/Cas13a system with transcription-mediated signal amplification to achieve highly sensitive detection of the important tumor immunotherapy response marker IL-15 mRNA. These studies demonstrate the potential of CRISPR/Cas-based biosensing in detecting nucleic acid and non-nucleic acid biomarkers,⁸⁰ greatly expanding the detection applications of gene editing technology and promoting rapid development in this field.^{81,82} With the gradual improvement in detection sensitivity, it is foreseeable that CRISPR-Cas technology has the potential to achieve precise diagnosis and prediction of tumors at an early stage.^{83,84}

Related CRISPR applications in TME analysis

TME is the intra- and extra-cellular microenvironment surrounding tumor tissues, including various stromal cells, immune cells, and the vascular system coexisting with tumor cells, as well as non-cellular components such as extracellular matrix, cytokines, and chemotactic factors.⁸⁵ The TME has complex interactions with tumor cells and provides a suitable soil for tumor initiation, development, invasion, and metastasis, playing a crucial role in the progression of tumors.⁸⁶ Dhainaut *et al.*⁸⁷ developed a gene knockout screening system on tissue slices to preserve tissue structure and microenvironmental information. By conducting gene knockouts in human and mouse tumor samples, they used image analysis to evaluate changes in the microenvironment and identified several potential regulators of the TME. This study demonstrated the potential of combining CRISPR gene editing technology with histological analysis for detecting the TME. Dervovic *et al.*⁸⁸ used in vivo CRISPR gene knockout screening to identify genes Serpinb9 and Adam2 that are involved in regulating the immune response to lung cancer immunotherapy. In addition, there are studies that utilize gene editing technology to modify the TME, including targeting T cells, CAR-T cells,⁸⁹ tumor-associated macrophages,⁹⁰ etc., to enhance the effectiveness of tumor immunotherapy or identify key genes regulating tumor immunotherapy.⁹¹ These efforts provide important insights and tools for the development of more effective precise immunotherapies.

Prospects

CRISPR/Cas-based biosensing holds great potential in advancing tumor diagnosis. By combining programmable target recognition with different signal amplification strategies, CRISPR/Cas systems can facilitate sensitive detection of tumor-associated nucleic acids, proteins, and other biomarkers in blood or other biological samples. Advantages of CRISPR/Cas system in detecting exosomes, ctDNA, CTCs, and tumor markers are summarized in Table 1.^{30,31,43,44,56,58,71,72} However, there are still several bottlenecks remain before clinical translation. Firstly, molecular diagnosis is not equal to clinical diagnosis currently. False-positive and false-negative results can lead to misdiagnosis, and clinical diagnosis requires other methods for synthetic judgments. Secondly, there is a need to improve the specificity and sensitivity of cancer biomarkers in the CRISPR/Cas system. Finally, the ethical considerations should not be overlooked. One of the ethical challenges and mitigation strategies is privacy protection: the use of CRISPR technology may involve obtaining and modifying personal genetic information. To protect patient privacy, researchers should comply with relevant ethical guidelines and regulations, using measures such as anonymization and encryption. The second is patient informed consent: Patients should fully understand the potential risks and benefits of CRISPR technology. Transparent communication and comprehensive patient education are key to addressing ethical issues, and informed consent should be obtained before any gene-editing procedure is undertaken. These issues should be addressed to support the responsible clinical translation of CRISPR/Cas-based biosensing.

Limitations

This narrative review has several limitations. The literature was not identified using a predefined systematic search or formal quality-assessment process; therefore, some selection bias may be present. In addition, the included studies used heterogeneous targets, sample types, assay designs, and performance measures, which limits direct comparison among different CRISPR/Cas-based biosensing strategies.

Conclusions

CRISPR/Cas-based biosensing strategies are rapidly developing for the detection of tumor-derived exosomes, ctDNA, CTCs, and tumor biomarkers. Current studies demonstrate promising analytical sensitivity and flexible integration with different signal amplification platforms; however, further standardization and clinical validation are needed before these approaches can be routinely applied to cancer screening or diagnosis. Continued development and validation may facilitate the translation of CRISPR/Cas-based biosensing toward precision cancer diagnosis.

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Table 1. Advantages of CRISPR/Cas system in detecting exosomes, ctDNA, CTCs, and tumor markers

Title	Method	Objects	Detection capability	Ref
Universal DNzyme walkers-triggered CRISPR-Cas12a/Cas13a bioassay for the synchronous detection of two exosomal proteins and its application in the intelligent diagnosis of cancer	Cas12a/Cas13a	exosome	limits of detection as low as 30.00 pg/mL for serum amyloid A-1 protein and 200.00 pg/mL for coagulation factor V	30
Fluorescent aptasensor based on the MNPs-CRISPR/Cas12a-TdT for the determination of nasopharyngeal carcinoma-derived exosomes.	Cas12a	exosome	linear range between 500 to 5×10^4 particles mL ⁻¹ and the limit of detection of 100 particles mL ⁻¹	31
Spherical nucleic acid reporter-based cascade CRISPR/Cas12a amplifier for stable and sensitive biosensing of circulating tumor DNA	Cas12a	ctDNA	Improved stability and sensitivity (5 orders of magnitude)	43
Proximity hybridization-regulated CRISPR/Cas12a-based dual signal amplification strategy for sensitive detection of circulating tumor DNA	Cas12a	ctDNA	detection limit of 5.43 fM	44
Multivalent Duplexed-Aptamer Networks Regulated a CRISPR Cas12a System for CTC Detection	Cas12a	CTCs	The detection limit is 26 cells/ml	56
FINDER: A Fluidly Confined CRISPR-Based DNA Reporter on Living Cell Membranes for Rapid and Sensitive Cancer Cell Identification	CRISPR	CTCs	The FINDER rapidly identified target cells in only 20 min, and achieved over 80 % recognition efficiency with only 0.1 % of target cells presented in clinical blood samples	58
Platinum nanoparticles-based CRISPR/Cas12a platform for detection of nucleic acid and protein in clinical samples	Cas12a	tumor biomarkers	The lowest detection concentration was 0.01 ng/mL	71
CRISPR-powered biosensing platform for quantitative detection of alpha-feto-protein by a personal glucose meter	CRISPR	tumor biomarkers	The detection sensitivity is as low as 10 ng/mL	72

Cas12a/Cas13a, CRISPR-associated protein 12a/CRISPR-associated protein 13a; ctDNA, circulating tumor DNA; CTCs, circulating tumor cells; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; FINDER, a fluidly confined CRISPR-based DNA reporter; MNPs, magnetic nanoparticles.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Literature review and manuscript writing (QLY and XYZ), drafting figures (LMZ and HZ), and topic conception and critical revision (SWH). All authors have made significant contributions to this study and approved the final manuscript.

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