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Review Article

Advances in CRISPR Based Biosensing Strategies for Cancer Diagnosis

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Abstract

With the development of gene editing technology, its application in tumor diagnosis is becoming increasingly widespread. The CRISPR/Cas system is an important gene editing tool that can significantly improve the early detection rate and precision diagnosis level, enabling high-throughput and high-sensitivity detection of tumors. This article focuses on CRISPR/Cas system for detecting various tumor-related targets and elaborates on its applications in tumor diagnosis from five aspects: (1) detection of tumor-derived exosomes: by recognizing the surface proteins or nucleic acids of exosomes secreted by tumor cells into blood or other samples through adaptors, the CRISPR system is activated, achieving non-invasive liquid biopsy of tumors; (2) detection of circulating tumor DNA tumor cells disseminate DNA into the circulatory system to trigger nucleic acid reactions involving gene editing enzymes, enabling the monitoring of tumor dynamic states; (3) detection of circulating tumor cells (CTCs): by using aptamers to recognize surface proteins of tumor cells or directly detecting tumor-related nucleic acids, the integrated CRISPR system allows for the detection of circulating tumor cells even in trace amounts, achieving precise diagnosis; (4) detection of tumor markers: high sensitivity is achieved through the coupling of various tumor marker aptamers and gene editing systems; (5) detection and identification of tumor microenvironments: by activating gene editing enzyme activity through differential factors in the tumor tissue microenvironment and triggering nucleic acid reactions, the diagnosis and dynamic monitoring of tumors can be achieved. The progress and bottlenecks of the CRISPR/Cas system in tumor diagnosis in the future are also discussed.

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 enables 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. This provides a more accurate and robust method for detecting PD-L1 exosomes in complex biological samples. In addition, recent advances in metal composite sensors,[8] such as the electrochemical-fluorescent dual-mode homogeneous sensor and the self-calibrating magnetic aptamer sensor,

Keywords: Gene editing technology; 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, Fluoroquinolone; GCE, Glassy Carbon Electrode; GR, Glucocorticoid Receptor; gRNAs, guide RNAs; HA, Hemagglutinin; HCR, hybridization chain reaction; HDPC, High Definition Photoconductor; MMP, Matrix Metalloproteinase; MNPs, magnetic nanoparticles; NLS, Nuclear Localization Signal; NUC, Nucleotide Excision Repair; PAM, protospace adjacent motif; PD-L1, Programmed Death-Ligand 1; REC, Recombination; 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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contribute to innovation in oncological diagnostics.[9] 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 treatment responses. The exploration of new diagnostic strategies holds significant value in tumor treatment in the future.

Gene editing technology, with its efficient and precise ability to manipulate genes, has demonstrated great potential in the field of tumor diagnosis with its fast and accurate detection capabilities.[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 Protospace 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, gene editing technology has significant advantages in operational procedures, detection time, and sensitivity. This not only enables precise tumor diagnosis but also predicts tumor occurrence and development, determines prognosis, and guides the selection of treatment plans, thereby greatly improving the level of tumor diagnosis and treatment.[21,22]

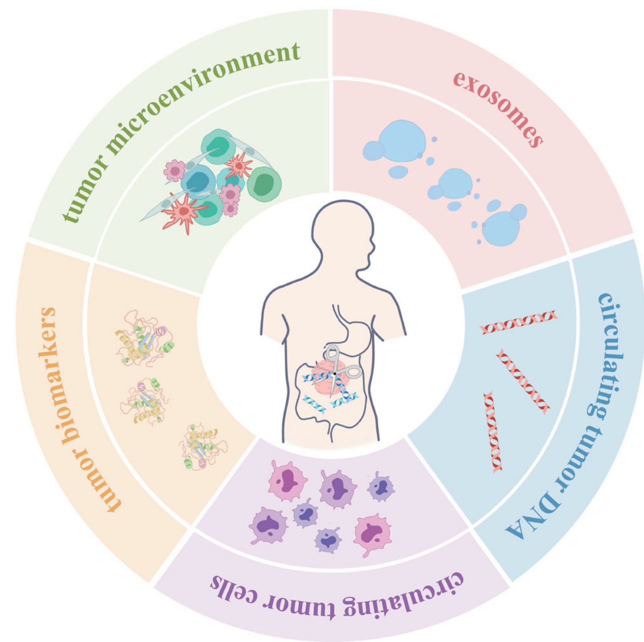

Fig. 1. Applications of gene editing technique in tumor diagnosis.

This paper systematically 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 gene editing technology for promoting the development of 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 con-

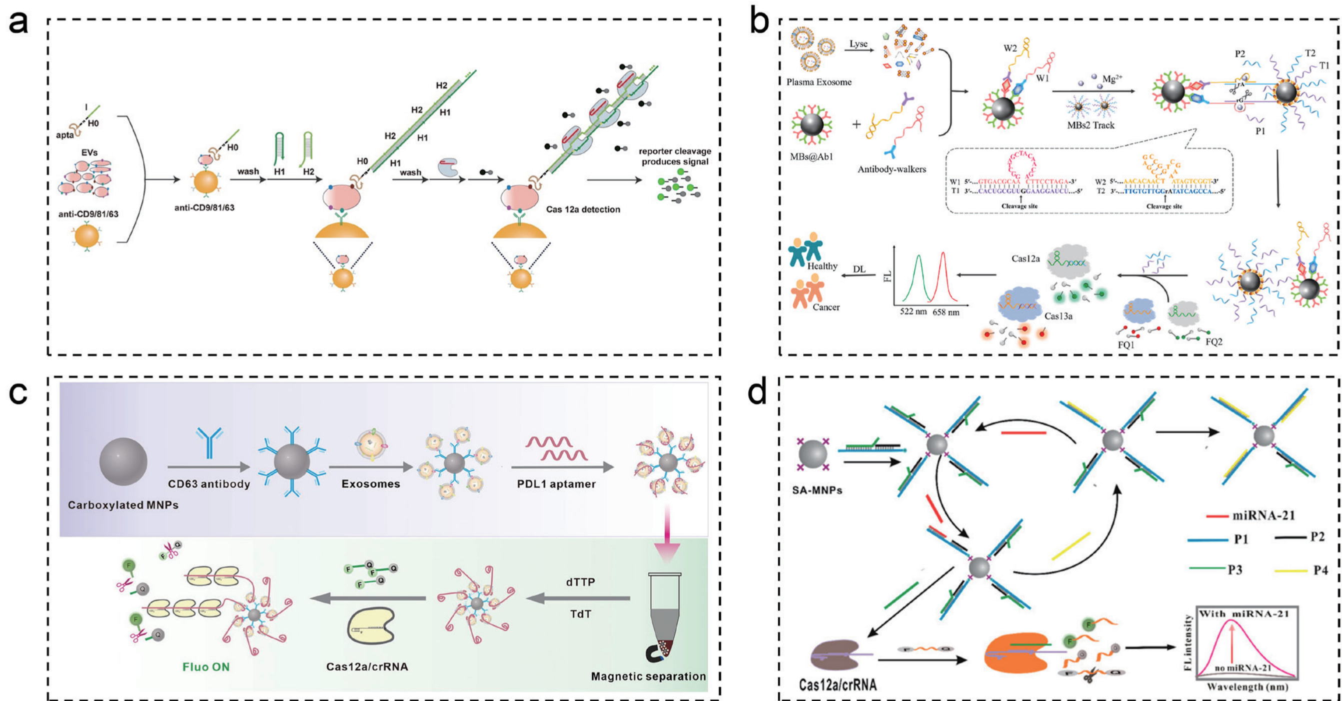

Fig. 2. The CRISPR-Cas system combined with multiple signal amplification strategies can detect tumor exosome components. (a) Utilizing HCR and CRISPR-Cas12a double amplification technique, a highly sensitive apta-HCR-CRISPR method was devised for the detection of TEV protein.[29] (b) A novel CRISPR-Cas12a/Cas13a 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, Fluoroquinolone;PD-L1, Programmed Death-Ligand 1. (a) Copyright 2020, Theranostics. (b) Copyright(2023), with permission from Elsevier; (c) Reproduced with permission from Springer Nature.(d) Copyright 2022, Multidisciplinary Digital Publishing Institute.

tains 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] Gene editing-driven 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 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

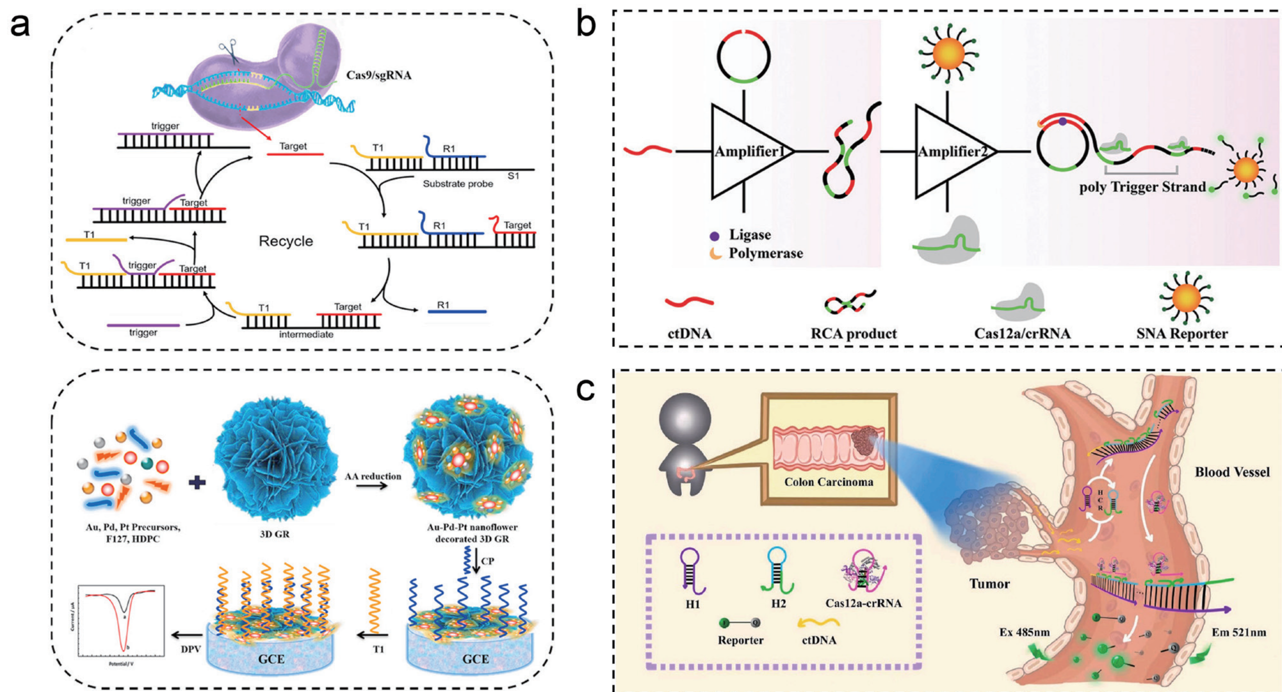

Fig. 3. The gene editing-based recognition and cutting model, in conjunction with signal amplification and detection techniques such as electrochemistry and fluorescence, was able to identify ctDNA. (a) A novel 3D GR/AuPtPd nanoflower sensing platform, based on CRISPR/Cas9 cleavage-triggered ESDR, has been developed for the effective detection of ctDNA.[42] (b) A 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 Voltammetr; ESDR, entropy-driven strand displacement reaction; GCE, Glassy Carbon Electrode; GR, Glucocorticoid Receptor; HCR, hybridization chain reaction; HDPC, High Definition Photoconductor. (a) Copyright(2020), with permission from Elsevier; (b) Copyright 2022, Royal Society of Chemistry. (c) Copyright(2020), with permission from Elsevier.

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 crucial indicator for liquid biopsy of tumors, although its concentration in samples is relatively low.[41] Xu *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

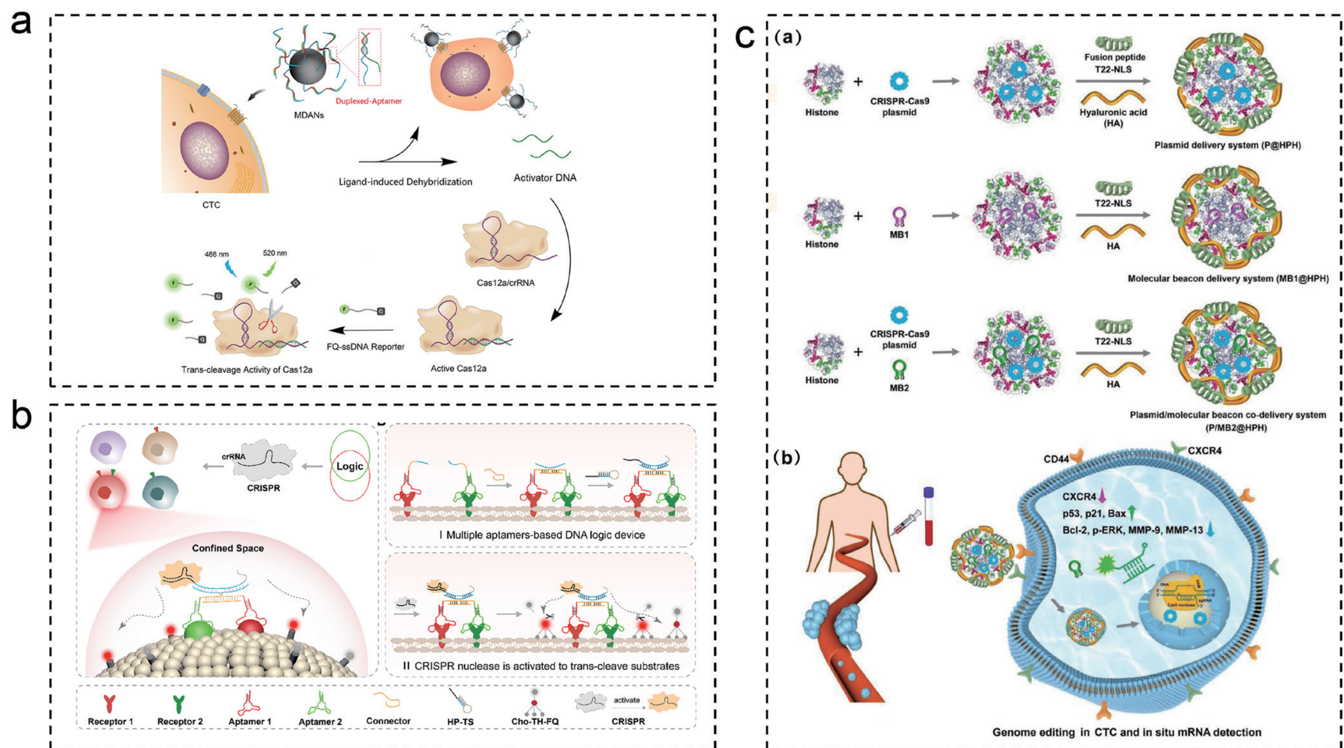

Fig. 4. An innovative CTC detection system, utilizing DNA aptamers and CRISPR-Cas effectors, was devised to attain precise capture and precise detection of CTC. (a) A sensor that detects CTCs using crispr-cas12 MDANs has been reported.[56] (b) A FINDER was successfully developed on living cell membranes, enabling the rapid and sensitive identification of cancer cells in clinical blood samples.[58] (c) The reprogramming of CTCs with in situ detection on 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) Copyright {2021} American Chemical Society. (b) Copyright 2023, Wiley. (c) Copyright 2022, Wiley.

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. Han *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 recognition and cleavage models based on gene editing, 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]

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] Gene editing technology holds significant potential for the detection of CTCs in cancer research.[54,55] Yang *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. Kong designed a dual-nucleic acid adapter DNA network to enhance the specific capture of CTCs.[57] 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 detection 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. Cheng *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

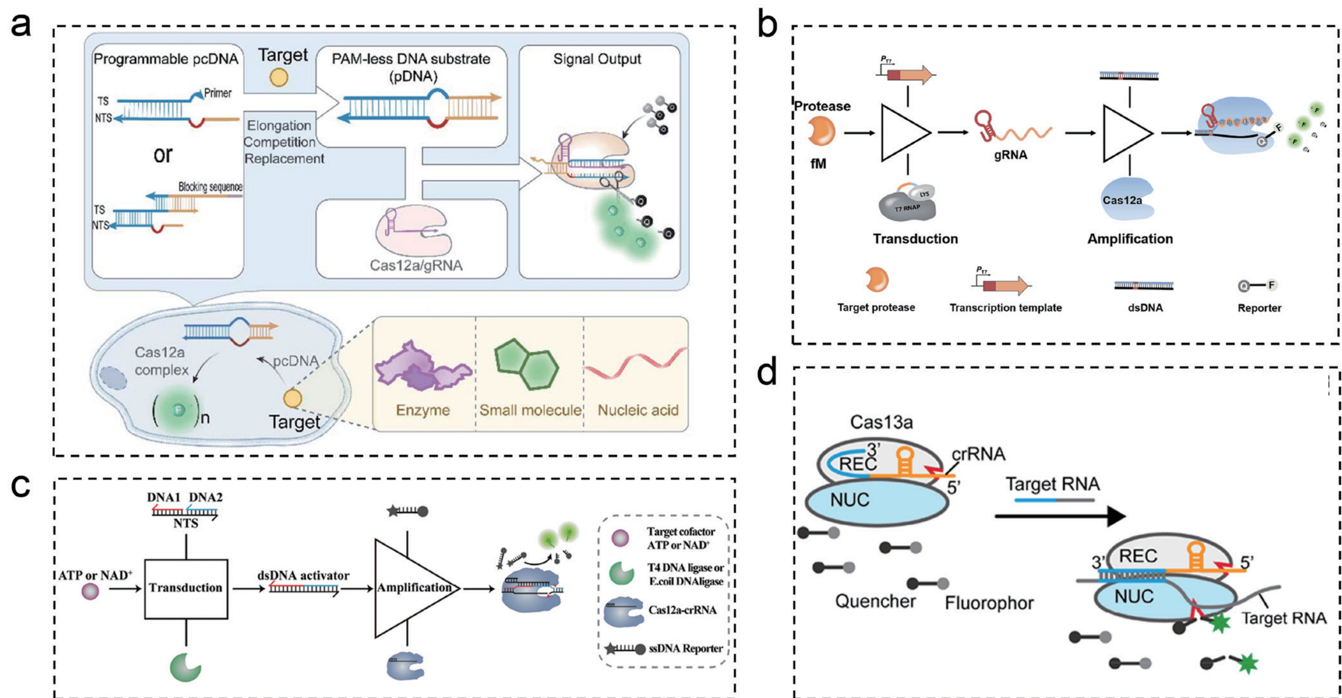

Fig. 5. The innovative combination of CRISPR/Cas technology with immunoassay, DNA nanotechnology, and multiple signal amplification technologies provides a fundamental platform for tumor marker monitoring. (a) A CRISPR-Cas12a system was designed that uses a generalized stimulus-response switching mechanism 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 gene editing 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/ Cas13-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, Nucleotide Excision Repair; REC, Recombination; pcDNAs, PAM-less conditional DNA substrates; PAM, protospacer-adjacent motif; PNK, polynucleotide kinase; VEGF, vascular endothelial growth factor. (a) Copyright 2022, Royal Society of Chemistry. (b) Reproduced with permission from Springer Nature. (c) Copyright 2021, Royal Society of Chemistry. (d) Copyright {2020} American Chemical Society.

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 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.[64,65] Combining gene editing technology with immunodetection, DNA nanotechnology, and various signal amplification techniques, ultra-sensitive detection of key tumor biomarkers can be achieved.[66,67] 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 Ca-

s12a, 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. Liu *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's 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. Xiaoming Zhou and others combined CRISPR/Cas13a with immunoassay technology to design a dual signal amplification mechanism, greatly improving the detection sensitivity of biomarker proteins.[75] The conceptual diagram is visually represented in Figure 5d. Srikanth Singamaneni and his team integrated the CRISPR/Cas13a system with gold nanoparticles technology, achieving direct detection of low-abundance RNA in cells without nucleic acid amplification or labeling.[76] Lixin Zhang and colleagues proposed a multifunctional biosensing platform integrating the CRISPR-Cas12a system with apatamers, which can detect a variety of target molecules.[77] Yuyang Jiang and others integrated CRISPR-Cas12a with rolling circle amplification, significantly improving the detection sensitivity of circular non-coding RNA.[78] WanLi Liu and colleagues 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.[79] These studies demonstrate the unique advantages of gene editing technology 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]

Detection of TME

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] Brian D Brown and colleagues developed a gene knockout screening system on tissue slices to preserve tissue structure and microenvironmental information.[87] 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. Daniel Schramek and his team 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.[88] 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

Gene editing technology holds great potential in advancing tumor diagnosis. Selective modification of genes enables a deeper understanding of the genetic factors that contribute to the development and progression of tumors. Targeting specific genes or genetic mutations associated with cancer holds promise for identifying novel diagnostic markers and refining diagnostic methods. The identification of oncogenes and tumor-suppressor genes has the potential to reveal the prepatent cancers. Non-invasive diagnostic methods for tumor-specific genetic material, such as ctDNA, CTCs, or exosomes, in blood samples or other bodily fluids, can facilitate the accurate diagnosis and gene editing technology plays a crucial role in improving the sensitivity and specificity. 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 that need to be addressed in the development of gene editing technology for tumor diagnosis. 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, off-target effects of gene editing tools can pose a challenge. Ensuring specific gene editing without inadvertently affecting other genes is crucial to avoid potential side effects. In addition, there is a need to improve the specificity and sensitivity of cancer biomarkers in the CRISPR/Cas system. The critical issue of in vivo delivery requires the development of more efficient vectors that can be used to deliver gene-editing tools precisely to target tissues. Finally, the ethical considerations surrounding gene editing technology 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. The application

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

Title	Method	Objects	Detection capability	Ref
Universal DNAzyme 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. MNPs, magnetic nanoparticles.	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: ctDNA, circulating tumor DNA	Stability and sensitivity are 5 orders of magnitude	43
Proximity hybridization-regulated CRISPR/Cas12a-based dual signal amplification strategy for sensitive detection of circulating tumor DNA	Cas12a	ctDNA: ctDNA, circulating tumor DNA	detection limit of 5.43 fM	44
Multivalent Duplexed-Aptamer Networks Regulated a CRISPR Cas12a System for CTC Detection	Cas12a	CTCs: CTCs, circulating tumor cells	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. FINDER, A Fluidly Confined CRISPR-Based DNA Reporter	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-fetoprotein 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.

of gene editing requires careful thought and consideration to ensure responsibility and ethical use.

Conclusions

Gene editing technology technology is undergoing a remarkable explosion, which can increase convenience for individuals in the field of tumor diagnosis. Applications of biosensing strategies involving tumor-derived exosomes, ctDNA, CTCs, tumor markers, and TMEs are blooming. However, there is still a long way for them to meet the standards for accurate diagnosis and precision medicine.

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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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