Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Review

CRISPR systems in cancer therapeutics and diagnostics

Mohsen Almakrami [1], Zubair Ahmed [2], Ghadah M Alali [3], Abdulmajeed Alqurashi [4 *]

[1] *Ministry of Heath Regional Lab and central Blood Bank Najran 66271, Saudi Arabia*
[2] *Ministry of Heath Central Blood Bank Najran 66271, Saudi Arabia*
[3] *Department, College of Science and Health Professions, - Al Ahsa, King Saud bin Abdulaziz University for Health Sciences, Saudi Arabia*
[4] *Department of Biology, College of Science, Taibah University, Medina, Saudi Arabia*

Article Info

Article history:

Received: November 07, 2025
Accepted: February 0, 2026
Published: March 31, 2026

Use your device to scan and read the article online

Abstract

Cancer is the leading cause of morbidity and mortality globally, underscoring the need for precise diagnostic approaches and effective therapeutic strategies. The invention of the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system has transformed cancer research by enabling accurate and efficient genome editing. This review provides a comprehensive overview of CRISPR-based applications in cancer therapeutics and diagnostics, beginning with an introduction to cancer biology and the CRISPR/Cas9 mechanism of action. The therapeutic potential of CRISPR is discussed with a focus on targeting oncogenes, restoring tumor suppressor gene function, and advancing cancer immunotherapy through approaches such as CAR-T cell engineering. The use of CRISPR-based screening platforms to identify genes associated with drug resistance is also examined, along with current challenges including off-target effects, delivery limitations, and ethical considerations, as well as future directions for clinical translation. In addition, the diagnostic capabilities of CRISPR technologies are highlighted, including the detection of circulating tumor DNA, cancer-associated extracellular vesicles, protein biomarkers, and microRNAs. Recent advances in CRISPR/Cas-based strategies aimed at improving detection sensitivity and specificity are summarized. Overall, this review highlights the expanding role of CRISPR systems in cancer diagnosis and therapy and emphasizes their potential to contribute to the development of more personalized and effective cancer management strategies.

Keywords: Introduction, Diagnostic potential of CRISPR, Therapeutic potential of CRISPR.

1. Introduction of cancer and CRISPR

Cancer is a disease marked by abnormal, uncontrolled cell division and the ability to invade nearby tissues or spread (metastasize) to other parts of the body. It can develop in almost any tissue and results from a combination of genetic mutations and environmental factors (1). Finding a cure for cancer continues to be one of the greatest medical challenges today. In some circumstances, a mix of therapies may be applied to obtain the best results. However, despite advances in cancer treatment, there is still much to be done in the quest to find a cure for cancer (2). Currently, there is no universal treatment for cancer, as management depends on factors such as the patient's age, general health and the specific type and stage of the disease. Available treatment options include radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, and surgery (3). Ongoing research is aimed at improving our knowledge regarding biology of cancer and developing better therapies.

The invention of a gene-editing technique called CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) has already revolutionized the field of biotechnology (4). This technique is based on a biological protection mechanism bacteria employ in safeguarding themselves against viral attack (5). CRISPR uses an RNA molecule to direct an enzyme to a specific site on the DNA sequence, which can be Cas9 or any other nuclease. When the target site is reached, the enzyme chop-chops the DNA, which enables the researchers to insert the right material, deletion or replacement of a particular gene (6). CRISPR, in the diagnosis of cancer, is applicable in identifying specific genetic changes that are associated with cancer. CRISPR-based diagnostics are able to identify cancer-causing mutations using small biopsies like blood, saliva, or other tissues due to the creation of tests identifying the cancer-causing mutations in a patient during their DNA (7). As an example, the CRISPR/Cas system can be used to develop a particular kit that will diagnose a given cancer-related mutation. In addition to disease progression tracking in diagnosed patients, this method may assist in diagnosing the individuals at high risk of getting cancer as well (8).

Cancer treatment strategies can be broadly classified

* Corresponding author.
E-mail address: aqurashi@taibahu.edu.sa (A. Alqurashi).
Doi: http://dx.doi.org/10.14715/cmb/2025.72.3.2

into three main approaches. The first approach involves conventional therapies, such as chemotherapy and radiotherapy, which primarily target rapidly dividing cancer cells but often lack specificity and cause substantial side effects. The second approach includes targeted therapies and immunotherapies that focus on specific molecular pathways or enhance immune recognition of tumor cells, improving treatment precision, CRISPR based strategy is 3rd and most recent approach for treating cancer. CRISPR can be used to specifically target mutations in a particular patient by eliminating them and their cancerous cells (9) this can be seen in picture 1. The potential application of CRISPR in the diagnosis and cure of cancer is overall an exciting area of study that has immense possibilities of raising our levels in such involvement. The possible applications of CRISPR in cancer treatment would be to deactivate cancer causing genes or also known as oncogenes. With the help of disabling such genes, it is possible to prevent the progression of cancer cells. The third approach is manipulating the genes that regulate immune response, like T- cell receptors or T- cell checkpoint genes, in order to enhance the immune system response to cancerous cell (10). This could enhance the immunity system in recognizing and destroying cancer cells. RISPR have also found an ability to generate CAR-T cell therapy, which is the genetically edited T cell that can target and kill the cancer cells (11). During these therapies, T cells are subjected to CRISPR editing where they are made to express a chimeric antigen receptor (CAR) to bind and destroy cancer cells. Recent developments in CRISPR based technologies have opened up new avenues regarding the growth of cancer diagnosing and treatment methodologies.

2. Literature search methodology

In this review, we shall outline the recent findings regarding the possible use of CRISPR as a tool in the battle against cancer in terms of both diagnosis and treatment. A comprehensive literature search was performed to identify peer-reviewed studies on CRISPR-based cancer therapeutics and diagnostics. Major databases, including PubMed, Web of Science, and Scopus, were searched for publications in English from 2015 to 2024. Studies were included if they focused on therapeutic applications, gene editing of oncogenes or tumor suppressors, immunotherapy, or diagnostic uses of CRISPR, while non-cancer studies, non-peer-reviewed articles, and duplicates were excluded.

3. Mechanism of action for CRISPR/Cas-9

CRISPR/Cas9 is a very precise genome-editing method that allows a particular form of genetic modification through the use of naturally occurring bacterial defense against viral DNA. In the framework of this system is the Cas9 protein that binds or cuts specific DNA dependent on a single-guide RNA (sgRNA). The sgRNA has two important parts, the crRNA, which represents a texture of the targeted DNA and the trans-activating CRISPR RNA (tracrRNA), which plays a role as a scaffold where Cas9 binds. The combination of all these constitutes the ribonucleoprotein (RNP) complex that guides Cas9 towards the locus of interest (12, 13). On binding, the Cas9-gRNA complex searches through the DNA sequence to identify a short sequence known as protospacer adjacent motif (PAM), which is required to identify the target. The most popular Cas9 protein variant (SpCas9) is based on Streptococcus pyogenes bacterium and recognizes the PAM sequence 5-NGG-3 - on the non-target strand of the DNA (14, 15). Once the PAM is identified, the crRNA within the sgRNA pairs with the complementary DNA strand, positioning Cas9 to introduce a double-strand break (DSB) approximately 3 nucleotides upstream of the PAM. This cleavage is facilitated by two nuclease domains in Cas9: the HNH domain, which cuts the target DNA strand (complementary to the crRNA), and the RuvC-like domain, which cleaves the non-target strand (16, 17). The resulting DSB triggers the cell's DNA repair mechanisms primarily non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ is an error-prone process that often introduces insertions or deletions (indels), leading to gene disruptions or knockouts. In contrast, HDR uses a homologous DNA template (such as a donor sequence) to enable precise gene corrections or insertions, making it useful for therapeutic applications (18-20). Due to its precision and versatility, CRISPR/Cas9 has become a revolutionary tool in genetic engineering, allowing researchers to edit genomes with unprecedented accuracy for applications ranging from gene therapy to agricultural biotechnology (21, 22). Figure 1 illustrates the mechanism of CRISPR/Cas9-mediated genome editing. While Cas9 remains the primary tool for DNA editing, CRISPR/Cas12a (also known as Cpf1) and CRISPR/Cas13 have broadened the functional spectrum of CRISPR technologies. Cas12a, like Cas9, is a DNA endonuclease but differs in several key aspects. It requires only a short crRNA (no tracrRNA), recognizes a TTTV PAM, and generates staggered 5′ overhangs rather than blunt-ended breaks. Notably, Cas12a exhibits collateral single-stranded DNA cleavage activity, a property leveraged in molecular diagnostics such as DETECTR for rapid pathogen detection. Cas12a is particularly advantageous for editing AT-rich regions, supports efficient multiplexed genome editing by processing its own crRNA array, and often shows reduced off-target effects compared to Cas9. In contrast, CRISPR/Cas13 represents a fundamentally different branch of the CRISPR family, functioning as an RNA-targeting endonuclease. Cas13 binds a single crRNA and recognizes a protospacer flanking site (PFS) on the target RNA rather than DNA. Upon activation, Cas13 not only cleaves the intended single-stranded RNA (ssRNA) but also exhibits collateral trans-RNA cleavage activity, a feature that underpins the SHERLOCK (Specific High-sensitivity Enzymatic Reporter unlocking) diagnostic platform. Cas13 is uniquely suited for post-transcriptional regulation, RNA knockdown, antiviral therapies targeting viral genomes,

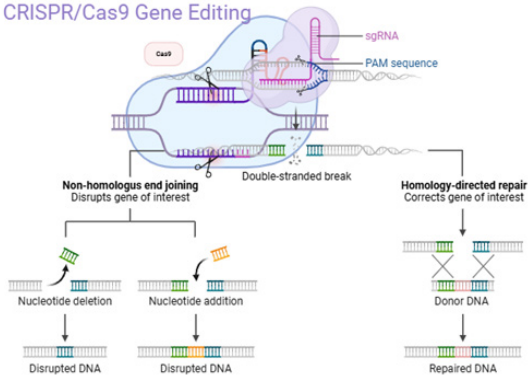


Fig. 1. Generalmechanism of CRISPR/Cas9 gene editing. (23).

and rapid, highly sensitive molecular diagnostics, with the additional benefit of avoiding permanent DNA modifications. Table 1 compares three common CRISPR-associated proteins (Cas9, Cas12a and Cas13) in terms of their properties and characteristics.

4. Therapeutic potential of CRISPR for cancer

CRISPR has emerged as a transformative tool not only for genetic diagnosis but also as a promising therapeutic strategy for cancer treatment. By precisely targeting cancer-causing mutations in a patient's DNA, CRISPR holds the potential to eliminate malignant cells and cure the disease (25). Preclinical studies have demonstrated its feasibility, such as selectively targeting *TP53*-mutated cancer cells (26). It can also suppress tumor growth in mouse models of liver cancer (27). Despite existing therapies, drug resistance remains a major challenge, particularly in estrogen receptor (ER)-positive cancers, necessitating novel approaches (28, 29). CRISPR/Cas9 offers a rapid and precise method for editing oncogenes or correcting tumor suppressor gene defects, paving the way for more effective treatments.

One of the most exciting applications of CRISPR in cancer therapy is its role in immunotherapy. Immune checkpoint inhibitors, such as *PD-1* and *PD-L1* blockers, have significantly improved survival rates in certain cancers, as evidenced by drugs like pembrolizumab (30). Building on this, researchers have employed CRISPR to disrupt the *PD-1* gene in T cells from patients with metastatic non-small cell lung cancer (NSCLC), enhancing their anti-tumor activity before reinfusion (NCT02793856) (31). This approach exemplifies how CRISPR can enhance existing immunotherapies by genetically modifying immune cells to better combat cancer.

Beyond immunotherapy, CRISPR enables personalized treatment strategies by directly inactivating oncogenic mutations. For instance, in (Epidermal Growth Factor Receptor) EGFR-mutant lung cancer, CRISPR can disrupt the mutated region, potentially overcoming treatment resistance (32). Similarly, in human papillomavirus (HPV) driven cervical cancer, CRISPR has been used to target the oncogenic E7 protein, leading to tumor cell apoptosis and reduced malignancy (33). A phase I clinical trial is currently evaluating CRISPR/Cas9-mediated disruption of HPV E6 and E7 in cervical cancer patients, highlighting its therapeutic potential.

Another breakthrough lies in CRISPR-enhanced CAR-T cell therapy, which has shown remarkable success in hematologic malignancies like acute lymphoblastic leukemia and B-cell lymphomas (34). While traditional CAR-T therapy relies on a patient's own T-cells, CRISPR now allows the use of donor-derived T cells, expanding access for patients with insufficient T cell counts (35, 36). However, challenges such as graft-versus-host disease (GVHD) and immune rejection remain (37).

From genome-wide screening to targeted interventions, CRISPR is revolutionizing cancer treatment by enabling efficient, personalized therapies. Its ability to identify and correct cancer-driving genetic abnormalities positions it as a powerful tool in the fight against drug resistance and treatment limitations. With ongoing clinical trials and rapid advancements, CRISPR-based therapies are poised to redefine the future of oncology. Table 2 summarizes suc-

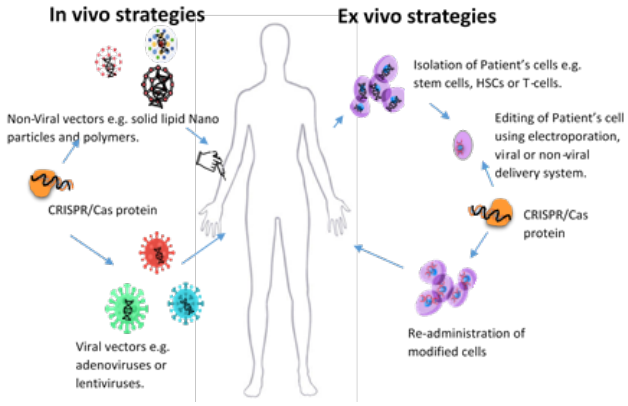


Fig. 2. Different strategies that can be followed in CRISPR-based cancer treatment. (19).

Table 1. Comparison of CRISPR-Cas9, CRISPR-Cas12a, and CRISPR-Cas13 Systems: Mechanisms, Features, and Applications (24).

Feature	CRISPR-Cas9	CRISPR-Cas12a	CRISPR-Cas13
Type of Nuclease	DNA endonuclease (cuts dsDNA)	DNA endonuclease (cuts dsDNA)	RNA endonuclease (cuts ssRNA)
Guide RNA	crRNA + tracrRNA (or sgRNA)	crRNA only (shorter)	crRNA only
PAM Requirement	NGG (SpCas9)	TTTV (for LbCas12a/AsCas12a)	Protospacer Flanking Sequence (PFS)
Cleavage Mechanism	Blunt-ended dsDNA breaks	Staggered (5' overhang) cuts	Cuts ssRNA (collateral activity)
Target	DNA	DNA	RNA
Multiplexing	Moderate (requires multiple gRNAs)	High (processes its own crRNA array)	High (can process multiple targets)
Collateral Activity	No	Yes (trans-ssDNA cleavage)	Yes (trans-ssRNA cleavage)
Key Differences	- Requires tracrRNA - Blunt cuts - High off-target risk	- No tracrRNA needed - Staggered cuts - Better for AT-rich regions	- Targets RNA only - No DNA damage risk - Useful for viral RNA
Applications	- Gene knockout - HDR-based repair - Base editing - Therapeutic (e.g., sickle cell therapy)	- DNA editing (AT-rich regions) - Diagnostics (DETECTR) - Multiplexed genome engineering	- RNA knockdown - Antiviral therapy - Diagnostics (SHERLOCK) - mRNA regulation

Table 2. Applications of CRISPR-Cas systems in cancer research: therapeutic, diagnostic, and functional studies across cancer types.

Cancer Type	Targeted approach followed in the research	CRISPER Cas protein used	Standard approach followed as a reference	Purpose and Impact	Reference
Prostate Cancer	Editing *PTEN*, *AR*, *TP53*	Cas9	Hormone therapy, chemo, immunotherapy	Functional study for future drug development	(38)
Bladder Cancer (Therapeutic)	Editing *FGFR3*, *RB1*, *CDKN2A*, *PIK3CA*	Cas9	Intravesical therapy, Chemotherapy	Identification of resistance markers	(39)
Bladder Cancer (Diagnostic)	CRISPR/Cas12a detection of exosomal lncRNA RMRP	Cas12a	Combined with RT-Recombinase-Aided Amplification and Nuclear Matrix Protein 22 biomarker	Rapid (30 min), non-invasive urine-based diagnosis with higher AUC than RT-qPCR	(40)
Esophageal Cancer	Editing *TP53*, *CDKN2A*, *TERT*, *NOTCH1*, *AJUBA*, *PIK3CA*, *CASP8*	Cas9	Surgery, palliative care, chemo, immunotherapy	Identification of functional genes with therapeutic implications	(41)
Hepatocellular Carcinoma (HCC)	Knockout of *CDK7* & *SGOL1*	Cas9	Transarterial Chemoembolization, Radiofrequency Ablation, Microwave Ablation	Loss of *SGOL1* decreases sorafenib cytotoxicity (drug response study)	(42)
HCC (Gene-specific therapy)	NF-κB-responsive Cas13a targeting *TERT*, *EZH2*, *RelA* via Adeno-Associated Virus	Cas13a	AAV-mediated delivery, NF-κB decoy promoter	Tumor-specific knockdown, inhibited tumor growth in xenografts, no effect on normal cells	(43)
CD19$^+$ Leukemia & Lymphoma	Allogenic UCART019 (CRISPR-edited CAR-T cells)	Cas9	Chemotherapy, stem cell transplant, CAR-T	"Off-the-shelf" CAR-T therapy development	(43)
Lung Cancer (Therapeutic)	Knockout of *EGFR*, *KRAS*, *P53*, *LKB1*	Cas9	Chemo, radiotherapy, targeted therapy, immunotherapy	Promoted apoptosis, inhibited tumor growth, suppressed MYC	(44)
Lung Cancer (Gene Activation)	CRISPR activation of *TNC*, *S100A10*, *S100A11*	Cas9	Chemo, radiotherapy, targeted therapy, immunotherapy	Showed enhanced metastatic potential (functional study)	(45)
Lung Cancer (Diagnostic)	CRISPR-based fluorescent bioassay for exosomal miR-21	Cas12a	Chemo, radiotherapy, targeted therapy, immunotherapy	Highly sensitive (161 fM) and specific detection	(46)
Breast Cancer	CRISPR target screening of *PHF5A*	Cas9	Surgery, radiotherapy, chemotherapy	Suppressed proliferation, migration, and tumor formation	(47)
Liver Cancer (Tumorigenesis Study)	Pten knockout + Nras activation	Cas9	Liver transplant, radiotherapy, chemotherapy	Induced liver tumorigenesis (cancer modeling)	(48)
Colorectal Cancer	Genome-wide CRISPR knockout of *NADK*, *NHK*, *ABCB1*	Cas9	Surgery, radiotherapy, chemotherapy	Overcame multidrug resistance (MDR)	(49)
Glioblastoma	Gene editing of *FGFR3-TACC3*	Cas13a	Surgery, chemotherapy	Precision tumor therapy	(50)

cess of CRISPR technology in various cancers diagnosis and treatments, while Figure 2 illustrates in vivo and ex vivo CRISPR strategies for cancer therapy.

5. Targeting oncogenes and repairing tumor suppressor genes

CRISPR-Cas9 has revolutionized cancer therapy and make possible precise targeting of oncogenes and restoring the function of tumor suppressor genes. Oncogenes such as *MYC*, *KRAS*, and *BCL-2* drive uncontrolled cell proliferation, and CRISPR has been used to disrupt these genes in various cancers. A previously reported study state that CRISPR-mediated knockout of *KRAS* in pancreatic cancer cells significantly reduced tumor growth in mouse models (51). Similarly, CRISPR has been employed to repair tumor suppressor genes like *TP53* and *PTEN*, which are frequently mutated in cancers. Researchers also restored *TP53* function in glioblastoma successfully which resulted in apoptosis and reduced tumor progression (52). These approaches highlight CRISPR's potential for preci-

sion oncology.

6. CRISPR in immunotherapy (e.g., CAR-T Cell Engineering)

CRISPR has caused significant advancement in cancer immunotherapy, particularly in engineering chimeric antigen receptor (CAR) T cells. Traditional CAR-T therapies face limitations such as immune rejection and off-target effect, but CRISPR allows for precise modifications and enhance efficacy. For example, CRISPR was used to knock out the *PD-1* gene in CAR-T cells for improving their ability to attack PD-L1-expressing tumors (53). Additionally, CRISPR-edited CAR-T cells targeting *CD19* have shown remarkable success in treating refractory B-cell malignancies, with clinical trials reporting complete remission rates of up to 90% (54). Beyond CAR-T cells CRISPR is being explored for natural killer cell engineering, offering a promising alternative for solid tumors (55). In addition to T cells and NK cells, CRISPR-based immunotherapy is being explored in macrophages and dendritic cells to enhance antigen presentation and overcome immunosuppressive signals within the tumor microenvironment. For instance, CRISPR can be used to knock out inhibitory receptors or signaling pathways in these cells, promoting a more pro-inflammatory and tumoricidal phenotype. Multiplex CRISPR editing allows simultaneous modifications in multiple genes, enabling combination strategies such as checkpoint blockade, cytokine expression, and enhanced immune recognition within a single cell population. Both in vivo approaches, where CRISPR components are delivered directly into patients, and ex vivo strategies, involving editing patient-derived immune cells before reinfusion, are under investigation to improve safety and efficacy. These innovations aim to address challenges such as tumor heterogeneity, immune evasion, and persistence of therapeutic cells, pointing toward the development of next-generation cellular immunotherapies with higher precision and potency (56, 57).

7. Identifying genes involved in drug resistance

Drug resistance is a major problem for cancer treatment. Commonly, these mechanisms are driven by mutations in the genes involved in drug resistance mechanisms. CRISPR-based screens have been used to identify many genes associated with resistance to chemotherapy and targeted therapies. Studies led by Lord & Ashworth (2016) mapped gene expression across the genome and identified *BRCA1 and BRCA2*, *TP53*, *RIF1* and *PAXIP1* as possible drivers of resistance to Poly (ADP-ribose) Polymerase inhibitors in ovarian cancer (58). Another finding identifies amplification of EGFR as a likely mechanism of resistance in patients with lung cancer treated with tyrosine kinase inhibitors (59). In addition to classic mechanisms of drug resistance, CRISPR-based functional genomic screens have been instrumental in uncovering resistance drivers across various cancer types. Genome-wide CRISPR/Cas9 knockout libraries have been used to identify genes whose loss confers resistance to chemotherapy drugs in colorectal, breast, and lung cancer cell models, revealing broad sets of resistance mediators and pathways linked to chemoresistance phenotypes (60). More recently, genome-wide CRISPR/Cas9 screens have continued to identify specific mediators of drug resistance across diverse cancer types, providing functional insights into underlying mechanisms and potential therapeutic targets. For example, CRISPR screens in esophageal squamous cell carcinoma revealed that loss of PFKFB3 enhances resistance to 5-fluorouracil by modulating metabolic and cell cycle pathways, suggesting a role for metabolic reprogramming in chemoresistance (61). Additionally, CRISPR approaches in EGFR-mutant lung cancer using combined activation and knockout libraries have delineated key regulators of resistance to EGFR inhibitors, highlighting pathways such as PI3K and MAPK in mediating reduced drug sensitivity. In clear cell renal cell carcinoma, a genome-wide CRISPR/Cas9 screen identified PTGR2 as a potential determinant of resistance to the tyrosine kinase inhibitor sunitinib, offering a novel target to overcome acquired resistance (62). These findings exemplify how CRISPR functional genomics can define novel resistance drivers and pathways, advancing the development of combination strategies and personalized therapeutic regimens to counteract drug resistance in cancer.

8. Challenges and future directions

CRISPR as a cancer therapy While it has shown promise, the treatment faces several challenges. These include off-target effects, inefficiencies in production of the CRISPR/Cas protein, and immune responses against Cas9.Undesirably, CRISPR/Cas proteins have shown no apparent biosafety benefit with a number of case reports involving genome modifications that do not appear to have been intended. It is even under discussion whether CRISPR specificity or CRISPR efficiency may be limited by viral delivery approaches or nanoparticle delivery strategies (63). The future roadmap will need to explore further ways to improve CRISPR specificity using base and prime editing. This will need to be combined with studies on in vivo use of CRISPR for early detection and diagnosis of cancer.

9. Diagnostic potential of CRISPR

By making it possible to identify specific genetic abnormalities linked to cancer, CRISPR has the potential to completely transform how cancer is diagnosed. Researchers are using CRISPR/Cas proteins to create diagnostic tests that can detect the presence of cancer-causing mutations in a patient's DNA (8, 64). These tests can reveal whether and what kinds of cancer-causing mutations are present in a small sample of a patient's blood, saliva or other tissue. Compared to Cas9, which has a limited diagnostic domain and limited PAM sequences (DETECTR assay) as well as an off-target effect, Cas12a is widely used for DNA-based detection. Its trans-cleavage activity can be combined with the ability to detect RNA in genomic sequences using highly sensitive enzyme-linked immunosorbent assays known as DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) (65). Meanwhile, Cas13 is an excellent choice for RNA targeting diagnostics to capture and target the purified RNA of virus agents using collateral cleavage (SHERLOCK) (66). Both systems have unique advantages and disadvantages that determine which one best fits a diagnosis application.

10. Detection of circulating tumor DNA

Circulating tumor DNA (ctDNA), a diagnostic for cancer, is frequently released into the circulation by cancer cells that undergo apoptosis or necrosis (67). A promising

biomarker for early detection and prognosis of many malignancies as well as for predicting the efficacy of cancer treatment is ctDNA. Identification of *EGFR* gene mutations in this regard has been more crucial in order to inform the choice of the most suitable treatments. Recently, Jiang et al. developed crRNAs targeting *EGFR790* and *EGFR858* mutations using the CRISPR/Cas12a system, which could enable accurate detection of mutant EGFR genes in patients with cancer. Such an approach may have significant implications for personalized cancer care (68).

A recent study has developed a novel method for the detection of breast cancer gene-1 using CRISPR/Cas12a. This technique eradicates the signification of amplification and also uses two different magnitudes of gold nanoparticles (AuNPs) to identify the target DNA sequence. In particular, two of the single-stranded DNA molecules were immobilized on top of the surface of the AuNPs, and one was an end-modified with the fluorophore, which was quenched by bigger-sized AuNPs. The aforementioned two morphologies of AuNPs were connected by partial hybridization of the single-strand DNA. These two different systems of AuNPs are segregated in which the colour of the lotion of AuNPs turned from purple to red-purple due to cleavage of the unpaired single stranded DNA (ssDNA) by the CRISPR/Cas12a after being stimulated by target ctDNA. Also, the success of the target gene detection was further confirmed by Metal-Enhanced Fluorescence signal. This was the procedure done all in the space of 30 minutes only and the process has been a promising new way of fast and effective screening of breast cancer gene (69). To be able to detect ctDNA, 3D GR/AuPtPd nanoflower sensing platform has been developed. In the system, CRISPR/Cas9-cleaved entropy-driven strand displacement reaction (ESDR) is used. The technique is non-destructive and very specific in detecting a single-nucleotide polymorphism. It enables the induction of low ctDNA levels, and this characteristic renders it applicable to medical and diagnostic applications. The method possesses massive potential to be used in disease diagnosis and prediction of the prognosis (70).

11. Cancer causing extracellular vesicles detection

Detection of a cancer early is significant within enhancing the possibility that the disease would be defeated. It is unfortunate that most cancers are discovered at later stages and surgery is of no considerable use. This is usually because there may be no signs and symptoms of advanced stages of solid tumor and these develop into metastatic aspersions. The usage of biomarkers in the circulating extracellular vesicles allows identifying early-stage malignancies which can be treated more effectively. Thus, the discovery and subsequent use of these biomarkers may help to improve the outlook of cancer patients (71). Extracellular vehicles (EVs), small lipid-based vesicles produced by cells, have been discovered as a potential new way of diagnostics in cancer. The role of these vesicles in intercellular communication is known to be considerable and they may also form positive microenvironments to support the growth of cancer cells. Most recently published data showed that certain biomarkers contained in the EVs are able not only to be used to detect that cancer exists, but are also capable of monitoring the progression of this disease. This renders EVS as an appealing target to deliver innovative cancer treatment and diagnostics (72).

Various methods have been given to examine the proteins on Extracellular Vesicles, which are of high significance in cell-to-cell communication. An encouraging method is to leverage CRISPR/Cas12a which has already been chosen by Liu et al. to identify with precision the proteins of the EV in nasopharyngeal cancer. This method can be used to capture the target EV by antibody-coated microplate and then labeling relevant CD109 protein with CD109 aptamer. Once the excess aptamer is removed in the wash, the CD109 that remains is increased using PCR, which can activate the CRISPR/Cas12a system. This causes the cleavage of a reporter ssDNA, therefore, resulting in detection of fluorescence. All told, the method can be used tremendously to expand our knowledge on the kind of proteins on the EVs and the functions of these proteins in biological processes (73).

Exosomes are extracellular vesicles (within the size range of 30-150 nm) and have proven to be a potentially decisive component in the early detection of cancer. In the recent developments of CRISPR, it has been able to detect cancer by application of the exosome as a cancer detecting mechanism based on genetic modification specific detection. The resulting innovation can transform the process of cancer diagnosis and treatment, leading to a quicker and more precise way of identifying the cancerous condition (74). The CD63 aptamer with an included blocking agent was used to modify the surface of magnetic beads (MB) and be able to detect exosomes. When the blocking agent was removed, the CRISPR/Cas system was turned on and could cut the reporter ssDNA when the CD63 aptamer bound to CD63 on the exosome (75). The measurement of exosomes has occurred with the levels between 3,103 and 6,107 particles/milliliter. To make the detection of exosome proteins more sensitive, a number of amplification methods have been suggested. Enriching exosomes using MBs which are treated with targeted antibodies is one of such methods. It has been identified that PD-L1 can be found present in the exosomes which can bind to the PD-L1 aptamers causing the initiation of RPA amplification. The RPA amplicon triggers the transcription-mediated amplification (TMA) reaction and produces the transcripts of RNA that is required to induce the CRISPR/Cas13a mechanism. The results have shown the potential of more efficient exosome-based diagnosis and therapy methods (76). Exosome miRNA as a cancer biomarker can be fair, and its LOD is 10 particles/mL. Isothermal detection of miRNAs with a lower LOD (90 fM) of multiple EVs by rolling circular amplification and CRISPR/Cas9 can be achieved. This method shows promise for dynamically tracking the advancement of immunotherapy. The stability of exosome miRNA due to exosome protection makes it more useful for cancer detection than plasma-derived circulating miRNA (77, 78).

12. Cancer-causing protein biomarker detection

The diagnosis can also include the detection of certain proteomic markers in the blood that could represent the presence of cancerous cells or overall markers of inflammation present in the body. These biomarkers are detected with the help of a number of laboratory tests and analyses which lead to proper identification of the condition. Although some of these biomarkers may be released specifically by the cancerous cells, others might be rather general in nature and have the causes in the reaction of the body

to inflammation. In general, early cancer diagnosis and the ability to treat those cases are achieved thanks to the identification of such biomarkers (79). A CRISPR/Cas13a was recently used in a study dealing with ELISA detection of human endothelial growth factor (VEGF) in a highly sensitive manner. The signal used in the ELISA method was based on a traditional colorimetric signal produced by a tag of a detection antibody conjugated to horseradish peroxidase, whereas, in this platform, the signal is a transcriptional signal produced by a tagged detection antibody tagged with double stranded DNA (dsDNA) containing the T7 promoter region which is bound to the enhancement effect of the target protein. This was used as a switch by the generation of RNA transcripts that enhanced fluorescence signal by further activating the CRISPR/Cas13a system. The technique obtained a limit of detection (LOD) of 32.27 fg/mL, making it more sensitive than the conventional ELISA methods. Nonetheless, the method did not actually make the complicated nature of ELISA that much easier. In general, this paper demonstrates the potential of a CRISPR/Cas13a protein detection system, combined with ELISA, which would exhibit a high level of sensitivity in detecting proteins (80).

One group of scientists generated a biosensor platform based on CRISPR/Cas mechanism and label-free fluorescence approach to identify substances that are related to the disease. They developed a target cycle and a trigger chain in order to amplify the signal. A biosensing platform is applicable in evaluating the proteins, small molecules or microRNA within the biological outcomes. The output signal of the system is a fluorescent signal based on ssDNA-Ag NCs, as this system is flexible and it does not need pre-marking. It provides a direct, low-cost, and speedy diagnostic platform (label-free sensing) to be used clinically (81).

In the recent research by Liu and his co-workers there has been a major breakthrough, in identifying a possible biomarker of hepatocellular cancer- the TGF -1, using an aptamer as the recognition entity. In this method, the aptamer was designed to have high affinity towards TGF-1 and cause a reaction of CRISPR trans-cleavage Cas12a. When exposed to TGF-1, the activity of this aptamer to CRISPR/Cas12a was low and it led to the alteration of the electrochemical signal. It would be of tremendous potential to the detection and cure of hepatocellular cancer in its early stage (82).

13. Cancer-causing Micro-RNA (miRNA) detection

MicroRNAs (miRNAs) are short RNA molecules, typically 20-24 nucleotides in length that are produced from endogenous transcripts with hairpin structures by the enzyme Dicer, which belongs to the RNase III family of nucleases. These messenger RNAs (mRNAs) can attach to noncoding RNAs (ncRNAs), which then control gene expression by either preventing translation or encouraging mRNA destruction (83, 84). The RNA-induced silencing complex (RISC) assembly and the miRNA couple up after unwinding, and this complex either suppresses the translation of the mRNA or cleaves and completely destroys the mRNA (85). MiRNAs possess a distinctive trait of regulating the expression of several protein-coding genes via binding to the promoter regions, 3' and 5' untranslated regions, and coding sections of target sequences. This regulation occurs at the transcriptional, post-transcriptional, and translational levels (85, 86). MiRNAs, which play a crucial role in regulating gene expression, can act as either tumor suppressors or oncogenic elements depending on the cell type and gene expression pattern. The complexity of miRNAs' role in the initiation and advancement of cancer is further increased by the discovery that they have a variety of impacts on cell survival, growth, and proliferation (87, 88). Circulating miRNA detection in human body fluids holds great promise for early cancer detection and improved patient survival rates (89). The CRISPR/Cas has a comparatively low detection sensitivity of a target nucleic acid. To counter this, there has to be nucleic acid amplification so as to make them more sensitive. Various suggestions involving the use of CRISPR/Cas method have been proposed to allow precise and sensitive miRNA to be identified. Most recent developments of molecular biology have driven the combination of the CRISPR/Cas12a system and PCR, which is the most massive technique in the nucleic acid amplification. It has been employed to detect the presence of the RNA of the miRNA let-7a, which is a highly important tumor suppressor gene that is usually down regulated in a lot of cancers like cervical cancer, prostate cancer and stomach cancer. introduce these two methods into a new combination to achieve encouraging outcomes in the early cancer diagnostics, and it may soon help transform the sphere of cancer diagnostics (90). The so-called colorimetric detection can be regarded as an extremely well-known form of visible recognition of analytes. In an experimental study, CRISPR/Cas13a was used to cut the reporter ssRNA to be able to visually distinguish the microRNA. In order to accomplish this, the reporter ssRNA was hybridized with the ssDNA that was modified in AuNPs which caused an aggregation of AuNPs. Due to ssRNA cleavage, the CRISPR/Cas13a system led to the redispersement of the AuNPs, changing the color of the latter to red (91).

14. The CRISPR/Cas approach's to increase the sensitivity of detection.

The CRISPR/Cas may be employed as a mechanism for improving detection accuracy and precision. With CRISPR/Cas system, one can identify and cut certain DNA sequences linked to a certain disease or pathogen. As a method of sensitizing detection, this represents a viable means of detection turn-down as the limits of the genetic material to be detected can be very small. It can also employ the use of other forms of detection like PCR so that the CRISPR/Cas can work better. As improvement in new CRISPR/Cas based detection tools, there can be a further improvement in sensitivity and accuracy of diagnostic tests, hence, resulting in improved control and management of the diseases. Stem-loop adapters were made to specifically bind to the 3’ end of let- 7a miRNA, and enable PCR amplification. When the resulting amplicons are detected, CRISPR/Cas12a system cleaves reporter single-stranded DNA to give out a reading (92). To make PCR a simplified process of operating and thermal cycling which is complex, a set of isothermal nucleic acid amplification methods have been suggested in lieu. miR-17 has been found to be a potential biomarker in the search of biomarkers of breast cancer. The researchers employed hyperbranching rolling circle amplification and the CRISPR/Cas13a to detect miR-17. It has been observed that the levels of miR-17 are often decreased in cases of highly

aggressive breast cancer (93). The detection of miR-17 was achieved through a series of steps. First, a hybridization reaction between miR-17 and a linear padlock probe occurred, followed by ligation of the resulting hybrid by a ligase enzyme. Rolling amplification was then initiated, resulting in the generation of repeat sequences that served as a template for strand displacement amplification. The amplified dsDNA was then changed into ssRNA, initiating CRISPR collateral Cas13a's cleavage activity. Finally, utilizing fluorescence signal readout, miR-17 was quantitatively detected with a LOD of 200 aM (94).

The detection method of CRISPR/Cas12a is more reliable and affordable as compared to CRISPR/Cas13a because the target and the reporter unit are both DNA. The undesirable background noise may also arise when Cas12a and crRNA assemblies take place prior to the detection. In order to resolve this problem, a state-of-the-art CRISPR/Cas12a-based sensing selective device has been invented, which eradicates all instances of a non-selective detection. It is interesting to note that miR-21 has been found overexpressed in various forms of cancer (95). A catalytic hairpin assembly (CHA) is a very efficient process of amplifying nucleic acid in isothermal conditions and thus enzymes are not required. A new DNA circuit founded on the CHA has been designed by the scientists that was combined with the CRISPR/Cas12a system with the generation of a strong miRNA sensing tool (96).

15. Future prospects

CRISPR technology has revolutionized cancer research and holds great promise for improving diagnosis and treatment. Its ability to target oncogenes, repair tumor suppressor genes, enhance immunotherapy (e.g., CAR-T cells), and identify cancer biomarkers provides enormous potential for improving patient outcomes. Ongoing challenges, such as off-target effects, delivery efficiency, and immune responses, must be addressed before broad clinical application. Ethical considerations and patient safety are also critical. Despite these challenges, continued research and innovation are likely to expand the therapeutic and diagnostic possibilities of CRISPR in oncology.

16. Conclusion

Recent advances in CRISPR-based diagnostics and therapeutics are transforming the landscape of cancer research and treatment. CRISPR technologies enable precise targeting of oncogenes, repair of tumor suppressor genes, enhancement of immunotherapy strategies (such as CAR-T cells), and identification of biomarkers associated with drug resistance. These capabilities offer the potential for more personalized, effective, and less invasive cancer therapies. Despite the remarkable progress, challenges remain, including off-target effects, delivery efficiency, immune responses, and ethical considerations. Continued research, optimization, and clinical validation will be essential to fully realize the promise of CRISPR in cancer management and to translate these findings into improved patient outcomes.

References

1. Yadav AR, Mohite SK (2020) Cancer-A silent killer: An overview. Asian J Pharm Res 10(3):213-6. doi: 10.5958/2231-5691.2020.00036.2
2. Gao Q, Feng J, Liu W, Wen C, Wu Y, Liao Q, et al. (2022) Opportunities and challenges for co-delivery nanomedicines based on combination of phytochemicals with chemotherapeutic drugs in cancer treatment. Adv Drug Deliv Rev 114:445. doi: 10.1016/j.addr.2022.114445
3. Abbas Z, Rehman S (2018) An overview of cancer treatment modalities. Neoplasm:1139-57. doi: 10.5772/intechopen.76558
4. Kato-Inui T, Takahashi G, Hsu S, Miyaoka Y (2018) Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 with improved proof-reading enhances homology-directed repair. Nucleic Acids Res 46(9):4677-88. doi: 10.1093/nar/gky264
5. Khadempar S, Familghadakchi S, Motlagh RA, Farahani N, Dashtiahangar M, Rezaei H, et al. (2019) CRISPR–Cas9 in genome editing: Its function and medical applications. J Cell Physiol 234(5):5751-61. doi: 10.1002/jcp.27476
6. Katti A, Diaz BJ, Caragine CM, Sanjana NE, Dow LE (2022) CRISPR in cancer biology and therapy. Nat Rev Cancer 22(5):259-79. doi: 10.1038/s41568-022-00441-w
7. Zhang H, Qin C, An C, Zheng X, Wen S, Chen W, et al. (2021) Application of the CRISPR/Cas9-based gene editing technique in basic research, diagnosis, and therapy of cancer. Mol Cancer 20(1):126. doi: 10.1186/s12943-021-01431-6
8. Dubey AK, Kumar Gupta V, Kujawska M, Orive G, Kim N-Y, Li C-z, et al. (2022) Exploring nano-enabled CRISPR-Cas-powered strategies for efficient diagnostics and treatment of infectious diseases. J Nanostruct Chem 12(5):833-64. doi: 10.1007/s40097-022-00472-7
9. Di Carlo E, Sorrentino C (2024) State of the art CRISPR-based strategies for cancer diagnostics and treatment. Biomark Res 12(1):156. doi: 10.1186/s40364-024-00701-x
10. Moses C, Garcia-Bloj B, Harvey AR, Blancafort P (2018) Hallmarks of cancer: The CRISPR generation. Eur J Cancer 93:310-8. doi: 10.1016/j.ejca.2018.01.002
11. Mollanoori H, Shahraki H, Rahmati Y, Teimourian S (2018) CRISPR/Cas9 and CAR-T cell, collaboration of two revolutionary technologies in cancer immunotherapy, an instruction for successful cancer treatment. Hum Immunol 79(12):876-82. doi: 10.1016/j.humimm.2018.09.007
12. Mir A, Edraki A, Lee J, Sontheimer EJ (2018) Type II-C CRISPR-Cas9 biology, mechanism, and application. ACS Chem Biol 13(2):357-65. doi: 10.1021/acschembio.7b00855
13. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012) A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science 337(6096):816-21. doi: 10.1126/science.1225829
14. Cong L, Zhang F (2014) Genome engineering using CRISPR-Cas9 system. Chromosomal mutagenesis. Springer. p.197-217. doi: 10.1007/978-1-4939-1862-1_10
15. Hirano S, Nishimasu H, Ishitani R, Nureki O (2016) Structural basis for the altered PAM specificities of engineered CRISPR-Cas9. Mol Cell 61(6):886-94. doi: 10.1016/j.molcel.2016.02.018
16. Gasiunas G, Barrangou R, Horvath P, Siksnys V (2012) Cas9–crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proc Natl Acad Sci U S A 109(39):E2579-E86. doi: 10.1073/pnas.1208507109
17. Song F, Stieger K (2017) Optimizing the DNA donor template for homology-directed repair of double-strand breaks. Mol Ther Nucleic Acids:753-60. doi: 10.1016/j.omtn.2017.02.006
18. Haapaniemi E, Botla S, Persson J, Schmierer B, Taipale J (2018) CRISPR–Cas9 genome editing induces a p53-mediated DNA damage response. Nat Med 24(7):927-30. doi: 10.1038/s41591-018-0049-z
19. Lino CA, Harper JC, Carney JP, Timlin JA (2018) Delivering

CRISPR: a review of the challenges and approaches. Drug Deliv 25(1):1234-57. doi: 10.1080/10717544.2018.1474964
20. Lin S, Staahl BT, Alla RK, Doudna JA (2014) Enhanced homology-directed human genome engineering by controlled timing of CRISPR/Cas9 delivery. eLife 3:e04766. doi: 10.7554/elife.04766
21. Román-Rodríguez FJ, Ugalde L, Álvarez L, Díez B, Ramírez MJ, Risueño C, et al. (2019) NHEJ-mediated repair of CRISPR-Cas9-induced DNA breaks efficiently corrects mutations in HSPCs from patients with Fanconi anemia. Cell Stem Cell 25(5):607-21.e7. doi: 10.1016/j.stem.2019.08.016
22. Branzei D, Foiani M (2008) Regulation of DNA repair throughout the cell cycle. Nat Rev Mol Cell Biol 9(4):297-308. doi: 10.1038/nrm2351
23. Doudna JA, Charpentier E (2014) The new frontier of genome engineering with CRISPR-Cas9. Science 346(6213):1258096. doi: 10.1126/science.1258096
24. Hillary VE, Ceasar SA (2023) A review on the mechanism and applications of CRISPR/Cas9/Cas12/Cas13/Cas14 proteins utilized for genome engineering. Mol Biotechnol 65(3):311-25. doi: 10.1007/s12033-022-00567-0
25. Bhattacharjee G, Gohil N, Khambhati K, Mani I, Maurya R, Karapurkar JK, et al. (2022) Current approaches in CRISPR-Cas9 mediated gene editing for biomedical and therapeutic applications. J Control Release 343:703-23. doi: 10.1016/j.jconrel.2022.02.005
26. Zhu G, Pan C, Bei J-X, Li B, Liang C, Xu Y, et al. (2020) Mutant p53 in cancer progression and targeted therapies. Front Oncol 10:595187. doi: 10.3389/fonc.2020.595187
27. Kong H, Ju E, Yi K, Xu W, Lao YH, Cheng D, et al. (2021) Advanced nanotheranostics of CRISPR/Cas for viral hepatitis and hepatocellular carcinoma. Adv Sci 8(24):2102051. doi: 10.1002/advs.202102051
28. Muley H, Fado R, Rodriguez-Rodriguez R, Casals N (2020) Drug uptake-based chemoresistance in breast cancer treatment. Biochem Pharmacol 177:113959. doi: 10.1016/j.bcp.2020.113959
29. Vasan N, Baselga J, Hyman DM (2019) A view on drug resistance in cancer. Nature 575(7782):299-309. doi: 10.1038/s41586-019-1730-1
30. He J, Hu Y, Hu M, Li B (2015) Development of PD-1/PD-L1 pathway in tumor immune microenvironment and treatment for non-small cell lung cancer. Sci Rep 5(1):13110. doi: 10.1038/srep13110
31. ClinicalTrials.gov (2021) Study of CRISPR-Edited T Cells in Patients With Advanced Cancers. [Available from: https://clinicaltrials.gov/study/NCT02793856]
32. Wang M, Herbst RS, Boshoff C (2021) Toward personalized treatment approaches for non-small-cell lung cancer. Nat Med 27(8):1345-56. doi: 10.1038/s41591-021-01450-2
33. Sreedurgalakshmi K, Srikar R, Rajkumari R (2021) CRISPR-Cas deployment in non-small cell lung cancer for target screening, validations, and discoveries. Cancer Gene Ther 28(6):566-80. doi: 10.1038/s41417-020-00256-7
34. Liu Y-C, Cai Z-M, Zhang X-J (2016) Reprogrammed CRISPR-Cas9 targeting the conserved regions of HPV6/11 E7 genes inhibits proliferation and induces apoptosis in E7-transformed keratinocytes. Asian J Androl 18(3):475-9. doi: 10.4103/1008-682x.157399
35. Razeghian E, Nasution MK, Rahman HS, Gardanova ZR, Abdelbasset WK, Aravindhan S, et al. (2021) A deep insight into CRISPR/Cas9 application in CAR-T cell-based tumor immunotherapies. Stem Cell Res Ther 12(1):428. doi: 10.1186/s13287-021-02510-7
36. Miliotou AN, Papadopoulou LC (2018) CAR T-cell therapy: a new era in cancer immunotherapy. Curr Pharm Biotechnol 19(1):5-18. doi: 10.2174/1389201019666180418095526
37. Al Saber M, Biswas P, Dey D, Kaium MA, Islam MA, Tripty MIA, et al. (2021) A comprehensive review of recent advancements in cancer immunotherapy and generation of CAR T cell by CRISPR-Cas9. Processes 10(1):16. doi: 10.3390/pr10010016
38. Brennen WN, Zhu Y, Coleman IM, Dalrymple SL, Antony L, Patel RA, et al. (2021) Resistance to androgen receptor signaling inhibition does not necessitate development of neuroendocrine prostate cancer. JCI Insight 6(8). doi: 10.1172/jci.insight.146827
39. Tong Z, Sathe A, Ebner B, Qi P, Veltkamp C, Gschwend JE, et al. (2019) Functional genomics identifies predictive markers and clinically actionable resistance mechanisms to CDK4/6 inhibition in bladder cancer. J Exp Clin Cancer Res 38:1-14. doi: 10.1186/s13046-019-1322-9
40. Prime S, Cirillo N, Cheong S, Prime M, Parkinson E (2021) Targeting the genetic landscape of oral potentially malignant disorders has the potential as a preventative strategy in oral cancer. Cancer Lett 518:102-14. doi: 10.1016/j.canlet.2021.05.025
41. Sun W, He B, Yang B, Hu W, Cheng S, Xiao H, et al. (2018) Genome-wide CRISPR screen reveals SGOL1 as a druggable target of sorafenib-treated hepatocellular carcinoma. Lab Invest 98(6):734-44. doi: 10.1038/s41374-018-0027-6
42. Smirnov S, Petukhov A, Levchuk K, Kulemzin S, Staliarova A, Lepik K, et al. (2021) Strategies to circumvent the side-effects of immunotherapy using allogeneic CAR-T cells and boost its efficacy: results of recent clinical trials. Front Immunol 12:780145. doi: 10.3389/fimmu.2021.780145
43. Platt RJ, Chen S, Zhou Y, Yim MJ, Swiech L, Kempton HR, et al. (2014) CRISPR-Cas9 knockin mice for genome editing and cancer modeling. Cell 159(2):440-55. doi: 10.1016/j.cell.2014.09.014
44. Zheng Y-Z, Xue M-Z, Shen H-J, Li X-G, Ma D, Gong Y, et al. (2018) PHF5A epigenetically inhibits apoptosis to promote breast cancer progression. Cancer Res 78(12):3190-206. doi: 10.1158/0008-5472.can-17-3514
45. Gao M, Liu D (2017) CRISPR/Cas9-based Pten knock-out and Sleeping Beauty Transposon-mediated Nras knock-in induces hepatocellular carcinoma and hepatic lipid accumulation in mice. Cancer Biol Ther 18(7):505-12. doi: 10.1080/15384047.2017.1323597
46. Liu B, Saber A, Haisma HJ (2019) CRISPR/Cas9: a powerful tool for identification of new targets for cancer treatment. Drug Discov Today 24(4):955-70. doi: 10.1016/j.drudis.2019.02.011
47. Lu Z, Ni W, Liu N, Jin D, Li T, Li K, et al. (2023) CRISPR/Cas12a-based fluorescence biosensor for detection of exosomal miR-21 derived from lung cancer. Microchem J 187:108370. doi: 10.1016/j.microc.2022.108370
48. Gao Y, Zhang X, Wang X, Sun R, Li Y, Li J, et al. (2024) The clinical value of rapidly detecting urinary exosomal lncRNA RMRP in bladder cancer with an RT-RAA-CRISPR/Cas12a method. Clin Chim Acta 562:119855. doi: 10.1016/j.cca.2024.119855
49. Wu Y, Jin W, Wang Q, Zhou J, Wang Y, Tan Y, et al. (2021) RETRACTED: Precise editing of FGFR3-TACC3 fusion genes with CRISPR-Cas13a in glioblastoma. Mol Ther 29(11):3305-18. doi: 10.1016/j.ymthe.2021.07.002
50. Gao J, Luo T, Lin N, Zhang S, Wang J (2020) A New Tool for CRISPR-Cas13a-Based Cancer Gene Therapy. Mol Ther Oncolytics:1979-92. doi: 10.1016/j.ymthe.2021.07.002
51. Sánchez-Rivera FJ, Papagiannakopoulos T, Romero R, Tammela T, Bauer MR, Bhutkar A, et al. (2014) Rapid modelling of cooperating genetic events in cancer through somatic genome editing. Nature 516(7531):428-31. doi: 10.1038/nature13906
52. Rajesh Y, Pal I, Banik P, Chakraborty S, Borkar SA, Dey G, et al. (2017) Insights into molecular therapy of glioma: current challenges and next generation blueprint. Acta Pharmacol Sin 38(5):591-613. doi: 10.1038/aps.2016.167
53. McGowan E, Lin Q, Ma G, Yin H, Chen S, Lin Y (2020) PD-1

disrupted CAR-T cells in the treatment of solid tumors: Promises and challenges. Biomed Pharmacother 121:109625. doi: 10.1016/j.biopha.2019.109625
54. Zanetti SR, Velasco-Hernandez T, Gutierrez-Agüera F, Díaz VM, Romecín PA, Roca-Ho H, et al. (2022) A novel and efficient tandem CD19- and CD22-directed CAR for B cell ALL. Mol Ther 30(2):550-63. doi: 10.1016/j.ymthe.2021.08.033
55. Peng L, Sferruzza G, Yang L, Zhou L, Chen S (2024) CAR-T and CAR-NK as cellular cancer immunotherapy for solid tumors. Cell Mol Immunol 21(10):1089-108. doi: 10.1038/s41423-024-01207-0
56. Chehelgerdi M, Chehelgerdi M, Khorramian-Ghahfarokhi M, Shafieizadeh M, Mahmoudi E, Eskandari F, et al. (2024) Comprehensive review of CRISPR-based gene editing: mechanisms, challenges, and applications in cancer therapy. Mol Cancer 23(1):9. doi: 10.1186/s12943-023-01925-5
57. Hao J, Han T, Wang M, Zhuang Q, Wang X, Liu J, et al. (2018) Temporary suppression the sequestrated function of host macrophages for better nanoparticles tumor delivery. Drug Deliv 25(1):1289-301. doi: 10.1080/10717544.2018.1474965
58. Harvey-Jones E, Raghunandan M, Robbez-Masson L, Magraner-Pardo L, Alaguthurai T, Yablonovitch A, et al. (2024) Longitudinal profiling identifies co-occurring BRCA1/2 reversions, TP53BP1, RIF1 and PAXIP1 mutations in PARP inhibitor-resistant advanced breast cancer. Ann Oncol 35(4):364-80. doi: 10.1016/j.annonc.2024.01.003
59. Peng D, Liang P, Zhong C, Xu P, He Y, Luo Y, et al. (2022) Effect of EGFR amplification on the prognosis of EGFR-mutated advanced non–small-cell lung cancer patients: a prospective observational study. BMC Cancer 22(1):1323. doi: 10.1186/s12885-022-10390-0
60. Zhong C, Jiang W-J, Yao Y, Li Z, Li Y, Wang S, et al. (2024) CRISPR screens reveal convergent targeting strategies against evolutionarily distinct chemoresistance in cancer. Nat Commun 15(1):5502. doi: 10.1038/s41467-024-49673-4
61. Zhang B, Chen Y, Chen X, Ren Z, Xiang H, Mao L, et al. (2024) Genome-wide CRISPR screen identifies ESPL1 limits the response of gastric cancer cells to apatinib. Cancer Cell Int 24(1):83. doi: 10.1186/s12935-024-03233-4
62. Chang W, Zhang B, Yang S, Zhang X, Zhang L, Zhang S, et al. (2025) Genome-wide CRISPR/Cas9 screening identifies PTGR2 as a potential therapeutic target for sunitinib resistance in clear cell renal cell carcinoma. Sci Rep 15(1):26263. doi: 10.1038/s41598-025-12192-3
63. Agrawal P, Harish V, Mohd S, Singh SK, Tewari D, Tatiparthi R, et al. (2023) Role of CRISPR/Cas9 in the treatment of Duchenne muscular dystrophy and its delivery strategies. Life Sci 330:122003. doi: 10.1016/j.lfs.2023.122003
64. Behrouzian Fard G, Ahmadi MH, Gholamin M, Amirfakhrian R, Saberi Teimourian E, Karimi MA, et al. (2024) CRISPR-Cas9 technology: As an efficient genome modification tool in the cancer diagnosis and treatment. Biotechnol Bioeng 121(2):472-88. doi: 10.1002/bit.28603
65. Son H (2024) Harnessing CRISPR/Cas Systems for DNA and RNA Detection: Principles, Techniques, and Challenges. Biosensors 14(10):460. doi: 10.3390/bios14100460
66. Kordyś M, Sen R, Warkocki Z (2022) Applications of the versatile CRISPR-Cas13 RNA targeting system. Wiley Interdiscip Rev RNA 13(3):e1694. doi: 10.1002/wrna.1694
67. Crowley E, Di Nicolantonio F, Loupakis F, Bardelli A (2013) Liquid biopsy: monitoring cancer-genetics in the blood. Nat Rev Clin Oncol 10(8):472-84. doi: 10.1038/nrclinonc.2013.110
68. Tsou J-H, Leng Q, Jiang F (2020) A CRISPR test for rapidly and sensitively detecting circulating EGFR mutations. Diagnostics 10(2):114. doi: 10.3390/diagnostics10020114
69. Choi J-H, Lim J, Shin M, Paek S-H, Choi J-W (2020) CRISPR-Cas12a-based nucleic acid amplification-free DNA biosensor via Au nanoparticle-assisted metal-enhanced fluorescence and colorimetric analysis. Nano Lett 21(1):693-9. doi: 10.1021/acs.nanolett.0c04303
70. Chen M, Wu D, Tu S, Yang C, Chen D, Xu Y (2021) CRISPR/Cas9 cleavage triggered ESDR for circulating tumor DNA detection based on a 3D graphene/AuPtPd nanoflower biosensor. Biosens Bioelectron 173:112821. doi: 10.1016/j.bios.2020.112821
71. Hinestrosa JP, Kurzrock R, Lewis JM, Schork NJ, Schroeder G, Kamat AM, et al. (2022) Early-stage multi-cancer detection using an extracellular vesicle protein-based blood test. Commun Med 2(1):29. doi: 10.1038/s43856-022-00088-6
72. Ma C, Jiang F, Ma Y, Wang J, Li H, Zhang J (2019) Isolation and detection technologies of extracellular vesicles and application on cancer diagnostic. Dose-Response 17(4):1559325819891004. doi: 10.1177/1559325819891004
73. Li H, Xing S, Xu J, He Y, Lai Y, Wang Y, et al. (2021) Aptamer-based CRISPR/Cas12a assay for the ultrasensitive detection of extracellular vesicle proteins. Talanta 221:121670. doi: 10.1016/j.talanta.2020.121670
74. Mubthasima P, Pande K, Prakash R, Kannan A (2021) CRISPR Cas/exosome based diagnostics: future of early cancer detection. Rural Health. IntechOpen. doi: 10.5772/intechopen.99900
75. Zhao X, Zhang W, Qiu X, Mei Q, Luo Y, Fu W (2020) Rapid and sensitive exosome detection with CRISPR/Cas12a. Anal Bioanal Chem 412(3):601-9. doi: 10.1007/s00216-019-02211-4
76. He Y, Wu Y, Wang Y, Wang X, Xing S, Li H, et al. (2020) Applying CRISPR/Cas13 to construct Exosomal PD-L1 Ultrasensitive Biosensors for dynamic monitoring of tumor progression in Immunotherapy. Adv Ther 3(10):2000093. doi: 10.1002/adtp.202000093
77. Wang R, Zhao X, Chen X, Qiu X, Qing G, Zhang H, et al. (2019) Rolling circular amplification (RCA)-Assisted CRISPR/Cas9 cleavage (RACE) for highly specific detection of multiple extracellular vesicle MicroRNAs. Anal Chem 92(2):2176-85. doi: 10.1021/acs.analchem.9b04814.s001
78. Zhang G, Zhang L, Tong J, Zhao X, Ren J (2020) CRISPR-Cas12a enhanced rolling circle amplification method for ultrasensitive miRNA detection. Microchem J 158:105239. doi: 10.1016/j.microc.2020.105239
79. Kling A, Dirscherl L, Dittrich PS (2023) Laser-assisted protein micropatterning in a thermoplastic device for multiplexed prostate cancer biomarker detection. Lab Chip 23(3):534-41. doi: 10.1039/d2lc00840h
80. Wang Z, Liu N, Feng F, Ma Z (2015) Synthesis of cadmium, lead and copper alginate nanobeads as immunosensing probes for the detection of AFP, CEA and PSA. Biosens Bioelectron 70:98-105. doi: 10.1016/j.bios.2015.03.015
81. Mu X, Wang X, Qin Y, Huang Y, Tian J, Zhao S (2023) A novel label-free universal biosensing platform based on CRISPR/Cas12a for biomarker detection. Talanta 251:123795. doi: 10.1016/j.talanta.2022.123795
82. Wang J, Yang X, Wang X, Wang W (2022) Recent advances in CRISPR/Cas-Based biosensors for protein detection. Bioengineering 9(10):512. doi: 10.3390/bioengineering9100512
83. Ambros V, Bartel B, Bartel DP, Burge CB, Carrington JC, Chen X, et al. (2003) A uniform system for microRNA annotation. RNA 9(3):277-9. doi: 10.1261/rna.2183803
84. Fromm B, Billipp T, Peck LE, Johansen M, Tarver JE, King BL, et al. (2015) A uniform system for the annotation of vertebrate microRNA genes and the evolution of the human microRNAome. Annu Rev Genet 49:213-42. doi: 10.1146/annurev-genet-120213-092023

85. Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. Cell 136(2):215-33. doi: 10.1016/j.cell.2009.01.002
86. Shukla GC, Singh J, Barik S (2011) MicroRNAs: processing, maturation, target recognition and regulatory functions. Mol Cell Pharmacol 3(3):83. doi: 10.1016/j.cell.2009.01.002
87. Jung J, Yeom C, Choi Y-S, Kim S, Lee E, Park MJ, et al. (2015) Simultaneous inhibition of multiple oncogenic miRNAs by a multi-potent microRNA sponge. Oncotarget 6(24):20370. doi: 10.18632/oncotarget.4827
88. Calin GA, Croce CM (2006) MicroRNA signatures in human cancers. Nat Rev Cancer 6(11):857-66. doi: 10.1038/nrc1997
89. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. (2008) Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA 105(30):10513-8. doi: 10.1073/pnas.0804549105
90. Pu J, Liu M, Li H, Liao Z, Zhao W, Wang S, et al. (2021) One-step enzyme-free detection of the miRNA let-7a via twin-stage signal amplification. Talanta 230:122158. doi: 10.1016/j.talanta.2021.122158
91. Yuan C, Tian T, Sun J, Hu M, Wang X, Xiong E, et al. (2020) Universal and naked-eye gene detection platform based on the clustered regularly interspaced short palindromic repeats/Cas12a/13a system. Anal Chem 92(5):4029-37. doi: 10.1021/acs.analchem.9b05597.s001
92. Wang J, Bing T, Zhang N, Liu X, Shangguan D (2020) FnCas12a/crRNA assisted dumbbell-PCR detection of IsomiRs with terminal and inner sequence variants. Chem Commun 56(69):10038-41. doi: 10.1039/d0cc04348f
93. Yu Z, Willmarth NE, Zhou J, Katiyar S, Wang M, Liu Y, et al. (2010) microRNA 17/20 inhibits cellular invasion and tumor metastasis in breast cancer by heterotypic signaling. Proc Natl Acad Sci USA 107(18):8231-6. doi: 10.1073/pnas.1002080107
94. Huang M, Huang R, Yue H, Shan Y, Xing D (2020) Ultrasensitive and high-specific microRNA detection using hyper-branching rolling circle amplified CRISPR/Cas13a biosensor. Sens Actuators B Chem 325:128799. doi: 10.1016/j.snb.2020.128799
95. Chen S, Zhao J, Xu C, Sakharov IY, Zhao S (2021) Absolute quantification of microRNAs in a single cell with chemiluminescence detection based on rolling circle amplification on a microchip platform. Anal Chem 93(26):9218-25. doi: 10.1021/acs.analchem.1c01463
96. Tian W, Liu X, Wang G, Liu C (2020) A hyperbranched transcription-activated CRISPR-Cas12a signal amplification strategy for sensitive microRNA sensing. Chem Commun 56(87):13445-8. doi: 10.1039/d0cc06034h