Clinica Chimica Acta 569 (2025) 120179

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Review

# CRISPR integrated biosensors: A new paradigm for cancer detection

Arzoo Saini [a], Neeraj Dilbaghi [b], Neelam Yadav [a,*]

[a] Department of Biochemistry, School of Interdisciplinary & Applied Sciences, Central University of Haryana, Jant-Pali, Mahendergarh-123031, India
[b] Department of Biotechnology, Guru Jambheshwar University of Science & Technology, Hisar-125001, India

ARTICLE INFO

*Keywords:*
Cancer
Cancer biomarkers
Cancer diagnostics
Nanomaterials
Biosensors
CRISPR-Cas system

ABSTRACT

Cancer remains one of the leading causes of morbidity and mortality globally, necessitating need for advancements of technologies for early therapeutics. Conventional detection methodologies often lag behind in terms of sensitivity, specificity, and cost-effectiveness, leading to delayed diagnosis and inadequate treatment. The need of advanced diagnostic techniques has considerably increased and led to the development of biosensors. Biosensing technologies offer several advantages over conventional methods hence, overcome limitations and improve diagnostic accuracy. Biosensors, particularly CRISPR-Cas based biosensors have emerged as a revolutionary technology for oncology diagnostics due to their high precision and adaptability. CRISPR-based biosensors provide remarkable precision, sensitivity, multiplexing capabilities, specificity, and rapidness for developing a cost-effective and portable point of care diagnostic device for cancer detection. In this review, we have discussed cancer pathogenicity, assessed the traditional detection techniques, and explored the advancements and advantages of biosensors, particularly CRISPR-based biosensors, in the detection of some major cancer types, namely lung, liver, colorectal, prostate, and cervical cancers. CRISPR-based biosensors represent a significant potential in cancer diagnostics, offering precise, cost-effective, and rapid detection of cancer biomarkers. The integration of CRISPR technology with biosensors holds substantial promise for enhancing early detection and improving patient outcomes in cancer diagnostics.

## 1. Introduction

At present, cancer is recognized as one of the foremost life-threatening pathologies, impacting human health and survival. It is characterized with the abnormal growth and division of the cells. This uncontrolled proliferation culminates in the malignant form resulting in the invasion to other body parts [1]. Cancer disease at the genome level causes amplification of oncogenes and inhibition of tumor suppressor genes. It results in the changes in cells of metabolism resulting in their growth in hostile environments, at the metabolic level [2].

As claimed by the recent estimates of the GLOBOCAN by International Agency for Research on Cancer (IARC) approximately 20 million new cases were reported in the year 2022 and about 9.7 million people died due to cancers [3]. In majority of all types of cancers, survival rates of the patient increase with effective and efficient treatment if the disease is diagnosed at early stages [4]. Hence, timely screening plays a very important role in reducing cancer associated morbidity and mortality. Conventionally, distinct detection strategies have been employed for cancer detection including imaging techniques (Magnetic Resonance Imaging (MRI), X-rays based techniques, ultrasound, microwave tomography, radar-based imaging, Computed Tomography (CT) scans); genomic and proteomic detection methods such as southern blot, DNA sequencing, polymerase chain reaction (PCR), liquid chromatography mass spectrometry (LC-MS); immunoassay techniques like enzyme-linked immunosorbent assay (ELISA) and other methods like biopsies. These common standard detection strategies are associated with their own set of limitations by exhibiting less sensitivity, expensive, requirement of specialized equipment, laborious etc. [5].

Therefore, biosensors are being developed to overcome the limitations conferred by these conventional detection methods. Biosensors are the advanced technical devices which detect the presence of a specific analyte in the test sample hence, providing a non-invasive and direct detection of specific type of cancer [6]. Although biosensing devices offer advantages by being highly specific, sensitive, less detection time and user friendly. But still there exists some of the shortcomings like complexity in development and manufacturing, technical challenges,

* Corresponding author at: Department of Biochemistry, School of Interdisciplinary & Applied Sciences, Central University of Haryana, Jant-Pali, Mahendergarh, 123031, India.
*E-mail address:* neelamindia12@rediffmail.com (N. Yadav).

https://doi.org/10.1016/j.cca.2025.120179
Received 25 December 2024; Received in revised form 29 January 2025; Accepted 29 January 2025
Available online 1 February 2025

lack of sensitivity in detecting subtle genetic changes etc. [7]. Integrating CRISPR-Cas system with biosensing technology can enhance the functionality of biosensors. In the recent times clustered regularly interspaced short palindromic repeats (CRISPR) – CRISPR-associated protein (Cas) technology has equipped the attention of the scientific community. CRISPR is the system used by bacteria to protect themselves from the viruses and Cas is an enzyme capable of cutting DNA strands at the specific locations [8]. Together they form a revolutionary technique that enables precise gene editing within an organism. The designing of CRISPR-Cas based biosensors has increased the specificity and sensitivity of existing biosensors. CRISPR technology can be designed to target specific nucleic acid sequences with high specificity hence, reducing the likelihood of false positives and increasing the precision of biosensors. The capability of CRISPR system to amplify the target sequence allow even minute quantity of detection [9]. CRISPR based biosensors also exhibit multiplexing capabilities and allow for the detection of various pathogens, genetic mutations, or biomarkers with minimal redesign efforts.

Moreover, the usage of CRISPR-Cas integrated biosensors has paved the way for developing portable on-site cancer detection rapid diagnostic devices. By offering non-invasive, cost-effective, and rapid diagnostic solutions, CRISPR-based biosensors make early cancer detection more accessible, particularly in resource-limited settings. In this review we will discuss pathogenicity associated with cancer, traditionally adopted diagnostic strategies, as well as new age biosensing technologies. Also, the utility of CRISPR-Cas as a promising tool in the development of biosensors for detection of some major potent cancers (lung cancer, liver cancer, colorectal cancer, prostate cancer, and cervical cancer), along with the associated limitations, and future prospects would be described.

## 2. Cancer pathogenicity

Cancer progression is attributed to numerous factors ranging from lifestyle factors, environmental factors to genetic factors [10]. Cancer usually gets triggered due to the mutations in the healthy cells. These mutations are induced due to the exposure to some of the carcinogens like tobacco, high-energy radiations, and certain chemicals [11]. The mutations further affect the cells at genetic level by overexpressing oncogenes and down regulating tumor suppressor genes [12]. The cell now undergoes rapid growth and division by evading apoptosis. Cancer cells when start to invade the distant organs through circulatory and lymphatic system, this process is called as metastasis [13]. At this stage cancer becomes hard and complex to treat, even reducing the survival chances of patient. The progression of normal cell to the malignant tumor is depicted in Fig. 1.

There are multiple risk factors which leads to progression of different types of cancer in different organs. A number of pathways and genetic mutations are also involved in cancer progression. A comparative analysis of risk factors, pathways and genes involved in progression of some of the major types of cancer (lung cancer, liver cancer, colorectal cancer, prostate cancer, and cervical cancer) are described in Table 1.

## 3. Cancer detection

Early-stage detection of cancer remains a big challenge. Technological advancements are needed to reduce the mortality rate of patients by early diagnosing particular cancer. This will not only provide the effective treatment but also reduce the economic burden. Herein, we discussed various techniques used for cancer biomarkers detection:

### 3.1. Conventional techniques for cancer detection

Numerous conventional as well as modern diagnostic techniques are being used to increase survival rate of patients. Table 2 provides for the detailed comparative analysis of the conventional techniques used for cancer detection.

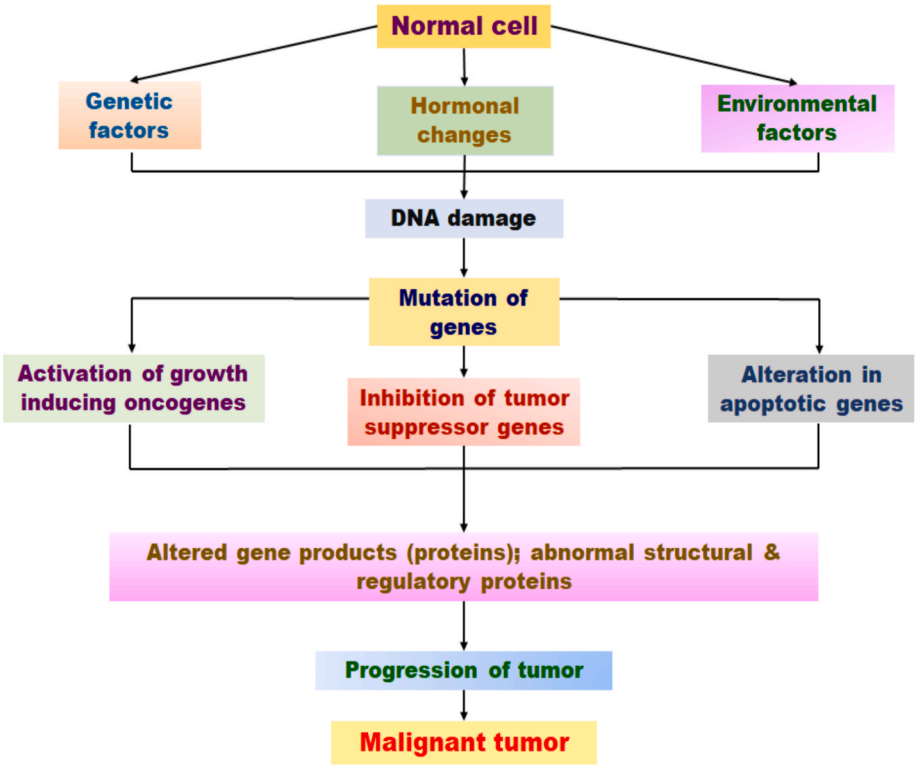


**Fig. 1.** Schematic representation of different steps involving cancer progression.

**Table 1**
A comparative analysis of major risk factors, pathways and genes involved in progression of some of the major types of cancer.

| Type of cancer | Risk factors | Pathways associated | Genes involved | References |
|---|---|---|---|---|
| **Lung cancer** | Tobacco<br>Air pollution<br>Occupational exposureHereditary susceptibilityRadiation | Epidermal Growth Factor Receptor (EGFR) Pathway; Kirsten rat sarcoma (KRAS) Pathway | *EGFR*<br>*KRAS* | [14],<br>[15,16] |
| **Liver cancer** | Alcohol and tobacco consumption<br>Exposure to toxins like- aflatoxin, arsenic etc.<br>Chronic viral hepatitisLifestyle factorsGenetics | WNT/β-catenin signalling pathway; MAPK/ERK signalling pathway; NF-κB Pathway | Catenin Beta 1 (*CTNNB1)* and Axis inhibition protein 1 (*AXIN1*)<br>*TP53* | [17,18<br>19,20] |
| **Colorectal cancer** | Hereditary mutations in genes (*APC, MLH1, PMS2, PTEN* etc.)<br>Radiation exposure<br>Cholecystectomy<br>Obesity & physical inactivityDietSmoking, Alcohol | EGFR/MAPK signalling pathway;<br>Notch pathway;<br>PI3K/Akt pathway;TGF-β signalling pathway;Wnt signalling pathway. | *EGFR*<br>*Hes-1, Deltex, NICD*<br>*Akt*<br>*TGF-β*<br>*c-MYC, c-Jun, CCND1* | [21,22] |
| **Prostate cancer** | Older age<br>African descent<br>Family history<br>Genetic risk loci<br>ObesitySmokingPhysical inactivity | PI3K-AKT-mTOR signalling pathway | Androgen Receptor (*AR*) gene,<br>*PTEN,*<br>*DEPTOR* | [23,24,25] |
| **Cervical cancer** | Multiple sexual partners<br>Long-term use of oral contraceptives<br>Genetic factorsTobacco use | Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signalling pathway | Active oncogenes- *PIK3CA, ATAD2 and CRNDE*; tumor suppressor genes- *p53*, Ras association domain family 1 isoform A and *NOL7* | [26,27] |

### 3.2. Biosensing techniques for cancer detection

Although, the conventional techniques are very significant in diagnosing cancer but they lacked in sensitivity and specificity; hence, there is need to find better alternatives. Increasing cancer burden attributed with approximately 1,670 deaths that had occurred in a day and estimated death of 6,09,280 people in the year 2023 in the U.S. itself is startling, and situation is even more alarming for low-middle income countries [43]. Hence, there is an urgent need for advancements in oncology diagnostics and developing a technique with potential of improving survival outcomes of the patients by detecting cancer at early stages with high specificity and sensitivity. Biosensing techniques lay the foundation for development of a robust detection method which would be highly specific, with quick response time, miniaturized, cost-effective and capability to be used as a point of care (POC) devices.

#### 3.2.1. Principle and working of biosensors

Biosensors are the advanced analytical devices which can detect even minute concentrations of target analyte from a complex mixture [44]. The detection of target analyte is made through the binding with corresponding specific bioreceptor, such as antibodies, aptamers, enzymes, DNA, RNA, peptides etc. The signal hence generated from bio-recognition site is further converted to optical, electrochemical, magnetic, electrical etc. signal by transducer, which is further detected by detector and presented in a user-friendly response [45]. In detail principle and working of a biosensor is explained through Fig. 2.

#### 3.2.2. Classification of biosensors

Several types of biosensors are being fabricated for the diagnosis of cancers. The biosensors are categorized depending on the types of transducers used. These different types of transducers generate distinct analytical signals such as, current, potential, absorbance, fluorescence, conductance, and impedance [46]. Table 3 provides a comparative analysis of different kinds of biosensors based on their principle, merits, and demerits.

Although, existing biosensors have made notable contributions to the field of cancer diagnostics, several challenges persist which limit their applicability in clinical settings. Many biosensors fail to achieve ultra-high sensitivity and specificity which are required to detect early-stage cancer biomarkers at very low concentrations. Conventional biosensors, like piezoelectric types have slower response times, optical biosensors use expensive and costly instrumentation. Certain biosensors also face challenges in terms of non-specific interferences and environmental sensitivity. Although, electrochemical biosensors can address these limitations of optical and piezoelectric biosensors however; there still remains a scope of improvement in analytical performance and diagnostic applications. Hence, there is a need of advancing the existing biosensing techniques and integrating them with advanced molecular techniques to improve cancer diagnostics. Integration of CRISPR technology with biosensors offers a promising and transformative solution to enable early, precise, non-invasive, and personalized approach for cancer detection. These CRISPR-Cas integrated biosensors are more sensitive, specific, rapid, and accurate than the conventional biosensors. They have multiplexing potential and have potential to be used in point-of-care (POC) applications in resource-limited settings.

### 3.3. CRISPR-Cas system for cancer detection

The molecular diagnostic tools such as PCR although sensitive but faces challenges in the form of cost-effectiveness and requirement of specialized equipment and trained personnel. A budding choice to existing detection tools for early-stage cancer detection is CRISPR-Cas based sensing technology. CRISPR-Cas system was originally present in the bacteria as an effective immune response strategy against alien nucleic acids [8]. The function of CRISPR-Cas is attributed to its component CRISPR RNA (crRNA) and Cas protein. The crRNA binds with the complementary gene sequence of the invading pathogen and ultimately results in the degradation by Cas proteins. Hence the complete system serves as genetic magical scissors. In detail components and cleavage mechanism of CRISPR-Cas system is explained through Fig. 3a.

#### 3.3.1. Classification of CRISPR-Cas system

CRISPR-Cas is categorized in two classes, Class 1 CRISPR-Cas systems and Class 2 CRISPR-Cas systems depending on the catalytic property of Cas protein [54]. Class 1 comprises of numerous effector Cas proteins performing single function, while Class 2 involves single protein performing multiple functions in the CRISPR system [8]. Both the classes are further divided into various types and sub-types as depicted in the Fig. 3b. Class 1 encompassing, types I, III and IV, and Class 2 comprising type II, V, and VI. CRISPR-Cas system belonging to Class 2 have much

**Table 2**
Comparative analysis of conventional techniques used for cancer diagnostics.

| Conventional Detection method | Principle | Advantages | Disadvantages | References |
|---|---|---|---|---|
| **Imaging technology** | | | | |
| Magnetic Resonance Imaging (MRI) | This technique is based on high-powered magnetic fields and radio waves to create detailed 3D images of organs and tissues in the body | It shows high sensitivity, quality and 3D images are obtained.Moreover, it is painless procedure and use non-ionizing radiations | It takes long scan time and expensive | [28] |
| X-rays | X-rays based imaging like mammogram uses X-ray system to produce image of the inside of the body tissues and organs | It is relatively fast and used as the first-hand technique for regular check-upas it is easily available | It produces low-contrast and grainy image. Moreover, it gives false negative, use ionizing radiations, not convenient as it uses limited dynamic range | [29] |
| Ultrasound | In the ultrasound imaging high frequency sound waves are used to produce images of internal structure. | It is easily available, cost-effective, portable, versatile, quick, painless and use nonionizing radiation. | It produces lower resolution, low sensitivity, specificity and require trained personnel. | [30] |
| Microwave tomography | This technology uses microwaves of the range between 300 MHz to 300 GHz and detects by using inversion scattering | It uses non-ionizing radiation.It is a non-invasive technique and economical. | It is complex method and easily not available | [31] |
| Radar-based imaging | It utilizes ultra-waveband (UWB) microwave pulse to illuminate the target tissue. | It is simple, fast good signal processing, and 2D images can be generated. | It requires computational power needed and not easily available | [31] |
| Computed tomography (CT) scans | Computed tomography scan uses X-rays to provide the detailed image of the inside of the tissues and organs. | It produces 3D images, minimum background noises and more sensitive to determine the size of tumor. | It shows poor soft-tissue contrast resolution, poor ability to distinguish between benign and malignant lesions and long-term exposure of radiations. | [32] |
| **Genomic and proteomic detection methods** | | | | |
| Southern blot | It uses the procedures of gel electrophoresis and blotting of separated DNA strands on a membrane to detect specific DNA sequences. | This can identify specific target sequence and used for quantification. | It is labour-intensive, non-specific binding of analyte and background noises can interfere with the results | [33] |
| DNA sequencing | It includes high-throughput sequencing of nucleic acids to determine any variations, genetic mutations or change in expression profiles. | It is used in targeted therapy due to high sensitivity and can analyse multiple genes simultaneously. | It is expensive, time consuming andrequires extensive data analysis. | [34] |
| Liquid chromatography mass spectrometry (LC-MS) | LC-MS combines liquid chromatography and mass spectrometry for the detection as well as quantification of certain molecules like peptides, proteins and metabolites which can serve as a potential biomarker for cancer. | These techniques exhibited high sensitivity, specificity and used for both qualitative and quantitative analysis | These techniques exhibited high complexity, expensive and requires extensive data analysis. | [35] |
| Polymerase chain reaction (PCR) | PCR includes amplification and detection of specific DNA/RNA sequences and changes in their expression profile associated with cancer. | It is highly sensitive and specific. It can detect changes even at early stages | It requires specialized equipment, require trained personnel and prior knowledge of target sequences | [36] |
| **Cell-based techniques** | | | | |
| Flow cytometry | This technique is used for analysing physical and chemical properties of cells in a fluid suspension with the use of lasers and fluorochrome-conjugated antibodies. | It is a high throughput technique with the ability to identify multiple parameters simultaneously.It can detect rare cells such as circulating tumor cells. | It requires expensive instruments and trained professionals. | [37] |
| Cytopathology | It involves the examination of cell morphology using cytological stains to detect malignancy. It includes techniques such as pap smear (for cervical cancer) and fine needle aspiration cytology (FNAC). | It is a simple and cost-effective procedure that is widely employed in clinical practice. | It provides limited molecular information and is subjective depending on the interpretation by pathologist. | [38] |
| **Immunoassay techniques** | | | | |
| Enzyme-linked immunosorbent assay (ELISA) | ELISA is a plate-based immunoassay technique which can detect and quantify soluble cancer biomarkers in blood, urine and tissue samples. | It is simple, quick and cost-effective. | It requires purified antigen, exhibit low sensitivity and produce false positive results | [39] |
| Immunohistochemistry (IHC) | IHC is used to detect specific antigens/proteins in the tissue sections with the help of specific antibodies. | It allows for precise detection of the localization of individual proteins within tissue samples. | This technique is effective only for detecting the specific known antigens. Also, the interpretation can be subjective depending on the observer. | [40] |
| **Other methods** | | | | |
| Fluorescence In Situ Hybridization (FISH) | FISH involves the use of fluorescently labelled probes to detect and localize specific nucleic acid sequences. | It is effective in detecting chromosomal abnormalities and allows spatial visualizations of the DNA/RNA sequences within the cells. | This technique is often expensive, complex, and labor-intensive. | [41] |
| Biopsies | This technique involves surgical removal of a portion or the entire tumor for examination. | It provides precise information about tumor histology and composition. | It is invasive and surgical risks cannot be used in all anatomical locations. | [42] |

utility in the biosensing technology due to the ease of handling as well as higher efficiency [55].

### 3.4. CRISPR-Cas integrated biosensing techniques for cancer detection

The use of CRISPR-Cas system in the biosensing platforms has gained the attention of scientific community from the recent past. The first reported CRISPR-Cas based comprehensive fluorescence-based biosensor with applicability was SHERLOCK (specific high sensitivity enzymatic reporter unlocking) [56]. A recent innovative and promising use of CRISPR-Cas system includes its potential to be used in biosensing platforms for early detection and monitoring of cancer [57].

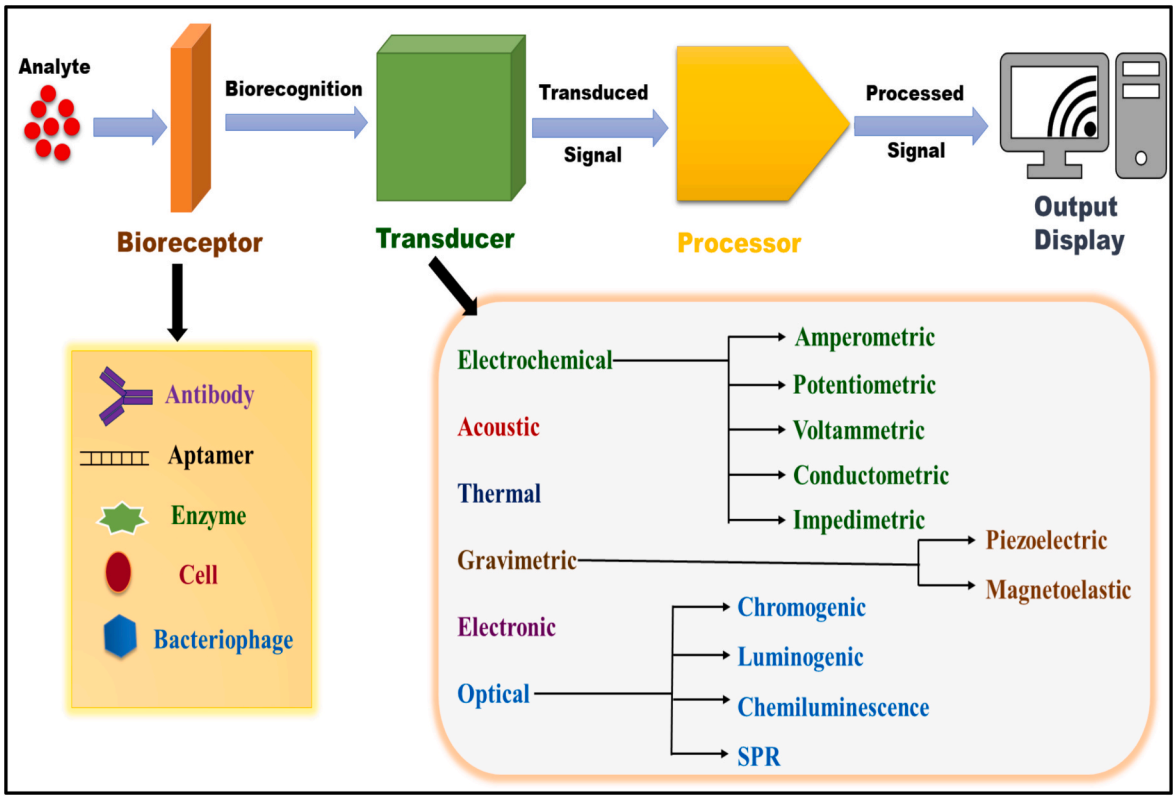


**Fig. 2.** Diagrammatic illustration of working and principle of a biosensor.

*3.4.1. Advantages of CRISPR-integrated biosensors over existing biosensors for cancer detection*

These CRISPR-integrated biosensors have edge over the existing biosensors in effective detection of cancer biomarkers. They use the programmable nature of the guide RNAs for the detection of genetic mutations, microRNAs, circulating tumor DNA (ctDNA) while conventional biosensors relied on surface recognition and signal transduction. This advancement had led to improved accuracy when dealing with the complex matrices such as, blood or serum [58]. Moreover, CRISPR-based biosensing techniques utilize *trans*-cleavage activities of Cas proteins and integrate a powerful signal amplification mechanism to enable ultra-sensitive detection. This detection is in the range of femto molar or atto molar which surpasses the limit of detection of many biosensors [59]. Moreover CRISPR-Cas based biosensing systems have additional advantages in the form of enhanced specificity, high accuracy, and precise detection [60]. When combined with the nanomaterials, CRISPR-biosensors exhibit enhanced analytical performance [61]. These biosensors also allow multiplex detection, allowing more comprehensive analysis [62]. Additionally, CRISPR-Cas methods are highly suitable for being used in point-of-care diagnostics [63]. Their adaptability and versatility also make them preferable to be used with microfluidic platforms. Hence, compared to the existing biosensors, CRISPR-integrated biosensors have more dynamic advantage in monitoring cancer progression and provide insights for precision oncology.

## 4. Applications of CRISPR-Cas integrated biosensors for cancer detection

CRISPR-Cas system is being used in the field of biomedical research ranging from genome editing to developing specific diagnostic tools. CRISPR-Cas integrated biosensing is an advanced technique which provides effective diagnosis for the cute detection of cancers. It also has varied applications in detecting genetic mutations and cancer specific biomarkers [64]. CRISPR-Cas plays a pivotal role in designing ultra-sensitive biosensors for the early and precise detection of detrimental cancers including; lung, liver, colorectal, prostate, and cervical cancers. Numerous biosensing platforms have been fabricated using the CRISPR-Cas system for detecting these cancers. The wide implementation of these next generation diagnostics are required to reduce mortality and healthcare burden. Herein, we discussed applications of CRISPR-Cas integrated biosensors for the detection of different types of cancers:

### *4.1. CRISPR-Cas integrated biosensors for lung cancer detection*

Lung cancer is the leading cause of cancer-associated morbidity in the world with 2.5 million new cases and 1.8 million reported deaths in the year 2022. The male to female lung cancer mortality ratio is around 2 and is the most common cancer in men and second most common cancer in women [3]. Depending upon the morphology of the cancer cells under microscope, it is characterized as non-small cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC). NSCLC is found to be associated with more than 85 % of lung cancer cases [65]. Depending on the histology NSCLC is further classified as adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. SCLC linked with nearly 15 % of the cases is characterized with rapid metastasis, poor prognosis, and is associated with smoking especially in men [66]. The progression of lung cancer through different stages is depicted in Fig. 4. These different stages are characterized by the size of tumors and spread to the distant organs. The stage 1 represents the confinement of tumor in lung tissues. In the stage 2, the tumor may have spread into the lymph nodes inside of the lung. Stage 3 is characterized when the size of tumor is increased to more than 6 cm and has spread in the middle of chest. At last, the stage 4 is the metastatic stage where the cancer has spread to distant organs [67].

Lung cancer if detected at early stage can help in reducing morbidity and increasing the survival outcomes. CRISPR-Cas based biosensors offer a rapid, sensitive, specific, and non-invasive technique for lung cancer detection in early stages. For the effective diagnosis of lung

**Table 3**
Comparison of various types of biosensors used for detection of cancer.

| Types of biosensors | Principle | Merits | Demerits | References |
|---|---|---|---|---|
| **Electrochemical biosensors** | | | | |
| **Amperometric biosensors** | These biosensors measure the current generated due to the redox reactions occurring between recognition element and target analyte onto working electrode. | They are simple, highly selective, sensitive and generate quick response for detecting cancer biomarkers. | They are less stable, highly susceptible to oxidative environment, and require routine calibration. | [47] |
| **Potentiometric biosensors** | Potentiometric biosensors are analytical devices that are used to detect specific biological substances by measuring the changes in potential or voltage at working electrode. | Detection of cancer biomarkers by these biosensors are easy to operate, modify, and cost-effective. | They are limited to the use of ionic analytes and are sensitive to signal drift with time. | [48] |
| **Impedimetric biosensors** | These biosensors measure changes in electrical impedance caused by binding of target analyte at the surface of a recognition element on the electrode. | They provide label-free, rapid detection with no harmonic distortions. | They are sensitive to environmental fluctuations. Also, the electrode surface is susceptible to fouling which degrade sensor's performance over time. | [49] |
| **Conductometric biosensors** | Conductometric biosensors function by monitoring the changes in conductivity as an analytical signals resulting with the generation of ion during biochemical reactions between the target analyte and a recognition element immobilized on the sensor. | They are simple to design, rapid, versatile, and cost-effective to produce. | They have limited specificity and sensitivity. Factors such as, temperature, pH, and presence of other ions can influence conductivity, which can affect accuracy. | [50] |
| **Optical biosensors** | | | | |
| **Fluorescence biosensors** | Fluorescence biosensors work on the principle of detection of fluorescence as analytical signals produced by the interaction of analyte and fluorescent probes. | They have good sensitivity, specificity, versatility, and multiplexing capabilities. | They use expensive equipment and are prone to photobleaching. Moreover, fluorescent probes have limited stability and potential toxicity. | [51] |
| **Colorimetric biosensors** | These biosensors detect target biological substances through apparent color changes made by analyte-probe interactions. They utilize chemical or enzymatic reactions to produce a visible signal. | These biosensors use simple approach and are rapid, selective, portable, and cost-effective. | They are prone to interference from colored or turbid samples. Also, they might not be able to detect very low concentrations of analyte and also face issues with quantitative analysis. | [52] |
| **Mass based biosensors** | | | | |
| **Piezoelectric biosensors** | Piezoelectric biosensors are based on piezoelectric effect, by which certain materials generate an electrical change when incur mechanical stress. These biosensors measures changes in viscosity or mass using surface acoustic waves to detect target analyte. | They have high sensitivity to mass changes and are suitable for real-time analysis. | They require highly stable conditions and can get affected by non-specific binding. The fabrication process of these biosensors can be complex and costly. | [53] |

cancer biomarkers such as, EGFR, hOGG1, FEN1, PD-L1, and other RNA/DNA biomarkers various CRISPR-integrated biosensors have been developed. Circulating Tumor DNA (ctDNA) is also one such potent biomarker for precise lung cancer diagnostics. For instance, Qi et al. (2024) fabricated a CRISPR-Cas14a system-based electrochemical biosensor for the detection of circulating Tumor DNA (ctDNA) in NSCLC patients [68]. The biosensor was able to specifically detect ctDNA EGFR L858R mutation. The detection mechanism involved primer exchange reaction (PER) for isothermal amplification of target ctDNA. CRISPR-Cas14a system got activated by PER reaction, resulting cleavage of substrate ssDNA-MB. This cleavage then affected the redox signal on the gold working electrode surface, that are detected electrochemically. The electrochemical biosensor was highly sensitive with low detection limit of 0.34 fM and a linearity range of 1 fM to 1 μM. This biosensor with a novel combination of PER and CRISPR-Cas14a for ctDNA detection holds promising applications for clinical diagnosis and personalized therapies for NSCLC. However, this highly complex method requires careful optimization and use of highly-quality reagents. In another study by Wang et al. (2023) a CRISPPR-Cas12a based biosensor was developed for detecting the circulating tumor DNA (ctDNA) mutations in NSCLC [69]. The detection mechanism involved amplification of the target ctDNA to detectable levels by recombinase polymerase amplification (RPA). Artificially inserted protospacer adjacent motif (PAMs) or sub-optimal PAMs were used to unlock the PAM restriction. CRISPR-Cas12a system was utilized to identify and trans-cleave the target DNA for fluorescent signal generation. The intensity of fluorescence signal was correlated with the amount of target ctDNA present in the sample. It was able to detect mutations within 50 min, with a LOD of 100 aM and was able to identify mutations with a variant allele frequency as low as 0.02 %. Overall, this fluorescence biosensor was rapid, highly sensitive, and specific method for ctDNA detection. It has a universal application with the introduction of artificial PAMs, that would overcome the requirement of PAM sequence hence, allowing for the detection of a wide range of mutations. However, designing and optimizing RNA primers and crRNA would be technically complex and there is a possibility of off-target effects. Moreover, the method might be not suitable for identifying unknown mutations as it is designed for targeting only specific ctDNA mutations.

Abnormal expression of human 8- oxoguanine DNA glycosylase (hOGG1) is found to be associated with various cancers (breast, lung, orolaryngeal, colon etc.) and various diseases. The detection of the aberrant expression of hOGG1 becomes crucial for early-stage cancer diagnostics. Zhang et al. (2022) used (A549 cells) human lung adenocarcinoma cell line to provide for a universal biosensor for the detection of hOGG1 as well as formamidopyrimidine [fapy]-DNA glycol-sylase (Fpg). The biosensor used a hairpin probe specific to the target enzymes (hOGG1 and Fpg), which undergoes conformational changes on binding of the enzyme [70]. The target DNA then undergoes multiple rounds of amplification through a process called as strand displacement amplification (SDA). The amplified product was then recognized by CRISPR-Cas12a system which caused subsequent cleavage of the ssDNA resulting signal generation. The signal was then visualized through fluorescence and the biosensor allowed for the detection with a limit of $4.24 \times 10^{-9}$ U/μL. The biosensor was found to be highly specific and rapid with the entire detection process completion within 40 min.

Specific RNA biomarkers are also being used for providing the accurate diagnosis of lung carcinoma. In a study by Lu et al. (2023) a CRISPR-Cas 12a-based fluorescence biosensor was developed. It had a potential of providing early diagnosis of lung cancer through liquid biopsy. They were able to specifically detect exosomal miR-21 associated

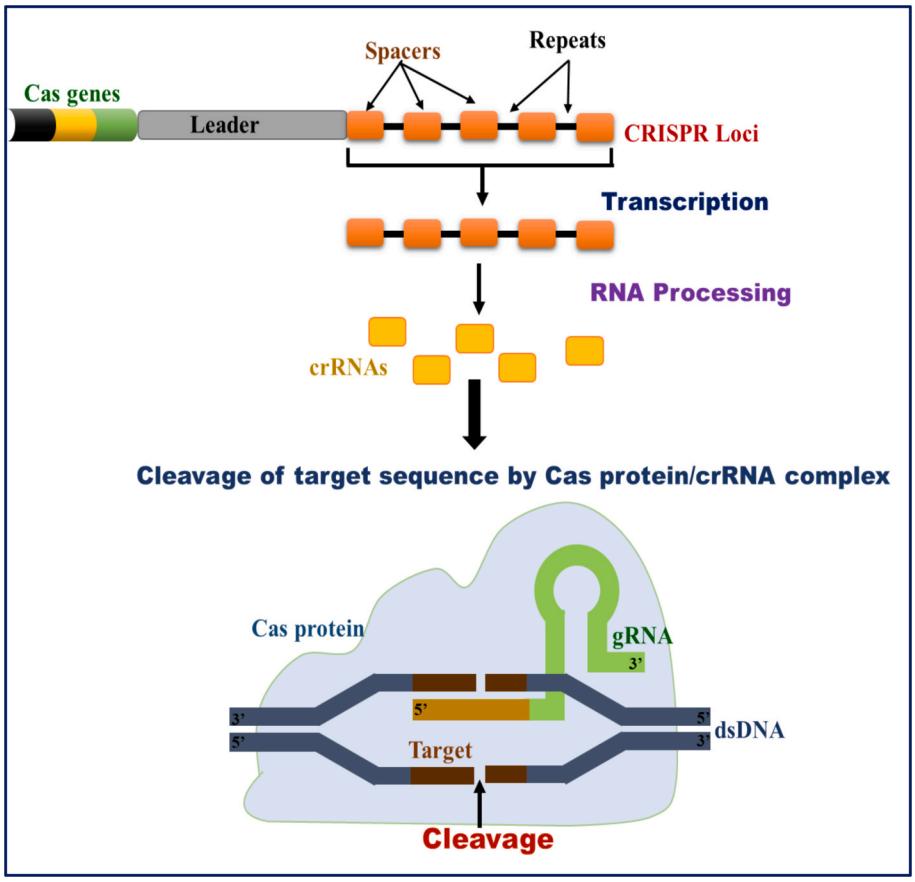


**Fig. 3a.** Diagrammatic illustration of components and cleavage mechanism of CRISPR-Cas system.

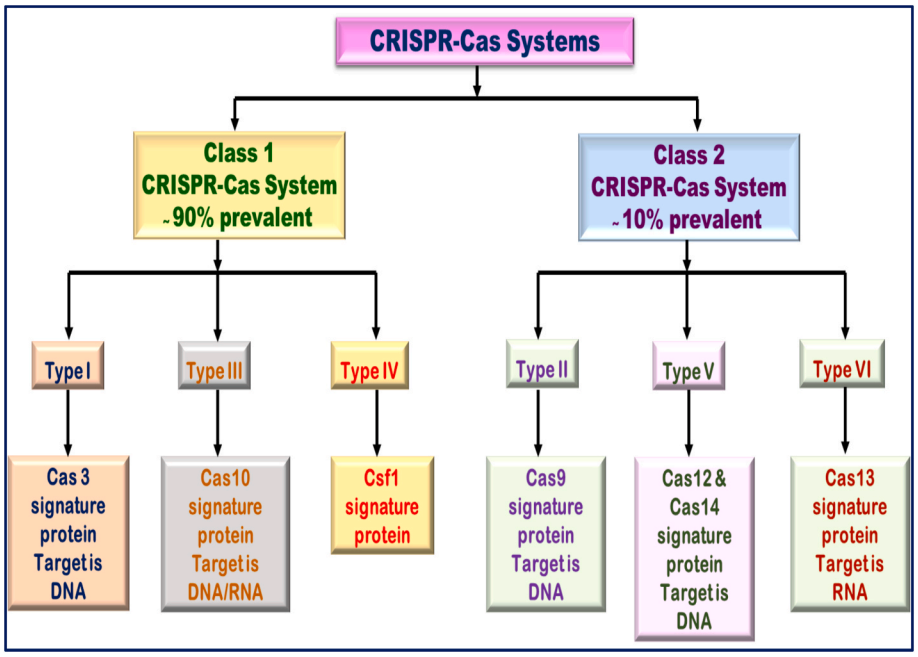


**Fig. 3b.** Schematic representation of classification of CRISPR-Cas systems.

with lung cancer in blood samples [71]. The biosensor exhibited a highly precise and sensitive approach to detect the presence of miR-21. It involved coupling of CRISPR/Cas12a system with a strand displacement reaction (SDR) as depicted in Fig. 5. The fabricated biosensor also used terminal deoxynucleotidyl transferase (TdT), and streptavidin-linked magnetic nanoparticles (SA-MNPs). The presence of miR-21 in the sample triggered the SDR, which caused release of DNA. The released DNA is used for amplifying the signal by TdT, which resulted in the

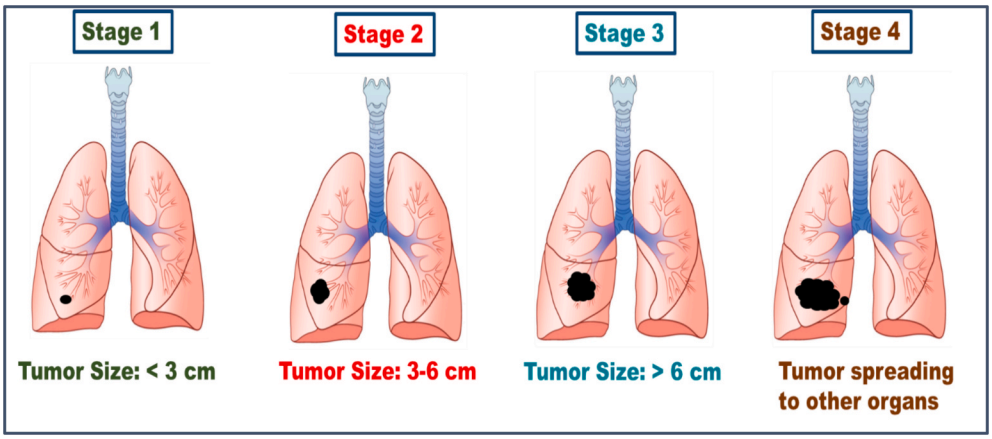


**Fig. 4.** Diagrammatic representation of progression of lung cancer through various stages.

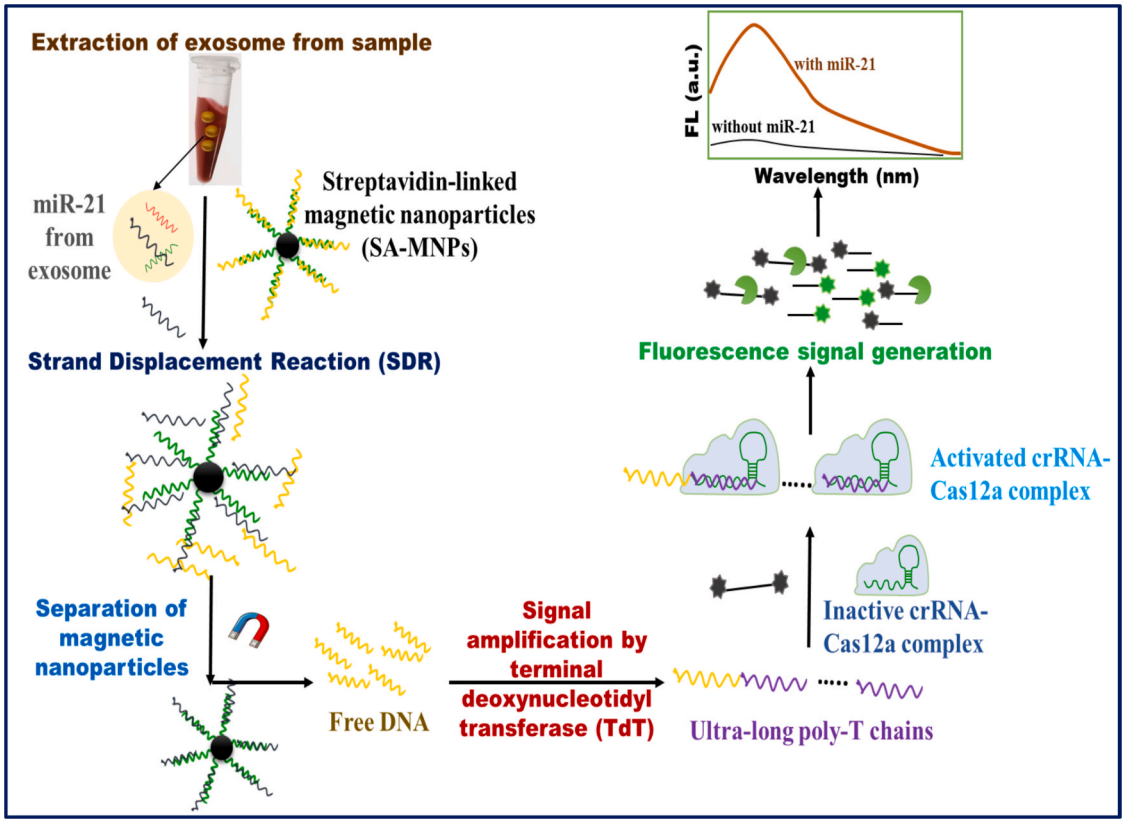


**Fig. 5.** Schematic representation of detection of exosomal miR-21 associated with lung cancer by using SDR and CRISPR-Cas12a based fluorescence biosensor.

production of ultra-long poly-T chains. The poly-T chains get bound with multiple CRISPR RNAs (crRNAs) which are complexed with Cas12a, specifically cleaved the target sequence, resulting in further amplification of signal. Magnetic nanoparticles are used to separate the target product from other interfering substances. The combination of these technologies allowed for the detection of miR-21 through fluorescence signaling with a detection limit of 161 fM. The linearity range as obtained was in between 500 fM to 500 pM. When compared with the gold standard detection method of quantitative reverse transcription polymerase chain reaction (qRT-PCR) the results of the biosensor were found to be statistically significant. Also, the biosensor was able to differentiate between the samples of cancer patients from healthy person hence, providing for a potential lung cancer detection technique.

Specific micro RNAs and messenger RNAs like miR-17, miR-155, TTF-1 mRNA, miR-19b, miR-210 and EGFR mRNA are found to be closely associated with the NSCLC. Detection of these potential biomarkers at early stages would lead to significant improvement of patient outcomes. Sheng et al. (2021) fabricated a reusable highly sensitive electrochemical biosensor for the detection of RNAs specific to NSCLC [72]. The biosensor used a dual amplification strategy by integrating CRISPR-Cas 13a system and catalytic hairpin DNA circuit (CHDC). Activation of CRISPR-Cas 13a system by the target RNA caused cleavage of specific single stranded nucleic acid trigger molecule. This signal is further enhanced by trigger through initiation of CHDC. The biosensor used screen-printed electrode (SPE) with gold working electrode, a platinum (Pt) counter electrode and silver/silver chloride (Ag/AgCl) as a reference electrode. The electrochemical signals are generated through the redox reactions between electrode and methylene blue (MB) and

ferrocene (Fc) redox reporters. This Cas-CHDC-powered electrochemical RNA-sensing technology (COMET) was able to detect minute concentration with a limit of detection (LOD) of 50 aM, specificity of 0.900 and sensitivity of 0.952. The total readout time was only 6 min and only small volume of analyte of 10 μL was used. The biosensor had reusable sensor surface and could perform 37 sequential detections on a single sensor chip hence making it cost-effective. This COMET biosensor offers a highly rapid, sensitive and inexpensive specific approach for the detection of NSCLC specific RNAs.

A novel, ultrasensitive biosensing platform, aptamer-RPA-TMA-Cas13a Assay (ARTCA) was developed by He et al. (2020) for the detection of programmed cell death receptor 1 (PD-L1) protein biomarkers on exosomes [73]. The CRISPR/Cas13-based biosensor captured exosomes by using a mixture of anti-CD63, anti-CD81, and anti-CD9 antibodies bound to beads. The exosomes were obtained from A549 cells. PD-L1 on the exosomes get bound to the specific aptamers, which on elution served as DNA amplification targets. The amplification is achieved twice through isothermal nucleic acid amplification strategies of recombinase polymerase amplification (RPA) along with transcription-mediated amplification (TMA). The amplified RNA transcripts are detected through CRISPR-Cas13a system which at last resulted in generation of fluorescence signal by cleaved quenched fluorescence RNA probes. The fluorescence intensity is directly proportional to the PD-L1 concentration. The LOD of the fabricated biosensing platform was found to be 10 particles/mL and linear range obtained between 10–$10^5$ particles/mL. The study was clinically significant and the biosensor was able to detect PD-L1 level from NSCLC patients' blood samples. This method proves to be a potential non-invasive, sensitive, and rapid method for PD-L1 protein detection in tumor progression. A detailed analysis of some of the other notable CRISPR-based biosensors for the detection of lung cancer is provided in Table 4.

The lung cancer diagnostics faces significant challenges due to the heterogeneity of the disease. The detection of key biomarkers and specific genetic mutations is crucial for improving patient outcomes. Also, the role of ctDNA, miRNAs, and protein biomarkers (such as CEA) are also being explored. For instance, the CRISPR-Cas14a based biosensor developed by Qi et al. (2024) was able to detect minute concentration of ctDNA of 0.34 fM with high accuracy. Conventional diagnostic platforms are often expensive, time-consuming, or have limited sensitivity for early-stage detection. The development of CRISPR-Cas based biosensors have offered significant advantages in detecting critical biomarkers such as, EGFR, hOGG1, PD-L1, and ctDNA with high sensitivity. The CRISPR-biosensors also offers the potential for multiplex detection as reported by Cheng et al. (2023) for hOGG1, FEN1 simultaneous detection; Liu et al. (2022) for ctDNA, EGFR, and L858R mutation; Gong et al. (2022) for miR-205 and miR-944 dual detection. This multiplexing capability is particularly advantageous for providing a more comprehensive assessment of the disease by addressing the associated heterogeneity of lung cancer. The integration of CRISPR technology with advanced biosensors can lead to earlier intervention and improved survival rates of the lung cancer patients.

### 4.2. CRISPR-Cas integrated biosensors for liver cancer detection

According to the 'global cancer statistics 2022′ by GLOBOCAN, liver cancer is ranked third on the basis of mortality and sixth on the basis of incidence with a total of 7,57,948 deaths in the year 2022 [3]. The risk of liver cancer is progressively rising attributing to the increased alcohol abuse, non-alcoholic fatty liver disease, exposure to toxins like aflatoxin, chronic hepatitis B and C infections etc. [17]. Fig. 6 depicts the development of liver cancer on progression from fatty liver to fibrosis, cirrhosis, and ultimately leading to liver cancer. Fatty liver is a reversible stage in which due to the fat deposition in liver cells, oxidative stress and inflammation is triggered. The chronic inflammation from fatty liver leads to fibrosis in which damaged liver tissue is replaced by scar tissue. Extensive scarring can lead to loss of normal liver structure and function characteristic of cirrhosis [80]. This stage is often irreversible and a major risk factor for progression to liver cancer where malignant cells proliferate into nodules. Hepatitis infections can also accelerate this transition.

Early detection is important for increasing patient outcomes, despite conventional detection methods generally lacks sensitivity and specificity for early detection. In the recent times advances in technology have opened new horizons in the field of diagnostics and detection techniques. One such is incorporation of CRISPR-Cas technology with the biosensors to enhance the sensitivity, modularity, accuracy, and efficiency of liver cancer detection [81].

Specific microRNAs (miRNAs) are often considered as one of the crucial biomarkers for the diagnosis and treatment of cancer. Certain specific miRNAs like miRNA-21 are often associated with HCC. A CRISPR-Cas12a based colorimetric biosensor was developed by Luo et al. (2024) for detecting miRNA-21[82]. The detection mechanism involved triggering of strand displacement amplification (SDA) by target miRNA which resulted in the production of numerous DNA promoters. The DNA promoters in turn activated CRISPR-Cas12a system which cleaves ssDNA linkers. These ssDNA linkers were attached with magnetic beads linked Pt@Au nanozymes having peroxidase like activity. The trans-cleavage activity of Cas12a resulted in the release of nanozymes catalysing colorimetric reaction with TMB. The detection platform was able to achieve LOD of 0.5 pM and 10 pM for colorimetric and visual readouts, respectively. A linear detection range of 1–500 pM was obtained for miRNA-21 and the method was found reliable for clinical settings. This method has advantages in the form of visual and colorimetric readouts which facilitate easy result interpretation without the need for sophisticated equipment. miRNA-122 also serves as one of the important biomarkers associated with liver cancer and its detection could serve an important role in the early diagnosis of liver carcinoma. Lv et al. (2023) fabricated a cathodic photoelectrochemical (PEC) biosensor [83]. The biosensor used a NiO photocathode sensitized by a $[(C6)_2Ir(dcbpy)]^+PF_6^-$ complex. On the illumination of photocathode, the sensitizer absorbed light which generated electron-hole pairs. These generated electrons were used in the reduction reactions at the photocathode. $Bi_2S_3$ quantum dots (QDs) were used as an n-type semiconductor and were deposited over the NiO photocathode. In the absence of the target miRNA, QDs quenched the photocurrent by capturing the photoexcited electrons. The biosensor also utilized the trans-cleavage activity of CRISPR-Cas12a and hairpin DNA probe with both miRNA-specific sequence and a DNA activator. Hence, in the presence of target miRNA, hairpin probe gets exposed and activated the CRISPR system which resulted in the removal of QDs from the photocathode. Due to the cleaved QDs, photocurrent is increased in proportionality to the concentration of miRNA. The biosensor was able to achieve a LOD of 36 aM and linearity range of 0.1 fM to 10 nM. The biosensor uses photoelectrochemical sensing technology which has low background noise, hence enhanced detection performance. However; the use of multiple components and processes can make the designing process complex and costly. Although, the sensor shows satisfying stability and selectivity but its performance might be sensitive to the environmental conditions such as light intensity and temperature.

An important protein biomarker glypican-3 (GPC3), overexpressed in HCC was targeted by Xiao et al. (2024). The researchers fabricated a CRISPR-Cas13a based immunosensor for the direct detection of GPC3 on extracellular vesicles (EVs) [84]. The detection mechanism involved capturing of EVs from the samples by using mixture of anti-CD63, CD9, and CD81 antibody conjugated magnetic beads. Anti-GPC3 antibody linked to ssDNA formed complex on binding with captured EVs. The complex was amplified using recombinase polymerase amplification. At last, CRISPR-Cas13a was activated generating a detectable fluorescent signal. This method was highly sensitive owing to the detection of minute concentration of 10 particles/mL and doesn't require any specialized equipment hence, would be suitable for regular clinical

**Table 4**
Detailed analysis of CRISPR-Cas integrated biosensors for the detection of lung cancer.

| Type of biosensor | CRISPR-Cas System type | Detection mechanism | Type of electrode used | Target analyte | Type of lung cancer | Limit of detection (LOD) | Sample investigated | References |
|---|---|---|---|---|---|---|---|---|
| Fluorescence biosensor | CRISPR-Cas12a System | CRISPR-Cas12a System combined with self-recruiting crRNA and Rolling Circle Transcription (RCT) | – | hOGG1 (human 8-oxo-guanine DNA glycosylase)FEN1 (flap endonuclease 1) | NSCLC | hOGG1: 0.0013 U/mLFEN1: 0.0052 U/mL | Human serum and A549 cells | [74] |
| Dual-mode biosensor (fluorescent and electrochemical) | CRISPR/Cas12a | Combination of rolling-circle strand displacement amplification (RC-SDA) and CRISPR/Cas12a | Electrochemical probe with T-rich sequence and 5′ methylene blue modification | EGFR 19del mutation | NSCLC | Fluorescent Biosensor: 18.62 fM. Electrochemical Biosensor: 0.13 fM. | Various cells with wild-type and mutant *EGFR* genes | [59] |
| Fluorescent Biosensor | CRISPR/Cas12a | Metal-Organic Frameworks (MOF) (UiO-66-NH2) loaded with Cy5 fluorescein enhances fluorescence signal upon target ctDNA binding. | – | Circulating Tumor DNA (ctDNA) | Lung Adenocarcinoma | 5.6 fM | Clinical Serum Samples | [75] |
| Colorimetric Biosensor | CRISPR/Cas12a | AND Logic-Gate design, triggering CRISPR-Cas12a to cleave ssDNA on magnetic beads, causing glucose oxidase (GOx) separation and a detectable color change. | – | miR-205 and miR-944 | – | 36.4 fM | Human Serum Samples | [76] |
| Electrochemical biosensor | CRISPR/Cas12a | Electrochemical signal measured based on the binding of methyleneblue to the nanocomposite (MB/$Fe_3O_4$@COF/PdAu) | – | ctDNA EGFR L858R mutation | NSCLC | 3.3 aM | Plasma samples from *NSCLC* patients | [77] |
| Electrochemical Biosensor | CRISPR-Cas12a | Trans-cleavage activity of CRISPR-Cas12a system results in cleavage of MB-aptamer DNA and decrease in the electrochemical signal. | Gold Nanoparticle (AuNP) based electrode functionalized with ultra-thin 2D Covalent Organic Framework (COF-367) Nanosheets | PD-L1 Positive (PD-L1 + ) Exosomes | NSCLC | 38 particles/μL | Clinical serum samples | [78] |
| Aptasensor | CRISPR/Cas12a | CD63 aptamer recognition and signal amplification by CRISPR/Cas12a | – | Exosomes | – | $3 \times 10^3$–$6 \times 10^7$ particles/μL | Clinical Samples | [79] |

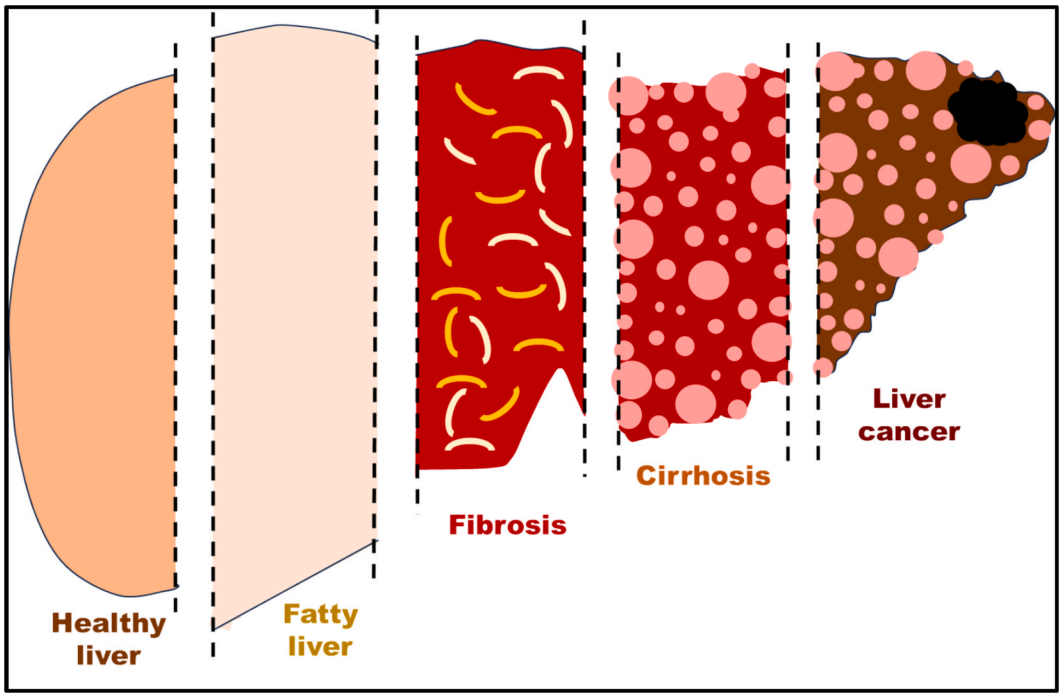


**Fig. 6.** Diagrammatic representation of development of liver cancer through subsequent stages.

diagnostics.

Alpha-fetoprotein (AFP) is also considered as one of the most common serological biomarkers for the hepatocellular-carcinoma (HCC). AFP is normally produced in embryonic stages and its level remain low throughout the lifespan in healthy cells [85]. However, overexpression of AFP in adults is commonly associated with HCC. Hence, various studies have been carried out for the successful early detection of AFP by using CRISPR-based biosensing platforms. In a study, Jia et al. (2023) reported the optimization of a CRISPR-powered personal glucose meter (PGM) biosensor for the detection of AFP [86]. They utilized the collateral ssDNA cleavage activity of CRISPR-Cas12a along with the high affinity aptamers specific to AFP. The binding of AFP with the aptamers resulted in the activation of CRISPR-Cas12a system. Once activated, collateral cleavage activity resulted in the release of enzyme invertase, which catalysed the conversion of sucrose to glucose. The produced glucose was then quantified by using PGM, here the glucose level correlated with the amount of AFP present in the sample. The detailed mechanism of detection is depicted in Fig. 7. The LOD obtained was 10

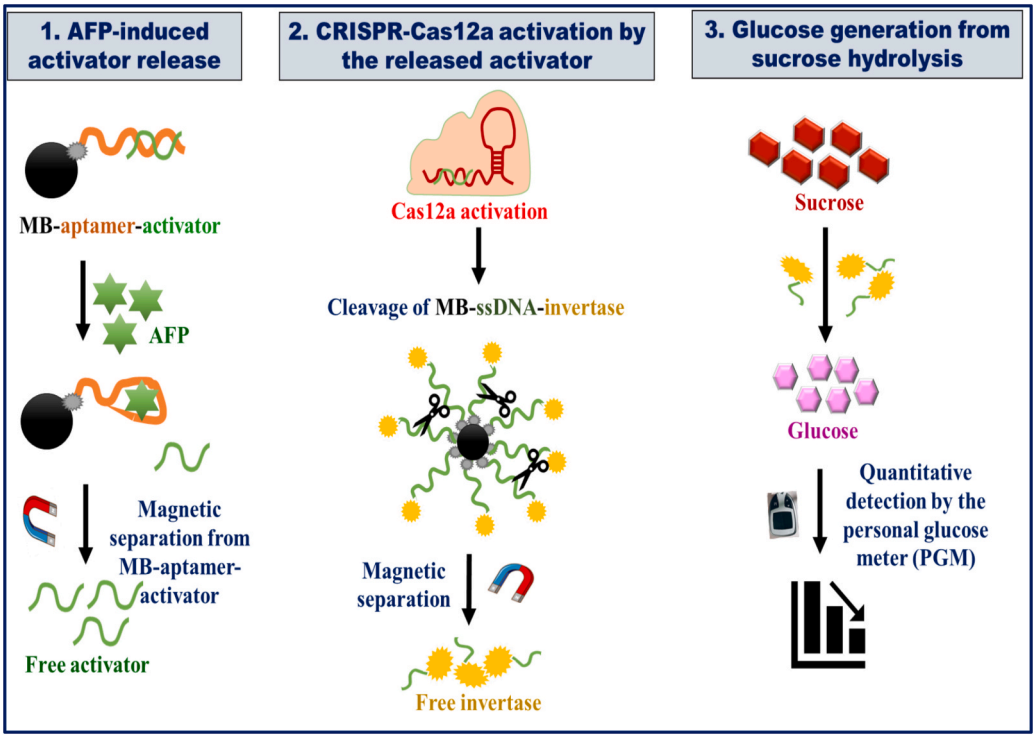


**Fig. 7.** Diagrammatic illustration of quantitative detection of AFP by using CRISPR-Cas12a and personal glucose meter based biosensing platform.

ng/mL and linearity range of 100 μg/mL to 0.01 μg/mL. The biosensor was simple, cost-effective, with a detection time of 60 min. Although, the biosensor is highly sensitive, specific, portable, and provide quantitative results but there is a need to improve the detection operation, detection time and multiplexing capabilities.

In other study, Liu et al. (2022) fabricated a magnetic bead separation platform combined with CRISPR-Cas12a biosensor for the identification of AFP in clinical samples from hepatoblastoma patients [87]. The developed biosensor involved binding of AFP with the specific aptamers, that triggered hybridization chain reaction (HCR), which when detected by CRISPR-Cas12a sensor got amplified. The amplified signal then produced a measurable fluorescence output. The HCR-CRISPR-Cas12a were regulated by functionalized magnetic nanoparticles. The biosensor was more sensitive than traditional ELISA with a limit of detection of 0.170 ng/mL and was able to detect AFP within the concentration range of 0.5–$10^4$ ng/mL. However, preparation and functionalization of magnetic beads and subsequent detection steps may be more complex as compared to the simple immunoassays. But still this method offers a sensitive, specific, and cost-effective alternative to ELISA for use in clinical diagnostics. Similarly, Zhao et al. (2021) developed a fluorescence biosensing platform based on CRISPR-Cas12a for the diagnosis of AFP in serum samples [88]. This biosensor had multiplexing capabilities and they used protein- AFP and small molecule- drug cocaine as the target analytes. The biosensor used aptamers specific to the analytes, the binding of target analyte with the aptamers activated the CRISPR-Cas12a system. The activation of cleavage activity of Cas12a resulted in generation of fluorescence signal due to the separation of fluorophore from the quencher. The aptasensor was versatile with a simple biosensing platform and was able to detect AFP and cocaine with a detection limit of 0.07 fmol/L and 0.34 μmol/L respectively. The linearity ranges for AFP was 0.24–977 fmol/L and for cocaine was 0.5–15 μmol/L. This detection was cost-effective and rapid with a detection time of only 18 min. This detection platform is highly useful for real-life settings owing to its potential for plug-and-play design, high modularity, and robustness. Potential limitations include the need for optimization for different analytes and challenges in the translation of technology to a commercial setting. Another application ability of the detection of miRNA-17 was tested by Zhou et al. (2020) with an electrochemiluminescence (ECL) chip based CRISPR/Cas13a biosensor[89]. In this study, target miRNA activated the Cas13a which resulted in cleavage of pretrigger (PT) into a mature trigger (MT), initiating exponential amplification (EXPAR). The EXPAR produced large amounts of dsDNA which on interaction with $[Ru(phen)_2dppz]^{2+}$ produced a luminescent signal which was detected by ECL platform. The biosensor was able to detect minute concentration of $1 \times 10^{-15}$ M of miRNA-17 from various human tumor cells including breast carcinoma cell lines (MDA-MB-231 and MCF-7) and human HCC cell line (HepG2). The developed biosensor can be a valuable platform for point-of-care cancer diagnosis in clinical settings.

Hepatitis infection is also considered as one of the important risk factors for HCC, Table 5 provides a comparative analysis of some of the biosensors developed in the recent times for the hepatitis viral detection.

Liver cancer is one of the leading causes of cancer-related deaths in the world. The poor prognosis of liver cancer can be attributed to late-stage diagnosis as early symptoms are generally absent or non-specific. Moreover, commonly used biomarkers like AFP, ctDNA, and other RNA biomarkers exist in very low concentrations in complex biological matrices, making their detection challenging. The traditional diagnostics methods face various challenges in detecting early-stage tumors which can be addressed by the introduction of CRISPR-based biosensors. These advanced biosensing techniques enable the detection of liver cancer biomarkers with high sensitivity and specificity. The CRISPR-biosensors offer unparallel precision in identifying tumor-specific genetic and epigenetic changes with high accuracy and provide rapid results.

### 4.3. CRISPR-Cas integrated biosensors for colorectal cancer detection

Colorectal cancer (CRC) has been ranked second on the basis of mortality and is third most prevalent carcinoma [97]. A total of 9,03,859 deaths and 1,926,118 new cases were reported of CRC according to the global cancer statistics 2022 by International Agency for Research on Cancer (IARC) [3]. CRC development is often attributed to a variety of risk factors such as, alcohol consumption, diet, genetics, exposure to toxins, age etc. [98]. Fig. 8 depicts the progression of colorectal cancer through successive stages. CRC progresses through various cellular and molecular stages namely, stage 1, 2, 3, and 4 [99].

A significant number of CRC cases are often diagnosed at later stages significantly hampering patient outcomes and decreasing survivability. The current diagnostic methods, including colposcopy and stool-based tests are generally invasive, costly, lack the sensitivity to detect early-stage CRC, and require significant patient preparation, which leads to low compliance rates. CRC exhibits various genetic and epigenetic alterations such as, aberrant DNA methylation, miRNA expression, and mutations. Detection of these biomarkers in a non-invasive and reliable manner is the need of hour. CRISPR-Cas based biosensing platforms offer a significant potential for early, sensitive, multiplexed, rapid, and point-of-care detection of CRC biomarkers. Detection of DNA methylation at specific sites can be a promising approach for the detection of CRC. This was exploited by Ding et al. (2024) in designing a ratiometric fluorescence biosensor for the detection of CRC patients [100]. This biosensor was able to differentiate between CRC, precancerous tissue as well as healthy people. Methylation-sensitive restriction enzyme (MSRE) was used to distinguish between methylated and non-methylated DNA targets. The process further involved recognition of target sequence by Cas12a guided by crRNA and exhibition of trans-cleavage activity. The activators generated by Cas12a triggered catalytic hairpin assembly reaction which caused structural changes in the hairpin probes. This reaction at last led to generation of a ratiometric fluorescence signal with enhanced sensitivity and specificity. The fabricated biosensor was able to detect even minute concentration of target sequence of 2.02 fM. Overall, while this method offers high sensitivity and specificity with significant potential for clinical applications, it also poses challenges in terms of complexity, time consumption, and cost-effectiveness. In another study by Li et al. (2024), a CRISPR-Cas12a based platform integrated with multiplex amplification and nanoparticle-stabilized fluorescence was developed for the improved detection of DNA methylation biomarkers [101]. The biosensor utilized cost-effective mesoporous silica nanoparticles. Oligo reporters are electrostatically co-assembled with oppositely charged nanoparticles, enhancing stability and fluorescence efficiency. The proximity of nucleic acids on nanoparticles further increases the catalytic efficiency and signal intensity. The detection principle involved Cas 12a collateral cleavage activity, producing fluorescence signal. The biosensor was validated in a CRC model and was able to detect DNA methylation in the atto molar levels within 40–60 min. The biosensor also had multiplexing capabilities with improved diagnostic accuracy and screening efficiency.

Chen et al. (2023) developed a one-pot detection platform for CRC specific non-coding RNAs (ncRNAs) like piRNA-54265 and miRNA21 [102]. The biosensor used a combination of CRISPR-Cas12a cleavage activity along with branched rolling circle amplification (BRCA) as shown in Fig. 9. BRCA utilized primers specific to ncRNA sequences to hybridize with circular probes generating dendritic DNA products. These dendritic DNA products in turn activated the trans cleavage activity of Cas12a resulting in fluorescent signal generation. A detection limit of 0.76 fM was obtained for piRNA-54265 and 0.87 fM for miRNA21. Although, the biosensor was able to simplify the detection process by combining all the steps into a single reaction however; the involvement of multiple steps in one-pot may increase the risk of contamination that can affect the accuracy of results. Despite that, it is a promising tool for rapid and practical diagnostic and prognostic screening of CRC in clinical settings.

**Table 5**
Comparative analysis of some of the CRISPR integrated biosensors for the detection of hepatitis infection associated with liver cancer.

| Type of biosensor | CRISPR-Cas System | Detection mechanism | Type of electrode used | Nanomaterial used | Detection target | LOD | Linearity range | Response time (Minutes) | Sample investigated | References |
|---|---|---|---|---|---|---|---|---|---|---|
| Fluorescence biosensor | CRISPR/Cas12a | Rolling Circle Amplification (RCA); CRISPR/Cas12a Activation; high-dimensional hybridization chain reaction (HCR). | – | – | Hepatitis B Virus (HBV)-DNA | 1.502 pM | – | – | – | [90] |
| Fluorescence biosensor | CRISPR-Cas12a | Cas12a's *trans*-cleavage activity | – | Gold nanoclusters (AuNCs), silver nanoclusters (AgNCs), and copper nanoclusters (CuNCs) | HBV- DNA | – | – | 25 min | Human serum samples | [91] |
| Colorimetric biosensor | CRISPR-Cas12a | CRISPR-Cas12a cleavage and DNA Metallization | – | Ag/Pt nanoparticles modified-$Ti_3C_2T_x$ Mxene nanostructures | HBV DNA | Subpicomolar levels | – | – | Human serum samples | [92] |
| Surface-Enhanced Raman Spectroscopy (SERS) based biosensor | CRISPR/Cas12a | CRISPR/Cas12a cleavage and SERS | – | Gold nanoparticles (AuNPs) | HBV DNA | 0.1 pM | 0.1 pM to 1 nM | 50 min | HBV DNA from clinical samples | [93] |
| Electrochemiluminescence (ECL) biosensor | CRISPR/Cas12a | Hybridization Chain Reaction (HCR); CRISPR/Cas12a activation; ECL Signal Modulation; | – | Ruthenium Bipyridine-doped Silica Nanoparticles (Ru@SiO2 NPs) | HBV DNA | 7.41 fM | 10 fM to 10 nM | – | Clinical samples | [94] |
| Indium Tin Oxide-based Extended Gate Field-Effect Transistor (ITO EG-FET) biosensor | CRISPR/Cas12a | Loop-Mediated Isothermal Amplification (LAMP) and CRISPR/Cas12a Cleavage | Indium Tin Oxide (ITO) electrodes | Indium Tin Oxide (ITO) | Hepatitis C Virus (HCV) RNA | 1 genomic copy per reaction | – | 20 min | – | [95] |
| Lateral Flow Test Strip | CRISPR/Cas12a | LAMP Amplification; CRISPR/Cas12a Activation | – | – | HBV DNA | 1 copy/μL | – | Fluorescence Readout: 13 min. Lateral Flow Test Strip: 20 min. | Serum samples | [96] |

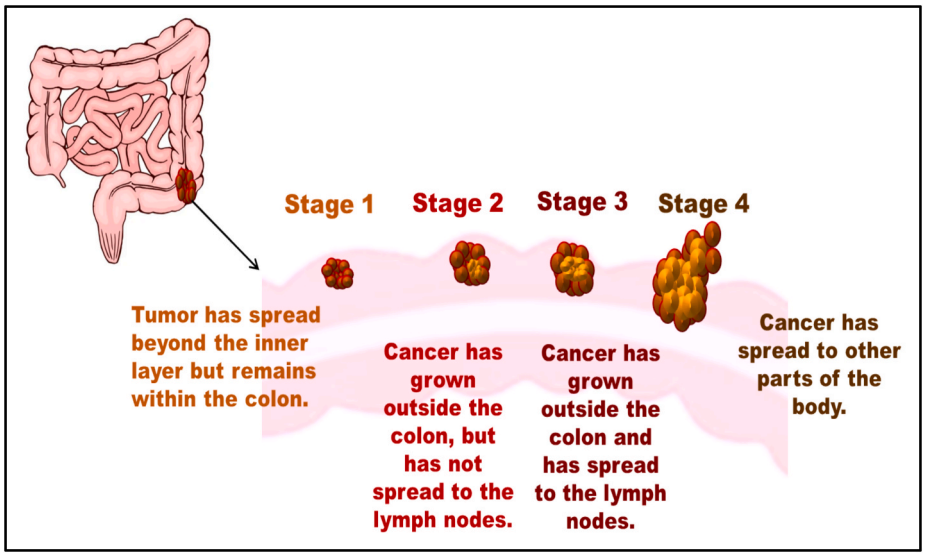


**Fig. 8.** Diagrammatic illustration of progression of colorectal cancer.

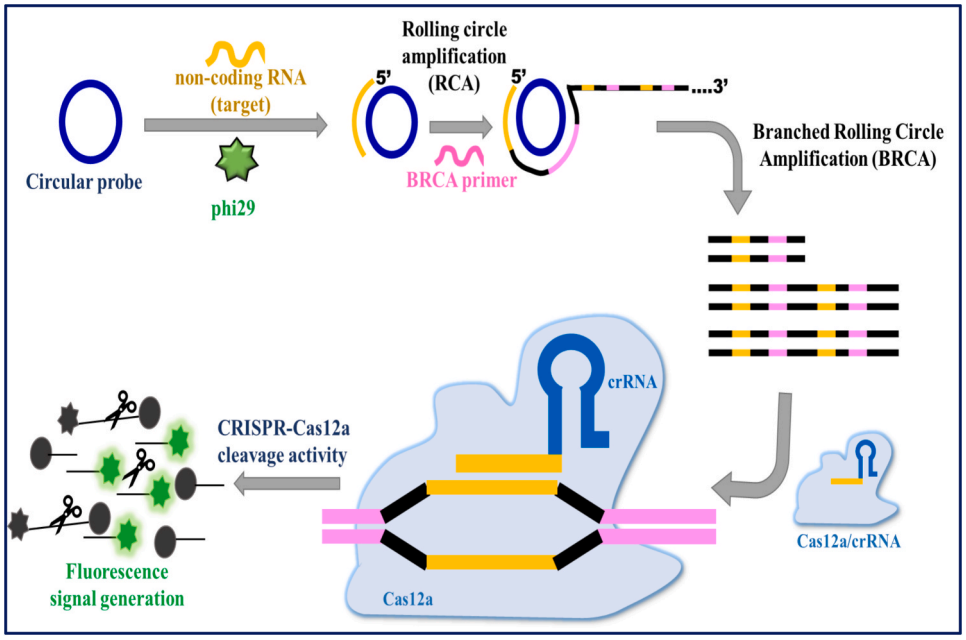


**Fig. 9.** Schematic illustration of detection of colorectal cancer specific ncRNAs by using a CRISPR-Cas12a based fluorescence biosensor.

These reported CRISPR-Cas-based biosensors have the potential to revolutionize CRC early diagnosis by providing reliable, cost-effective and non-invasive platform to enhance the survival rate of patient.

### 4.4. *CRISPR-Cas integrated biosensors for prostate cancer detection*

Prostate cancer often remains asymptomatic in the early-stages and there is limited accuracy of current biomarkers. It has been projected in various studies that the number of prostate cancer cases will increase from 1.4 million in 2020 to 2.9 million by 2040 [103]. Prostate cancer commonly affects men with ages 45–60 and the risk is more common in low- and middle-income countries where access to healthcare resources is limited [104]. Confirmative diagnosis of prostate cancer usually requires invasive transrectal ultrasound-guided prostate biopsies, which are discomforting and poses risk of infections. Hence, blood-based or urine-based detection strategies are needed to improve patient compliance. Prostate cancer develops through a series of stages involving molecular, cellular, and structural changes in prostate tissue. Fig. 10 depicts progression of prostate cancer.

In the stage of benign prostate as depicted in Fig. 10, tissue is healthy and composed of glandular and stromal elements. Proliferative inflammatory atrophy (PIA) is characterized by presence of small, atrophic glands, with persistent inflammation. After PIA, prostatic intraepithelial neoplasia (PIN) stage follows which includes presence of cytological abnormalities and nuclear enlargement. At the last stage, malignant prostate epithelial cells breach the basement membrane and invade surrounding stromal tissue, representing prostate cancer [105]. There are various conventional diagnostic methods available for prostate cancer detection such as, prostate-specific antigen (PSA) testing, digital rectal examination [106]. These conventional techniques although widely opted, often lack the necessary sensitivity and specificity which leads to false positives and negatives [107]. Hence, to overcome the highlighted limitations, CRISPR-Cas based biosensors are serve as an effective solution for early, rapid, and on-site diagnostic tool for the

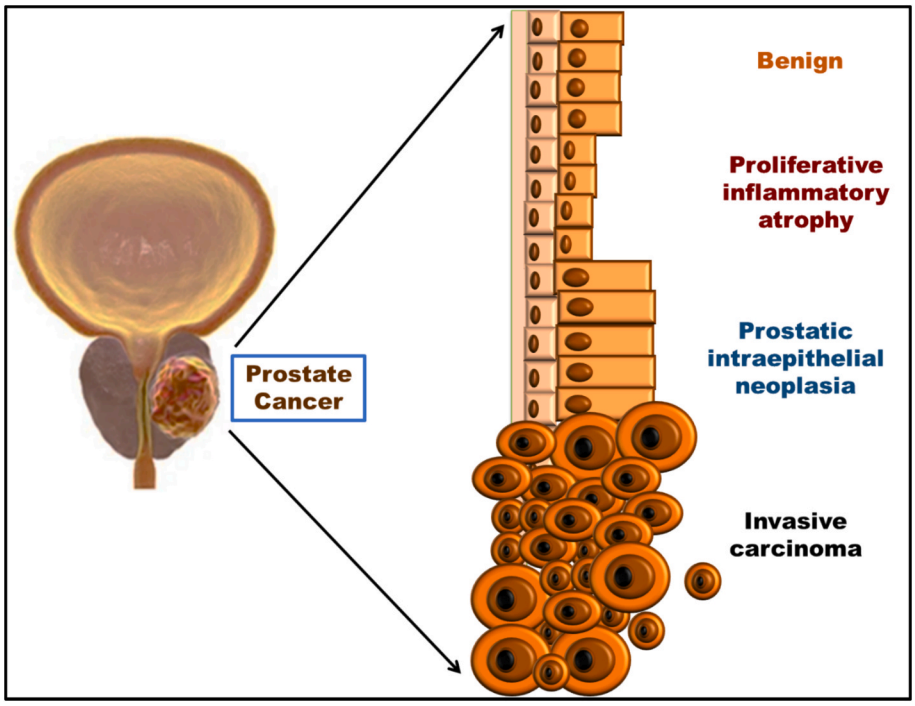


**Fig. 10.** Diagrammatic representation of prostate cancer progression by depicting its different stages.

detection of prostate cancer. Elevated levels of PSA are commonly associated with prostate cancer and various CRISPR-based biosensors are being fabricated for its detection. For instance, a ratiometric fluorescent immunosensor was developed by Ling et al. (2023) for the specific and early detection of PSA for increasing the survivability outcomes of the patient [108]. This immunosensor utilized CRISPR-Cas12a

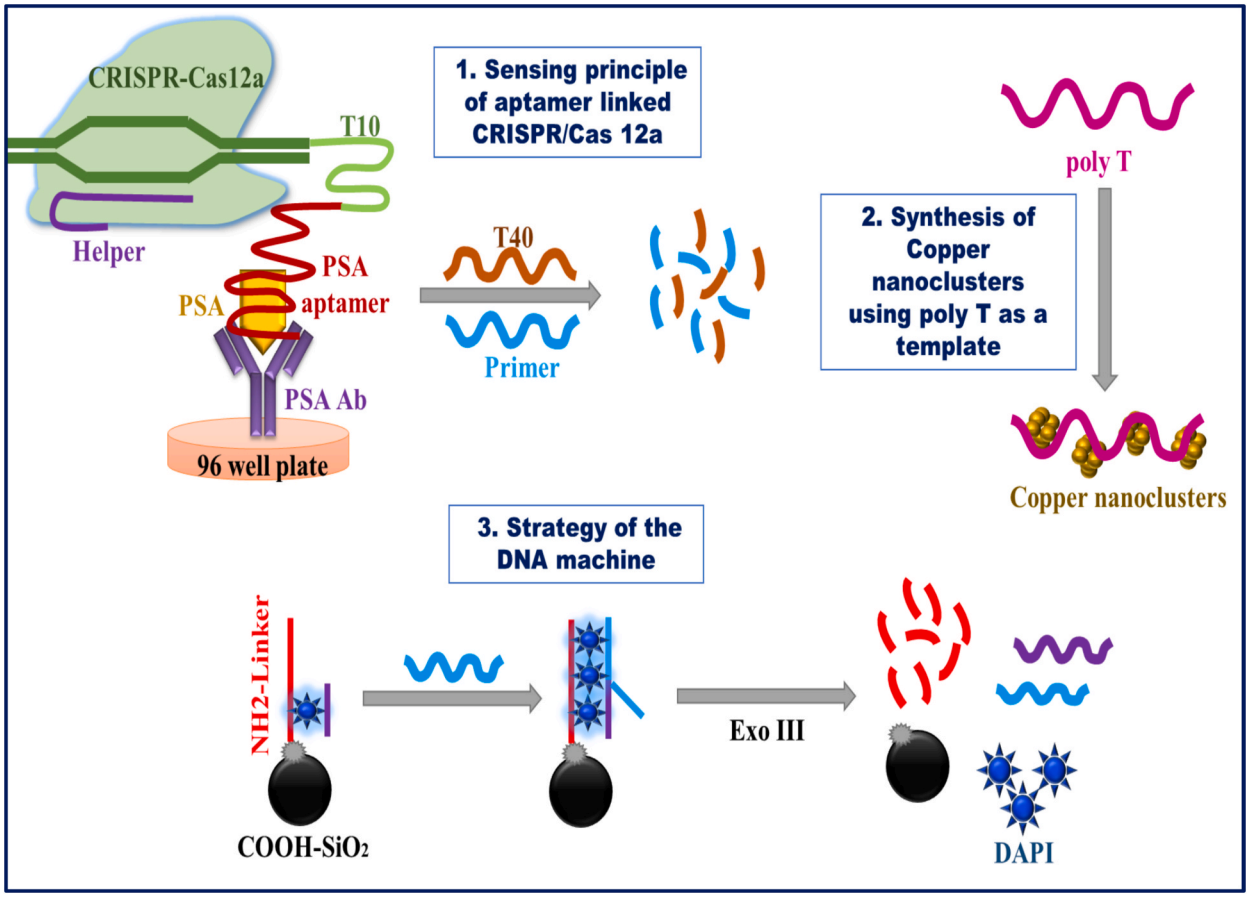


**Fig. 11.** Diagrammatic representation of PSA detection by using a CRISPR-Cas12a and 3D DNA walker mechanism based biosensing platform.

system as well as a 3D DNA walker mechanism as depicted in Fig. 11. The aptamer-linked activator DNA after binding with PSA underwent conformational change that activated the CRISPR-Cas12a and resulted in cleavage of oligonucleotides. The cleavage resulted in the quenching of fluorescence from copper nanoclusters (Cu NCs) and halted amplification of DNA walker. The 3D DNA walker mechanism was powered by exonuclease III (Exo III) activity to produce ssDNA from dsDNA. The immunosensor contained silica nanospheres loaded with dsDNA to track the emitted fluorescence signals on the movement of DNA walker. The accurate detection by the biosensor relied on the generation of two types of fluorescence signals, one is generated on the cleavage by poly (thymine)-templated Cu NCs which is quenched on cleavage by CRISPR-Cas12a and the other is produced by DAPI (4′,6-diamidino-2-phenylindole). The ratio of both the fluorescence signals gave a reliable readout and the specific change in the fluorescence ratio was used to determine the presence and concentration of PSA. The detection limit obtained was 0.5 ng/L and linear range was obtained between 2.0–200 ng/L. The biosensor was highly sensitive and showed negligible interference to other analytes. It was able to show the recovery range between 95.93 %-110.95 % when checked for the applicability in real sample of spiked 10-fold diluted healthy human serum samples. This fabricated immunosensor holds advantage in providing a label-free detection platform. Hence, reducing the need of antibodies, simplifying the procedures, reducing the cost, and simultaneously enhancing the sensitivity and reliability of PSA detection.

Similarly, diagnosis of non-nucleic acid targets such as PSA and estradiol (E2) was carried out by a fluorescence/colorimetric dual biosensor based on CRISPR-Cas12a by Su et al. (2023) [109]. The non-nucleic acid targets were first converted into DNA signals by using specific aptamers, and then amplified by catalysed hairpin assembly (CHA). This amplified dsDNA then activated the CRISPR-Cas12a system. The fluorescence detection was carried out by using up conversion nanoparticles (UCNPs) as an alternative fluorophore to enhance fluorescence stability and decrease background interferences. A DNA hydrogel-encapsulated Metal-Organic Framework (MOF) biomimetic enzyme is used for colorometric detection. The CRISPR-Cas12a induced controlled lysis of the DNA hydrogel-coated MOF, which resulted in the release of MOF-catalyzed hydrogen peroxide for color development. The detection limit observed was 0.015 ng/mL for E2 and 0.14 ng/mL for PSA. The percentage analytical recovery obtained for PSA in serum was 84.12 %− 116.01 %. This method uses CHA for effective signal amplification without the need of enzymes, hence significantly reducing the cost. Also, this biosensor hold potential for diagnosis of multiple targets by using different aptamers and nanomaterials.

Other than the PSA, miR-141, miR-375 and miR-21 have been investigated as the biomarkers for prostate cancer. SMARTLOCK (specific multifunctional DNA tetrahedron nanostructure unlocking), a versatile biosensing diagnostic tool was developed by Zhu et al. (2023) for the detection of these prostate cancer specific RNAs [110]. The biosensor used a non-linear hybridization chain reaction (HCR) for the activation of CRISPR-Cas12a crRNA system. Efficient binding of the HCR with the Cas12a complex was ensured by connecting with DNA tetrahedron. This binding resulted in the activation of Cas12a and generation of a fluorescence signal. The multifunctional characteristic of this biosensor was attributed to its different lock configurations, i.e. double-safety dumbbell lock and triple-safety dumbbell lock for dual-target and triple-target detection respectively. To check the clinical utility of the biosensor in prostate cancer diagnostics, miR-141 and miR-375 was detected in human serum from healthy person, prostate cancer stage I-II and III-IV patients. SMARTLOCK showed a high sensitivity of 95 % and accuracy of 92.5 %. It was highly specific, with specificity observed of 93.3 %. Along with the technical advantages, SMARTLOCK was highly cost-effective with the total cost of performing each test to be $ 0.35. Along with the prostate cancer specific RNAs, it was able to detect ATP, cortisol, and insulin thus enhancing its multiplexing capabilities and increasing utility in detection of other life-threatening diseases as well.

**Table 6**
Detailed analysis of some of the CRISPR integrated biosensors for the detection of prostate cancer.

| Type of biosensor | CRISPR-Cas System | Detection techniques used | Type of electrode used | Nanomaterial used | Detection target | LOD | Linearity range | Response time | Sample investigated | References |
|---|---|---|---|---|---|---|---|---|---|---|
| Optical biosensor | CRISPR/Cas12a | Dual signal amplification: DNA signalling network and strand displacement amplification (SDA) | – | – | miR-141 | 34 aM | 100 aM – 1 pM | – | Human serum samples | [111] |
| Colorimetric biosensor | CRISPR/Cas12a | Hybridization chain reaction (HCR) Process | – | Gold nanoparticles (AuNPs) | PSA | 0.1 ng/mL | – | – | Serum samples, including spiked and clinical samples | [112] |
| Fluorescence aptasensor | CRISPR-Cas12a | Formation of DNA cruciform structure | – | – | PSA | 4 pg/mL | – | – | Serum samples | [113] |
| Photoelectrochemical biosensor | CRISPR/Cas13a | Direct RNA detection and photoelectrochemical Output | – | – | miRNA-21 | 1 fM | 1 fM to 5 nM | – | Human serum samples and cell lysates | [114] |
| Paper based test strip | CRISPR-Cas9 | Reverse transcriptase-recombinase aided amplification (RT-RAA) | – | Gold nanoparticles (AuNPs) | Prostate cancer antigen 3 (PCA3) and kallikrein related peptidase 3 (KLK3) genes | PCA3- 500 fg/μL and KLK3-50 fg/μL | – | – | Urine and peripheral blood specimens | [115] |

Table 6 provides a detailed description of some of the other CRISPR-Cas-based biosensing platforms reported for the detection of prostate cancer.

Aforementioned CRISPR-based biosensors can accurately detect PSA, PCA3, and other genetic alterations in biological samples. These biosensors enable non-invasive, multiplexed, and rapid detection of prostate cancer specific biomarkers. CRISPR-biosensors hence, have potential to serve as point-of-care devices in the clinical settings and reduce the burden on healthcare systems.

### 4.5. CRISPR-Cas integrated biosensors for cervical cancer detection

Cervical cancer is ranked fourth most prevalent cancer among with 6,04,000 new reported cases and 3,42,000 deaths in the year 2020 [116]. Human papillomavirus (HPV) infection to the cervical cells has lead to genomic instability and mutation, consequently transform the normal cells to neoplastic cells leading to cervical cancer [117]. Usually, high risk type HPVs are associated with almost 100 % cervical cancer cases [118]. The disease progression can involve various stages such as HPV infection, koilocytosis, Cervical Intraepithelial Neoplasia (CIN) 1, CIN 2, CIN 3 and at last invasive carcinoma. Koilocytosis indicates active HPV infection and the cellular changes are characterized by enlarged nuclei, perinuclear halos, and irregular nuclear borders. Further pre-cancerous changes in the cervical epithelium are classified into three grades (CIN 1, 2, and 3) based on increasing severity of disease. At last, cancer cells invade through basement membrane and metastasize to distant organs [119]. Fig. 12 further describes these various steps involved in cervical cancer progression.

Cervical cancer is easily preventable if diagnosed at early stages. Despite significant advancements in prevention through vaccines; early and precise detection of cervical cancer remains essential for effective intervention, particularly in low resource settings. Routine screening is required to detect cervical cancer in initial stages. The conventional detection strategies are less-sensitive, less-specific, and invasive causing lot of discomfort to the patients. Hence, the CRISPR-based biosensor provides for a promising point of care device to detect the HPV infection at early stage. For instance, Park et al. (2024) constructed a colorimetric biosensor based on CRISPR-Cas12a with a potential to be used as a POC device for sensitive detection of HPV-16 and HPV-18 in human serum [120]. The biosensor involved use of gold-coated iron oxide ($Au@Fe_3O_4$) nanohybrids (NHs) to bind with ssDNA. These ssDNAs were modified with biotin and thiol groups to facilitate binding with AuNHs and were linked with HRP (Horseradish Peroxidase) for signal amplification. With the binding of viral DNA, CRISPR-Cas12a system got activated and caused trans-cleavage reaction which led to release of multiple HRPs. The presence of HRP in the supernatant catalyzed the oxidation of TMB (3,3′,5,5′-Tetramethylbenzidine), which resulted in color change. This one-pot system was able to visually indicate the presence of target HPV DNA with a LOD of 0.25 nM and linear range from 256 nM to 250 pM. The use of AuNHs facilitated easy magnetic separation which further simplified the detection process. This colorimetric biosensor guarantees ease of use and enhanced sensitivity, hence demonstrating its potential to be used in cervical cancer diagnostics and public health.

In another study Bao et al. (2023) combined polydimethylsiloxane (PDMS) micropillars and CRISPR-Cas12a technique for the sensitive detection of HPV nucleic acid targets [121]. The study involved use of quantum dots (QDs) as FRET donor and ssDNA with lowa RQ linkers as acceptor. When target HPV DNA got bound with Cas12a it resulted in cleavage of the neighboring ssDNA molecules and release of quencher. The emitted fluorescence from the QDs indicated the presence of target DNA. The use of micropillars had enhanced the sensitivity of the biosensor and the micropillars with a pitch distance of 109 μm were able to detect HPV with a LOD of 0.1 ng/ μL. The use of CRISPR-Cas12a system ensured less possibility of false positive results as only specific target DNA can activate the fluorescence signal. Overall, this study provides a novel approach of using micropillars for sensitive detection of HPV DNA and paves the way for integrating the detection mechanism in a microfluidic device. A simple, stand alone, and automatic microfluidic biosensor based on CRISPR-Cas12a and recombinase polymerase amplification (RPA) was developed by Zhou et al. (2023). The biosensor was capable of detection of HPV subtypes 16 and 18 with a detection sensitivity of 0.5 nM and $1 \times 10^{-18}$ M for unamplified and amplified plasmids respectively [122]. RPA was used for isothermal amplification of target nucleic acids at constant temperature. The amplified targets were then detected by using guide RNA through CRISPR-Cas12a system. The sample detected were crude DNA extracts from human cervical swab samples. This low cost and portable platform allowed simultaneous detection of multiple samples within 30 min by using fluorescence and lateral flow dipstick (LFD) readouts. Both the signal readouts methods were able to discriminate HPV (16 and 18) positive and

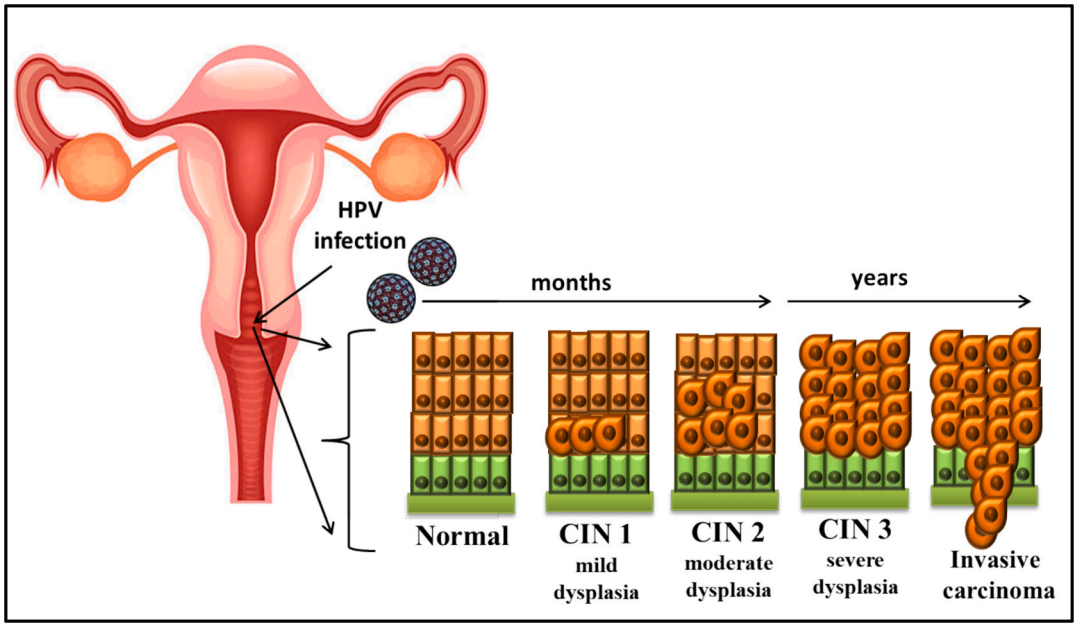


**Fig. 12.** Diagrammatic illustration of progression of cervical cancer through a series of stages.

negative samples. This highly sensitive platform is highly advantageous for rapid and on-site detection of HPV in resource limiting settings.

A research by Su et al. (2023), presented a novel detection method for HPV DNA detection by combining CRISPR/dCas9 technology with surface-enhanced Raman scattering (SERS) and enzyme-catalyzed signal amplification [123]. The biosensor used CRISPR/dCas9/sgRNA complex anchored on magnetic bead to capture target biotinylated HPV DNA sequences. Streptavidin-modified horseradish peroxidase (HRP) bridged to the magnetic bead then catalysed the reaction with its substrate 3,3′,5,5′-tetramethylbenzidine (TMB) resulted in production of a measurable product. The measurement of SERS spectra of the oxidative product of TMB was carried out by using gold nanostars with a silica shell. The combination of enzyme catalysis and SERS significantly enhanced the sensitivity and specificity of detection. This highly sensitive and rapid detection method has potential for clinical applications in providing early diagnosis of cervical cancer. This biosensor has also provided adaptability for different DNA targets by changing the sgRNA sequence. However, the integration of multiple technologies may increase the complexity and cost of the assay.

In a different study by Ganbaatar et al. (2022), the effect of extending 5′ and of the crRNA on CRISPR detection was studied and a biosensing technique named 5′ eNd EXTension CRISPR ("NEXT CRISPR") was developed [124]. The biosensing platform uses CRISPR-Cas12a system with 5' end extended crRNA and incorporated RPA for target nucleic acid amplification. The detection of HPV 16 from clinical swab samples was carried out with high sensitivity in atto molar ranges and within a short time of 30 min. The detailed detection mechanism is explained in Fig. 13. This nucleic acid biosensor utilized fluorescence or lateral flow strips-based detection and was able to achieve comparable performance to PCR-based methods. Some of the other reported noteworthy CRISPR-based biosensors for the detection of cervical cancer are explained in Table 7.

Hence, CRISPR-Cas-based biosensors with their unique sensitivity, specificity and flexibility are emerging as revolutionary tools in cervical cancer diagnostics. Multiplexing capabilities of these novel biosensors have been used for the simultaneous detection of HPV types 16 and 18 which allow a comprehensive diagnostic profiling. Moreover, CRISPR-biosensors can be used to detect other novel biomarkers such as DNMT1 this can lead to improving specificity for identifying the risk of cervical cancer progression. These biosensors offer non-invasive analysis of cervical samples, vaginal samples, serum, or urine, allowing patient friendly screening. The ability of CRISPR-biosensors to detect HPV and other biomarkers with high sensitivity and rapidity provides a transformative solution for early detection of this preventable disease.

## 5. Challenges associated with CRISPR-biosensing for cancer detection

Applications of CRISPR integrated biosensors in the diagnosis of various cancers, including lung, liver, colorectal, prostate, and cervical cancers, have tremendously revolutionized the oncological diagnostics. These biosensors are able to effectively deal with the limitations of conventional biosensors and provide more sensitive, specific, and rapid detection of cancer associated biomarkers. CRISPR integrated biosensors, although promises unique advantages however; also faces some challenges and limitations which need to be addressed. For instance, CRISPR-Cas technology in diagnostics often rely on the pre-amplification of the target nucleic acid to enhance the signal of low-abundant target biomarkers. This additional step increases the cost, time, technical expertise, and complicates the workflow. The reproducibility of results from CRISPR-biosensors can be inconsistent due to the potential variability in the preparation and functioning of reagents, reaction conditions, or user handling. Also, there is a limited knowledge about the robustness of the components of CRISPR-system (cas proteins, guide RNAs, and reporter systems) in different clinical conditions, like extreme temperatures or long-term storage. The current CRISPR-technology for biosensing often require trained personnel, laboratory infrastructure, precise handling and mixing of the reagents. These requirements make them less accessible for use in low-resource or point-of-care settings. The guide RNA's specificity is most crucial for

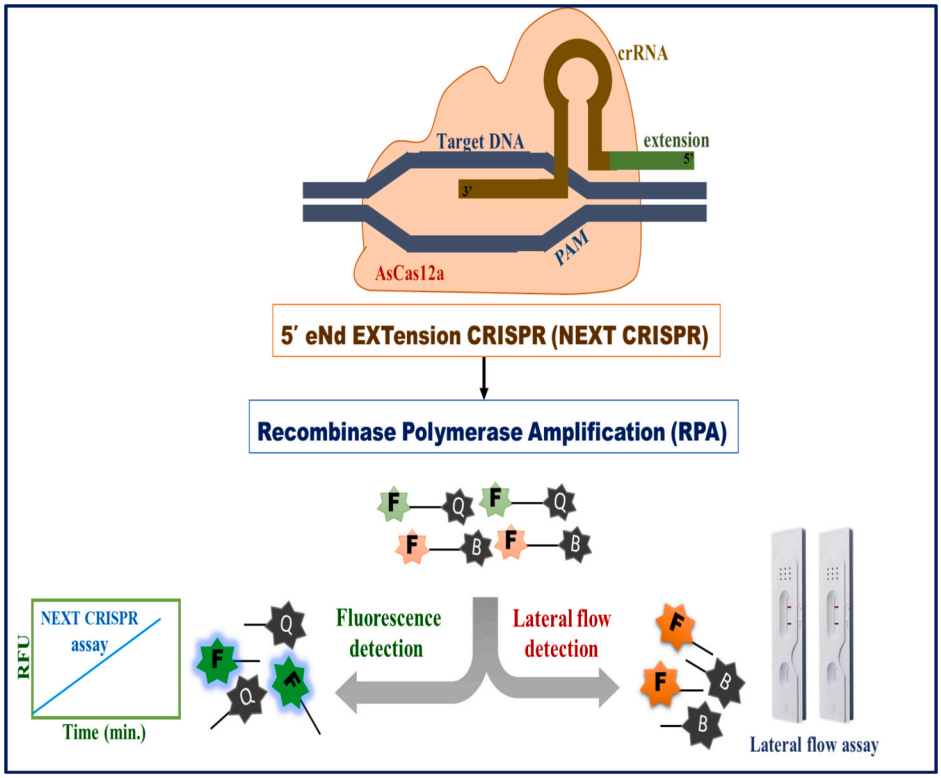


**Fig. 13.** Diagrammatic illustration of detection mechanism of "NEXT CRISPR", a CRISPR-Cas12a based biosensor for the detection of HPV-16.

**Table 7**
A comparative analysis of prominent CRISPR integrated biosensors for the detection of cervical cancer.

| Type of biosensor | CRISPR-Cas System | Detection techniques used | Type of electrode used | Nanomaterial used | Detection target | Limit of detection (LOD) | Linearity range | Response time | Sample investigated | References |
|---|---|---|---|---|---|---|---|---|---|---|
| Fluorescence biosensor | CRISPR/Cas12a | Recombinase polymerase amplification (RPA) | – | Nanomagnetic beads | HPV types (16, 18, 31, 33, 35, and 45) | 1 copy/μL for HPV DNA | – | 45 min | Cervical fluid samples | [125] |
| Fluorescence biosensor | CRISPR-Cas12a | Recombinase Polymerase Amplification (RPA); Transcription reaction | – | – | HPV types 16 and 18 | 1 aM | – | 80 min | Clinical samples | [126] |
| Photoelectrochemical (PEC) Biosensor | CRISPR-Cas12a | G-quadruplex-mediated biocatalytic reaction | Screen-Printed Electrode (SPE) modified with $In_2O_3$-$In_2S_3$ | Hollow $In_2O_3$-$In_2S_3$ | HPV-16 | 1.2 pM | 5.0 to 5000 pM | – | – | [127] |
| Photoelectrochemical (PEC) Biosensor | CRISPR-Cas12a | Quantum size-controlled engineering; Z-scheme heterojunction structure for enhancing photo-to-electron conversion | Z-scheme heterojunctions | Quantum dots | HPV-16 | 1.0 pM | 3.0 pM to 600 nM | – | HPV-16 DNA | [128] |
| Electrochemiluminescence (ECL) Biosensor | CRISPR-Cas12a | Electrochemiluminescence Signal | Au Nanoparticle-Deposited Glassy Carbon Electrode | DNA Tetrahedron Nanostructures (TDNs); Au Nanoparticles; $Ru(bpy)_3^{2+}$ (Ruthenium complex) | HPV-16 | 8.86 fM | 100 fM-10 nM | 100 min | Human serum samples | [129] |
| Aptasensor | CRISPR-Cas12a | CAED (Catalytic and Entropy-driven DNA Network) Method | – | – | DNA Methyltransferase 1 (DNMT1) | 90.9 fM | – | – | Plasma samples; Cervical tissue samples | [130] |
| Lateral flow biosensor | CRISPR-Cas12a | Photothermal and photoelectric signaling | $CuCo_2S_4$/$ZnIn_2S_4$-sulfur vacancy electrode | Polydopamine-coated gold nanoparticles (Au-PDA) | HPV types 16 and 18 | HPV-18—0.21 pM; HPV-16–42.92 pM | – | – | – | [131] |

accurate results which can be hampered by unintended binding to similar sequences, leading to false positive results. Finally, reliance of some CRISPR-biosensors on labelled signal reporters further complicates the process, increases costs, and hinder portability of the device. The application of CRISPR-Cas technology in biosensing is in nascent stage, to be suitable for POC use, it must integrate all steps of sample preparation, detection, data collection, and result analysis into a single user-friendly device.

## 6. Future prospects

Cancer remains one of the significant causes of mortality and morbidity around the world hence, there is a need for the development of advanced diagnostic tools for early detection and treatment. Integration of CRISPR-Cas with biosensors hold immense potential to transform oncological diagnostics. The fabrication of portable, user-friendly, and point of care CRISPR-based biosensors will enable real time monitoring and personalized diagnostic tools even in low resource settings. The personalized cancer diagnostics would further enhance precise monitoring and planning of treatment strategies tailored to individual genetic profiles. To advance the clinical readiness of CRISPR-biosensors, nanotechnology can be exploited for improving the analytical performance. Various nanomaterials like, graphene, gold, silver, carbon nanotubes, and metal–organic frameworks can enhance signal transduction and refine sensor development. Future innovations in sensor design could focus on multiplexing capabilities, label-free detection, and portability. The structural design of CRISPR-Cas molecular components can be optimized to improve their efficiency, reduce off-target effects and enhance target recognition. The detection system can also be coupled with enzymes (such as, polymerase) and other amplification methods (such as, loop-mediated isothermal amplification) that would lead to more efficient target amplification. Different macromolecules from the extremophiles could also be explored to provide high stability to these biosensors under varying conditions.

These biosensors when integrated with liquid biopsy techniques would remove the need of invasive tissue biopsies. Additionally, the integration of CRISPR-biosensors with microfluidics can enhance the miniaturization potential, allow automated sample preparation, and can be incorporated with smartphone readouts or wearable biosensors. The wearable biosensors would allow real-time monitoring of cancer biomarkers hence, patient can be continuously updated of their health status. The CRISPR-based biosensors can be combined with artificial intelligence (AI) and advanced machine learning models for improved and accurate real-time data analysis. This would further enhance biomarker identification, improve detection accuracy, and enable predictive insights for better decision-making in clinics. The use of quantum sensing and quantum computing would allow the enhanced sensitivity, detection limits, and improving signal-to-noise ratio hence, enabling comprehensive cancer profiling with greater ease and speed in a single assay. With the increasing complexity of biosensor-generated datasets, advanced techniques like big data analytics, integration of multi-omics data, and error correction algorithm would be essential. As these innovations progress, further research and advances are needed to make CRISPR-based biosensors a holistic, effective, personalized, and accessible point of care diagnostic tool for cancer detection.

## 7. Conclusion

Biosensing techniques offer numerous applications in the fields of biosciences, medicine, toxicology, food safety, drug delivery, disease diagnostics and progression. The incorporation of CRISPR-Cas technology in biosensors have led to an immense growth in biosensing technology. In this review article, we have discussed various reported CRISPR-based biosensors for the detection of some of the deadliest cancers (lung cancer, liver cancer, colorectal cancer, prostate cancer, and cervical cancer). Limitations and the drawbacks of the conventional cancer detection methods have laid the foundation for the development of more specific and sensitive diagnostic tools based on CRISPR-type II (Cas9), V (Cas12), and VI (Cas13) systems. This expansion of CRISPR-Cas technologies had enabled more efficient, non-invasive, rapid, real-time, and multi-plexed capabilities of biosensors for early-stage cancer detection. However, challenges and limitations are to be considered. The complex design, reagent stability, ease of use, and tedious optimization steps still pose hurdles. In addition to many successful advancements, CRISPR-Cas based biosensors still need to be checked for a larger section of patients to ensure their effectiveness. Despite these challenges CRISPR-based biosensors offer sensitive, specific and rapid early-stage detection of cancer; hence, significantly improving the chances of patient survival. These biosensors exhibit the potential for non-invasive, real-time, cost-effective, and personalized oncology diagnostics. While transitioning from laboratory to successful clinical use, these biosensing techniques promises the transformation of cancer diagnostics to reap real life benefits.

## 8. Ethical issues

Not required.

## 9. Research data for this article

All the data supporting the outcomes of this review are available within the article.

## CRediT authorship contribution statement

**Arzoo Saini:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Neeraj Dilbaghi:** Writing – review & editing, Visualization, Validation, Supervision, Formal analysis. **Neelam Yadav:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Formal analysis, Data curation, Conceptualization.

## Acknowledgement

Arzoo Saini is sincerely grateful to UGC-granted Junior Research Fellowship (UGC-JRF), [grant number 221610196566] for providing financial assistance for this publication.

## Data availability

All the data supporting the outcomes of this review are available within the article.

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