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Research on the Biosensing Mechanisms of CRISPR/Cas9 Systems and the Applications in Detection of Non-Infectious Disease

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Abstract:

The CRISPR/Cas9 system has become a groundbreaking technology in molecular diagnostics due to its precise nucleic acid recognition capabilities. This paper comprehensively analyzes the molecular mechanisms of CRISPR-based biosensing platforms, emphasizing target specificity and signal transduction. It reveals how engineered Cas9 variants and optimized guide RNA enhance detection sensitivity, with applications in non-infectious disease monitoring (e.g., early cancer biomarkers). CRISPR-mediated platforms achieve remarkable performance, with detection limits at femtomolar concentrations. The review discusses challenges like off-target effects and signal amplification, proposing solutions involving nanotechnology and microfluidics. Chronic non-communicable conditions challenge global healthcare. CRISPR/Cas9 comprises gRNA and Cas9, with class 2 systems (e.g., Type II) prominent. Cas9 identifies sequences via protospacer adjacent motifs, inducing DNA breaks. Traditional methods are labor-intensive; CRISPR Class 2 platforms enable sensitive, precise detection at lower costs. Emerging Cas variants (e.g., Cpf1) offer enhanced capabilities. Challenges include sample processing and non-specific binding. Future research should simplify protocols and conduct clinical trials. CRISPR/Cas9 advances precision genetic analysis.

Keywords: CRISPR/Cas9; Non-Infectious Disease; Mechanism.

1. Introduction

Chronic conditions such as cancer, diabetes, and cardiovascular disorders have emerged as predominant global health challenges. While infectious diseases remain prevalent causes of morbidity and mortality in low-income nations according to Global Burden of Disease (GBD) research findings, these non-infectious pathologies now represent the leading mortality drivers worldwide, particularly affecting marginal-

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ized populations. The pervasive nature of related health risks highlights a striking disparity in healthcare resource allocation between combating infectious agents and addressing chronic illnesses [1].

Such gaps highlight the urgent need for high sensitivity and rapid biosensing devices. Among currently available detection platforms, new generation of CRISPR/Cas9-based biosensing sensors are promising due to its modular development and specificity. CRISPR/Cas9 is originally from a bacterial immune system that was first discovered in Escherichia coli in 1987 and it developed as a cutting-edge genetic-engineering tool [2].In 2012, the Cas9 enzymes were proven to work for DNA-targeted cleavage within a system setup under controlled environments, after which several follow-up efforts have demonstrated not only the molecular modularity of this system, but also its operational diversity, rendering it one of the preferred experimental and clinical genome editing technology options to use in a variety of eukaryotic organisms [3].These distinct properties have been exploited by various researchers to exploit to design enzymes where the enzyme recognizes nucleic acids and enzymatic amplification of the signal.

Other than genetic editing, CRISPR has also been employed in diagnostic tests for chronic illnesses. Researchers have used the CRISPR mechanism, which is referred to as the SHERLOCK system, to precisely detect tumour protein signatures and circulating tumour DNA (ctDNA) related to some of the early stages of cancer130, which has provided more individualised therapies for cancer treatment.Recent CRISPR-based diagnosis methods such as the CRISPR-SNP-Chip assay and polymerase chain reaction can facilitate the detection of WD as it is a genetic disorder of copper homeostasis due to mutations in ATP7B that interfere with copper transport. The techniques provide specificity and sensitivity at the molecular level greater than standard laboratory methods.

This review provides a comprehensive analysis of CRISPR/Cas9-enabled biosensing platforms and their clinical implementation prospects for non-communicable disease detection. Drawing upon historical research findings and documented clinical evidence, the paper evaluates existing CRISPR diagnostic capabilities while identifying technological limitations requiring refinement. Although CRISPR-derived detection methodologies show remarkable potential, their clinical adoption necessitates rigorous validation through extensive clinical validation and longitudinal investigations to confirm operational safety and diagnostic reliability.

2. Mechanisms of CRISPR/Cas9 Systems

The CRISPR-Cas system can be categorized into two main classifications. Class 1 systems, representing approximately 90% of documented CRISPR-Cas genetic arrangements across bacterial and archaeal species, operate through coordinated multi-protein complexes that execute defensive functions [4]. In contrast, Class 2 systems account for about 10% of identified configurations and feature autonomous effector proteins capable of performing multiple biochemical activities, demonstrating superior adaptability for genetic engineering purposes [5]. Among these, the type II CRISPR mechanism employing the Cas9 nuclease has emerged as the predominant research focus due to its precision in creating targeted double-stranded DNA breaks, revolutionizing genome editing methodologies [6].

The CRISPR-Cas9 platform has emerged as a predominant technology for accurate genetic modification. This molecular machinery comprises two key elements: a guide RNA (gRNA) component and the Cas9 enzymatic protein. The cas9 is a bilobed domain structure.Recognition lobe (REC) consists of three adjacent α-helical domains stabilized by bridging helix, and nuclease lobe (NUC) is composed of many subdomains – RuvC catalytic domain, HNH catalytic domain, topology interacting domain and a Cterminal domain (CTD) [7].These regions are necessary for important processes, such as sequence identification, binding interaction and cleavage activity, and where different coordination take place between structurally defined motifs and the corresponding gRNA sequence. The designed hybrid gRNA nucleic molecule consists of CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA). The crRNA sequence dictates the specificity for DNA by the engineered protein while the tracrRNA assists in the stabilization of the complex Cas9 protein and nucleic acid combination [3].

Once Cas9 binds to the protospacer adjacent motif (PAM) - usually described as 5‘-NGG-3’ where N may be any nucleotide - that abuts the target sequence of DNA, Cas9 is structurally stabilized [8]. At this point, guide RNA (gRNA) hybridizes with the DNA strand via complementary base pairing, which induces Cas9 enzyme function. In DNA, the precise cutting activity of the Cas9 RuvC and HNH catalytic activity is responsible for the specific cleavage of the DNA phosphodiester bonds in both DNA strands - the non-complementary one (and its complementary strand) [9]. These two strands are specifically cleaved immediately before the PAM in three nucleotides 3’ to the PAM with blunt ends and resulting double-strand breaks (DSBs) that are resolved via one of the two endogenous repair pathways: homology-directed repair (HDR)

or non-homologous end joining (NHEJ) [10].HDR-dependent repair needs a homologous donor template and peaks at late S–G2 phase in the cell cycle to allow precise genome editing applications such as substitution of single nucleotides or insertions of particular sequence [11].

HDR exhibits a degree of accuracy, however, HDR activation is rare. NHEJ, conversely, is a repair mechanism that dominates cell cycles, regardless of the presence or absence of a homologous template, which rejoins a blunt broken DNA ends, often resulting in small insertions or deletions that can cause a frameshift mutation, and/or truncated proteins [3].Increased understanding of the molecular process underlying CRISPR-Cas9 has lead to applications beyond molecular biology in medicine, agriculture, biotechnology, and environmental research, where several Cas9-assisted therapies are in clinical trial for many diseases with promising, if still limited outcomes. From a therapeutic standpoint, it is also clear that gene editing holds tremendous promise for personalized and precision medicine.

3. CRISPR-Based Diagnostic Approaches for NCDs

3.1 Progress in Tumor Biomarker Detection Based on CRISPR-Cas System

In a relatively short period, CRISPR-Cas based diagnostic tools have been rapidly advanced towards detecting biomarkers of tumors. Via capitalizing on specific biochemical characteristics of CRISPR, these promising tools empower their capabilities to interrogate the tumor biomarkers, i.e. the ctDNA, tumor-associated proteins or RNA signitures in liquid biopsies, in a minimally-invasive manner.This capability will be paramount for advancing both early cancer diagnosis, monitoring cancer growth kinetics, and personalized management - all without needing invasive tumor biopsies [12].

Circulating tumour DNA (ctDNA) is one of the different biomarkers that could be used in liquid biopsy as a clinically relevant biomarker for cancer diagnosis and monitoring as it can be accessed easily in the different available body fluid, such as blood and urine. The team of Gootenberg developed a novel system using the integration of nucleic acids amplification with the CRISPR/Cas to be able to detect with high sensitivity and high specific the different cancer-related genetic alterations [13]. The research team led by Zhang demonstrated successful identification of rare mutations such as EGFR L858R and BRAF V600E in synthetic cfDNA samples containing 0.1% mutant alleles. Subsequent technical improvements enhanced the platform‘s signal sensitivity by 3.5-fold, achieving an ultra-low detection threshold of 2 attomolar concentration.

Jang et al. designed a CRISPR-Cas system with strong mutation detection ability for circulating EGFR mutagenesis in plasma specimens which were reported to have stronger sensitivity in T790M detection as compared to digital PCR and allowing real-time analysis of plasma specimens without DNA purification prior to testing [14]. In the next study a nanomaterial modified electrochemical biosensor were combined with CRISPR-Cas and resulted in higher long term response dynamic range and the impressive detection limit for EGFR L858R characterization. The platform exhibited 92% consistency with digital PCR results in clinical validations. Parallel investigations using CRISPR-inspired detection frameworks have validated comparable accuracy in identifying BRAF V600E alterations and PIK3CA genetic variations [15].

In addition to ctDNA analysis, advancements in protein biomarker analysis have achieved notable breakthroughs. CRISPR-Cas biosensing platforms have been successfully implemented for prostate-specific antigen (PSA) analysis, where innovative integration of multiple technological approaches has enabled highly sensitive colorimetric measurements and fluorescence-based identification. Certain systems demonstrate substantially enhanced detection thresholds relative to conventional commercial assays, though current methodologies remain constrained by operational complexity and prolonged processing durations.

However, several practical challenges hinder widespread adoption of this technology, particularly in resource-limited settings. High costs and infrastructure requirements pose significant barriers, as advanced diagnostic tools are often inaccessible in such environments. The multi-stage sample preparation process presents additional obstacles due to its technical complexity and time-intensive nature, frequently requiring specialized instruments and skilled operators. These factors collectively elevate operational expenses while amplifying potential procedural inaccuracies.

3.2 Diagnosis of Metabolic Disorders (Wilson’s disease) Based on the CRISPR/Cas System

Wilson‘s Disease (WD), clinically termed Hepatolenticular Degeneration (HLD), represents an inherited autosomal recessive condition characterized by disrupted copper homeostasis. This metabolic disorder stems primarily from genetic alterations in the ATP7B gene, which encodes a critical copper-transporting ATPase. The pathogenic variants compromise biliary copper excretion, leading to pathological copper retention. The metal initially deposits

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in hepatic tissues, inducing hepatocellular injury, before spreading systemically to accumulate in vital organs including cerebral structures, ocular tissues (particularly the cornea), and renal systems.

Nevertheless, existing diagnostic approaches remain prohibitively expensive and fail to detect ATP7B gene mutations that are essential for accurate diagnosis and genetic counseling. While conventional genetic testing methods like Sanger sequencing and next-generation sequencing (NGS) serve as gold standards, they present operational challenges: Sanger sequencing struggles with throughput for rare mutations, whereas NGS requires sophisticated infrastructure with analysis costs ranging from $500-$5000 and turnaround times exceeding 7-14 days. Such limitations risk postponing time-sensitive therapies like copper-chelating agents and zinc supplementation, which could prevent irreversible neurological and hepatic damage if administered promptly. The emerging CRISPR-based diagnostic platforms address these limitations through enhanced detection capabilities.

Class 2 CRISPR-Cas systems (encompassing Types II, V, and VI) demonstrate notable efficiency in identifying ATP7B point mutations. The Type II category, exemplified by Cas9 nucleases, are adaptable for both enzymatic cleavage and molecular binding approaches. Notable applications include RGEN-RFLP methodology that achieves results within 90 minutes using standard laboratory tools, alongside CRISPR-SNP-Chip technology employing electrical impedance variations for genetic variant recognition. Systems utilizing Cas12a (Type V) and Cas13a (Type VI) leverage their collateral cleavage properties, with detection platforms like DETECTR (Cas12a-dependent) and SHERLOCK (Cas13a-powered) generating amplified fluorescent signals capable of identifying low-frequency mutations, rendering them ideal for decentralized diagnostic settings [16]. When targeting specific ATP7B genetic alterations in Wilson‘s disease cases, distinct CRISPR-Cas platforms exhibit distinct performance characteristics. This operational diversity highlights the necessity of choosing optimal detection systems tailored to particular mutation profiles.

The optimal Cas protein selection depends on specific clinical requirements and mutation profiles. For example, when patients present with both the c.2072G>T (p.G691V) and c.2108G>A (p.C703Y) variants in Wilson‘s Disease, SpCas9 demonstrates effective detection capability for the c.2072G>T alteration. However, alternative systems become necessary for variants like c.2108G>A (p.C703Y) where SpCas9 shows reduced recognition efficiency. The V-B AacCas12b enzyme from *Alicyclobacillus acidoterrestris*, which targets a 5‘-TTN PAM sequence positioned upstream of DNA targets, presents a promising diagnostic alternative for such cases [17]. While current CRISPR diagnostic platforms face technical constraints including multi-guide RNA array optimization challenges, along with issues of nonspecific binding and inconsistent enzymatic activity, this approach still outperforms conventional methods through rapid turnaround (60-120 minutes), economical implementation, and adaptability for use in areas with limited resources. These advantages position CRISPR technology as a transformative diagnostic solution for Wilson‘s Disease detection.

This can potentially allow for more timely and ameliorative treatments of diseased patients. However, there are still considerable challenges, most importantly, depending on which locus and different ATP7B haplotype a patient is found, it may not possess a sequence recognized by SpCas9 (5′-NGG PAM). In this case, other nickase enzymes should be used in the genome editing approach, e.g. AacCas12b that acts upon 5’-TTN PAMs[18].

Since its emergence, the CRISPR/Cas9 system has emerged as a powerful molecular technique for pinpointing oncogenes and critical molecular regulators, finding widespread application in oncology research. This groundbreaking methodology has empowered researchers to unravel genetic underpinnings across diverse aspects of cancer and non-communicable diseases. Notably, CRISPR/Cas9 demonstrates remarkable potential for identifying synergistic gene interactions that could combat therapeutic resistance, offering innovative approaches to treatment challenges [8,9]. Recent advancements in CRISPR-based functional genomics platforms further enable comprehensive tracking of transcriptional alterations during therapies and precise identification of genetic factors driving drug resistance. These technological breakthroughs create novel opportunities to elucidate carcinogenesis mechanisms and develop personalized biomarkers for targeted therapies [18].

4. Conclusion

To conclude, the CRISPR/Cas9 technology allows to control non communicable diseases due to its efficiency and flexibility. Technologies such as SHERLOCK represent CRISPR potential for the clinical use in oncology, allowing to monitor blood-derived ctDNA or proteins biomarker with high sensitivity early cancer detection and personalized therapy monitoring.Innovations based on CRISPR technology, such as that developed by SNPChip and amplification, can analyse the SNPs causing genetic disorders in cases, such as WD, by detecting mutations in ATP7B directly. Consequently, such advanced screening tools not only overcome disadvantages of existing metabolic disorder analyses but also achieve rapidness for the

genetic mutations-caused disease.

Despite this, numerous barriers still exist in this field such as intensive sample processing operations, less translational nature in under-resources countries and poor diagnosing performance. We anticipate that the researchers will focus efforts on the robust workflow construction of these platforms, and also more multicenter clinical trials and long-term observational studies to better assess the robustness and therapeutic performance of the platform. With advancing of CRISPR/Cas9, it seems that the approach of personalized health is going to bring revolutionary changes in medicine to achieve more efficient, precise and friendly testing method for various NCD.

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