CRISPR-Based Technologies in Diagnostics and Cancer Research: Advances, Applications, and Future Perspectives

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Abstract

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology has revolutionized molecular biology, enabling precise genome editing and highly sensitive diagnostic applications. This review integrates recent advances in CRISPR-based diagnostics and gene editing, particularly focusing on point-of-care (POC) diagnostics and cancer research. CRISPR-Cas systems such as Cas9, Cas12, and Cas13 have demonstrated exceptional potential in detecting infectious and non-infectious diseases with high specificity and sensitivity. Furthermore, CRISPR/Cas9-based gene editing has emerged as a powerful tool in cancer research for understanding tumorigenesis, identifying therapeutic targets, and overcoming drug resistance. This review highlights current developments, applications, and future directions of CRISPR technology in diagnostics and oncology.

Keywords: CRISPR, CRISPR-Cas9, genome editing, molecular diagnostics, point-of-care diagnostics

1. Introduction

The CRISPRISPR-Cas system, initially identified as a prokaryotic adaptive immune system (Kas, et al. 2007), has also been exploited for genome editing and nucleic acid detection (Barrangou, et; al 2007). These developments may enable a transformation in cancer diagnosis as they offer the prospect of rapid, inexpensive and portable tests, particularly at limited-resource and rural environments (Fang Y, et;al2024). Novel virus outbreaks such as SARS-CoV-2, H1N1 influenza, avian influenza, and dengue virus have led to a global economic meltdown (Bartoletti et;al 2018)(Ghouneimy et;al 2024) The increasing need for rapid, sensitive, and economical diagnostic devices, particularly after global health crises such as COVID-19, has driven the development of CRISPR-based diagnostic systems.

Meanwhile, cancer continues to be a significant cause of death in the world, calling for novel strategies including CRISPR-mediated gene editing based diagnosis and therapy. Several rapid nucleic acid diagnostic kits are commercialized now. CRISPR, a gene-editing technology, has been transformed into a diagnostic tool thanks to its high specificity, sensitivity, and simplicity of use (3). The optimized CRISPR-Cas9 and CRISPR-Cas12/13 systems to detect cancer biomarkers with excellent sensitivity have further applied CRISPR-based diagnostics to both viral and nonviral biomarkers.

1.1.CRISPR as a Therapeutic Tool

CRISPR-Cas systems are demonstrating great promise as therapeutics for infectious diseases by targeting pathogen genomes for disruption or editing at unprecedented levels of specificity. This selectivity is especially useful in the treatment of antibiotic-resistant bacterial diseases, for which traditional therapies are mostly ineffective. In case of viral infection, CRISPR can cut out the viral genomes integrated into host DNA, enabling potential cures for chronic diseases like HIV or hepatitis B (Lee HL et; al 2018,9). CRISPR provides a transformative platform for precision treatment of infectious diseases. Nevertheless, there still remain challenges in achieving phage-host specificity, immune responses, and regulatory acceptance. With phage therapy attracting interest once again in the post antibiotic era, CRISPR-phage approaches offer a hybrid option that may change the treatment of multidrug-resistant infections (Krishnan A et; al 2024). NHEJ is the dominant repair mechanism in most stages of the cell cycle, including G1, S, and G2 phases, while HDR is mainly active during the late S and G2 phases (Heyer et al., 2010). These natural repair processes have been successfully harnessed by researchers for genome editing in mammalian cells using CRISPR-Cas9 systems (Fu et al., 2013).

In practice, NHEJ often results in small insertions or deletions (InDels) at the DNA break site, which can disrupt gene function. This approach has been shown to achieve relatively high editing efficiencies, ranging from about 2% to 25%, making it useful for gene knockout applications (Fu et al., 2013). When properly designed donor templates are provided—such as plasmid DNA with long homology arms or short single-stranded oligodeoxynucleotides—both small and large genomic modifications can be introduced with high precision (Capecchi et al., 1989). However, HDR-based CRISPR editing is still less efficient and not as fully optimized as NHEJ. Because NHEJ is error-

prone, it can introduce frameshift mutations that produce non-functional proteins or trigger nonsense-mediated mRNA decay, ultimately leading to gene disruption (Bibikova et al., 2001).

2. Pros and Cons of CRISPR-Based Diagnosis Systems

2.1 Pros:

Due to the use of isothermal amplification technologies, both the SHERLOCK and DETECTR rapid test systems can be finished in less than 30 minutes compared to the greater than 1 hour typically required for an RT-PCR test. Isothermal amplification eliminates the need to denature the DNA (the first step of an RT-PCR test), as is performed with a denaturing agent like a heat source (Li et al., 2019). The two assays are compatible with lateral flow dipsticks for detection, unlike large (thermo-cycler) and/or cumbersome (detection) equipment used in RT-PCR testing. Reduced time to complete diagnostic tests combined with low equipment costs make the new class of CRISPR-based diagnostic tests very strong for candidates for use as rapid diagnostic tests. In addition, the CRISPR-based DETECTR rapid diagnostic test may be combined with a Microfluidic(s) or Surface Plasmon Resonance (SPR), to create a portable, rapid test that can be performed on-site to a patient. This type of module is not feasible for other tests, including qRT-PCR, which require large, expensive instruments. CRISPR-Cas diagnostics systems have one of their few key advantages from multiplexing capability. Pathogen-specific crRNAs may be designed from a common portion of a pathogen's genome. The multiplex diagnostic capability has made it possible to distinguish between several viral pathogens or even different virulent variants of the same virus within the same sample (Myhrvold C et al, 2018).

2.2 Cons:

Current diagnostic strategies, such as serologic and nucleic acid-based tests, have disadvantages that prompted researchers to develop CRISPR-based techniques, DETECTR, and SHERLOCK assays. DETECTR assays target DNA, while SHERLOCK assays target RNA. Both are able to meet the requirements mentioned above and offer unique multiplexing capabilities. The CRISPR-based DETECTR assay has the same accuracy as a qRT-PCR assay but is also faster. Therefore, the limitations noted for qRT-PCR assays would also apply to a CRISPR-based diagnostic assay at this point. However, some of the

additional benefits of a CRISPR-based diagnostic assay over a qRT-PCR assay are the speed of results, isothermal signal amplification (no need for thermocycling), single nucleotide target specificity (no cross-reactivity), no need for complex laboratory setup, and accessible reporting methods (e.g., lateral flow strips). Furthermore, CRISPR systems can be rapidly reorganized to diagnose newly emerging viral infectious diseases, as evidenced by the rapid creation of the DETECTR assay for diagnosing SARS-COV-2 infection (Huang C et al,2018)

3.Applications in Infectious Diseases

Cancer survival rates have improved significantly over the past few decades, mainly due to better methods for early detection and more effective treatment strategies. However, many existing diagnostic techniques still face limitations in terms of sensitivity, specificity, and speed, which highlights the need for more accurate and rapid molecular-level detection approaches. In this context, identifying cancer-related genetic mutations through advanced diagnostic tools has become highly important for improving early diagnosis and patient outcomes. This system uses the Cas13a enzyme, which can be programmed to detect specific RNA sequences. Once the target is identified, Cas13a activates a collateral cleavage activity that breaks nearby fluorescent RNA reporters, producing a detectable signal. This approach has shown high sensitivity in identifying important cancer-related mutations such as BRAF V600E and EGFR L858R in mammalian systems (Gootenberg et al., 2017).

This system has been effectively used for rapid and cost-efficient detection of viral infections, including high-risk human papillomavirus (HPV) types such as HPV16 and HPV18. Overall, these CRISPR-based diagnostic technologies demonstrate strong potential for fast, accurate, and affordable detection of disease-related genetic markers, particularly in cancer and infectious diseases (Chen et al., 2018).

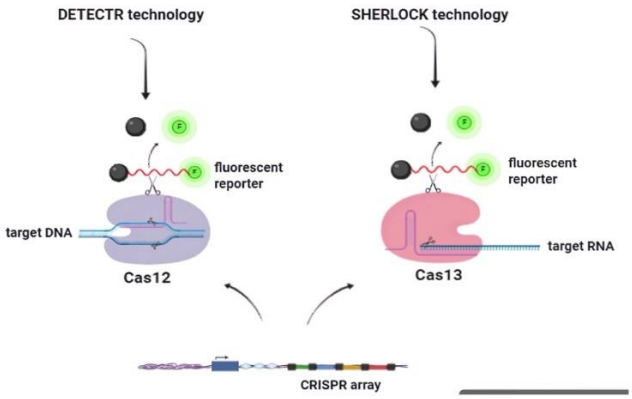

Figure 3: CRISPR-Based Diagnosis of Infectious Diseases

4. CRISPR in Cancer Research and Therapy

CRISPR is the instrument of choice for cancer biology and therapy because of the potential to directly and accurately alter the genes that contribute to tumor formation and growth as well as resistance to cancer therapies. The Cas9 protein is guided by the guide RNA (gRNA), cleaving both DNA strands, resulting in a double-strand break (DSB) that will be repaired by the cell, which may lead to inactivation, modification or substitution of the target genes. In cancer, this technology is most widely applied to forward genetics and reverse genetics of oncogenes and tumor suppressors, exemplifying how mutations acquired over the course of a lifetime can lead to cancer initiation and metastasis. CRISPR has been used to study key genes, including EGFR, KRAS, TP53, and PTEN, in multiple cancers. It's also good for studying mechanisms of chemo resistance by taking out genes that help cancer cells endure treatment. CRISPR-based technologies also provide intriguing opportunities to pursue these and other strategies, including the correction of deleterious mutations, the knockout of cancer-promoting genes, and the enhancement of immune responses to tumors, for use in cancer therapy. A very exciting line of investigation is CRISPR-engineered immunotherapy that modifies immune cells such as T-cells to better recognize and kill cancer cells.Although off-target effects, delivery, and ethical concerns need to be addressed, CRISPR is constantly developing as a potential technique for the next generation of cancer diagnosis, targeted treatment, and precision oncology.

Cancers/ Genetic diseases	Mutations that can be corrected with CRISPR/Cas9	CRISPR/Cas9 Gene targets	Refere
Lung	exon 19 deletion and L858R	EGFR	[11]
Breast	G309A, D769H, D769Y, V777L, P780ins, V842I, and R896C and BRCA1/2 mutations	HER2/Neu, BRCA	[12]
Thyroid	C228T and C250T	TERT promoter	[13]
β- Thalesemia	IVS2-654 (C > T)	HBB	[14]
Huntington	p.(Gln302) and p.(Tyr539Cys)	RNF216	[15]
Limb girdle muscular dystrophies types 2B and 2D	c.5713C>T; p.R1905X, and missense c.229C>T; p.R77C	Dysferlin, and alpha-sarcoglycan	[16]
Alzheimer's	H214N, R220P	Presenilin 1	[17]

Table 1: Cancers, genes, mutations, and CRISPRs' editing ability

5. Future Perspectives

The expectation for future CRISPR technology is very high, and advancements are continually being made which supports the science of CRISPR applications increasing in volume over time for diagnostics, therapeutics, and personal medicine, As CRISPR technology continues to be developed, advancements in delivery methods and the specificity associated with

these delivery methods will result in safer and more effective clinical applications for the next generation of CRISPR systems currently being developed. In the area of diagnostics, CRISPR-based diagnostic platforms will soon be commonplace as point-of-care devices used to quickly (in minutes) identify infectious agents, biomarkers for cancer, and genetic abnormalities through portable devices or using a smartphone app. The integration of artificial intelligence (AI), microfluidics, and nanotechnology with CRISPR technology increases the possibilities for automated processes in CRISPR-based diagnostic devices providing greater sensitivity and improved data analysis capabilities. In cancer, CRISPR-based therapeutics could be used to generate truly personalized therapy through options like correcting mutations, engineering immune cells, or developing novel drug targets. Innovative techniques, including base editing and prime editing, may surpass the limitations of traditional CRISPR methods enabling precise edits to genes without having to create double-stranded breaks in DNA. CRISPR technology may ultimately become a critical element in the global fight against infectious disease by developing a rapid diagnostic test and/or developing antiviral treatment modalities. In an impressive display of speed and efficiency, CRISPR's rapid progress in parts of the world, however, improvements to ethical, regulatory and safety aspects of the CRISPR gene-editing tool need continued study and technological investment. Continued research and technology development may establish CRISPR as a primary tool for developing medical and life sciences focused on providing exact, contemporary, and future-ready healthcare.

CRISPR-Cas9 technology was originally developed as a genome-editing tool, but it has quickly gained importance in the field of disease diagnostics due to its high specificity, programmability, and ability to accurately identify nucleic acid sequences. Because of these features, it is now regarded as a powerful approach for fast, cost-effective, and reliable detection of infectious diseases, genetic disorders, and cancers (Hsu, Lander, & Zhang, 2014).

In recent developments, CRISPR-based diagnostic systems have been integrated into simple and portable platforms such as paper-based strips, microfluidic devices, and handheld biosensors, making them suitable for use in clinical settings as well as in remote or resource-limited areas (Kellner et al., 2019). Some CRISPR diagnostic tools have

also shown the capability to detect viral genetic material within an hour, highlighting their strong potential for rapid field-based testing and real-world application (Broughton et al., 2020).

6. Conclusion

Since the technology behind CRISPR has fundamentally changed diagnostic and therapeutic research, the technology allows for the quick identification and editing of genetic information, making it one of the most important tools available in modern-day medical care. Even though there are challenges, developing better CRISPR systems and new technologies such as AI will hopefully lead to elimination of the current limitations experienced with CRISPR systems and should lead to many of them being used in clinical practice universally.

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