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Popular Article

The Potential of Crispr-Cas Technology In Combating Infectious Diseases

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Abstract

Infectious diseases continue to pose a worldwide threat, causing excess morbidity and mortality each year, with the potential for destabilizing pandemics. Improved understanding of bacterial, viral, fungus, and parasite pathogenesis and quick identification and treatment of infections are critical for improving infectious disease outcomes globally. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) together with CRISPR-associated endonuclease (Cas) have facilitated new developments in genetic engineering. Due to its high specificity, sensitivity, and simplicity, the CRISPR-Cas system has been employed for molecular diagnosis and therapy of infectious diseases. Numerous bacteria and archaea have native CRISPR-Cas systems, which act as an adaptive immune system by degrading the genome of an invasive pathogen in a sequence-specific manner. This system has been modified for application in gene expression control, drug development, diagnostics, cancer prevention and treatment, and the management of genetic and infectious illnesses. DNA- and RNA-targeting nucleases provided by the CRISPR toolset serve as dual weapons against infectious diseases. When used for nucleic acid detection in the context of emerging infectious diseases, the CRISPR-Cas system, as a quick, accurate, and user-friendly genome editing tool, provides a quick, cost-effective, and highly sensitive diagnosis.

Introduction

Genomic manipulation offers humanity a chance to heal various tumors, infections, and degenerative disorders in addition to curing monogenic diseases. Research and clinical uses for clustered regularly interspaced short palindromic repeats (CRISPR) and the associated CRISPR-associated (Cas) proteins have expanded quickly as a result of a better understanding of their structure and function. In Escherichia coli, a genomic structure with five almost identical repetitions of 29 nucleotides, separated by 32-nucleotide spacers, was found in 1987. (Ishino Y *et al.,*1987). Mobile

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genetic elements (MGEs) that include such as plasmids and bacteriophages, transposons, and their spacer sequences were discovered. In the years that followed, bacteria and archaea discovered a source of both heritable and adaptive immunity. (Ishino Y, Krupovic M, Forterre P. 2018). Another important discovery in the field of CRISPR-Cas biology was that Cas9 nucleases produce blunt double-stranded DNA breaks when directed by complexes of the CRISPR RNA (crRNA) and trans-activating RNA (tracrRNA). (Garneau *et al*.,2010 and Deltcheva *et al*.,2011). To date, CRISPR-Cas systems are divided into two classes, with six types and several subtypes. System types I, III, and IV of CRISPR-Cas use an interference apparatus composed of numerous Cas proteins, whereas types II, V, and VI of CRISPR-Cas systems use a single Cas protein for interference. The capacity to edit genes had previously been possible through the use of *zinc-finger nuclease* (*ZFN*), transcription activator-like effector nuclease (*TALEN*) but with the advent of the CRISPR-Cas system, this capability has become more precise and adaptable Additionally, the CRISPR-Cas technology shows great promise for use in preclinical disease models such cultured human cells and animal models. There are numerous ways to use this system therapeutically, but the best technique will depend on the target tissue, the administration route, and the type of editing (Urnov, F. D. *et al*.,2018 and Ross C Wilson, Luke A Gilbert.,2018). The National Institutes of Health (NIH) advisory group approved the first human experiment using CRISPR gene editing for use in cancer therapy. The first application of CRISPR genome-wide searching to find a gene that benefits cells fight flavivirus infection was reported by researchers from the UT Southwestern Medical Centre. (Reardon S.,2016 and Richardson *et al*.,2018).

2. Applications for Infectious Diseases

2.1 Identifying Functions of Genes in Pathogens

Utilizing CRISPR/Cas9 technology, screen crucial bacterial genes and determine their chemical vulnerabilities and virulence factors. A CRISPR-mediated knockdown screen by (Peters *et al*., 2006) identified a network of whole-genome connections in *Bacillus subtilis.* Tao *et al*.,2016) identified Frizzled family (FZDs) members as toxin B (TcdB) receptors using genome-wide CRISPR-Cas9 screens on *Clostridium difficile*. Zheng *et al*.,2018) directed the Zika virus was discovered utilizing nucleic acid sequence-based amplification (NASBA) with the insertion of a synthetic trigger sequence and T7 primer, followed by CRISPR-Cas9 cleavage of the sequence close to the protospacer adjacent motif (PAM) site (Pardee *et al.* 2016). Gootenberg *et al*., 2017 recognized

E. coli and *Pseudomonas aeruginosa*, separated *K. pneumoni*ae isolates with various resistance genes, The CRISPR-Cas13-based specialized high-sensitivity enzymatic reporter unlocking (SHERLOCK) test has been applied to detect dengue, Zika, HIV, West Nile, and yellow fever viruses (Myhrvold *et al.* 2018).

2.2 Diagnostics using CRISPR-Cas9

Recent CRISPR applications make an effort to develop diagnostics for infectious diseases. Use of collateral cleavage generated by the Cas12 and Cas13 nucleases. The first of these technologies combines isothermal recombinase polymerase amplification (RPA) or reverse transcription (RT)-RPA with Cas13a cleavage and is known as SHERLOCK. Muller *et al*.,2016 used CRISPR-Cas9 in conjunction with another application that uses optical DNA mapping to find the genes responsible for bacterial antibiotic resistance. Guk *et al*.,2017 used CRISPR-Cas9 in conjunction with fluorescent in situ hybridization (FISH) to detect methicillin-resistant *Staphylococcus aureus* (MRSA). Isothermal RPA and Cas12a enzymatic activity are combined in a related technique known as DNA endonuclease-targeted CRISPR trans reporter (DETECTR) (Chen *et al*.,2018). Broughton *et al*., 2020 recently selected two crRNA targeting the E and N genes specific to SARS-COV-2 and were able to identify COVID-19 within 30 minutes using the DETECTR assay.

2.3 Emerging therapeutic applications

The human immunodeficiency virus (HIV) epidemic has caused an estimated 35 million deaths worldwide since it began, and a comparable number of people are still living with the disease today (World Health Organization. 2018). The main targets of newly developed CRISPR-based infectious disease therapies are drug-resistant bacteria and persistent viral diseases, such as HIV and hepatitis B virus (HBV).

2.3.1 Focusing on microorganisms that are harmful and drug-resistant.

The development of antimicrobials that are targeted and titrable to get rid of pathogenic bacteria has been suggested as a potential use for CRISPR technology. In trials using pure and mixed cultures, a subtype, I-E CRISPR-Cas system was used to specifically eradicate particular strains of Salmonella enterica and E. coli. To cause the least amount of harm to the natural microbiota, CRISPR technology has also been studied in vitro for the removal of drug-resistant genetic components and restoration of antibiotic susceptibility while maintaining bacterial viability.

2.3.2 Targeting chronic viral infection

Several viral pathogens produce persistent infection after initial infection through the integration of their genome into host chromosomal DNA or retaining it as found episomally in host cells. HIV, hepatitis viruses, herpes viruses, and papillomaviruses are illustrations of viral pathogens that lead to recurrent infections. In vitro and in animal models, CRISPR technology has recently been used to reduce or eradicate persistent viral infections providing new hope for therapies for latent and chronic viral infections. Additional herpesviruses that have been in vitro targeted by CRISPR include Epstein-Barr virus (EBV), which predisposes people to specific lymphomas and nasopharyngeal malignancies, and human cytomegalovirus (CMV), which causes severe sickness in immunocompromised people.

3. Problems in this Field

3.1 Microbial Communities: A Problem

Identifying distinct even mobile genetic components such as plasmids holding within a single species, a variety of resistance genes is difficult, therefore assessing the real-world environment would be even more complex. Another significant problem in the use of CRISPR-Cas-based antimicrobials is predicting community-wide reactions to perturbations Furthermore, the effects of CRISPR/Cas-mediated microbial community elimination are unknown.

3.2 Resistance to CRISPR-Cas

Inactivation of CRISPR-Cas loci could result in resistance. Resistance to CRISPR-Cas can be caused by changes in the cas genes that are responsible for deleting or cleaving target spacers. According to studies, the delivery of flawed CRISPR systems is more likely to result in target sequence modifications.

3.3 CRISPR-Cas System Distribution

Delivery vehicles and how to get around obstacles are another issue with the usage of CRISPR-Cas systems. Although resistance genes are present in many different bacterial species, they are encoded in different places. Furthermore, phages are potent vectors, but their host ranges are limited, posing a significant difficulty in the delivery of CRISPR-Cas systems. Conjugative plasmids are an additional delivery vector., which may be transported between bacteria. However, this delivery technique has certain drawbacks, including a limited host range, low conjugation effectiveness, and absorption of plasmids is challenging.

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Conclusions

CRISPR-Cas systems have proven to be extremely effective tools for gene editing, viral knockdown, and pathogen identification Until now, early research on CRISPR-based therapeutics for infectious diseases has concentrated on the prevention and treatment of chronic viral infections and drug-resistant pathogenic microorganisms. CRISPR-based diagnostics are simple to use, portable, and require less time than the current real-time PCR-based assay. CRISPR-Cas for lethal-self targeting, eradication of particular bacterial strains with care, or the fight against antibiotic resistance and virulent genes could be a future antimicrobial resistance solution. Furthermore, nuclease-deactivated Cas9 opens the door to a new method of interfering with the Expression of genes in bacteria. Despite the hurdles, there is a chance that this technology will be useful in infectious illness therapy.

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