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Clustered Regularly Interspaced Short Palindromic Repeats/ Cas9 Genetic Engineering: Robotic Genetic Surgery

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Abstract

As a novel technology that utilizes the endogenous immune defense system in bacteria, CRISPR/Cas9 has transcended DNA engineering into a more pragmatic and clinically efficacious field. Using programmable sgRNA sequences and nucleases, the system effectively introduces double strand breaks in target genes within an entire organism. The applications of CRISPR range from biomedicine to drug development and epigenetic modification. Studies have demonstrated CRISPR mediated targeting of various tumorigenic genes and effector proteins known to be involved in colon carcinomas. This technology significantly expands the scope of gene manipulation and allows for an enhanced modeling of colon cancers, as well as various other malignancies.

Keywords

CRISPR/Cas9; DNA Engineering; Genetics; Robotics; Biotechnology

Introduction

DNA engineering and selective mutation targeting has been at the forefront of disease modeling and therapeutic intervention techniques. Its applications have a significant impact on understanding the genetic basis behind various carcinomas. Techniques such as miRNA mediated targeting have allowed for a better means of disease diagnosis and prognosis, specifically in colon carcinomas [1, 2]. However, due to the rising costs and tedious

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procedural concerns of DNA engineering, such methods present themselves as an option to only a small niche in the biomedical research community. Recently, the field of gene therapy was revolutionized with the advent of novel programmable nucleases, notably the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system [3, 4].

An innate immune defense mechanism in bacteria

The CRISPR/Cas system is endogenously found in a wide range of bacteria and serves as a defense mechanism against invading viruses. The CRISPR portion of the system functions as an “immune memory” which stores the unique viral DNA sequence in-between sections of regularly interspaced palindromic repeated segments [5]. The CRISPR-associated proteins (Cas) portion of the system, its encoding genes located in close proximity to CRISPR genes, serves to snip the DNA of invading viruses [6]. The relationship between these two components of the system determines the efficacy and potency of the underlying DNA editing. CRISPR converts the unique viral DNA amongst its repeated segments to two RNA moieties: tracrRNA and crRNA, the former determining enzymatic activity and the latter substrate specificity [7]. Cas enzymes bind to this complex and traverse through the entire cell, tightly latching on to any genetic material from invading viruses that match the crRNA. Upon binding, the Cas enzymes cleave the target DNA, preventing further viral replication [8].

Modified use of CRISPR/Cas9 in biomedicine

The precision and potency of this system makes it an incredibly viable tool for biomedical and therapeutic advances. Utilizing a unique Cas enzyme (Cas9) from *streptococcus pyogenes* [9], and a chimeric single guide RNA (sgRNA) containing crRNA and tracrRNA [10, 11], the CRISPR system can be programmed to virtually target any sequence throughout a cell. Specifically, a unique plasmid is created which entails the necessary targeting and modifying instructions for the system on how to behave within *in vivo* cells (Fig 1). In order for Cas9 to bind to and cleave the target substrate, base pairing between target DNA and the 5' end of sgRNA has to occur [11]. Thus, by modifying the sequence at the 5' end of sgRNA, Cas9 can essentially be programmed to target a broad spectrum of sequences and trigger DNA double strand breaks (Fig 2). The nuclease-induced double stranded breaks can be repaired by both homology-directed repair (HDR) and nonhomologous end joining (NHEJ) pathways [12]—allowing for variable length insertion or deletion mutations along the code, ultimately regulating gene expression [7].

CRISPR gene therapy in colon carcinomas

Genome editing via the CRISPR/Cas9 system can potentially easily and efficiently modify genes in a broad spectrum of cell types that have historically been difficult to genetically manipulate—including malignant or pre-malignant cell types. The application of CRISPR as a potential tumor suppressor tool in carcinomas, specifically colon derived, has been a rapidly advancing area in recent literature. Genome wide association studies (GWAS) have identified various nucleic acid changes associated with an increased risk for colorectal cancers [13]. In a recent study by Farnham et al, 66 risk-associated enhancers and

promoters, specifically ones adjacent to the tumorigenic MYC gene, were identified as potential targets due to CRISPR therapy [13]. Using Cas9 nucleases, specific enhancers that harbored risk SNPs for colorectal cancer were deleted and downstream gene deregulation was assessed.

Employing the precise CRISPR guide technology, researchers are able to better analyze potential colorectal carcinoma promoting factors—especially those located in non-coding regions [14]. Additionally, CRISPR mediated genome editing can also be utilized for reverting loss-of-function (LOF) allele mutations. For instance, Newton et al were able to successfully revert the PKC β A509T LOF mutation in DLD1 colon cancer cells resulting in a reduction in tumor volume [15]. The specific CRISPR/Cas9 sequence used was able to effectively restore PKC activity, reducing the severity of colon cancer tumor growth.

The application of CRISPR/Cas9 can transcend beyond pre-clinical stages and even be utilized in reducing radiation sensitivity in colon carcinomas. Studies have used a unique CRISPR/Cas9 variant that targets membrane heat shock protein 70 (mHsp70), which is often overexpressed in the cytosol of tumor cells [16]. It was demonstrated that nuclease-induced down-regulation of the membrane protein had a significant impact on colon carcinoma radiation resistance [16].

CRISPR/Cas9 applications in other fields

In addition to targeting and modulating gene expression in specific subtypes of colon carcinomas, the CRISPR/Cas9 system has a myriad of other applications. Many studies have documented *in vivo* CRISPR-guided genome editing for modeling cancers in which tumor-suppressor genes and oncogenes are targeted in hepatocarcinomas, lung adenocarcinomas, and haematopoietic malignancies [17-20]. The technology also allows researchers to better understand the functional organization of the genome and determine connections between genetic variation and phenotype. A few of the recent advances and potential applications utilizing the CRISPR/Cas9 system are outlined in Table 1.

Concerns with the technology

The immense usability and power of CRISPR inevitably yields concerns and ethical issues underlying its applications. Gene editing and modulation, although with its numerous benefits, also present with many apprehensions throughout the biomedical community. One of the major issues with CRISPR is the ability to ensure that only the target gene is edited and changes hazardous to the individual's health are not introduced throughout the genome. Unwanted off-target site mutations could invariably lead to further malignancies and undesirable phenotypes [25]. In a recent study by Huang et al researchers have demonstrated the ability to perform human embryo editing with CRISPR, potentially triggering ethical dilemmas across the community [26, 27]. Studies have also successfully created CRISPR gene drives, allowing the instantaneous spread and evolution of an edited gene throughout populations. Such discoveries, albeit profound in the field of gene therapy, posit unavoidable political and ethical concerns for commercial usage as well as development.

Future Directions

Recent studies have developed a further means of bettering the CRISPR technology. As an effort to essentially make a sharper “molecular DNA scalpel” of CRISPR, Zhang and colleagues have discovered a novel RNA-guided DNA nuclease known as CPF1 (cite). The advantage of CPF1 over Cas9 is demonstrated through the CPF1 enzyme's ability to unevenly excise target DNA strands using minimal amounts of helper RNA, with even more enhanced precision (cite). The potential cost benefits of using reduced guide RNA, and the experimental implications of enhanced molecular precision will indubitably play a role in future of CRISPR technology.

Conclusion

The CRISPR/Cas9 system boasts precision and ease of use while being an applicable form of therapy for a variety of diseases. The ability to modify genes and their downstream targets allows CRISPR to be greatly effective in assessing colon carcinomas. By using sgRNA guides, tumorigenic colon cancer genes and SNPs in coding as well as non-coding regions can be studied and potentially destroyed. In addition, colon carcinoma promoting proteins such as deregulated protein kinase C isozymes, MYC effector proteins, and various inflammatory promoters, are viable targets for the programmable nucleases [3, 28, 29]. By understanding the implications behind its uses as well as concerns, CRISPR/Cas9 stands at the forefront of biomedical advances and serves to change the landscape of genetic engineering in a broad spectrum of clinical malignancies.

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Fig.1. Essential elements of a CRISPR/Cas9 Plasmid

The CRISPR/Cas9 plasmid is designed to be efficient and specific in its mechanism of action. In order for optimal gene targeting, the plasmid needs to entail the cleaving enzyme from *streptococcus pyogenes*, Cas9. The plasmid also contains the counterpart that dictates the specific sequence Cas9 will bind to, known as the guide RNA. Lastly, the included target sequence ensures that the cas9-guide RNA complex will bind only to elements containing the given sequence.

Fig.2. Schematic representation of the CRISPR/Cas9 mode of action

[A] The designed single-guide RNA (sgRNA) contains the target gene (crRNA) as well as a guide element (tracrRNA).

[B] Cas9 cleaving enzyme recognizes and binds to the sgRNA, creating a complex that facilitates binding of sgRNA and the endogenous target gene.

[C] All endogenous genes containing the target sequence are located and the CRISPR/Cas9 complex binds and cleaves the specific DNA.

[D] Upon excision, the broken endogenous target gene undergoes attempted repair via Non-Homologous End Repair (NHEJ). However, the error prone method results in a non-functional sequence, rendering the target gene as well as its downstream mediators inactive.

Table 1
Applications of genomic engineering through CRISPR/Cas9 technology

Field	Gene Targets	Method Used	Application
Biotechnology – Agriculture [21]	ZmPDS, ZmIPK1A, ZmIPK, ZmMRP4; <i>Zea mays</i> (Maize)	CRISPR/Cas induced mutations in <i>Z. mays</i> protoplasts	Maize genome modification
Medicine – Drug Development [22]	C528S mutation in the XPO1 gene confers cells with resistance to anti-cancer drug Selnexor	C528S specific nuclease; CRISPR	Understanding modes of drug resistance and drug-target interactions
Medicine – Epigenetic Control [23, 24]	Cas9 epigenetic effectors (epiCas9s)	Artificially remove and install epigenetic marks at various loci via Cas9	More precise epigenetic modification than currently used zinc finger proteins and TAL effectors