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The engineered CRISPR-CAS system is a beneficial biological tool for detecting and combating antibiotic resistance microbes

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Nowadays, the quick detection of antibiotic resistance bacteria causes a major problem in the field of the development of new antibiotics against the resistant bacteria. To overcome this problem, genome editing tools like clustered regularly interspaced short palindromic repeats (CRISPR) can be used. The CRISPR-CAS system is useful for targeting and killing antibiotic-resistant bacteria by cleaving resistance genes. It is also used to detect antibiotic resistant bacteria. CRISPR is made up of a single guide RNA and the CAS 9 protein. The single guide RNA is used to guide toward the target sequence, and the CAS 9 protein is an enzyme that cuts DNA and is used in conjunction with the guide RNA. This modified sgRNA contains a complementary sequence to that of the target resistance gene and recognizes the target resistance sequence; therefore, it is cleaved by CAS-9 protein, and the removal of the resistance gene turns bacteria into antibiotic-sensitive ones. One of the delivery systems of CRISPR into bacteria is via bacteriophage.

Keywords: Antibiotic-resistance bacteria, resistance gene, CRISPR-CAS system, CAS 9 protein, single guide RNA, genome editing tool

Introduction

The discovery of antibiotics in the nineteenth century was one of the most significant achievements in medicine, helping the human race overcome the problem of bacterial infection. The discovery of antibiotics was made by Alexander Fleming. At present, the misuse of antibiotics has led to the origin of antibiotic resistance and the bacteria that have the capability to show multidrug resistance is one of the challenges for the scientists. To control antibiotic resistance bacteria, the first step is the accurate detection of antibiotic-resistance bacteria. There are some detection methods available, such as PCR and immunoassay, but they are time-consuming, complex, and require specialized instruments. Also, to solve this problem, CRISPR is used to provide a promising solution. CRISPR was first discovered in E. coli as a defense mechanism for bacteria against foreign DNA by researchers. The CRISPR guide RNAs in the engineered CRISPR-CAS system are designed to direct the CAS 9 protein toward the resistance or virulence gene, where it will cleave the resistance gene, reverting bacteria to antibiotic sensitive bacteria.

CRISPR structure

Clustered regularly interspaced short palindromic repeats-CAS9 is a genome editing tool that consists of a single-guide RNA with two components and a CRISPR-associated endonuclease.

SgRNA: A single guide RNA is a combination of tracrRNA and crRNA. crRNA is a spacer sequence that directs the CRISPR-CAS9 system to the target site and binds to tracr RNA. When tracr RNA binds to crRNA, a functional guide RNA is formed for CAS 9 to recognize and provide guidance.

CAS 9: CAS 9 is an endonuclease enzyme that functions as a pair of molecular scissors for the target DNA sequence. CAS 9 contains six domains: Rec I, Rec II, the bridge helix, Ruv C, HNH, and Pam.

Mechanism of CRISPR against the antibiotic resistance gene

Clustered regularly interspaced short palindromic repeats, which contains single-guide RNA, and CAS 9 endonuclease play a very important role in manipulating genes. In the case of an antibiotic-resistant microbe, the CRISPR-CAS system's single guide RNA is modified to be complementary to the antibiotic-resistance plasmid and gene of the bacteria.

The engineered single guide RNA will guide the CAS9 endonuclease toward the target antibiotic resistance gene or plasmid. The delivery of CRISPR CAS 9 to the microbial population by a polymer CRISPR nanocomplex or bacteriophages following successive entry into an antibiotic resistant microbe, the CRISPR's guide RNA directs the CRSPR-CAS system to the target site of the antibiotic resistance gene, where the Ruv C and HNH domains of the CAS9 protein cleave the antibiotic-resistance gene, resulting in the removal of the antibiotic-resistance gene and reversion of the microbe to antibiotic sensitivity.

Conclusion

Engineered CRISPR is going to be a useful tool in the upcoming future, and it got significant attention for killing antibiotic-resistance bacteria and reverting them into antibiotic-sensitive bacteria. Moreover, this CRISPR- CAS system can target only pathogens with the help of modifications in a single guide RNA according to the target gene and preserve beneficial bacteria in the microflora.

Author contributions

The author confirms being the sole contributor of this work and has approved it for publication.

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