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Perspective

Exploiting CRISPR/Cas system in controlling antibiotic resistance of bacteria

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Abstract
The emergence and spread of antimicrobial resistance is a threat to human health, therefore there is a need to explore new strategies to combat antibiotic-resistant bacteria. Antimicrobial resistance genes are mainly transmitted through horizontal gene transfer (HGT) mediated by transposable elements, phages, and plasmids, which leads to the emergence of multidrug-resistant bacteria. Clustered Regularly interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated proteins (Cas) protect archaebacteria and eubacteria from potentially harmful foreign DNA such as plasmids, transposable elements, and phages. Several studies have introduced the CRISPR/Cas system as an innovative and effective strategy to trigger antibiotic resistance and virulent genes in bacteria. Therefore, this study aimed to introduce the CRISPR-Cas system in controlling antibiotic resistance of bacteria. We hope exploiting CRISPR/Cas could provide a bright future for the treatment of disastrous infections with resistant and virulent organisms.
Keywords: CRISPR/Cas system, Antibiotic-resistant bacteria, Genome editing; Resensitization

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CRISPR-Cas systems and antimicrobial resistance

The emergence and spread of antimicrobial resistance is a threat to human health, therefore there is a need to explore new strategies to combat antibiotic-resistant bacteria. Antimicrobial resistance genes are mainly transmitted through horizontal gene transfer (HGT) mediated by transposable elements, phages, and plasmids, which leads to the emergence of multidrug-resistant bacteria.[1] Clustered Regularly interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated proteins (Cas) protect archaebacteria and eubacteria from potentially harmful foreign DNA such as plasmids, transposable elements, and phages.[2,3] Several studies have introduced the CRISPR/Cas system as an innovative and effective strategy to trigger antibiotic resistance and virulent genes in bacteria.[4-6] However, there are several challenges when using CRISPR-based research to eliminate antibiotic-resistant bacteria including lack of knowledge, off-target effects, delivery methods, resistance developments, and ethical considerations. Bacteria are live organisms and could have the ability to develop resistance to CRISPR-based treatments. They can evolve mechanisms to evade the CRISPR system, making it less effective over time. It is needed to stay ahead of bacterial resistance and continuously adapt the CRISPR system to overcome these challenges. In addition, CRISPR-Cas9 has the potential to cause unintended genetic changes in bacteria, leading to off-target effects. Ensuring the specificity of the CRISPR system and minimizing off-target effects is a significant challenge. There is still much to learn and need a deeper understanding of the mechanisms involved and how to optimize the system for different bacterial strains. Genomic sequence analysis of pathogenic bacteria has shown that there is a significant inverse association between the acquisition of antibiotic-resistance genes and the presence of CRISPR loci in some hosts.[1,7] However, the inverse association demonstrated that the use of antibiotics instinctively selects antibiotic-resistant strains with dangerous genomic protection.[8] The results demonstrated that CRISPR loci could be the main barrier against transferring selective pressure traits. The CRISPR loci can be transferred into the target cells by plasmids, extracellular vesicles, nanoparticles, and phages. Unlike traditional antibiotics, the CRISPR-Cas system could eradicate pathogenic and resistant bacteria without affecting other bacteria in the bacterial complex population. The CRISPR system (i.e., Cas9, Cas12, Cas13, and Cas14) can be used to detect and limit bacterial resistance to antibiotics.

Cas9 and Cas13 enzymes

The most popular and widely used *cas* genes are *cas9* and *cas13a*. The difference between these genes was that CRISPR-Cas13a is a sequence-specific bacteria-killing drug on both their chromosome and a plasmid, but CRISPR-

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Cas9 kills target genes on bacterial chromosomes. In addition, Cas13a targets bacterial mRNA, but Cas9 directly cleaves bacterial DNA. Therefore, Cas13a has excellent potential as an antibacterial agent due to its ability to target mRNA, which has lower mutagenic activity.[9] Delivery of the CRISPR-Cas system to bacteria still faces challenges such as difficulty in introduction and degradation by bacterial intracellular nucleases or proteases. In addition, effective delivery to the target bacteria plays an important role in the bactericidal effect of the CRISPR-Cas system. Simultaneous targeting conserved sequences and inhibiting multiple endogenous genes in a single cell by the CRISPR-Cas system could solve the problem of high diversity of antibiotic resistance genes and a wide range of bacteria. Plasmids can facilitate bacterial adaptation to environmental stressors. Small high-copy plasmids (100–300 copies/cell) play a critical role in antibiotic resistance. Tagliaferri et al[10] demonstrated that the CRISPR-Cas9 system only eliminated high-copy plasmids from some bacteria, and persistent cells remained in this bacterium which is due to persistent cells in an analogy to bacteria persisting antibiotic treatment.

Antimicrobial perspective

Targeted antibacterial plasmids (TAPs) carry the CRISPR-Cas system and the gRNA sequence. They are effectively transferred to bacteria and affect bacteria containing the DNA sequence complementary to the carried gRNA. In addition, designing reliably identifying gRNA that could trigger one or more target strains can enable TAPs to be used for a wide range of bacteria. In the future, modification of intestinal flora using CRISPR-Cas is proposed as an antibacterial agent. In addition, several studies have shown that bacteria are immunized to resist the acquisition of antibiotic resistance by carrying CRISPR-Cas.[9,10] CRISPR-carrying pheromone-responsive plasmids (PRPs) could be used to target resistant bacteria in the gut flora and eliminate antibiotic resistance.[11] PRPs are specified for *Enterococcus faecalis* with naturally high rates of conjugation and comprehensively infiltrate *E. faecalis* populations.[12] Therefore, designing a CRISPR-Cas-based “vaccine” could be used to prevent the transmission of antimicrobial resistance through HGT, which is a method worthy of in-depth study by scientists. VADER, an environmentally aimed degradation system for eliminating antimicrobial resistance genes, was designed based on the cas9 and the broad-spectrum IncP conjugation machinery system. VADER was used in a conjugation reactor to sensitize antibiotic resistance bacteria with 100% efficiencies.[13]

Conclusion

CRISPR-Cas system may have potential applications to promote sensitizing drug-resistant pathogens and control drug resistance gene transfer. Designing efficient methods to reduce any off-target could overcome limitations in the application of the CRISPR-Cas system. As well as, an efficient delivery system plays an important role in the effectiveness of this system in clinical interventions, and advances in nanoparticles and the use of phages may provide a better solution. Exploiting CRISPR/Cas could provide a bright future for the treatment of disastrous infections with resistant and virulent organisms. These systems could develop innovative antibiotics that can effectively combat multidrug-resistant infections and differentiate between valuable and pathogenic bacteria.

Authors’ Contribution

Conceptualization: Pourya Gholizadeh.
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Methodology: Pourya Gholizadeh, Hossein Samadi Kafil.
Supervision: Hossein Samadi Kafil.
Writing–original draft: Pourya Gholizadeh, Elnaz Faghfuri, Hossein Samadi Kafil, Khudaverdi Ganbarov.
Writing–review & editing: Pourya Gholizadeh, Elnaz Faghfuri, Hossein Samadi Kafil, Khudaverdi Ganbarov.

Competing Interests

None to declare.

Ethical Approval

This study was approved by Local Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran with reference number IR.TBZMED.REC.1397.188.

Data Availability Statement

Not applicable.

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