OPINION PIECE

OPEN ACCESS

Evading Evasion: How Phages get around CRISPR-Cas

Mostafa Mohammed Elsabagh

McMaster University, Biomedical Discovery and Commercialization, Class of 2020

ARTICLE INFORMATION

Recieved 14 October 2018
Accepted 11 November 2018

Available online:
26 November 2018

Editors:
Ishita Paliwal
Caitlin Reintjes

Layout Editor:
Youssef El-Sayes

ABSTRACT

The CRISPR-Cas9 system has paved the way for realising gene-editing, but its main weakness lies in its potential for off-target effects. Studies into phages reveal that they express "anti-CRISPR" proteins which if harnessed, could provide us with the solution to this lack of control.

Keywords: Phage, CRISPR-Cas, anti-CRISPR, antimicrobial resistance

INTRODUCTION

Today, the CRISPR-Cas system has become the most promising source of enthusiasm in biotechnology. It has had a fair share of attention with wide-ranging, controversial discussions of gene-editing. While the CRISPR-Cas system may be the gene-editing tool we had hoped for, we should not overlook the prospect of off-target effects. Writing in Nature Microbiology, Hynes et al.[1] reports how this problem may soon be surmounted by studying how phages evolved a method to evade CRISPR-Cas.

WHAT IS CRISPR?

The CRISPR-Cas system is a bacterial defence mechanism against a prophage, the integrated genome of an invading phage[2]. It accomplishes this by recognising the foreign DNA sequence and cleaving it from the bacterium's genome[2]. The CRISPR-Cas is a riboprotein complex, made up of a crRNA, which recognises foreign DNA sequences by binding to them, and Cas, an endonuclease which cleaves DNA[2]. Scientists have been able to modify the crRNA to cut any sequence they choose, the essence of gene-editing.

CRISPR's PROBLEMS

However, the CRISPR-Cas system may occasionally cleave sequences they are not intended to. This occurs despite the absence of full complementarity, and for biological research and gene therapy, these off-target effects are a serious concern[3]. In human cell studies, up to five mismatches can occur between the target and crRNA without a noticeable change in editing activity [3]. As a result, many scientists have been developing methods to detect off-target effects[3]. However, modification of the guide RNA to lower mutation rates can cause a decrease in binding or cuts by Cas in intended regions[3]. Hence, Hynes et al.[1] looked to another avenue to overcome this issue.

SEARCHING FOR ANTI-CRISPRs

While bacteria battle phage infections with the CRISPR-Cas system, many phages possess genes coding for anti-CRISPR (Acr) proteins to inhibit CRISPR-Cas[1]. Hynes and colleagues sought to find Acr's in phages targeting *Streptococcus thermophilus*, a member of the same genus of the original source of CRISPR-Cas. Using the phage-first method, they screened phages for their ability to bypass bacterial immunity conferred by CRISPR-Cas[1]. *S. thermophilus*, is frequently challenged by phages to produce phage-resistant variants[1]. This is analogous to "immunizing" them against strains of phage. It was observed that some phages did not result in immunized bacteria, and thus research was focused on five of these phages[1].

These candidates looked promising, but failure of CRISPR-Cas in the bacteria is not the only possible reason for their death. It could be that the phages had taken over the bacteria faster than CRISPR's ability to defend against them[1]. Hence, Hynes and colleagues created a new strain of *S. thermophilus* containing genes common to the five phages, such that its CRISPR-Cas system would readily prevent invasion from any of these phages. Despite this, one phage: D4276, was consistently successful in destroying this strain[1]. This was the candidate Hynes was looking for.

Having narrowed down the search, Hynes and colleagues created strains of *S. thermophilus*, each with acrIIA5, a novel Acr. The effect of this gene was a six-fold increase in sensitivity[1], indicating high efficacy. Hynes and colleagues attempted to challenge the Acr-bearing strain against other unrelated phages, to which it was immune, and they observed sharp increases in sensitivity[1]. The findings were clear and promising: acrIIA5 is an effective anti-CRISPR. Hynes and colleagues are now working on the analysis of acrIIA5's mechanism of action and structure[4].

ARE ANTI-CRISPRs THE RIGHT PATH?

While their discovery illustrates evolution resulting from phage-host interaction, Acr's could be essential in the development of several biotechnological applications. This includes gene-editing, as Acr's will allow for modulation of CRISPR-Cas systems. Studies in human cells have shown that with a correct time delay, adding an Acr can lead to a significant reduction in off-target edits by Cas9, while retaining target specificity, leading to improved accuracy in gene-editing[5,6].

It is natural to rush towards Acr's as a solution to the off-target effects. However, an area of exploration with low activity is antimicrobial resistance by bacteria. CRISPR-Cas evolved in bacteria to destroy invading phages to prevent plasmid integration. However, studies have shown that some bacterial species lose this ability if it impedes acquisition of beneficial bacterial DNA, including those coding for antimicrobial resistance[6]. In fact, some lineages do not even possess a CRISPR-Cas system[6]. Therefore, if some lineages benefit from the absence of this system, it may be possible that a bacterial strain with a CRISPR-Cas system acquiring an Acr could improve its ability to attain and integrate plasmids from antimicrobial-resistant neighbours. This is alarming when we consider a field of CRISPR-Cas antimicrobials. This endeavor harnesses CRISPR-Cas gene-editing to remove genes conferring antimicrobial-resistance in bacteria[7]. If, through the increased use of Acr's in one application, strains of bacteria acquire these Acr genes to evade CRISPR-Cas antimicrobials, will we have closed a newly opened avenue in the battle against antimicrobial-resistance? It becomes clear that additional research into these phenomena is necessary and that Acr's will provide us with great power, but clearly, with great power comes great responsibility.

REFERENCES

(1) Hynes, A. P. et al. An anti-CRISPR from a virulent streptococcal phage inhibits Streptococcus pyogenes Cas9. Nat. Microbiol. 2, 1374–1380 (2017).

(2) Jiang, F. & Doudna, J. A. CRISPR – Cas9 Structures and Mechanisms. Annu.Rev.Biophys 46, 505–529 (2017).

(3) Peng, R., Lin, G. & Li, J. Potential pitfalls of CRISPR/Cas9-mediated genome editing. FEBS J. 283, 1218–1231 (2016).

(4) Hynes, A. P. et al. Widespread anti-CRISPR proteins in virulent bacteriophages inhibit a range of Cas9 proteins. Nat. Commun. 9, 1–10 (2018).

(5) Shin, J. et al. Disabling Cas9 by an anti-CRISPR DNA mimic. Sci. Adv. 3, (2017).

(6) Pawluk, A., Davidson, A. R. & Maxwell, K. L. Anti-CRISPR: discovery, mechanism and function. Nat. Rev. Microbiol. 16, 12 (2017).

(7) Pursey, E., Sünderhauf, D., Gaze, W. H., Westra, E. R. & van Houte, S. CRISPR-Cas antimicrobials: Challenges and future prospects. PLoS Pathog. 14, e1006990 (2018).