CRISPR/Cas9: Revolutionary gene editing tool requires regulation

M. Atta, A. Clapperton, Y. Lagman, D. Phan, C. Reyes, A. Shankar

Department of Biological Sciences, University of Toronto Scarborough

Introduction

CRISPR/Cas9 is a bacterial immune system that cleaves DNA of infecting viruses (1). This allows the bacteria to recognize and target the virus' DNA during the next infection (1). The CRISPR/Cas9 system has been modified to edit the genomes of other organisms. A single guide RNA (sgRNA) guides Cas9, an endonuclease, to cleave a specific sequence (1). Once the DNA has been cut, it can be repaired or replaced with a modified sequence (1). This technique has been researched as a possible treatment for HIV and has potential for use in conservation biology and pathogen control.

CRISPR & HIV treatment

Current treatment for HIV is antiretroviral therapy (2). This is not a cure as the HIV provirus remains integrated in the host DNA (2). CRISPR/Cas9 can be used to excise the HIV provirus from infected cells (2). Research on potential off-target effects in the human genome has been conducted (Fig. 1) (3). Adeno-Associated Virus (AAV) can be used as a vector for delivery of saCas9/sgRNA (2). This was successful in excising the HIV provirus and reducing mRNA expression of HIV proteins in mice (Fig. 2) (2).

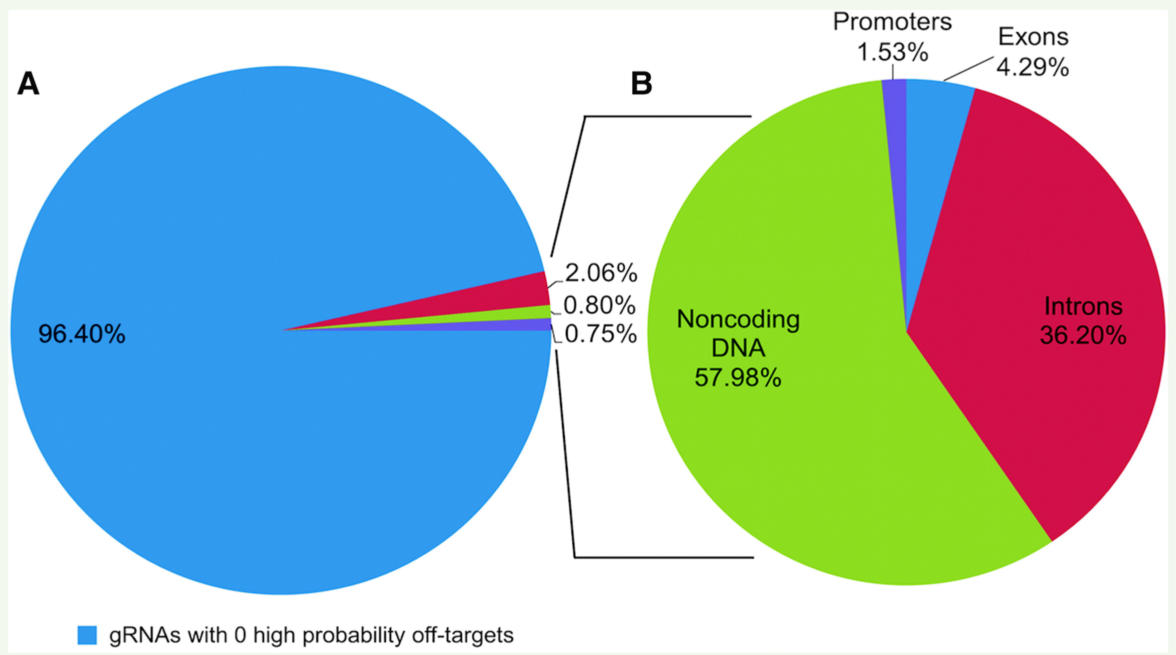

Fig 1. Probability of off-target effects of sgRNA targeting the HIV-1 provirus in the human genome (3).

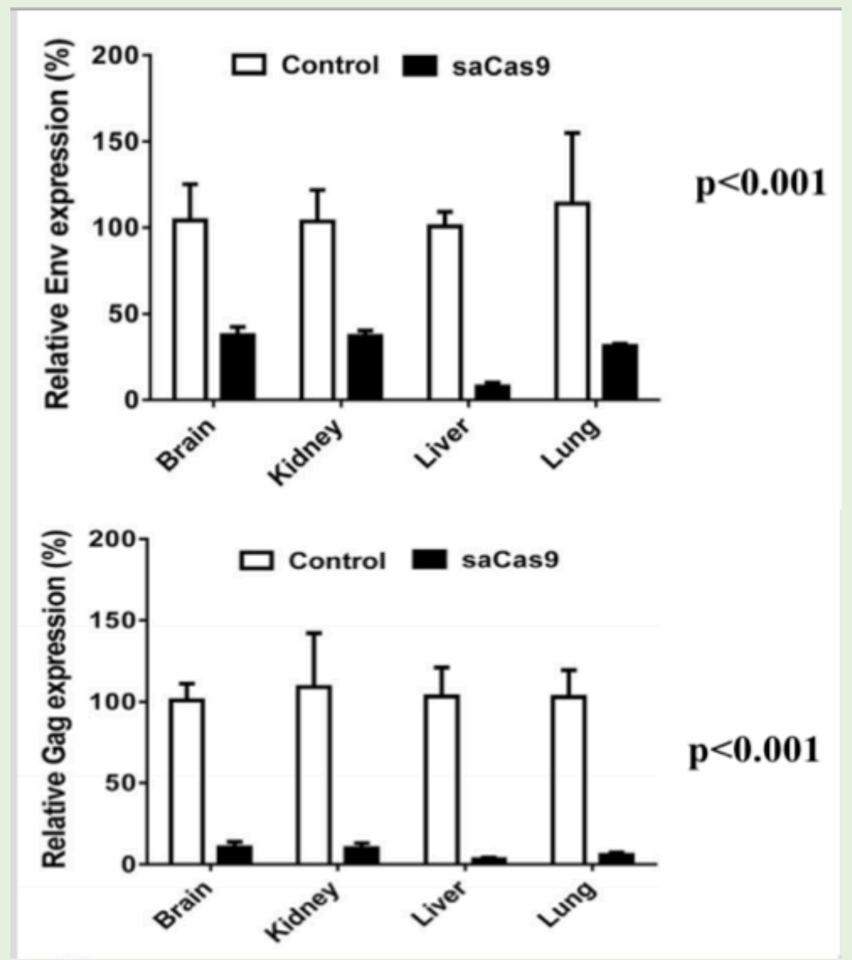

Fig 2. Expression of mRNA encoding HIV proteins Env and Gag in tissues of HIV infected mice. The experimental group was treated with saCas9/sgRNAs in an AAV vector (2)

CRISPR & plant breeding

CRISPR can also be used in modifying plant genomes for plant breeding. One study used Cas9/sgRNA to induce mutations in the apple phytoene desaturase (PDS) gene (4). A mutated PDS gene results in albinism (Fig. 3) (4). The study discovered that mutations should be induced in the sub-epidermal layer to ensure the traits are expressed in the fruit (4). While there are safety measures currently in place for transgenic plants, regulations must be created for CRISPR-mutated plants (5).

Fig. 3 Left: wild-type apple plant, Middle & Right: mutations introduced in PDS gene by Cas9/sgRNA (4).

CRISPR & pathogen control

CRISPR can be used to edit genomes of pathogens to control their virulence. In a recent study, Cas9/sgRNAs were injected into the zoospore vectors of *Aphanomyces invadans*, a pathogen that causes epizootic ulcerative syndrome (EUS) in fish. Their aim was to induce a mutation in the genome of *A. invadans* in order to inhibit the production of its virulence factors (6). The fish inoculated with the Cas9/sgRNA treated zoospores did not show any signs of EUS (Fig. 4) (7).

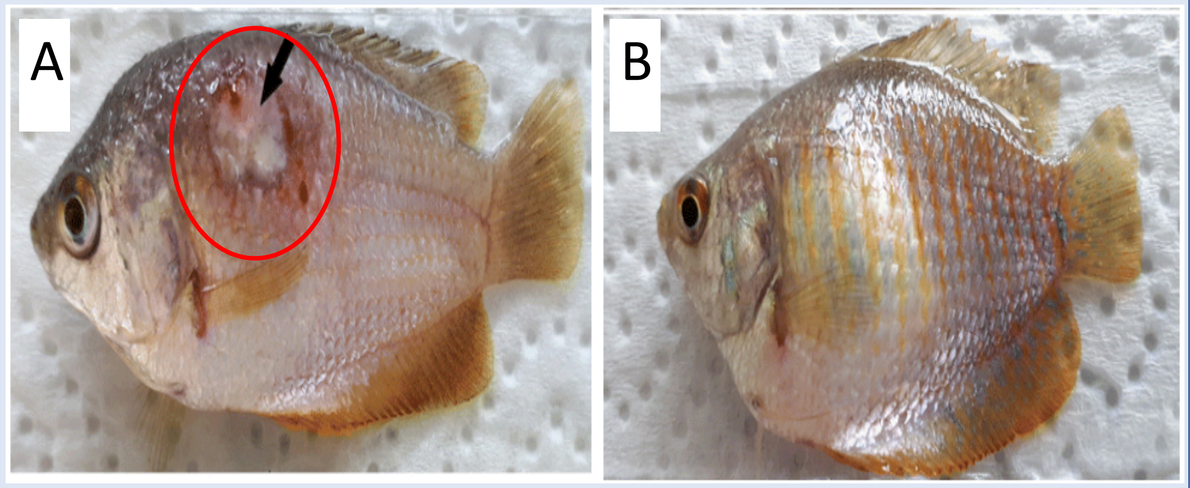

Fig. 4 *T. lalius* are injected with *A. invadans* zoospores. (A) Fish injected with untreated zoospores show evidence of EUS after 7 days. (B) Fish infected with sgRNA/cas9 treated zoospores show no signs of EUS infection after 7 days (7)

CRISPR & conservation biology

Low genetic diversity is a challenge faced by populations that have experienced bottleneck, as seen with the black-footed ferret (Fig. 5). While this species was rescued through captive breeding programs, their low heterozygosity makes them highly susceptible to sylvatic plague in the wild (8). CRISPR can be used to edit alleles conferring resistance in domestic ferrets into the genome of these re-established populations (8). Without CRISPR, this would normally require selection for resistance traits when breeding; which can cause the loss of evolved traits and decrease their adaptation to the current environment (8).

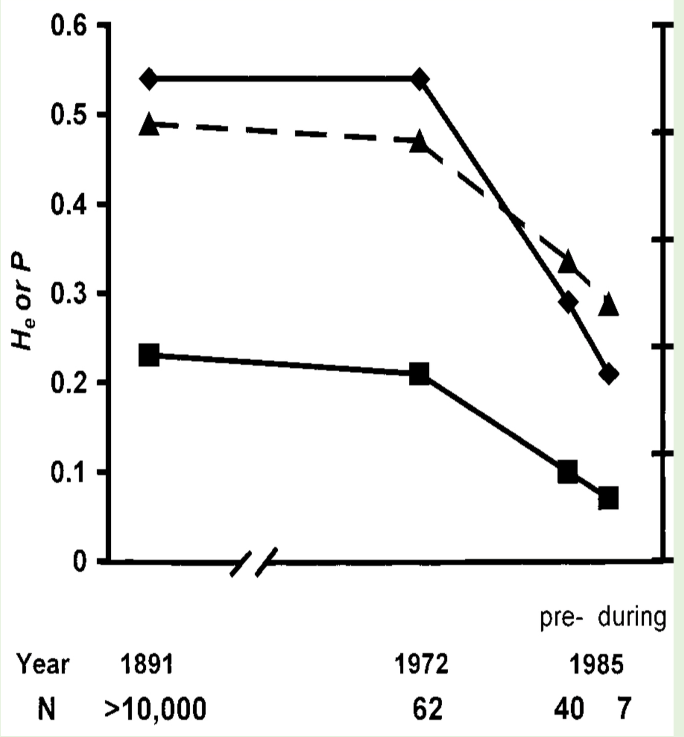

Fig 5. Genetic diversity estimates of black-footed ferret population using frequency of heterozygosity (H) (squares) and polymorphism (P) (diamonds). Severe bottleneck occurred due to heavy hunting and viral infections (9).

Conclusion

CRISPR has resulted in a major expansion of biological research. CRISPR can be used to improve the resilience of crops and species with low genetic diversity. Additionally, it has significant applications in controlling infections by viruses and other pathogens. Further research involves developing efficient and accurate delivery mechanisms to reduce off-target mutations. As well, an increased understanding of the downstream effects of using CRISPR to create changes in the germline will help move research to human clinical trials.

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