Gene drive system based on CRISPR-Cas9 in mosquito control

Yutian Liu [1, †], Liru Wang [2, †] and Yaoran Yu [3, *, †]

[1] School of Hong Kong Polytechnic University, Hong Kong

[2] School of life sciences and technology, Harbin Normal University, Harbin, China

[3] School of life sciences, Nanjing Agricultural University, Nanjing, China

* Corresponding Author Email: 11319101@njau.edu.cn

[†]These authors contributed equally.

Abstract. Malaria, which is primarily transmitted by female mosquitoes, is increasing recognized as a global public health problem. Since the number of malaria cases remains high, with more than 200 million cases globally in 2019 alone, the development of new drugs and corresponding prevention and control measures are in urgent need. With the development of CRISPR/Cas9 technology in recent years, gene editing technology plays an increasingly significant role in mosquito control. A new generation of gene drive based on CRISPR/Cas9 has been applied into improving the heritability of specific genes in mosquito populations, which can be exploited to control mosquito and combat diseases transmitted by mosquito. However, this technology still faces challenges of ecological environment safety, ethical safety and technical effectiveness. This review introduces the current research status of gene drive systems for mosquito control using the CRISPR/Cas 9 system, and summarizes the current problems and solutions in this research field.

Keywords: CRISPR/Cas9; gene drive; mosquito control; disease control.

1. Introduction

The main vector of malaria transmission is *Anopheles gambiae* in sub-Saharan Africa. They carry a large number of malaria parasites and have extremely strong reproductive ability. Millions of people are infected with malaria every year after being bitten by this kind of mosquito, which causes the most deaths in humans among animals [1]. Scientists around the world are working to develop a new technology to eradicate malaria, reduce human deaths and free the African continent from the horrors of malaria.

During transmission, the malaria parasite uses the mosquito as a vector to interact with its intestine, hemolymph and salivary glands. Since only female Anopheles gambiae mosquitoes transmit malaria, while males have no effect, scientists have explored a way to improve the sex ratio. They use gene-drive technology to make mosquitoes produce more male offspring, resulting in fewer or even no female mosquitoes to eradicate Anopheles gambiae.

1.1. Gene driving system

The first gene driving system proposed was the gene driving system based on HEG (homing endonuclease genes) [2]. HEG is an endonuclease that can recognize long specific sequences. As long as the recognition sequence is complete, it can cleave and integrate its coding sequence into the cleavage site. The sequence of the integrated HEG cannot be cut again because its recognition site is destroyed, while the sequence of another homologous chromosome is recognized and cut because its recognition site is complete, and then the chromosome of the integrated HEG is used as a template for repair. Finally, both homologous chromosomes have HEG series. This process is also called homing [3]. Because any nuclease has a long recognition sequence, it can be used to build a similar gene drive system, and subsequent gene drive systems including CRISPR / cas9 can be built by similar principles. However, the poor stability of HEG technology itself leads to its low efficiency.

With the improvement of CRISPR/Cas9 technology, the current CRISPR technology gradually has the assets of simple operation, inexpensive, strong stability and high efficiency when compared

with the traditional HEG technology [4]. The CRISPR/Cas9 based gene drive system utilizes the homologous recombination repair (HDR) of CRISPR/Cas9 technology (Fig.1), and can identify and cleave sites corresponding to the protospacer adjacent motif (PAM) structures on target genes by designing suitable single guide RNAs (sgRNAs) in the experiment, which has greatly expanded the usable range of this technology in practical applications [5]. Current approaches to prevent mosquito from spreading diseases using the CRISPR/Cas9 gene drive system include some different research ideas: by inhibiting the mosquito population to directly reduce the number of harmful female mosquitoes; by controlling the way of pathogen-infected mosquitoes; by changing the growth state and phenotypic modification of mosquitoes to inhibit pathogenic infection, etc.

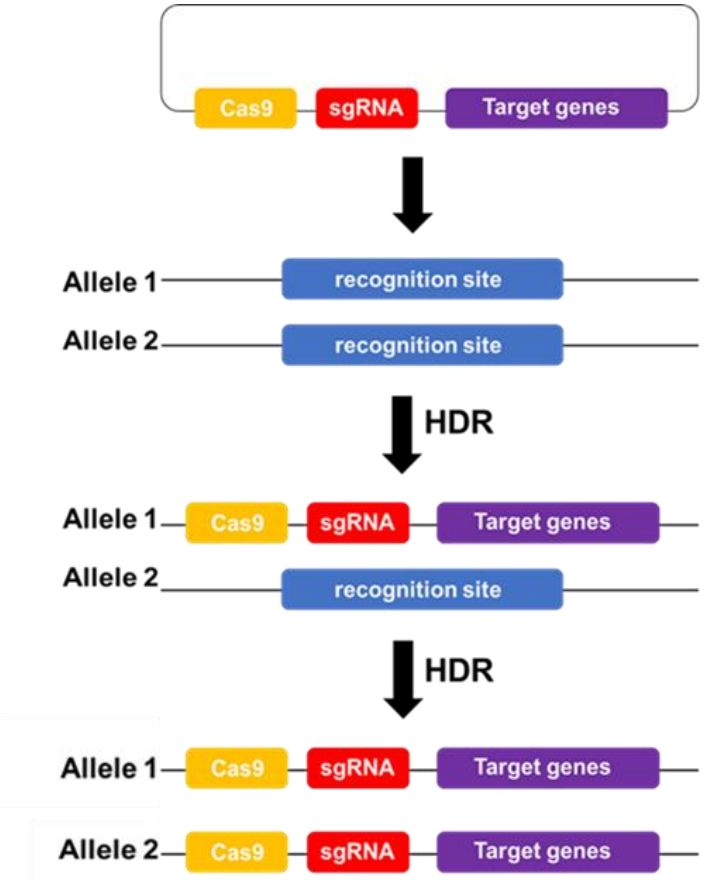

Fig. 1 The principle of gene drives based on CRISPR/Cas9 system

1.2. CRISPR/Cas9 system

The CRISPR / Cas9 system includes three parts: specific CRISPR-derived RNA (crRNA), Trans-activating crRNA(tracrRNA), CRIPSR associated sequences 9(Cas9). Overall, the CRISPR-Cas reaction includes 3 stages. The first is called the adaptation stage. The Cas1-Cas2 protein complex excises the protospacer in the target DNA sequence and inserts it between the CRISPR repeats at the 5' end to generate a new spacer. The second stage is the expression and processing stage. The CRISPR sequence transcribes the precursor crRNA (Pre- crRNA) together with the new spacer, and is processed into mature crRNA by the special Cas9. The third stage is the interference stage, crRNA binds with cas9 protein to form a complex that recognizes the target sequence before PAM and activates the HNH(Histine-Asparagine-Histine) and RuvC domains in cas9 to generate cleavage functions, thereby generating double DNA strand breaks (DSBs), then, targeted insertion, knockout, or modification of the genome can be achieved through self-repair methods such as non-homologous end joining (NHEJ) or homology-directed repair (HDR) [6].

The development of the CRISPR-Cas9 system has enabled engineered nucleases to cut in specific genomes. The guide RNA complementing the DNA target site can direct the activity of the Cas9 endonuclease to this sequence, thus, without complex protein engineering and screening process, any

selected DNA sequence can be tested. edit. In addition, the specificity and adaptability of CRISPR-Cas9 enables faster control of insect vectors. Compared with traditional gene drive systems, CRPSR-Cas9-based gene drive sites have greater flexibility [7].

2. CRISPR/Cas9 system in mosquito control

2.1. Use CRISPR to modify and screen mosquito phenotypes

Li et al. studied four species of mosquitoes in 2018, including *A.coluzzii* wild *A.gambiae* white-eye mutant strain M2 (MRA-105), *A.albimanus* wild strain STECLA (MRA-126) and *A.funestus* wild strain FUMOZ (MRA-127). The edited sgRNA was injected into the egg by microinjection to obtain the mosquito embryos after incubation. Therefore, mature mosquitoes were analyzed by PCR to assess whether the gene editing was successfully targeting the eye-specific gene White protein (The ID of the gene scripts of the three anopheles are:ALB006905-RA, ACOM037804-RA, AFUN003538-RA） to produce mosaic effect. The result also confirms that the CRISPR-Cas9 system is able to be applied into editing those previously uneditible species [8].

Meanwhile, Liu et al. firstly used the CRISPR-Cas9 technology in Aedes albopictus to target two genes of canine hydroxylase (kynurenine hydroxylase, kh) and dopa pigment-convertingase (yellow) and produced a feasible Cas9 system in Ae with visible phenotypes in 2018 [8]. After Cas9 and sgRNA were injected into the back end of the anterior embryo, 30%-50% of infertile individuals produced alleles that could not comprise existing kh and yellow mutations. Both pupae and adults of G1 have complete loss of eye and body pigments, indicating that the gene is highly heritable. This proves that the CRISPR-Cas9 system is more efficient compared with earlier gene editing technology such as HEG, which determines the basis for the practical application of other gene-driven technologies in related species.

2.2. CRISPR-Cas9 gene-driven system for inhibiting mosquito populations

The main mode of disease transmission is via pathogens carried by individuals who are infected by mosquitoes bites. The replication of pathogens in mosquitoes gives them the ability to spread diseases to other uninfected individuals by biting, and thus causes the spread of diseases. In the mosquito population, only female mosquitoes will bite after mating, leading to the spread of the disease. Therefore, the most effective approach to restrain the spread of diseases is controlling the number of female mosquitoes [9].

Hammond et al. destroyed three genes of Anopheles gambiae (AGAP005958, AGAP011377 and AGAP007280) in 2016, resulting in the death of X-carryosomal sperm. Meanwhile they inserted each locus CRISPR-Cas9 gene-driven construct to give recessive female sterility phenotypes, thus greatly reducing the number of fertile females [10]. They observed the gene-driven system on the target gene and found that its genetic rate was 91.4% to 99.6% [5].Later in the experiment, it was confirmed that the CRISPR-Cas9 construct for one of the locus AGP007280 met the minimum requirements for female reproduction in insect populations through population modelling and cage experiments.

Crisanti et al. destroyed the reproductive gene named doublesex of a group of Gambian Anopheles mosquitoes with a 100% efficiency in 2018. In human Plasmodium, Agdsx is composed of two distinct gene segments, dsx-female (AgdsxF) and dsx-male (AgdsxM). Unlike men, the female gene contains one exon (exon 5), the sequence of which is very conserved in all mosquitoes. By blocking the 4-exon 5 junction, the CRISPR-Cas9 system prevents AgdsxF production without affecting male development and reproduction.Females with destroyed allele homozygous showed bisexual phenotype and complete infertility [11].

2.3. CRISPR-Cas9 gene-driven system for controlling pathogen infections of mosquitoes

Plasmodium have a complex journey in the mosquito vectors infected by them to successfully be spread, including interaction with many molecules in the vector's intestine, hemolymph and salivary

glands. This process relies on many host factors. Modifying host factors in mosquito-borne organs through a gene-driven system can effectively reduce the transmission of malaria parasitesquito vectors and reduce the incidence of infection.

Dong et al. studied the fibrinogen-associated protein family (FREP, also known as fibrinogen domain immunolectin [FBN]), the gene silence of FREP1 leads to the inhibition of the development of Plasmodium falciparum in the middle intestinal tissue in 2018 [8]. FREP1 antibody can inhibit Plasmodium vivax and Plasmodium falciparum in Anopheles mosquitoes, suggesting that FREP1 is a promising immunosuppressant (TBV). Although this TBV has been exploited, we here use disruption of the FREP1 gene to explore its use as a host factor, and its potential as a transmission-blocking pathway, to develop a novel strategy against malaria. In this study, knocking out the FREP1 gene effectively reduced the susceptibility of mosquitoes to human malaria parasites, and had a large negative impact on mosquitoes' own growth and blood collection ability [12].

2.4. Other relevant CRISPR-Cas9 gene-driven system research

Lanzar et al. reported the results of the 1,280 genomes of Anopheles mosquitoes in the Gambia in 2020. About 90% of the protein-coded CGD target gene in the natural population have been determined to include at least one target point without DRA

(DR-alpha) at a frequency of ≥ 1.0%. This gene leads to the abundance of conservative targets in the mosquito genome and the inherent flexibility inherent in CGD design, which exist in permanent genetic variations of mosquito populations. The problem that the DRA will not be conducive to the deployment of this technology in the population modification strategy has been solved, eliminating concerns for the subsequent further use of conservative sites in relevant mosquitoes and the development of corresponding effective gene-driven systems.

Kyrou et al. reported to target the destruction of the male Anophet 4-exon 5 boundary in the female Anophere gene transcript by using CRISPR-Cas9 technology to prevent the formation of the bisexual gene AgdsxF, which has no impact on the reproductive ability of male Anopheles in 2018. However, the sabotaged female homozygous represent a bisexual epigenesity and complete infertility [4]. The cage test reached a 100% prevalence rate in the 7-11th generation, leading to the collapse of the population.

Some authors have suggested that CRISPR-Cas9-based gene drives (CGDs) are likely to fail due to persistent genetic mutations. This includes non-truncated alleles that are not directed to RNA recognition. A study of the effects of DRA on CGD in a natural population of wheat beetles showed that specific rare alleles can suppress or eliminate driver efficiency. In general, genetic variants have a greater impact on CGD than de novo mutations. Because of the interest in CGD research, there is a need to study the polymorphisms of these important mosquito genomes and to evaluate possible targets encoded by CRISPR-Cas9 [13].

3. Conclusions

The CRISPR/Cas9 system has played an important role in mosquito management and mosquito-borne disease control. It can screen mosquitoes by modifying mosquito phenotypes, such as mosquito eye color and fluorescence characteristics, and evaluate sgRNAs at the same time. Whether it can successfully act on mosquito DNA. From the perspective of malaria control, gene editing can also reduce the survival rate of Plasmodium in mosquitoes, which has a greater negative impact on the mosquito's own growth and blood collection capabilities. In addition, some other side studies, such as the study of 1280 genomes in Anopheles gambiae, have determined that about 90% of the protein-coding CGD target genes in the natural population include at least one target site without DRA for subsequent further utilization of conservation in related mosquitoes loci and the development of corresponding efficient gene drive systems eliminates concerns.

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