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The Method and Prospects of Changing Mosquito Genes with CRISPR-Cas9

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Abstract CRISPR-Cas9 technology, as a precise and efficient gene editing tool, has attracted much attention in the field of mosquito gene editing. The aim of this review is to explore the potential of CRISPR-Cas9 application in mosquito gene editing, the challenges and the importance of environmental health balance. This review discusses how this technology can provide new strategies for controlling mosquito-borne diseases, such as modulating antiviral genes and reproductive capacity, through targeted editing of mosquito genes, and also recognizes technical challenges such as guide RNA design and non-targeted editing, as well as ecological risks that may be triggered by editing. Against this background, this review emphasizes the need to balance scientific and technological development with environmental health to ensure that the application of editing technologies does not cause irreversible impacts on the environment and ecosystems, and that while advancing scientific and technological progress, the balance between technological development and environmental health must be carefully weighed in order to achieve the dual goals of human health and ecological sustainability.

Keywords CRISPR-Cas9; Mosquito gene editing; Gene alteration; Disease control

Infectious diseases transmitted by mosquitoes have long been a serious public health problem in human societies, threatening human health and social stability on a global scale. Dengue fever, Zika virus and malaria, as representatives of these mosquito-borne diseases, have caused millions of infections and serious health consequences. Traditional control methods have been effective to some extent, but outbreaks and transmission of these diseases have long continued to occur, especially in tropical and subtropical regions, owing to the short life cycle, adaptability and complex transmission routes of mosquitoes.

With the rapid development of modern biotechnology, gene editing technology has become a new way of exploring solutions to the problems of these diseases.CRISPR-Cas9 technology, as a highly precise gene editing tool, offers an unprecedented opportunity to intervene in the genome of mosquitoes. By targeting specific genes for precise editing, scientists can attempt to reduce the ability of mosquitoes to spread disease or even cause them to dwindle in their natural environment. However, research and applications in this field face numerous technical challenges, ecological risks, and ethical and moral issues.

The aim of this review is to explore in depth the application of CRISPR-Cas9 technology in mosquito gene editing, and to comprehensively analyze the current and future status of this cutting-edge field, from the technical approach to the scientific outlook, and from the ecological risks to the ethical and legal issues. Through the analysis of existing studies and cases, as well as in-depth consideration of possible impacts, it is hoped that a clearer understanding will be provided to readers, prompting in-depth discussions on how to find a balance between science and technology and human health. By gaining a deeper understanding of the methods and challenges of CRISPR-Cas9 technology in mosquito gene editing, it may be possible to find more forward-looking and sustainable health solutions.

1 Principle and Application of CRISPR-Cas9 Technology

1.1 Basic principles of CRISPR-Cas9

CRISPR-Cas9 is a revolutionary gene editing technology that derives its name from the acronyms "Clustered Regularly Interspaced Short Palindromic Repeats" and "CRISPR-associated protein 9". associated protein 9". The

principle of CRISPR-Cas9 technology can be summarized in the following key steps, using a special protein called Cas9 and a custom-designed guide RNA to precisely edit specific regions of an organism's genome.

Bootstrap RNA design and pairing. A guide RNA is a short RNA sequence designed by scientists to pair with a specific DNA sequence in the target genome. A guide RNA consists of two parts: crRNA (CRISPR RNA) and tracrRNA (trans-activating CRISPR RNA). In practice, these two components are often combined into a single guide RNA molecule for more simplified handling. The design of the guide RNA determines the gene or genomic region to be targeted by the CRISPR-Cas9 system.

The cleavage function of Cas9 protein.Cas9 protein is a nuclease with specific DNA cleavage ability (Figure 1). Upon binding of the guide RNA to the Cas9 protein, the sequence of the guide RNA will guide the Cas9 protein to localize to the target DNA sequence. Once Cas9 binds to the DNA, it recognizes the complementary base pairing of the guide RNA to the target DNA sequence and forms a "cut" site on the target DNA.

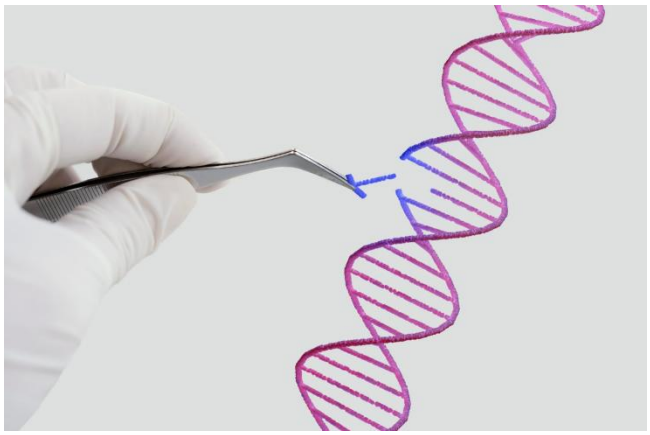

Figure 1 DNA cleavage

Intervention of the DNA repair mechanism. Once Cas9 creates a shear site on the target DNA, the cell's internal repair mechanism will intervene. Cells have two main DNA repair pathways: (non-homologous end joining, NHEJ) and (homology-directed repair, HDR). NHEJ usually triggers insertion or deletion errors, leading to gene knockouts or mutations. HDR, on the other hand, allows the introduction of exogenous DNA templates at DNA shear sites, thus enabling precise gene editing.

Gene editing results are produced. Through the NHEJ or HDR repair mechanism, the cell will complete the repair of the target DNA. In the case of NHEJ, this could result in inserted or missing bases, which could lead to gene mutations (Johansen, 2021). In the HDR case, an exogenous DNA template would be used to precisely replace or repair the target DNA sequence. This approach can be utilized to achieve gene knock-in, repair disease-causing mutations, and introduce new functional genes.

1.2 Design and selection of guided RNA

Selection of the appropriate target site is critical. This usually involves identifying the gene or genomic region to be edited and the region associated with the desired function or property. To avoid unwanted effects, shear sites located within exonic regions should often be avoided.

The design of the guide RNA sequence is an important factor in determining editing efficacy (Ishibashi et al., 2020). The guide RNA sequence usually has 20~30 bases at the target site, which is complementarily paired with the target DNA sequence to guide the Cas9 protein for precise localization. And the 5' end of the guide RNA needs to contain a specific triplet sequence (PAM) for Cas9 recognition.

The key to this is to avoid non-specific binding to minimize off-target effects. Design to ensure that the guide RNA matches the target site and is not complementary to other DNA sequences. This helps to avoid editing occurring at unintended sites, thus ensuring specificity of the editing result. In order to confirm the efficacy of the guide RNA, efficacy validation is often required. This can be accomplished by experimenting with in vitro or cell line systems. The purpose of the validation is to ensure that the guide RNA is able to successfully guide the Cas9 protein to localize to the target site and trigger editing. Validation ensures the efficiency and viability of the selected guide RNA.

1.3 Cas9 protein mediated DNA cleavage and repair mechanism

The Cas9 protein has nuclease activity and can recognize and cleave DNA double strands through its "nuclease active site" (Liu et al., 2021). This active site is usually located in the nucleic acid binding domain of the Cas9 protein. The sequence in the guide RNA will direct the complementary pairing of the Cas9 protein with the target DNA sequence. When the guide RNA and the target DNA form a stable complementary pair, the Cas9 protein will form a complex on the target DNA that is precisely localized to the specific site. At the region where the guide RNA is complementarily paired with the target DNA, the Cas9 protein will mediate the cleavage of the DNA double strand to form a double strand break (DSB).The DSB activates the intracellular DNA repair mechanism, which in turn leads to the realization of gene editing.

There are two main DNA repair pathways in cells: non-homologous end joining (NHEJ) and homologous recombination (HDR). NHEJ is a fast but imprecise repair pathway. Cells attempt to rejoin broken DNA ends, but this often results in insertion or deletion errors that can trigger mutations.HDR, on the other hand, requires an exogenous DNA template, usually a DNA sequence that is identical or similar to the target locus. During cellular replication and repair, this template is used to precisely repair the cut site, enabling specific gene editing.

Under the NHEJ repair mechanism, editing results may be insertion deletions, heterozygous insertions, base insertions or deletions, and so on. These results usually lead to alteration or inactivation of gene function. Under the HDR repair mechanism, exogenous DNA templates will be used to accurately replace or repair cut sites for precise gene editing, such as gene knock-in and repair of disease-causing mutations.

2 Successful Cases of Antiviral Gene Editing

2.1 Selection and importance of antiviral genes

Antiviral genes are genes that play a role in the immune response of an organism and whose encoded products recognize, inhibit, or clear viral infections. In mosquitoes, the selection and function of antiviral genes is important for the control of mosquito-borne diseases.

In the process of gene selection, genes with potential antiviral functions are selected as targets, which may encode antiviral proteins, antiviral signaling molecules, or molecules associated with immune responses (Trogu et al., 2020). Antiviral genes can modulate the immune response of mosquitoes, including activation of immune signaling pathways, promotion of synthesis of antiviral proteins, and modulation of apoptosis, which can help enhance mosquito defense against viruses. Expression of antiviral genes can limit the replication and spread of viruses, thereby reducing the ability of mosquitoes to act as disease vectors (Figure 2), and is thus essential for preventing the spread of disease.

Viruses evolve to adapt to their host environments, and selecting and maintaining antiviral genes can force viruses to adapt to more stringent pressures, slowing down their adaptive evolution and thus maintaining resistance to viruses. Editing mosquito genes using CRISPR-Cas9 technology could strengthen or introduce antiviral genes, thereby enhancing their immune response and helping to reduce the mosquito's ability to act as a disease vector (Lu et al., 2020). Editing based on antiviral genes could provide new ideas for sustainable control of mosquitoes, and by reducing disease transmission, the reliance on chemical pesticides could be reduced, thereby reducing the burden on the environment and human health.

Figure 2 Vector Mosquito Blood Test

2.2 Methods and strategies for implementing antiviral gene editing

Antiviral gene editing aims to enhance an organism's immune response and reduce viral infection and transmission. In mosquitoes, the realization of antiviral gene editing using technologies such as CRISPR-Cas9 can be achieved using a variety of approaches and strategies.

For gene selection and design, genes related to mosquito antiviral immune response, such as antiviral proteins and immune signaling molecules, should be selected, and guide RNAs should be designed so that they can precisely target selected antiviral genes (Kyrou et al., 2018). Knockdown of antiviral genes using CRISPR-Cas9 technology blocks the immune response suppressed by the virus, and activation of genes related to antiviral immunity by CRISPR activation technology enhances the immune response.

For gene repair and modification, CRISPR-Cas9 technology can be used to repair damaged antiviral genes to restore their normal function, and to modify antiviral genes to have stronger antiviral ability, such as enhancing the stability or activity of antiviral proteins (Mak et al., 2022). New antiviral genes, such as immune-related genes derived from other organisms, can also be knocked in to enhance mosquito immunity, and antiviral genes can be precisely integrated into the mosquito genome through CRISPR-Cas9-mediated homologous recombination.

Using cellular experiments and mosquito models, assess the performance of edited antiviral genes in cellular immune responses and overall immune function, and analyze the resistance of edited mosquitoes to viral infection and transmission. When implementing antiviral gene editing, consider the impact on the environment to ensure that the editing will not affect the ecological niche of mosquitoes and the balance of the ecosystem. By adopting the above methods and strategies, the realization of antiviral gene editing can improve the resistance of mosquitoes to viruses, reduce the risk of disease transmission, and provide innovative solutions to control mosquito-borne diseases.

3 Challenges and Limitations

3.1 Challenges in editing efficiency: technical challenges in specific gene editing

Despite the enormous potential offered by gene editing technologies such as CRISPR-Cas9, there are still some technical difficulties in specific gene editing that limit the efficiency and precision of its application. The design of guide RNAs is crucial, but in certain gene regions, especially those rich in repetitive sequences or highly

conserved, the selection of suitable guide RNAs may become difficult, which may lead to low editing efficiency or off-target effects.

Despite proper design of the guide RNA, editing tools may still have off-target effects at non-target sites, which may cause unexpected mutations that affect regions other than the target gene (Xuan, 2017). During DNA repair, cells may use different repair pathways at the same time, such as non-homologous end joining and homologous recombination. This may lead to unpredictable editing results that affect the desired editing effect. Certain gene regions may have low editing efficiencies, which may be related to factors such as chromatin structure, DNA replication status, etc., and low editing efficiencies can lead to the need for a large number of attempts to successfully edit a target gene.

Different cell types may respond differently to gene editing, and an editing strategy that is efficient in one cell type may not achieve the same results in other cell types. Certain gene types, such as gene families and non-coding RNAs, may be more difficult to edit. This may involve guide RNAs that are difficult to select or that are more difficult to edit due to the particular structure of the gene.

The editing process may lead to changes in the expression or regulation of genes, which may have unexpected effects on cellular functions and organisms and require careful follow-up studies. Although gene editing technology is constantly evolving, these challenges still exist and need to be overcome by further research and innovation to achieve efficient and precise specific gene editing.

3.2 Potential issues of incomplete dominant mutations and adaptive changes in mosquito populations

An incomplete dominant mutation is a mutation that expresses a different phenotype in the heterozygous state than in the pure state. In mosquito gene editing, incomplete dominant mutations are introduced to reduce the risk of mosquito-borne diseases using methods such as gene drive systems. However, this strategy may pose some potential problems and may also cause adaptive changes in mosquito populations.

Incomplete dominant mutations may be unstable in heterozygous individuals, leading to phenotypic differences in different generations, thus affecting the long-term stability of gene editing effects. The role of gene editing tools is not limited to the target gene, and incomplete dominant mutations may increase the likelihood of editing and mutation of non-target genes, leading to unintended effects (Qiu et al., 2022). Incomplete dominant mutations may produce side effects in mosquito physiology, such as undesirable survival, reproductive, or behavioral traits. The effects of incomplete dominant mutations may vary under different environmental conditions, and in different environments, mutations may manifest themselves to different extents and in different ways, which may have unintended effects on the ecosystem.

Introduction of incomplete dominant mutations may cause changes in the adaptability of mosquito populations. Incomplete dominant mutations may alter the role of mosquitoes in the ecosystem, resulting in a change in their position in the food chain; mosquitoes may adapt to antibiotics or chemicals in the environment by means of incomplete dominant mutations, leading to the emergence of new resistance problems; and the introduction of incomplete dominant mutations may provide mosquitoes with a survival advantage in certain conditions, thereby expanding their proportion in the population.

Thus, while the introduction of incomplete dominant mutations may provide some benefits in mosquito gene editing, there are also potential problems and changes in population fitness. When implementing such strategies, in-depth research and evaluation are needed to ensure stability of editing effects, minimize adverse effects, and consider the overall impact on the ecosystem.

3.3 Risk assessment of non target editing and off target effects

Off-target editing and off-target effects are potential risks to be concerned about in gene editing techniques, especially when editing with the CRISPR-Cas9 system. These risks may lead to unexpected genetic alterations

that affect the editing effect and the stability of the organism. Therefore, it is crucial to perform a comprehensive risk assessment of off-target editing and off-target effects.

The risk assessment process requires the use of computational tools to compare guide RNA sequences with the genome to predict potential off-target sites, which can help identify possible off-target editing risks. Experiments are performed at the cellular level to verify that computationally predicted off-target sites are actually edited, thus providing more accurate information on whether the editing effect extends to non-target sites. Perform genome-wide analysis of edited cells or organisms using high-throughput sequencing to detect off-target editing events. This can help detect possible off-target effects, especially when editing multiple genes. Experiments at the whole organism level to detect phenotypic changes and genetic stability in edited individuals can help assess the overall impact of off-target editing on the organism. Consider the impact of genetic changes introduced by editing on the environment and ecosystems. This includes the possibility of affecting ecological chains, food chains, and other populations of organisms.

Risk assessment is useful to safeguard the accuracy of editing effects, and assessment of off-target editing and off-target effects can help to confirm that the editing has been successful in achieving the desired effect and to reduce unintended results. Assessing risk can also help identify possible adverse effects and avoid instability or undesirable genetic changes introduced by editing. In gene editing, understanding potential risks can help make trade-off decisions about whether to proceed with editing and how to minimize potential risks. Risk assessment of off-target editing and off-target effects is an integral part of gene editing research and applications. Experiments and analyses from multiple perspectives can accurately assess the possible risks introduced by editing, thereby better guiding the design and implementation of editing strategies.

3.4 Ecosystem impact and environmental security considerations

When conducting research on and applying gene editing and genetic modification, there is a need to give full consideration to their potential impact on ecosystems and environmental safety. In terms of impact on the ecosystem, gene editing may affect the interactions between organisms and other species, such as predatory and competitive relationships, as it may lead to the perturbation of the ecological chain, which in turn affects the balance of the entire ecosystem. Gene editing may change the ecological niche of an organism, its role in the ecosystem, which may affect the structure of the food chain and biodiversity. The new traits introduced by editing may give the organism a competitive advantage in the new environment, leading to its overpopulation in that environment, which may trigger biological invasion problems.

For environmental safety considerations, edited organisms may escape from the laboratory environment into nature, leading to unanticipated ecological and environmental problems. Edited genetic alterations may spread into wild populations through hybridization or genetic dispersal, thereby introducing new genetic variation in nature. Editing organisms may have an impact on the food chain, affecting the species and ecological processes with which they are interconnected. Editing may also lead to the spread or invasion of new species, which can cause damage to existing ecosystems and habitats.

Considering these risks and potential impacts, the protection of ecosystems and environmental security becomes crucial. At the beginning of the design of the editing strategy, potential ecological risks should be considered and appropriate precautions should be put in place to reduce the possibility of unintended impacts. In the laboratory and in practical applications, measures are taken to ensure that editing organisms do not escape and that the necessary monitoring measures are in place. To continuously monitor the impacts of editing organisms after their introduction into the environment, and to identify and respond to potential problems in a timely manner. Communication with the public and relevant stakeholders is required to ensure that they are aware of the risks and safety measures. Only after the potential ecological risks are fully understood and assessed can effective safety strategies be developed to ensure that the impact of edited organisms on the ecosystem and the environment is minimized and controlled.

4 Conclusion

In the field of mosquito gene editing, CRISPR-Cas9 technology has revealed compelling applications. Through this precise and efficient gene editing tool, people have been able to realize the purposeful modification of mosquito genomes, opening new pathways for controlling diseases transmitted by them, such as dengue fever and Zika virus. These diseases have long plagued public health on a global scale, however, the emergence of CRISPR-Cas9 technology has provided a means to precisely intervene in mosquito populations. By enhancing the expression of antiviral genes, it is expected to reduce the ability of mosquitoes to transmit diseases, thereby reducing the extent and impact of disease transmission. In addition, by regulating reproductive capacity, control of mosquito population size can be achieved, providing a long-term mosquito vector management strategy for infected areas.

However, despite the encouraging prospects for the application of CRISPR-Cas9 technology in mosquito gene editing, there are a series of challenges and potential risks that need to be taken seriously. During the editing process, the design and selection of guide RNAs are crucial for editing efficiency and precision. Not only that, editing tools may have off-target effects at non-target sites, leading to unintended gene alterations. Such unintended gene editing may affect the normal physiological and ecological functions of the organisms, thus triggering undesirable ecological and environmental effects. In addition, the balance of ecosystem and environmental health is a central issue that must be carefully considered. Editing mosquito genes may have effects on ecosystems such as predator-prey relationships, ecological niche allocation, etc. If not properly managed, these changes may lead to disturbances in the ecological balance, which in turn may affect the stability of the entire ecosystem. At the same time, edited mosquitoes may have an impact on their habitats or even lead to changes in the ecosystem, and these changes need to be fully considered in the decision-making and implementation process.

Risks must therefore be carefully assessed and managed during the application process. In addition to emphasizing the innovation and potential of editing technology, it is also important to stress the importance of balancing scientific and technological development with environmental health. Only when the potential risks in the editing process are fully assessed and managed to ensure that edited organisms do not cause irreversible ecological and environmental problems can a truly sustainable mosquito control and disease prevention and control strategy be realized. Collaboration and knowledge sharing across borders is crucial in this process, and scientists, policy makers, the community and the public globally must work together to build a sustainable bridge for applications in the field of gene editing. While pursuing innovation, the ethical and social implications of science and technology should also be considered. Only when scientific and technological development is balanced with environmental health can the application of mosquito gene-editing technology be truly realized and make a useful contribution to public health and the health of the ecosystem.

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