VOLUME-9, ISSUE-4, APRIL -2020 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper | Community Medicine

GENE DRIVE: AN INNOVATIVE GENE-EDITING TECHNIQUE FOR MALARIA CONTROL

Dr PMP Singh	DADH, HQ 41 Artillery Division C/o 56 APO
Dr MS Mustafa*	ADH,HQ 17 Corps, C/0 99 APO *Corresponding Author
Dr Nitin Kumar Sharma	MO, HQ 17 Corps, C/0 99 APO

ABSTRACT

Introduction: Development of insecticide resistance by the mosquito coupled with drug resistance by the parasite has made the prevention of malaria even more challenging and resource intensive.

What is Gene Drive Technique? : Gene drive is a technique whereby the DNA is engineered in such a manner that the gene is passed from a parent to its offsprings almost cent percent. When two organisms of the same species mate, the offsprings inherit two copies of a particular gene - one from the male and the other from the female parent. Hence, each gene gets passed on to about 50% of the offsprings.

CRIPSR/Cas9 Technology : CRIPSR/Cas9 is a "search and replace" technology wherein mutations are created in the vector genome that get transmitted to the next generation almost completely; in contradiction to Mendelian laws.Gene drive technology using a specific autonomous CRISPR/Cas 9 has been applied in Anopheles stephensi mosquitoes, adapted from the mutagenic chain reaction.

Safety Issues and Challenges : Gene drive systems depend on a guide RNA to ensure that the DNA sequence is cut at the precise location. Any guidance error may damage the non - target genes with catastrophic effects on the organism.

Another plausible effect is that interbreeding amongst sibling species may lead to transmission of CRISPR genes from vector species to harmless species with deleterious effects.

Conclusion : CRISPR/Cas9 is a simple, cheap and efficient system of gene editing. In the long run, the main aim would be the release of an engineered vector as an adjunct to the already existing tools for malaria control.

KEYWORDS : Gene drive, CRISPR/Cas9, DNA, Vector

Introduction

Malaria is a serious public health concern with approximately 3.4 billion people at risk of contracting the disease worldwide. 198 million cases of symptomatic malaria were reported across the globe in 2013.[1] Of special concern is P falciparum malaria which is life threatening. Source reduction, insecticide spray and personal protective measures have met with little success in control of the disease. Development of insecticide resistance by the mosquito coupled with drug resistance by the parasite has made the prevention of the disease even more challenging and resource intensive.

The above pitfalls have led to newer options in malaria control; of which genetic engineering has become an integral part. Though a beginning has been made by creating transgenic mosquitoes, the main drawback is that only about 50% of the offspring inherit get infected; thereby taking multiple generations for the effect to be visible.[2] The new concept of gene drive circumvents this drawback. The name is based on the fact that these genes "drive" themselves rapidly through populations over many generations.

What is Gene Drive Technique?

Gene drive is a technique whereby the DNA is engineered in such a manner that the gene is passed from a parent to its offsprings almost cent percent. When two organisms of the same species mate, the offsprings inherit two copies of a particular gene - one from the male and the other from the female parent. Hence, each gene gets passed on to about 50% of the offsprings. This is in consonance with Mendel's laws of inheritance. Gene drive technique introgresses a gene that switches the other copy of the gene into the same version of the gene. Hence, following each mating, almost the entire offsprings will inherit two identical copies of the same gene.[3] In this way, a genetic change can rapidly spread through the entire population of a particular species. This forms the basis of the gene drive phenomenon.

CRIPSR/Cas9 Technology

CRIPSR/Cas9 is a "search and replace" technology wherein mutations are created in the vector genome that get transmitted to the next generation almost completely; in contradiction to Mendelian laws. This complex comprises Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and a DNA repair enzyme called Cas9 which target specific DNA sequences under RNA guidance.[4]

This genome editing CRISPR/Cas9 gene drive complex selects a particular site in the genome, following which, it introduces a copy of the entire DNA required to reproduce additional copies of the same CRISPR/Cas9 complex. Thus, when a vector carrying the gene drive mates with a non-gene drive carrying vector in the wild, the gene drive inherited by the offspring from the carrier parent carries out genetic editing in the non-carrier chromosome, thereby ensuring that both chromosomes of almost 100% of the offspring inherit the gene drive; thus making the offspring homozygous. Gene drive complex studies in Drosophila melanogaster have established a 97% propagation rate in contrast to just 50% inheritance as per Mendelian principle.[5] As the entire generation of offsprings gets a copy of the mutation, the gene thus modified gets transmitted rapidly amongst vectors in the wild. CRISPR/Cas9 exploits the nuclease activity and target-site specificity inherent to the system.

Gene drive technology using a specific autonomous CRISPR/Cas 9 has been applied in Anopheles stephensi mosquitoes, adapted from the mutagenic chain reaction.[6] This system results in offsprings of males and females derived from transgenic males showing a high frequency of germ-line gene conversion. The system copies a 17 kb construct from its site of insertion to its homologous chromosome. The marker anti-Plasmodium falciparum effector genes and the autonomous gene drive components are introduced into 99.5% of the

progeny following outcrosses of transgenic lines to mosquitoes in the wild. The effector genes remain transcriptionally inducible on blood feeding.[6]

Safety Issues and Challenges

The main risk of this controversial intervention is the ethical and safety factor, like any other genetic engineering modality, wherein possible harmful effects of the CRISPR/Cas9 system cannot be ruled out. To cope up with this contingency, a back-up package of reversal genes has been proposed to remove the potentially harmful gene and replace it with the original version in case of an unexpected occurrence. Gene drive systems depend on a guide RNA to ensure that the DNA sequence is cut at the precise location. Any guidance error may damage the non - target genes with catastrophic effects on the organism.[7]

It is possible that mutations in the vector at a later stage may remove that specific portion of DNA which is used as target by the CRISPR/Cas9 system; thereby nullifying the effect. Another plausible effect is that interbreeding amongst sibling species may lead to transmission of CRISPR genes from vector species to harmless species with deleterious effects.

A rare possibility of accidental escape of the gene drive from the lab with resultant infection of other species cannot be ruled out. However, the silver lining is that as the CRISPR/Cas9 technique targets a genetic sequence specific to P falciparum alone, the same is more or less ruled out. As adult mosquitoes as well as their larvae serve as prey for other animals, there is a possibility about the potential knock-on effects of releasing genetically driven vectors which may affect the predatory animal populations.

Genetically engineered male only mosquitoes are already being released in the wild as a part of the anti-malaria strategy. If this approach is executed inside a gene drive, it may lead to complete extermination of vector mosquito populations. However, the same is less desirable as compared to just reducing the disease transmitting capacity of the vector; since vectors who acquire resistance to the gene drive through mutations would not be affected and would fill up the ecological niche vacated by the gene drive susceptible vectors. As of now, CRISPR/Cas9 edited mosquitoes are restricted to the laboratory and not scheduled for release for field trials. Further testing is needed to confirm efficacy and safety of this system before trials in the field.

Conclusion

CRISPR/Cas9 is a simple, cheap and efficient system of gene editing.[8] However, it is still in the teething stages and requires international regulations to prevent undesirable and hitherto unknown consequences. Intersectoral coordination involving ethical, technical and political leadership needs to be put in place. Besides, the community needs to be involved to allay fears about release of gene drive manipulated vectors in the field.

In the long run, the main aim would be the release of an engineered vector as an adjunct to the already existing tools for malaria control. For this purpose, the gene drive manipulated vector needs to be released in the wild using mathematical modelling programmes to assess various public health issues such as the number of modified mosquitoes to be released, the time it would take for the entire vector population to be affected and the time to be taken for the prospective protective effect to be visible.

The gene drive technology, if approved ethically and from the safety view point, may be of colossal benefit to the Armed Forces in the future as the engineered mosquitoes would be able to traverse inaccessible regions and propagate malaria resistance genes in thick forest canopies, especially in the North-Eastern region of the country, where malaria is widespread, but unfortunately human effort cannot be applied due to hostile terrain.

References

1. WHO. World Malaria Report 2014. Geneva, Switzerland http://www.who.int/malaria/publications/world_malaria_report_2014/report/en/ (Accessed on December 18, 2014).
2. Curtis C F, Coleman P G, Kelly D. Advantages and limitations of transgenic vector control: sterile males versus gene drivers. In: Genetically modified mosquitoes for malaria control. Boete C (Ed). Landes Bioscience, Georgetown, Texas; 2006 : 60.
3. Mali P, Yang L, Esvelt K M, et al. RNA-guided human genome engineering via Cas9. Science 2013; 339 : 823 - 26.
4. Jiang W, Bikard D, Cox D, Zhang F, Marraffini L. CRISPR-assisted editing of bacterial genomes. Nat Biotechnol 2013; 31: 233 - 39.
5. DiCarlo J E, Chavez A, Dietz S L, Esvelt K E, Church G M. Safeguarding CRISPRCas9 gene drives in yeast. Nat Biotechnol 2015; 33: 1250.
6. Gantz V M, Jasinskiene N, Tatarenkova O, et al. Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito Anopheles stephensi. PNAS 2015; 112 (49) : E6736 - E6743.
7. Farasat I, Salis H M. A biophysical model of CRISPR/Cas9 activity for rational design of genome editing and gene regulation. PLoS Comput Biol 2016; 12(1): e1004724.
8. Doudna J A, Charpentier E. The new frontier of genome engineering with CRISPRCas9. Science 2014; 346: 6213.