Curbing the spread of vector-borne diseases to mitigate the impact of human activities on the environment using CRISPR

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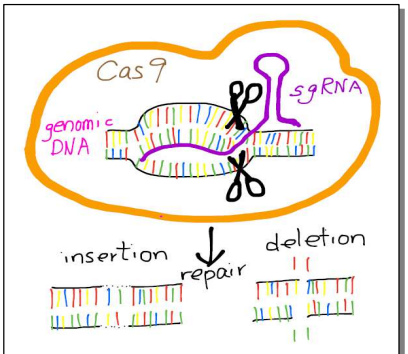
In the last decades many vector-borne diseases are emerging or re-emerging e.g. Dengue, West Nile virus, Bluetongue, Zika, mainly as a consequence of human activities (global warming, deforestation, invasion of habitation, urbanization and international travel etc). This has become a heavy burden to the healthcare system and the World Health Organization. Even the Rio Olympics had suffered with many top athletes pulled out because of the Zika scare. To mitigate the impact of human activities on the spread of vector-borne diseases it is urgent that we can find a way to curb these re/emerging diseases.

Because a vector is required to transmit the disease to human, it is logical to think that the best way to break the transmission cycle is to eliminate the vector. From 1950 to 1980 the insecticide DDT was widely used in agriculture and to control mosquitoes. We now know the cost of DDT on the environment. Not only that the toxic chemical endangered wildlife and human, some mosquitoes had become resistant to DDT.

There is a pressing need to find new ways to combat vector-borne diseases. Genetically modified (GM) mosquitoes have proven to have the potential. The UK company OXITEC has engineered suicide mosquitoes which have been released into the field with some success [1]. Another genetic approach is to generate pathogen-resistant mosquitoes by engineering resistant genes or silencing genes that are essential for infection e.g. host receptor protein that must be recognized by the virus before infection can ensue.

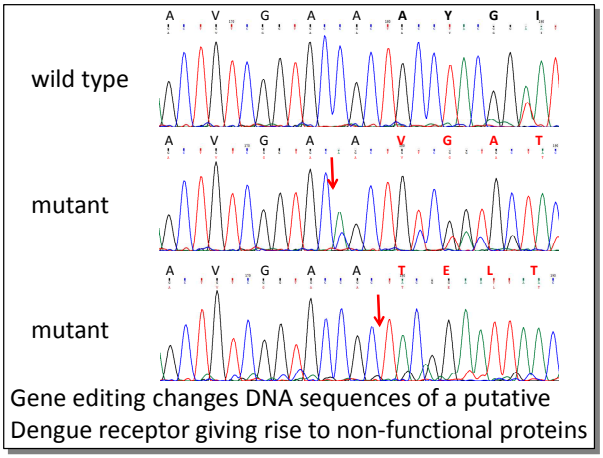
A powerful technique called CRISPR has emerged recently and is tipped to win the Nobel Prize [2]. CRISPR has swept the entire scientific and medical community by surprise because of its simplicity and versatility. CRISPR is a genome editing tool to silent specific genes. The CRISPR/Cas9 system was discovered in bacteria/Archaea as a defence mechanism against phage (virus) infections. It has now been repurposed as a gene editing tool in various organisms throughout the tree of life. The principle is that by using an RNA guide complementary to a target genomic DNA sequence, the endonuclease Cas9 will recognize specific genomic DNA sequence to create double-stranded DNA breaks. Repairing of these DNA breaks is often imprecise, thus generating insertions/deletions (INDELs) resulting in disruption of reading frames and the production of non-functional proteins.



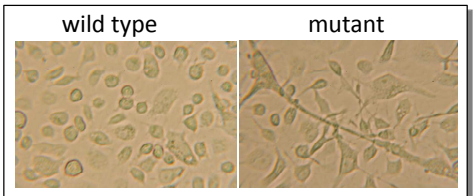
The CRISPR/Cas9 system consists of a sgRNA and a Cas9. sgRNA binds to target genomic DNA sequence and guides the endonuclease Cas9 to make double-stranded DNA breaks. Imprecise repair of the DNA strands gives rise to insertions or deletions of the nucleotides.

CRISPR has just been given the green light to edit human embryo in a UK first. Only recently CRISPR was successfully used to generate GM mosquitoes belonging to the species *Anopheles*

stephensi and *Aedes aegypti* [3-4]. Here we use Dengue virus as a model virus and the host entry step as a model target. To infect the host cell virus needs to gain entry by binding to one or more receptors on the cell surface. By knocking out the receptor(s) the host cell will become resistant to infection. Using CRISPR we have successfully knocked out a putative receptor from an insect cell line derived from the Asian tiger mosquito *Aedes albopictus*. Our next step is to validate resistance of the insect cells to Dengue virus and other virus infections. The results will generate information in help creating GM mosquitoes that will become resistant to a number of virus infections as CRISPR can be multiplexed.



Gene editing changes DNA sequences of a putative Dengue receptor giving rise to non-functional proteins



Knocking out of a putative Dengue receptor gives rise to a distinctive population of mutant cells which display different growth characteristics to that of the wild type cells.

With new technology comes new worry. The release of such suicide and engineered mosquitoes could have tremendous, unpredictable impact on the evolutionary and ecological fronts and must be vigorously debated, tested, regulated and monitored.

About the author: Shiu-Wan Chan is a virologist working on hepatitis C virus and vector-borne diseases. She owns multiple worldwide patents on hepatitis C. She is a lecturer at the University of Manchester and an Associate Chief Editor of the Frontiers in Microbiology.

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Mosquito image: https://phil.cdc.gov/phil/details.asp?pid=4490