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Role of CRISPR/Cas9 gene editing tool in parasitology: A review

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Abstract
CRISPR/Cas9 is the newly discovered revolutionary genome editing tool by which to manipulate the DNA of variety of organism and that allows the scientist to studies on functional genome of parasites. It play important role in improvement of science of Parasitology. Structural and functional genomics information helps in investigation of the relationship between parasite and their host. CRISPR/Cas9 based gene editing studies have been shown and found to be successful on several parasites including *Leishmania donovani*, *Toxoplasma gondii*, *Trypanosome cruzi*, *Strongyloides stercoralis*, *Cryptosporidium* species and Mosquitoes. Data from these studies could provide the experimental basis for applications related to early diagnosis, treatment and disease prevention.

Keywords: CRISPR/Cas9, genome editing tool, DNA, parasitology

1. Introduction
Parasites are recognised as a major constrain to live stock and human that affect large proportions of the world's population [1] and a major cause of morbidity [2, 3], greater economic and social deprivation among humans and animals. Most of the parasite control programs are based upon a combination of chemotherapy, grazing management, nutritional management, biological control, vaccination and ethno veterinary medicine (EVM) treatment [4] but still parasitic infections are the primary cause of productivity losses in livestock worldwide [5]. This is because there is lack of proper diagnostic methods, new anthelmintic drugs and vaccines development is limited by poor tractability of parasite.
CRISPR is the newly discovered revolutionary genome editing tool that allows the scientist to work inside the cells that make any change at any DNA sequence in a precise manner [6, 7]. Along with structural genomics (sequencing), studying of functional genomics of parasite allows better understanding about the parasite's biology, physiology and biochemistry [8]. It play important role in improvement of science of parasitology. Structural and functional genomics information helps in investigation of the relationship between parasite and their host. Understanding of relationship could lead to new diagnostic methods for parasitic diseases, development of new drug targets as well new anthelmintic drugs, development of effective vaccine against parasite and new control measure.
In 2017, Jennifer Doudna and Emmanuel Charpentier were awarded the Japan Prize for their revolutionary invention of CRISPR-Cas9 in Tokyo, Japan [9]. Unlike any other previously developed techniques of gene editing, CRISPR is remarkably simpler, faster and cheaper. It is two part system comprising of guide RNA (gRNA) which guide the Cas9 protein to make double strand break (DSB) in matching DNA sequence [10]. When Cas9 cuts the DNA it triggers the cells natural repair enzymes to fix these breaks. The target gene can be modified by adding, disruption or inserting of new gene means through this technique we can knock out target gene and simultaneously knock in functional gene [7, 11]. Researches already have been done in variety of eukaryotes including mice, human cells, bacteria and nematodes. This review mainly focus on use of CRISPR/Cas9 system in parasites and how it will be helpful in future research.

2. Mechanism of CRISPR/ Cas9 system
Scientist didn't design CRISPR themselves, indeed they borrowed from microbes and it was first identified in *E. coli* [12]. CRISPR is the abbreviation of Clustered Regularly Interspaced Short Palindromic Repeats. CRISPRs were described as it is a region of bacterial genome that contain short DNA (20 bp) which repeats over and over with

unique spacer in between [13, 14]. In a palindromic repeat, the sequence of nucleotides is the same in both directions. These bacteria use CRISPR system as defence mechanism against invading virus or bacteriophages [15] or it is an acquired immunity of bacteria [16]. Each repetition is followed by short segments of spacer DNA from previous exposures to virus DNA. When a virus attack a bacterium, virus introduce their DNA into the bacteria, some virus use DNA to highjack the bacterium cellular machinery and more copies of themselves and they eventually burst out of the bacterial cell but with CRISPR/Cas9 the bacterium can fight back. Apart from these small clusters of *cas* (CRISPR-associated system) genes are located next to CRISPR sequences responsible for Cas9 protein that works like molecular scissor, bacterium use this Cas protein to cut foreign DNA and disabling the virus [14, 17]. The bacteria insert the section of viral DNA into their own genome at particular area so over time bacteria use this Cas protein and guide RNA for recognition of virus DNA when they attack in future. Bacterium sequence transcribed into a sequence called CRISPR RNA guide RNA (gRNA) each guide RNA combined with Cas9 protein, when they encounter with a piece of DNA (foreign DNA) or virus DNA this gRNA matches the sequence. If this gRNA combine the piece of DNA (foreign DNA) or virus DNA then Cas 9 protein cut piece of DNA (foreign DNA) or virus DNA at specific site and disable it. Emmanuella Charpentier awarded the 2015 Luise Jeante prize for her contribution in harnessing an ancient mechanism of bacterial immunity into powerful technology for genome editing. Jennifer Doudna and Emmanuel Charpentier realized that this technique takeout from microbes and use as gene editing tool in any organism. We can take a Cas9 protein and design our own gRNA to which match with the gene which we want to edit. If we introduce this gRNA along with Cas9 protein into a cell this gRNA will recognise and matches with the target DNA and Cas9 protein will cut that targeted gene.

3. Application of CRISPR/Cas9 in Parasitology

There is significant advancements have been made in genome editing of *Leishmania, Trypanosoma cruzi*, and T*oxoplasma gondii*, as well as vectors like the mosquito and water flea. *Leishmania donovani* is the causative agent of fatal visceral leishmaniosis. To understand *Leishmania* infection and pathogenesis and identify new drug targets for control of leishmaniosis, more-efficient ways to manipulate this parasite genome are required. Successful use of the Cas9 endonuclease to knockout a specific gene targeting a complex locus made of three tandomely arrayed genes family, the paraflagellar rod-2 locus. It is possible to get results in single transfection in as well as in short period of time (> 1month) [18]. Similarly, recent study generated loss-of-function insertion and deletion mutations in *L. donovani*, identified a novel single point mutation caused by CRISPR-Cas9 in *LdMT* (M381T) that led to miltefosine resistance, a concern for the only available oral antileishmanial drug [19].

In *Trypanosoma cruzi*, which is responsible for Chagas disease in humans. In this parasite CRISPR/Cas9-based system has been used to knockouts of high-capacity repeats led to decreased expression of the β- galactofuranosyl Glycosyltransferase family of enzymes, by targeting all 65 known members of the gene family with as few as three rounds of transfection [20]. Another study was conducted to knockouts of Pfr1, Pfr2, and Gp72 gene using CRISPR/Cas9 which revealed that these genes were responsible for flagellar attachment and cell motility [21].

In *Toxoplasma gondii*, the CRISPR/Cas9-based successful knockout of leucine aminopeptidase (TgLAP) gene, it has important role in parasite attachment, invasion, replication and growth of parasite. TgLAP deficiency decrease parasite virulence [22]. CRISPR/Cas9 system also has been used to guide destruction of RNA genes. Gene editing was applied to knock out the Rop18 gene and then use the mutant to transform type I GT1 cells [23].

In case of *Cryptosporidium* spp. there is no vaccine and only single approved drug is available. Drug and vaccine development is limited because lack of cell culture. To avoid this problem CRISPR/Cas9 based sporozoites developed by introducing genetic reporter, it helps in to study about pathogenesis, infection, role of parasite in pathogenesis [24].

In *Strongyliodes stercoralis* (human thread worm), disruption of twitchin gene unc-22 (Ss unc-22) using CRISPR/Cas9 results in sever motility defect in parasite and this mutation is heritable transfer into their progeny [25, 26].

CRISPR/ Cas9 system can enable gene editing in mosquito vectors. Application of this technique in vectors is valuable as a means to study vector parasite interactions, and to potentially introduce genetic changes that block the parasite's life cycle [27]. Kistler *et al*. [28] found that the CRISPR/Cas9 system can be use to integrate exogenous gene sequences of mosquitoes in a precise, targeted way, and that sgRNAs play an important role in investigating directional change of fatal genes of mosquitoes.

In *Anopheles stephensi* Cas9-mediated synthetic system known as a gene drive, which passes a malaria-resistance gene onto both the male and female offspring of the mosquito have been developed [29]. In genetics, gene drive is the phenomenon in which the inheritance of a particular gene or set of genes is favourably biased. Engineered gene drives have been proposed to provide an effective means of genetically modifying populations or even whole species. Applications of gene drive include preventing the spread of insects that carry pathogens (in particular, mosquitoes that transmit some viral as well as parasitic disease like *Dirofilaria* spp, *Woucheria* spp. and *Brugia* spp.) controlling invasive species, or eliminating herbicide or pesticide resistance gene. The technique can be used for adding, disrupting, or modifying genes, such as to cause a crash in the populations of a disease vector by reducing their reproductive capacity of mosquitoes transmit not only malaria [30] but also chikungunya, yellow fever, and dengue viruses; thus, the technology can also be apply on to those that transmit parasites [31].

In addition, CRISPR/Cas9 based gene editing has been successful in water fleas, which transmitt several parasites like *Dracunculus medinensis, Spirometra* spp. Targeting a functionally conserved regulator of eye development, the *eyeless* gene into the eggs of water fleas (Daphnia magna), 18–47% of the surviving larvae showed eye abnormalities as juveniles. After these juveniles matured, 8.2% produced offspring which also showed deformities resulting from eyeless gene mutations [32].

The CRISPR technique have been implemented on *Leishmania, Trypanosoma cruzi, Toxoplasma gondii, Cryptosporidium, Strongylodis*, water fleas, mosquito and parasitic model organism *C. Elegans* [33] and achieved positive or directional results so this technique readily can be adapted for studies on other parasite genome structure and function.

4. Advantages of CRIPR/ Cas9 protein over other existing tools

Until lately three major genome editing technologies were there for genome editing i.e., ZFNs (Zinc Finger Nucleases), Meganucleases and TALENs (Transcription Activator-Like Effector Nucleases) [34] However, these earlier approaches had their like being protein-based systems these are very expensive and difficult to customize and synthesize and also require very high end expertise and experimental setups.

CRISPR/Cas9 being a simple and adaptable technology offers several advantages over the other genome editing tools. One of the major advantage of CRISPR/Cas9 is that it doesn't required protein engineering which is a challenging task as in case of other genome editing tools. Testing and designing of gRNA is straight forward and simpler than engineering a protein for a specific target site [25, 35]. Moreover, by using only 20 nucleotide of gRNA a huge library of gRNA can be generated which can be used for high-throughput functional genomics applications. Also, CRISPR/Cas9 is highly suitable for multitasking. Practically along with a single Cas9 any number of gRNA, having varied specificities, can be introduced into a cell to cleave multiple target sites. Finally it is cost effective technology can be use by researchers having limited research budgets. All these advantages have made this revolutionary technology a mainstream method across the research laboratories working in the areas of genome editing.

5. Limitations

Though CRISPR/Cas9 is a breakthrough technology it does has some inherent constraints. The most potent problem with CRISPR/Cas9 is the 'off target effect' Mutation can be induced by a gRNA at sites similar, but not identical, to the target site [17, 36]. These off targets are difficult to identify and the only way possible would be a genome wide survey for the sites showing sequence similarity to the target site. Nevertheless, efforts are being directed to rectify the problem of 'off target' pertaining to the successful application of this technology in near future.

6. Conclusion

The CRISPR/Cas9 technique has been applied for various study purpose. It has several advantages over other traditional gene editing techniques (TALENs/ZFNs), the CRISPR/Cas9 system is more efficient, more accurate, cheaper, easier and has less off target effect. It has higher knockout efficiency and more accurate gene editing. Further, the technique will provide more opportunity for functional genomics research related to parasites.

This technique has single drawback that is off target effect However, to achieve wide application, further refinement is needed. Particular areas of optimization include reducing the off-target, unnecessary mutation problems, and improving the efficiency of homologous recombination and directional insertion of a gene segment so it can be more efficiently and successfully apply for the large and complex genomes of parasites.

This technique will help to understand the genome function, expression, and regulatory mechanisms of parasite, by replacing the gene it will change the activity of gene in biological pathway and examine the impact of this change and can find the novel therapeutic target, receptor of a specific protein and drug resistance gene in a certain parasite. The application of CRISPR/Cas9 system as genome editing tool have been used for studies of basic features of model organisms and studies on the genetic basis of behavior of mosquitoes, and for genetic strategies to control vector population or disease competence. Data from these studies could provide the experimental basis for applications related to early diagnosis, treatment and disease prevention.

7. References

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