CRISPR/Cas9-programmed editing of two key genes in *Schistosoma mansoni*.

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Background

- Schistosomiasis is first on the scale of devastating parasitic helminth diseases and is primarily caused by *Schistosoma mansoni*, *S. japonicum* and *S. haematobium*. It causes infection of 230 million people and approximately 280, 000 deaths annually.
- No effective vaccine is available and the clinical treatment relies on a single drug, praziquantel, leading to a constant threat of developing praziquantel-resistant worms [5]. Therefore, there is an urgent need to identify new drug targets against schistosomiasis and to develop alternative and effective therapies.
- Complete genome sequences for the 3 major schistosome species: *S. mansoni*, *S. japonicum* and *S. haematobium* have been released. The genomes comprise ~11,000 protein-encoding genes, but very few have been characterized.
- CRISPR/Cas9-mediated genome editing has been proved to be feasible in *S. mansoni.* We propose to explore functions of two key genes in schistosoma life stages using CRISPR/Cas9-mediated genome editing system. The outcomes will provide an improved understanding of key gene function in this parasitic flatworm, thereby supplying more information about schistosoma vaccine developing.
- *SmAChE* (encodes *S. mansoni* acetylcholinesterase) plays pivotal roles in the neuromuscular movement of *S. mansoni* and has long been an attractive intervention target for developing novel drugs and vaccines. *SmfgfrA* (encodes *S. mansoni* fibroblast growth factor receptor) was found preferentially expressed in *S. mansoni* stem cells and it plays important roles in long-term maintenance of neoblast-like cells in *S. mansoni*.

Research Methods and Results

➢ **1. Design sing-guided RNA (sgRNA) and single-stranded oligodeoxynucleotide (ssODN) donor template.**

A sgRNA targeting exon 5 of *SmAChE* (named X5) and X5ssODN were designed for programmed editing of *SmAChE*. SgRNA3 and 3ssODN, sgRNA4 and 4ssODN were designed targeting site 3, and site 4 in exon one of *SmfgfrA*, respectively. *Sm*fgfrA 34ssODN is supposed to replace the sequence between the double stand breaks (DSBs) in site 3 and site 4.

➢ **2. Insert sgRNA into lentiCRISPRv2-mCherry vector.**

Sanger sequencing results showed the double-stranded DNA sequence complementary to the sgRNA X5, sgRNA3 and sgRNA4 was successfully inserted into lentiCRISPRv2-mCherry vector, respectively.

Research Methods and Results

➢ **3. Produce infectious lentiviral particles by transfection of HEK293T cells with CRISPR vectors.** HEK293T cells were cultured and transfected with the reconstructed vector and two additional vectors: one vector (pCMVdR8.91) that express HIV structural and packaging genes and another (pCMVVSVG) that express the pseudotyping envelope protein Vesicular Stomatitis Virus Glycoprotein (VSVG). Mcherry red fluorescence signal was observed in transfected cells (Figure 1A). The virion titer was measured. Results showed prepared lentivial particles targeting *SmAChE*, *SmfgfrA* site 3 and *SmfgfrA* site 4 have more than 5 x 10^5 IFU/ml infective units (two pink color bands) (Figure 1B).

➢ **4. Deliver CRISPR components into *S. mansoni* parasites by lentiviral transduction and electroporation.** liver eggs (isolated from infected mice livers), Day1 eggs (collected from *in vitro* cultured worms at 24 h after perfusion) and schistosomula were used for the transduction. MCherry fluorescence was observed in eggs (Figure 1A) and schistosomula (Figure 1B) transduced with lentiviral particles, indicating the successful delivery of CRISPR components into these parasites.

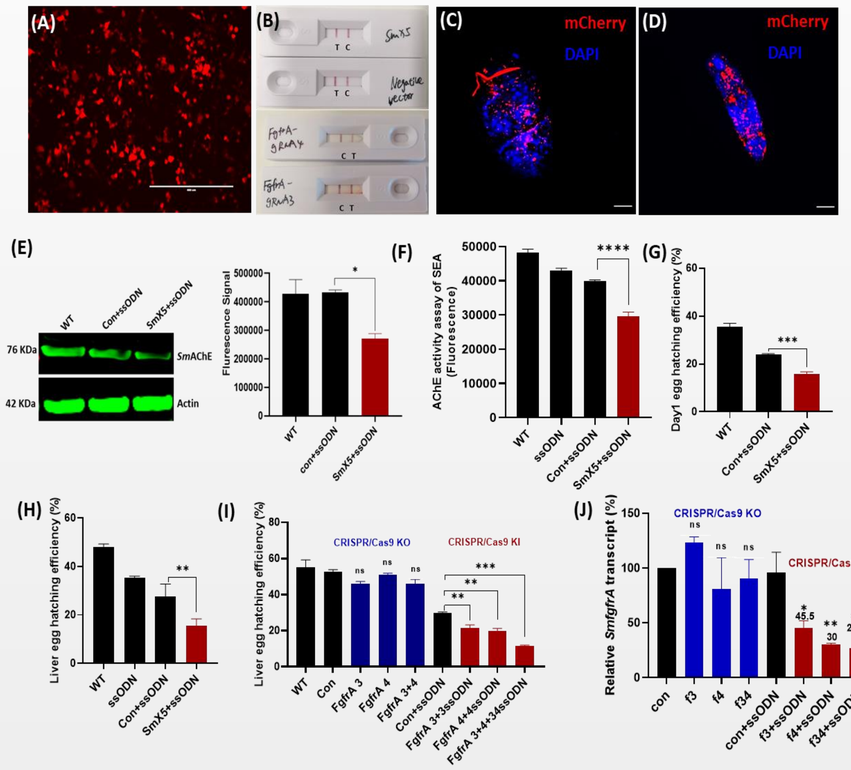

Figure 1. CRISPR/Cas9-mediated editing of *SmAChE* and *SmfgfrA*.

(A) mCherry red fluorescence signal was observed in transfected HEK293T cells. Sale bar: 400 μm. (B) Two pink color bands indicating the virion titer is higher than 5x10^5 IFU/ml infective units. 'T' test sample, 'C' positive control. (C) mCherry red fluorescence was observed in egg. (D) mCherry fluorescence was observed in schistosomula. Scale bars in (C-D) : 20 μm. (E) Reduced AChE expression in eggs treated with SmX5 lentirial particles and ssODN (*SmAChE*-edited eggs) determined by western blot. (F) Decreased AChE activity in *SmAChE*-edited eggs. (G) Declined hatching ability of *SmAChE*-edited Day1 eggs. (H) *SmAChE*-edited liver eggs demonstrated a 43.7% reduction of egg hatching. (I) Integrate a transgene in *SmfgfrA* site 3 and 4 generated diminished egg hatching ability. (J) Marked downregulation of *SmfgfrA*-specific transcript in eggs confer knockin (KI) in *SmfgfrA*.

Research Methods and Results

➢ **5. Determine gene mutation efficiency:**

➢ **1) genomic level:** Integration of the transgene at target sites were confirmed by PCR assays. Samples were prepared for Next Generation Sequencing.

➢ **2) transcriptional level:** *SmfgfrA*-specific transcript in *S. mansoni* liver eggs with knockin of a transgene in *SmfgfrA* target 3, target 4, and replacing the sequence between DSBs of site 3 and 4, was markedly downregulated by 54.5%, 70%, and 73.15%, respectively (Figure 1J).

➢ **3) translational level:** The expression of AChE in eggs confer knockin at X5 site was significantly decreased by 37%. A remarkable decrease of AChE activity was observed in these eggs (reduced by 25.6%) (Figure 1E, 1F).

➢ **4) phenotypic level:** The hatching ability of *SmAChE*-edited liver eggs and Day1 eggs was declined by 43.7% and 34.2%, respectively (Figure 1H, 1G). Knockin of a transgene at target site 3 and 4 of *SmfgfrA* generated 28.9% and 33.7% reduction of egg hatching, respectively. Significantly, replacing the sequence between site 3 and site 4 of *SmfgfrA* by transgene induced a 62% reduction of egg hatching (Figure 1I).

Conclusion

We employed CRISPR/Cas9-mediated gene editing to explore the functions of *SmAChE* and *SmfgfrA* in *S. mansoni*, a parasitic helminth that causes schistosomiasis. A reconstructed plasmid containing sgRNAs complementary to target gene and Cas9, was delivered into *S. mansoni* eggs and schistosomula by lentiviral transduction. Some parasites were also transfected with a single-stranded oligodeoxynucleotide (ssODN) donor template including a six-stop-codon transgene. The knock-in of transgene in target locus has been confirmed by PCR assays. Diminished AChE expression and enzymatic activity were observed in *SmAChE*-edited parasites. Marked downregulation of *SmfgfrA*-specific transcript was observed in eggs confer knock-in at *SmfgfrA* target site 3 and 4. Significantly, when the sequencing between *SmfgfrA* locus 3 and 4 was replaced by a transgene, a 73% reduction of *SmfgfrA* transcript was obtained. Inspiringly, depleting *SmAChE* and *SmfgfrA* in *S. mansoni* eggs generated markedly decreased egg hatching ability, indicating the important roles of these two genes to the development and movement of this parasite. The outcomes will provide an improved understanding of key gene functions in this parasitic flatworm, thereby supplying more information about schistosoma vaccine developing.