Determining the Function of MHC Class I in *Xenopus laevis* Using CRISPR/Cas9 Gene Editing

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ABSTRACT

The MHC Class I protein plays a critical role in the immune system of *Xenopus laevis*. This protein functions in the determination between self and non-self. The MHC Class I protein is an important immune gatekeeper identifying foriegn pathogens and ensuring that infected cells are killed by CD8 T cells. Also, it's important to ensure that normal cells are not attacked by the immune system. In *Xenopus laevis*, it has been shown that MHC Class I molecules are found active in adult frogs, but have not been detected in tadpoles even though they are immunocompetent. We are interested to see if MHC Class I is critical for immune function in *Xenopus* tadpoles despite low to undetectable levels. By knocking out the MHC Class I gene in tadpoles we aim to determine its function. To do this, we utilized the CRISPR/Cas9 gene editing tool. Cas9 creates a break in the dsDNA at the location of the gene by using specific guide RNAs, and the cell attempts to fix the break. This leads to mutations in the gene sequence that will inactivate the gene. We have generated multiple transgenic tadpoles using two sets of guide RNAs and are currently in the process of extracting DNA from both transgenic and control samples. We will use DNA sequencing to verify successful knockout of the MHC Class I gene and will generate more transgenic animals to monitor the effects of gene inactivation on the phenotype of individual tadpoles.

INTRODUCTION

We use *Xenopus laevis* as our model organism. It is an ideal candidate for several reasons. We are able to induce ovulation in the females at any time by injection of the hCG hormone. The eggs are easily manipulated as they are laid externally and we are able to inject the transgenic solution within an hour of them being laid. Importantly, the immune system of the *Xenopus laevis* functions in a similar way to the immune system of mammals, which allows for comparison between the two. We study the MHC class I protein. This protein prevents autoimmune destruction by distinguishing between self and nonself, which allows the targeting of foreign bodies while avoiding their own cells. We study the MHC class I protein because it is present in adult frogs but not present in tadpoles. Although tadpoles do not have this protein, they are still immunocompetent. Since both the tadpoles and adult frogs have a functioning immune system, it is unclear what the function of the MHC class I protein is. To study it, we used the CRISPR/Cas9 genome editing system to target our gene of interest. The DNA of *Xenopus laevis* is targeted by the Cas9 protein with the use of guide RNAs (sgRNAs). The sgRNA allows CRISPR to target the gene of interest, cut the DNA, and introduce a double stranded break. To fix the double stranded break, cells use non-homologous end joining (NHEJ) which inserts and deletes random nucleotides causing a mutation, therefore inactivating the gene.

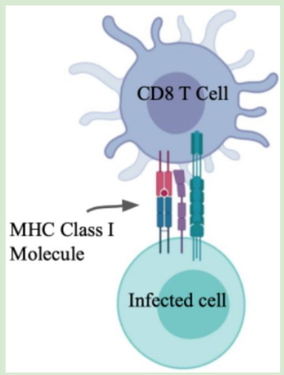

Figure 1: Education of the CD8 T cell by the MHC class I protein. Created in BioRender.

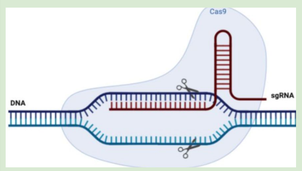

Figure 2: Cas9 protein function. Created in BioRender.

RESULTS

Figure 3. MHC class I protein and MHC class I sgRNA. (A) MHC class I protein showing the different domains and conserved regions. Image created using BioRender. (B) MHC class I domains α_1 and α_2 and their interaction with antigen. Image created using BioRender. (C) The sequence for the 3′ designed MHC class I sgRNA for both the α_1 and α_2 is shown as well as the target sequence in the DNA for each region. Design tool used CRISPRdirect. (D) Left: The α_1 domain sgRNA run on an agarose gel. Right: The α_2 domain sgRNA run on an agarose gel. The lanes, left to right, contain the DNA marker and the α_1 or α_2 domain sgRNAs.

Table 1. Survival rates of injected eggs and de-jellied controls for transgenic experiments using different sgRNAs

Target: MHC Class I, α_1 domain			
Group	Starting Population	3 Days Post-Injection	Survival Rate (%)
Injected	149	39	26.2
De-jellied Control	1189	217	18.3

Target: MHC Class I, α_1 domain			
Group	Starting Population	3 Days Post-Injection	Survival Rate (%)
Injected	34	0	0
De-jellied Control	300	0	0

Target: MHC Class I, α_1 and α_2 domain			
Group	Starting Population	3 Days Post-Injection	Survival Rate (%)
Injected	108	13	12
De-jellied Control	361	119	33

Target: MHC Class I, α_1 and α_2 domain			
Group	Starting Population	3 Days Post-Injection	Survival Rate (%)
Injected	143	8	5.6
De-jellied Control	84	5	5.9

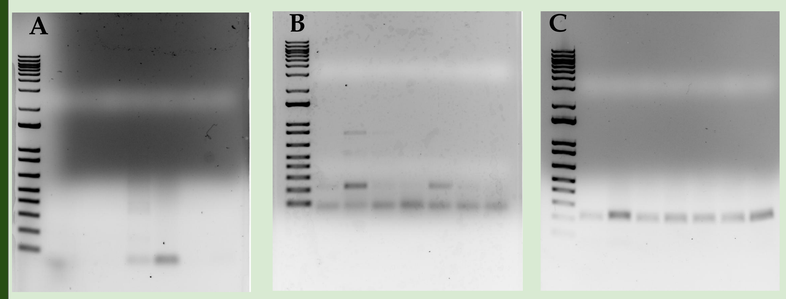

Figure 4. Testing transgenic DNA samples. Agarose gel electrophoresis using (A) MHC Class I Alpha 1 primers, (B) MHC Class I Alpha 2 primers, and (C) EF-1 Alpha primers. Lane assignments from left to right: DNA ladder, water control, 6 different transgenic samples.

METHODS & MATERIALS

sgRNA Design: sgRNAs were designed using the CRISPR direct design tool (https://crispr.dbcls.jp/) and selected for the presence of a PAM sequence, 50% GC content, and annealing temperature around 70℃. The sequences were run through BLAST to ensure that they matched *Xenopus laevis* alpha 1 and 2.

sgRNA Preparation: sgRNAs were prepared using annealing PCR. The sgRNAs concentration and purity were confirmed using NanoDrop Spectrophotometer and gel electrophoresis.

Transgenesis: Transgenesis was done at the University of Rochester. Freshly fertilized one cell stage eggs were de-jellied in HCL-cysteine in 0.1X MBS and washed extensively. Dejellied eggs were injected with 10 nL mixture containing 8 ng of Cas9 protein and 200 pg/nL of sgRNA.

The transgenic and control dejellied embryos were allowed to hatch. The surviving tadpoles were left to develop normally. Tadpoles that died were collected for DNA extraction and knockout analysis.

PCR: MHC class I alpha 1 and 2 domains and EF-1 alpha were amplified by PCR. We used gel electrophoresis to ensure that the primers are specific and that the genes of interest were amplified.

CONTINUING RESEARCH

- Use Exo SAP-IT PCR clean up reagent to purify our PCR products
- Send samples for sequencing to verify the knockout of the MHC Class I gene in both the Alpha 1 and Alpha 2 regions.
- Extract DNA from and test the new transgenic samples.
- Create more transgenic samples at the University of Rochester in order to expand our pool of samples and determine that our results are repeatable.

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