Reducing Feline Immunodeficiency Viral Loads Using CRISPR: A Proof of Concept

Tatiana Wolf, Brian G. Murphy DVM, PhD.
School of Veterinary Medicine, University of California, Davis
Department of Pathology, Microbiology and Immunology

INTRODUCTION

- Feline Immunodeficiency Virus (FIV) infection is a common and incurable infectious disease of domestic cats and invariably results in death once it progresses to the stage of feline acquired immunodeficiency syndrome (FAIDS).
- FIV is a lentivirus, a type of retrovirus with a tropism for CD4+T cells.
- FIV is capable of integrating into the cell's genome and persist as a permanent proviral DNA reservoir.
- FIV infected cells are capable of evading the immune system resulting in chronic lifelong infections, referred to as latency.
- There are no current medical therapies that consistently and efficiently eliminate lentiviral reservoirs from infected latent cells.
- CRISPR (clustered, regularly interspaced, short palindromic repeats) is a molecular technique based on a bacterial antiviral defense mechanism to locate and excise integrated viral genes.
- Proviral FIV is flanked by LTR sequences containing regions with compatible sites for CRISPR applications (Figure 1).

HYPOTHESIS: Feline cells infected *in vitro* with FIV and treated with the anti-FIV CRISPR gene editing tool will demonstrate reduced proviral loads and virus production relative to non-CRISPR treated FIV-infected feline cells.

Anti-FIV CRISPR MODEL

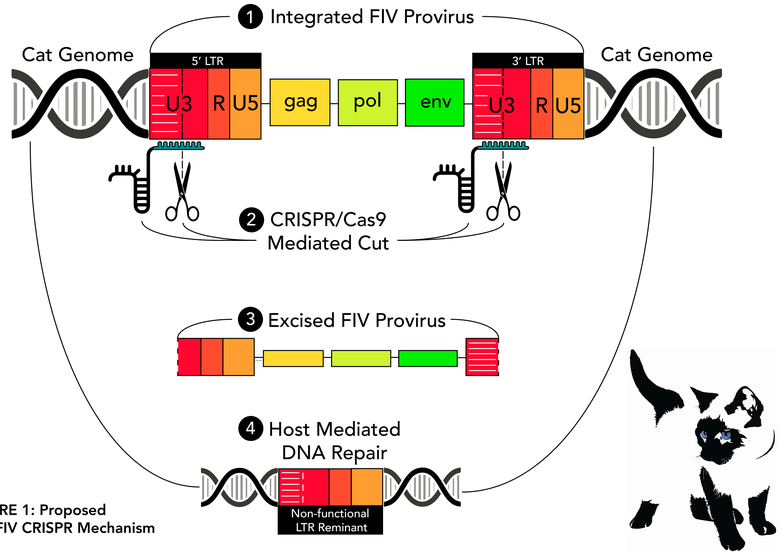


FIGURE 1: Proposed anti-FIV CRISPR Mechanism

MATERIALS & METHODS

- A recombinant lentivirus was designed to deliver the anti-FIV CRISPR components (Figure 2).
- The CRISPR lentivirus integrates into a cell's genome as DNA to express two guide RNA sequences (Thing 1 & Thing 2) specific to the FIV U3 LTR proviral sequence. Additionally, the CRISPR lentivirus codes for the Cas9 endonuclease, which is co-transcribed with a Puromycin resistance gene.
- Two main experiments were conducted and performed in triplicates. Simplified models outlined by Figures 3 and 4.
- Experiments performed on MCH5-4 cells (immortalized feline CD4+ T-cells).
- FIV clade C virus was utilized to infect cells.
- Integration of proviral DNA was quantitated using real-time PCR to measure FIV gag DNA copies normalized to copies of feline GAPDH.
- Isolated FIV viral RNA from cell-free culture media was quantitated by RT real-time PCR measuring gag copies normalized to volume.

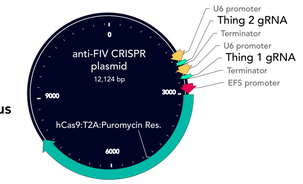


FIGURE 2: Plasmid Design for Recombinant CRISPR Lentivirus

EXPERIMENTAL DESIGN

EXPERIMENT#1: FIV Infected Cells Treated with anti-FIV CRISPR

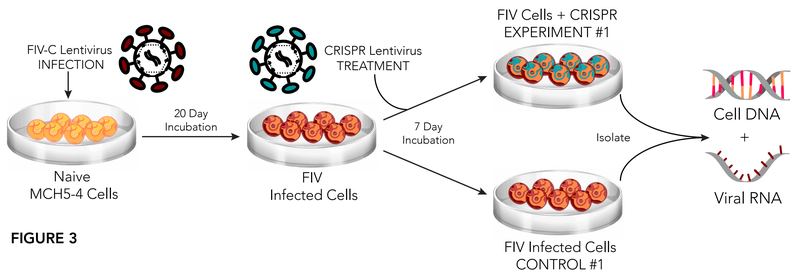


FIGURE 3

EXPERIMENT #2: anti-FIV CRISPR Treated Cells Infected by FIV

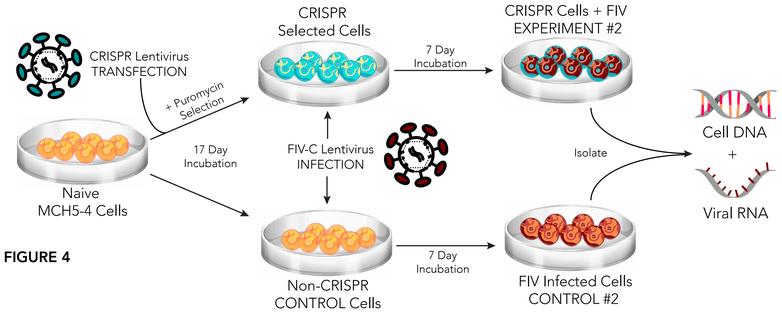
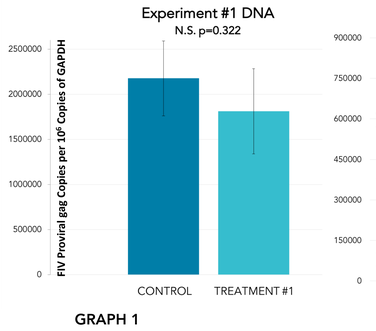
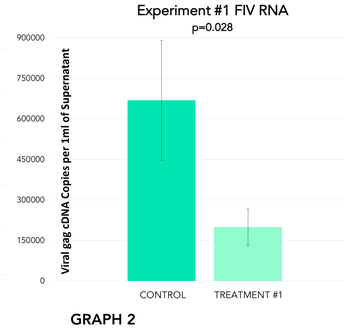


FIGURE 4

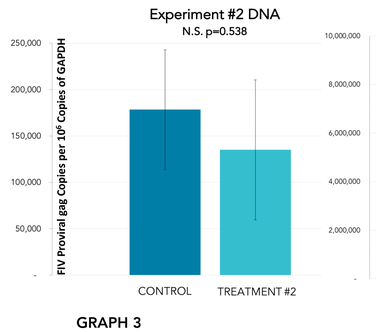
RESULTS



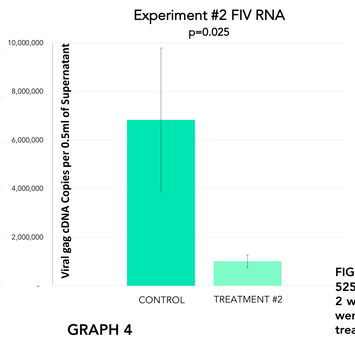
GRAPH 1



GRAPH 2



GRAPH 3



GRAPH 4

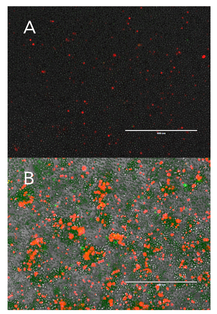


FIGURE 5: MCH5-4 cells infected with recombinant lentivirus coding for Puromycin resistance. Day 2 of infection (A) prior to puromycin selection. Selection Day 6 (B) culture in puromycin.

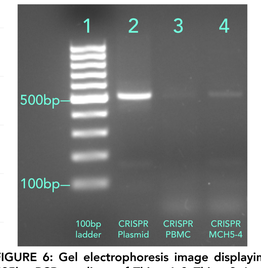


FIGURE 6: Gel electrophoresis image displaying 525bp PCR amplicon of Thing 1 & Thing 2. Lane 2 was a positive control (Figure 2). Lane 3 and 4 were DNA samples extracted from Exp #2 treatment cells on Day 17 of Puromycin selection (Figure 4).

DISCUSSION

- Real-time PCR results from Experiment #1 and #2 are supportive of our hypothesis that CRISPR treatments have an effect on reducing viral loads.
- Graph 2 and Graph 4 demonstrate significantly lower viral loads in Experiment #1 & 2 CRISPR treatment groups when compared to controls.
- Higher FIV titers correspond with higher cell infectivity.
- Proviral DNA loads were not significantly different between control and treatment groups (Graph 1 & 3). We considered the possibility that our sample size was too small to observe a significant change or excised proviral DNA was not degraded and remained stable for real-time PCR gag amplification.
- Figure 5 were results from a preliminary experiment testing the recombinant lentivirus compatibility with MCH5-4 cells. Red fluorescence corresponded with Thing 1 & Thing 2 expression and green fluorescence corresponded with Cas9 expression. Photo B confirmed we could select for CRISPR only cells that co-transcribe Cas9 with the puromycin resistance gene.
- Figure 6 provides evidence that MCH5-4 CRISPR treated cells contain integrated CRISPR lentivirus DNA sequences based on the presence of Thing 1 and Thing 2 gRNA.
- Data not shown are results from Experiment 1 and 2 using feline peripheral blood mononuclear cells (PBMC) harvested from specific pathogen free cats. PBMC results were mixed and inconclusive, possibly due to the mixed population of cell types and difference in FIV and/or CRISPR lentivirus infectivity. Additionally, cellular RNA was collected at the end of the experiments but real-time PCR results were mixed and inconclusive.

FUTURE DIRECTIONS

- Repeat experiments using a lower FIV titer and higher anti-FIV CRISPR lentivirus titer to observe effects on proviral load
- Perform an infectivity assay on FIV-free cells using the cell-free virus harvested at the end of the experiments

ACKNOWLEDGEMENTS

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