How Does it FHIT – A Study of the Effects of FHIT on 5'UTRs

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Background and Introduction

- We often see the deletion of certain loci early in the cancer development process, of these loci is Histidine Triad Diadenosine Triphosphatase (FHIT), a known tumor suppressor
- Previous studies have shown that FHIT regulates the translation of a small number of mRNAs by acting on their 5' untranslated regions (UTRs)

Abstract and Summary

The aim of this study was to observe the effects of FHIT on the translation of 26 different 5'UTRs and study its tumor suppressant effects. Our methodology used a novel 2 color reporter assay system and flow cytometry to observe results. Preliminary results have shown large effects in expression based on the 5'UTR present and validated the usage of TrypLE in our novel 2 color reporter assay.

Hypothesis

- We believe that the introduction of FHIT to the 5'UTRs will cause an increase, decrease, or no effect translation of downstream sequences

Methodology

- A total of 26 different 5'UTRs were tested using a genetically engineered 2 color reporter assay.
- Reporter plasmid contained the sequence for the red fluorescent protein mCherry, whose brightness was used as a transfection control, and Enhanced Green Fluorescent Protein (EGFP) whose brightness was used to determine the effects of FHIT and the 5'UTR on translation
- Molecular cloning was done to create 26 experimental plasmids and one control plasmid
- Plasmid screening was done using Colony Polymerase Reaction (PCR) and gel electrophoresis
- Plasmids were then transfected into FHIT knockout HEK293 cells, engineered to express either wild-type or H96N mutant FHIT in presence of doxycycline
- Flow Cytometry was done to assess effects on translation

Figure 1. Map of 2 color reporter plasmid

Results

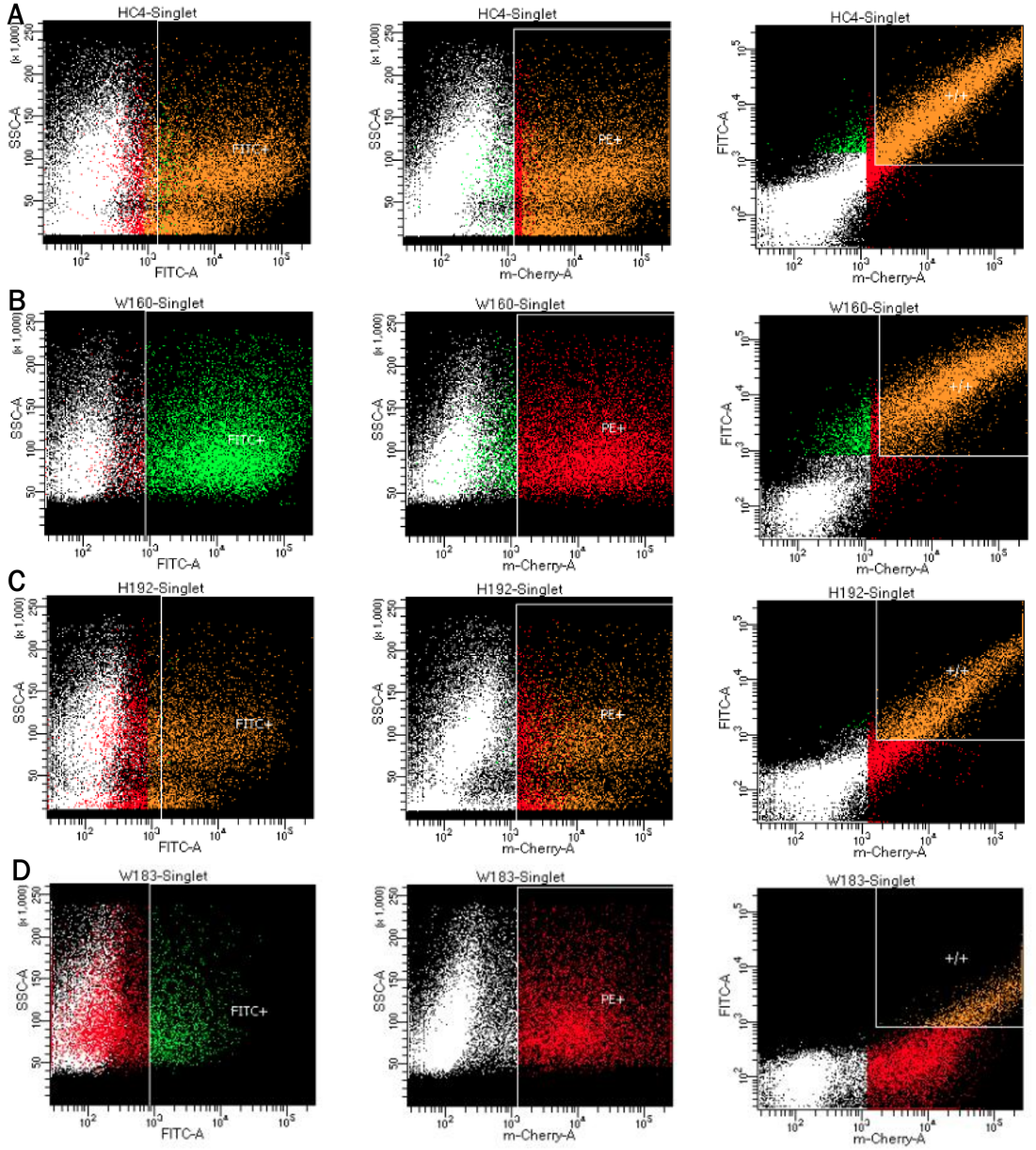

Figure 2: Selection of flow cytometry data from H96N and Wild-Type FHIT samples, representing different levels of expression The graph to the far right is EGFP, which demarks the result from our test, generally, the more green present, the more the protein was able to be expressed. The middle is our mCherry signal, demarking our transfection control, ensuring that there is plasmid present. The left graph is the combined signal the 2. Each graph is given with an exponentially scaling x-axis representing the luminosity. **A.** Flow cytometry results from the control plasmid with H96N FHIT added **B.** Flow cytometry results from IGSF9 5'UTR without FHIT introduction **C.** Flow cytometry results from MGLL 5'UTR with H96N FHIT introduction **D.** Flow cytometry results from MECP2 5'UTR with Wild Type FHIT introduction

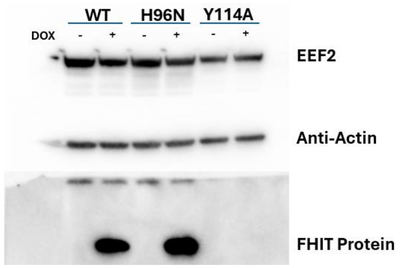

Figure 3. Western Blots showing FHIT induction with Doxycycline.
Crispr knockout HEK293 cells were induced with doxycycline (+) or left uninduced(-). Whole cell lysates were harvested and analyzed by Western Blotting. Anti-bodies for EEF2 and Actin serve as loading controls.

	Samples	High Expression	Medium Expression	Low Expression	Uncounted Controls
H96N	110	16	41	47	6
Wild Type	118	44	41	27	6
Totals	228	60	82	74	12

Figure 4. Table showing the total amount of samples tested and categorization into high, medium, and low expression

Results and Discussion

- Large differences (20% increase in expression in Wild Type) were observed with TrypLE usage vs no dissociation reagent
- We were able to observe large differences in expression based on the 5' UTR in the plasmid
  - There was a 22% increase in the amount of high expressors in Wild-Type FHIT, however, the same amount of medium expressors
- Several samples indicated a large increase in the expression of mCherry (or control reference gene) which will need to be examined further to determine the cause

Future Actions

- Further data analysis to control for mCherry expression
- Redo the results for H96N FHIT using TrypLE methodology to reduce cell shearing
- Repeat with the experiment with more replicates, with the goal of getting a total 6 replicates per condition
- Compare the effects of Wild Type FHIT vs H96N on the translation of EGFP 5' UTR reporters
- Screen additional Y114A lines to validate inducible expression
- Conduct experiment with Y114A mutant FHIT expressors

References

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