Pediatrics Internship Program at Stanford

Stanford MEDICINE School of Medicine — Department of Pediatrics

Potential Role of Loss of RFX3 in Hyperinsulinism

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Background

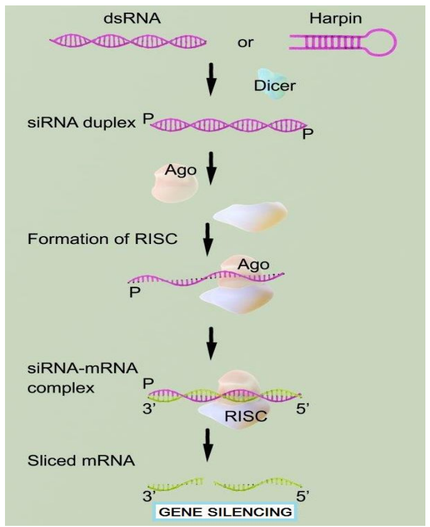
- Patients with **hyperinsulinism (HI)** suffer from **persistent hypoglycemia** (low blood sugar), fatigue, feeding issues, cardiomyopathy, and developmental delays.
- **Dysregulated insulin secretion** by the beta cells in the pancreas is the cause for HI.
- Chromosome 9p deletions, a region with 38 protein coding genes, have been identified as an important cause of HI.
- Of these 38 genes, based on information in public gene expression databases, pLI scores from gnomAD and the role of the RFX transcription factor family in islet-cell development and identity, **loss of *RFX3*** could play a role in causing HI.

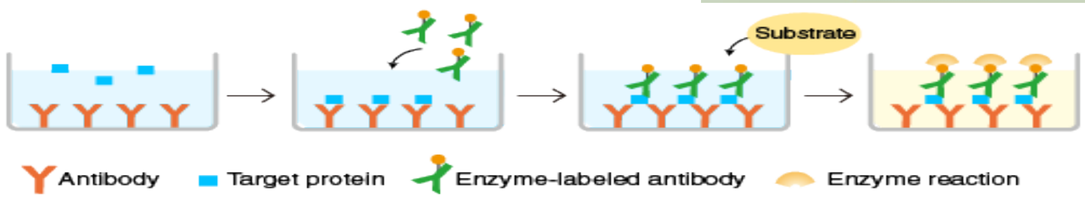
Objectives

- Determine the effects of loss of *RFX3* on insulin secretion in a human pancreatic beta cell model (EndoC-βH1 cells).

Methods

- **siRNA-mediated knockdown** of the *RFX3* gene in the EndoC-βH1 cells was done to mimic the loss of *RFX3* expression in beta cells *in vitro.*
- This was followed by **glucose-stimulated insulin secretion (GSIS)** to trigger insulin secretion at varying glucose concentrations.
- **Enzyme-linked immunosorbent assay (ELISA)** was done to measure the amount of insulin secreted by the cells during GSIS.
- **Quantitative PCRs (qPCRs)** and **Western Blots** were used to verify the efficacy of the siRNA-mediated knockdown at the RNA and protein levels.





Results

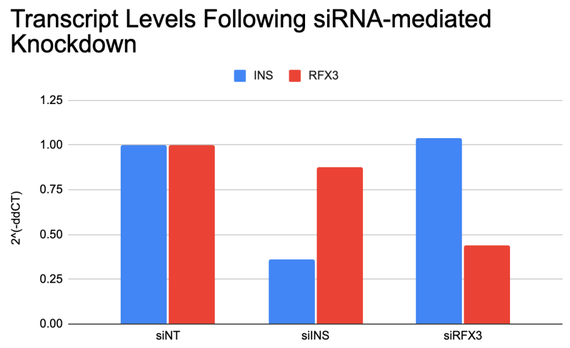


Figure 1.
INS and *RFX3* transcript levels following siRNA- mediated knockdown in EndoC-βH1 cells, normalized to the housekeeping genes *TBP* and *PPIA*. (n=1)

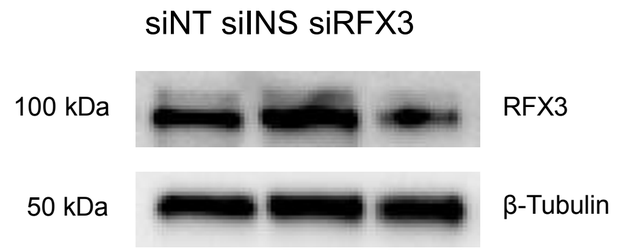


Figure 2.
Representative western blot of RFX3 (100 kDa) and β-Tubulin (50 kDa) in siNT, siINS, and siRFX3 EndoC-βH1 cells.

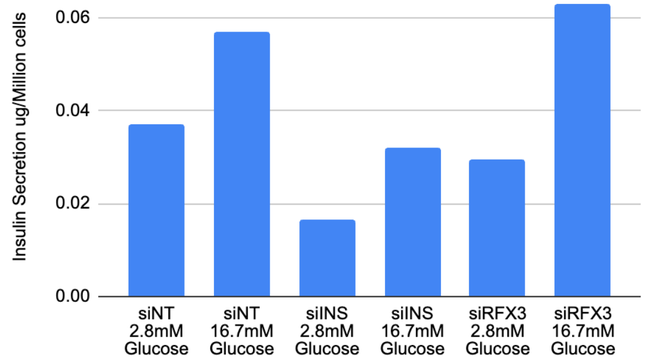


Figure 3.
Glucose-stimulated insulin secretion (ug/Million cells) measured in siNT, siINS, and siRFX3 EndoC-βH1 cells. (n=1)

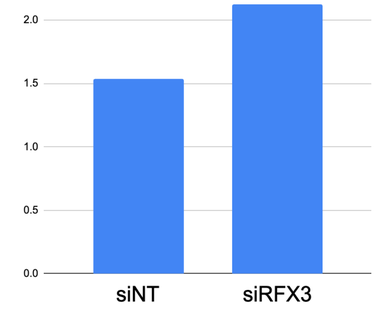


Figure 4.
Fold change in insulin secretion between exposures to 2.8 and 16.7 mM glucose concentrations in siNT and siRFX3 EndoC-βH1 cells. (n=1)

Limitations

- Culturing of EndoC-βH1 cells in a dish may not exactly simulate what is happening in the beta cells in the human body.
- Multiple genes in the chromosome 9p region could together be responsible for HI.
- siRNA-mediated knockdown was not able to completely eliminate *RFX3* expression.

Conclusions / What I've Learned

- siRNAs targeting RFX3 were able to **reduce expression of RFX3** at the RNA and protein levels in EndoC-βH1 cells.
- Loss of RFX3 expression was seen to cause an **increase in insulin secretion** compared to normal cells under exposure to the same glucose concentrations.
- **Loss of RFX3 could be a cause for HI**, which is characterized by elevated levels of insulin in the bloodstream.

Future Directions

- Knockdown of the other 37 genes in the chromosome 9p region to see if they affect insulin secretion as well.
- Examining the effects of loss of *RFX3 in vivo*.
- The experiments need to be repeated two more times to get an n of 3

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wellcome

NIH National Institute of Diabetes and Digestive and Kidney Diseases