Exploration of Harmine and p57 in Relation to Diabetes

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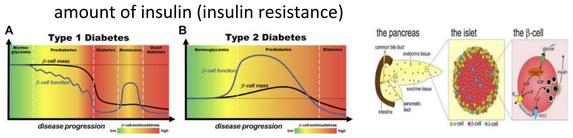
Background

Introduction to Diabetes:

- Diabetes affects more than 422 million people worldwide and the prevalence of diabetes has been rapidly increasing for the past several decades
- Worldwide, diabetes is the sixth leading cause of death, with 80% of the deaths occurring in low and middle-income countries.
- Diabetes is a chronic health condition that prevents the body from producing insulin or utilizing insulin, causing high blood sugar levels for prolonged periods of time
- Without proper care and medication, diabetes can lead to various complications like heart disease, vision loss, kidney disease, amputation, stroke, hearing impairment, and more

Pancreatic Beta Cells:

- Those who have either type 1 or type 2 diabetes are unable to create an adequate number of functional β cells.
  - In type 1 diabetes, the β cells are attacked or destroyed by the immune system (hardly any beta cells)
  - In type 2 diabetes, the β cells are unable to produce a sufficient amount of insulin (insulin resistance)



Current Treatment/Research:

- Current, widely-prescribed diabetic treatments that focus on pancreatic β cells (Glipizide, Gliclazide, Glyburide, and Repaglinide) work by stimulating insulin secretion from remaining β cells
- However, an interesting area of new research focuses on β cell proliferation instead
- The highest β cell proliferation rate occurs very early in life after birth in humans (a brief window of time)
- β cell replication rates are very low in both embryonic life and after early childhood.
- Researchers have proposed using organ transplantation, stem cell differentiation, and small molecule inhibitors as potential methods to stimulate β cell proliferation into adulthood.
- The Stewart Laboratory discovered that small doses of harmine, an alkaloid, can be used to proliferate β cells.

Research Questions

1. Harmine is revolutionary as it is the first molecule to be found to induce beta cell proliferation. How does it affect transcription factor genes in beta cells?
2. p57 plays an important structural role in beta cell physiology. We have demonstrated it's upregulated in harmine treated cells. What variations can we make on this gene?
3. While research has shown that a combination of harmine and GLP-receptor agonists can proliferate pancreatic beta-cells to a level where it's possible to cure T2D, the exact mechanisms of harmine are unclear. Recent research has shown that Notch pathways help regulate beta cell proliferation. How does the presence of harmine effect the signaling pathway?

Methods

PCR:

- Treated islet cells in 2 Treatments: DMSO (our control) and Harmine
- The cDNA from islet cells were extracted
- Picked 3 transcription factors for beta-cells: PDX1, NKX6.1, MAFA , GLUT2, P57, GLP1R
- Used 1 control: Cyclophilin A
- Mixture of 5 microliters of SYBR Green (nucleic acid stain) + 1 microliter of primer + 3 microliters of H_2O + 2 microliters of cDNA
- Inserted into Real-Time PCR machine

p57:

After we tranduced our 3 adenoviruses into our islet cells, we stained our cells.

- Aspirate 250 microliters of PBS (Phosphate Buffer Solution -- washes out wells)
- 300 microliters of 4% PFA in each well for 10 minutes
- Used 2mL of blocking buffer + 100 microliters of PBS and placed on a shaker for an hour
- Rinsed out twice with 250 microliters + left in 4 degree Celsius freezer
- Add 4.0 microliters of C peptide antibody and 6.7 microliters p57 antibodies
- Aspirate + add 250 microliters of buffer + DAPI

Notch:

- similar to p57 staining method except with rounds of both primary and secondary antibodies

Results- PCR

	Angela	Jeffrey	Olivia
Cyclin	1	1	1
MAFA	2.127	1.362064102	1.194704601
NKX6.1	0.8841260032	1.726957221	2.377149194
GLUT2	3.397788575	3.202771406	1.03100909
P57	5.829893498	1.631759083	1.368040939
PDX1	3.605243746	3.8310551	0.9318056693
GLP1R	4.216079538	2.662022586	1.987830693

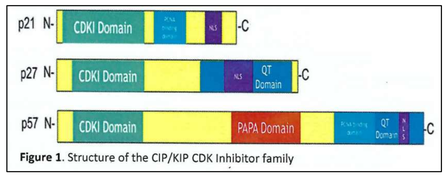
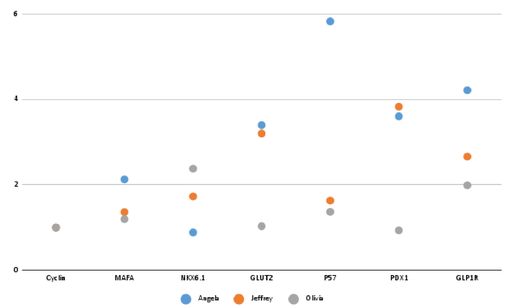
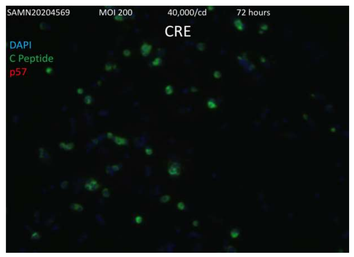


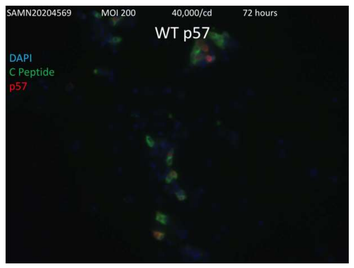
Figure 1. Structure of the CIP/KIP CDK Inhibitor family



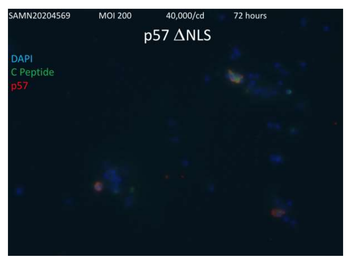
Results- p57 Transduction



In this image, CRE, our control, only has two stainings: DAPI and C Peptide. DAPI (blue) stains for all cells and C Peptide (green) stains for insulin.



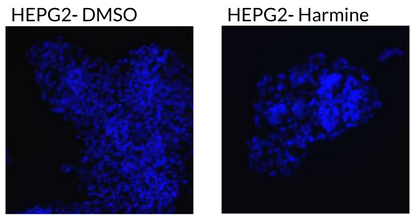
This image shows the WT (wildtype) p57 gene without any deletions. We can see all three colors—DAPI, C Peptide, p57—present. Also, the red p57 coloring is in the nuclei of all the beta cells.



This is an enlarged version of the image to the left. In this image, you can clearly see how the red p57 coloring is surrounding the nuclei of the beta cells.

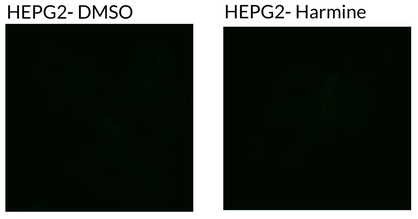
Results- Notch Antibodies

DAPI (1:500) + Notch2 (1:100) **40,000/cd**



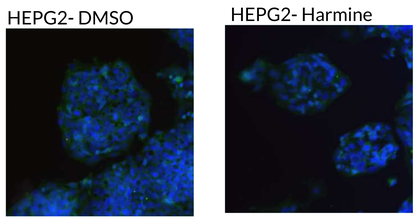
There appears to be no Notch 2

Notch2 (1:100) **40,000/cd**



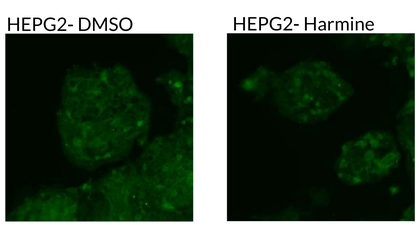
Notch 2 alone

DAPI (1:500) + Notch 3(1:100) **40,000/cd**



Notch3 staining in the vesicle/cytoplasm (seen there because it is being secreted)

Notch 3(1:100) **40,000/cd**



Notch3 appears duller in harmine treated cells

Discussion/Conclusions

Harmine/DYRK1A Inhibitors:

- Dr. Stewart and Dr. Wang partnered with Mount Sinai to perform a high throughput small molecule (can be taken orally) screen of 102,300 small molecules to test for beta cell proliferation
- Only one molecule, harmine, statistically significantly caused beta cells to proliferate (2% per day)
- Harmine is a DYRK1A inhibitor (Dual Specificity Tyrosine-phosphorylation-Regulated Kinase 1A is an enzyme encoded by the *DYRK1A* gene)
- Later experiments found interactions between GLP-1 (a current on the market diabetes treatment that causes living beta cells to secrete insulin) and harmine (5-6% proliferation per day)

PCR

In our results, we saw that p57 increased the greatest out of all the transcription factors. So we concluded that p57 plays an important structural role in beta cell physiology.

p57

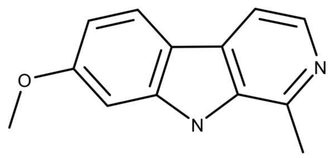
In our experiment, we transduced 3 adenoviruses into our islet cells: CRE, WT p57, and ΔNLS

- CRE - Our control
- WT p57 - Contains the full p57 gene (NO deletion)
- ΔNLS - Contains the full p57 gene WITHOUT the NLS domain

In the **Δ**NLS images, there was no red p57 coloring in the nuclei of the islet cells. However, in the WT p57 images, there was p57 located inside the nuclei. So, our prediction of the location of the nuclear localization sequence was correct.

Notch

We have confirmed the effective dilution of our lab's antibody for Notch3 and shown the antibody for Notch2 to be ineffective in cell lines. This will be useful information for our lab as they continue to investigate the relationship between Notch and beta cell replication.



To the left is an image of the chemical compound of Harmine ($C_{13}H_{12}N_2O$).

Future Work

- Harmine is currently in human trials.
- P57 merits more research.
- Currently undergoing experiments deleting other sections of the virus like PAPA and CDKI.
- Also currently undergoing research in combination with harmine and other DYRK1A inhibitors.
- Researching various other antibodies, both in the Notch family and not
- Further confirm the antibodies work with negative controls and western blotting

Selected References

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