University of Utah
UNDERGRADUATE RESEARCH JOURNAL

INVESTIGATING THE ROLE OF A PTF1A TARGET GENE, FGF21, IN MAINTAINING DIFFERENTIATED PANCREATIC CELLS

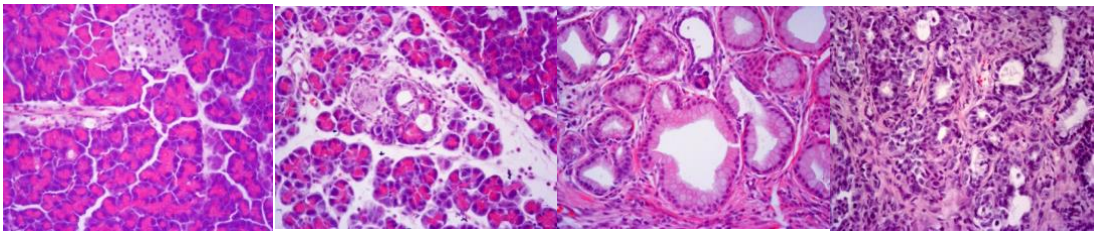
Ella Gregory (Diane Hernandez, Dr. Charles Murtaugh)
Department of Human Genetics

Introduction

Pancreatic cancer is the fourth leading cause of cancer death in men and women (Ilic et al 2016). The most common form is pancreatic ductal adenocarcinoma (PDAC), which has an extremely low survival rate. PDAC forms epithelial tumors that arise from acinar cells, which normally produce and secrete digestive enzymes to aid in digestion (Rooman and Real, 2012; Murtaugh, 2014). PDAC initiation is characterized by the transition of healthy acinar cells to acinar-to-ductal metaplasia (ADM), pancreatic intraepithelial neoplasms (PanINs), and finally pancreatic cancer. Mutations in the KRAS gene have been linked to the development of pancreatic cancer, and have shown to drive the formation of PanINs (Di Magliano and Logsdon, 2013). However, mutant KRAS is not the sole driver of PDAC initiation and is not sufficient in activating the pathway needed for aggressive cell proliferation (Murtaugh, 2014). We are interested in what other factors could be playing a role in this cancer initiation.

Our lab studies the transcription factor Ptf1a and its role in PDAC progression. Ptf1a is a transcription factor important in the maintenance and function of healthy acinar cells. Loss of acinar cell identity results in acinar cell reprogramming, in which the cells lose their normal pattern of gene expression and adapt a more duct like phenotype (Rooman and Real, 2012; Murtaugh, 2014). Knocking out Ptf1a in a mutant Kras background resulted in exacerbated ADM and PanIN formation compared to control mutant Kras mice. This work showed that Ptf1a inhibits mutant Kras reprogramming when it is present (Krah et al., 2015).
Work from our lab studying the role Ptf1a plays in the initiation of pancreatic cancer has found that Ptf1a is turned off in PanINs and that ADM and PanIN growth were extremely high in mice that had lost Ptf1a and expressed mutant KRAS. This led us to hypothesize that Ptf1a has protective properties and works to inhibit reprogramming of acinar cells or maintain differentiated acinar cells. To discover this potential protective factor, our lab analyzed RNA sequencing data comparing pancreata from wild-type mice and Ptf1a knockout mice. Through this method, fibroblast growth factor 21 (FGF21) was identified as a possible candidate for several reasons. FGF21 is highly expressed in the pancreas, it is significantly downregulated in Ptf1a KO mice, and has anti-inflammatory properties in the pancreas (Fon Tacer et al, 2010). Therefore, we hypothesize that the Ptf1a target gene, FGF21, works to maintain acinar cell differentiation and inhibit reprogramming.

1. Differentiated Acinar Cell | 2. Acinar-to-Ductal Metaplasia | 3. PanIN | 4. Pancreatic Cancer

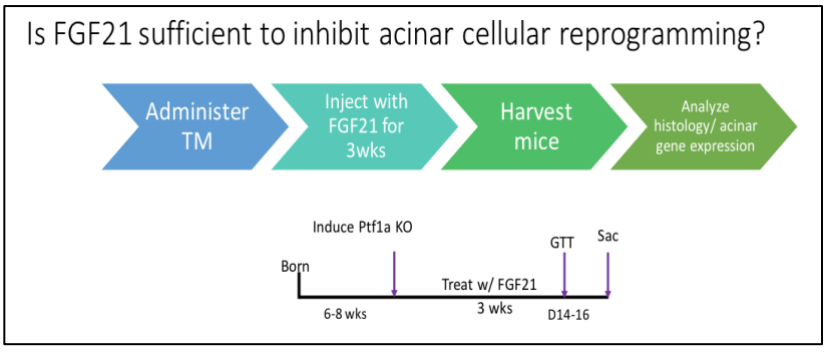


This figure represents the progression of the precancerous stages from healthy to cancerous through H&E staining. Figure 1 shows healthy acinar cells in clusters with nuclei at the edges of the cell and large pink cytoplasm. Figure 2 shows ADM formation where acinar cells lose their shape and become more cuboidal/duct-like. Figure 3 shows PanIN formation, these lesions can form large structures in which cells become more columnar in shape. Figure 4 shows pancreatic cancer with loss of normal tissue, fibrosis, and cellular reprogramming.

Experimental Design

To test if FGF21 is sufficient in inhibiting acinar cell reprogramming, Tamoxifen (TM) will be administered to Ptf1a cKO and Ptf1a cKO; KrasG12D mice to induce Ptf1a KO. FGF21 will then be injected to the mice for three weeks. The mice will then be harvested and their tissue will be analyzed through histology/ acinar gene expression. We will look for less ADM/PanINs in the Ptf1a KO mice and collect data. If there is no change in the amount of ADM/PanINs quantified, then we may be able to reject our hypothesis that FGF21 works to maintain acinar cell differentiation and inhibit reprogramming.

Figure 5: Timeline of Experiment

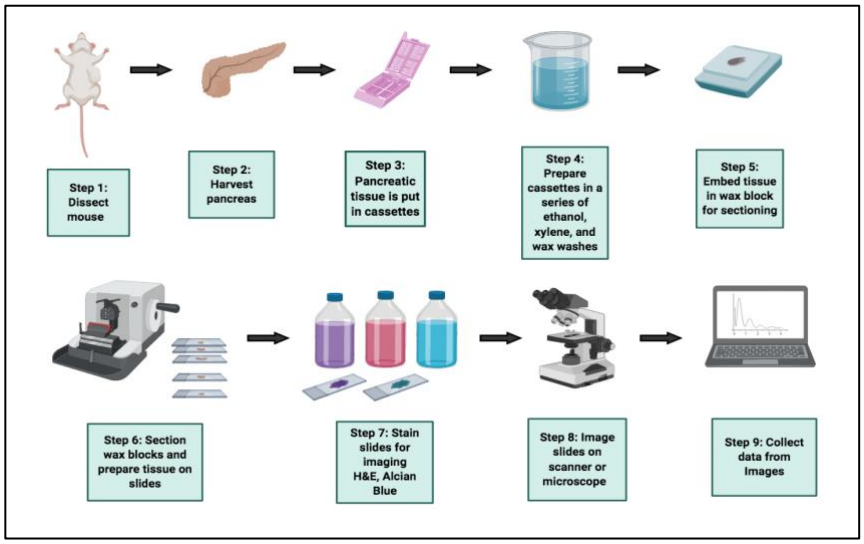


Methods

My role in this project begins in the fixation and embedding process of the tissue. I assist hands-on in the dissection of the mouse when harvesting the pancreas, in which I take note the weight and appearance of the pancreas. Following the dissection, I then fix the tissue in a series of rinses and washes of ethanol, xylene, and hot wax. After doing so, I will embed the tissue in a wax block for sectioning. I then section the tissue and prepare it on slides for histology staining and imaging. I will be looking for the formation of ADM and PanINs. These are distinguishable

from healthy pancreatic tissue by their structure and by using different stains that will allow us to look at markers for healthy acinar cells, ADM, and PanINs. I will then quantify the number of these cellular changes, analyze overall findings from the imaging and draw conclusions.

Figure 6: Methods of Experiment



In the lab, most of my time is dedicated to sectioning and staining. H&E staining is the most common stain used in histology. Within the tissue, the nucleus and other RNA-containing structures of the cells will stain purple and the cytoplasm will stain pink. This will help us to better see the morphology of the tissue (Paxton 2003).
Alcian Blue staining is used to identify PanINs. Alcian blue stains acidic sulfate groups in mucins that are produced by PanINs, staining them blue. This allows for better PanIN identification within the tissue (Myers 2010).

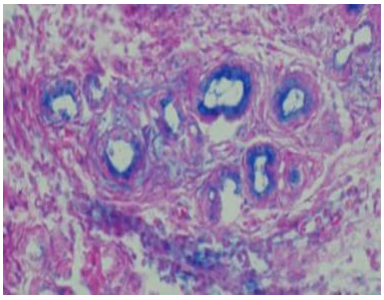


Figure 7: Alcian Blue Staining from *FGF21 Kras Exp 1*, image of PanINs in tissue

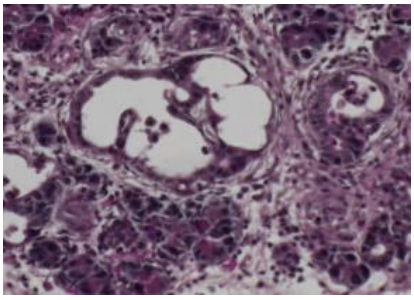


Figure 8: H&E Staining from *FGF21 Kras Exp 1*, image of PanINs and ADM

Results

Fgf21 treatment does not prevent ADM/ PanIN formation

Figure 9

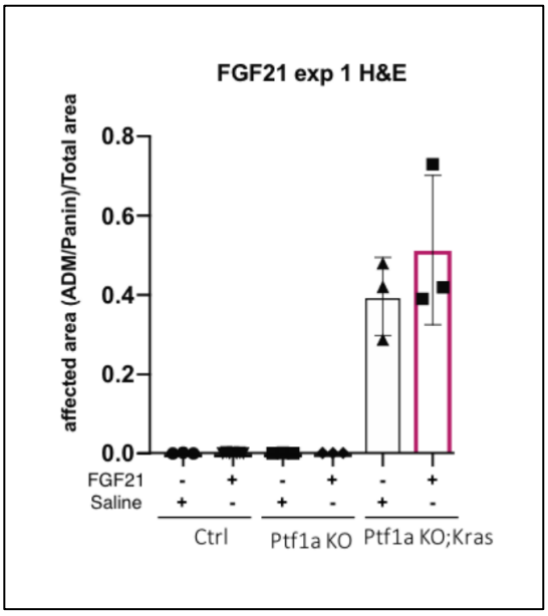


Figure 10

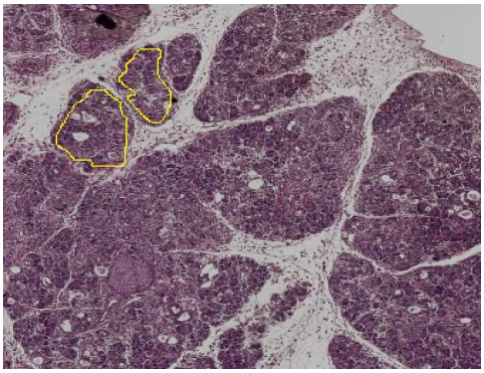


Figure 11

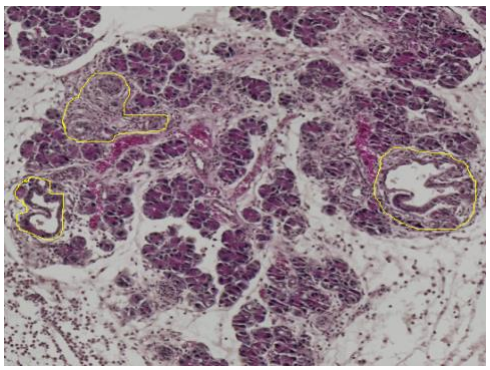


Figure 9 is a graph showing the histological quantification results of *FGF21 exp 1* in control and mutant mouse models. Affected area in Ptf1a KO; Kras is high despite the administration of FGF21. Figure 10 is an H&E stained image of a Ptf1a KO; Kras mouse *without* the administration of FGF21. PanIN/ADM (highlighted in yellow) formation is high. Figure 11 is an H&E stained image of Ptf1a KO; Kras mouse *with* the administration of FGF21. PanIN/ADM (highlighted in yellow) formation is high, similar to Figure 8. Both Figure 10 and 11 are from *FGF21 exp 1*.

Conclusion

To test our hypothesis FGF21 administration is sufficient in inhibiting acinar cell reprogramming, we administered TM to induce Ptf1a KO and then injected mice with FGF21 for three weeks before the mice were harvested. We expected to see a significant decrease in PanIN/ADM formation in the mice that received FGF21 injections compared to the ones that did not. However, there was no significant change in PanIN/ADM formation between the two. Both conditions, with FGF21 and without, had similar results. These results can be seen in Figure 10, 11, and 12. Figure 10 shows there is no change in affected area of the Ptf1a Ko; Kras mice. Pancreata from these conditions show severe PanIN/ADM formation but no difference (figure 11,12).

There are several reasons that could explain why this experiment was unsuccessful. This was the first experiment conducted that tested this hypothesis. Therefore, mistakes could have been made and our methods or experimental design may need to be altered in order to yield different results. Another factor in explaining the lack of effect of FGF21 could be that there were inconsistent levels of recombination within the different experimental groups. A different possibility could be that this hypothesis is incorrect and FGF21 is insufficient in suppressing PanINs on its own and the FGF21 protein is ineffective. In order to test this and draw conclusions, we plan to analyze immunofluorescence staining. We also plan to test this hypothesis in future experiments in similar and different mouse models as well as re-analyze our data. If similar results are reproduced in future experiments, a new hypothesis may need to be tested in order to determine if the mice are responding to FGF21 administration.

Acknowledgements

I would like to thank the University of Utah Office of Undergraduate Research for supporting my work and for funding this research. Also, special thanks to Diane Hernandez for her mentorship and help throughout this experiment. Figures 5 and 9 are taken from her presentation on this research. Lastly, I would like to thank Charles Murtaugh for allowing me to research in his lab and help improve my knowledge and understanding in scientific experimentation and research.

References

Aier, I., Semwal, R., Sharma, A., & Varadwaj, P. K. (2019). A systematic assessment of statistics, risk factors, and underlying features involved in pancreatic cancer. *Cancer epidemiology*, *58*, 104-110.

Di Magliano, M. P., & Logsdon, C. D. (2013). Roles for KRAS in pancreatic tumor development and progression. *Gastroenterology*, *144*(6), 1220-1229.

Fon Tacer, K., Bookout, A. L., Ding, X., Kurosu, H., John, G. B., Wang, L., ... & Kliewer, S. A. (2010). Research resource: comprehensive expression atlas of the fibroblast growth factor system in adult mouse. *Molecular endocrinology*, *24*(10), 2050-2064.

Hammad, S., Othman, A., Meyer, C., Telfah, A., Lambert, J., Dewidar, B., ... & Gao, Y. (2018). Confounding influence of tamoxifen in mouse models of Cre recombinase-induced gene activity or modulation. *Archives of toxicology*, *92*(8), 2549-2561.

Hezel, A. F., Kimmelman, A. C., Stanger, B. Z., Bardeesy, N., & DePinho, R. A. (2006). Genetics and biology of pancreatic ductal adenocarcinoma. *Genes & development*, *20*(10), 1218-1249.

Ilic, M., & Ilic, I. (2016). Epidemiology of pancreatic cancer. *World journal of gastroenterology*, *22*(44), 9694.

Jain, R., Fischer, S., Serra, S., & Chetty, R. (2010). The use of cytokeratin 19 (CK19) immunohistochemistry in lesions of the pancreas, gastrointestinal tract, and liver. *Applied Immunohistochemistry & Molecular Morphology*, *18*(1), 9-15.

Jensen, J. N., Cameron, E., Garay, M. V. R., Starkey, T. W., Gianani, R., & Jensen, J. (2005). Recapitulation of elements of embryonic development in adult mouse pancreatic regeneration. *Gastroenterology*, *128*(3), 728-741.

Keefe, M. D., Wang, H., De La O, J. P., Khan, A., Firpo, M. A., & Murtaugh, L. C. (2012). β-catenin is selectively required for the expansion and regeneration of mature pancreatic acinar cells in mice. *Disease models & mechanisms*, *5*(4), 503-514.

Krah, N. M., De La O, J. P., Swift, G. H., Hoang, C. Q., Willet, S. G., Pan, F. C., ... & Murtaugh, L. C. (2015). The acinar differentiation determinant Ptf1a inhibits initiation of pancreatic ductal adenocarcinoma. *Elife*, *4*, e07125.

Lankadasari, M. B., Mukhopadhyay, P., Mohammed, S., & Harikumar, K. B. (2019). TAMing pancreatic cancer: combat with a double edged sword. *Molecular cancer*, *18*(1), 48.

Murtaugh, L. C. (2014). Pathogenesis of pancreatic cancer: lessons from animal models. *Toxicologic pathology*, *42*(1), 217-228.

Myers, R. (2010). Special stain techniques for the evaluation of mucins. *Journal [serial on the Internet]. Available from: http://www. leicabiosystems. com/pathologyleaders/special-stain-techniques-for-the-evaluation-ofmucins.*

Paxton, S., Peckham, M., & Knibbs, A. (2003). The Leeds Histology Guide.

Rooman, I., & Real, F. X. (2012). Pancreatic ductal adenocarcinoma and acinar cells: a matter of differentiation and development?. *Gut*, *61*(3), 449-458.