Understanding Pancreatic Development Using a Ferret Model

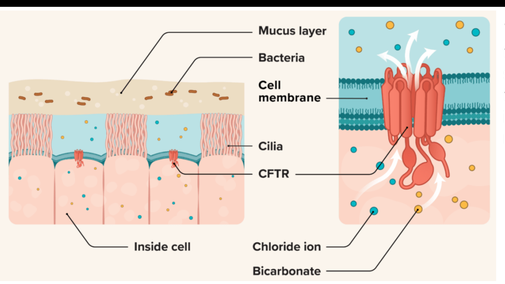
Rachael Branscomb[1,2], Bryce Hopwood[1], John Engelhardt[3], Lori Sussel[1]

[1]Barbara Davis Center, [2]University of Colorado School of Medicine, [3]University of Iowa

Abstract

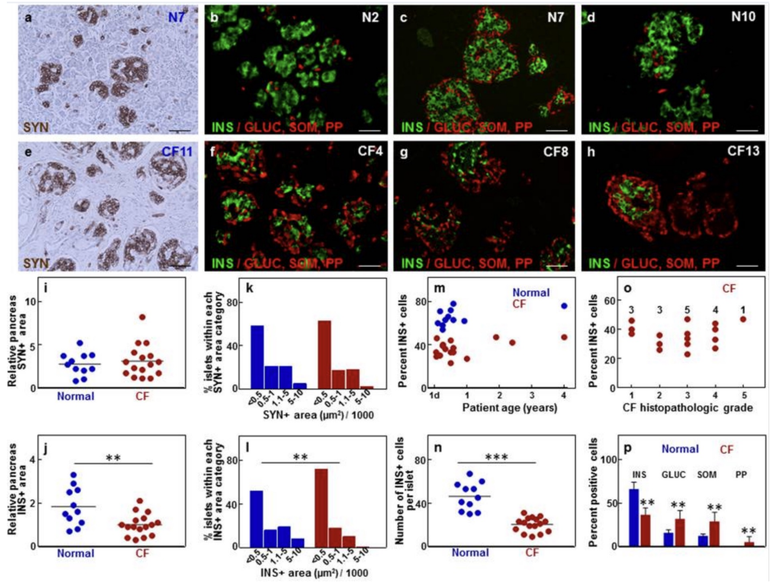
Cystic fibrosis-related diabetes (CFRD) is the most significant comorbidity of CF, impacting >50% of adult patients. Studies in young children with CF indicate that perinatal defects in islet function is an early clinical feature of CF, but the cause of this dysfunction remains controversial. To begin to understand the potential origins of CFRD, it would be optimal to study an animal model; however, CFRD is not well-modeled in mice and other animal models pose their own challenges. Alternatively, CFRD occurs spontaneously in the ferret model of CF, suggesting this would be a useful model to characterize whether there is a developmental origin of pancreas dysfunction in patients with CF. Because the development of the fetal ferret pancreas has not yet been characterized, the purpose of this project is to characterize wild type ferret pancreas development as a baseline for future comparison with a CF ferret model. Immunofluorescent staining was employed to identify key markers of development and islet hormone expression patterns in fetal ferret tissues. In this study, we demonstrate that WT ferret and human islet formation appear similar, and both species diverge from mouse pancreatic morphology. Future studies are underway to determine whether CF ferrets display altered pancreatic islet development and hormone expression.

CFTR Mutation Leads to Ductal Obstruction in the Pancreas



The CFTR protein is highly expressed in the apical membrane of epithelial cells in small pancreatic ducts. The resultant thick, inspissated mucous leads to intraluminal destruction of ducts with resultant progressive pancreatic damage.

Pancreatic Remodeling Occurs in Young CF Patients



Analysis of endocrine cell distribution and islet structure in CF patients <4 years of age compared to control patients. Immunofluorescence microscopy and quantification of islet cell types indicated remodeling of islets (Bogdani, 2017).

Acknowledgements

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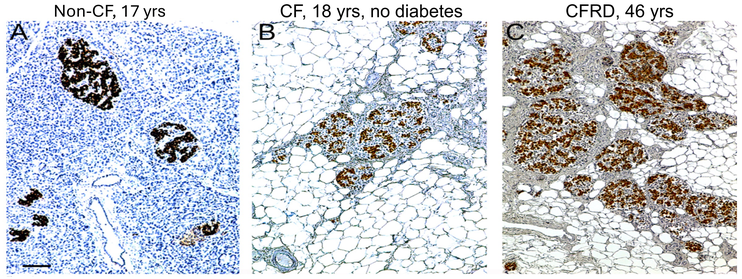
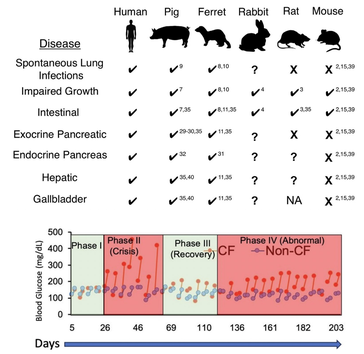
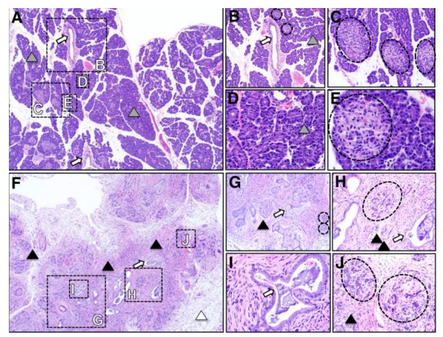
Cystic Fibrosis-Related Diabetes (CFRD) is the Most Significant Comorbidity of CF



Immunohistochemical staining demonstrates progressive widespread acinar destruction, fatty replacement, and appearance of extensive fibrosis in the pancreas of patients with CF and CFRD (courtesy of Engelhardt lab).

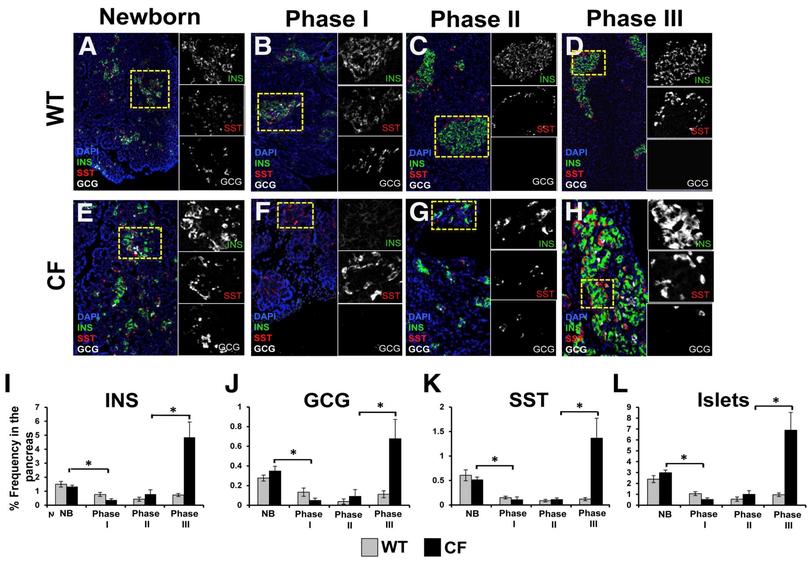
CF Ferrets Recapitulate Human Disease





A-E: WT ferret pancreas demonstrates abundant exocrine pancreatic tissue, multiple ducts, and interspersed islets of Langerhans. F-J: CF ferret pancreas with loss of exocrine tissue and replacement by fibrosis and adipose tissue, dilation of ducts, and clumping of islets (Rotti, 2018)

CF Ferrets Undergo Pancreatic Remodeling Early in Life



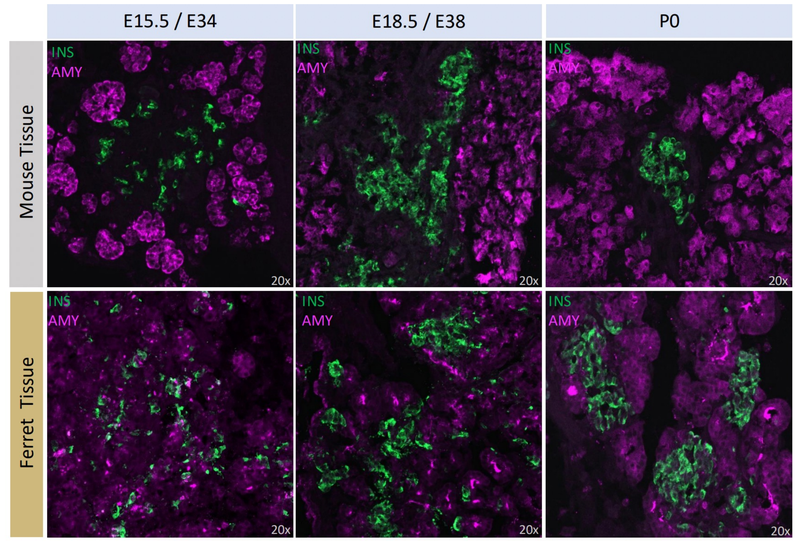
Immunofluorescence microscopy demonstrating differences in islet cell distribution and remodeling in young CF ferret pancreas compared to WT ferrets (Rotti, 2018).

Methods

We used n = 6 wild-type ferret embryos which were dissected for the pancreas, fixed, mounted, and sectioned. Immunofluorescence staining was performed as per a standard protocol. Image acquisition was performed using a Zeiss confocal microscope.

Comparative Analysis of Ferret Pancreatic Development

1.



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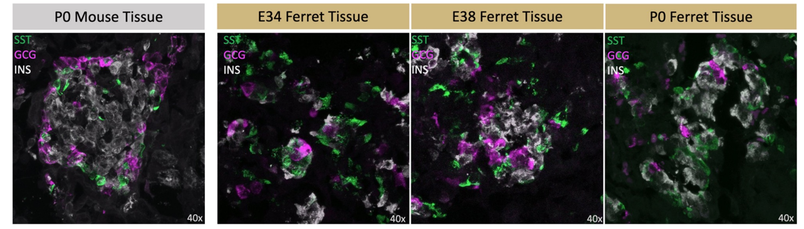

1. Immunofluorescence microscopy comparison of exocrine and endocrine structure of WT ferret pancreas compared to WT mouse. 2. Developmental progression of islet architecture in WT ferret.

Conclusions

- Given the many similarities in islet morphology, pathology, and disease progression seen in CF ferrets and CF humans, ferrets appear to be a promising candidate as a model for the study of CF and CFRD.
- Our preliminary immunofluorescence data indicates differences in WT ferret development as compared to WT mouse development and appears to be more similar to published human data.

Future Directions

- Single cell RNA-sequencing using SPLiT-sequencing is underway to perform comprehensive comparative analyses between the ferret, human, and murine transcriptomes.
- Optimize protocol to improve primary antibody staining.
- Perform immunofluorescence staining at other time points in development and in CF ferrets