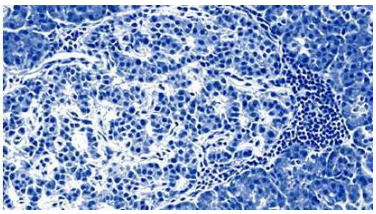
nPOD
Network for Pancreatic Organ Donors with Diabetes



3D Visualization of the Dynamic and Regional Variation of Pancreatic Innervation in Diabetes

AUTHORS

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PURPOSE

The endocrine pancreas is densely innervated, and neural signals play a significant role in glucose regulation by modulating pancreatic hormone release. However, relatively little is known about the anatomical relationships between islets and nerves across the whole pancreas. Since thin filamentous structures, such as nerves, are difficult to quantify and trace over large volumes using thin section histology, there is a need for high resolution imaging and rendering of intact tissue in 3D. Here, we used optical clearing, whole organ imaging, and 3D rendering to quantify innervation across the whole pancreas in healthy mice, in two mouse models of diabetes, and in pancreatic samples from nondiabetic and diabetic human donors.

METHODS

Whole-mount staining and clearing was performed using iDISCO+ to quantify innervation, defined by the neuronal marker neurofilament 200 kDa, and beta cells in pancreata from C57Bl/6 mice, non-obese diabetic (NOD) mice, streptozotocin (STZ)-treated mice, and in pancreatic samples from nondiabetic and diabetic human donors. Z-stacked optical sections were acquired with an Ultramicroscope II at 4x or 12x magnification. Imaris was used to create digital surfaces covering the NF200+ innervation and islets to automatically determine innervation density and islet/nerve interactions.

SUMMARY OF RESULTS

Tissue clearing and volume imaging of the pancreas provided several new insights. First, innervation of the endocrine pancreas is significantly enriched compared to the surrounding exocrine pancreas with regional variation. Next, islets are closely associated with pancreatic innervation and decrease in size with increasing distance from nerves in both mouse and human pancreatic tissue. Innervated islets are relatively preserved in models of diabetes. Finally, islet innervation and expression of neural markers are higher in human samples from diabetic patients and in mouse models of diabetes with temporal and regional differences.

CONCLUSIONS

3D imaging and unbiased analysis across the whole pancreas provides comprehensive measurement of pancreatic nerve volumes and distribution. It allows detailed analysis of the anatomical relationship between nerves and islets, which reveals a close association that is maintained across species. The relative enrichment of innervated islets in diabetes and dynamic changes in islet innervation during the development of diabetes suggest further work is need to examine the role of pancreatic nerves in preserving and protecting beta cells.