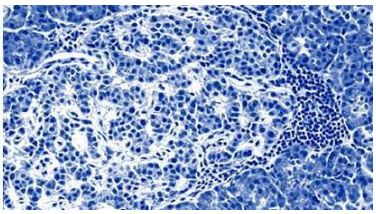
nPOD
Network for Pancreatic Organ Donors with Diabetes



Normal cellular prion protein is highly expressed in beta cells of type 1 diabetes human pancreata

AUTHORS

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PURPOSE

The abnormally folded isoform of prion protein (PrP^{SC}) has been mostly studied for its role in the development of transmissible spongiform encephalopathies (TSEs) and for its critical role in pathogenesis of neurodegenerative disorders like Alzheimer's disease (AD). However, loss of the normal prion protein (PrP^{C}) function in the brain has shown to be an integral part of neurodegenerative processes. Over the years PrP^{C} has been associated with a wide array of different cellular mechanisms and many interacting partners have been suggested. Some studies have proposed a role in copper and iron homeostasis in the brain. More recent studies have confirmed its participation in physiological processes such as regulation of cellular differentiation and proliferation, and control of cell morphology. As neurons and pancreatic β cells share abundant functional similarities, we examined PrP^{C} expression, a protein found predominately in neurons, in type 1 diabetes (T1D) and non-diabetic (ND) human pancreas. Moreover, we addressed differences between the expression of PrP^{C} in insulin positive (INS^{+}) versus insulin negative (INS^{-}) pancreatic islets in short duration T1D pancreata.

METHODS

Pancreata for this study were recovered from T1D and ND organ donors with informed consent from next of kin and processed by the Network for Pancreatic Organ donors with Diabetes (nPOD) program. PrP^{C} mRNA expression levels were measured by means of RT-qPCR in pancreata from 30 T1D and 30 ND. Normalization factors were applied to the analysis to report quantitative changes in gene expression between non-diabetics and T1D pancreata, with the fold difference (FD) reported as the ratio of the means (T1D/ND).Paraffin embedded pancreatic tissue sections from 12 T1D (with residual insulin positive islets) and 12 ND organ donors were stained for PrP^{C}, insulin (INS), glucagon (GCG) and somatostatin (SOM) by immunofluorescence. Imaging was performed using a BZ-X710 Keyence florescence microscope. Immunofluorescence brightness was measured to assess a degree of prion protein expression in INS^{+} and INS^{-} pancreatic islets and it was calculated by means of Integration (value of multiplying the area of extracted part by the mean brightness before binarization) using the Hybrid Cell Count software from Keyence®. Consecutive tissue sections, were stained with Thioflavin-t and Congo red to determine the presence of

amyloid fibrils and aggregates. To assess metal deposition in T1D versus ND pancreas histochemical stains for copper and iron were performed using commercially available kits.

SUMMARY OF RESULTS

With total mRNA isolated from each pancreas using gene specific oligonucleotide primer sets, expression levels of the PrP^C gene in T1D and ND donors was tested. Normalized RTqPCR data revealed significant increase in PrP^C expression in T1D versus ND pancreata, with a mean FD of =10.85 (*p=<0.0001*).

At the cellular level and based on immunofluorescence analysis (IF), PrP^C was found to be homogeneously distributed in the cytoplasm of T1D and ND donors. Foremost, we found that in ND pancreas as well as in residual INS^+ islets of T1D pancreata, PrP^C was expressed mostly in β cells. In contrast, in INS^- islets we found that PrP^C localized generally with α-cells. Interestingly, in both INS^+ and INS^- islets we observed PrP^{C+} cells that were negative for insulin, glucagon and somatostatin. Based on our IF quantitative analysis we found that the overall intensity of PrP^C expression was significantly higher in INS^+ versus INS^- islets in T1D pancreas with residual INS^+ islets. In addition, we found that within INS^+ islets T1D donors, PrP^C intensity in INS^+ cells was significantly higher than in adjacent PrP^{C+}/INS^- cells.

In neurodegenerative disorders such as TSEs and AD it has been shown that the accumulation of the modified form of prion protein (PrP^{SC}) into amyloid deposits is one of the leading reasons for the onset of these diseases. To assess the presence of amyloid aggregates and their possible correlation with PrP^{SC}, the pancreatic tissue sections from T1D organ donors were stained with Thioflavin-t and Congo red. Only 3/12 T1D cases showed potential amyloid deposits within the islets, which most interestingly did not display co-expression with PrP^C. Finally, histochemical staining showed no positivity for either copper or iron in T1D or ND donors, which conforms with new literature suggesting a weak involvement of PrP^C in copper/iron hemostasis and stress protection

CONCLUSIONS

To our knowledge, this is the first study to examine the presence of normal cellular prion protein in human pancreata, specifically in donors with type 1 diabetes. We observed a significant increase in PrP^C mRNA level in T1D donors, suggesting a possible link between T1D and PrP^C. Our staining results indicate that PrP^C localizes and is expressed mostly within β cells in either ND or in residual INS^+ islets of T1D donors. Most strikingly, in both INS^+ and INS^- islets of T1D pancreata, we observe PrP^C expression in cells lacking prominent endocrine cell markers. Future studies in live human pancreatic slice cultures will attempt to address the mechanistic implications of our findings and the potential role of PrP^C in type 1 diabetes pathogenesis.