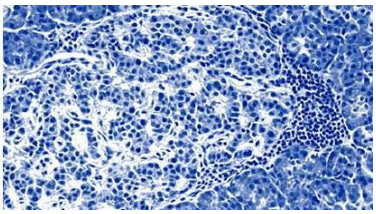
nPOD
Network for Pancreatic Organ Donors with Diabetes



Investigating the Role of Nuclear versus Cytoplasmic Hyperphosphorylated Tau in Human β cells During Ageing and Diabetes

AUTHORS

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PURPOSE

Two common haplotypes, H1 and H2, of the *MAPT* gene, which encodes the Tau protein, are associated with susceptibility and protection, respectively, to neurodegenerative diseases, including Parkinson's. Although the molecular pathways that translate genetic risk into disease susceptibility are incompletely understood, Tau hyperphosphorylation (pTau) and aggregation are thought to be drivers of neuronal death. The lower risk of H2 carriers has been associated with higher activity of a polymorphic antioxidant response element NRF2-binding site and, in neurons, with a higher expression of exon 3-containing *MAPT* isoforms, thought to encode less aggregation-prone isoforms of Tau. The H2 *MAPT* haplotype is also protective for T1D and therefore we asked whether Tau, often referred to as neuron-specific, and its presumed pathological form, pTau, are also expressed in islet β cells. Given that we now know that the answer is unequivocally, yes, we are currently investigating if they are functionally implicated in β-cell dysfunction, in the context of ageing or diabetes. We use a combination of the human EndoC-βH1 and -H3 cell lines, human pancreatic islets isolated from organ donors and pancreatic tissue sections from nPOD (University of Florida, USA) and IsletCore (University of Alberta, Canada).

METHODS

A panel of total Tau and pTau antibodies were validated and optimised for use in western blotting (WB), co-immunoprecipitation (co-IP), immunocytochemistry (ICC) on cultured cells and immunohistochemistry (IHC) on FFPE tissue sections protocols. EndoC-βH1 and -H3 were used to study the expression, subcellular localisation and co-localisation with various compartment and proliferation markers of total Tau and pTau variants by WB and ICC-IF. To examine the localisation and levels of pTau in human β cells *in situ*, 4 μm FFPE human donor pancreas sections were obtained from nPOD and IsletCore. So far, sections from a total of 128 donors have been analysed: of which 77 non-diabetic donors, 28 T2D donors and 23 T1D donors. All sections were stained using standard IF protocols using an antiserum raised against pTau. Anti-insulin, anti-glucagon or the nuclear marker DAPI were also included. High-resolution images of 20 islets/case were then processed using a custom Matlab script to define the subcellular localisation (cytoplasmic or nuclear) and quantify the intensity of pTau staining. Sections from a subset of donors were also stained for other anti-

pTau antibodies, anti-total Tau antibodies or the Ki67 proliferation marker. Clinical data for nPOD donors were retrieved from the nPOD database. Information on donor genotype at the rs1052553 SNP (A>T), which tags the H1 and H2 main *MAPT* haplotypes, was also obtained from nPOD and IsletCore, and was used to assess the effect of donor genotype on pTau in β cells. To gain insights into the role of Tau and pTau variants in β cells, Tau-interacting proteins in β cells were identified through a series of co-IP experiments using total and pTau antibodies on lysates from both, EndoC-βH1 and EndoC-βH3 cultured cells, and also from human pancreatic islets isolated from non-diabetic organ donors. Sub-cellular fractionation protocols were employed to study the differences in Tau-interacting partners between the cytoplasmic and nuclear compartments. Gene ontology enrichment analysis was carried on the resulting list of proteins using publicly-available tools.

SUMMARY OF RESULTS

Using FFPE pancreas sections and a validated panel of anti-Tau antibodies, we found that both total Tau and "pathogenic" pTau variants are present in human endocrine cells from non-diabetic donors *in situ*. Tau and pTau presence were further confirmed in human islets by WB and mass-spectrometry, and in cultured EndoC-βH1 and EndoC-βH3 immortalised human β cells by WB, ICC-IF and mass-spectrometry. High-resolution confocal imaging revealed that pTau, phosphorylated at several pathological epitopes, is present in β cells under basal culture conditions, and that pTau variants localise preferentially to the nucleus, in contrast to the mainly cytoplasmic distribution of total Tau. Using human donor pancreas sections, we have confirmed that findings, including the unexpected nuclear localisation of pTau variants, also translate to human β cells *in situ*. In non-diabetic donors (BMI<31), we further identified an effect of ageing on the subcellular localisation of pTau variants. Specifically, we found that, whereas in younger individuals (<10 yr) pTau localises predominately to the nucleus, with increasing age, nuclear pTau levels decrease accompanied by an increase in cytosolic pTau levels. This relationship correlated directly with age such that all individuals >35 yr had the majority of the pTau present within the islet localised to the cytosol. Using high-resolution confocal microscopy, it was further observed that pTau immunostaining intensity levels varied among β cells within the same islet, and that pTau and insulin are detected in distinct, mutually exclusive cytoplasmic locations. We note that pTau also localises to the nucleus in acinar cells, but maintains its nuclear localisation throughout the different age sub-groups, thus widening the relevance of pancreatic Tau studies beyond β-cell biology but also pointing to features of an ageing β cell that do not occur in other cell types, including islet δ and α cells. To gain insight into the role of total Tau and pTau variants in human β cells, we have optimised and carried out a series of Tau co-IP experiments in both cultured EndoC-βH1 cells and human donor islets, using either whole-cell lysates or nuclear vs. cytoplasmic fractions. Gene ontology enrichment analyses have revealed that nuclear pTau interacting partners are significantly enriched in RNA-binding proteins and spliceosome components, suggesting a novel role for pTau in control of gene expression and alternative splicing. Using EndoC-βH3 cells treated with chemical modulators of kinase and phosphatase inhibitors, we have found evidence that Tau phosphorylation controls the protein's subcellular localisation.

CONCLUSIONS

pTau variants, widely regarded in neurodegenerative diseases as cytotoxic, are also present in human β cells *in situ*, even in the absence of diabetes, and in human β cells cultured under basal conditions. Furthermore, we find that pTau variants localise predominantly to the nucleus, at least in younger individuals, despite the fact that Tau lacks a nuclear localisation signal. Through co-IP experiments we have characterised the Tau-interacting partners in human β cells, thus revealing novel potential roles for Tau in the control of RNA metabolism, alternative splicing and gene expression. Ongoing analyses include analysis of nPOD pancreas sections from patients with T1D, with T2D and non-diabetic donors with a range of BMIs (controlling for age effects), and understanding the changes that lead to the decrease in nuclear pTau and accumulation of pTau in the cytoplasm of β cells with increased age. A progress update on these aspects of the work will also be presented. Given our findings, we hypothesise that *MAPT* is a T1D causal gene in the chromosomal region 17q21.31, and that the loss of nuclear pTau and the accompanying increase in cytosolic pTau that occurs in β cells with age and increased BMI (as our preliminary data suggest) may be associated with reduced insulin secretory capacity and increased β-cell fragility.