Increased vimentin in human α- and β-cells in type 2 diabetes

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Abstract

Type 2 diabetes (T2DM) is associated with pancreatic islet dysfunction. Loss of β-cell identity has been implicated via dedifferentiation or conversion to other pancreatic endocrine cell types. How these transitions contribute to the onset and progression of T2DM in vivo is unknown. The aims of this study were to determine the degree of epithelial-to-mesenchymal transition occurring in α and β cells in vivo and to relate this to diabetes-associated (patho)physiological conditions. The proportion of islet cells expressing the mesenchymal marker vimentin was determined by immunohistochemistry and quantitative morphometry in specimens of pancreas from human donors with T2DM (n = 28) and without diabetes (ND, n = 38) and in non-human primates at different stages of the diabetic syndrome: normoglycaemic (ND, n = 4), obese, hyperinsulinaemic (HI, n = 4) and hyperglycaemic (DM, n = 8). Vimentin co-localised more frequently with glucagon (α-cells) than with insulin (β-cells) in the human ND group (1.43% total α-cells, 0.98% total β-cells, median; P < 0.05); these proportions were higher in T2DM than ND (median 4.53% α-, 2.53% β-cells; P < 0.05). Vimentin-positive β-cells were not apoptotic, had reduced expression of Nkx6.1 and Pdx1, and were not associated with islet amyloidosis or with bihormonal expression (insulin + glucagon). In non-human primates, vimentin-positive β-cell proportion was larger in the diabetic than the ND group (6.85 vs 0.50%, medians respectively, P < 0.05), but was similar in ND and HI groups. In conclusion, islet cell expression of vimentin indicates a degree of plasticity and dedifferentiation with potential loss of cellular identity in diabetes. This could contribute to α- and β-cell dysfunction in T2DM.

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Key Words
- dedifferentiation
- EMT
- non-human primate
- islet
- amyloid
- insulin
- glucagon
Introduction

Phenotypic plasticity of fully differentiated cells in adult pancreatic islets has been demonstrated in several rodent models of diabetes \textit{in vivo} (Thorel \textit{et al.} 2010, Talchai \textit{et al.} 2012, Brereton \textit{et al.} 2014, Piran \textit{et al.} 2014, Wang \textit{et al.} 2014, Kim-Muller \textit{et al.} 2016), in human islet cells \textit{in vitro} (Bramswig \textit{et al.} 2013, Spijker \textit{et al.} 2013) and in human pancreatic islets \textit{in vivo} (Sijpker \textit{et al.} 2015, Butler \textit{et al.} 2016, Cinti \textit{et al.} 2016). These cellular transitions of one mature endocrine cell type to a progenitor-like state or another islet cell type demonstrate that fully differentiated islet cells in adult pancreas have the capacity for conversion. This is likely to affect hormone secretion of individual cells (Brereton \textit{et al.} 2014), but it is as yet unknown whether it affects islet function to a clinically relevant degree in humans.

In rodent models, transcription factors that are important in islet cell development and embryonic differentiation (e.g. Pdx1, Ngn3, MafA, Foxo1, Arx, Pax4) are involved in the regulation of these processes and can be triggered by stimuli including hyperglycaemia and cellular stress (Jonas \textit{et al.} 1999, Dhawan \textit{et al.} 2011, Papizan \textit{et al.} 2011, Yang \textit{et al.} 2011, Szabat \textit{et al.} 2012, Talchai \textit{et al.} 2012, Al-Hasani \textit{et al.} 2013, Courtney \textit{et al.} 2013, Gao \textit{et al.} 2014, Nishimura \textit{et al.} 2015). Recently, our group reported reprogramming of β-cells in human pancreatic islets \textit{in vitro} and \textit{in vivo} (Spijker \textit{et al.} 2013, 2015); both bihormonal cells and cells transitioning from β- to α-cells were identified to be associated with changes in relevant transcription factors. Similar transitioning phenotypes and endocrine cells expressing the mesenchymal protein vimentin have been described in Foxo1-knockout mice in combination with metabolic stress (Talchai \textit{et al.} 2012); this was associated with the onset of hyperglycaemia, suggesting that β-cells can convert to a mesenchymal-like phenotype in diabetes.

Human β-cells can undergo an epithelial-to-mesenchymal transition (EMT) (including expression of vimentin) when cultured as a monolayer \textit{ex vivo} (Gershengorn \textit{et al.} 2004, Russ \textit{et al.} 2009). Vimentin expression in a subset of islet cells has been described as a limited number of human post-mortem pancreas specimens (Rukstalis & Habener 2009, Guo \textit{et al.} 2013, Gao \textit{et al.} 2014). In addition, case reports of 3 subjects proposed that vimentin expression was linked to aetiology of type 2 diabetes (T2DM) (White \textit{et al.} 2013). However, it is not known if this aberrant expression of a mesenchymal protein is a more widespread feature of T2DM or how vimentin expression is linked to the aetiology of T2DM and islet (patho)physiology \textit{in vivo} (White \textit{et al.} 2016).

The aim of this study was to determine the proportions of vimentin-positive endocrine cells in islets \textit{in vivo}, using quantitative immunohistochemistry in post-mortem samples of human and non-human primate pancreas and to determine if this was associated with the onset and pathophysiology of T2DM.

Materials and methods

Human pancreas and islets

Pancreatic tissue was obtained from subjects at post-mortem examination, including human organ donors. Samples were taken from subjects with a documented history of type 2 diabetes \((n=28)\) and without diabetes \((n=38)\) (Supplementary Table 1, see section on supplementary data given at the end of this article). Pancreata from organ donors were procured through donor programmes in The Netherlands and in Oxford, UK. Post-mortem and multi-organ donor tissue was obtained according to National laws and National and Local ethics guidelines and permissions. Pancreatic tissue or islets from the donor programmes were only studied if they could not be used for clinical purposes (pancreas or islet transplantation), and if research consent had been obtained. Tissue samples from the pancreatic tail region were fixed overnight in 4% formaldehyde and embedded in wax. For quantitative light microscopy, analyses were performed on 4-μm thick sections of pancreas. For electron microscopy, human isolated islets were fixed in 2.5% glutaraldehyde and embedded in resin. Human islet isolations were performed in the Good Manufacturing Practice facility in Leiden and in the Diabetes Research & Wellness Foundation Human Islet Isolation Facility in Oxford.

Pancreatic tissue from non-human primates

Macaca mulatta and Macaca fascicularis, non-human primates, were selected from a larger colony that has been extensively characterised for features of diabetes and metabolism (Hansen & Bodkin 1986). The animals were maintained in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (USA).
Animals for this study were divided into three groups based on the metabolic profile at killing, being normoglycaemic and normoinsulinaemic, normoglycaemic and hyperinsulinaemic (fasting immunoreactive insulin (IRI) >600 pmol/L) or overtly diabetic (fasting plasma glucose (FPG) >7.8 mmol/L) (Supplementary Table 2). Samples of the pancreatic tail were taken during killing, fixed in 4% formaldehyde and processed for wax embedding; 4-μm sections were cut for analysis.

Immunofluorescent labelling and quantification

Labelling of tissue sections and cell quantification were performed independently in Leiden and Oxford laboratories on different materials using similar but not identical methodologies. Tissue sections were rehydrated, and antigen retrieval was performed by heating slides in 0.01 M citrate buffer (pH = 6.0). Primary antibodies against insulin, glucagon, vimentin, Nkx6.1, Pdx1, N-cadherin, smooth muscle actin and active caspase 3 were used. Fluorescent secondary antibodies were chosen to allow three antigens to be co-visualised (Supplementary Table 3 for more information on antibody sources and methods). The tyramide amplification system (Invitrogen, Thermo Fisher) was used as a secondary antibody system to improve antigen visualisation in Oxford. Sections were mounted in antifade reagent (VECTASHIELD, Vector Laboratories, Burlingame, CA, USA).

Sections were imaged using LSM 7 MP (Zeiss) or Biorad, Radiance 2100 (Biorad) confocal microscopes with pinhole set at 1 Airy unit. Images of at least 25 islets per post-mortem specimen were quantified; the labelled pancreatic section was scanned in sequential but not overlapping fields and images of all islets in the section were collected for analysis. The frequency of all double- (vimentin plus one other label) and triple-positive (vimentin plus two other labels) cells was quantified by semi-automatic image analysis systems (AxioVision, Zeiss) and Zen, Zeiss and ImageJ, and expressed as percentages of the total number of insulin- or glucagon-positive cells examined/subject. Cells that expressed a clear nuclear Pdx-1 or Nkx6.1 staining were considered as Pdx-1 or Nkx6.1 positive. To establish that two/three antigens were located in the same cell, each colour image was examined separately. Importantly, the investigators were blind to the presence of diabetes or other clinical features during quantification.

The association of vimentin positivity (vim+) with β-to α-cell conversion was assessed by the examination of cells expressing both insulin and glucagon (vim+ins+glu+). Vim+ cell proportion was examined in glu+ or ins+ cells, in relation to diabetic status, age, gender, BMI and in organ donors, the status of the donor and duration of cold ischaemic time of the organ donation.

To examine the relationship of vim+ cells with islet amyloid deposits in pancreatic tissue samples of patients with T2DM, sections were stained for amyloid with thioflavin s (Sigma Aldrich) before undertaking the immunolabelling. The proportion of vim+ cells was assessed in each islet noting the presence of thioflavin s–stained amyloid. In addition, the vim+/endocrine-positive cell proportion for each subject (and each animal) was examined in relation to the total pancreatic amyloid prevalence (percentage of islets containing amyloid throughout the pancreas).

Electron microscopy

Isolated islets from human donors with (n=2) and without (n=3) T2DM (Supplementary Table 4) were contrast enhanced with 2% uranyl acetate (Agar Scientific, Stanstead, UK), dehydrated and embedded in London Resin Gold (LRG) (Agar Scientific) without osmium post-fixation to preserve antigenicity. Sections were immunolabelled with antibodies to vimentin (Dako) or the lysosomal marker cathepsin D (Sigma) using biotin-conjugated secondary antibodies (Dako) and streptavidin gold (vimentin) or protein A gold conjugates (BioCell, Cardiff, UK). Sections were contrasted with 2% uranyl acetate (Agar) and lead citrate and were examined in a Joel 1010 electron microscope fitted with a digital camera (Gatan, CA, USA).

Flow cytometry

Isolated islet preparations (purity >60%) from 4 donors with T2DM (Supplementary Table 4) were dispersed into single cells using a 0.025% trypsin solution containing 10 μg/mL DNase (Pulmozyme, Genentech) at 37°C. Cells were fixed with 4% formaldehyde (Klinipath BV, The Netherlands) for 15 min at RT, and 0.1% saponin (Sigma) was added to permeabilise the cells for an additional 30 min. Primary antibodies against insulin (1:200; Santa Cruz Technologies) and vimentin (1:100; Dako) were used and incubated for 20 min at 4°C. Anti-mouse and anti-rabbit Alexa Fluor-488 and Alexa Fluor-647 (1:500, Molecular Probes) were used as secondary antibodies. Cells were analysed with LSRII Flow Cytometer (BD Biosciences).
Statistical analysis

All data are expressed as median and interquartile range (IQR), unless stated otherwise. Statistical significance of difference between groups was assessed by Mann–Whitney U test, or Kruskal–Wallis and Dunn’s Multiple Comparison Test when more than two groups were compared. P < 0.05 was considered to be statistically significant.

Results

General characteristics of the ND and T2D human tissue samples

Human pancreatic tissue samples were obtained from post-mortem subjects, including organ donors, diagnosed with T2DM (n = 28; Oxford n = 17, Leiden n = 11) or without diabetes (ND) (n = 38; Oxford n = 30, Leiden n = 8) (Supplementary Table 1). The characteristics of the subjects were not different in the two centres (Supplementary Table 1); T2DM group; age range 34–86 years, BMI 22–54 kg/m², 9 females; ND group; age 27–78 years, BMI 19–38 kg/m², 19 females. The median age was significantly less in the ND group compared to that in the T2DM group (56.0 vs 61.5 years, ND vs T2DM, P < 0.05), but the BMIs were similar between groups. The duration of diabetes ranged between 1 and 28 years and age of onset between 30 and 76 years, median 57.5 years. There were more organ donations from donation after brain death (DBD) than donation after circulatory death (DCD) in the ND group than that in the T2DM group (ND: DBD 23 and DCD 18; T2DM: DBD 13 and DCD 14).

Expression of vimentin is increased in α- and β-cells in T2DM

Confocal imaging showed that vimentin (vim+) is expressed in a subset of insulin-positive (ins+) and glucagon-positive (glu+) cells. Vimentin positivity was usually in small patches in the cytoplasm of the endocrine cells and rarely spread throughout the cell (Fig. 1A, B, C and D). Investigations were performed independently in Leiden and Oxford on different materials using slightly different methods; the characteristics of the patients and the data derived were not different and therefore the data sets were merged for analyses. The proportion of ins+ cells coexpressing vimentin (vim+ins+) was significantly higher in the T2DM group compared to that in the ND group (1.43 (0.74–2.32)% (median (IQR)) vs 0.98% (0.61–1.87)% for ND and T2DM subjects respectively).

Figure 1

The mesenchymal protein vimentin is expressed in a subset of human α- and β-cells in T2DM. (A, B) Islet from non-diabetic (ND) subject labelled for vimentin (white) and (A) insulin (red) and (B) glucagon (green). Insets show cells double labelled for the islet peptide and vimentin. (C, D) Islet from subject with type 2 diabetes (T2DM) labelled for vimentin (white) and (C) insulin (red) and (D) glucagon (green). Insets show cells double labelled for the islet peptide and vimentin. Scale bars = 50 µm. (E, F) Quantitative data of the frequency of vimentin+insulin+ cells (E) and vimentin+glucagon+ cells/subject (F) in pancreatic sections from subjects without (ND) and with T2DM. Graphs represent individual values (as scatter plots) and median values ± interquartile range (as box plots, *P < 0.05). Vimentin+glucagon+ cells were more frequent than vimentin+glucagon+ cells in both ND and T2DM subjects (note the differences in axis values).
Increased α- and β-cell vimentin in diabetes

Ultrastructural identification of vimentin in human α- and β-cells

To determine the ultrastructural location of the vim+ labelling, human isolated islets were examined by electron microscopy. Immunogold labelling for vimentin was present in α- and β-cells in human isolated islets from donors with type 2 diabetes. No labelling for vimentin was found in islets from ND subjects (Fig. 2A and B). In addition, gold labelling for vimentin was present on intermediate filaments in residual islet capillary endothelial cells and islet perivascular macrophages (mesenchymal cells) in islets from ND and T2DM subjects as expected, confirming the antibody specificity (Supplementary Fig. 2A). The intermediate filaments were in bundles or small patches in the cytoplasm in islets isolated from both subjects with T2DM (Fig. 2C). In β-cells in T2DM, cytoplasmic vim+ labelling was less frequently observed than in α-cells and was located primarily in lysosomes (Fig. 2D). Primary lysosomes were identified by labelling with antibodies to the lysosomal enzyme cathepsin D (Supplementary Fig. 2B). The morphology of endocrine cells containing cytoplasmic vim+ intermediate filaments or vim+ lysosomes was not different from vim− cells in terms of the secretory granule population, apoptotic nuclei or swollen ER.

Vimentin-positive islet cells are not apoptotic and rarely express β-cell transcription factors

Immunofluorescence for active caspase 3 identified only rare islet cells undergoing apoptosis, and none of them expressed vimentin. This suggests that vim+ins+ and vim+glu+ cells

(0.29–1.42)% respectively $P<0.05$, Fig. 1E). The proportion of islets containing at least one vim+ins+ cell in a given subject was variable and was not different between ND and T2DM groups (28.0 (0–73.3)% (median (IQR)) vs 33.3 (4.0–93.3)% respectively).

The frequency of glu+ cells coexpressing vimentin (vim+glu+) cells was also significantly higher in the T2DM than that in the ND group (4.53 (2.78–9.11)% vs 2.53 (1.39–5.71)% respectively $P<0.05$, Fig. 1F). Moreover, the proportion of vim+glu+ cells was significantly higher than that in the number of vim+ins+ cells in both the ND and T2DM groups. The proportion of islets containing at least one glu+vim+ cell in a given subject was also highly variable but significantly higher in the T2DM group than that in the ND group (53.8 (9.5–93.3)% (median (IQR)) vs 72.0 (24.0–100)% total islets respectively, $P<0.05$). In the whole cohort, no significant correlation was found between the proportion of either vim+ins+ or vim+glu+ cells and donor age, BMI, age of onset or duration of T2DM, the donor organ procurement technique (DBD or DCD and duration of ischaemic time) or gender in either the ND or T2DM groups (data not shown).

To demonstrate that this double labelling was not an artefact of post-mortem pancreatic material embedded in wax, the presence of vim+ins+ cells in human islets from 4 donors with T2D ($n=4$) was confirmed by flow cytometry of human dispersed islet cells labelled for each peptide in an islet collagenase digest. A small population of vim+ins+ cells was identified in each preparation in the total population of cells labelled for insulin or vimentin (Supplementary Fig. 1A, B, C, D, E and F).
are not undergoing apoptosis (Supplementary Fig. 3A, B, C and D). This was confirmed by electron microscopy where no vim+ cells had apoptotic nuclei.

Most ins+ cells (~70%) in both ND and T2DM groups were positive for the β-cell transcription factor Nkx6.1 in nuclei (Fig. 3A, white arrow head; Fig. 3B, left panel). However, cells coexpressing vimentin (vim+ins+ cells) rarely (<10%) expressed Nkx6.1 (Fig. 3A, white arrow, Fig. 3B, right panel, Supplementary Fig. 4A). Similarly, the transcription factor Pdx1 was identified in the nucleus of most ins+ cells in ND and T2DM islets. However, ins+ cells coexpressing vimentin and Pdx1 were very rare in both ND and T2DM (<0.1% of ins+ cells) (Fig. 3C, Supplementary Fig. 4B). In our previous study using many of the same samples of T2DM and ND subjects (Spijker et al. 2015), we were unable to find cells positive for the transcription factor neurogenin 3, which is associated with primordial β-cells in foetal pancreatic development and dedifferentiation (Rukstalis & Habener 2009). The mesenchymal protein, smooth muscle actin (SMA), was present in islets but not co-localised with vimentin in islet cells. However, the mesenchymal matrix protein, N-cadherin, was present in the extracellular matrix in islets and was expressed in vim+ins-negative cells as well as in vim+ins-positive cells in islets (Supplementary Fig. 3E, F, G and H).

No correlation between frequency of vimentin-positive cells and bihormonal endocrine cells or islet amyloid deposition in human T2DM

To determine whether the presence of vimentin in islet cells was associated with bihormonality, (insulin and glucagon granules in the same cell), vim+ins+glu+ (i.e. vimentin+bihormonal) cells were counted in eight subjects from the Oxford cohort (ND n=3; T2DM n=5). The frequency of ins+glu+ cells was, as expected, very low and variable (range 0.4–5% median 0.6%, of total insulin-positive cells (all subjects)). Importantly, of these bihormonal cells, less than 15% also expressed vimentin (range 0–14.3%, median 0.0% of ins+glu+ cells) (Fig. 3D). Finally, no correlation was found between vim+ins+ or vim+glu+ cells and bihormonal cell proportions in any subject. Altogether, these data suggest that vimentin expression (potential EMT) and dual expression of insulin and glucagon are independent processes.

Islet amyloid deposition in T2DM disrupts the islet architecture. We hypothesised that this could affect the extracellular matrix adjacent to endocrine cells and thereby trigger EMT. The proportion of vim+ cells associated with amyloid was assessed. There was no relationship between the prevalence of islet amyloid (determined as the proportion of islets affected throughout the pancreas

Figure 3
Vimentin+insulin+ cells rarely express the key β-cell transcription factor Nkx6.1. (A) Islet labelled for vimentin (white), insulin (red) and Nkx6.1 (green). Insets show a cell double labelled for insulin and vimentin (arrow), as well as an insulin+Nkx6.1+ (white arrow head) and insulin+Nkx6.1− cell (yellow arrow head). Scale bar 20 µm. (B) Quantification data on samples from the Leiden cohort. The majority of insulin+ cells express Nkx6.1 (B, left panel), whereas most vimentin+insulin+ cells were Nkx6.1 negative (B, right panel). (C) Islet labelled for vimentin (white), insulin (red) and Pdx1 (green). Most vimentin+insulin+ cells were Pdx1 negative. Scale bar 50 µm. Insets show a cell double labelled for insulin and vimentin. (D) Islet from a subject with T2DM labelled for vimentin (green), insulin (blue) and glucagon (red). Vimentin-positive cells (arrows) were positive for glucagon but not insulin. Scale bar 20 µm.
(range 0–90%) and the proportions of vim+ins+ or vim+glu+ cells in eight donors with T2DM (data not shown). In a set of 6 donors with T2DM possessing islet amyloidosis, the proportion of vim+ins+ or vim+glu+ cells was not significantly different in the amyloid-free compared to the amyloid-containing islets (amyloid-free islets: vim+ins+%ins, 1.14±0.69%; vim+glu+%glu, 2.18±1.08%; amyloid-containing islets: vim+ins+%ins, 0.45±0.14%, vim+glu+%glu, 3.37±2.274%, mean±s.e.m.) (Supplementary Fig. 3I, J, K and L).

Frequency of vimentin-positive endocrine cells is increased in diabetic but not in hyperinsulinaemic non-human primates

To determine whether the expression of vimentin in islet cells occurs before the onset of diabetes and could be an aetiological factor for islet dysfunction, pancreatic tissue specimens from primates were studied. These animals develop a syndrome of diabetes with characteristics similar to T2DM in humans, and the natural history of diabetes has been described (Hansen & Bodkin 1986, Guo et al. 2013).

Animal tissue of normoglycaemic and normoinsulinaemic (ND; n=4), normoglycaemic and hyperinsulinaemic (HI; n=4) and overtly diabetic (DM; n=8) monkeys was analysed. The average age (17.1±14.5 vs 22.8±5.8 vs 21.8±4.3, ND vs HI vs DM), weight (7.7±3.0 vs 12.3±3.7 vs 10.7±2.1) and percentage of body fat (20.0±2.8 vs 35.1±5.7 vs 31.5±11.1) were similar between the three groups (Supplementary Table 2).

The vim+ins+ cell proportion was larger in DM animals compared to that in the ND group (6.85 (5.03–17.45)% (median (IQR)) vs 0.50 (0.10–0.75)%, respectively, P<0.05) (Fig. 4A, C and E). The frequency of these cells in HI animals, which are considered to be prediabetic, was not different from the ND group (0.40 (0.33–1.7)% (median (IQR)) vs 0.50 (0.10–0.75)%) (Fig. 4E). A trend towards an increased %vim+glu+ cells, although not statistically significant, was observed in the DM compared to that in ND and HI groups (Fig. 4B, D and F). In addition, we reported previously that the frequency of bihormonal cells (ins+glu+) was increased in DM compared to that in ND and HI animals (Spijker et al. 2015). Importantly, as observed for the human samples, the large majority of these bihormonal cells (all animals) did not coexpress vimentin (vim+/ins+/glu+ve, median <7% ins+/glu+ve), and the frequency of bihormonal cells expressing vimentin in DM was not different from ND.
or HI groups (data not shown). Finally, no association was found between the presence of amyloidosis and the frequency of vim+ins+ or vim+glu+ cells in the HI and DM groups (data not shown).

Discussion

The main findings of our observational study are that in diabetes there is an increased proportion of vimentin-positive α- and β-cells in pancreatic islets. Increased vimentin-positive islet cell proportions was associated with diabetes rather than with prediabetic conditions (obesity or insulin resistance) as shown in a cohort of spontaneously diabetic non-human primates. Vimentin positivity was not associated with islet amyloidosis or with cells expressing both insulin and glucagon.

The presence of vim+ islet cells in adult human histological specimens has been described in a few isolated cases (Gershengorn et al. 2004, Fanjul et al. 2010) including a recent examination of 3 subjects where it was concluded that vimentin islet cell positivity was absent in non-diabetic subjects and related to early-onset diabetes and metabolic stress (White et al. 2013). Our two-centred study, including data from 28 patients with T2DM and 38 normoglycaemic subjects of similar ages and BMI showed vim+ islet cells in ND subjects and increased vim+ endocrine cells to be associated with diabetes but with no correlation with age of onset or duration of diabetes. Human post-mortem or donor tissue has some degree of hypoxia, which could affect some islet features (Sullivan et al. 2015). However, there were no correlations of vim+ α- or β-cell proportions with any feature of tissue collection, age or BMI in the non-diabetic subjects. This suggests that the higher proportion of vim+ cells was associated only with diabetes. This was confirmed by data from the metabolically well-characterised non-human primates; the frequency of insulin+vimentin+ cells was increased in diabetic but not in hyperinsulinaemic (prediabetic) animals. Thus, we suggest that the elevated expression of vimentin is associated with, rather than a cause of diabetes. Recently, a single-cell transcriptome atlas of the human pancreas was generated by our group and others (Muraro et al. 2016, Segerstolpe et al. 2016). Segerstolpe and coworkers also examined a few cases of donors with T2DM (Segerstolpe et al. 2016). From their online database, it is evident that although expression is very variable; overall there was an increased expression of vimentin in donors with T2DM (Supplementary Fig. 5).

This confirms our findings using an antibody-independent technique.

The presence of vimentin in endocrine cells could represent an attempt to regenerate islet cells, either from mesenchymal cells or pericytes resident in islets (Carlotti et al. 2010, 2011) or from duct cells through an EMT stage, as proposed by Collombat and coworkers (Al-Hasani et al. 2013, Pfeifer et al. 2013). However, in this case, insulin expression would be preceded by or accompanied with Nkx6.1 and Pdx1 expression (Russ et al. 2011, Pagliuca et al. 2014, Rezania et al. 2014). Thus, the lack of β-cell-specific transcription factors in vim+ins+ cells suggests that these cells are losing rather than acquiring their endocrine phenotype. This is in line with the findings from a transgenic mouse study that associated expression of vimentin expression and β-cell dedifferentiation in diabetes (Talchai et al. 2012). β-cell dedifferentiation has been associated with expression of neurogenin 3 (Ngn3) (a marker for β-cell precursor cells) in a number of mouse models (Talchai et al. 2012, Wang et al. 2014). We did not find any Ngn3 positivity in adult human islets although cells were positive in foetal tissue (Spijker et al. 2015). However, both the downregulation of key transcription factors (Hay & Docherty 2006, Butler et al. 2012, Szabat et al. 2012, Guo et al. 2013, Schaffer et al. 2013, Gao et al. 2014) and the expression of vimentin in islet cells (Gershengorn et al. 2004, Russ et al. 2009) have been associated with β-cell dysfunction and/or diabetes in several studies. Altogether, we propose that the presence of vimentin in endocrine cells is likely to represent a dedifferentiation stage that could contribute to islet cell dysfunction in T2DM.

Vimentin+ cell proportion was significantly higher (×4) in α-cells than that in β-cells. It has been postulated that the potential to alter identity is greater in α-cells than that in β-cells (Bramswig et al. 2013, van der Meulen & Huising 2015). The influence of aberrant expression of transcription factors in α-cells has been less extensively studied than that for β-cells, but T2DM is associated with dysfunctional stimulus secretion coupling of glucagon (Zhang et al. 2013). Whether these vim+ endocrine cells undergo the full transition to mesenchymal cells by EMT cannot be determined in this snapshot of cells in vivo. Mesenchymal cells typically express not only vimentin but also smooth muscle actin and N-cadherin. However, vim+ endocrine cells in islets did not express smooth muscle actin, and N-cadherin was present in insulin-positive cells in the absence of vimentin (Parnaud et al. 2011). Our data
suggest that epithelial endocrine cells can express the so-called specific mesenchymal proteins (Ye & Weinberg 2015) and that if vimentin expression is a component of EMT, the process may be only partial and possibly reversible as in some cancer cells (Nieto 2013).

We previously identified that bihormonal cells were increased in T2DM (Spijker et al. 2015). In the current study we show, both in humans and in non-human primates, that bihormonal cells were not associated with vimentin positivity and that the infrequent bihormonal cells expressing vimentin were not present in higher proportions in diabetic vs non-diabetic groups. Altogether, these data indicate that vimentin positivity and bihormonality are two independent processes. Whether the higher number of vim+glu+ cells could represent previous β-cells that have transdifferentiated cannot be excluded from this study.

Conversion of β-cells towards mesenchymal cells in vitro is induced by changes in the extracellular matrix induced by isolation and monolayer culture (Gershengorn et al. 2004, Russ et al. 2009). However, extracellular matrix changes induced by islet amyloid formation in vivo in T2DM were not associated with significantly increased vim+ islet cells. Therefore, it appears that human islet cells can undergo several different forms of identity changes in T2DM in vivo. Functional studies are not possible on post-mortem fixed samples. Studies on human isolated islets in vitro can act as surrogates for some in vivo observations. However, the artificial environment including the process of isolation and culture (particularly in relation to EMT) precludes direct translation of experiments in vitro to the in vivo situation (Gershengorn et al. 2004, Russ et al. 2009, Negi et al. 2012).

To conclude, we propose that vimentin expression in islet cells is increased in T2DM. If this aberrant protein expression results in irreversible EMT, the potential loss of islet cell identity could contribute to the loss of islet cell function that occurs in diabetes. It is apparent that the cellular phenotype of mature islet cells can be challenged by several different mechanisms many of which are increased in type 2 diabetes.

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