Pluripotency-associated stem cell marker expression in proliferative cell cultures derived from adult human pancreas

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Abstract

The source of new β -cells in adult human pancreas remains incompletely elucidated with recent studies on rodents providing evidence for neogenesis from progenitor cells in addition to self-replication. The aim of this study was to investigate the expression of pluripotency-associated stem cell markers in proliferative cultures derived from adult human pancreas. Human pancreatic tissue was obtained from deceased donors following ethical approval and relative consent. Islet-enriched fraction was separated from the retrieved organ by digestion and density gradient centrifugation. Dissociated cells were seeded in adherent culture forming proliferative 'islet survivor cells' (ISCs). These were characterised at fifth passage by RT-PCR, immunofluorescence staining, FACS, western

blot and transfection studies with an OCT4 promoter-driven reporter. Nuclear expression of the pluripotency-associated stem cell marker complex OCT4/SOX2/NANOG was confirmed in ISCs. The phenotype constituted $\sim 8\%$ of the overall population. OCT4 biosynthesis was confirmed by western blot and activation of an exogenous OCT4 promoter. Co-expression of pluripotency-associated markers has been confirmed in proliferative primary cells derived from adult human pancreas. Further studies are required to elucidate whether these cells possess functional stem cell characteristics and assess potential for differentiation into pancreatic cell lineages including new β -cells.

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Introduction

There is considerable evidence to support self-replication of existing β -cells as the predominant source of new β -cells in young growing rodents (Dor *et al.* 2004) and humans (Meier *et al.* 2008). In established adults (Manesso *et al.* 2009) and in response to injury (Dor *et al.* 2004, Xu *et al.* 2008), however, the potential for non- β -cell sources including facultative endocrine progenitor cells has been proposed in convincing studies. Several groups have identified and isolated putative stem cells from adult pancreas (Seaberg *et al.* 2004, Suzuki *et al.* 2004, Eberhardt *et al.* 2006), but the expression of the pluripotency-associated embryonic stem (ES) cell markers has only been reported in exploratory studies on mice (Zuba-Surma *et al.* 2009) and unmanipulated human pancreas (Zhao *et al.* 2007, Al-Turaifi *et al.* 2008).

In humans, maintenance of the pluripotent circuitry within ES cells is coordinated by three transcription factors: OCT4, SOX2 and NANOG (Boyer *et al.* 2005). These proteins are essential for generation and maintenance of the ES cell identity by forming an auto-regulatory network that controls the expression and repression of pluripotency- and

differentiation-associated genes respectively (Boyer *et al.* 2005). Although a key pluripotency mediator in ES cells, several studies have reported the expression of OCT4 in unmanipulated and cultured somatic tissues (Zhao *et al.* 2007, Kristensen *et al.* 2010, Pacini *et al.* 2010).

In common with many important human developmental genes, multiple isoforms of OCT4 are generated by alternative mRNA splicing. The two main isoforms, OCT4A and OCT4B, have been reported as having differential expression patterns with OCT4A localising to the nuclei and OCT4B detected within the cytoplasm (Atlasi *et al.* 2008). Whilst OCT4A has been confirmed as the isoform responsible for maintaining the ES cell identity, OCT4B fails to confer the self-renewal and pluripotency of ES cell and is thought to be involved in cell stress responses (Lee *et al.* 2006). In addition to OCT4 splice variant expression, the presence of transcribed pseudogenes that demonstrate significant homology to the OCT4A sequence has been reported (Liedtke *et al.* 2007). Collectively, potential pseudogene and splice variant expression generate the potential for false-positive results and subsequently the detection and role of OCT4 within the soma has come under scrutiny.

By using the necessary controls (ES cells (positive) and human skin fibroblasts (negative)), primers that only amplify the *OCT4A* transcript, and demonstration of nuclear protein expression with both western blotting and immunocytochemistry, we here provide robust evidence for *OCT4A* expression in proliferative cells derived from adult human pancreas. This is supported by transfection studies in which we identified a population of cells within the proliferative culture that were capable of activating the transgenic OCT4 promoter as demonstrated by GFP expression. Importantly, we have demonstrated that OCT4A-positive cells co-express SOX2 and NANOG, a phenotype that has not been previously reported in proliferative cultures derived from adult human pancreas.

Materials and Methods

Human islets, fibroblasts and ES cells

Pancreases were retrieved from deceased donors following formal ethical approval and written informed relative consent. Organs were handled exactly as for a whole pancreas transplant including cooling of the lesser sac and were transferred into University of Wisconsin medium to the King's College Hospital islet isolation facility.

Islets were separated from exocrine tissue by Liberase HI digestion in a Ricordi chamber and a continuous density gradient according to the published methods (Huang *et al.* 2004). Purified islets were established in adherent culture in CMRL-1066 medium (Gibco, Invitrogen) including 10% foetal bovine serum and 1% insulin–transferrin–selenium supplement (Gibco). The medium was changed every 48–72 h and cells passaged at 80% confluence.

The MIN6 mouse β-cell line was available within the group. Primary human skin fibroblasts were generously provided by Dr Matthew Wright and undifferentiated human ES cells (H9) from Dr Linda Lako (both Newcastle University UK).

RNA analysis

Total RNA was extracted using the RNeasy microkit 50 (Qiagen) and treated with RQ1 RNase-Free DNase (Promega) to remove contaminating genomic DNA. cDNA was synthesised using superscript III reverse transcriptase (Invitrogen) according to the manufacturer's instructions. The PCR contained 1 µl cDNA (500 ng/µl), 1·25 µl RedTaq polymerase (Sigma–Aldrich), 2·5 µl PCR buffer, 1 µl forward primer (10 pmol/l), 1 µl reverse primer (10 pmol/l), 1 µl dNTPs (10 mM) (Promega) and 17·75 µl nanopure water. Parallel PCRs with RNA alone served as negative controls. PCR primer sequences and cycle number are given in Table 1. RT-PCR for *OCT4* was validated in serial dilutions of the H9 human ES cell line, confirming semi-quantification at 35 cycles. Semi-quantitative PCR was also validated for *GAPDH* as a reference gene at 32 cycles.

Immunofluorescence

Frozen sections cut from human pancreas blocks; accutase-dissociated single cells obtained from isolated islet fractions (IF) and cytopun onto slides; and cells grown on sterile coverslips were fixed with 4% paraformaldehyde for 20 min and permeabilised with 1% t-octylphenoxy-polyethoxyethanol (Triton, Sigma–Aldrich) for 45 min. To block non-specific binding, the slides were treated with blocking buffer (Roche Applied Science) for 1 h at room temperature.

Primary antibodies diluted in blocking buffer, at concentrations given in Table 2, were added and incubated for 1 h at room temperature or overnight in a humid chamber at 4 °C. In all studies, parallel no primary antibody negative controls were undertaken. Secondary antibodies (Table 3) were applied for 1 h at room temperature in the dark. Quantification of OCT4-expressing cells was done by capturing 12 random fields at 200× magnification and expressing proportion of OCT4-positive cells as a percentage of total (DAPI-positive) cells per field. Images were captured using either a Nikon Eclipse 400 inverted fluorescence, Nikon Eclipse TE2000-5 transmission fluorescence or Leica SP2 AOBs UV confocal microscope.

Table 1 PCR primers. All genes shown are human genes

Gene	Sequence		No. of cycles
	Forward	Reverse	
<i>GAPDH</i>	AAGGGCTCATGACCACAGTCCAT	CAGGAAATGAGCTTCACAAAGTT	30
Insulin	CATGGCCCTGTGGATGCGC	AGTTGCAGTAGTTCTCCAG	35
Glucagon	CCCAAGATTTGTGCAGTGGTT	GCGGCCAAGTTCTTCAACAAT	35
Somatostatin	GACCCAGACTCCGTCAGTTTC	GCCTCATTTATCCTGCTAGC	35
<i>PP</i>	CAATGCCACACAGAGCAGATC	TGGGAGCAGGGAGCAAGC	35
<i>CK19</i>	AGGTGGATTCCGCTCCGGGCA	ATCTTCCTGTCCCTCGAGCA	35
Amylase	GGTTCAGGTCTCTCCACCAA	TCCTGCACTCACAGCGTTAC	35
<i>OCT4</i>	GAAGTGGAGAAGGAGAAGCTG	CAAGGGCCGCAGCTTACACATGTT	35
<i>SOX2</i>	CGCCCCAGCAGACTTCACA	CTCCTCTTTTGACCCCTCCCATTT	35
<i>NANOG</i>	CATCGGGCCCGCCACCATGAGTGTGGATCCAGCTG	GATCGAGCTCCATCTTCACAGCTCTTCAGGTTG	35

Table 2 Primary antibodies

	Species raised in	Clonality	Specificity	Dilution	Supplier
Antibody					
Anti-Ki67	Mouse	Monoclonal	H/M/R	1:100	Novocastra Laboratories (Milton Keynes, UK)
Anti-Oct4	Rabbit	Polyclonal	H/M/R	1:50	Abcam Ab18976 (Cambridge, UK)
Anti-Nanog	Goat	Polyclonal	H	1:100	R&D AF1997 (Abingdon, UK)
Anti-Sox2	Rabbit	Polyclonal	H/M	1:50	Abcam Ab15830
Anti-CK19	Mouse	Monoclonal	H/M	1:200	DAKO M088801 (Cambridge, UK)

H, human; M, mouse; R, rat; Cy3, cyanine 3; TR, Texas Red.

Fluorescence-activated cell sorting

Cells were fixed with 4% paraformaldehyde for 20 min, permeabilised by 1% saponin (Sigma–Aldrich) for 20 min and treated with blocking buffer for 30 min (all at room temperature). Primary antibody (Table 2), at a concentration of 1:50, diluted in blocking buffer was then added with incubation for 60 min. Following washing with 1% saponin in PBS, secondary antibody (Table 3) was added at a concentration of 1:50 in blocking buffer with incubation in the dark for 30 min. Following further washes, cells were resuspended in PBS for FACS analysis.

Western blotting

Nuclear or whole cell extracts were prepared by performing western blotting as described previously (Macfarlane *et al.* 2000). Protein concentration was determined by Bradford protein assay and 30 µg of each sample were fractionated by SDS–PAGE. Following electrotransfer to a nitrocellulose membrane and blocking with 10% dried skimmed milk, the membrane was incubated overnight with primary antibody (GAPDH or OCT4 (Table 2) prepared in 1:200 dilutions) at 4 °C and then with secondary antibody (HRP-conjugated anti-rabbit 1:2000 dilution, Sigma–Aldrich) for 1 h at room temperature. Immunoreactivity was detected using a chemiluminescence kit (ECL, Amersham).

Transgene expression studies

Transfections were performed using 10 µl Lipofectamine 2000 (Invitrogen) and 3 µg plasmid DNA (pIRES-eGFP (available within the group) and OCT4–eGFP (provided by Dr Wei Cui, Imperial College London)) following the manufacturer's instructions.

Results

Expression of *OCT4* was initially demonstrated in unmanipulated human pancreas by semi-quantitative RT-PCR in comparison to human ES cells and primary human skin fibroblasts (Fig. 1A). Following isolation and purification, expression of the pluripotency-associated markers *OCT4*, *SOX2* and *NANOG* (Fig. 2A) could be detected by RT-PCR

in the IF in addition to the classical differentiated pancreatic phenotypic markers (insulin, glucagon, somatostatin, pancreatic polypeptide, *CK19* (ductal) and amylase (exocrine)) (Fig. 2A and B).

Isolated human islet preparations of >70% purity were established in proliferative monolayer culture conditions. Islets and single cells with a range of morphologies adhered to the flask over the first 48–72 h, with proliferation over subsequent days including outgrowth of cells from the islets as described previously (Hardikar *et al.* 2002). Following passage, proliferative cells with a fibroblast-like morphology became the predominant phenotype. These cells were termed islet survivor cells (ISCs).

Characterisation by RT-PCR of ISCs in culture demonstrated loss of differentiated endocrine phenotypic marker expression including insulin, glucagon, somatostatin and pancreatic polypeptide over time with all being consistently undetectable at fifth passage (Fig. 2A and B). Whilst expression of the exocrine marker amylase was also lost, the specific ductal marker *CK19* was maintained (Fig. 2A).

Expression of the classical pluripotent stem cell markers *OCT4*, *SOX2* and *NANOG* appeared to diminish over time with increased expression in ISCs at fifth passage (Fig. 2A). H9 human ES cells and human skin fibroblasts were used as positive and negative controls respectively. Consistent *OCT4* expression in ISCs was confirmed by semi-quantitative RT-PCR in three repeated studies, expressed at ~30% of the level in ES cells at fifth passage (Fig. 2C).

Table 3 Secondary antibodies (all supplied by Jackson Immuno-Research, Newmarket, UK). Specificity of all IgG. Dilution of all 1:300

	Species raised in	Conjugation	Supplier code number
Antibody against			
Goat	Donkey	TR	705-095-003
Rabbit	Donkey	TR	711-076-152
Rabbit	Donkey	FITC	711-095-152
Rat	Donkey	FITC	712-095-153
Mouse	Donkey	Cy3	715-165-151

H, human; M, mouse; R, rat; Cy3, cyanine 3; TR, Texas Red.

Figure 1 OCT4 expression in unmanipulated adult human pancreas. (A) RT-PCR analysis of *OCT4* expression in human pancreas (HP) compared to human embryonic stem cells (hESCs) and human skin fibroblasts (HSF). (B) Immunofluorescence staining of OCT4 (red) in human pancreas sections. Size bar = 100 µm. (C and D) Confocal images of small OCT4 (red)-positive cells (~2–3 µm). Size bars = 3.07 µm (C) and 2.23 µm (D). Nuclei are stained with DAPI blue. Data are representative of results from four separate human pancreases.

Immunofluorescence studies demonstrated OCT4 staining in a small number of cells throughout the pancreas (Fig. 1B) with confocal microscopy confirming that stained cells were very small (1.5–3 µm) and nucleated with cytoplasmic OCT4 localisation (Fig. 1C). Cytoplasmic localisation was also demonstrated in IF cells with no evidence of co-localisation with insulin (Fig. 3A) or CK19 (Fig. 3B).

Following culture, the expression pattern of OCT4 was altered with nuclear localisation of the transcription factor evident in $8.6 \pm 2.2\%$ (mean $\pm$ s.d. of > 500 cells from each of the three donors) of ISCs at fifth passage (Fig. 3C). There was no co-localisation of OCT4 and CK19 (Fig. 3D), with protein expression of insulin and all other end-differentiated endocrine markers lost (data not shown).

Nuclear co-localisation of NANOG (Fig. 4A) and SOX2 (Fig. 4B) in OCT4-positive cells was confirmed by confocal microscopy. H9 human ES cells were used as a positive OCT4, SOX2 and NANOG control and human skin fibroblasts as negative control showing no positive staining (data not shown).

OCT4 protein expression in ISCs was quantified by flow cytometry (Fig. 5B), with no OCT4 positivity detected in the absence of primary antibody (Fig. 5A) or in control human skin fibroblasts (data not shown).

Approximately 8% of ISCs at fifth passage expressed OCT4 (Fig. 5C). Nuclear OCT4 protein localisation in ISCs was further demonstrated by western blot with a band corresponding to that in H9 human ES cells (Fig. 5D).

No OCT4 protein expression was seen in human skin fibroblasts. Western blot densitometry (corrected for GAPDH band intensity) demonstrated nuclear expression in ISCs at 48% of that in human ES cells.

ISCs were transfected with a plasmid designed to label undifferentiated pluripotent ES cells in which the *GFP* reporter gene is expressed under the control of the human OCT4 promoter. Control transfections with the pIRES-GFP plasmid in which GFP is driven by the constitutive cytomegalovirus demonstrated the capability of ISCs to express exogenous GFP with a transfection efficiency of ~5% (Fig. 6A1). Expression of eGFP under the control of the OCT4 promoter in ISCs was confirmed in ~0.5% of cells (Fig. 6A2) with expression of nuclear OCT4 (Fig. 6B) and NANOG (Fig. 6C) in all GFP-positive cells indicating the accurate designation of putative stem cells in these reporter gene studies. Furthermore, the absence of Ki67 staining in all GFP-positive ISCs demonstrated that OCT4-expressing cells showed no evidence of active proliferation

Figure 2 Expression of differentiated and pluripotency-associated genes in ISCs. (A) Analysis of insulin, *CK19* and pluripotency-associated gene expression in islet-enriched fraction (IF) and cultured ISCs over ten passages. Human embryonic stem cells (hESCs) and human skin fibroblasts (HSFs) were included as positive and negative controls respectively. (B) Expression of endocrine and exocrine genes in ISCs at P5 in comparison to IF and primary HSFs. *GAPDH* was used as a reference gene control in all RT-PCR experiments. (C) Percentage expression of *OCT4* mRNA relative to *GAPDH* expression in HSFs, ISCs (P5) and hESCs determined by densitometry. Data are mean $\pm$ s.d.; * $P < 0.05$; ** $P < 0.01$ (one-way ANOVA). Results are representative of those from three separate human pancreases (all of which are included in the data comprising the graph in panel C).

Figure 3 Immunofluorescence staining for OCT4 in IF and cultured ISCs. (A and B) Cytoplasmic expression of OCT4 (red) in islet fraction with no evidence of co-localisation with insulin (green) or CK19 (green). (C) Nuclear expression of OCT4 in ISCs at P5. (D) CK19-expressing ISCs (green) at P5 show no co-localisation with Oct4 (nuclear)-expressing cells. Size bar = 50 μ m. Data are representative of cultures derived from three separate donor pancreases.

(Fig. 6D). Stem cell specificity was evidenced by OCT4–GFP expression in transfected H9 human ES cells but absence of expression in transfected MIN6 mouse β -cells and primary human skin fibroblasts (data not shown).

Discussion

Several groups have previously published evidence of proliferative cultures derived from purified human islet preparations, with lineage tracing studies confirming the contribution of de-differentiated β -cells (Gershengorn *et al.* 2004, Kayali *et al.* 2007, Russ *et al.* 2008, 2009). However, these islet-enriched fractions are not entirely pure as evidenced by initial expression of ductal and exocrine markers in these studies. Following culturing to the fifth passage, exocrine and differentiated endocrine marker expression was lost, although the ductal marker CK19 was maintained with evidence of proliferation by Ki67 positivity.

Collectively, OCT4, SOX2 and NANOG form the core transcriptional network responsible for maintaining

pluripotency in ES cells (Boyer *et al.* 2005). Although a key ES cell regulator, OCT4 expression has been detected in multiple somatic tissues including the liver, kidney, bone marrow and umbilical cord blood (Tai *et al.* 2005, Seo *et al.* 2009, Wer & Shen 2011). Cells expressing OCT4 termed ‘very small embryonic-like stem cells’ have recently been isolated from murine pancreas (Zuba-Surma *et al.* 2009), and it has been proposed that these may provide a quiescent pool of stem cells for replenishing tissue-specific progenitors in times of extensive tissue injury. The presence of cells expressing OCT4 and SOX2 in unmanipulated human pancreas has previously been reported (Zhao *et al.* 2007). In this study, we have confirmed the size of OCT4-positive cells as only ~ 1.5 – 3 μ m, in keeping with murine ‘very small embryonic-like stem cells’. Furthermore, we have demonstrated the presence of larger OCT4-expressing cells in purified islet-enriched fractions, with no co-localisation with insulin or CK19-positive cells.

Expression of OCT4 in human pancreas-derived primary cell cultures has been previously reported (Tai *et al.* 2005, Gorjup *et al.* 2009). However, here we have demonstrated for the first time evidence for the nuclear co-localisation of OCT4, SOX2 and NANOG, a phenotype that constitutes $\sim 8\%$ of the overall population following five passages. Critically, through specific experimental design, we have provided novel evidence for expression in human pancreatic derived proliferative cells of OCT4A, the only isoform capable of maintaining pluripotency.

Two main OCT4 isoforms have been described, *OCT4A* and *OCT4B* (Lee *et al.* 2006, Atlasi *et al.* 2008). Since *OCT4A* is the only isoform capable of conferring pluripotency, considerable concerns have been expressed regarding the potential for falsely designating expression of *OCT4B* as *OCT4A* in cells derived from adult tissues. Given that

Figure 4 Confocal images of ISC immunofluorescence staining for stem cell markers. (A) Nuclear co-localisation of OCT4 in green and NANOG in red. (B) Nuclear co-localisation of SOX2 in green and NANOG in red. Nuclei are stained with DAPI blue. Size bars = 50 μ m (A) and 25 μ m (B). Data are representative of cultures derived from three separate donor pancreases.

Figure 5 Flow cytometry and western blotting of ISCs for OCT4 protein expression. (A) Absence of signal in negative control lacking primary antibody. (B) Confirmation of OCT4 expression in ISCs (P5) by flow cytometry. (C) OCT4 expression in $8.3 \pm 0.3\%$ of ISCs. Data expressed as mean $\pm$ s.d. ($n=3$ donors). The gate was set to exclude cell debris. (D) OCT4 expression by western blotting in human embryonic stem cells (hESC) and in the nuclear but not cytoplasmic fraction of ISCs. *GAPDH* was used as a reference gene.

the *OCT4B* mRNA transcript is truncated without exon 1 of the original *OCT4A* isoform (Atlasi *et al.* 2008), we were able to eliminate false-positive RT-PCR results by designing the 5' primer to recognise a sequence within the first exon. Additionally, the potential for pseudogene expression was eliminated by treating all RNA samples with DNase and conducting parallel RNA only PCR-negative control reactions.

In all of these studies, H9 ES cells were used as the gold standard positive control. Moreover, no OCT4 gene or protein expression was detected in human skin fibroblasts used as a negative control. OCT4 protein expression was confirmed by immunostaining, flow cytometry and western blotting. Nuclear localisation provided further evidence for functional OCT4A, as opposed to OCT4B expression, which has been reported as being expressed exclusively within the cytoplasm (Atlasi *et al.* 2008). In addition, activation of the OCT4 promoter in a reporter gene construct was seen in H9 ES cells and ISCs but not in control differentiated β -cells or human skin fibroblasts.

Recently, expression of OCT4B in peripheral blood mononuclear cells has been reported (Papamichos *et al.* 2009). This study also found the regulatory sequences of the human OCT4 promoter in these cells to be densely methylated and hence the OCT4 locus transcriptionally inactive (Papamichos *et al.* 2009). This paradox suggests that transcription of the OCT4A isoform is controlled by the promoter region upstream of exon 1 while OCT4B transcription may be regulated by an alternative promoter possibly located within the *OCT4A* gene, i.e. exon 1. The

OCT4 promoter sequence within our reporter construct comprised the full-length promoter sequence upstream of the transcription start site (Okazawa *et al.* 1991, Minucci *et al.* 1996, Yeom *et al.* 1996). This reporter construct has been extensively characterised with several reports demonstrating its stem cell-specific expression (Gerrard *et al.* 2005). In particular, GFP expression has been shown to closely correlate with endogenous OCT4 expression in human ES cells (Gerrard *et al.* 2005). Furthermore, utilisation of this reporter construct has enabled groups to identify false-positive detection of OCT4 in adult tissues. For example, by using several published primers, *OCT4* expression was detected in a number of adult tissues; however, the only cells capable of activating GFP expression under the control of the OCT4 promoter were embryonic carcinoma cells (positive control) (Redshaw & Strain 2010). Sequencing of each mRNA transcript identified *OCT4A* expression in EC cells only, with all adult cells demonstrating *OCT4* pseudogene or splice variant expression.

Figure 6 Oct4-eGFP expression in ISCs. (A1) Control transfections of ISCs (P5) with pIRES-eGFP. (A2) ISCs transfected with Oct4-eGFP. Oct4-eGFP-positive ISCs co-staining with nuclear (B) Oct4 (red) and (C) NANOG (red) antibodies. (D) Non co-localisation of proliferative (Ki67 positive (red)) ISCs with Oct4-eGFP-positive cells. Nuclei stained with DAPI blue. Size bars = 100 μ m (A1 and A2), 50 μ m (B and D) and 25 μ m (C). Data are representative of cultures derived from three separate donor pancreases.

Demonstration of OCT4–GFP-expressing ISCs with nuclear co-localisation of OCT4, NANOG and SOX2 and absence of co-localisation with any differentiated markers provides robust evidence for the existence of a pancreatic phenotype, which has not previously been adequately characterised. Although the origin of these cells remains unknown, migratory mesenchymal stem cells (MSCs) are a possible candidate with studies indicating the presence of cells within pancreatic primary cultures expressing markers characteristic of MSCs, including CD90 and CD105 (Carlotti *et al.* 2010). Indeed, expression of OCT4, SOX2 and NANOG has been previously demonstrated in MSCs derived from adult tissues (Riekstina *et al.* 2009) with OCT4 and NANOG RNA expression in pancreas-derived cells (Gorjup *et al.* 2009). Further studies are planned to explore the origin of these cells and to undertake *in vitro* lineage tracing studies to determine whether these are derived from cells in unmanipulated pancreas expressing OCT4 in the cytoplasm, whether they possess functional stem cell characteristics and potential for differentiation into mature pancreatic cell types.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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