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SHORT COMMUNICATION

Gene expression heterogeneity in human islet endocrine cells in vitro: the insulin signalling cascade

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Abstract
Aims/hypothesis Insulin secretion is a highly regulated mechanism involving a complex insulin-dependent network of communication between alpha, beta and delta cells. However, whereas the role of insulin in beta cells has been well documented, very little is known about its role in alpha and delta cells. Having recently demonstrated heterogeneity of insulin receptor (*INSR*) isoform expression in these three endocrine cell types, our current study aimed to characterise the expression pattern of the multiple isoforms involved in the insulin signal transduction cascade in human alpha, beta and delta cells in vitro.
Materials and methods cDNA samples prepared from single human islet cells were subjected to nested PCRs.
Results Of 706 cells analysed, 15% were alpha cells, 28% beta cells, 8% delta cells and 46% non-endocrine cells. Profiling of expression of the insulin signalling cascade elements showed a heterogeneity between islet cell types, although at least one member of each protein family was expressed in the three populations of endocrine cells. Thus, the mRNAs coding for *INSR-B*, phosphoinositide-dependent protein kinase-1 and the human homologue of v-akt murine thymoma viral oncogene homologue 1 (*AKT1*) could not be detected in alpha cells, but were expressed by beta and delta cells. In addition, while the insulin receptor substrates *IRS1* and *IRS2*, phosphoinositide-3-kinase, catalytic, beta polypeptide (*PIK3CB*) and *AKT2* were expressed with relatively low frequencies in alpha and delta cells (<17% for *IRS1*, *IRS2*, *PIK3CB*; <25% for *AKT2*), their frequencies of expression in beta cells were 50, 33, 33 and 100%, respectively.
Conclusions/interpretation Our results suggest that insulin signalling cascade elements in human alpha, beta and delta cells have distinct expression patterns.

Keywords Alpha cells · Beta cells · Delta cells · Gene expression · Human islets · Insulin signalling · Inter-islet cells heterogeneity · Islet hormones · Single-cell RT-PCR

Abbreviations

AKT	v-akt murine thymoma viral oncogene homologue
INSR	insulin receptor
IRS	insulin receptor substrate
PDK1	phosphoinositide-dependent protein kinase-1
(SC)-PCR	single-cell polymerase chain reaction

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Introduction

The discovery that the insulin receptor (*INSR*) and *IRS1* are expressed in rodent islets [1] and the recent observations that *INSR*, *IRS2* and the v-akt murine thymoma viral oncogene homologue (*AKT*)2 expression levels are altered in islets isolated from humans with type 2 diabetes [2] suggest a key autocrine role for insulin and its signalling

cascade in the regulation of beta cell function. Consistent with these observations, glucose-induced insulin secretion is impaired in mice lacking the beta cell *INSR* [3] and in human islets carrying the Gly^{972}→Arg *IRS1* polymorphism [4].

However, beta cell function and insulin secretion are not only regulated at the beta cell level, but also involve a complex inter-cellular network of communication between alpha, beta and delta cells. For instance, while glucose stimulates insulin secretion from beta cells, it also inhibits glucagon secretion from alpha cells and stimulates somatostatin secretion from delta cells, both phenomena contributing to reduced insulin release. Conversely, hypoglycaemia-stimulated glucagon secretion is inhibited in the absence of insulin [5] and requires the expression of *INSR* in alpha cells [6].

We recently demonstrated a high heterogeneity of expression of *INSR* in human islet cells, with *INSR-A* (Exon 11^{-}) being expressed in ~20% of the alpha, beta and delta cell populations and *INSR-B* expression being absent in alpha cells, present in 11% of delta cells and highly expressed in beta cells [7]. These results, and those showing that *AKT2*, but not *AKT1* expression is decreased in islets isolated from type 2 diabetes patients [2], support the concept of cell-type heterogeneity in expression of the multiple insulin signalling cascade isoforms.

The aim of this study was therefore to characterise by single-cell (SC)-PCR the pattern of gene expression of the multiple classes and isoforms of the insulin signalling cascade in isolated human islet cells.

Materials and methods

Reagents Fetal bovine serum, L-glutamine, streptomycin/penicillin, PBS and trypsin-EDTA were obtained from Sigma (Dorset, UK). CMRL 1066 was from Invitrogen (Paisley, UK). Taq DNA polymerase, RNase A, rRNasin and mmLV-RT were obtained from Promega (Southampton, UK). Primers used in this study were from Operon Biotechnology (Cologne, Germany).

Isolation and maintenance of human islets of Langerhans Human islets were isolated under aseptic conditions at the King's College Hospital Islet Transplantation Unit with appropriate ethical approval and maintained in CMRL 1066 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin for 2 days before single cells were isolated.

Single cell RT-PCR Isolation of single human islet cells and RT-PCR amplification of mRNAs were performed as previously described [7]. Primer sequences and technical conditions are given in the Electronic supplementary material, (ESM) Table 1). RT-PCR amplifications using 0.5 μl of the culture medium after RNase A treatment and 0.5 μl of the last PBS wash were routinely performed to ensure that products were derived from intact single cells and not from extracellular mRNAs released from damaged cells. In addition, to confirm that RT-PCR products were not amplified from genomic DNA, negative control PCR reactions were performed in the absence of mmLV-RT. The threshold of (SC)-PCR amplification sensitivity was determined by performing serial dilutions of mRNA isolated either from an individual or ten pooled beta cells. In these experiments the threshold for detection was between 8 and 4% of the content of a single cell. The following amounts (% from a single cell) were used in all (SC)-PCR reactions described in this study: 20% (preproinsulin [*PPI*], preproglucagon [*PPG*], preprosomatostatin [*PPS*]) or 33% (insulin transduction elements). Consequently, each cell provided sufficient material for analysis of islet hormone gene expression and two insulin signalling elements.

Results

Human islet cell populations (SC)-PCR analysis of 706 cells isolated from seven preparations of human islets indicated that 15% were positive for *PPG*, 28% for *PPI*, 8% for *PPS* and 46% did not express *PPG*, *PPI* or *PPS*. These cells that did not express *PPG*, *PPI* or *PPS* were designated as non-endocrine cells. We also observed the presence of hormone co-expressing cells (Fig. 1) with 2.1% being positive for both *PPI* and *PPG*, and 0.8% expressing both *PPG* and *PPS* mRNAs.

Heterogeneity of expression of the insulin signal transduction cascade isoforms in human islet endocrine cells Expression of mRNAs coding for *INSR-A*, *INSR-B*, *IRS1*, *IRS2*, phosphoinositide-3 kinase, catalytic subunits alpha, beta, gamma and delta (*PI3KCA, PIK3CB, PIK3CG* and *PIK3CD*), phosphoinositide-3 kinase, class 2 alpha, beta and gamma (*PIK3C2A*, *PIK3C2B* and *PIK3C2G*), phosphoinositide-dependent kinase-1 (*PDK1*) and *AKT* isoforms 1, 2 and 3 was analysed in alpha, beta and delta cells. In all, 182 cells were used in this part of the study: 67 alpha cells, 63 beta cells and 52 delta cells.

The results displayed in Table 1 demonstrate that at least one isoform of each insulin signalling element is expressed in the three endocrine cell types. However, our data also suggest the existence of a high degree of heterogeneity in expression of the insulin signalling cascade elements. Whereas *INSR-B* (exon 11^{+}), *PDK1* and *AKT1* mRNAs were all amplified from beta cells, mRNA expression of

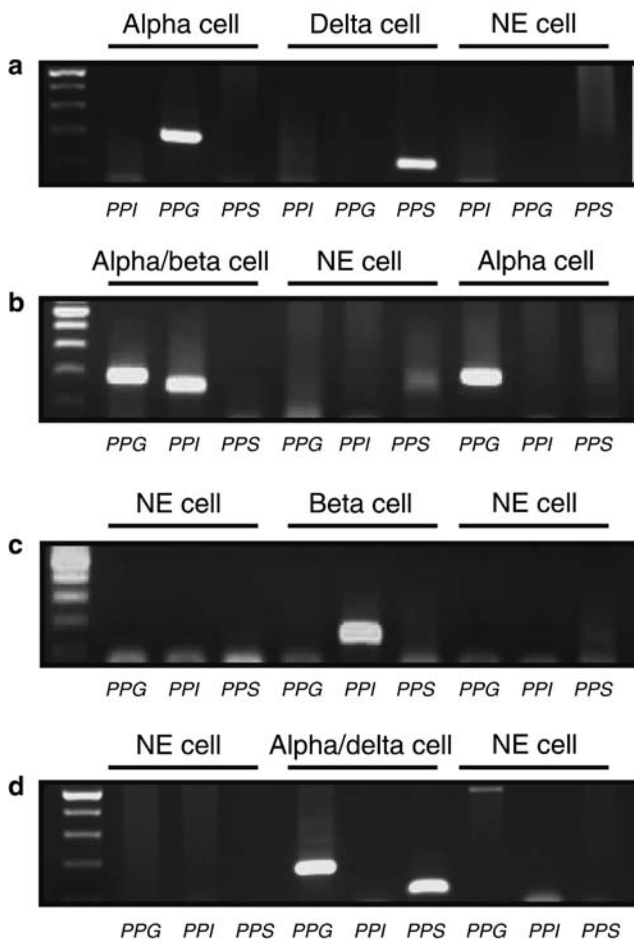

Fig. 1 Cell-type characterisation of human islet cells. Isolated single cells from human islets were subjected to two-rounds of PCR using two pairs of primers. Insulin-, glucagon- and somatostatin-secreting cells were identified by reactions using primers for *PPI*, *PPG* and *PPS*. Cells that did not express *PPI*, *PPG* or *PPS* were designated as non-endocrine (NE) cells. *Blots* show the presence of: **a** one alpha cell, one delta cell and one NE cell; **b** one alpha cell, one NE cell and one *PPI* and *PPG* co-expressing cell; **c** one beta cell and two NE cells; and **d** one *PPG* and *PPS* co-expressing cell and two NE cells

these isoforms in alpha cells was below the (SC)-PCR detection limit. This heterogeneity of mRNA expression was also demonstrated by the distribution analysis between each cell-type where *INSR-B* and *AKT1* were expressed with a higher frequency in beta cells than in delta cells. Thus, *INSR-B* and *AKT1* mRNAs were amplified from 64 and 55% beta cells, but from only 11 and 13% delta cells, respectively. In a similar manner, *IRS1*, *IRS2*, *PIK3CB* and *AKT2* expression frequencies were also higher in beta cells than in alpha or delta cells, with respective expressions that were 15, 13, 13 and 25% in alpha cells; 50, 33, 33 and 100% in beta cells; and 17, 14, 17 and 13% in delta cells. In contrast to this high degree of heterogeneity, the mRNAs coding for *INSR-A*, *PIK3CA*, *PIK3CG*, *PIK3CD*, *PIK3C2B* and *AKT3* were expressed with a relatively similar frequency in the three endocrine cell types.

Table 1 Gene expression pattern of insulin signalling cascade elements in human islet alpha, beta and delta cells

Isoform	Alpha cells[a]	Beta cells[a]	Delta cells[a]
INSR-A(exon 11$^-$)	3/12 (25)	2/11 (18)	2/9 (22)
INSR-B(exon 11$^+$)	0/12 (0)	7/11 (64)	1/9 (11)
IRS1	2/13 (15)	6/12 (50)	1/6 (17)
IRS2	1/8 (13)	3/9 (33)	1/7 (14)
PIK3CA	2/8 (25)	2/7 (29)	2/7 (29)
PIK3CB	1/8 (13)	3/9 (33)	1/6 (17)
PIK3CG	1/11 (9)	1/11 (9)	1/10 (10)
PIK3CD	1/11 (9)	0/11 (0)	1/9 (11)
PIK3C2A	3/9 (33)	7/9 (78)	3/6 (50)
PIK3C2B	1/8 (13)	2/9 (22)	1/8 (13)
PIK3C2G	3/6 (50)	4/6 (66)	1/6 (16)
PDK1	0/11 (0)	2/8 (25)	2/6 (33)
AKT1	0/12 (0)	5/9 (55)	1/8 (13)
AKT2	2/8 (25)	7/7 (100)	1/8 (13)
AKT3	6/8 (75)	7/7 (100)	4/7 (57)

[a] Values: positive cells / tested cells (% of cells testing positive).

Discussion

Several recent studies have suggested an autocrine role for insulin in the regulation of beta cell function [3, 4, 7]. It has also been proposed that dysfunction in the insulin signalling cascade might play an important role in the development of type 2 diabetes [2, 7, 8]. However, the expression pattern of the multiple insulin signal transduction cascade isoforms by human islet endocrine cells is as yet unknown.

Here, we demonstrated by (SC)-PCR that at least one isoform of each insulin signalling cascade element was expressed in human alpha, beta and delta cells, suggesting the presence of a functional insulin signalling pathway in these cells. However, our results also demonstrated for the first time the existence of inter-islet cell heterogeneity, which was characterised by the inability to detect *INSR-B* (exon11$^+$), *PDK1* and *AKT1* mRNAs in alpha cells, whereas these were amplified from beta and delta cells.

In vitro, we also observed heterogeneity in expression of the insulin signal transduction cascade isoforms between the islet endocrine cells at the level of their frequency of expression. We found that *INSR-B*, *IRS1*, *IRS2* and *AKT2* expression frequencies were higher in beta cells than in alpha and delta cells. These results suggest that INSR-B and AKT2 might play key roles in regulating beta cell function and confirm previous reports showing the critical roles played by IRS1 and IRS2 in beta cells [8]. The possibility that AKT2 is crucial for beta cells to function correctly is consistent with observations that: (1) its

expression level is decreased in islets isolated from humans with type 2 diabetes, with no changes in *PPI*, *PPG* or *PPS* expression levels [2]; and (2) *AKT2*-deficient mice exhibit marked hyperglycaemia and loss of pancreatic beta cells [9].

Finally, this study also demonstrated co-expression of multiple islet hormones in individual cells [10]. This observation is unlikely to be an artefact subsequent to mRNA contamination from damaged cells during the isolation process, since: (1) in this case all three mRNAs coding for *PPI*, *PPG* and *PPS*, and not only two of them, would have been amplified by PCR; (2) the cell suspensions were treated with RNase A prior to the isolation procedure; and (3) PCRs performed using 4 μl of the last wash did not give any product. Therefore, we propose that those cells that co-express two of the three hormones might be intermediate progenitors of newly synthesised endocrine cells. The isolation and characterisation of these cells may give novel insights into the differentiation processes of human pancreatic progenitors into specialised islet endocrine cells, and more importantly into beta cells.

In conclusion, our results demonstrate for the first time the existence of heterogeneity in expression of the insulin signalling cascade elements among human islet endocrine cells. The islets had been maintained in culture for 2 days prior to analysis, so it should be borne in mind that nutrients or growth factors in the culture medium may have influenced gene expression patterns and we are currently profiling insulin signal transduction element expression in freshly isolated and cultured human islets. Our findings also suggest that in human islets, insulin signals through two distinct cascades: one involving stimulation of INSR-B, PDK1 and AKT1/AKT2 and preferentially operating in beta cells; the other involving AKT3 activation subsequent to INSR-A stimulation and preferentially operating in alpha cells.

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Duality of interest The authors are not aware of any conflict of interest.

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