Epidermal growth factor induces adult human islet cell dedifferentiation

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Abstract

Given the inherent therapeutic potential of the morphogenetic plasticity of adult human islets, the identification of factors controlling their cellular differentiation is of interest. The epidermal growth factor (EGF) family has been identified previously in the context of pancreatic organogenesis. We examined the role of EGF in an in vitro model whereby adult human islets are embedded in a collagen gel and dedifferentiated into duct-like epithelial structures (DLS). We demonstrated that DLS formation was EGF dependent, while residual DLS formation in the absence of added EGF was abrogated by EGF receptor inhibitor treatment. With respect to signaling, EGF administration led to an increase in c-Jun NH2-terminal kinase (JNK) phosphorylation early in DLS formation and in AKT and extracellular signal-regulated kinase (ERK) phosphorylation late in the process of DLS formation, concomitant with the increased proliferation of dedifferentiated cells. In the absence of EGF, these phosphorylation changes are not seen and the typical increase in DLS epithelial cell proliferation seen after 10 days in culture is attenuated. Thus, in our model, EGF is necessary for islet cell dedifferentiation, playing an important role in both the onset of DLS formation (through JNK) and in the proliferation of these dedifferentiated cells (through AKT and ERK).

Journal of Endocrinology (2011) 211, 231–239

Introduction

Recent studies of adult human islets have reported their ability to undergo dedifferentiation, proliferation, and redifferentiation (Gershengorn et al. 2004, Gao et al. 2005, Jamal et al. 2005, Lechner et al. 2005, Ouziel-Yahalom et al. 2006, Hanley et al. 2008). While still controversial, current research now focuses on identifying factors responsible for controlling the morphogenetic plasticity of these cells, once thought to be terminally differentiated. Initial examination suggests similarities in pancreatic endocrine development with respect to transcriptional regulators and the expression profile of the intermediate precursor-type cells (Gao et al. 2005, Jamal et al. 2005, Lechner et al. 2005). Accordingly, interest has been directed toward factors identified as critical to pancreatic organogenesis. To this end, we have implicated transforming growth factor β (TGFβ) in the control of human islet plasticity (Hanley & Rosenberg 2007). Likewise, epidermal growth factor (EGF) ligands contribute to islet development and therefore may represent candidates for modulating the differentiated state of adult human islet cells.

Fetal pancreatic explants respond to EGF with an increase in epithelial cell proliferation and a decrease in endocrine differentiation, while pharmacologic inhibition of the extracellular signal-regulated kinase (ERK) pathway, a known mechanism of EGF signaling, elicits an opposite effect (Cras-Meneur et al. 2001). While a certain degree of redundancy likely exists within such a large family of molecules (Erickson et al. 1997), individual EGF ligands have differential effects on specified pancreatic endocrine progenitors; betacellulin (BTC) appears to direct cells toward a β-cell phenotype, while neuregulin-4 seems crucial to the normal development of δ-cells, with both of these phenotypes developing at the expense of the α-cell (Huotari et al. 2002). EGF receptor (EGFR)−/− mice have disrupted pancreatic growth, most notably with respect to epithelial proliferation and branching. Moreover, β-cell frequency is significantly decreased during development, as is post-natal β-cell proliferation. The remaining islets are closely associated with ducts, and bud outward, suggesting that islet migration is also adversely affected by defective EGF signaling (Miettinen et al. 2000). Finally, it has been shown that EGF can stimulate the formation of duct-like epithelial structures (DLS) in mice pancreatic rudiments embedded in collagen (Sanvito et al. 1994).

The importance of EGF signaling in the maintenance of adult islet function has also recently been highlighted. Pancreas-specific expression of dominant-negative EGFR leads to overt diabetes caused by decreased β-cell proliferation.
and function, along with increased β-cell apoptosis (Miettinen et al. 2006). Several EGF ligands have been reported as β-cell mitogens (Huotari et al. 1998), with BTC postulated as a signaling intermediate for the mitogenic effects of glucagon-like peptide-1 (Buteau et al. 2003). However, EGF ligands are not only mitogenic to endocrine cells but also induce duct cell proliferation (Vinter-Jensen et al. 1997, Rescan et al. 2005, Suarez-Pinzon et al. 2005a). In fact, this latter effect appears to prime duct cells for islet regeneration. It is therefore noteworthy that several studies have drawn attention to the regenerative effect of gastrin and EGF in combination (von Herrath 2005). Individually, these factors act mainly as duct cell mitogens (Bockman & Merlino 1992, Vinter-Jensen et al. 1997, Rooman et al. 2001), whereas co-administration (or the production of double-transgenic animals) leads to a significant increase in β-cell mass (Wang et al. 1993, Brand et al. 2002, Suarez-Pinzon et al. 2005b). The role of EGF ligands in this model, however, appears to be the generation of regeneration-competent metaplastic DLS (Wagner et al. 1998, Means et al. 2005), rather than the β-cell regeneration observed in response to gastrin alone (Rooman et al. 2002). In fact, EGFR signaling leads to the metaplastic transformation of acini into DLS, both in vivo (Wagner et al. 1998) and in vitro (Means et al. 2005, Lipsett et al. 2007).

With respect to cellular transformation, EGF signaling is linked to pancreatic adenocarcinoma (Korc et al. 1992, Murphy et al. 2001, Miyamoto et al. 2003), characterized by expression of duct epithelial markers. There is debate as to the cell of origin (Pour et al. 2003), suggesting that EGF signaling may again induce cellular dedifferentiation into a duct-like phenotype. Interestingly, studies on isolated islets suggest that EGF ligands have little mitogenic effect in culture (Sjoholm 1996). However, in vivo β-cell-specific overexpression of EGF leads to increased β-cell proliferation (Krakowski et al. 1999). These contrasting effects may reflect a differential response of the islet to the microenvironment. For example, isolated human islets, when cultured in suspension, maintain a quiescent endocrine phenotype (Schmied et al. 2000); however, embedding islets in a collagen matrix facilitates cellular proliferation (Metракos et al. 1994), and culture with added EGF and cholera toxin induces dedifferentiation into DLS (Yuan et al. 1996). Although we have characterized the roles of extracellular matrix and cholera toxin in the model of islet dedifferentiation (Wang et al. 2001), we have not fully elucidated the role of EGF. In this paper, we sought to determine the contribution of EGF to the dedifferentiation of cultured human islets as well as to identify the signaling pathways involved.

Materials and Methods

Islet isolation and culture

Pancreata from adult human cadaveric organ donors were obtained through the local organ procurement organization (Table 1). Islets were isolated according to the established protocols (Ricordi et al. 1988). Briefly, organs were flushed and stored in cold ViaSpan solution (Barr Laboratories, Pomona, NY, USA) until islet isolation. The main pancreatic duct was cannulated and perfused with a solution of Liberase BTC postulated as a signaling intermediate for the mitogenic effects of glucagon-HI (Roche Diagnostics). The distented organ was placed in a closed system (Bio-Rep, Miami, FL, USA) and heated to 37 °C to activate the enzyme blend. Following the appearance of free islets in samples, the system was cooled and free tissues were collected, washed, and separated by continuous Ficoll density gradient centrifugation in a cell processor (COBE DCT, Denver, CO, USA). Free islets, determined to be ≥75% pure by real-time staining with dithizone (Sigma), a Zn2+ chelator, were collected, washed, and counted as islet equivalents (IE, islet with diameter of 150 μm).

Isolated islets were cultured in suspension overnight in DMEM/F12 (Gibco) containing penicillin, streptomycin, fungizone, 10% FBS (Montreal Biotech, Montreal, QC, Canada), 1 μM dexamethasone, 10 ng/ml EGF (Sigma), and 24 mU/ml insulin (Lilly, Toronto, ON, Canada). Islets were then embedded in type I rat tail collagen, as per previous reports (Yuan et al. 1996, Wang et al. 2001, Jamal et al. 2003), at a density of 2000 IE/25 cm², and cultured for 8 days in serum-free DMEM/F12 with penicillin, streptomycin, fungizone, 1 μM dexamethasone, 24 mU/ml insulin, and 200 ng/ml cholera toxin (Sigma), with media being changed every other day. EGF was added over a range of concentrations, from 0-001 to 100 ng/ml. When added, CB5233705, U0126, SP600125, and SB203580, pharmacologic inhibitors of AKT phosphorylation, ERK kinase, c-Jun NH2-terminal kinase (JNK), and p38 respectively, were used at 10 μM (Calbiochem, San Diego, CA, USA; Davies et al. 2000, Bain et al. 2003, Aikin et al. 2004, 2006). The EGFR inhibitor AG1478 was used at 5 μM (Sigma). Inhibitors were prepared so that the final concentration of dimethylsulfoxide (DMSO) was 0-1% and vehicle controls were used. To remove the tissue from collagen, samples were incubated with 0-25 mg/ml collagenase XI (Sigma) for 30 min at 37 °C.

Immunofluorescence

Samples were stored at 4 °C in phosphate-buffered formalin until cell pellets (representing 5000 IE) were embedded in 2%
agrose (Gibco). Following routine processing and embedding, 4 μm sections were cut and dewaxed in xylene and petroleum ether. Slides were permeabilized by incubation in Tris-buffered saline + 0.1% Triton X-100 for 5 min, prior to antigen retrieval by heating for 10 min in 0.1 M citrate buffer, pH 6. Slides were then incubated in blocking buffer (Zymed, San Francisco, CA, USA) for 15 min before overnight incubation with primary antibody in blocking buffer. Following washing in PBS, slides were incubated for 1 h with secondary antibody in blocking buffer and reprobed with a different primary antibody. Again, following washing, slides were incubated with secondary antibody before washing and coverslipping with mounting medium containing 4',6-diamidino-2-phenylindole (DAPI; Vector, Burlingame, CA, USA). The primary antibodies used were mouse α-EGFR (Abcam), rabbit α-insulin (Santa Cruz Biotechnology, Santa Cruz, CA, USA), α-glucagon, α-somatostatin, and α-pancreatic polypeptide (Dako, Carpinteria, CA, USA), along with FITC-conjugated α-mouse and rhodamine-conjugated α-rabbit secondary antibodies (Abcam).

**Quantitative real-time PCR**

Briefly, samples (representing 5000 IE) were stored in RLT buffer (Qiagen) until the time of RNA isolation. Following RNA extraction, as per the manufacturer’s protocol, cDNA was reverse transcribed using an Omniscript RT kit (Qiagen). Custom primers (Invitrogen) were synthesized (Table 2; Adam et al. 1999, Rescan et al. 2005), and quantitative real-time PCR was performed using a QuantiTect SYBR green kit (Qiagen). RNA extraction, as per the manufacturer’s instructions on substrate-specific phospho-protein ELISAs for AKT, ERK, JNK, and p38, namely PathScan phospho-AKT1 (Ser473), phospho-p44 MAPK (Thr202/Tyr204), phospho-SAPK/JNK (Thr183/Tyr185), and phospho-p38 MAPK (Thr180/Tyr182) ELISA kits (Cell Signaling, Danvers, MA, USA) respectively. Results are presented relative to time-matched or pre-treatment controls, as indicated in the figures.

**ELISAs**

To determine protein phosphorylation levels, samples (representing 5000 IE) were washed thoroughly in PBS before sonication in lysis buffer (50 mM Tris–HCl, pH 8, 1·37 mM NaCl, 0·1 mM Na3VO4, complete protease inhibitor tablet (Roche), 1% v/v nonidet P-40, and 10% v/v glycerol). Cell lysates were then centrifuged and supernatant transferred to a fresh tube. Protein concentration was determined using the Bradford assay (Bio-Rad) and 0·25 mg/ml cell lysate per reaction was processed according to the manufacturer’s instructions on substrate-specific phospho-protein ELISAs for AKT, ERK, JNK, and p38, namely PathScan phospho-AKT1 (Ser473), phospho-p44 MAPK (Thr202/Tyr204), phospho-SAPK/JNK (Thr183/Tyr185), and phospho-p38 MAPK (Thr180/Tyr182) ELISA kits (Cell Signaling, Danvers, MA, USA) respectively. Results are presented relative to pre-treatment controls.

**Table 2** Quantitative real-time PCR primers and conditions

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers</th>
<th>Annealing (°C)</th>
<th>Amplicon (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTC</td>
<td>5'-TTCACTGTGTTGGCAGATG-3'</td>
<td>58</td>
<td>350</td>
</tr>
<tr>
<td></td>
<td>5'-AACGATGTTGCACTGCAAC-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGF</td>
<td>5'-TGGGAATGTTGCTTAC-3'</td>
<td>58</td>
<td>399</td>
</tr>
<tr>
<td></td>
<td>5'-TGGAGGCACTGACTCAG-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGFa</td>
<td>5'-TTCTGGGAACTCCAT-3'</td>
<td>58</td>
<td>317</td>
</tr>
<tr>
<td></td>
<td>5'-GGGAGACGAGATTTTTCG-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGFR</td>
<td>5'-CATGTCGATGAACTCCAG-3'</td>
<td>58</td>
<td>125</td>
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<tr>
<td></td>
<td>5'-GGGCCACAGATGATTCTGGTC-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ErbB2</td>
<td>5'-ACTGGCTGCTCCCTACAATA-3'</td>
<td>58</td>
<td>194</td>
</tr>
<tr>
<td></td>
<td>5'-ATATGGCACTGTAACCTGC-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ErbB4</td>
<td>5'-GTCACTGTTAGTGGCTCT-3'</td>
<td>58</td>
<td>311</td>
</tr>
<tr>
<td></td>
<td>5'-ACCTCACCATCTGAACTAC-3'</td>
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<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>5'-TGAGAACCAGGAAACCCTTCA-3'</td>
<td>58</td>
<td>218</td>
</tr>
<tr>
<td></td>
<td>5'-TCACAACCATGACGAACTAG-3'</td>
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**DLS proliferation**

To assess their proliferation, DLS were cultured for two additional days ± EGF. Samples (representing 5000 IE) were then processed for immunofluorescence as described above, using mouse α-proliferating cell nuclear antigen (PCNA; Dako) and rabbit α-cytokeratin-19 (CK19, Proteintech, Chicago, IL, USA) primary antibodies, along with FITC-conjugated α-mouse and rhodamine-conjugated α-rabbit secondary antibodies (Abcam), following the same protocol. Slides were mounted in Vectashield mounting medium with DAPI to counterstain DNA.

A minimum of 500 CK19+ nuclei were counted per sample, and proliferation was determined as the proportion of PCNA+ nuclei, expressed in relation to baseline DLS proliferation at day 8 of culture.

**Statistical analysis**

All experiments were paired such that control and treatment groups contained tissue from the same donor. Results are expressed as mean ± s.e.m. Statistical significance was determined by one-way ANOVA with post-hoc Bonferroni’s test, or paired Student’s t-test, where appropriate. Differences were considered significant when P<0·05.
Results

EGF induces islet dedifferentiation

Following overnight suspension culture, islets embedded in a type I rat tail collagen matrix and cultured with 10 ng/ml EGF and 200 ng/ml cholera toxin undergo islet cell dedifferentiation into DLS, consisting of a layer of flattened or low cuboidal epithelium of one to several cells thickness (Fig. 1A), at a rate of 63±3% (Yuan et al. 1996, Hanley & Rosenberg 2007). We have previously correlated the morphological evaluation of DLS formation with changes in endocrine and ductal cell frequencies (Hanley & Rosenberg 2007), as well as the cells involved in this process (Hanley et al. 2008).

To further investigate the role of EGF in the dedifferentiation process, islets were embedded and cultured in the absence of EGF. Under these circumstances, DLS formation was significantly reduced (19±4% of control, \( P<0.001 \)). Moreover, EGF induced DLS formation in a concentration-responsive manner (Fig. 1B), with an EC\(_{50}\) of \(~0.05 \text{ ng/ml}\). This concentration is significantly less than that reported by others culturing fetal pancreatic and embryonic stem cells (Cras-Meneur et al. 2001, Yasada et al. 2002, Yoshida et al. 2002, Humphrey et al. 2003), suggesting that cells of the isolated adult islet are highly sensitive to EGF ligands. Nevertheless, in keeping with previous work, a concentration of 10 ng/ml EGF was used for subsequent experiments.

While addition of EGF significantly improved DLS formation, we did observe minimal spontaneous DLS formation in the absence of added EGF. To determine whether this was the result of endogenous EGF ligands, islets were embedded and cultured as described, with the addition of AG1478 (Fig. 1C), a pharmacological inhibitor of the EGFR tyrosine kinase (Lin et al. 1997). As expected, some DLS formation was observed in vehicle-treated samples (47±6% of control, \( P<0.001 \)), while addition of EGF induced a significant increase in DLS formation (96±5%, NS). However, inhibition of EGFR tyrosine kinase activity blocked DLS formation to a greater extent than simply omitting EGF (8±2%, \( P<0.001 \)), suggesting that endogenous EGF ligands may be acting in this system. Co-administration of EGF and AG1478 also led to reduced DLS formation (16±5%, \( P<0.001 \)), confirming the specificity of the inhibitor.

These experiments show that EGF is necessary for islet dedifferentiation into DLS. We then assessed whether there was a specific cell type within the islet that was responsive to EGF.

Islet endocrine cells express EGFR

To determine the cellular target of EGF ligands within islets, we performed immunofluorescence localization studies of EGFR (Fig. 2). Double immunofluorescence indicated that EGFR is in fact expressed by the four principal endocrine cells of the islet, as indicated by co-expression of EGFR with insulin, glucagon, somatostatin, and pancreatic polypeptide. This finding is in keeping with the fact that each of the four endocrine cell types is implicated in the process of DLS formation (Hanley et al. 2008).

Islets produce endogenous EGF ligands

We have previously reported that islets produce various factors in response to isolation and culture (Aikin et al. 2006, Hanley et al. 2006, Hanley & Rosenberg 2007). Given that the aforementioned studies with AG1478 suggested that islets were indeed producing an EGF ligand, we sought to establish the nature of such a ligand and whether production was responsive to embedding and culture. Accordingly, we examined mRNA expression levels of the EGF family ligands BTC, EGF, and TGF\(\alpha\) as well as the EGFR family proteins EGFR, ErbB2, and ErbB4. The effects of embedding \( \pm \) EGF administration were examined at 24 h, given that DLS formation appears to be initiated within this time period (Wang et al. 2001).

Based on cycle threshold values, EGF ligands were highly expressed by isolated human islets, even prior to embedding
or EGF administration, while EGFRs were moderately expressed (data not shown). In both suspension and embedded cultures, exposure to EGF for 24 h did not induce any significant changes in gene expression, compared with pre-treatment control (Fig. 3). In cultures without EGF, the act of embedding islets significantly decreased BTC expression at 24 h, while EGFR expression showed a similar trend. In EGF-treated cultures, embedding significantly reduced EGF and ErbB4 expression at 24 h, while EGFR and ErbB2 expression displayed similar trends. The greatest changes in gene expression were incurred by islet embedding and EGF treatment, as these conditions led to significant reductions in BTC, EGF, and ErbB4 expression, while EGFR and ErbB2 were decreased, though not significantly. These results show that islets do produce EGF ligands in response to the act of embedding islets significantly decreased BTC expression at 24 h, while EGFR expression showed a similar trend. In EGF-treated cultures, embedding significantly reduced EGF and ErbB4 expression at 24 h, while EGFR and ErbB2 expression displayed similar trends. The greatest changes in gene expression were incurred by islet embedding and EGF treatment, as these conditions led to significant reductions in BTC, EGF, and ErbB4 expression, while EGFR and ErbB2 were decreased, though not significantly. These results show that islets do produce EGF ligands in response to embedding and EGF.

We then sought to identify which were the signaling pathways involved in EGF action and when they were activated over the course of DLS formation.

**AKT and ERK signaling mediate EGF-induced islet dedifferentiation**

With respect to the signaling pathways mediating DLS formation, we have previously observed an increase in JNK phosphorylation concomitant with the observed wave of cellular apoptosis, followed by an increase in AKT and ERK phosphorylation at the time of duct epithelial cell proliferation (Jamal et al. 2003). We hypothesized that the effect of EGF in this system was likely the activation of the latter two kinases, as these are known to act downstream of EGF binding (Rescan et al. 2005, Miettinen et al. 2006). Moreover, the effect of EGF on pancreatic tissue appears to be the induction of cellular dysplasia and proliferation (Wagner et al. 1998, Means et al. 2005).

To first assess the direct signaling effects of EGF, cultured islets were treated with 10 ng/ml EGF for 15, 30, 60, 120, and 240 min, and AKT1 (Ser473), ERK (Thr202/Tyr204), JNK (Thr183/Tyr185), and p38 (Thr180/Tyr182) phosphorylations were assessed by ELISA (Fig. 4A). Under these conditions, AKT and ERK phosphorylation peaked early, at 30 and 15 min, respectively, before returning to baseline levels. Neither JNK nor p38 phosphorylation was affected by EGF administration over this time window. At 24 h, no differences in protein phosphorylation were observed for AKT, ERK, JNK, or p38 (data not shown).

To further clarify signal transduction events during DLS formation, we sampled cultures on 0, 2, 4, 6, and 8 days post-embedding (Fig. 4B) and assessed AKT1 (Ser473), ERK (Thr202/Tyr204), JNK (Thr183/Tyr185), and p38 (Thr180/Tyr182) phosphorylation by ELISA (Fig. 4C). Our data show that the phosphorylation of p38 did not change during the time course of DLS formation. The earliest event observed is a spike in JNK phosphorylation at day 2 of culture, the period of maximal DLS formation, thereafter returning to basal levels. In contrast, AKT phosphorylation did not change significantly until day 6 of culture, by which time phosphorylation was double that was found in unembedded islets. By day 8, the level of AKT phosphorylation was already returning to baseline. ERK phosphorylation was significantly decreased at day 2 of culture and slowly recovered, peaking at day 8 of culture.

Thus, JNK phosphorylation occurs early, at the time of maximal DLS formation, whereas increased AKT and ERK phosphorylations were observed after DLS formation, but during the period of peak DLS cell proliferation (Yuan et al. 1996, Jamal et al. 2003), suggesting a role for EGF in this process.

To further confirm this observation, we next examined phosphorylation events during islet culture in the absence of EGF (Fig. 5A). Without added EGF, JNK, and AKT phosphorylation levels do not significantly deviate from baseline throughout the culture period, suggesting that these events are EGF dependent (Jamal et al. 2003).

To confirm that EGF-mediated activation of these signaling pathways was in fact essential for the process of DLS formation, we next examined DLS formation in the

![Image of EGFR expression](https://www.endocrinology-journals.org)

**Figure 2** EGFR is expressed by endocrine cells. Immunofluorescent co-localization of EGFR with insulin, glucagon, somatostatin, or pancreatic polypeptide in freshly isolated human islets (bar = 100 μm, representative of n=3 individual donors).

*www.endocrinology-journals.org*
presence of pharmacological inhibitors of AKT, ERK, JNK, and p38 (Fig. 5B). Inhibition of AKT phosphorylation, ERK, or JNK significantly reduced DLS formation rates, while inhibition of p38 did not. These results are in keeping with our previous observations of JNK-mediated islet cell apoptosis resulting in the initial transformation of islets into DLS, with AKT and ERK activity contributing to the proliferation of cells within the newly forming DLS (Jamal et al. 2003, 2005). However, the time course and inhibition studies also suggest that the effect of EGF on JNK phosphorylation during early DLS formation may not be immediate or direct, but perhaps permissive. Conversely, it appears that the effect of EGF on AKT phosphorylation in the newly forming cells of the DLS may be more immediate and may contribute to their proliferative state.

We have previously evaluated cellular proliferation over the course of DLS formation, starting from virtually no proliferation in islets to over 80% in DLS at day 12 (Yuan et al. 1996, Jamal et al. 2003). To characterize the role of EGF in DLS cell proliferation, we assessed proliferation rates in DLS cultures incubated ± EGF for 2 days (Fig. 5C). Compared with pre-treatment levels, there was a statistically significant increase in DLS cellular proliferation with the addition of EGF (51 ± 1% vs 45 ± 1%, P<0.01) while culture without EGF decreased proliferation rates (37 ± 1% vs 45 ± 1%, P<0.01). Although the absolute change in proliferation rates would appear to be unimpressive, subjective assessment of cultures without EGF would suggest that the DLS that do form are smaller in size, suggesting a decrease in cell proliferation. Moreover, with a high baseline proliferation rate, cells within the DLS may in fact produce their own EGF ligands in a feed-forward loop.

Discussion

We have previously reported that islets do not simply react passively to the ex vivo environment following isolation but can in fact produce autocrine factors to modulate the islet response to these new surroundings. These signals can act to enhance islet cell survival and to promote maintenance of the differentiated phenotype, as with insulin secretion in islets differentiated phenotype, leading to islet cell dedifferentiation and the formation of highly proliferative precursor-like cells (Hanley et al. 2008).

Our studies on the islet response to collagen embedding ± EGF administration suggest that simple EGF administration induces little in the way of transcriptional feedback control of EGF ligands and receptors. While embedding of islets induced somewhat stronger transcriptional changes, these changes were still relatively minimal, even with concomitant addition of EGF. In fact, expression of ErbB4, a protein with limited

Figure 4  EGF induces differential JNK, AKT, and ERK phosphorylation. (A) Short-term time course of the AKT1 (Ser473), ERK (Thr202/Tyr204), JNK (Thr183/Tyr185), and p38 (Thr180/Tyr182) phosphorylation states of islets following EGF administration, compared to time-matched controls (n=6 individual donors). (B) Time course of DLS formation, assessed relative to maximal DLS formation in the presence of 10 ng/ml EGF (n=4 individual donors). (C) Evaluation of the AKT1 (Ser473), ERK (Thr202/Tyr204), JNK (Thr183/Tyr185), and p38 (Thr180/Tyr182) phosphorylation states of cultures over the course of DLS formation, compared to pre-treatment islets (*P<0.05 and †P<0.01 vs day 0, n=3 individual donors).

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affinity for EGF (Jones et al. 1999) and not required for EGF-mediated activation of the AKT and ERK signaling pathways (Deb et al. 2001), was the only receptor transcript to be significantly downregulated in this context. Thus, EGF ligands are constitutively expressed by isolated islets, even after embedding and/or EGF administration. These results do not appear to reflect differences in expression levels between the differentiated endocrine and primitive precursor cell phenotypes, as preliminary studies suggest that the latter dedifferentiated cells overexpress EGFRs relative to the islets from which they are derived (data not shown).

The role of EGF ligands in the control of islet morphogenetic plasticity is far from clear. Studies of pancreatic development have highlighted these proteins as essential for pancreatic duct epithelial development (Cras-Meneur et al. 2001) and the subsequent differentiation and migration of endocrine cells (Miettinen et al. 2000). Knockout studies in adult animals have indicated that EGFR expression is required in β-cells to maintain function and allow the limited proliferation that these cells undergo in the adult (Miettinen et al. 2006). However, several groups have now reported that the culture of isolated islets in medium containing EGF leads to the subsequent dedifferentiation of adult islet cells (Gershengorn et al. 2004, Jamal et al. 2005, Lechner et al. 2005). Moreover, in this paper, we have presented evidence that suggests a critical role for EGF in this process, inducing the signaling pathways that lead to the subsequent proliferation of these dedifferentiated cells. However, the time course of events leading to DLS formation suggests that the main effect of EGF may be to modulate events later in the progression, although it seems to also have a slight effect in the first days. Accordingly, we have identified the key signal transduction responses of islet cells to EGF administration as ERK and AKT phosphorylation and we have demonstrated the absolute requirement for these signals for islet cell dedifferentiation to occur. However, while time course studies of isolated islets suggest that these pathways are activated early after EGF exposure, with peaks within 30 min, such activation signals are not observed during the transition from islet to DLS until at least day 6, that is, until after cellular dedifferentiation has occurred and when DLS cell proliferation is maximal. Thus, while essential for DLS formation, it appears that the primary role of EGF is the induction of proliferation of the dedifferentiating cells. While EGF is also known to activate the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway, use of the EGFR tyrosine kinase inhibitor AG1478 confirmed the receptor dependence of the EGF signal (Egeblad et al. 2001), while JAK/STAT activation is not known to require such receptor kinase activity (Deb et al. 2001).

EGF also modulates JNK phosphorylation at the beginning of islet dedifferentiation, suggesting a possible role for EGF in the induction of the islet cell apoptosis that also appears to be necessary for DLS formation (Jamal et al. 2003, Hanley et al. 2008).

EGF and other ligands of the same family seem to play contradictory roles in the control of pancreatic β-cell mass. In islet cells, EGF signaling appears to be key to the maintenance of β-cell function and survival in the native pancreas. However, in islet cells removed from the in vivo environment, EGF ligands act to induce islet cell dedifferentiation, highlighting the importance of the local microenvironment, namely the extracellular matrix, in directing the nature of the islet response to EGF. This niche-mediated effect of EGF ligands is not specific to mature islet cells. For example, EGF acts on primitive pancreatic ductal epithelium to induce proliferation and branching morphogenesis during pancreatic organogenesis, while later inducing islet cell differentiation from this same primitive pancreatic epithelium.
(Miettinen et al. 2000). In fact, studies on transgenic and TGFβz-administered animals suggest that EGF ligands may continue to act as inducers of islet differentiation in adults, through an apparent priming of otherwise differentiated ductal epithelium, which can then respond more strongly to a true differentiation stimulus (Wang et al. 1993, Brand et al. 2002, Suarez-Pinzon et al. 2005b).

Finally, EGF signaling is also implicated in pancreatic carcinogenesis (Korc et al. 1992, Murphy et al. 2001, Miyamoto et al. 2003). Here again, however, the mechanism of EGF is difficult to interpret, especially given that the cell of origin of pancreatic carcinogenesis remains a subject of debate. Regardless of an endocrine or duct epithelial origin, however, there is ample evidence to implicate EGF signaling in the metaplastic conversion of pancreatic tissues.

While work continues on identifying the factors and mechanisms that act to control the morphogenetic plasticity of adult human islets, it is tempting to speculate that the manipulation of such variables may allow the reprogramming of the endogenous pancreas to re-establish pancreatic β-cell mass. Such a feat would be invaluable in the treatment of diabetes. However, any manipulation of the proliferative potential and differentiation state of cells also carries with it the risk of neoplasia, and so care must be taken to fully understand the complex interrelationships of mechanisms that may be involved (Lipsett et al. 2006).

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.

Funding

This work was supported in part by the Canadian Institutes of Health Research (CIHR). S C H is supported by fellowships from the Canadian Diabetes Association (CDA)/CIHR and Fonds de la Recherche en Santé du Québec (FRSQ). L R is a Chercheur National (national scientist) of the FRSQ.

Acknowledgements

We thank Emily Austin, Jieping Ding, Deborah Driver, Daniel Ellis, Mimi He, Xinling Li, Maria Petrovavlovskaia, and Ryan Scott for technical assistance, as well as Québec-Transplant for coordination of organ availability.

References


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Hanley S & Rosenberg L 2007 Transforming growth factor-β is a critical regulator of adult human islet plasticity. Molecular Endocrinology 21 1467–1477. (doi:10.1210/me.2007-0045)


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EGF induces islet cell dedifferentiation

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Received in final form 24 August 2011
Accepted 20 September 2011
Made available online as an Accepted Preprint 20 September 2011

Journal of Endocrinology (2011) 211, 231–239

www.endocrinology-journals.org