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Dipeptidyl peptidase-4 (DPP-4): Localization and activity in human and rodent islets

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ABSTRACT

Dipeptidyl peptidase 4 (DPP-4) was recently found to be expressed in human and mouse islets with different expression patterns. However, whether species-dependent expression pattern is a generalized phenomenon and whether islet DPP-4 activity is regulated are not known. This study was conducted to investigate DPP-4 localization in several different species, and to examine the impact of glucose, incretin hormones, and insulin on islet DPP-4 activity. It was shown by immuofluorescent staining that there were two distinct species-specific expression patterns of islet DPP-4. The enzyme was expressed exclusively in α-cells in human and pig islets, but primarily in β-cells in mouse and rat islets. INS-1 832/13 cells also expressed DPP-4, and inhibition of DPP-4 enhanced insulin secretion in the presence of glucagon-like peptide-1 (GLP-1) in the cells. DPP-4 activity was remarkably robust when cultured with high glucose, incretin hormones, and insulin in mouse and human islets as well as INS-1 832/13 cells and islet DPP-4 activity and expression pattern was not altered in double incretin receptor knockout mice, compared to wild type mice. We conclude that islet DPP-4 is species-specifically expressed in α-cell and β-cell dominant patterns in several species and both patterns remained robust in enzyme activity during short-term metabolic challenge.

1. Introduction

Dipeptidyl peptidase-4 (DPP-4) is a widely distributed enzyme which exists both in a membrane bound form in many tissues, and in a soluble form in the circulation [1]. The enzyme has gained considerable clinical interest during recent years because it rapidly inactivates incretin hormones, i.e. glucose dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) and its inhibition results in glucose dependent stimulation of insulin secretion and inhibition of the inappropriate glucagon elevation in type 2 diabetes [2,3]. Thus, several pharmaceutical DPP-4 inhibitors have been developed to control hyperglycemia in type 2 diabetes [4].

We recently showed that DPP-4 is expressed also within endocrine cells of the pancreatic islets. In human islets, DPP-4 was found to be largely expressed in α-cells, whereas in mouse islets, DPP-4 was expressed primarily in β-cells [5]. Expression of DPP-4 in islet endocrine cells is of interest since the bioactive forms of both GIP and GLP-1 have been shown to be produced by pancreatic α-cells [6,7] and therefore a local production and inactivation system of these hormones may exist. Local GLP-1 is produced from cleavage of proglucagon by proconvertase 1/3 expressed in α-cells [8]. It has been shown that GLP-1 secreted by α-cells is enhanced by high glucose [6], cytokines (IL-6 and SDF-1α) [9,10] and during the development of diabetes in rodent models [11,12]. Furthermore, inhibition of DPP-4 has been shown to increase glucose-induced insulin secretion and reduce cytotoxicity caused by hyperglycemia, lipids and cytokines via stabilization of paracrine GLP-1 in isolated islets [5,13]. However, the functional role and its regulation factors of islet DPP-4 activity are not fully understood particularly in view of the species-specific expression of islet DPP-4. In this study, we examined whether islet DPP-4 is expressed in a species-specific manner in several species and whether its activity is regulated by glucose, insulin or the incretin hormones.

Abbreviations: DPP-4, dipeptidyl peptidase 4; GLP-1, glucagon-like peptide-1; GIP, glucose dependent insulinotropic polypeptide; DIRKO, double incretin receptor knockout; SDF-1, stromal cell-derived factor-1.

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2. Materials and methods

2.1. Pancreatic tissue samples

Human pancreas tissue samples were collected from 5 non-diabetic donors in Cisanello Hospital of University of Pisa, Italy. Sprague Dawley rats were purchased from Charles River Laboratories (Germany). Ten-week old female C57BL6/JBomTac mice were purchased from Taconic Europe (Skensved, Denmark). Pancreas biopsies from mixed race pigs were obtained from the Lund University Medical School. Double incretin receptor knockout (DIRKO) mice and single GLP-1 receptor deficient mice were derived as previously described [5]. Human islets were obtained from cadaver courtesy of the Nordic Network of Clinical Islet Transplantation (www.nordicislets.org) accessed in July 30, 2014, Uppsala University. Ethical Approvals from regional ethical committees in Lund, Pisa, and Uppsala were obtained.

2.2. Immunohistochemistry

Pancreatic issue samples from humans, pigs, mice and rats were fixed in formaldehyde, embedded in paraffin and sectioned 5 μm in thickness. After antigen retrieval in sodium citrate buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0) and permeabilization with PBS containing 0.25% TRITON X-100 and 0.1% BSA, sections were exposed to primary antibodies at 4 °C overnight. The primary antibodies were: polyclonal goat anti-mouse DPP-4/CD 26 antibody, (R&D Systems, Minneapolis, MN, USA) for mice DPP-4, polyclonal goat anti-human DPP-4 antibody, (R&D, Minneapolis, MN, USA) for human and pig DPP-4, polyclonal rabbit anti-CD26 (Abcam, Cambridge, UK) for rat DPP-4; polyclonal Guinea pig anti-insulin antibody (EuroProxima, Arnhem, Netherlands) for insulin, monoclonal mouse anti-glucagon antibody (Abcam, Cambridge, UK) for glucagon. Afterwards, Sections were incubated with secondary antibody for 1 h, and fluorescent images were captured and processed with Olympus DP72 microscope and Cell sens dimension software package.

2.3. Culture and glucose stimulated insulin secretion (GSIS) of islets

Human islets were isolated from the donors using collagenase and cultured as previously described [16]. Upon arrival in Lund the islets were transferred to RPMI1640 medium (Life technologies, New York, USA) containing 0.1% bovine serum albumin and

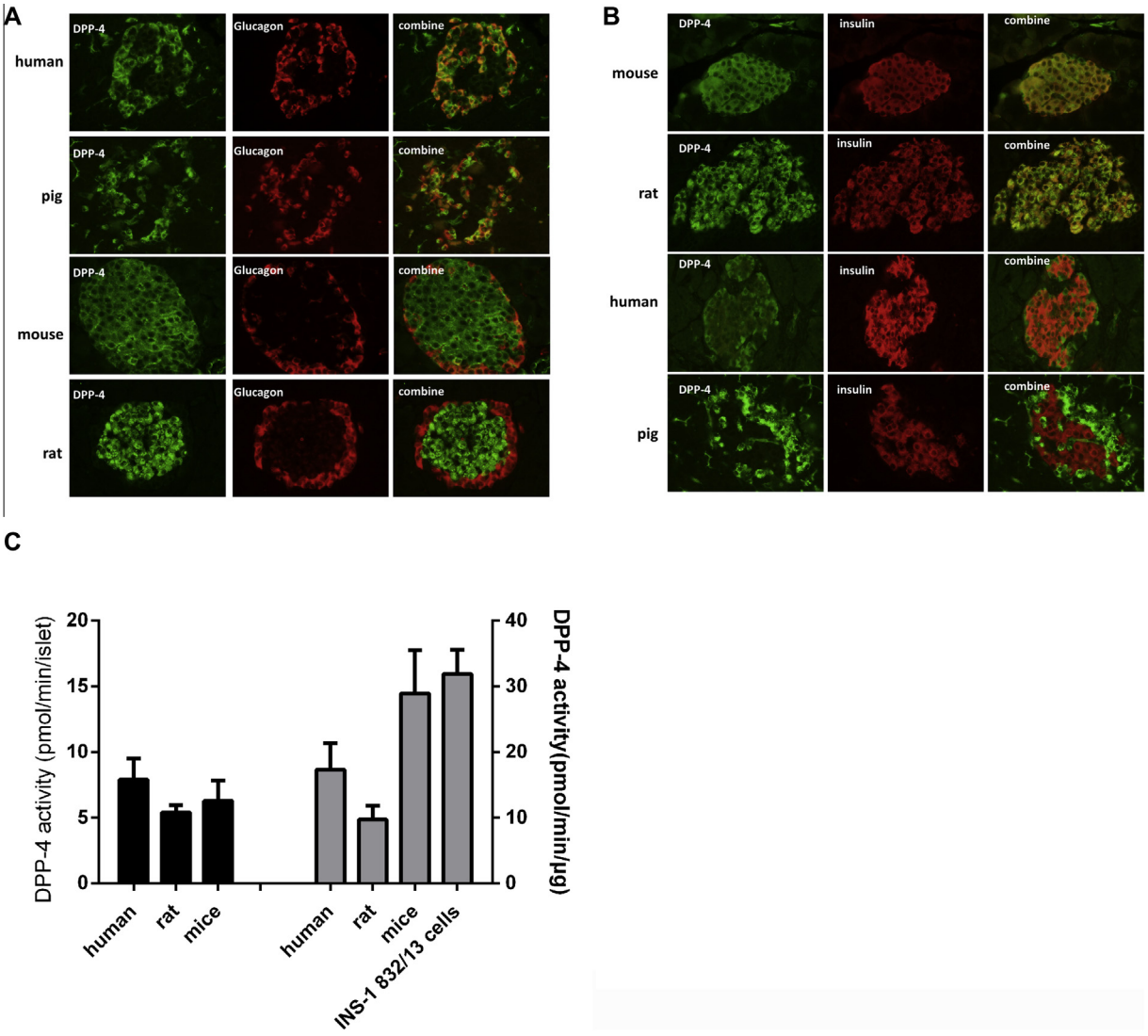

Fig. 1. Species difference of DPP-4 localization in mouse, rat, pig and human islets. (A) Immunofluorescent co-staining of DPP-4 and glucagon. (B) Immunofluorescent co-staining of DPP-4 and insulin. (C) DPP-4 activity in different models (n = 4–10).

1% penicillin/streptomycin (Sigma–Aldrich, St. Louis, MO, USA) at 37 °C (5% CO_2). Mouse and rat islets were isolated by collagenase digestion and handpicked. For in vitro culture, mouse islets were incubated in the same medium described above for human islets.

For determination of DPP-4 activity, islets were incubated for 72 h with 5.6–33.3 mmol/L glucose, physiological glucose level (10.0 mmol/L glucose for mice islets and 5.6 mmol/L glucose for human islets) plus 10 nmol/L GLP-1 (Sigma–Aldrich, St. Louis, MO, USA), 10 nmol/L GIP (Bachem, Germany), or 10 nmol/L insulin (Novo Nordisk, Bagsværd, Denmark). Fresh medium was replaced every 24 h.

For determination of insulin secretion, mouse islets were cultured for 3 days and then incubated in groups of three for 1 h in 200 μL of HEPES balanced salt solution (125 mmol/L $MgCl_2$, 25 mmol/L HEPES, and 0.1% fatty acid free BSA, pH 7.4) with 2.8 or 16.7 mmol/L glucose. Afterwards, supernatant was collected for insulin measurements.

2.4. Culture, immunofluorescent staining and GSIS of INS-1 832/13 cells

INS-1 832/13 cells (kindly provided by Professor Hindrik Mulder, Department of Clinical Science, Lund University, Sweden, passage number of 70–75) were cultured with RPMI-1640 medium containing 10.0 mmol/L glucose, 10% fetal bovine serum, 100 unit/mL penicillin G, 100 μg/mL streptomycin, 10 mmol/L glutamine, 1 mmol/L sodium pyruvate, 10 mmol/L HEPES, and 50 μm β-mercaptoethanol. Cells were exposed to different glucose and hormone treatment conditions, as indicated in the results section, and incubated for 7 days with medium changed every 24 h. For insulin secretion, cells were cultured in 24 well plates to reach ⩾90% confluence then washed with secretion assay buffer (SAB, consist of 114 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L KH_2PO_4, 1.16 mmol/L $MgSO_4$, 20 mmol/L HEPES, 2.5 mmol/L $CaCl_2$, 25.5 mmol/L $NaHCO_3$, 0.2% Bovine serum albumin, PH 7.2) supplemented with 2.8 mmol/L glucose. Afterwards, cells were incubated in 2.8 mmol/L glucose SAB with or without 100 nmol/L vildagliptin (Novartis Pharmaceutical, East Hannover, NJ) for 2 h. Cells were then incubated in SAB supplemented with varying concentrations of glucose in the presence or absence of vildagliptin and the GLP-1 receptor antagonist Exendin 9–39. Insulin in supernatant was measured with rat insulin ELISA kit (Mercodia, Uppsala, Sweden) and normalized with total protein content (Bradford protein assay).

2.5. DPP-4 measurements

DPP-4 activity was measured using Gly-Pro-pNitroaniline as the substrate as previously described [5]. Briefly, islets or cells were sonicated in buffer containing 50 mM TES, 2 mM EGTA, 1 mM EDTA, 250 mM Sucrose and 40 mM Phenylphosphate, pH 7.4. Twenty micro liter of lysate was mixed with 100 μL of 1 mM Gly-Pro-pNitroaniline and subsequently incubated at 37 °C for 60 min. DPP-4 activity was calculated according to the released pNitroaniline by measuring the absorbance at 405 nM with 50 nM nitroaniline as the standard.

2.6. Statistical analysis

Data were expressed as mean ± SEM unless otherwise indicated. Comparison between treatments groups were performed using One-way ANOVA, and post hoc Bonferroni correction was applied if the difference reached statistical difference. $P < 0.05$ was defined as statistical significance. All analyses were carried out with Prism 6.0 software package (Graphpad, San Diego, CA).

3. Results

3.1. Functionally active DPP-4 was expressed in two species-specific distinct localization patterns within the islets from several species

Fluorescent images from pancreatic sections of different species showed that islet DPP-4 was expressed in two distinct patterns (Fig. 1). In islets from humans and pigs, DPP-4 was expressed in α-cells and was therefore co-localized nearly exclusively with glucagon. In contrast, in islets from the two rodent models, DPP-4 was expressed mainly in β-cells. Rat islets expressed DPP-4 in a more β-cell dominant way with little DPP-4 fluorescent signals in α-cells compared with mouse islets, in which weak co-staining of glucagon and DPP-4 could still be found. Intriguingly, in all species and regardless of α- or β-cell expression, DPP-4 was expressed in small particles within the cells. INS-1 832/13 cells, which originated from radiation induced rat β-cell tumor cells, expressed DPP-4 in a way similar to rodent models (supplementary data). Human islets possessed higher DPP-4 activity per islet when compared with mouse and rat islets, but when it turned to DPP-4 activity per microgram of protein in islet lysate, INS-1 832/13 cells had the highest activity (Fig. 1C).

3.2. Inhibition of DPP-4 in the presence of GLP-1 enhanced insulin secretion in INS-1 832/13 cells

We have previously shown that inhibition of DPP-4 potentiates GSIS in isolated mouse and human islets in a GLP-1 dependent manner [5]. As high activity of DPP-4 was found in INS-1 832/13 beta cells, and INS-1 cells have been reported to produce GLP-1 [14], we proceeded to evaluate the effect of DPP-4 inhibition on insulin secretion in INS-1 832/13 cells. High glucose stimulated a 1.9-fold increase of insulin secretion of baseline, while adding vildagliptin alone did not further potentiate the GSIS. Cells treated with 16.7 mmol/L glucose in the presence of 10 nmol/L GLP-1 exhibited a 5.1-fold increment of GSIS, and 100 nmol/L vildagliptin further potentiated it by 22.0%. The potentiation of insulin

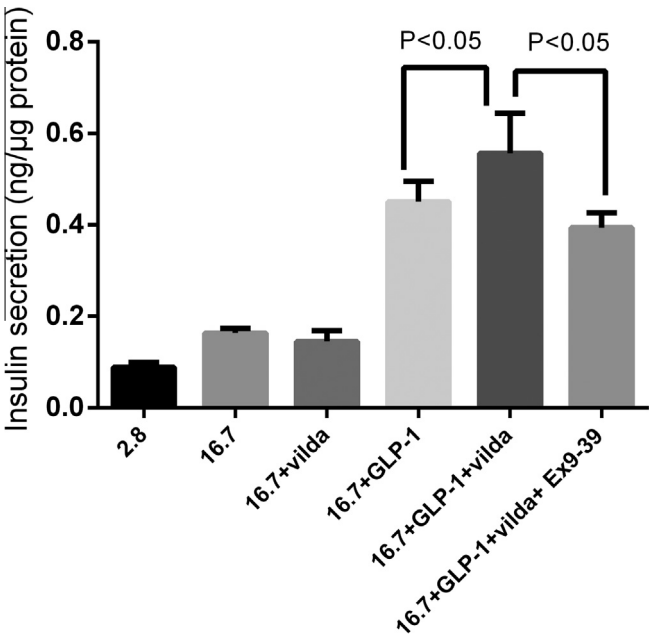

Fig. 2. The insulinotropic effect of DPP-4 inhibition in the presence of GLP-1 in INS-1 832/13 cells. Insulin secretion was measure in cells incubated with 2.8 or 16.7 mmol/L glucose, with or without 10 nmol/L GLP-1, 100 nmol/L vildagliptin (vilda), 100 nmol/L exendin 9–39 (Ex9–39) or combined for 1 h, $n = 8$.

secretion by vildagliptin was reversed by the GLP-1 receptor antagonist exendin 9–39 (Fig. 2).

3.3. The impact of glucose, incretin hormones and insulin on islet DPP-4 activity in islets with α-cell or β-cell DPP-4 expression

In order to exclude the effect of in vitro culture over time on intra-islet DPP-4 activity, we first incubated mouse islets and human islets in physiological concentrations of glucose (for mouse islets, 10.0 mmol/L, for human islets, 5.6 mmol/L) for 72 h. There were no significant differences in DPP-4 activity induced in either human or mouse islets by 72 h of culture (Fig. 3A). We then incubated mouse islets in different levels of glucose for 72 h as the representative of β-cell dominant pattern. DPP-4 activity in islets cultured in 10.0, 16.7, 33.3 mmol/L glucose were similar, while DPP-4 activity in islets cultured with 5.6 mmol/L glucose were lower than other groups (Fig. 3B). Since the lower DPP-4 activity in islets incubated in 5.6 mmol/L glucose might be caused by poor integrity with poor function, we compared GSIS in islets cultured in 5.6 mmol/L and 16.7 mmol/L glucose. Islets cultured in 5.6 mmol/L glucose showed poorer insulin secretory responses to glucose stimulation than those cultured with 16.7 mmol/L glucose

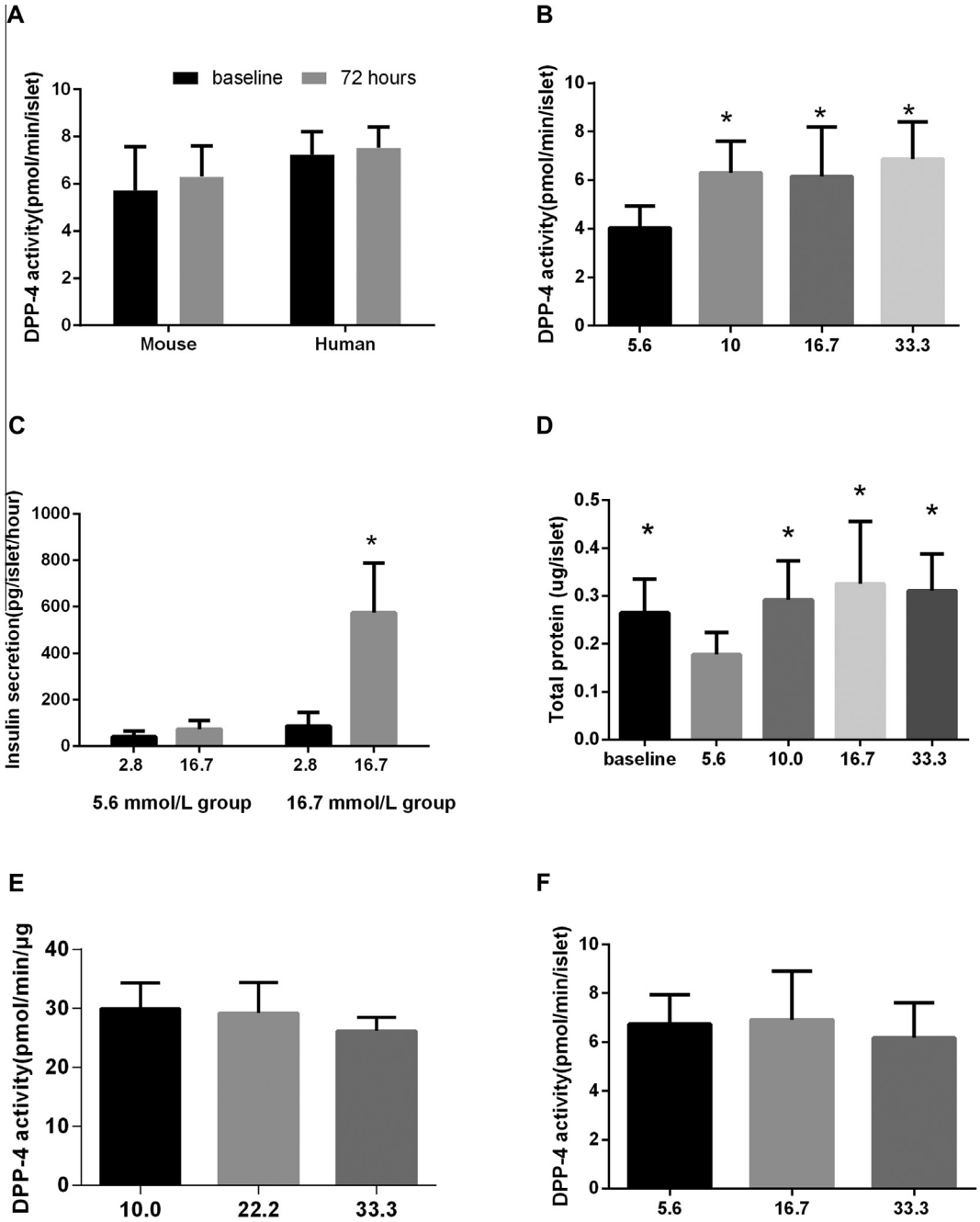

Fig. 3. The effect of glucose on islet DPP-4 activity. (A) DPP-4 activity was not changed by 72-h incubation with physiologic level of glucose (5.6 and 10.0 mmol/L for human ($n = 4$) and mouse islets ($n = 6$) respectively). (B) DPP-4 activity after 72-h culture with different glucose levels in mouse islets. $n = 6–10$, $^*P < 0.05$ compared with 5.6 mmol/L group. (C) GSIS of mouse islets after 72-h culture with 5.6 or 16.7 mmol/L glucose. $^*P < 0.05$ compared with 5.6 mmol/L group, $n = 3$. (D) Protein content in islets cultured with different glucose levels for 72 h. $^*P < 0.05$ compared with 5.6 mmol/L group. $n = 6–10$. (E) DPP-4 activity in INS-1 832/13 cells after cultured with various glucose concentrations for 7 days. $n = 8$. (F) DPP-4 activity of human islets after cultured with 5.6, 16.7, and 33.3 mmol/L glucose for 72 h, $n = 4$.

(Fig. 3C). Poorer functional integrity in islets cultured in 5.6 mmol/L glucose was further indicated by lower protein content in this group (Fig. 3D). As a pure β-cell model, INS-1 832/13 cells incubated with 10.0, 22.2, and 33.3 mmol/L glucose for up to 7 days had similar DPP-4 activity (Fig. 3E). In human islets, the representative model of α-cell dominant pattern, DPP-4 activity was also not significantly modified after 72-h culture with either 5.6, 16.7, or 33.3 mmol/L glucose (Fig. 3F).

3.4. Islet DPP-4 is not altered by incretin hormones and insulin

To study whether compromised incretin receptor signals affect islet DPP-4 activity, we investigated DPP-4 activity and expression pattern in incretin receptor deficient mice. The overall islet DPP-4 staining in DIRKO mice was similar to that in wild type mice (primarily in β-cells) except that there seemed to be more cells showing co-expression of glucagon and DPP-4 in DIRKO mice (Fig. 4A). DPP-4 activity was similar in islets from wild type, GLP-1 receptor knock out, and DIRKO mice (Fig. 4B). To investigate the effect of exogenous incretin hormones and insulin on islet DPP-4, islets from humans and mice (72 h) and INS-1 832/13 cells (7 days) were incubated in the presence of GLP-1, GIP or and insulin (10 nmol/L). None of GLP-1, GIP and insulin altered DPP-4 activity in human islet, mice islets or INS-1 cells (Fig. 4C–E).

4. Discussion

In this study, we show that among several species, there are two distinct species-specific patterns for islet DPP-4 cellular localization: the α-cell dominant pattern in humans and pigs and the β-cell dominant pattern in rats and mice. We also demonstrated that the clonal insulinoma cell line (rat), INS-1 832/13, retain high DPP-4 activity. Although INS-1 cells were reported to express GLP-1 [14], the fact that inhibition of DPP-4 alone did not potentiate the GSIS was likely due to the secreted GLP-1 from INS-1 cells being too minor in amount to elicit any insulinotropic effect. Similar to our previous study on human and mouse islets [5], GSIS was increased in INS-1 cells in the presence of GLP-1, indicating that DPP-4 in situ was biologically functional in truncating incretins in spite of different expression patterns. These findings extend our knowledge on species difference of DPP-4 localization within the islets.

The exact function of islet DPP-4 and the importance for its species-specific distribution are not completely understood. Based on the relatively low enzyme activity compared with that in circulation [5], islets are not likely the major location for systemic incretin degradation. Since inhibition of DPP-4 exerted similar effect in facilitating insulin secretion in α-cell dominant and β-cell dominant patterns, other hypotheses might be required to explain the distribution discrepancy among species. In rodent islets, β-cells are located in the center and surrounded by a mantle consisting of α, δ and PP cells, whereas islets of human and pig are more lobulated, with more non-β-cells scattered throughout the islets [15]. Therefore, it could be hypothesized that different DPP-4 localization might play a role in the formation and maintenance of different islet architectures. Frerker et al. demonstrated that the islet size of DPP-4 deficient Dark Agouti rats was smaller than that of wild type rats, which supports this hypothesis [16]. Some substrates of DPP-4 are known to be involved in islet development. For example, peptide YY, which is inactivated by DPP-4, is known to be expressed in a very early stage in embryonic pancreas and may play a role in endocrine cell differentiation [17,18]. GLP-1 is also able to induce ductal precursor cells into insulin producing cells [19]. Prohormone convertase 1/3, the critical enzyme that converts proglucagon into GLP-1, was shown to be expressed in fetal mice α cells but not in fetal human α cells [20,21]. On the other hand, DPP-4 mediated cellular adhesion by interacting with fibronectin, which is an important molecule in organ development and cell organization [22]. Further research is required to elucidate the role of DPP-4 during pancreatic ontogenesis.

We found that islet DPP-4 activity was remarkably robust and did not change in response to high glucose, incretin hormones or insulin in either localization patterns in vitro. Therefore, acute regulation of islet DPP-4 activity might not be a major adaptation to instant insulin demand. In our previous study, islet DPP-4 activity was positively associated with insulin secretion capability, and

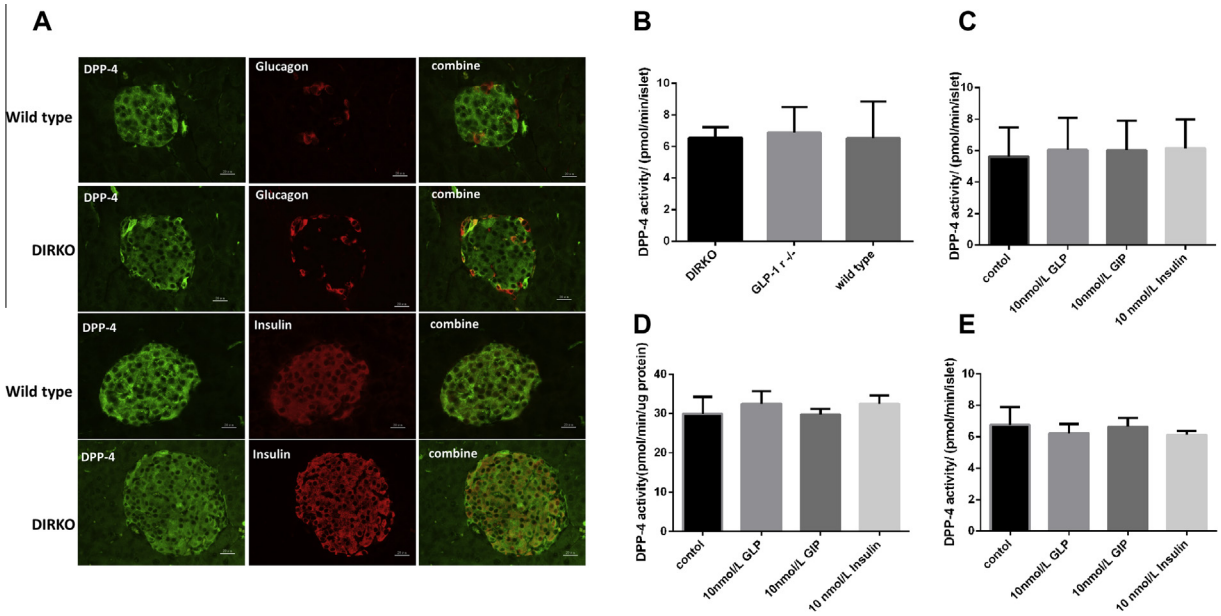

Fig. 4. The impact of incretin hormones and insulin on islet DPP-4. (A) Double incretin receptor knock out (DIRKO) mice and wild type mice showed a similar localization pattern of islet DPP-4. (B) The DPP-4 activity was similar in DIRKO, GLP-1 receptor knockout and wild type mice ($n = 3$). Incubation with GLP-1, GIP or insulin did not alter DPP-4 activity in mouse islets ((C), $n = 6$), INS-1 832/13 cells ((D), $n = 8$) and human islets ((E), $n = 4$).

DPP-4 activity was lower in islets from subjects with type 2 diabetes [5]. In our current study we also found that both DPP-4 activity and GSIS were lower in mouse islets chronically cultured in sub-physiological glucose. A similarity between islets from subjects with type 2 diabetes and mouse islets chronically cultured in sub-physiological glucose is loss of functional integrity. This would suggest that DPP-4 activity is reduced in concordance with the development of islet dysfunction.

The long-term impact of hyperglycemia on islet DPP-4 activity is unclear. Previous studies on potential regulation of DPP-4 activity have shown only modest or subtle influences of glucose which sometimes are not consistent throughout the literature. Transient excursion of glucose such as OGTT and post-meal glucose fluctuation did not show any acute effect on DPP-4 activity in circulation [23,24]. DPP-4 activity was shown to increase in type 2 diabetes, but whether it is higher in type 1 diabetes is unclear [25,26]. DPP-4 activity, however, is more closely associated with factors related to obesity and insulin resistance than to glucose level in type 2 diabetic patients [24,25,27]. Moreover, elimination of hyperglycemia did not change the plasma DPP-4 activity in patients with type 2 diabetes [28]. These data implied that hyperglycemia per se might not be a major regulator of DPP-4 activity in the circulation. On the other hand, membrane-bound DPP-4 in different tissue types responds differently to glucose. In high glucose milieu, DPP-4 activity was increased in human microvascular epithelium cells, unchanged in human macrovascular epithelium cells, and decreased in intestinal epithelium cells [29,30]. The finding of this study adds to the knowledge of tissue-specific response to glucose of DPP-4, and the underlying mechanism is yet to be clarified.

Since DPP-4 inactivates multiple substrates including incretin hormones, it was hypothesized that DPP-4 substrates functioned as feedback regulators of the enzymes activity. However, a main finding of this study did not support this hypothesis. GLP-1 and GIP did not show any effect on islet DPP-4 activity in both models and the fact that DIRKO mice, genetically deficient in both GLP-1 and GIP receptors, had a normal islet DPP-4 activity also indicates that islet DPP-4 activity is not regulated by incretin hormone signaling.

In conclusion, islet DPP-4 is expressed in α-cell and β-cell dominant patterns in different species, and both patterns remained robust during short-term metabolic challenge. The functional difference and possible regulators of the two patterns are pending to be explored in ongoing research.

Competing interest

Authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.09.096.

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