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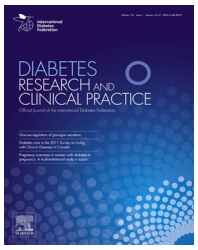

Localization of dipeptidyl peptidase-4 (CD26) to human pancreatic ducts and islet alpha cells

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ABSTRACT

Aim: DPP-4/CD26 degrades the incretins GLP-1 and GIP. The localization of DPP-4 within the human pancreas is not well documented but is likely to be relevant for understanding incretin function. We aimed to define the cellular localization of DPP-4 in the human pancreas from cadaveric organ donors with and without diabetes.

Methods: Pancreas was snap-frozen and immunoreactive DPP-4 detected in cryosections using the APAAP technique. For co-localization studies, pancreas sections were double-stained for DPP-4 and proinsulin or glucagon and scanned by confocal microscopy. Pancreata were digested and cells in islets and in islet-depleted, duct-enriched digests analyzed for expression of DPP-4 and other markers by flow cytometry.

Results: DPP-4 was expressed by pancreatic duct and islet cells. In pancreata from donors without diabetes or with type 2 diabetes, DPP-4-positive cells in islets had the same location and morphology as glucagon-positive cells, and the expression of DPP-4 and glucagon overlapped. In donors with type 1 diabetes, the majority of residual cells in islets were DPP-4-positive.

Conclusion: In the human pancreas, DPP-4 expression is localized to duct and alpha cells. This finding is consistent with the view that DPP-4 regulates exposure to incretins of duct cells directly and of beta cells indirectly in a paracrine manner.

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Abbreviations: APAAP, alkaline phosphatase-anti-alkaline phosphatase; DPP-4, dipeptidyl peptidase IV; FCS, fetal calf serum; GAD Ab, glutamic acid decarboxylase 65 antibody; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide 1; SSC, side scatter.

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1. Introduction

Dipeptidyl peptidase-4 (DPP-4) is a serine exopeptidase that cleaves X-proline dipeptides from the N-terminus of polypeptides. DPP-4 is identical with the leukocyte surface antigen CD26 and adenosine deaminase complexing protein 2 [1–3]. It is expressed on the surface of many cell types and has multiple functions, including the processing of peptide hormones, chemokines and neuropeptides, binding of adenosine deaminase and co-stimulation of T cells [4–7]. In addition, DPP-4/CD26 is a marker of cancer stem cells and has been implicated in malignant transformation [8].

DPP-4 plays a major role in glucose metabolism [9]. It degrades the incretin hormones glucagon-like peptide 1 (GLP-1) and gastric inhibitory peptide (GIP) that are released from the gut into the bloodstream in response to ingested glucose [10,11]. Both incretins enhance glucose-stimulated insulin secretion, suppress glucagon secretion and reduce gastric emptying, but have short half-lives due to their degradation by DPP-4 [4,10,11]. DPP-4 inhibitors that increase incretin concentrations and extend their duration of action are a new class of oral hypoglycaemic drugs [9].

Earlier studies detected DPP-4 in the exocrine pancreas of rabbits [12] and in the endocrine pancreas of mice [13] and pigs [14]. In pig islets, DPP-4 was detected in secretory granules of alpha cells [13,14]. The cellular localization of DPP-4 in the human pancreas has been poorly documented but may have implications for understanding the physiology of incretins. Dinjens et al. in 1989 [15], reported DPP-4 expression in the luminal membranes of intra- and interlobular pancreatic ducts but not in islets or pancreatic acini. In 2013 Bramswig et al. [16] detected DPP-4 gene expression by RNA-Seq in human glucagon-secreting alpha cells. At the same time Omar et al. [17] and ourselves [18,19] investigated DPP-4 expression in the human pancreas by immunochemistry. Recently, Omar et al. [20] reported the expression of DPP-4 in islets. Here, we localize DPP-4 expression to ducts and alpha cells in human pancreas tissue obtained from cadaveric donors without diabetes and with type 1 and 2 diabetes.

2. Materials and methods

2.1. Pancreatic tissue processing

Human pancreata were obtained through the Australian Islet Transplant Consortium and trained coordinators of DonateLife, from heart-beating, brain-dead donors, with informed written consent of next-of-kin. Copies of the consent documents were retained by the next-of-kin, DonateLife and the researchers. The Human Research Ethics Committees of St. Vincent's Institute of Medical Research, Western Sydney Local Health District and the Walter and Eliza Hall Institute of Medical Research approved the consent procedure and the research on organ donor tissue.

Tissue samples were taken from the mid-section of the pancreas, cleaned of fat, embedded in OCT and snap frozen. Human islets were purified using a modified Ricordi method and cultured in Miami 1A Media (Mediatech, Inc., Manassas, VA) supplemented with 2 mmol/L L-glutamine, in a 37 °C, 5% CO_2 humidified incubator for 2 days as previously described [21,22]. To obtain single islet cells, 1000–1500 islet equivalents were incubated in 1 mL Accutase solution (Innovative Cell Technologies, Inc., San Diego, CA) at 37 °C for 10 min, then mechanically dispersed by gentle pipetting. Islet cells were washed and recovered in CMRL 1066 media (Invitrogen, Grand Island, NY, USA) for 1 h at 37 °C [21].

To recover non-islet cells, pancreas digests depleted of islets by density gradient centrifugation [21,22] were seeded at a density of 1×10^6 cells per 5 cm^2 into tissue culture flasks (Corning Incorporated, Corning, NY, USA) coated with gelatin (0.1%). The digest was cultured in human tonicity DMEM with Ham's nutrient mixture F-12 (DMEM/Ham) containing 5% fetal calf serum (FCS), 100 mM non-essential amino acids, 2 mM glutamine, 5×10^{-5} M 2-mercaptoethanol and 100 IU/mL penicillin and 100 μg/mL streptomycin. After incubation overnight the floating dead cells were gently decanted without disturbing attached cells and the medium replaced. The cells were cultured for 2–5 days at 37 °C in a 5% CO_2 humidified chamber, and the medium replaced daily. To obtain single cells for flow cytometry the flasks were gently rinsed with PBS and the attached cells dissociated with trypsin (0.2%) at 37 °C before stopping the reaction by addition of medium containing 10% FCS. Islet cells and cells in islet-depleted duct-rich digests were characterized by flow cytometry with monoclonal antibodies [23] that recognize surface antigens on distinct subsets of human pancreas cells. Overlays of labeled cells were created in FlowJo v9.3.3. The majority of islet cells expressed the human islet marker Hpi1 [23] and a minority the ductal cell marker Hpd1 [23]; on the other hand, duct-rich cells expressed the duct (Hpd1, CA19-9) and exocrine (Hpx2) markers but very little Hpi1 (Fig. 1A and B).

2.2. Immunohistochemistry

Cryostat 6 μm sections of pancreas were fixed in acetone for 10 min, washed once with HT-PBS and blocked with DAKO® Protein Block (DAKO, Glostrup, Denmark) for 10 min, followed by Biotin Blocking System (DAKO) when biotinylated GS9A8 mouse monoclonal antibody (a gift from O.D. Madsen, Denmark) was used to detect human proinsulin [24]. Sections were incubated overnight at 4 °C with mouse anti-human mouse DPP-4 monoclonal IgG1 antibody (clone M-A261; BD Biosciences) diluted 1/1000 in HT-PBS supplemented with 5% FCS and detected using the Alkaline Phosphatase-Anti-Alkaline Phosphatase (APAAP) technique (DAKO).

For double-staining experiments, sections were blocked with mouse serum 2% for 2 h. After washing, beta cells were identified with biotinylated GS9A8 antibody [24] diluted 1/200, detected by horseradish peroxidase labeled streptavidin (DAKO). APAAP staining was visualized with FastRed or BCIP/NPT (Sigma, Castle Hill, NSW, Australia). For each antibody, species-matched non-immune immunoglobulin and secondary antibody alone were tested as negative controls. Staining was visualized with an upright microscope from Zeiss (Axio Imager, Oberkochen, Germany) equipped with a digital camera.