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Nestin-Expressing Cells in the Pancreatic Islets of Langerhans

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The pancreatic islets of Langerhans produce several peptide hormones, predominantly the metabolically active hormones insulin and glucagon, which are critical for maintaining normal fuel homeostasis. Some evidence exists that pancreatic endocrine cells turn over at a slow rate and can regenerate in certain conditions. This could be due to the presence of pluripotent cells residing in the pancreas. Recently the intermediate filament protein nestin has been identified to be a marker for a multipotent stem cell in the central nervous system. Given the similarity between the pancreatic islets and neuronal cells, we hypothesized that stem cells expressing nestin might be present in the pancreas. Here we present evidence that a subset of cells in the pancreatic islets express the stem cell marker nestin. These cells might serve as precursors of differentiated pancreatic endocrine cells.

Diabetes mellitus is a devastating disease accounting for significant morbidity and mortality in industrialized populations. The main hallmark of diabetes mellitus is a relative lack of insulin to meet peripheral demands. Type 1 diabetes is due to an immune-mediated destruction of pancreatic insulin-producing β-cells, whereas in type 2 diabetes the endocrine pancreas produces insufficient amounts of insulin to compensate for peripheral insulin resistance.

The β-cells in the adult pancreas has a life span of approximately 50 days, after which it undergoes apoptosis [1].The senescent cells are replaced by replication and neogenesis of new β-cells [2] derived from progenitor cells. Identification of these progenitor cells may further the understanding of islet cell regeneration and ultimately provide a potential for treatment of diabetes mellitus.

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Pancreatic endocrine cells share characteristics with neuronal cells such as electrical excitability, neuronal adhesion molecules (i.e., NCAM), and communication between cells through specialized molecules (i.e., cadherins) [3]. Furthermore, transcription factors implicated in development, phenotype determination and maintenance of function of islet cells are also implicated in the development of the central nervous system (CNS) [3].

Recently, cells have been identified in the CNS which are capable of differentiating into different cell lineages within the CNS [4–6]. These cells express the intermediary filament protein nestin, that is considered to be a marker for stem cells [6]. Furthermore, these cells have recently been shown to be able to differentiate into bone marrow cells [7] and may thus have a multipotent capacity to differentiate into even more other tissues.

Given the regenerative capacity of the endocrine pancreas and the resemblance of its cells to neurons, we hypothesized that the islets of Langerhans might harbor stem cells identifiable with by their expression of the neural stem cell marker nestin. Here we report detection within the pancreatic islets a subset of cells that express the stem cell marker nestin.

MATERIALS AND METHODS

Isolation of mouse pancreas and islets. Mouse pancreata were removed from a 4-week-old male C57/B6 mouse (Charles River) and fixed in buffered formalin at 4°C overnight, before embedding in paraffin for sectioning. Mouse pancreatic islets were isolated according to reference [8] with minor modifications. In brief, pancreata of anesthetized 4-week-old male C57/B mice were injected with DNase I (2 mg/ml) and collagenase P (1 mg/ml) (Boehringer Ingelheim) with a syringe (22 gauge) before removal of the entire organ. After digestion at 37°C for approximately 20 min, the tissue was washed and centrifuged several times, resuspended in 10 ml Histopaque (Sigma), overlayed with Hanks balanced salt solution and centrifuged for 20 min at 4000*g*. After centrifugation islets from the interphase were hand-picked.

Reverse transcription (RT)–polymerase chain reaction (PCR). RNA was extracted with the TRIZOL reagent (GIBCO-BRL) according to the instructions of the manufacturer. Reverse transcription

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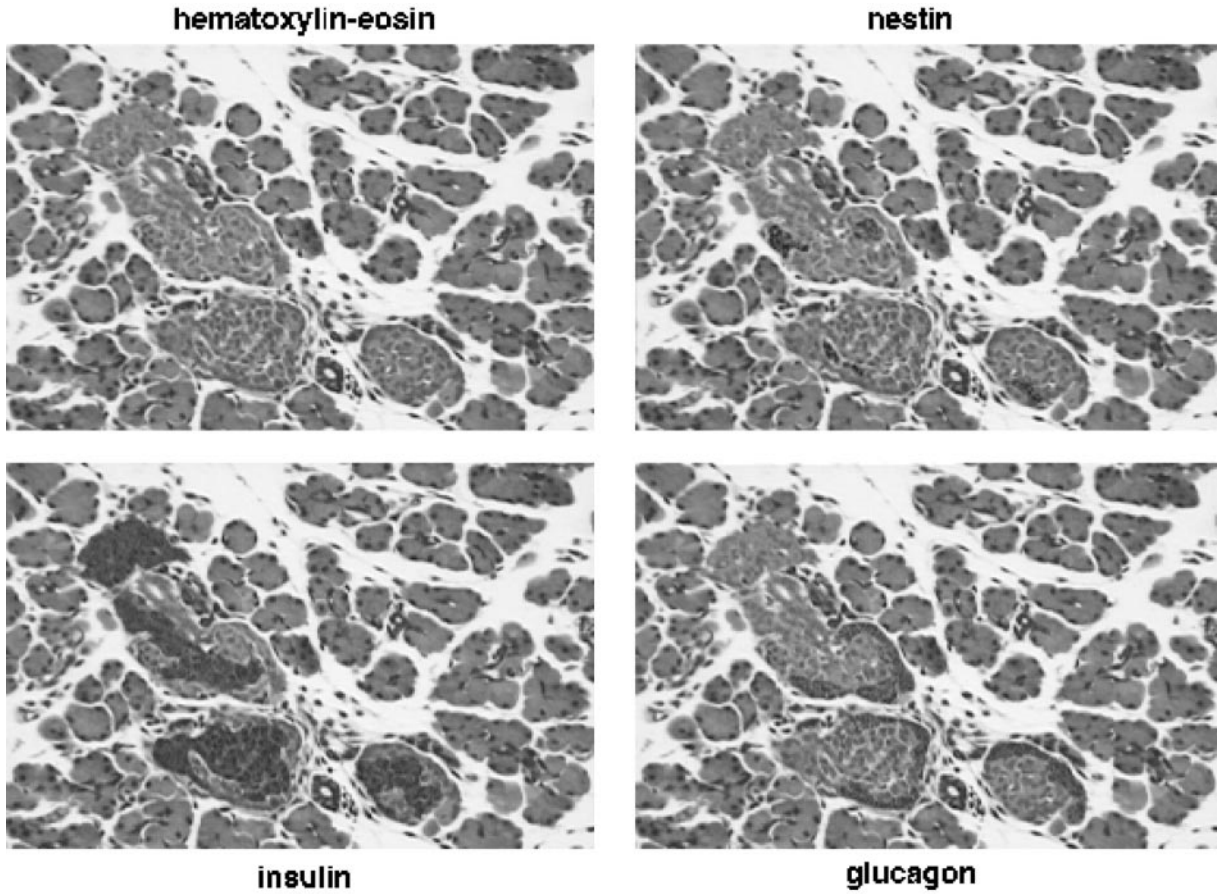

FIG. 1. *In situ* hybridization for insulin, glucagon, and nestin in mouse pancreas. A subset of insulin and glucagon-negative cells expresses nestin. Serial sections were hybridized with different probes. *In situ* results were overlayed digitally onto a hematoxylin–eosin stain for orientation.

was performed with Superscript kit (GIBCO-BRL). PCR was performed in an automated cycler using Andvantage 2 PCR kit (Clontech). The conditions were for all PCRs: 95°C for 1 min, 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s. Transcripts were T/A cloned (Invitrogen) and inserts were sequenced for verification. Primers used were β-tubulin (fw: 5′-TGGCCAGATCTTCAGACCAG-3′; rv: 5′-GTAAGTTCAGGCACAGTGAG-3′), insulin-I (fw 5′-TGCTGGTGCAGCACTGATC-3′; rv: 5′-CAGCCCTTAGTGACCAGCT-3′), glucagon (fw 5′-CCAGTTGATGAAGTCTCTGG-3′; rv 5′-ATTCACAGGGCACATTCACC-3′), and nestin (fw: 5′-TTCCCTTCCCCCTTGCCTAATACC-3′; rv: 5′-TGGGCTGAGCTGTTTTCTACTTTT-3′).

In situ hybridization. Sections (8 μm) of paraffin-embedded mouse pancreas were collected on slides and hybridized with ^{35}S-labeled antisense and sense (negative controls) riboprobes. *In situ* hybridization was performed essentially as in Ausubel *et al.* [9]. Probes for insulin, glucagon and nestin were generated by RT-PCR and T/A cloning (Invitrogen). Sense and inverse probes were generated by using the appropriate selection of the respective recombinant polymerase (T7 or SP6, Promega). Hybridization signal was detected with a Video Imaging Film MRF 34 Clear (Du Pont). Sense probes did not give any signal at all. A serial section adjacent to the hybridized sections was stained with hematoxylin–eosin (H/E) for orientation. Images of the *in situ* signals were digitally overlayed to the H/E section for orientation.

Immunohistochemistry. Paraffin-embedded tissue was sectioned at a thickness of 5 μM and collected on microscope slides. Immunohistochemical staining was performed on deparaffinized sections. Immunohistochemical staining was carried out using the Vector DAB Substrate Kit (Vector Laboratories, Burlingame CA). The anti-rat-nestin (1:500, Clone Rat-401, Developmental Hybridoma Bank, University of Iowa), guinea-pig anti-insulin (1:2000, Linco) and rabbit anti-glucagon (1:1000, Linco) were used as a primary antibodies. The sections were counterstained with H/E.

RESULTS

RT-PCR from mouse islets detected, as expected insulin and glucagon and, most importantly, nestin, which was confirmed by T/A cloning and sequencing. Sequences matched those deposited in GenBank (NCBI) for the respective cDNAs. Next we utilized the T/A cloned cDNA fragments of insulin, glucagon and nestin to perform in-situ hybridization of rat pancreatic tissue to localize the different transcripts. As shown in Fig. 1 insulin transcript localized generally in the center, whereas glucagon transcript was detected at the periphery of the islet. Surprisingly, nestin transcript in the islet was detected in cells, that were neither positive for insulin nor glucagon (Fig. 1). An ad-

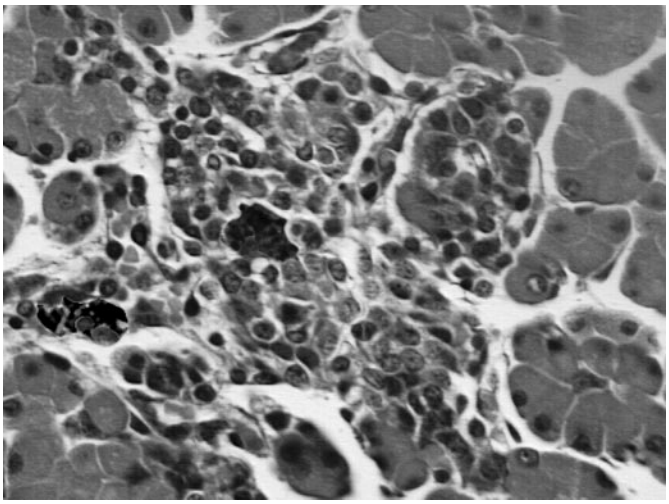

FIG. 2. Immunohistochemistry for nestin in mouse pancreas. A small subset of cells within the islet stain for nestin. These cells did not stain either for insulin nor for glucagon (not shown).

ditional *in situ* hybridization for somatostatin did not localize these nestin positive cells to pancreatic δ-cells (not shown). Sense probes did not yield any positive signal (not shown). Thus, the cells expressing nestin express neither insulin, glucagon, nor somatostatin. Immunohistochemistry to detect expression of nestin within the islets confirmed the findings of the *in situ* hybridization. Nestin was found in a subset of cells (Fig. 2) that did not express detectable insulin or glucagon (not shown). It is important to note that not all islet sections contained cells staining for nestin. Approximately 2–5% of all the islet sections reviewed (total of 100 sections) contained nesting staining cells. The figures shown in the present work are representative images of particularly good samples.

DISCUSSION

The present studies provide evidence that a subset of cells within the pancreatic islet express the intermediary filament nestin. Nestin is an intermediate filament protein that is the best available marker for brain stem cells [6, 10]. Cells that express nestin have been found at the ventricular border in mammalian brains [11]; and these cells give rise to neurons and glia in avian models [12]. More recently, nestin expressing cells have been shown to be ependymal cells [4]. The multipotent capacity of nestin expressing cells of the central nervous system has been demonstrated by the ability of such cells cultured *in vitro* to posses the ability to differentiate into different cells within the central nervous system, in skeletal muscle and in hematopoietic cells [7, 13].

Our findings suggest that a subset of cells within the pancreatic islet of 4-week-old mice express nestin. These cells do not express the main hormones, that are produced in the pancreatic islet. However, we have not tested whether these nestin expressing cells also express pancreatic polypeptide (PP). PP has been implicated to be expressed in cells which can further differentiate into α- and β-cells during development [14]. Further it must be mentioned that at in a 4 week old mouse the pancreatic islet cells have reported to still be in a phase of continued proliferation and architectural reorganization [15, 16]. Thus, the detection of cells in a phase of differentiation toward terminally differentiated, hormone-producing cells may be more likely than in an adult animal.

The turnover of β-cells approximately every 50 days in the rat [1]. Replacement of β-cells is considered to occur through proliferation of β-cells but also through neogenesis from precursor cells [2]. The nestin-expressing, hormone-negative cells might represent—at least in part—the population of cells, that provide the precursors for neogenesis of pancreatic endocrine cells. With the present work it is yet unclear whether these nestin-positive cells are generated within the pancreatic islets or whether they accumulate in the islets, being generated elsewhere in the organism. A recent report describes the isolation of pancreatic stem cells that could be cultured *in vitro* for prolonged periods and cured insulin-dependent diabetes in a mouse model [17]. Whether these cells express nestin remains as yet unclear.

In conclusion, this is the first description of nestin expressing cells in pancreatic islets in a subset of cells, which do not express detectable insulin, somatostatin or glucagon. Whether these cells are stem cells with a multipotent potential to differentiate into cells of different systems (i.e., bone marrow, cells of the CNS) or are precursor cells already committed to differentiate into pancreatic endocrine cells is currently being addressed. Isolation and further characterization of these cells may ultimately yield the possibility of generating pancreatic islet cells *in vitro.*

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