Stem Cell Marker Prominin-1/AC133 Is Expressed in Duct Cells of the Adult Human Pancreas

Jessy Lardon, PhD, Denis Corbeil, PhD,† Wieland B. Huttner, PhD, MD,‡ Zhidong Ling, PhD,§ and Luc Bouwens, PhD**

Objectives: Many efforts are spent in identifying stem cells in adult pancreas because these could provide a source of β cells for cell-based therapy of type 1 diabetes. Prominin-1, particularly its specific glycosylation-dependent AC133 epitope, is expressed on stem/progenitor cells of various human tissues and can be used to isolate them. We, therefore, examined its expression in adult human pancreas.

Methods: To detect prominin-1 protein, monoclonal antibody CD133/1 (AC133 clone), which recognizes the AC133 epitope, and the αhE2 antiserum, which is directed against the human prominin-1 polypeptide, were used. Prominin-1 RNA expression was analyzed by real-time polymerase chain reaction.

Results: We report that all duct-lining cells of the pancreas express prominin-1. Most notably, the cells that react with the αhE2 antiserum also react with the AC133 antibody. After isolation and culture of human exocrine cells, we found a relative increase in prominin-1 expression both at protein and RNA expression level, which can be explained by an enrichment of cells with ductal phenotype in these cultures.

Conclusions: Our data show that pancreatic duct cells express prominin-1 and surprisingly reveal that its particular AC133 epitope is not an exclusive stem and progenitor cell marker.

Key Words: pancreas, stem cells, AC133 epitope, prominin-1, duct, human

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Regulation of the pancreatic β-cell mass represents a critical issue in diabetes research.[1] Diabetes results from loss and/or dysfunction of islet β cells in the pancreas. The disease can be treated by islet cell transplantation, but this therapy is seriously hampered by the shortage of donor organs. Therefore, an important goal of diabetes research is to find additional sources of islet cells. Adult tissue stem cells could provide such a source but it remains a contentious issue whether stem cells reside in adult human pancreas. Stem cells are defined as clonogenic, self-renewing cells that can (re-)generate multiple types of differentiated progeny. It has been reported that cells that are clonogenic and self-renewing and have some degree of multipotency in differentiation can be isolated from rodent pancreas.[2–4] As for adult human pancreas tissue, it has been suggested that new insulin-producing islet cells can be generated in vitro from exocrine duct cells,[5,6] but it is not clear whether these cells represented stem cells. An alternative to stem cells as islet precursors is represented by dedifferentiated acinar or ductal exocrine cells that can transdifferentiate into β cells. The transdifferentiation capacity of acinar cells[7–9] makes it more difficult to interpret studies reporting neogenesis of islet cells from uncharacterized precursor cells.[10] It is generally considered that, in vivo, adult pancreatic stem cells reside in the exocrine ducts. However, a subset of duct cells that could represent stem cells has not yet been identified. In vitro, human pancreatic cytokeratin 19 (CK19)–expressing cells were reported to differentiate into islet cells.[6] Cytokeratin 19 is an intermediate filament protein that represents a good marker of human pancreatic duct cells[11] and that is also expressed by immature β cells in the fetal human pancreas.[12] However, duct-type cytokeratins are expressed by all cells lining the exocrine ducts and can also be expressed by dedifferentiated acinar cells.[9,13] Another candidate marker for pancreatic stem cells that may reside in rodent islets is the intermediate filament protein nestin.[14,15] However, nestin expression is also found in mesenchymal stellate cells and endothelial cells in the islets,[16] and because of this, it cannot be considered a prospective marker of pancreatic stem cells. The receptor for hepatocyte growth factor, c-met, was proposed as a marker for mouse pancreatic stem cells.[4] This receptor is also expressed by duct epithelial cells in the human pancreas.[17,18]

It must be concluded that the extensive search for a pancreatic stem cell has not yet revealed a way to identify stem cells that may reside in adult pancreas tissue (reviewed by Bonner-Weir and Sharma,[19] Zhang and Sarvetnick,[20] Holland et al,[21] and Street et al[22]). Finding

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From the *Cell Differentiation Unit, Diabetes Research Center, Vrije Universiteit Brussel, Brussels, Belgium; †Tissue Engineering Laboratories, BIOTEC, University of Technology Dresden, Dresden, Germany; ‡Max-Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; and §Cell Therapy Unit, Diabetes Research Center, Vrije Universiteit Brussel, Brussels, Belgium.

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Reprints: Luc Bouwens, PhD, Cell Differentiation Unit, Diabetes Research Center, Vrije Universiteit Brussel, Laarbeeklaan 103, B-1090 Brussels, Belgium (e-mail: lucbo@vub.ac.be).

a marker to immunohistochemically identify stem cells in pancreatic tissue, and preferably with which the stem cells could also be separated immunomagnetically or immunocytometrically, would represent an important step forward. For that reason, we studied the expression of prominin-1 in adult human pancreas.

Prominin-1 (alias CD133, AC133 antigen; for a nomenclature, see the work of Fargeas et al[23]) is a cholesterol-binding membrane glycoprotein that is localized in various plasma membrane protrusions.[24] Its precise physiological function is still unclear (for review, see Fargeas et al[25] and Jászai et al[26]). Human prominin-1 transcripts are found in various adult tissues, including the pancreas.[27,28] Prominin-1 protein is expressed in various differentiated cell types, including epithelial cells lining the kidney tubules, mammary gland lactiferous ducts, and epididymal duct.[28,29] It is also expressed in photoreceptor cells.[26] Several distinct glycoforms of prominin-1 have been described,[29] and a particular prominin-1 epitope recognized by the monoclonal antibody (mAb) AC133 appears structurally dependent on glysosylation.[27] Remarkably, this AC133 epitope has been detected exclusively in human somatic stem and progenitor cells, embryonic epithelia, and putative cancer stem cells so far,[30–32] leading to the wide use of mAb AC133 for the prospective identification and purification of prominin-1–positive stem and progenitor cells from distinct sources that include neural[33] and hematopoietic/endothelial[34,35] systems, prostate,[36] and kidney.[37]

We here report that the ductal cells of the human pancreas express prominin-1 and, surprisingly, its AC133 epitope.

MATERIALS AND METHODS

Sample Preparation and Cell Culture

Human pancreatic tissue samples were obtained from donor organs that were procured by transplant departments affiliated to Eurotransplant Foundation (Leiden, the Netherlands).[38] After collagenase digestion of the pancreas and Ficoll gradient centrifugation of the digest, the exocrine fraction consisting of 35% ductal cells and 55% acinar cells is recovered.[39] Cells were cultured in suspension for 4 to 7 days in RPMI-1640 medium (Invitrogen, Carlsbad, Calif) supplemented with 5% fetal bovine serum (Perbio, Erembodegem, Belgium) under 5% CO_2 atmosphere. The Ethical Committee of the Vrije Universiteit Brussel has approved the experiments.

Immunohistochemistry

For staining of the AC133 epitope (and combined stainings), pancreas samples were frozen in liquid nitrogen. Five-micrometer cryosections were fixed for 10 minutes in buffered 4% formaldehyde. For other stainings, tissue samples and cells were fixed in 4% buffered formaldehyde and embedded in paraffin. The indirect immunostaining method with fluorochrome-conjugated secondary antibodies was used.

The following primary antibodies were used: mouse mAbs CD133/1 (clone AC133) and CD133/2 (clone 293C3) (Miltenyi Biotec, Bergisch Gladbach, Germany) directed against the AC133 and AC141 epitopes of human prominin-1, respectively; rabbit αhE2 antiserum generated against human prominin-1 polypeptide[28]; rabbit polyclonal anti-carbonic anhydrase II (CA-II) and sheep polyclonal anti-CK19 (The Binding Site, Birmingham, UK).

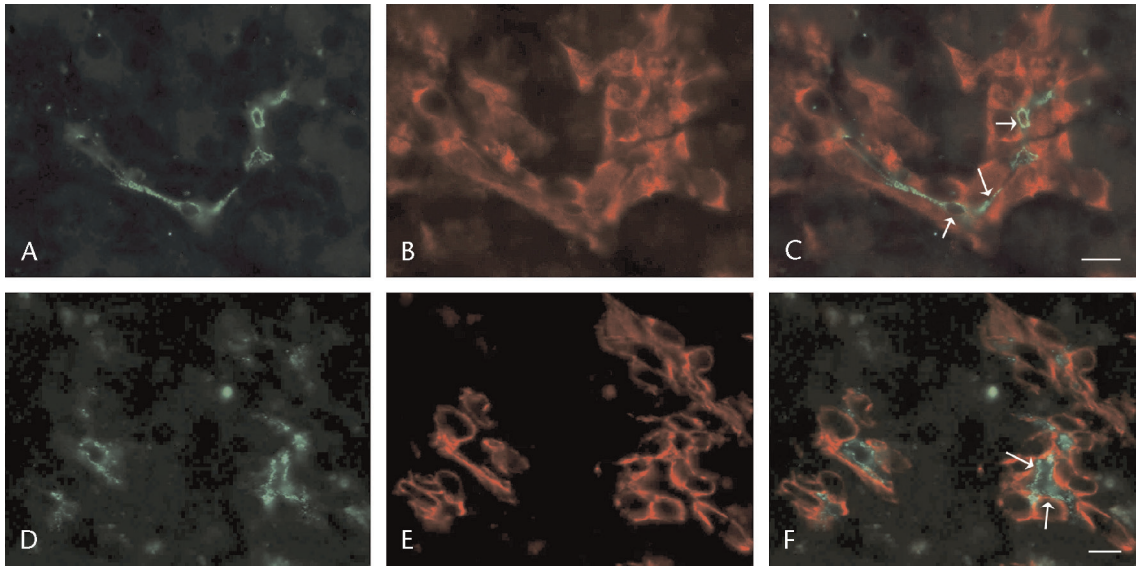

FIGURE 1. Expression of prominin-1 in the human adult pancreas. Pancreatic ducts were double-labeled for prominin-1 using either αhE2 antiserum (A) or mAb AC133 (D) and either CA-II (B) or CK19 (E) and analyzed by immunofluorescence microscopy. In the merged images (C, F), prominin-1 appears in green, whereas CA-II (C) or CK19 (F) is in red. Arrows show that the prominin-1 immunoreactivity is limited to the apical domain of all duct epithelial cells. Scale bar, 30 μm.

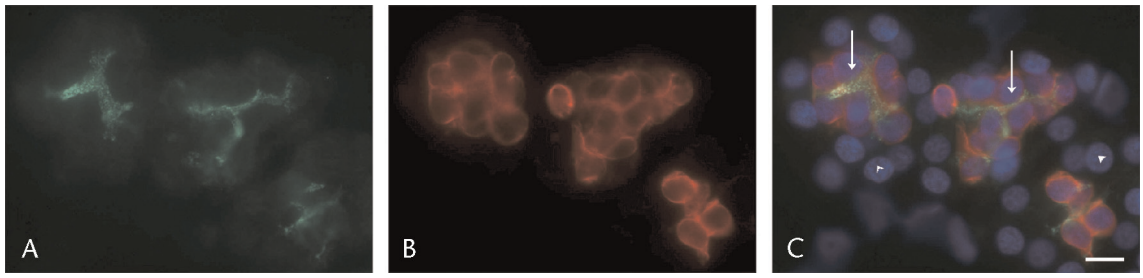

FIGURE 2. Immunofluorescence analysis of prominin-1 in freshly isolated human exocrine cells. A–C, The freshly isolated exocrine cells were double-labeled with prominin-1 using mAb AC133 (A, green) and CK19 (B, red) and analyzed by immunofluorescence microscopy. In the merged image (C), arrows show the ductal cells expressing prominin-1, whereas arrowheads indicate the acinar exocrine cells (CK19-negative cells). Nuclei were stained with DAPI (blue). Scale bar, 15 μm.

FITC- and TRITC-labeled anti-mouse, anti-sheep, and anti-rabbit antibodies (Jackson Immunoresearch, West Grove, Pa) were used as secondary antibodies.

All analyses were performed at least 3 times on different tissue samples.

Electron Microscopy

Cultured exocrine cells were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4), postfixed in 1% osmium tetroxide, stained with 2% uranyl acetate, dehydrated in a graded ethanol series, and embedded in Epon. Ultrathin sections were examined with a Philips Tecnai 10 electron microscope.

Real-time Polymerase Chain Reaction

Real-time polymerase chain reaction (RT-PCR) was performed to quantify the expression level of prominin-1 transcripts. Total RNA was isolated from cultured exocrine cells using the GenElute Mammalian Total RNA Miniprep kit (Sigma, St Louis, Mo). Complementary DNA was prepared from 500 ng of total RNA after DNase treatment and 10 ng of RNA equivalent used for PCR with specific primers (see below) in the presence of SYBR Green I. Polymerase chain reaction reagents were from Abgene (Epsom, UK). A melt curve analysis was performed at the end of each reaction. Values (mean ± SEM) are from 4 independent experiments. Expression levels were normalized to individual glyceraldehyde-3-phosphate dehydrogenase (GAPDH, internal control). Primers were designed to anneal to specific sequences of human prominin-1, 5′-GACCCATTGGCATTCTCTTT-3′ (forward) and 3′-CCCAGGACACAGCATAGAATAA-5′ (reverse), and GAPDH, 5′-ACAGTCAGCCGCATCTTCTTT-3′ (forward) and 3′-CGCCCAATACGACCAAAT-5′ (reverse). Statistical analysis was performed by analysis of variance.

RESULTS

Expression of Prominin-1 in Normal Pancreas Tissue

Normal pancreas tissue was subjected to an immunohistochemical analysis for the expression of prominin-1 using 2 distinct antibodies: the αhE2 antiserum, which recognizes the human prominin-1 polypeptide independently of its glycosylation profile,[28] and the mAb CD133/1 (AC133 clone; hereafter called mAb AC133), which reacts with the glycosylation-dependent AC133 epitope[27] that has been so far specific for undifferentiated cells. Interestingly, both antibodies revealed a prominin-1 immunoreactivity on the apical but not the basolateral side of all epithelial cells lining pancreatic ducts (Figs. 1C, F, see arrows). Both the small intercalated and the large ducts were labeled. (Another mAb directed against human prominin-1, CD133/2 [293C3 clone], gave the same results [data not shown], whereas no signal was detected when the anti–prominin-1 primary antibody was omitted [data not shown].) Double immunostaining for the duct cell markers CK19 (Fig. 1B) or CA-II (Fig. 1E) confirmed the duct cell nature of the prominin-1–expressing cells

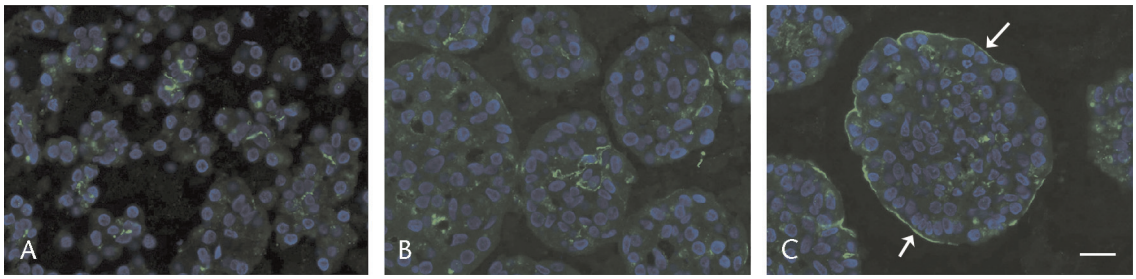

FIGURE 3. In vitro cultured human exocrine cells expressed prominin-1. Freshly isolated exocrine cells (A) and those cultured for 4 (B) or 7 (C) days were immunolabeled for prominin-1 using αhE2 antiserum (green). Nuclei were stained with DAPI (blue). In 7-day-old cultured duct cells, prominin-1 immunoreactivity is enriched at the outer surface of the cell aggregate (arrows). Scale bar, 15 μm.

(Figs. 1C, F). No prominin-1 staining was detected in endocrine cells in the islets of Langerhans, acinar exocrine cells, and connective tissue (data not shown). Human kidney samples were used as a positive control for stainings with the αhE2 antiserum.

Similar results were obtained after staining for prominin-1 in the mouse pancreas (J. Jászai, DVM and D. Corbeil, PhD, unpublished data, September 2006).

Expression of Prominin-1 in Primary Duct Cells

The nonendocrine cell fraction obtained after islet isolation from pancreas tissue was cultured for up to 7 days in suspension. We followed the expression of prominin-1 in this fraction during the culture period to investigate whether the increase in amount of ductal cells is concordant with an increase in prominin-1 expression.

Prominin-1 staining was seen in a fraction of the cells that coexpressed duct cell markers, as was shown with the mAb AC133 (Fig. 2, arrows), and that increased in number with time of culture. This relative increase in prominin-1–expressing cells (as is shown in Fig. 3 with the αhE2 antiserum) most likely resulted from the better survival of duct cells compared to the acinar cells, and possibly from transdifferentiation of some acinar cells into ductlike cells. At day 7 of culture, prominin-1 immunoreactivity was expressed mainly at the outer surface of the aggregates (Fig. 3C, arrows). In agreement with the exclusive subcellular localization of prominin-1 in microvilli,[24,40] transmission electron microscopy revealed that the epithelial cells at the outer surface of the aggregates contained numerous microvilli (Fig. 4).

Finally, expression of prominin-1 in the exocrine pancreas fraction was confirmed by RT-PCR analysis, showing an increase of more than 2-fold in the relative prominin-1 messenger RNA (mRNA) level after 1 week of culture (Fig. 5; $P < 0.05$).

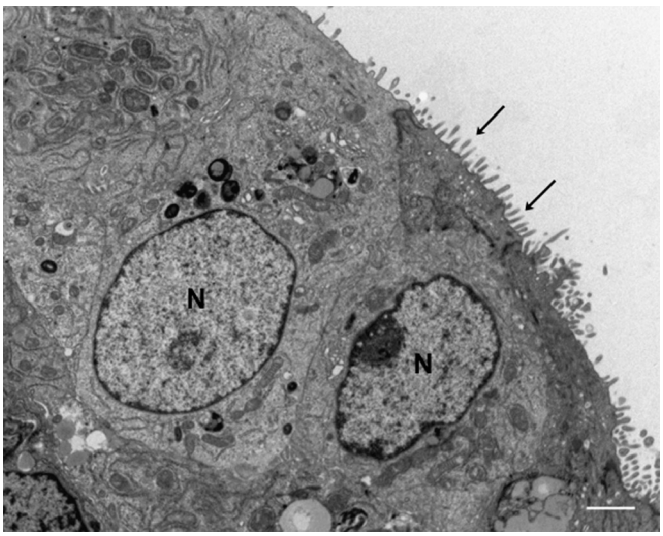

FIGURE 4. Electron micrograph of an aggregate of human exocrine cells with numerous microvilli at the outer surface after a culture period of 4 days. Arrow indicates microvillus; N, nucleus. Scale bar, 2 μm.

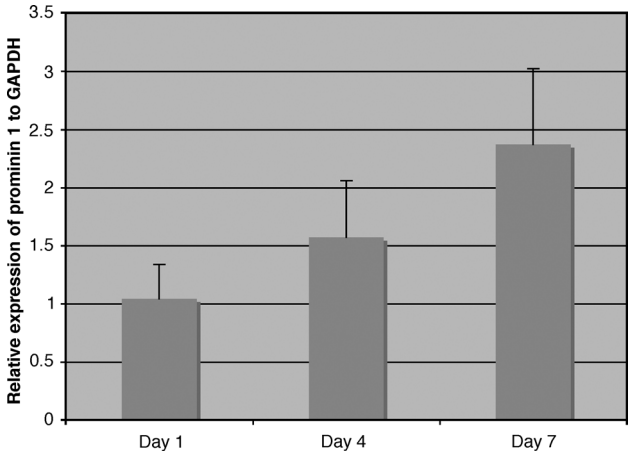

FIGURE 5. Prominin-1 transcripts are up-regulated upon cultivation of exocrine pancreatic cells. Expression profile of prominin-1 transcripts was determined by RT-PCR and normalized to GAPDH. Histogram bars correspond to culture days 1, 4, and 7, respectively. Values represent the mean ± SEM of 4 independent experiments ($P < 0.05$).

DISCUSSION

Several studies had reported the expression of prominin-1 mRNA in normal adult human pancreas[27,28,41] and in ductal cells from pancreatic juice of patients with pancreatic ductal carcinoma,[42] but evidence of the localization of the protein in the human pancreas was lacking. We here report that prominin-1 protein is actually present in ductal epithelial cells all along the pancreatic ducts. As expected, the protein was located exclusively at the apical membrane of the epithelial cells, facing the lumen. Most notably, and in contrast to other adult epithelia such as kidney,[28] the cells that react with the αhE2 antiserum also displayed the AC133 epitope, which had been reported to be restricted so far to stem and progenitor cells and to developing epithelia in normal individuals[27,28,32,34] and was, therefore, considered a stem cell–specific marker. It appears here that the AC133-positive pancreatic duct cells are fully specialized cells as they do express CA-II and cannot represent an undifferentiated stem cell population. Interestingly, it has been hypothesized that mature duct cells can transiently regain a less differentiated phenotype and, thereby, serve as a multipotent progenitor in conditions of tissue injury and regeneration. This type of progenitor was named functional or facultative stem cells.[19,43] In fact, this type of regeneration represents transdifferentiation (ie, a conversion of one differentiated cell type into another).[9,44] Both ductal cells and acinar exocrine cells in rodents can dedifferentiate and reexpress embryonic characteristics like the transcription factor Pdx1.[13,43] Acinar cells can transdifferentiate to insulin-producing β cells[8] or to hepatocyte-like cells.[45] Transdifferentiation, however, is clearly distinct from the derivation of cells from true and undifferentiated stem cells.

The relative increase with time of prominin-1 mRNA expression in primary cell culture of nonendocrine pancreas cells can be explained by an enrichment of cells with ductal phenotype in these cultures. After a few days of culture, acinar cells die, whereas duct cells selectively survive and, hence, reach a frequency greater than 90%.[39,46] In multicellular aggregates with ductal phenotype, prominin-1 was most strongly expressed at the outer surface of these aggregates.

In a previous study, Miraglia et al[27] failed to detect immunoreactivity for AC133 in paraffin sections from pancreas, despite a strong prominin mRNA level. The apparent discrepancy with our study is likely to be caused by differences in tissue processing; indeed, we could not obtain reproducible immunostaining with this antibody on paraffin sections, although it worked very well on cryosections. However, in agreement with our results, 2 recently published studies show flow cytometric sorting of ductal cells from the mouse fetal, neonatal, and adult pancreas using CD133 antibody.[47,48]

It has been estimated that between 0.02% and 0.03% of murine pancreatic islet and duct cells are clonogenic and could represent stem and progenitor cells.[3,4] In the human pancreas, approximately 30% of the cells are ductal cells[11] and express prominin-1. We conclude that the AC133 epitope is not an exclusive stem and progenitor cell marker but that it may also be expressed by certain differentiated cells, such as the pancreatic duct cells. Hence, in the pancreas, prominin-1 cannot be used as a simple stem cell marker.

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