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Cells with surface expression of $CD133^{high}CD71^{low}$ are enriched for tripotent colony-forming progenitor cells in the adult murine pancreas☆

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ABSTRACT

Progenitor cells in the adult pancreas are potential sources of endocrine beta cells for treating type 1 diabetes. Previously, we identified tri-potent progenitor cells in the adult (2–4 month-old) murine pancreas that were capable of self-renewal and differentiation into duct, acinar, and endocrine cells *in vitro*. These progenitor cells were named pancreatic colony-forming units (PCFUs). However, because PCFUs are a minor population in the pancreas (~1%) they are difficult to study. To enrich PCFUs, strategies using cell-surface marker analyses and fluorescence-activated cell sorting were developed. We found that $CD133^{high}CD71^{low}$ cells, but not other cell populations, enriched PCFUs by up to 30 fold compared to the unsorted cells. $CD133^{high}CD71^{low}$ cells generated primary, secondary, and subsequent colonies when serially re-plated in Matrigel-containing cultures, suggesting self-renewal abilities. In the presence of a laminin hydrogel, $CD133^{high}CD71^{low}$ cells gave rise to colonies that contained duct, acinar, and $Insulin^{+}$ $Glucagon^{+}$ double-hormonal endocrine cells. Colonies from the laminin hydrogel culture were implanted into diabetic mice, and five weeks later duct, acinar, and $Insulin^{+}$ $Glucagon^{-}$ cells were detected in the grafts, demonstrating tri-lineage differentiation potential of $CD133^{high}CD71^{low}$ cells. These $CD133^{high}CD71^{low}$ cells will enable future studies of putative adult pancreas stem cells *in vivo*.

1. Introduction

The pancreas plays a critical role in regulating metabolism and is composed of three major cell lineages: acinar cells, which secrete digestive enzymes such as amylase; duct cells, which transport digestive enzymes into the gut and secrete mucin to fend off pathogens; and endocrine cells, which secrete hormones such as insulin and glucagon that are important for glucose homeostasis.

Pancreas development follows a sequence of events regulated by transcription factors. Around embryonic day (E) 8.5, murine pancreatic endoderm expresses critical transcription factors, such as pancreas duodenal homeobox (Pdx) 1 and Sox9. Between E8.5 and E12.5, $Pdx1^{+}$ and $Sox9^{+}$ ductal cells are multi-potential and capable of giving rise to all three lineages in the adult pancreas (Gu et al., 2002; Zhou et al., 2007; Kopp et al., 2011). After E12.5, the lineage potential of the ductal epithelium becomes restricted (Gu et al., 2002; Zhou et al., 2007; Kopp et al., 2011), and the up-regulation of another key transcription factor,

Abbreviation: PCFU, pancreatic colony-forming unit; CD, cluster of differentiation; R2, region 2 cells.

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neurogenin (Ngn) 3 is necessary for subsequent differentiation into endocrine cells (Apelqvist et al., 1999; Gradwohl et al., 2000).

There has been an intense debate about whether the adult pancreas harbors stem and progenitor cells that can give rise to new insulin-producing beta cells. Because of their roles as progenitors in the early embryo, duct cells in the adult pancreas have long been the assumed source of new beta cells (Bonner-Weir et al., 2004). However, in mouse models using Cre-Lox lineage-tracing techniques, contradictory results either positively (Inada et al., 2008; Xu et al., 2008) or negatively (Kopp et al., 2011; Dor et al., 2004; Furuyama et al., 2011; Kopinke &

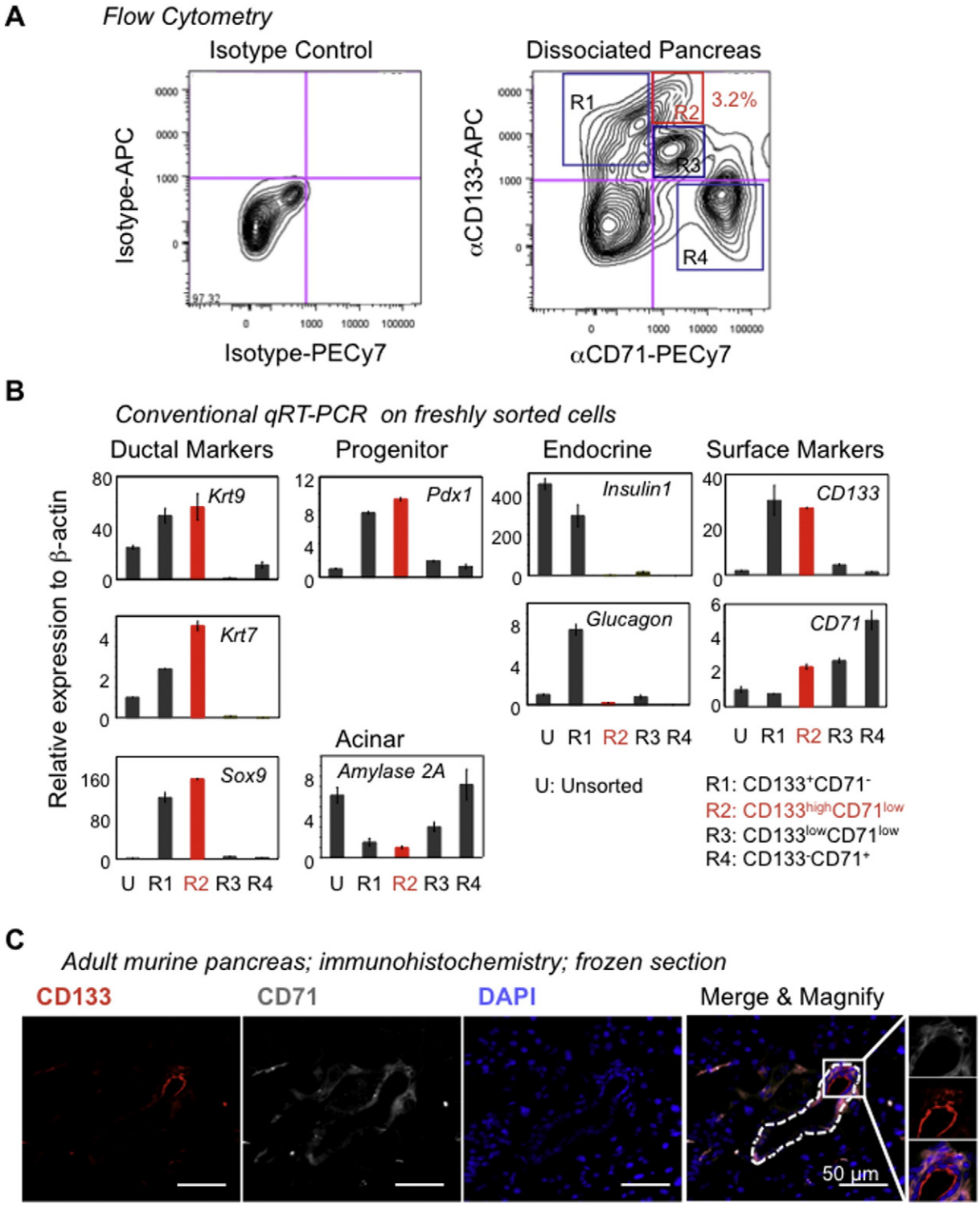

Fig. 1. CD133 and CD71 are expressed in the adult murine pancreas. (A) Flow cytometry analyses of dissociated pancreatic cells stained with antibodies against CD133 and CD71. CD71 subfractionates CD133-expressing ductal cells, suggesting heterogeneity of ductal cells. (B) Regions (R) 1 to 4 indicated in (A) were analyzed for gene expression by conventional qRT-PCR. (C) Double immunohistochemical staining on frozen sections showed that CD133 and CD71 expressions were located mostly in the ductal structures (outlined by a dotted line) in the adult murine pancreas. (D) Immunohistochemical staining on adjacent slides prepared from formalin-fixed, paraffin-embedded pancreas. Both primary antibodies for Sox9 and CD71 were rabbit antibodies. Results showed that Sox9^{+} ductal regions were also positive for CD71 staining. (E) Double immunostaining on frozen sections showed that Pdx1 protein was expressed in the CD133^{+} ductal cells with various intensities. The yellow arrow indicates a CD133^{+} duct cell expressing a higher level of Pdx1 than other duct cells. (F) Representative genes identified from RNA-seq analyses which are significantly up-regulated in R2 versus R1 cells.