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PCSK9-deficient mice exhibit impaired glucose tolerance and pancreatic islet abnormalities

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ABSTRACT

Proprotein convertase subtilisin/kexin type 9 (PCSK9), a liver-secreted plasma enzyme, restricts hepatic uptake of low-density lipoprotein (LDL) cholesterol by promoting the degradation of LDL receptors (LDLR). PCSK9 and LDLR are also expressed in insulin-producing pancreatic islet β cells, possibly affecting the function of these cells. Here we show that, compared to control mice, PCSK9-null male mice over 4 months of age carried more LDLR and less insulin in their pancreas; they were hypoinsulinemic, hyperglycemic and glucose-intolerant; their islets exhibited signs of malformation, apoptosis and inflammation. Collectively, these observations suggest that PCSK9 may be necessary for the normal function of pancreatic islets.

1. Introduction

Proprotein convertase subtilisin/kexin type 9 (PCSK9) belongs to a 9-member family of serine endoproteinases, structurally related to bacterial subtilisin and to yeast kexin. These enzymes are found in the secretory pathway of all cells, in varying combinations and levels. Collectively, they are responsible for the activation of a wide variety of precursor proteins by endoproteolysis after selected residues [1]. PCSK9 is predominantly produced and secreted by the liver [2,3]. It has no known physiological substrate.

The most studied property of PCSK9 is its ability to down regulate hepatic uptake of low-density lipoprotein-cholesterol (LDL-C) by promoting intracellular degradation of the LDL receptor (LDLR). According to the current prevailing model, the convertase attaches to the receptor and directs it to endosomes/lysosome-like compartments for degradation [4–6]. Inactivation of the *Pcsk9* gene in mouse increases hepatic LDLR content and the clearance of plasma LDL-C, leading to hypocholesterolemia [3,7]. Its overexpression in the liver of transgenic mice has the opposite effect [3,8–12].

Pancreatic β cells express significant amounts of LDLR [13,14]. These receptors can mediate the uptake of exogenous lipoproteins by established β cell lines and isolated islets in culture. Chronic exposure to high LDL or VLDL is lethal for these cells [14,15]. Death occurs through endogenous heavy metal-catalyzed oxidative stress since it can be abrogated by chelating agents and antioxidants [15]. A survey of established cell lines indicated that RIN-m5F and βTC-3 lines derived from insulin-producing pancreatic β cells contained substantial amounts of PCSK9 mRNA [2,3]. We hypothesized that PCSK9 protects these cells from the toxic effects of excessive cholesterol accumulation. Comparing wild type and PCSK9-null mice, we show in this report that male mutant mice exhibit impaired glucose homeostasis.

2. Materials and methods

2.1. Mice

129Sv;C57BL/6 (129;B6) *Pcsk9*$^{+/-}$ male mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and were backcrossed with B6 *Pcsk9*$^{+/+}$ females for eight generations. The B6-N8 *Pcsk9*$^{+/-}$ incipient congenic mice were then intercrossed to

Abbreviations: IB, immunoblotting; IP, immunoprecipitation; LDL, low-density lipoprotein; LDL-C, LDL-cholesterol; LDLR, LDL receptor; OGTT, oral glucose tolerance test; qRT-PCR, quantitative reverse transcriptase-polymerase chain reaction; PCSK9, proprotein convertase subtilisin/kexin type 9; sqIB, semi-quantitative immunoblotting; TBP, TATA-box binding protein

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generate *Pcsk9*$^{-/-}$ and *Pcsk9*$^{+/+}$ mice used in these studies at 4–5 months of age. Mice were treated according to guidelines of the Canadian Council on Animal Care under a protocol approved by an institutional Animal Care Committee. They were housed in temperature-controlled (25 °C) rooms with a 12-h light/dark cycle and given access to standard mouse chow and water *ad libitum*, except when overnight fasting (12–16 h) was required.

2.2. Oral glucose tolerance test (OGTT)

Mice were fasted overnight, weighed and blood (~0.05 ml) was collected by submandibular puncture and supplemented with EDTA to a final concentration of 10 mM. They were then fed glucose (1.5 mg/g body weight) by oral gavage and were bled as above after 15, 30, 60 and 120 min. Blood samples were centrifuged for 10 min at 1100×*g* to sediment cells; plasma was collected and stored at −80 °C until analysis.

2.3. Pancreatic islet isolation and culture

Mouse pancreatic islets were isolated following ductal injection of collagenase buffer (1.5 mg/ml) [16,17]. Briefly, mice were anesthetized and sacrificed by cervical dislocation; pancreata were injected with a collagenase solution; islets were handpicked under a microscope.

2.4. Glucose and insulin and lipid assays

Glucose levels were determined using the Beckman Coulter glucose analyzer and insulin levels were measured using the Ultra Sensitive Mouse Insulin ELISA Kit from Crystal Inc. (Downers Grove, IL).

2.5. Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR)

The levels of specific mRNAs were quantified in a PCR-based fluorogenic assay using the Taqman technology [18]. Briefly, total RNA was extracted using the RNeasy extraction kit from Qiagen (Mississauga, ON) and reverse-transcribed into cDNA using random hexameric primers and the Superscript II RNase H$^-$ Reverse Transcriptase (Invitrogen). The cDNA was used as a template to produce PCR amplicons using FastStart TaqMan ProbeMaster-Rox master mix, primer pairs and the appropriate fluorescent probe from the Universal Probe Library (UPL) (Roche, Laval, QC) (Supplementary Table 1S) in a Mx3005P thermocycler (Stratagene, LaJolla,

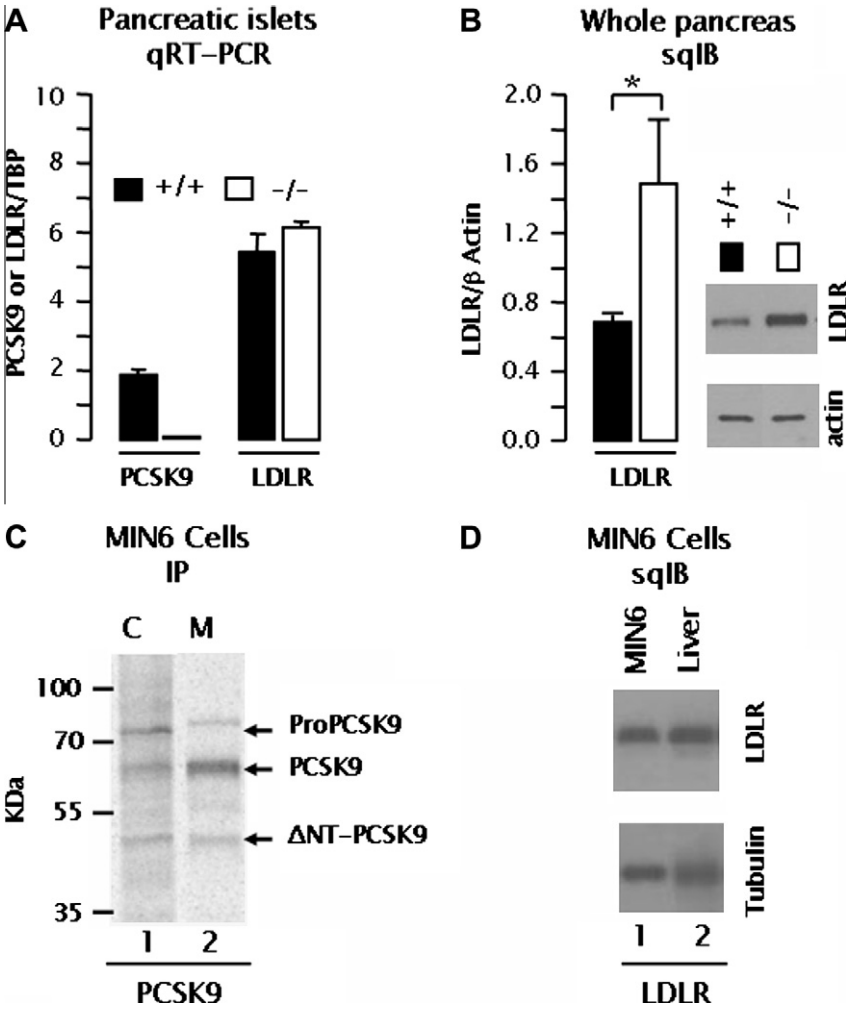

Fig. 1. Mouse pancreatic β cells express PCSK9 and LDLR. *Pcsk9*$^{+/+}$ or *Pcsk9*$^{-/-}$ male mice were used (n = 4/genotype). (A) Isolated pancreatic islets were used as the source of RNA for qRT-PCR for PCSK9 and LDLR and TBP mRNAs, the latter serving as normalizing internal control. (B) Whole pancreas extracts (n = 4 mice/genotype) were analyzed by sqIB for LDLR (120 kDa) and β actin (35 kDa), the latter serving as a normalizing internal control. *Pcsk9*$^{-/-}$ mice contained twofold ($P < 0.05$) more LRLR protein in their pancreas than the *Pcsk9*$^{+/+}$ mice. (C) MIN6 cells were metabolically labeled with [^{35}S]-Met/Cys for 6 h. The cell lysate and spent medium were analyzed by IP of PCSK9-related forms. (D) MIN6 cell lysates and liver extracts from *Pcsk9*$^{+/+}$ or *Pcsk9*$^{-/-}$ mice were analyzed by sqIB for LDLR (120 kDa) and α-tubulin (55 kDa). Note the increase in the receptor level in hepatic extracts from null mice.