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Growth Factors, Cytokines, Cell Cycle Molecules

β-Cell-Specific Ablation of the Hepatocyte Growth Factor Receptor Results in Reduced Islet Size, Impaired Insulin Secretion, and Glucose Intolerance

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Hepatocyte growth factor (HGF) and its c-met receptor consist of a paired signaling system that has been implicated in the regulation of pancreatic β-cell survival, proliferation, and function. To define the role of HGF/c-met signaling in β-cell biology *in vivo*, we have generated conditional knockout mice in which the c-met receptor gene was specifically inactivated in pancreatic β cells by the Cre-loxP system. Mice with β-cell-specific deletion of the c-met receptor (*βmet*$^{-/-}$) displayed slight growth retardation, mild hyperglycemia, and decreased serum insulin levels at 6 months of age when compared with their control littermates. Deficiency of the c-met receptor in β cells resulted in a complete loss of acute-phase insulin secretion in response to glucose and an impaired glucose tolerance. Glucose transporter-2 expression was down-regulated in the β cells of *βmet*$^{-/-}$ mice. Compared to controls, *βmet*$^{-/-}$ mice exhibited reduced islet size and decreased insulin content in the pancreas, but displayed normal islet morphology. Therefore, HGF/c-met signaling plays an imperative role in controlling islet growth, in regulating β-cell function, and in maintaining glucose homeostasis. *(Am J Pathol 2005, 167:429–436)*

Dysregulation of the β-cell mass and differentiated function in the pancreatic islets plays an important role in the pathogenesis of diabetes, a devastating disease that afflicts more than 100 million people worldwide.[1,2] Despite that the regulation of β-cell survival, proliferation, and function *in vivo* remains elusive, numerous studies have implicated peptide growth factors as key players in controlling β-cell development and function in the pancreatic islets.[3–5]

Hepatocyte growth factor (HGF) is a pleiotropic factor that plays an essential role in the regulation of proliferation, survival, migration, and differentiation in many kinds of cells.[3,6,7] These biological activities of HGF are mediated by a single specific receptor c-met, a transmembrane receptor tyrosine kinase encoded by *c-met* proto-oncogene.[8,9] In pancreatic islets, both HGF and its c-met receptor are expressed during islet development and in adult tissues.[10,11] Earlier studies from our laboratory and others have demonstrated that the delivery of exogenous HGF gene could preserve β-cell mass and mitigates hyperglycemia induced by streptozotocin.[4,12] Such beneficial effects of HGF are primarily mediated by preventing β-cell depletion through inhibition of apoptosis and by promoting β-cell proliferation.[4,13] Consistently, transgenic mice overexpressing exogenous HGF in pancreatic β cells show an increased insulin production and prevention from the genesis of diabetes mellitus after injury.[13,14] However, although these findings have implicated HGF as a regulator in controlling β-cell growth and survival under pathological conditions, the role of HGF signaling in β-cell biology and glucose homeostasis in the normal physiological setting remains primarily unknown.

Earlier studies indicate that the mice globally lacking either HGF or c-met by conventional knockout approach died before birth, owing to severe developmental defects in placenta and liver.[15–17] This precludes a comprehensive analysis of the role of HGF signaling in β-cell development and function. In the present study, we have generated a conditional knockout mouse model in which c-met receptor gene is specifically inactivated in pancre-

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atic β cells by using the Cre-loxP system. Mice lacking c-met receptor in a β-cell-specific manner show slight growth retardation, mild hyperglycemia, and decreased serum insulin levels. More interestingly, β-cell-specific ablation of c-met receptor leads to reduced expression of glucose transporter-2 protein in β cells, which results in complete loss of acute-phase insulin secretion in response to glucose, but not to L-arginine, and consequently causes glucose intolerance. In addition, these mice exhibit reduced islet size and decreased insulin content in pancreas. Thus, these studies provide direct evidence for a critical role of HGF signaling in regulating β-cell growth and function *in vivo*.

Experimental Procedures

Mice and Genotyping

The c-met floxed mice were created by homologous recombination using a c-met gene fragment with loxP sites flanking exon 16, as described elsewhere.[18] Transgenic mice expressing Cre recombinase under the control of a β-cell-specific rat insulin 2 promoter (Rip-Cre mice) were obtained from the Jackson Laboratory (Bar Harbor, ME). These mice were created using a fusion gene construct made of 668 bp of the rat insulin 2 promoter, nuclear localization sequence-modified Cre, and a fragment of the human growth hormone gene.[19,20] The mice were derived from a C57BL/6J genetic background. β-Cell-specific inactivation of c-met was achieved by cross-breeding Rip-Cre transgenic mice with mice carrying the c-met floxed gene. In all of the experiments, littermates from the same breeding pair were used as controls. Polymerase chain reaction (PCR) was used for c-met loxP and Cre genotyping. All mice with ages between 6 to 8 months were used in the study.

Blood Glucose and Insulin Detection

Blood glucose level was determined by the Accucheck active glucometer and test strips (Roche Diagnostic, Indianapolis, IN). For insulin detection, venous blood was collected in chilled heparinized tubes, immediately centrifuged, and the plasma stored at −80°C. Insulin levels were measured with ultrasensitive mouse insulin detection enzyme-linked immunosorbent assay kit (Crystal Chem Inc., Chicago, IL) with purified mouse insulin as a standard, as described previously.[4]

Intraperitoneal Glucose Tolerance Test

Mice were fasted for 24 hours and blood glucose was detected immediately before and 30, 60, 90, and 120 minutes after intraperitoneal injection of glucose at a concentration of 2 g/kg body weight. Serum was also collected before and 30 minutes after glucose for the insulin detection.

Insulin Sensitivity Test and Acute Insulin Release Test

In mice on a normal diet, blood glucose was detected before and 15, 30, and 60 minutes after intraperitoneal injection of synthesized porcine insulin (0.75 U/kg body weight). For the acute insulin release test, mice were fasted for 24 hours and injected intraperitoneally with glucose at 3 g/kg body weight or L-arginine (10 mmol/L). At 0, 2, 5, and 10 minutes after injection, blood was collected and serum insulin levels were measured with an ultrasensitive mouse insulin detection enzyme-linked immunosorbent assay kit.

Immunohistochemisty and Morphometric Analysis

Indirect immunofluorescence and immunohistochemical staining was performed using an established procedure as described previously.[4,21] Briefly, pancreatic cryosections or paraffin sections were prepared and stained with the specific primary antibodies using the Vector M.O.M. immunodetection kit according to the protocols specified by the manufacturer (Vector Laboratories, Burlingame, CA).[4] The primary antibodies used were as follows: Cre recombinase (Covance Inc., Denver, PA) c-met (Santa Cruz Biotechnology, Santa Cruz, CA), insulin (clone 2D11-H5; Ventana Medical Systems Inc., Tucson, AZ), Glut-2, glucagon (A0565), and somatostatin (A0566) (DAKO, Carpinteria, CA).

A computer-aided morphometric analysis was used for determining islet size and number. Briefly, a series of digital images was captured from the pancreatic sections stained with insulin. The areas of all islets and pancreas were measured by using morphometric analysis software (MetaMorph; Universal Imaging Corp., Downingtown, PA). The percentage of the islet area per pancreas area was calculated based on each individual animal, 8 animals per group ($n = 8$). For analyzing total islet number and islet size distribution, all islets in a given pancreas section were counted, and the islet size was estimated by counting insulin-positive cell number per islet. Pancreas size was measured from digital images by using morphometric analysis software. Data were expressed as islet numbers per 10-mm^2 pancreas area ($n = 8$). Cellular composition of the islets was estimated by counting the ratio of glucagon- or somatostatin-positive cells per insulin-positive cells.

Western Blotting

Mouse pancreatic islets were isolated by collagenase digestion followed by separation on Ficoll-Conray gradients, according to the procedure described previously.[4,13] The isolated islets were incubated with human recombinant HGF (20 ng/ml) (provided by Genentech, Inc., South San Francisco, CA) for various periods of time as indicated. The islets were then homogenized and protein concentration was determined using a bicinchoninic-acid protein assay kit (Sigma, St. Louis, MO). Pro-

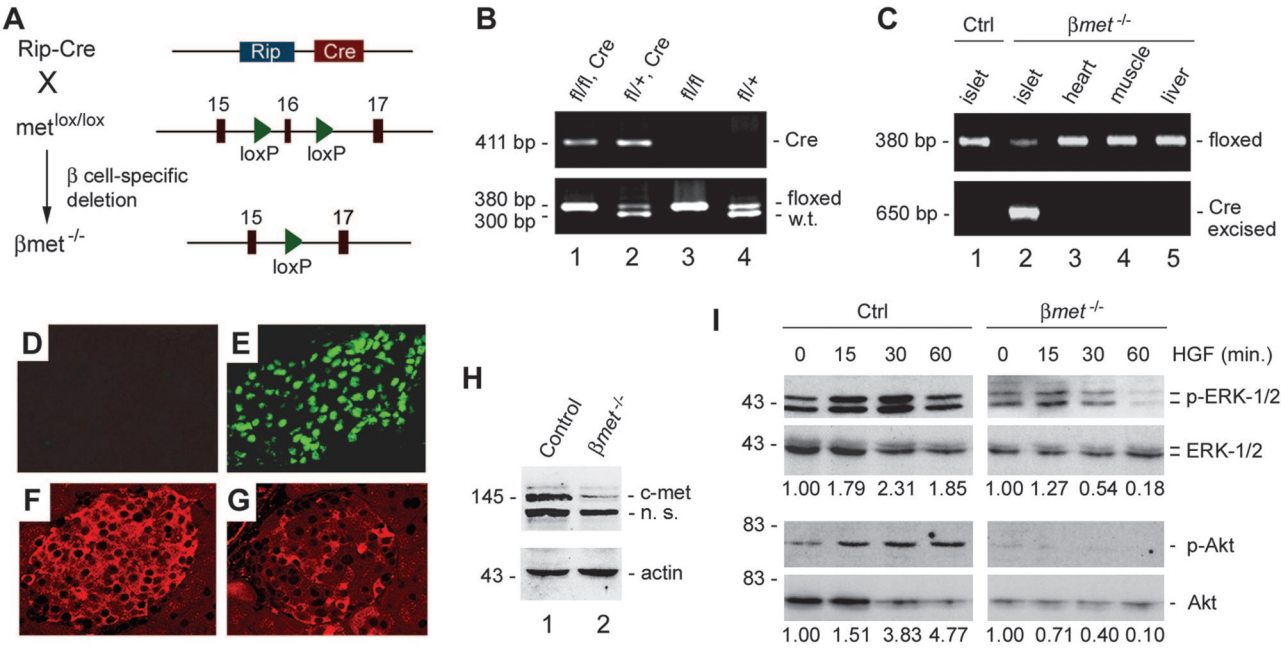

Figure 1. Tissue-specific ablation of the HGF receptor (c-met) in pancreatic β cells. **A:** Diagram illustrates the strategy of generating β-cell-specific c-met knockout mice. Shown is the deletion of exon 16 in the event of recombination of the c-met gene. **B:** Representative picture shows PCR analysis of the genomic DNA from tail clippings. The PCR bands of wild-type (300 bp), floxed (380 bp), and Cre (411 bp) are indicated. Genotypes of representative litters are indicated. fl, c-met floxed. **C:** Cre-mediated excision of the floxed c-met allele in a tissue-specific manner. DNA was isolated from various tissues of the control ($met^{lox/lox}$) and $\beta met^{-/-}$ ($met^{lox/lox}$, Cre) mice and analyzed by PCR with appropriate primers. A band after Cre excision was only observed in the isolated islets of $\beta met^{-/-}$ mice (**lane 2**). **D** to **G:** Immunofluorescence staining demonstrates c-met and Cre expression in pancreatic islets of the control and $\beta met^{-/-}$ mice. **D** and **E**, Cre (green); **F** and **G**, c-met (red); **D** and **F**, controls; **E** and **G**, $\beta met^{-/-}$ mice. The location of islets in pancreatic sections was confirmed by staining cell nuclei with PI (data not shown). **H:** Western blot analysis of c-met protein expression in the islets isolated from the control and $\beta met^{-/-}$ mice. Islet lysates were immunoblotted with antibodies against c-met and actin, respectively. The lower band (below 145 kd) is nonspecific (n. s.). **I:** Western blot analysis shows the activation of Erk-1/2 and Akt in the isolated islets in response to HGF stimulation. Pancreatic islets were isolated from the control and $\beta met^{-/-}$ mice, pooled from five different animals for each group and incubated with 20 ng/ml of HGF for various periods of time as indicated. Islet lysates were immunoblotted with antibodies against p-Erk-1/2, total Erk-1/2, p-Akt, and total Akt, respectively. Relative abundances (fold induction over 0 time point) of p-Erk1/2 and p-Akt after normalization are presented at the **bottom** of Western blot pictures.

tein expression in the isolated islets was analyzed by Western blot analysis according to the procedures described elsewhere.[4] The primary antibodies used were as follows: phospho-Erk-1/2, phospho-Akt, and total Akt (Cell Signaling Technology, Inc., Beverly, MA), Erk-1/2 (Sigma), c-met, Glut-2, and actin (Santa Cruz Biotechnology).

Reverse Transcriptase (RT)-PCR

For determination of insulin and Glut-2 mRNA expression, a semiquantitative RT-PCR was used.[22] Total RNA was prepared from the isolated pancreatic islets. After reverse transcription of the RNA, cDNA was used as a template in PCR reactions using gene-specific primer pairs.[13] Generally 20 to 25 cycles for amplification in the linear range were used. After quantifying bands by using densitometry, the relative steady-state level of mRNA was calculated and compared after normalizing to actin.

Statistical Analysis

Statistical analysis of the data were performed by using SigmaStat software (Jandel Scientific, San Rafael, CA). Comparison between groups was made using one-way analysis of variance followed by the paired *t*-test. A *P* value of less than 0.05 was considered significant.

Results

β-Cell-Specific Disruption of c-met Receptor Gene in Mice

We created a conditional knockout mouse model in which c-met receptor gene was specifically disrupted in the pancreatic β cells by using Cre-loxP-mediated recombination strategy.[23–25] Figure 1A shows the structure of loxP-flanked (floxed) c-met allele. LoxP sites were inserted downstream from exons 15 and 16 at the c-met locus through homologous recombination in embryonic stem cells (Figure 1A), as described previously.[18] By mating c-met floxed mice with Rip-Cre transgenic mice in which Cre recombinase was driven under rat insulin promoter, mice heterozygous for the c-met floxed allele were generated (genotype: $met^{lox/+}$, Cre). These mice were bred with the homozygous c-met floxed mice to inactivate both c-met alleles by Cre-mediated excision, whereby creating conditional knockout mice in which c-met gene was specifically disrupted in pancreatic β cells. These mice were hereafter referred to as $\beta met^{-/-}$ (genotype: $met^{lox/lox}$, Cre). This breeding also generated a littermate control group with different genotype ($met^{lox/lox}$, hereafter referred to as controls), which were confirmed by PCR analysis of the tail genomic DNA (Figure 1B). All animals were born normally at the expected Mendelian fre-

Table 1. Comparison of the Body Weight, Blood Glucose, and Insulin Level between Control and $\beta met^{-/-}$ Mice at 6 Months of Age

	Body weight (g)		Blood glucose (mg/dl)		Blood insulin (ng/ml)	
	Male	Female	Male	Female	Male	Female
Control	31.2 ± 1.7 (n = 6)	24.2 ± 3.4 (n = 8)	187.2 ± 7.2 (n = 6)	180.1 ± 27.2 (n = 8)	0.92 ± 0.23 (n = 6)	1.01 ± 0.12 (n = 8)
$\beta met^{-/-}$	28.8 ± 2.5* (n = 6)	21.4 ± 2.8* (n = 8)	208.8 ± 16.8* (n = 6)	203.7 ± 12.7* (n = 8)	0.54 ± 0.20* (n = 6)	0.70 ± 0.21* (n = 8)

*$P < 0.05$ versus controls.

quency, and no significant differences in their body weights at birth were observed among different groups.

Figure 1C shows the efficiency and specificity of recombination of the c-met floxed allele mediated by Cre expression. PCR analysis of genomic DNA from the isolated islets revealed that the excised floxed c-met allele was detectable only in $\beta met^{-/-}$ mice (Figure 1C, lane 2), but not in the controls (lane 1). Of note, a faint 380-bp band corresponding to the c-met floxed allele was still observable in the sample derived from the islets of $\beta met^{-/-}$ mice (Figure 1C, lane 2). This is apparently due to the fact that β cells only make up ~80 to 85% of the islet cell population. DNA from non-β-islet cells and some integrated vascular cells should not exhibit a recombination event. Excision of the floxed c-met allele only occurred in the pancreatic islets, DNA isolated from other tissues such as heart, muscle, and liver did not exhibit a Cre-mediated recombination (Figure 1C, lanes 3 to 5).

The c-met receptor was expressed at high levels in the pancreatic islets (Figure 1, D through H). Inactivation of c-met gene by Cre-mediated excision caused a reduction of the expression of c-met protein in β cells. Western blot analysis demonstrated that c-met protein level in the isolated islets was reduced by 80 to 85% in $\beta met^{-/-}$ mice, compared with the controls (Figure 1H). This is approximately proportional to the percentage of β cells in islet cells, suggesting a virtually complete loss of c-met in β cells of the islet. Immunofluorescence staining showed that Cre recombinase was expressed in almost all β-cell populations in the islets (Figure 1E); and accordingly c-met receptor protein was ablated in these cells in $\beta met^{-/-}$ mice (Figure 1G).

We further examined the responsiveness of the islet cells from control and $\beta met^{-/-}$ mice to HGF stimulation *in vitro*. As presented in Figure 1I, pancreatic islets isolated from the control mice clearly responded to HGF stimulation to activate major signal pathways. Both Erk-1/2 mitogen-activated protein (MAP) kinase and Akt/protein kinase B underwent rapid phosphorylation on HGF stimulation (Figure 1I). However, little Erk-1/2 and Akt activation was detected in the pancreatic islets from $\beta met^{-/-}$ mice after HGF incubation under the identical conditions, suggesting that specific ablation of c-met receptor have rendered the islet cells incapable of responding to HGF stimulation.

Physiological Features of βmet$^{-/-}$ *Mice*

We observed a slight, but statistically significant, reduction of the body weights in $\beta met^{-/-}$ mice at 6 months of age, when compared with the control littermates (Table 1). This decrease in body weight took place in both male and female mice. $\beta met^{-/-}$ mice at 2 months of age showed a slight tendency of growth retardation, but the difference in the body weights between $\beta met^{-/-}$ mice and controls did not reach statistical significance (data not shown).

We then examined the potential effects of β-cell-specific ablation of c-met on blood glucose homeostasis. As shown in Table 1, in normal diet state, a mild increase (~12%) in blood glucose level was observed in both male and female $\beta met^{-/-}$ mice at 6 months of age, compared with the controls. Such elevation of glucose level in $\beta met^{-/-}$ mice was age-dependent because no significant difference in glucose level was found between $\beta met^{-/-}$ mice and controls at 2 months of age (data not shown). After a 24-hour fast, blood glucose levels in $\beta met^{-/-}$ mice and controls were comparable, although there was tendency being higher in $\beta met^{-/-}$ mice. Table 1 also shows serum insulin levels in control and $\beta met^{-/-}$ mice at 6 months of age. Consistent with an elevated glucose, serum insulin was significantly decreased in $\beta met^{-/-}$ mice. Therefore, specific deletion of c-met in pancreatic β cells displays a progressive, age-dependent impairment of glucose homeostasis.

Impaired Glucose-Stimulated Acute-Phase Insulin Secretion in βmet$^{-/-}$ *Mice*

To evaluate the effect of c-met deletion on β-cell function, we examined acute-phase insulin secretion in response to glucose. As presented in Figure 2, both male and female $\beta met^{-/-}$ mice manifested a complete loss of glucose-stimulated acute-phase insulin secretion at 6 months of age. In controls, insulin secretion was increased by fourfold to fivefold at 2 minutes after intraperitoneal injection of glucose, and remained higher than baseline values at least to 10 minutes. However, the acute-phase insulin secretory response to glucose was virtually absent in both male (Figure 2A) and female $\beta met^{-/-}$ mice (Figure 2B). We further examined acute insulin release in response to L-arginine, another major insulin secretory stimulant that acts by a different mechanism.[26] In sharp contrast to the response to glucose, a fourfold to sixfold increase in insulin secretion was observed in $\beta met^{-/-}$ mice and controls after injection of arginine (Figure 2, C and D). Hence, β-cell-specific deletion of c-met leads to a major loss of acute-phase

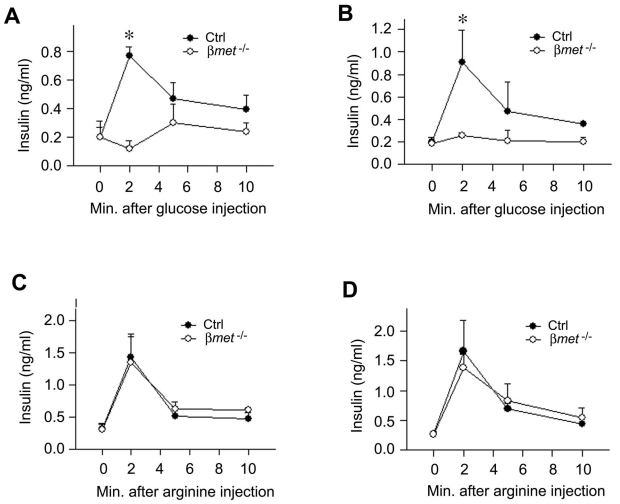

Figure 2. $\beta met^{-/-}$ mice display impaired acute-phase insulin secretion in response to glucose but not to L-arginine. Glucose and L-arginine were injected intraperitoneally, respectively, in the male (**A** and **C**) and female (**B** and **D**) control and $\beta met^{-/-}$ mice at 6 months of age after a 24-hour fast. A loss of acute-phase insulin secretion was observed in both male and female $\beta met^{-/-}$ mice in response to glucose (**A**, **B**), but not to arginine (**C**, **D**). $^*P < 0.05$, $n = 4$.

insulin secretion in response to glucose but not to arginine.

β-Cell-Specific Ablation of c-met Leads to Glucose Intolerance

We next assessed the impact of β-cell-specific deletion of c-met on the ability of mice to handle glucose challenge. After intraperitoneal injection of glucose, $\beta met^{-/-}$ mice exhibited higher blood glucose levels than the controls (Figure 3). Such glucose intolerance developed in both male (Figure 3A) and female $\beta met^{-/-}$ mice (Figure 3B). Insulin level at 30 minutes after glucose injection was significantly lower in $\beta met^{-/-}$ mice compared with the controls (1.06 ± 0.15 versus 0.69 ± 0.17, $P < 0.05$, $n =$ 6 to 8). Therefore, consistent with insulin secretory defect in response to glucose, mice lacking c-met receptor in pancreatic β cells display impaired glucose tolerance. Figure 3 also shows the results of the insulin sensitivity test. After injection of insulin, blood glucose levels dropped in a similar rate in $\beta met^{-/-}$ mice and controls. Except at the starting point, blood glucose levels were virtually identical in the male (Figure 3C) and female (Figure 3D) control and $\beta met^{-/-}$ mice at various time points after insulin injection.

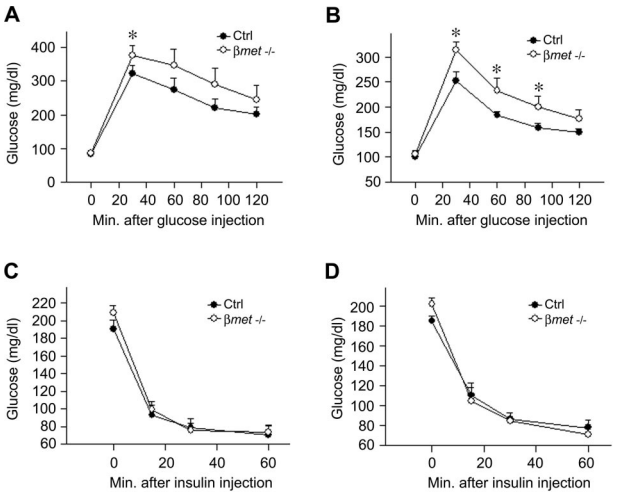

Figure 3. $\beta met^{-/-}$ mice show impaired glucose tolerance but normal insulin sensitivity. Glucose tolerance tests were performed in the male (**A**) and female (**B**) control and $\beta met^{-/-}$ mice at 6 months of age in a fasted state. Glucose intolerance was observed in both male and female $\beta met^{-/-}$ mice. $^*P < 0.05$, $n = 6$ to 8. Insulin-sensitive tests were performed in the male (**C**) and female (**D**) control and $\beta met^{-/-}$ mice at 6 months of age under normal diet conditions. $n = 3$.

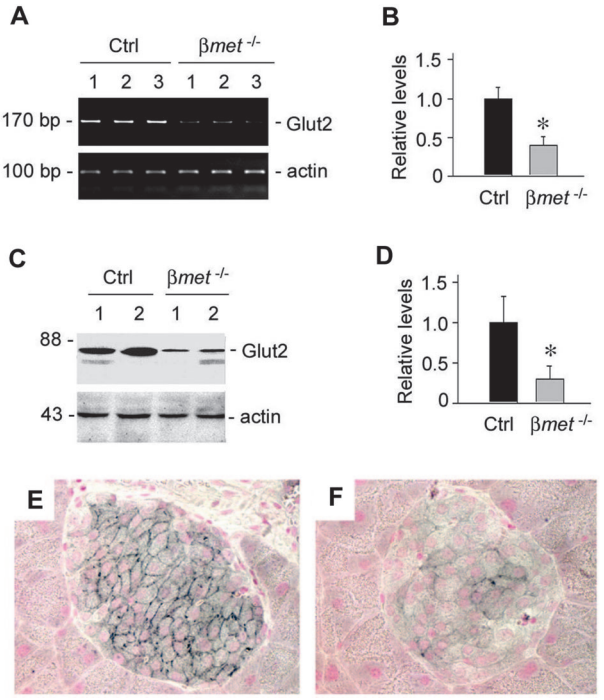

Figure 4. Specific deletion of c-met in mice decreased Glut-2 expression in pancreatic β cells. **A**, **B:** Semiquantitative RT-PCR demonstrates decreased Glut-2 mRNA levels in the pancreatic islets isolated from $\beta met^{-/-}$ mice, compared with the controls. Graphical presentation shows the relative Glut-2 mRNA abundance in the control and $\beta met^{-/-}$ islets after normalization to actin. $^*P < 0.05$, $n = 3$. **C**, **D:** Representative Western blot and graphical presentation show decreased Glut-2 protein levels in the pancreatic islets isolated from $\beta met^{-/-}$ mice, compared with the controls. $^*P < 0.05$, $n = 4$. **E**, **F:** Representative micrographs demonstrate localization and abundance of Glut-2 protein in the islets of control (**E**) and $\beta met^{-/-}$ mice (**F**).

Decreased Glucose Transporter-2 Expression in the β Cells of βmet$^{-/-}$ Mice

Glucose entry into the β cells is primarily mediated by glucose transporter-2 (Glut-2). To unravel the mechanism underlying the loss of glucose-stimulated insulin secretion in $\beta met^{-/-}$ mice, we examined the expression of Glut-2 in the pancreatic islets isolated from the $\beta met^{-/-}$ mice or controls. Figure 4A shows the results of semiquantitative RT-PCR for measuring the steady-state levels of Glut-2 mRNA. Compared to controls, Glut-2 mRNA in the islets of $\beta met^{-/-}$ mice was reduced by more than 60% (Figure 4B). Consistently, Glut-2 protein level was

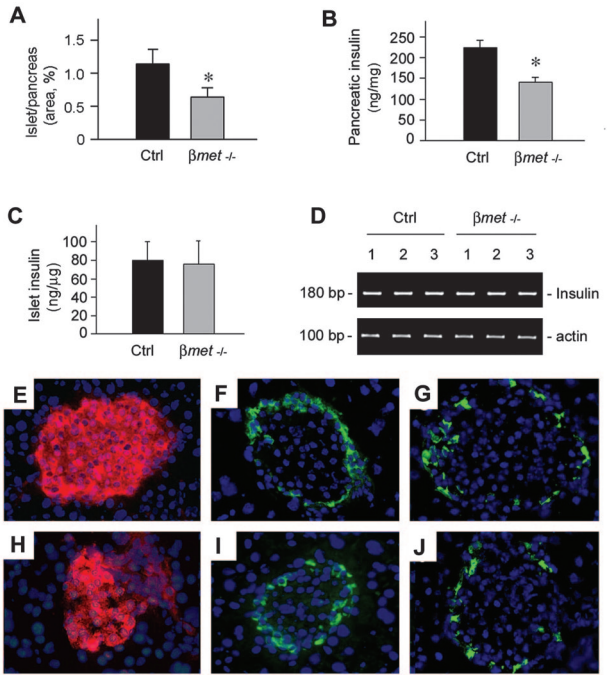

Figure 5. β-Cell-specific ablation of c-met results in reduced islet size but maintains normal islet structure at 6 months of age. **A:** Reduced islet area was observed in $\beta met^{-/-}$ mice at 6 months of age, compared to the controls. The percentage of islet/pancreas areas was measured in pancreatic sections stained with insulin and data were presented as mean ± SE. $^*P < 0.05$, $n = 8$. **B:** Pancreatic insulin content was decreased in $\beta met^{-/-}$ mice at 6 months of age, compared to the controls. Insulin levels were expressed as ng/mg pancreas. $^*P < 0.05$, $n = 4$. **C:** No difference in relative insulin content in the islets of the control and $\beta met^{-/-}$ mice was found. Islet insulin content was measured in acid-ethanol extracts and expressed as ng/μg protein ($n = 4$). **D:** No difference in relative abundance of insulin mRNA levels was observed in the islets of the control and $\beta met^{-/-}$ mice ($n = 3$). **E** to **J:** Representative micrographs show islet structure and morphology in the control (**E** to **G**) and $\beta met^{-/-}$ mice (**H** to **J**) after staining with various antibodies. **E** and **H**, insulin; **F** and **I**, glucagons; **G** and **J**, somatostatin.

also reduced in $\beta met^{-/-}$ mice by Western blot analysis of the islet homogenates (Figure 4, C and D). Figure 4, E and F, also shows the representative micrographs of immunohistochemical staining for Glut-2 in the pancreatic islets of the $\beta met^{-/-}$ mice and controls at 6 months. In the controls, Glut-2 protein was abundantly expressed and predominantly localized in the plasma membrane of β cells (Figure 4E). However, Glut-2 staining primarily disappeared in the islets of $\beta met^{-/-}$ mice, suggesting that specific deletion of c-met in β cells leads to a dramatic down-regulation of Glut-2 expression.

$\beta met^{-/-}$ Mice Show a Reduced Islet Size and Insulin Content but Display Normal Morphology

We measured the islet sizes in the pancreas of the $\beta met^{-/-}$ mice and controls at 6 months of age. In the pancreatic sections stained with insulin, the areas of all islets and pancreas in a given section were measured and the ratio of the islet per pancreas areas (%) was calculated. As shown in Figure 5A, the average ratio of the islet/pancreas areas in $\beta met^{-/-}$ mice at 6 months was significantly lower than in the controls. However, total islet number per pancreas area was not significantly different between the controls and $\beta met^{-/-}$ mice (Table 2), indicating a reduced islet size in the $\beta met^{-/-}$ pancreas. Table 2 also showed the distribution of different sizes of pancreatic islet in the controls and $\beta met^{-/-}$ mice. Large islets with more than 90 insulin-positive cells in $\beta met^{-/-}$ pancreas were significantly less than in the controls, whereas there was a tendency with an increasing number of small islets in $\beta met^{-/-}$ mice. Consistent with a reduced islet size, insulin content in the pancreas of $\beta met^{-/-}$ mice was significantly less than that of the controls (Figure 5B). The insulin content in the isolated islets with similar sizes, however, was almost identical between the controls and $\beta met^{-/-}$ mice (Figure 5C). Similar results on insulin mRNA levels of the isolated islets were obtained in the control and $\beta met^{-/-}$ mice (Figure 5D). This suggests that the ability of β cells in the islets to produce insulin is not affected by deletion of c-met receptor.

$\beta met^{-/-}$ mice exhibited normal islet morphology (Figure 5E). Despite being smaller in size, the composition and location of the islet cells as well as the ratio of the β cells versus non-β cells in $\beta met^{-/-}$ mice were similar to that in the controls (Figure 5, E through J). The intensity of insulin staining was same in the islets of controls and $\beta met^{-/-}$ mice (Figure 5E). Thus lack of c-met does not influence the overall structure of pancreatic islets in mice.

Discussion

Depletion and/or dysfunction of the insulin-producing β cells in pancreatic islets contribute to the development of different types of diabetes.[24,27–29] Although numerous factors have been linked to the regulation of β-cell biology *in vivo*, previous studies are primarily focused on the molecules in insulin action cascade, including insulin receptor,[19,25,30] insulin receptor substrate-1,[31] and insu-

Table 2. Comparison of the Islet Numbers between Control and $\beta met^{-/-}$ Mice at 6 Months of Age

	Total islet number per pancreas area (10 mm^2)	Islet size distribution (number/10 mm^2 pancreas)*				
		< 10	11 to 30	31 to 60	61 to 90	> 90
Control ($n = 8$)	9.5 ± 1.3	5.5 ± 0.9	1.5 ± 0.4	1.1 ± 0.2	1.0 ± 0.3	0.7 ± 0.1
$\beta met^{-/-}$ ($n = 8$)	10.5 ± 1.3	6.7 ± 1.3	2.1 ± 0.6	1.0 ± 0.3	0.5 ± 0.2	0.4 ± 0.2†

*Islet size was defined by counting insulin-positive cell number per islet. The distribution of different sizes of islet was expressed as numbers per 10-mm^2 pancreas area.
$^\dagger P < 0.05$ versus controls.

lin-like growth factor (IGF)-1 receptor.[32] In the present study, we have investigated the role of HGF, a growth factor that does not belong to a component of insulin action cascade, in β-cell growth and function by inactivating its sole receptor gene in a cell-type-specific manner. Mice lacking c-met receptor in pancreatic β cells manifest glucose intolerance due to loss of the glucose-stimulated acute-phase insulin release, which in turn possibly results from a diminished glucose sensing. In addition, β-cell-specific ablation of c-met leads to reduced islet size, decreased serum, and pancreatic insulin levels and mild hyperglycemia. These findings underscore an imperative role of HGF signaling in controlling β-cell growth and differentiated function in pancreatic islets, as well as in the maintenance of glucose homeostasis *in vivo*.

One of the important features in $\beta met^{-/-}$ mice is a reduced circulating insulin level under normal conditions (Table 1). This is in sharp contrast to a mild elevation in circulating insulin levels observed in mice with β-cell-specific deletion of insulin receptor or IGF-1 receptor.[19,32] Such reduction of serum insulin level in $\beta met^{-/-}$ mice was accompanied by a mild hyperglycemia and growth retardation (Table 1), whereas blood glucose levels are within the normal range in mice defective in insulin and IGF-1 signaling in pancreatic β cells.[19,32] In this regard, HGF signaling is at least as important as IGF-1 signaling in the maintenance of glucose homeostasis under normal conditions.

Several mechanisms could potentially account for a decrease of the circulating insulin level in $\beta met^{-/-}$ mice, including a decreased insulin production in β cells and/or a reduced β-cell mass. However, judged from the magnitude of insulin reduction in the circulation, which is approximately proportional to the decrease in islet size and insulin content in pancreas (Figure 5), it is conceivable that a retardation in the postnatal growth of β-cell mass and islet size, rather than a defect in insulin production, primarily contribute to hypoinsulinemia in $\beta met^{-/-}$ mice. Consistent with this view, insulin mRNA and protein content are almost identical in pancreatic islets derived from the controls or $\beta met^{-/-}$ mice with similar size (Figure 5). These observations underline the fact that HGF signaling is an essential factor for β-cell growth in pancreatic islets at postnatal stage under basal conditions. In accordance with it, transgenic mice with overexpression of HGF gene in β cells driven under rat insulin promoter leads to an increased islet size and a mild hypoglycemia.[14] In addition, supplementation of HGF by intravenous administration of HGF plasmid promotes β-cell proliferation in the pancreatic islets in streptozotocin-induced diabetic mice.[4]

The abnormality in serum insulin and glucose levels in $\beta met^{-/-}$ mice is less prominent at 2 months of age, and there is essentially no difference in these parameters at the time of birth between the controls and $\beta met^{-/-}$ mice. Such observation suggests that the impairment of glucose homeostasis observed in $\beta met^{-/-}$ mice is progressive and age-dependent. Furthermore, this finding is also consistent with the notion that HGF signaling may not be essential for the early, prenatal development of pancreatic β cells. However, because the $\beta met^{-/-}$ mice were created by using a Cre-mediated recombination driven under the rat insulin promoter, we cannot exclude the possibility that c-met receptor may be important for islet development before insulin expression at day 9 of embryogenesis.[32,33]

The regulation of glucose homeostasis is complex, in which both insulin secretion from β cells and peripheral tissue sensitivity to insulin play a crucial role. Another feature of $\beta met^{-/-}$ mice is manifested by the complete loss of the acute-phase insulin secretion in response to glucose (Figure 2). Such dysfunction of the pancreatic β cells deficiency in c-met receptor highlights a failure of the β cells to respond appropriately to the glucose stimulation, which inevitably leads to glucose intolerance after challenge (Figure 3). It is of interest to note that the defective insulin secretory response of β cells is stimulant-specific. Although there is a selective loss of the glucose-stimulated insulin secretion, the secretory response of β cells to arginine is apparently intact (Figure 2), a condition similar to the early phase of diabetes in humans.[19,34]

Although the mechanism underlying the loss of glucose-stimulated insulin secretion is not fully understood, a defect in glucose-sensing machinery likely plays a predominant role. We have observed a down-regulation of Glut-2 expression in the β cells lacking c-met receptor, suggesting that HGF signaling is vital for the maintenance of the glucose-sensing protein expression *in vivo*. This is consistent with a previous report demonstrating that HGF can induce pancreatic acinar AR42J cells to convert into insulin-producing cells and activates Glut-2 gene expression *in vitro*.[35] Likewise, pancreatic islets with overexpression of exogenous HGF gene also display an increased Glut-2 steady-state mRNA level.[13] Hence, it becomes clear that Glut-2 gene expression in β cells is primarily dependent on an intact HGF signaling, although the molecular details involved remain to be elucidated. Ablation of c-met receptor in β cells silenced Glut-2 expression by ~70% (Figure 4), which would hinder glucose entry into the β cells. Of note, reduced Glut-2 levels in the β cells have been previously linked to the loss of the acute-phase insulin secretion and hyperglycemia in some animal models of diabetes.[36] Therefore, disruption of HGF signaling via deletion of its receptor in pancreatic β cells triggers a cascade of events including down-regulation of the Glut-2 expression, attenuation of the glucose sensing, loss of the glucose stimulated insulin secretion, and finally glucose intolerance.

In summary, the results presented in this study demonstrate that mice with β-cell-specific ablation of c-met receptor exhibit a reduced islet size and decreased insulin levels, and display a complete loss of the glucose-stimulated acute-phase insulin secretion and glucose intolerance. These findings illustrate a direct functional role for HGF signaling in the islet β-cell biology and in the maintenance of glucose homeostasis. Whether a genetic alteration in HGF/c-met or the components of their signaling cascade contributes to the development of diabetes in humans awaits further investigation.

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