Roles of ATP-Sensitive K^+ Channels in Cell Survival and Differentiation in the Endocrine Pancreas

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To determine the roles of the ATP-sensitive K^+ (K_{ATP}) channels in endocrine pancreas more directly, two types of genetically engineered Kir6.2 mice were developed: mice expressing a dominant-negative form of Kir6.2 specifically in β-cells (Kir6.2G132S Tg mice) and mice lacking Kir6.2 (Kir6.2$^{-/-}$ or Kir6.2 null mice). The Kir6.2G132S Tg mice show severe impairment of K_{ATP} channel function only in the β-cells, whereas Kir6.2 null mice are completely defective in K_{ATP} channel function in all of the cells in which Kir6.2 is a constituent of the K_{ATP} channels, because of the disruption of Kir6.2. Both types of mice show abnormal architecture of the pancreatic islets. The number of β-cells in Kir6.2G132S Tg mice decreases markedly with age, whereas that in Kir6.2$^{-/-}$ mice decreases slightly. α-Cells, which are normally present only in the periphery of pancreatic islets, also appear in the center of the islets in both Kir6.2G132S Tg and Kir6.2$^{-/-}$ mice. Interestingly, the number of peptide YY (PYY) and glucagon-positive cells is markedly increased in Kir6.2 null mice, whereas the number of PP cells and δ-cells is not altered. Apoptotic cells are detected by the TdT-mediated dUTP nick-end labeling (TUNEL) method at a high frequency in both Kir6.2G372S Tg and Kir6.2$^{-/-}$ mice compared with the respective controls. Thus, studies of Kir6.2G372S Tg and Kir6.2$^{-/-}$ mice indicate that K_{ATP} channels play an important role in cell survival and differentiation in the endocrine pancreas. *Diabetes* 50 (Suppl. 1):S48–S51, 2001

ATP-sensitive K^+ (K_{ATP}) channels couple cell metabolism with membrane potential in many cells (1). Metabolic alterations induce changes in the concentrations of ATP and MgADP in cells that inhibit and activate the K_{ATP} channels, respectively. K_{ATP} channels thus function as ATP and ADP sensors in the regulation of many cellular functions, including insulin secretion from pancreatic β-cells, excitability of muscles and neurons, and K^+ recycling in renal epithelia (1). Classic plasma membrane K_{ATP} channels are now known to consist of two subunits: the inward rectifier K^+ channel member Kir6.2 (2,3) and a sulfonylurea receptor SUR1 (4) or SUR2 variant (5,6)—a member of the ATP-binding cassette transporter superfamily. Pancreatic β-cell K_{ATP} channels comprise Kir6.2 and SUR1 subunits, whereas cardiac and skeletal muscle K_{ATP} channels comprise Kir6.2 and SUR2A subunits (7–9). Because the β-cell K_{ATP} channel is a regulator of glucose- and sulfonylurea-induced insulin secretion, mutations of SUR1 or Kir6.2 could cause disorders of glucose homeostasis. Mutations of the SUR1 gene were first described in patients with persistent hyperinsulinemic hypoglycemia of infancy (PHHI) (10). PHHI (familial hyperinsulinism), formerly called pancreatic nesidioblastosis, is a rare disorder occurring in neonates and infants, characterized by excessive insulin secretion despite severe hypoglycemia (11). Abnormalities of β-cell K_{ATP} channel function are responsible for the pathophysiology of PHHI (12). Mutations of the *SUR1* or *Kir6.2* gene are now known to cause PHHI (13).

Although the K_{ATP} channel is thought to be a major determinant of membrane potential in β-cells, the direct consequences of its disruption were not known. In addition, electrophysiological studies find K_{ATP} channels also in α-cells , δ-cells, and pancreatic polypeptide (PP) cells (14–16). lmmunohistochemistry and in situ hybridization studies have revealed that both Kir6.2 and SUR1 are present in all the islet cells of some species (17,18). To directly elucidate the physiological roles of K_{ATP} channels in the endocrine pancreas, we generated two types of Kir6.2 genetically engineered mice: mice expressing a dominant-negative form of Kir6.2 specifically in pancreatic β-cells (Kir6.2G372S Tg mice) (19) and mice lacking Kir6.2 (Kir6.2$^{-/-}$ or Kir6.2 null mice) (20).

THE PHENOTYPE OF KIR6.2G372S Tg

Kir6.2 is a protein composed of 390 amino acids with two transmembrane domains (TM1 and TM2). The H5 region, between TM1 and TM2, is highly conserved in inward rectifier K^+ channels, and the motif Gly-Tyr (or Phe)-Gly in the H5 region is thought to be critical for K^+ ion selectivity (19). A substitution of the first residue of the Gly-Tyr-Gly motif with Ser (residue 156) is found in the G-protein–gated inward rectifier GIRK2 (Kir3.2) of the neurological mutant *weaver* mice (21). This substitution is responsible for the impairment of neuronal differentiation and development characteristic of *weaver* mice (22–25). By analogy with the *weaver* mutant, we replaced residue 132 Gly with Ser (Kir6.2G132S mutant) by in vitro mutagenesis. ^{86}Rb flux experiments (used to trace K movements) showed that Kir6.2G132S, when coexpressed with wild-type Kir6.2 and SUR1 in COS-1 cells, functions as a dominant-negative inhibitor. Mice expressing Kir6.2G132S

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8-OHdG, 8-hydroxy-2′-deoxyguanosine; $[Ca^{2+}]_i$, cytoplasmic calcium concentration; HNE, 4-hydroxy-2-nonenal; K_{ATP} channel, ATP-sensitive K^+ channel; NPY, neuropeptide Y; PHHI, persistent hyperinsulinemic hypoglycemia of infancy; PP, pancreatic polypeptide; PYY, peptide YY; TUNEL, TdT-mediated dUTP nick-end labeling.

TABLE 1
Phenotypes of Kir6.2G132S Tg and Kir6.2$^{-/-}$ mice

	Kir6.2G132S Tg	Kir6.2$^{-/-}$
Pancreatic β-cells		
K_{ATP} channel	Impaired	Absent
Membrane potential	Depolarized	Depolarized
Basal $[Ca^{2+}]_i$	Elevated	Elevated
Insulin response to glucose	Impaired	Impaired
Blood glucose levels		
Neonates	Hypoglycemia	Hypoglycemia
Young adults (4–16 weeks)	Hyperglycemia	Normoglycemia
Aged obese mice (50–55 weeks)	ND	Hyperglycemia

ND, not determined.

specifically in pancreatic β-cells were generated by using the human insulin gene promoter. The K_{ATP} channel currents of β-cells of transgenic mice are markedly impaired. In the basal state, the β-cells of Kir6.2G132S Tg mice are depolarized, and their cytoplasmic calcium concentration ($[Ca^{2+}]_i$) is significantly higher than that in controls. Kir6.2G132S Tg mice exhibit relatively high serum insulin levels despite hypoglycemia, suggesting a phenotype resembling PHHI; however, in adult age, they develop mild to severe hyperglycemia among the various transgenic lines. Glucose-induced insulin secretion is markedly reduced in Kir6.2G132S Tg mice with severe hyperglycemia. The phenotype of Kir6.2G132S Tg is summarized in Table 1.

THE PHENOTYPE OF KIR6.2$^{-/-}$

Because the Kir6.2 subunits form the K^+ ion-permeable pore of the K_{ATP} channel, we disrupted the Kir6.2 gene by homologous recombination to generate mice lacking K_{ATP} channels. Homozygous mice (Kir6.2$^{-/-}$) were generated by interbreeding heterozygous mice (Kir6.2$^{+/-}$). K_{ATP} channel activity is completely absent in the pancreatic β-cells, skeletal muscle, and heart of Kir6.2$^{-/-}$ mice, indicating that Kir6.2 is an essential subunit of the plasma membrane K_{ATP} channels in these tissues. Because there is little or no K_{ATP} channel activity, insulin secretion at low concentrations of glucose is not suppressed and leads to hypoglycemia in Kir6.2G132S Tg and Kir6.2$^{-/-}$ neonates, suggesting a mechanism that could account in part for the hypoglycemia seen in PHHI. Action potentials are already found in the basal state at low glucose concentrations in the β-cells of Kir6.2$^{-/-}$ mice, but the β-cell membrane cannot be hyperpolarized by K^+ channel openers such as diazoxide and pinacidil. Basal $[Ca^{2+}]_i$ in the β-cells of Kir6.2$^{-/-}$ is significantly elevated compared with controls, and acetylcholine or high K^+ stimulation increases $[Ca^{2+}]_i$ to levels comparable to those of controls. In contrast, neither glucose nor tolbutamide stimulation increases $[Ca^{2+}]_i$ in the β-cells of Kir6.2$^{-/-}$ or elicits significant insulin secretion from the perifused islets of Kir6.2$^{-/-}$. There is almost no glucose-dependent insulin secretion from the isolated islets of Kir6.2$^{-/-}$, and insulin response to intraperitoneal glucose loading is impaired but not completely abolished. Interestingly, there is only a slight impairment in glucose tolerance despite the severe defect in glucose-induced insulin secretion. Insulin tolerance tests show that the glucose-lowering effect of insulin at a relatively low dose is significantly increased in Kir6.2$^{-/-}$ mice compared with controls, suggesting that insulin sensitivity is enhanced in Kir6.2$^{-/-}$ mice.

There were no differences in random measurements of blood glucose or body weight between Kir6.2$^{-/-}$ mice and controls when the mice were fed normal diet until 20 weeks of age. However, fasting blood glucose was significantly elevated, and glucose intolerance became evident in those Kir6.2 mice that developed obesity during aging. In contrast, neither age-matched Kir6.2$^{-/-}$ mice without obesity nor age-matched control mice with obesity exhibited glucose intolerance, suggesting that both a primary defect in insulin secretion and insulin resistance secondary to environmental factors contributes to the development of diabetes. The phenotype of Kir6.2$^{-/-}$ mice is summarized in Table 1.

β-CELL DEATH IN KIR6.2G132S Tg AND KIR6.2$^{-/-}$ MICE

Although Kir6.2G132S Tg mice exhibited transient hypoglycemia at birth, they became hyperglycemic after 4 weeks of age, when the number of β-cells was significantly decreased and the architecture of the islets became markedly abnormal. This decrease in β-cell number could be due to accelerated β-cell death or to decreased β-cell proliferation. Bromodeoxyuridine incorporation into islet cells showed no difference between control mice and Kir6.2G132S Tg mice (T.I., unpublished data). However, apoptotic cells in the islets of Kir6.2G132S Tg mice were detected at a higher frequency than those in controls by TdT-mediated dUTP nick-end labeling (TUNEL) assay of serial sections of pancreases (19). These data suggest the decrease in β-cell number of Tg mice is due to cell death, probably apoptosis (19).

Before 16 weeks of age, the number of β-cells in Kir6.2$^{-/-}$ mice was similar to that of controls, but a decrease became evident in aged Kir6.2$^{-/-}$ mice (T.I., unpublished data). Apoptotic cells detected by the TUNEL method were more frequent in the islets of Kir6.2$^{-/-}$ mice than in those of control mice (26). These apoptotic cells are most probably β-cells because, among the islet endocrine cells, the β-cell is the only one that shows a significant decrease in number with age in Kir6.2$^{-/-}$ mice. The mechanisms of apoptosis in Kir6.2G132S Tg and Kir6.2$^{-/-}$ mice are not clear at present. In *weaver* mice, the *weaver* mutant Kir3.2 homomultimeric channels lose their selectivity for K^+ ions and probably cause chronic depolarization of neurons because of an increase in basal Na^+ currents, which leads to death in cerebellar granule cells (22–25,27). Unlike the *weaver* mutant channels, the Kir6.2G132S mutant channels do not exhibit any ionic currents (S. Gonoi, S.S., unpublished data). In the β-cells of Kir6.2$^{-/-}$ mice, there is no K_{ATP} channel activity. Therefore, in both Kir6.2G132S Tg and Kir6.2$^{-/-}$ mice, it is likely that the chronic depolarization and the abnormally high levels of basal $[Ca^{2+}]_i$ due to the loss of K_{ATP} channel function contribute to β-cell death. However, it appears that the β-cells of Kir6.2G132S Tg mice are more markedly affected than those of Kir6.2$^{-/-}$ mice. The Kir3.2G156S *weaver* mutant reduces channel activity when coassembled with Kir3.1 (22). Because Kir6.2 and Kir6.1 can form heteromultimers (28), overexpression of the Kir6.2G132S mutant in pancreatic β-cells may impair not only Kir6.2 function but also the function of other inward rectifiers such as Kir6.1 (which is expressed widely in tissues and possibly also present in β-cells) and lead to more severe β-cell damage. It has been suggested that K_{ATP} channels are involved in protection from cell death (29,30). In addition, Efanova et al. (31) recently

TABLE 2
Morphological changes in the pancreatic islets of Kir6.2G132S Tg and Kir6.2$^{-/-}$ mice

	Kir6.2G132S Tg	Kir6.2$^{-/-}$
α-Cells	Slightly increased in number; widely distributed in the islets	Markedly increased in number; widely distributed in the islets; most are also positive for PYY
β-Cells	Markedly decreased in number	Significantly decreased in number (the decrease is apparent in aged mice)
δ-Cells	Unchanged in number	Unchanged in number
Apoptosis*	Detected at higher frequency	Detected at higher frequency

*See the text for details about apoptosis.

reported that apoptosis of pancreatic β-cells is induced by high glucose or tolbutamide in a Ca^{2+}-dependent manner. Altogether, these findings suggest that K_{ATP} channels may play an important role in β-cell survival by regulating the membrane potential and $[Ca^{2+}]_i$.

It has recently been shown that oxidative stress deteriorates the β-cells of GK rats, an animal model of type 2 diabetes (32). Although chronic hyperglycemia promotes oxidative stress in GK rats, it is not known whether oxidative stress participates in the decrease of β-cell number in aged Kir6.2$^{-/-}$ mice. We therefore evaluated the oxidative stress of the β-cells of Kir6.2$^{-/-}$ mice by measuring the levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG)- and 4-hydroxy-2-nonenal (HNE)-modified proteins using quantitative immunohistochemical analyses with specific antibodies (33). In contrast to the GK rat, neither 8-OHdG nor HNE was detected in β-cells of aged Kir6.2$^{-/-}$ mice (data not shown). Accordingly, it is unlikely that oxidative stress contributes to the decrease of β-cells in Kir6.2$^{-/-}$ mice.

MORPHOLOGICAL CHANGES IN THE ISLETS OF KIR6.2G132S Tg AND KIR6.2$^{-/-}$ MICE

Islet architecture is abnormal in both Kir6.2G132S Tg and Kir6.2$^{-/-}$ mice. Some alterations are common to both mouse lines, but others are not (Table 2). The differences in morphological changes between Kir6.2G132S Tg and Kir6.2$^{-/-}$ mice are probably due to K_{ATP} channel function being defective only in the β-cells of Kir6.2G132S Tg mice, whereas it is defective in all the endocrine cells of the islets in Kir6.2$^{-/-}$ mice. The α-cells, which are normally located at the periphery of the islets, are also present in the central part of the islets in both Kir6.2G132S Tg and Kir6.2$^{-/-}$ mice. This abnormality in topographical arrangement could be due to the disruption of K_{ATP} channels in β-cells because the K_{ATP} channels in the other endocrine cells of the islets are not impaired in Kir6.2G132S Tg mice. This suggests that direct interaction by cell-to-cell contact or indirect interaction via unknown paracrine signals between β- and α-cells might be important for the maintenance of normal pancreatic architecture; disruption of the β-cell K_{ATP} channels could well impair such interactions. The number of δ-cells appears to be normal. We initially found that the number of islet cells stained by an anti-PP antibody was markedly increased in Kir6.2$^{-/-}$ cells. In addition, many of the PP$^+$ cells were also found to be positive for glucagon. However, since it is possible that anti-PP antibody cross-reacts with neuropeptide Y (NPY) or peptide YY (PYY), we also stained the islet cells with anti-NPY antibody and anti-PYY antibody. Although the PP$^+$ cells were not stained by the antibody specific for NPY, many of the "PP$^+$" cells were immunoreactive to the antibody specific for PYY (T.I., unpublished data). It is likely, therefore, that the number of PYY and glucagon double-positive cells is markedly increased in the islets of Kir6.2$^{-/-}$. Since it has been suggested that PYY and glucagon double-positive cells appear in the early stage of development of the endocrine cells of pancreatic islets (34,35), disruption of the Kir6.2 gene might impair the differentiation into mature α-cells.

CONCLUSIONS

Studies of these two mouse lines with impaired K_{ATP} channel function show clearly that the K_{ATP} channels in pancreatic β-cells are critical in both glucose-induced and sulfonylurea-induced insulin secretion. They also stress the important roles of K_{ATP} channels in β-cell survival and differentiation of islet endocrine cells. Further studies on the mechanisms of cell death and islet cell differentiation in such mice should provide more direct insight into the pathogenesis of type 2 diabetes as well as normal differentiation of islet endocrine cells.

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REFERENCES

1. Ashcroft FM: Adenosine 5′-triphosphate-sensitive potassium channels. *Annu Rev Neurosci* 11:97–118, 1988
2. Inagaki N, Gonoi T, Clement JP IV, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S, Bryan J: Reconstitution of IK_{ATP}: an inward rectifier subunit plus the sulfonylurea receptor. *Science* 270:1166–1170, 1995
3. Sakura H, Ämmälä C, Smith PA, Gribble FM, Ashcroft FM: Cloning and functional expression of the cDNA encoding a novel ATP-sensitive potassium channel subunit expressed in pancreatic β-cells, brain, heart and skeletal muscle. *FEBS Lett* 377:338–344, 1995
4. Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JP IV, Boyd AE III, Gonzalez G, Herrera-Sosa H, Nguy K, Bryan J, Nelson DA: Cloning of the β cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science* 268:423–426, 1995
5. Inagaki N, Gonoi T, Clement JP IV, Wang CZ, Aguilar-Bryan L, Bryan J, Seino S: A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. *Neuron* 16:1011–1017, 1996
6. Isomoto S, Kondo C, Yamada M, Matsumoto S, Higashiguchi O, Horio Y, Matsuzawa Y, Kurachi Y: A novel sulfonylurea receptor forms with BIR (Kir6.2) a smooth muscle type ATP-sensitive K^+ channel. *J Biol Chem* 271:24321–24324, 1996
7. Aguilar-Bryan L, Clement JP IV, Gonzalez G, Kunjilwar K, Babenko A, Bryan J: Toward understanding the assembly and structure of K channels. *Physiol Rev* 78:227–245, 1998

8. Ashcroft FM, Gribble FM: Correlating structure and function in ATP-sensitive K^+ channels. *Trends Neurosci* 21:288–294, 1998
9. Seino S: ATP-sensitive potassium channels: a model of heteromultimeric potassium channel/receptor assemblies. *Annu Rev Physiol* 61:337–362, 1999
10. Thomas PM, Cote GJ, Wohllk N, Haddad B, Mathew PM, Rabl W, Aguilar-Bryan L, Gagel RF, Bryan J: Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science* 268:426–429, 1995
11. Permutt MA, Nestrowicz A, Glaser B: Familial hyperinsulinism: an inherited disorder of spontaneous hypoglycemia in neonates and infants. *Diabetes Rev* 4:347–355, 1996
12. Kane C, Shepherd RM, Squires PE, Johnson PR, James RF, Milla PJ, Aynsley-Green A, Lindley KJ, Dunne MJ: Loss of functional K_{ATP} channels in pancreatic beta-cells causes persistent hyperinsulinemic hypoglycemia of infancy. *Nat Med* 12:1344–1347, 1996
13. Aguilar-Bryan L, Bryan J: Molecular biology of adenosine triphosphate-sensitive potassium channels. *Endocr Rev* 20:101–135, 1999
14. Bokvist K, Olsen HL, Hoy M, Gotfredsen CF, Holmes WF, Buschard K, Rorsman P, Gromada J: Characterisation of sulphonylurea and ATP-regulated K^+ channels in rat pancreatic A-cells. *Pflügers Arch* 438:428–436, 1999
15. Berts A, Ball A, Dryselius G, Gylfe E, Hellman B: Glucose stimulation of somatostatin-producing islet cells involves oscillatory Ca^{2+} signaling. *Endocrinology* 137:693–697, 1996
16. Liu YJ, Hellman B, Gylfe E: Ca^{2+} signaling in mouse pancreatic polypeptide cells. *Endocrinology* 140:5524–5529, 1999
17. Suzuki M, Fujikura K, Inagaki N, Seino S, Takata K: Localization of the ATP-sensitive K^+ channel subunit Kir6.2 in mouse pancreas. *Diabetes* 46:1440–1444, 1997
18. Suzuki M, Fujikura K, Kotake K, Inagaki N, Seino S, Takata K: Immuno-localization of sulphonylurea receptor 1 in rat pancreas. *Diabetologia* 42:1204–1211, 1999
19. Miki T, Tashiro F, Iwanaga T, Nagashima K, Yoshitomi H, Aihara H, Nitta Y, Gonoi T, Inagaki N, Miyazaki J-I, Seino S: Abnormalities of pancreatic islets by targeted expression of a dominant-negative K_{ATP} channel. *Proc Natl Acad Sci U S A* 94:11969–11973, 1997
20. Miki T, Nagashima K, Tashiro F, Kotake K, Yoshitomi H, Tamamoto A, Gonoi T, Iwanaga T, Miyazaki J-I, Seino S: Defective insulin secretion and enhanced insulin action in K_{ATP} channel-deficient mice. *Proc Natl Acad Sci U S A* 95:10402–10406, 1998
21. Patil N, Cox DR, Bhat D, Faham M, Myers RM, Peterson AS: A potassium channel mutation in weaver mice implicates membrane excitability in granule cell differentiation. *Nat Genet* 11:126–129, 1995
22. Slesinger PA, Patil N, Liao YJ, Jan YN, Jan LY, Cox DR: Functional effects of the mouse weaver mutation on G protein-gated inwardly rectifying K^+ channels. *Neuron* 16:321–331, 1996
23. Kofuji P, Hofer M, Millen KJ, Millonig JM, Davidson N, Lester HA, Hatten ME: Functional analysis of the weaver mutant GIRK2 K^+ channel and rescue of weaver granule cells. *Neuron* 16:941–952, 1996
24. Navarro B, Kennedy ME, Velimirovic B, Bhat D, Peterson AS, Clapham DE: Nonselective and G betagamma-insensitive weaver K^+ channels. *Science* 272:1950–1953, 1996
25. Hess EJ: Identification of the weaver mouse mutation: the end of the beginning. *Neuron* 16:1073–1076, 1996
26. Seino S, Iwanaga T, Mnagashima K, Miki T: Diverse roles of K_{ATP} channels learned from Kir6.2 genetically engineered mice. *Diabetes* 49:311–318, 2000
27. Slesinger PA, Stoffel M, Jan YN, Jan LY: Defective gamma-aminobutyric acid type B receptor-activated inwardly rectifying K^+ currents in cerebellar granule cells isolated from weaver and Girk2 null mutant mice. *Proc Natl Acad Sci U S A* 94:12210–12217, 1997
28. Zerangue N, Schwappach B, Jan YN, Jan LY: A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K(ATP) channels. *Neuron* 22:537–548, 1999
29. Terzic A, Jahangir A, Kurachi Y: Cardiac ATP-sensitive K^+ channels: regulation by intracellular nucleotides and K^+ channel-opening drugs. *Am J Physiol* 269:C525–C545, 1995
30. Amoroso S, Schmid-Antomarchi H, Fosset M, Lazdunski M: Glucose, sulfonylureas, and neurotransmitter release: role of ATP-sensitive K^+ channels. *Science* 247:852–854, 1990
31. Efanova IB, Zaitsev SV, Zhivotovsky B, Kohler M, Efendic S, Orrenius S, Berggren PO: Glucose and tolbutamide induce apoptosis in pancreatic beta-cells: a process dependent on intracellular Ca^{2+} concentration. *J Biol Chem* 273:33501–33507, 1998
32. Ihara Y, Toyokuni S, Uchida K, Odaka H, Tanaka T, Ikeda H, Hiai H, Seino Y, Yamada Y: Hyperglycemia causes oxidative stress in pancreatic beta-cells of GK rats, a model of type 2 diabetes. *Diabetes* 48:927–932, 1999
33. Toyokuni S: Reactive oxygen species-induced molecular damage and its application in pathology. *Pathol Int* 49:91–102, 1999
34. Upchurch BH, Aponte GW, Leiter AB: Expression of peptide YY in all four islet cell types in the developing mouse pancreas suggests a common peptide YY-producing progenitor. *Development* 120:245–252, 1994
35. Larsson LI: On the development of the islets of Langerhans. *Microsc Res Tech* 43:284–291, 1998