Downregulation of Fas activity rescues early onset of diabetes in \(\text{c-Kit}^{Wv/+}\) mice

Zhi-Chao Feng,1,2 Matthew Riopel,1,4 Jinming Li,1,2 Lisa Donnelly,1 and Rennian Wang1,2,3

1Children’s Health Research Institute, London, Ontario, Canada; 2Departments of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada; 3Department of Medicine, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada; and 4Department of Pathology, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada

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Feng Z, Riopel M, Li J, Donnelly L, RW. Downregulation of Fas activity rescues early onset of diabetes in \(\text{c-Kit}^{Wv/+}\) mice. Am J Physiol Endocrinol Metab 67: E557–E565, 2013. First published December 26, 2012; doi:10.1152/ajpendo.00453.2012.— c-Kit and its ligand stem cell factor (SCF) are important for \(\beta\)-cell survival and maturation; meanwhile, interactions between the Fas receptor (Fas) and Fas ligand are capable of triggering \(\beta\)-cell apoptosis. Disruption of c-Kit signaling leads to severe loss of \(\beta\)-cell mass and function with upregulation of Fas expression in \(\text{c-Kit}^{Wv/+}\) mouse islets, suggesting that there is a critical balance between c-Kit and Fas activity in \(\beta\)-cells. In the present study, we investigated the interrelationship between c-Kit and Fas activity that mediates \(\beta\)-cell survival and function. We generated double mutant, \(\text{c-Kit}^{Wv/+};\text{Fas}^{lpr/lpr}\) (\(\text{Wv}^{-/-}\)), mice to study the physiological and functional role of Fas with respect to \(\beta\)-cell function in \(\text{c-Kit}^{Wv/+}\) mice. Isolated islets from these mice and the INS-1 cell line were used. We observed that islets in \(\text{c-Kit}^{Wv/+}\) mice showed a significant increase in \(\beta\)-cell apoptosis along with upregulated p33 and Fas expression. These results were verified in vitro in INS-1 cells treated with SCF or c-Kit siRNA combined with a p33 inhibitor and Fas siRNA. In vivo, \(\text{Wv}^{-/-}\) mice displayed improved \(\beta\)-cell function, with significantly enhanced insulin secretion and increased \(\beta\)-cell mass and proliferation compared with \(\text{Wv}^{+/+}\) mice. This improvement was associated with downregulation of the Fas-mediated caspase-dependent apoptotic pathway and upregulation of the cFlip/NF-κB pathway. These findings demonstrate that a balance between the c-Kit and Fas signaling pathways is critical in the regulation of \(\beta\)-cell survival and function.

c-Kit; Fas; \(\beta\)-cell apoptosis; insulin secretion; diabetes

THE PREVALENCE OF DIABETES MELLITUS has been increasing at an alarming rate. During the progression of diabetes, pancreatic \(\beta\)-cells are often lost because the delicate balance between \(\beta\)-cell proliferation and death is disrupted (4, 5, 45). Recent accumulating evidence has suggested that c-Kit, with its ligand stem cell factor (SCF), not only plays an essential role in hematopoiesis, melanogenesis, and gametogenesis (1), but also affects multiple pro-survival downstream signaling pathways, including the phosphoinositide 3-kinase (PI3K) pathway (1). Our previous studies showed that \(\text{c-Kit}^{Wv/+}\) mice, which contain a point mutation in the kinase region (\(\text{Wv}\)) of c-Kit, exhibit severe \(\beta\)-cell mass loss (16) that was associated with a significant downregulation of phospho-Akt/glycogen synthase kinase-3β (Gsk3β)/cyclin D1 signaling (9). However, the underlying mechanisms involved in c-Kit-mediated effects on \(\beta\)-cell survival have yet to be determined.

The Fas receptor (Fas) belongs to the tumor necrosis factor family and requires the Fas ligand (FasL) for activation. Fas/FasL interactions result in activation of Fas-associated death domains (FADD) and cleavage of caspase 8, which triggers apoptosis. In diabetes, \(\beta\)-cells constitutively express Fas (24, 26). Fas/FasL interactions were suggested to be one of the major mechanisms leading to \(\beta\)-cell apoptosis in \(T\) cell-mediated autoimmune diabetes (7, 10, 40). In addition, cytokine-induced upregulation of the Fas apoptotic pathway is also involved in glucotoxicity and subsequent increases in \(\beta\)-cell death (25); meanwhile, deletion of Fas protects human islet amyloid polypeptide deposition-mediated \(\beta\)-cell apoptosis (36). In vivo studies have demonstrated that nonobese diabetic mice with nonfunctional Fas (global \(lpr/lpr\) mutation) show protection against diabetes (10, 40), and transgenic mice with \(\beta\)-cell-specific knockout of Fas exhibit increased \(\beta\)-cell insulin secretion function (8).

There have been numerous studies in hematopoietic cells, melanocytes, and germ cells indicating that Fas-mediated cell apoptosis can be prevented by upregulation of SCF/c-Kit interactions (20, 33). Conversely, in cells with deficient c-Kit signaling, downregulation of Fas can rescue cell death and dysfunction (11, 15, 29, 39, 44). In the present study, we generated \(\text{c-Kit}^{Wv/+};\text{Fas}^{lpr/lpr}\) (\(\text{Wv}^{-/-}\)) double mutation mice to understand the interrelationship between c-Kit and Fas with respect to \(\beta\)-cell survival and function.

MATERIALS AND METHODS

Generation of \(\text{c-Kit}^{Wv/+}\) and \(\text{c-Kit}^{Wv/+};\text{Fas}^{lpr/lpr}\) mutant mice. C57BL/6J/Kit\(^{Wv/+}\) (\(\text{c-Kit}^{Wv/+}\)) and B6.MRL-\(\text{Fas}^{lpr/lpr}\) (\(\text{Fas}^{lpr/lpr}\)) mice both on a C57BL/6J background were obtained from the Jackson Laboratory (Bar Harbor, ME; stock no. 000499; stock no. 000482, respectively). \(\text{c-Kit}^{Wv/+}\) and \(\text{Fas}^{lpr/lpr}\) mice were crossbred to generate six phenotypes, including \(\text{c-Kit}^{-/-};\text{Fas}^{-/-}\) (\(\text{WT}\)), \(\text{c-Kit}^{-/-}\);\(\text{Fas}^{lpr/lpr}\) (\(\text{WT}^{-/-}\)), \(\text{c-Kit}^{Wv/+}\);\(\text{Fas}^{lpr/lpr}\) (\(\text{Wv}^{-/-}\)), and \(\text{c-Kit}^{Wv/+};\text{Fas}^{lpr/lpr}\) (\(\text{Wv}^{-/-}\)) mice. In the present study, \(\text{Wv}^{-/-}\) and \(\text{Wv}^{+/+}\) were the experimental groups, while WT was designated as the control group. WT^{-/-} was not included in cellular and molecular analyses due to insignificant differences observed in glucose metabolism compared with WT. The c-Kit Wv mutation was distinguished by fur pigmentation: black for WT and piebaldism for \(\text{Wv}\) (16). Meanwhile, the Fas \((lpr)\) mutation was identified by PCR with DNA isolated from tails (Table 1). In this study, male mice were used, and all experiments were performed under an approved protocol by the Western University Animal Use Subcommittee in accordance with the guidelines of the Canadian Council of Animal Care.
Table 1. Sequences of primers used in PCR for B6 MRL-Fas\(^{0w/+}\) mutation genotyping

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5’-&gt;3’</th>
<th>Primer Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>oIMR1678</td>
<td>GTA AAT AAT TGT GCT TCG TGA G</td>
<td>Common</td>
</tr>
<tr>
<td>oIMR1679</td>
<td>TAG AAA GTG CCA CCG GTG TG</td>
<td>Mutant</td>
</tr>
<tr>
<td>oIMR1680</td>
<td>CAA ATC TAG GCA TTA ACA GTG</td>
<td>Wild type</td>
</tr>
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From the Jackson Laboratory, Bar Harbor, ME.

**INS-1 cell culture.** INS-1 (832/13) cells were cultured in RPMI 1640 medium with 1-glutamine containing 10% fetal bovine serum, wherein medium was replaced every other day (FBS; Invitrogen, Burlington, ON, Canada) (16). After cells reached near-confluence, the following experiments were undertaken.

For the SCF study, INS-1 cells were first cultured in serum-free RPMI 1640 medium plus 1% BSA for 3 h. Cell cultures were then treated with SCF vehicle (10 mM acetic acid) as a treatment control, SCF (50 ng/ml; ID Labs, London, ON, Canada), or SCF plus 100 nM wortmannin (Sigma, St. Louis, MO) for 24 h (48). For the c-Kit and Fas siRNA transfection studies, INS-1 cells were cultured in RPMI 1640 medium plus 10% FBS overnight. Cells were then treated for 48 h with either control siRNA (sc-37007, proprietary sequence), c-Kit(r) siRNA (sc-63633) with or without a p53 inhibitor, 5 μM pituitrin-α (PTT-α; Sigma), or combined c-Kit(r) siRNA and Fas(r) siRNA (sc-270241; Santa Cruz Biotechnology, Santa Cruz, CA) using a siRNA transfection protocol described by the manufacturer (Santa Cruz Biotechnology) (48). The sequences for c-Kit(r) and Fas(r) siRNAs are listed in Table 2.

At the end of the culture period, cells were harvested and prepared for Western blot and the TUNEL assay. Experiments were performed using at least three to four different cell passages per experimental group, representing \( n = 3 \rightarrow 4 \).

**Body weight and in vivo metabolic studies.** Body weight, fasting blood glucose level, and intraperitoneal glucose and insulin tolerance tests (IPGTT and IPITT, respectively) were performed in \( W_v/w^- \) mice and compared with WT and \( W_v/w^+/- \) littersmates at 8 wk of age (9, 16). For the IPGTT and IPITT, an intraperitoneal injection of glucose (2 mg/g) was administrated after 4 h of fasting, and blood glucose levels were then examined at 0, 15, 30, 60, and 120 min after injection. Body area under the curve (AUC) was used to quantify responsiveness (9, 16). A minimum of 12 random islets (at least 1,000 cells) in each pancreatic section per experimental group was analyzed (9, 16), and at least five to seven mouse pancreata per experimental group were measured.

**RNA extraction and real-time qRT-PCR.** Islet RNA was extracted using the miRNeasy kit (Qiagen, Germantown, MD) (9, 16). Real-time qRT-PCR analyses were performed using the iQ SYBR Green Supermix kit in Chromo4 Real Time PCR (Bio-Rad Laboratories, Mississauga, ON, Canada). Relative levels of gene expression were calculated and normalized to the internal standard gene 18S rRNA with at least four repeats per age per experimental group (9, 16).

**Protein extraction and Western blot analyses.** Isolated islet protein from WT, \( W_v/w^-/\) and \( W_v/w^+/- \) mice and INS-1 cells was extracted in a Nonidet P-40 lysis buffer (9, 16). Equal amounts of lysate protein were separated by 10% or 12% SDS-PAGE and transferred to a nitrocellulose membrane (Bio-Rad Laboratories). Membranes were incubated with primary antibodies, followed by the horseradish peroxidase (HRP)-conjugated secondary antibodies (Cell Signaling, Danvers, MA). Protein bands were detected, imaged, and analyzed by densitometric quantification (9).

**Statistical analysis.** Data are expressed as means ± SE. Statistical significance was determined by unpaired Student’s t-test or ANOVA followed by the Bonferroni-Dunn post hoc test. Differences were considered to be statistically significant when \( P < 0.05 \).

**RESULTS**

c-Kit\(^{W_v^-}\) mice showed increased β-cell apoptosis due to increased p53 signaling and resulting upregulated Fas activity. c-Kit\(^{W_v^-}\) mice had severe β-cell loss and dysfunction, which was associated with Akt/Gsk3β/cyclin D1 pathway downregulation (9). Significantly elevated p53 mRNA expression and protein levels were observed in the islets of c-Kit\(^{W_v^-}\) mice compared with c-Kit\(^{+/-}\) mice (\( P < 0.05 \); Fig. 1A). Fas mRNA expression was significantly elevated in c-Kit\(^{W_v^-}\) islets (Fig. 1B), which was corroborated with increased Fas protein level (\( P < 0.05 \); Fig. 1B) and Fas-expressing insulin positive

Table 2. Pool of 3 different c-Kit(r) and Fas(r) siRNA duplexes

<table>
<thead>
<tr>
<th>Sense</th>
<th>Antisense</th>
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<tbody>
<tr>
<td>c-Kit siRNA (sc-36533)</td>
<td>1) CCAUGUGGUAGGAUAAAGUUGAAtt</td>
</tr>
<tr>
<td>2) GAUGUGUGGUUCCUCAAATGtt</td>
<td>UUUUGACGACAAAGACAGCtt</td>
</tr>
<tr>
<td>3) GCCAAGAAGUACAGUUAAtt</td>
<td>UAAUAGGCAGCUAUCUGGtt</td>
</tr>
<tr>
<td>Fas siRNA (sc-270241)</td>
<td>1) CCGAAGAAGUUGCAUGAUGtt</td>
</tr>
<tr>
<td>2) GCAUUDUUGUGAUGCUAUUt</td>
<td>AAUAGGCAACUGAAAGUUt</td>
</tr>
<tr>
<td>3) CAGCAGUACUGUUCUAUUt</td>
<td>AAUAGGCAACUUAACUGGtt</td>
</tr>
</tbody>
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From Santa Cruz Biotechnology, Santa Cruz, CA.
cells ($P < 0.01$; Fig. 1D) relative to the c-Kit$^{+/+}$ group. However, there was no significant alteration of FasL among all groups (Fig. 1C). A significant increase in β-cell apoptosis, as indicated by elevated TUNEL staining in insulin-positive cells, was observed in c-Kit$^{+/+}$ mice ($P < 0.01$; Fig. 1E).

In vitro studies on INS-1 cells were used to further understand the relationship between c-Kit, p53, and Fas in the regulation of β-cell survival. The expression of c-Kit and Fas mRNA in INS-1 cells was measured by qRT-PCR and showed relatively higher c-Kit and lower Fas expression in INS-1 cells compared with normal mouse islets (data not shown). INS-1 cells treated with exogenous SCF showed a slight reduction in p53 and a significant decrease in Fas protein levels, which was attenuated by the addition of the P38 inhibitor wortmannin (Fig. 2A). However, when INS-1 cells were transfected with c-Kit siRNA, the protein levels of p53, Fas, and cleaved caspase-3 were significantly elevated compared with the controls (Fig. 2B). Interestingly, cells transfected with c-Kit siRNA and treated with PFT-α (p53 inhibitor) or cotransfected with Fas siRNA displayed significantly abated protein levels of p53, Fas, and cleaved caspase-3 compared with c-Kit siRNA-transfected INS-1 cells (Fig. 2B). Fas protein levels were significantly reduced in the Fas siRNA group compared with the control siRNA group (Fig. 2C). Furthermore, INS-1 cell apoptosis was measured by TUNEL staining, which demonstrated a significant increase in the c-Kit siRNA group compared with other experimental conditions (Fig. 2D).

Downregulation of Fas in c-Kit$^{+/+}$ mice led to improved β-cell function. At 8 wk of age, there was no difference in body weight (Fig. 3A), fasting glucose levels were significantly lower in Wv$^{-/-}$ mice than in Wv$^{+/+}$ mice ($P < 0.05$; Fig. 3B) but significantly higher than in their WT littermates ($P < 0.01$; Fig. 3B). Wv$^{-/-}$ mice had significantly improved glucose tolerance, demonstrated by decreased AUC relative to Wv$^{+/+}$ mice ($P < 0.05$; Fig. 3C). There were no significant changes observed in insulin tolerance among the three different groups (Fig. 3D). In vivo GSIS analyses demonstrated that insulin secretion in Wv$^{-/-}$ mice was significantly higher at all three time points (0, 5, and 35 min after glucose challenge) compared with Wv$^{+/+}$ mice ($P < 0.05$; Fig. 3E). This was further corroborated by our observations from the ex vivo GSIS assay, which showed a significant increase in insulin secretion from Wv$^{-/-}$ mouse islets after incubation in 22 mM glucose compared with Wv$^{+/+}$ mouse islets ($P < 0.05$; Fig. 3F). However, the ability of Wv$^{-/-}$ mice islets to respond to a 22 mM glucose challenge was significantly lower compared with islets from WT mice ($P < 0.05$; Fig. 3F). Insulin secretion from the ex vivo GSIS assay was comparable to measured insulin content, which was significantly higher in islets from Wv$^{-/-}$ mice relative to Wv$^{+/+}$ mice ($P < 0.05$; Fig. 3G), but not significantly lower than in WT mice (Fig. 3G). It was noted that the effect of glucose on islet insulin secretion was smaller in all experimental groups compared with previous reports, which may be associated with increased sensitivity when islets are freshly isolated.

Increased β-cell mass and proliferation and decreased β-cell apoptosis observed in c-Kit/Fas double mutant mice. Pancreatic weight was unchanged between experimental groups at 8 wk of age; however, Wv$^{-/-}$ mice displayed a significant increase in islet number compared with Wv$^{+/+}$ mice ($P < 0.05$; Fig. 4B) and no difference compared with WT mice (Fig. 4B). Wv$^{-/-}$ mice exhibited a doubling of β-cell mass compared with Wv$^{+/+}$ mice ($P < 0.05$; Fig. 4C), but α-cell mass was unchanged (data not shown). Furthermore, Wv$^{-/-}$ mice showed a significant increase in β-cell proliferation, determined by Ki67 labeling ($P < 0.001$; Fig. 4, D and F), with a twofold decrease in TUNEL-positive β-cells compared with Wv$^{+/+}$ mice ($P < 0.05$; Fig. 4, E and F).

Downregulation of Fas in the absence of c-Kit signaling activated the cFlip/NF-κB pathway and increased islet transcription factor expression. To further investigate the underlying mechanisms of improved β-cell survival and function in
**Wv**−/− mice, qRT-PCR analysis of insulin, glucagon, pancreatic and duodenal homeobox 1 (Pdx-1), and v-Maf musculoaponeurotic fibrosarcoma oncogene family protein A (avian) (Mafa) showed significantly increased mRNA levels in Wv−/− mice compared with Wv+/+ and WT mice (Fig. 5A). The mRNA levels of cFlip, Fadd, RelA, RelB, and NFκb2 in isolated islets of Wv−/− mice were significantly increased compared with Wv+/+ mice (Fig. 5B). However, no significant changes were found in the mRNA levels of Bax and ratio of Bcl-2 over Bax between Wv+/+ and Wv−/− mouse islets (Fig. 5C). Furthermore, Western blot analysis showed downregulation of Fas expression which also led to a significant reduction of cleaved caspase-8 and -3 protein levels in Wv−/− mouse islets (Fig. 6), and this was correlated with reduced β-cell apoptosis in Wv−/− mice compared with Wv+/+ group (Fig. 4E).

**DISCUSSION**

In the present study, we have demonstrated that the loss of β-cell mass in c-Kit**Wv**−/− mice is due to increased p53 and Fas levels, along with associated downstream caspase-mediated β-cell death in c-Kit**Wv**−/− mouse islets, as outline in Fig. 7. Mutation of Fas (lpr) rescued early onset of diabetes in c-Kit**Wv**−/− male mice by enhancing insulin-secretory function and increasing β-cell mass and proliferation. This improvement was associated with downregulation of the Fas-mediated extrinsic apoptotic pathway and upregulation of the cFLIP/ NF-κB pathway, as well as the key islet transcription factors Pdx-1 and Mafa. Therefore, the present findings suggest that a balance between the c-Kit and Fas signaling pathways is required to maintain β-cell mass and function.

Both p53 and Fas levels were upregulated in the islets of c-Kit**Wv**−/− mice, which was correlated with increased β-cell apoptosis. It has been documented that p53 is an important checkpoint protein that regulates cyclin D1 expression during cell cycle progression in many cell types (38, 41). Although the cellular mechanism by which c-Kit regulates p53 is unclear, we have previously demonstrated that decreased cyclin D1 levels are observed in c-Kit**Wv**−/− mouse islets (9), suggesting that inhibition of cell cycle progression by c-Kit deficiency may be p53 dependent. Not only is p53 involved in cell cycle arrest, it...
also plays a pivotal role in cell apoptosis. Several apoptotic genes are upregulated by p53 (27, 30, 31, 47), in particular, p53 induces Fas mRNA expression by binding to elements that are found in the promoter and first intron regions of the Fas gene (30). Also, overexpression of p53 may promote trafficking of the Fas to the cell surface via the Golgi apparatus (3). Thus, the elevated p53 levels observed in c-Kit<sup>Wv</sup>/H<sup>11001</sup> mouse islets suggest that p53 serves as a mediator in cell cycle arrest and may induce Fas-mediated apoptosis in the absence of c-Kit signaling in vivo. FasL levels were slightly increased in c-Kit<sup>Wv</sup>/H<sup>11001</sup> mouse islets, suggesting that FasL was already present at sufficiently high levels and that β-cell apoptosis is dependent on Fas expression in islets. The interrelation among c-Kit, p53, and Fas was further examined by stimulation of c-Kit via up- and downregulation assays in INS-1 cells. Activation of c-Kit by exogenous SCF reduced both p53 and Fas protein levels in a PI3K-dependent manner. When cells were transfected with c-Kit siRNA, there was upregulation of p53 and Fas protein levels, which was reversed by the addition of a p53 inhibitor or Fas siRNA transfection and led to reduced apoptosis. These data suggest that c-Kit plays a key role in regulating p53-induced Fas-mediated cell death. p53-mediated Fas upregulation has been proposed by several in vitro (3, 30, 43) and rodent studies (6, 14) and is likely responsible for increasing β-cell apoptosis in c-Kit<sup>Wv</sup>/H<sup>11001</sup> mice.

To further elucidate the physiological role of Fas in the absence of c-Kit signaling in vivo, we generated Wv<sup>H11002</sup>/H<sup>11002</sup> mice bearing both c-Kit<sup>Wv</sup> and Fas<sup>lpr</sup> mutations and found that antiapoptotic genes such as cFlip, NF-κB2, and both RelA and RelB were significantly upregulated in Wv<sup>−/−</sup> mouse islets. It
is well established that Fas-mediated apoptosis occurs through activation of procaspase-8, the most upstream caspase in the Fas apoptotic pathway, while cFlip, a protease-deficient caspase homolog to caspase-8, is able to modulate activation of procaspase-8 and trigger pro-survival signaling (18). cFlip exerts its physiological function by triggering the NF-κB signaling pathway, which plays an important role in regulating cell survival and proliferation (13). Therefore, in Wv−/− mouse islets, an upregulation of cFlip could have enhanced NF-κB activity by increasing NF-κB-inducing kinase and RelB, leading to improved β-cell function and survival.

We further investigated the signaling pathway downstream of Fas that regulates β-cell apoptosis and dysfunctions in c-KitWv−/− mice. Cleaved caspase-8 and -3 levels were significantly increased in c-Kit siRNA-treated INS-1 cells and c-KitWv−/− mouse islets. However, downregulation of Fas in c-Kit-deficient islets resulted in significantly decreased cleaved caspase-8 and -3 levels compared with controls. Previous ex vivo studies with human pancreatic β-cells have shown that glucotoxicity-induced β-cell apoptosis occurs via upregulation of the Fas-mediated apoptotic pathway, which could be rescued by downregulation of Fas (25, 36). Furthermore, deletion of Fas in rodent pancreatic β-cells has shown protection against FasL-induced apoptosis and increased in vivo insulin secretion (8). While much evidence has pointed to Fas as an important mediator of β-cell apoptosis, downstream caspase-8 and -3 are also well known for their roles as the principal executioners of β-cells. In vivo, deletion of either caspase-8 or -3 in mouse β-cells has shown to be protective against low doses of streptozotocin-induced cell death (22, 23). Taken together, these results suggest that a loss of c-Kit signaling promotes β-cell apop-
tosis and dysfunction, in part by activation of the Fas-dependent apoptotic pathway.

In this study, we demonstrated that c-Kit deficiency led to an increase in Fas expression and a mutation in Fas rescued defects associated with the c-KitWv/H11001 mutation. The correlation between c-Kit and Fas signaling coincides well with previous studies, which suggest that PI3K/Akt signaling via c-Kit suppresses Fas-mediated apoptotic signaling by downregulating the expression of Fas. However, several lines of evidence have also demonstrated that SCF-induced PI3K/Akt pathway signaling can inactivate proapoptotic transcriptional factor FKHRL-1 (28) as well as Bim, a proapoptotic member of the Bcl-2 family (2). Therefore, our findings suggest that Fas-mediated apoptotic signaling is directly involved in β-cell dysfunction, especially when Fas is upregulated due to the c-Kit Wv mutation, but we also cannot exclude the participation of other apoptotic factors that may be regulated by the disrupted c-Kit/PI3K/Akt pathway.

The biological role of Fas signaling is becoming increasingly complex. Controversial findings have reported both apoptotic and antiapoptotic roles for Fas in the β-cell in vivo. A previous study has reported that Fas is essential for β-cell insulin-secretory function but not in the regulation of β-cell mass (42). Another study has shown that islet-specific Fas deletion is protective against FasL- and ceramide-induced apoptosis ex vivo and enhances β-cell insulin secretion in vivo (8). These discrepancies may be linked to differences in genetic backgrounds of the mouse strains used in these two studies. In the present study, Wv/− mice were obtained by crossing c-KitWv/H11001 and Faslpr/lpr mice, both from a C57BL/6J background. By use of the same mutant mouse model, previous studies have reported that loss of Fas function led to reduced apoptosis and improved function in granulosa...
cells, oocytes, and germ cells of c-Kit<sup>W+v</sup> mice (29, 39). In agreement with these findings, we propose that a balance between c-Kit and Fas signaling is important for β-cell survival and function.

Through analyses of INS-1 cells and c-Kit<sup>W+v</sup>; Fas<sup>−/−</sup> mice, our results suggest that SCF/c-Kit interactions prevent Fas-mediated β-cell apoptosis and dysfunction. This study advances the understanding of the relationship between c-Kit-mediated survival signals and Fas-mediated death signals in β-cells, which will assist in the development of protocols that maintain β-cell survival and function essential for cell-based therapies in the treatment of diabetes.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Z.-C.F., J.L., and L.D. performed the experiments; Z.-C.F., M.R., J.L., and L.D. analyzed the data; Z.-C.F., M.R., and R.W. interpreted the results of the experiments; Z.-C.F. prepared the figures; Z.-C.F. drafted the manuscript; Z.-C.F., M.R., L.D., and R.W. reviewed the manuscript; Z.-C.F., M.R., L.D., and R.W. contributed to the conception and design of the research.

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