ORIGINAL ARTICLE

Stromal Cell–Derived Factor-1 (SDF-1)/CXCL12 Attenuates Diabetes in Mice and Promotes Pancreatic β-Cell Survival by Activation of the Prosurvival Kinase Akt

Tatsuya Yano, Zhengyu Liu, Jennifer Donovan, Melissa K. Thomas, and Joel F. Habener

OBJECTIVE—Diabetes is caused by a deficiency of pancreatic β-cells that produce insulin. Approaches to enhance β-cell mass by increasing proliferation and survival are desirable. We determined whether stromal cell–derived factor (SDF)-1/CXCL12 and its receptor, CX chemokine receptor (CXCR)4, are important for the survival of β-cells.

RESEARCH DESIGN AND METHODS—Mouse pancreata and clonal β-cells were examined for expression of SDF-1 and CXCR4, activation of AKT and downstream signaling pathways by SDF-1, and protection against apoptosis and diabetes induced by streptozotocin (STZ).

RESULTS—CXCR4 is expressed in β-cells, and SDF-1 is expressed in microvascular endothelial cells within the islets and in surrounding interstitial stromal tissue. Transgenic mice overexpressing SDF-1 within their β-cells (RIP-SDF-1 mice) are resistant to STZ-induced β-cell apoptosis and diabetes. In MIN6 β-cells, a CXCR4 antagonist (AMD3100) induces apoptosis, increases reactive oxygen species, decreases expression levels of the anti-apoptotic protein Bcl-2, and reduces phosphorylation of the proapoptotic protein Bad. Active phosphorylated prosurvival kinase Akt is increased both in the β-cells of RIP-SDF-1 mice and in INS-1 cells treated with SDF-1 and sensitive to AMD3100. Inhibition of AKT expression by small interfering RNA attenuates the ameliorative effects of SDF-1 on caspase-dependent apoptosis induced by thapsigargin or glucose deprivation in INS-1 β-cells. Specific inhibition of Akt activation by a soluble inhibitor (SH-5) reverses the anti-apoptotic effects of SDF-1 in INS-1 cells and mouse islets.

CONCLUSIONS—SDF-1 promotes pancreatic β-cell survival via activation of Akt, suggesting that SDF-1 agonists may prove beneficial for treatment of diabetes. ***Diabetes* 56:2946–2957, 2007**

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CREB, cAMP-responsive element binding protein; CXCR, CX chemokine receptor; GLP, glucagon-like peptide; PI, phosphatidylinositol; ROS, reactive oxygen species; SDF, stromal cell–derived factor; siRNA, small interfering RNA; SMA, smooth muscle actin; STZ, streptozotocin; TUNEL, transferase-mediated dUTP nick-end labeling.

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A contributing factor to the causation of diabetes is an insufficient mass of β-cells required to provide insulin in the amounts needed to maintain glucose homeostasis. The mass of β-cells is determined by the relative rates of β-cell formation, by neogenesis and/or replication, and by the rates of apoptosis. An understanding of the factors that control β-cell growth and survival could provide new rational approaches for the treatment of diabetes.

Stromal cell–derived factor (SDF)-1/CXCL12 is a peptide chemokine initially identified in bone marrow–derived stromal cells and now recognized to be expressed in stromal tissues in multiple organs (1–3). SDF-1 is 1 of over 40 chemokines for which there are 18 known receptors. The SDF-1 receptor, CX chemokine receptor (CXCR)4, is highly specific for SDF-1; the SDF-1/CXCR4 ligand-receptor pair is uniquely without crosstalk with other chemokines or receptors. SDF-1 and CXCR4 modulate cell migration and survival during development and tissue remodeling (4,5). A major function of the SDF-1/CXCR4 axis is chemoattraction during leukocyte trafficking and stem cell homing, in which local tissue gradients of SDF-1 attract circulating hematopoietic and tissue-committed somatic stem cells (3). The SDF-1/CXCR4 axis is involved in broader aspects of development, tissue repair and regeneration, and cancer (1–3). SDF-1 expressed in bone marrow inhibits the apoptosis of myeloid progenitor cells and promotes their survival (6,7). CXCR4 is expressed in human embryonic stem cells destined to become endoderm (8). Malignant cancer cells also express CXCR4, and their survival and migration to distant tissues is promoted by SDF-1 (9). Experimental disruption in mice of either the SDF-1 or CXCR4 genes results in late embryonic lethality with multiple generalized developmental defects in organogenesis. SDF-1 is expressed in both endothelial and mesenchymal cells (10–12). Cultured primary endothelial cells express SDF-1, where it is required for the regulation of branching morphogenesis (10). However, other reports suggest that endothelial cells display SDF-1 by the transcytosis of SDF-1 produced by perivascular fibroblast-like cells (13,14).

In the pancreas of γ-interferon transgenic mice, a model of pancreas regeneration, SDF-1 and CXCR4 are expressed within islets and in and around proliferating ductal epithelium (15). SDF-1 activates Src, Akt, and Erk in the ductal cells of NOD mice and induces migration and supports survival of duct cells, suggesting that SDF-1 may be obligatory for pancreatic regeneration from cells of ductal origin (15). However, the physiological relevance of the

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SDF-1/CXCR4 axis for differentiated β-cell functions in normal pancreatic islets remains unclear.

Several reports indicate that the serine/threonine kinase Akt/protein kinase B, a downstream mediator of insulin, IGF-1, and glucagon-like peptide (GLP)-1 signaling, is essential for the survival and proliferation of islet β-cells (16–19). However, mice with genetic inactivation of either the insulin receptor (BIRKO mice) (20) or the IGF-1 receptor (BIGFKO mice) (21) exhibit normal islet development, although BIRKO mice show a lag in normal age-dependent increases of β-cell mass. The incretin hormones GLP-1 and glucose-dependent insulinotropic polypeptide enhance Akt activity in islet β-cells (19,22), but GLP-1 and glucose-dependent insulinotropic polypeptide receptor null mice have normal islet development (23). Therefore, the regulation of Akt activity and the proliferation of β-cells by insulin/IGF-1 and incretin hormones may be primarily directed toward the maintenance of β-cell mass in the adult pancreas. SDF-1 also stimulates Akt activity via the coupling of phosphatidylinositol (PI)-3 kinase-γ to Gi (24). However, it is not yet known whether SDF-1 regulates Akt activity in adult differentiated β-cells.

Taking the earlier studies into consideration, we hypothesized that the survival of pancreatic β-cells may be supported by the SDF-1/CXCR4 axis. In this report, we define the sites of expression of SDF-1 and CXCR4 in the adult mouse pancreas and present evidence that SDF-1 promotes β-cell survival through the activation of Akt in cultured clonal β-cells, mouse islets, and transgenic mice.

RESEARCH DESIGN AND METHODS

The RIP-SDF-1 (transgenic mice overexpressing SDF-1 within their β-cells) model, in which murine SDF-1 is expressed under the regulation of the rat insulin-2 promoter (RIP), was reported earlier (25). Mice obtained from Dr. J. Cyster were bred under the regulations of the institutional animal care and use committee for the Massachusetts General Hospital. All experiments were conducted using young mice within 10 weeks after birth because of published observations that a subset of RIP-SDF-1 mice developed small lymphocytic infiltrates consisting of dendritic, plasma, and B- and T-cells within pancreatic islets after 4 months of age (25). For genotyping, genomic DNA was prepared from mouse tails using the Qiagen DNeasy Tissue Kit (Qiagen Sciences, Germantown, MD). PCR was performed using TaKaRa (Takara Bio, Otsu, Japan) recombinant *Taq*DNA polymerase and primers RIP7 (5′-CAACCCTGA CTATCTTCCAG-3′) and SDF-1 reverse (5′-CTTGTTTAAAGCTTTCTCCAGGT AC-3′).

Cell culture. MIN6 cells were cultured in Dulbecco's modified Eagle's medium containing 15% fetal bovine serum. INS-1 cells were cultured in RPMI-1640 with 10% fetal bovine serum. The treatment of MIN6 cells with the 12G5 monoclonal antibody was conducted in serum-free medium. An expanded version of RESEARCH DESIGN AND METHODS is available in an online appendix (available at http://dx.doi.org/10.2337/db07-0291).

RESULTS

Pancreatic expression of SDF-1 and CXCR4. To determine the sites of expression of SDF-1 and its receptor CXCR4, immunohistochemical analyses were conducted on pancreas sections from 10-week-old normal adult mice. CXCR4 is strongly expressed in normal adult islets and also in some ducts and unidentified extra-islet cells (Fig. 1*A*). All three major endocrine cells types of the islets—α-, β-, and δ-cells—express CXCR4. Only a minority of α-cells coexpress glucagon and CXCR4. These observations are in agreement with a previous report describing CXCR4 expression in pancreatic islets of the NOD mouse (15) and implicate the SDF-1/CXCR4 axis in the endocrine functions of pancreatic islets.

In contrast to CXCR4, which is expressed in cells thoughout the islets, SDF-1 is only expressed in a small subpopulation of islet cells in adult mice. The majority of SDF-1 expression in the pancreas is in cells within the interstitium surrounding the ducts and blood vessels of the exocrine parenchyma (Fig. 1*B*). By costaining the islets with BS-1 lectin, a specific marker of the microvascular endothelial cells (26), and anti–SDF-1 monoclonal antibody, the small subpopulation of SDF-1–expressing cells in the islets were identified as vascular endothelial cells (Fig. 1*C*). Pancreatic ducts and surrounding periductal interstitial and vascular tissues also were analyzed by immunostaining with *Dolichos biflorus* lectin (DBA) to identify ductal epithelium, von Willebrand factor to indicate vascular endothelium, anti–smooth muscle actin (SMA) to mark vascular smooth muscle and myofibroblasts, and anti–SDF-1 antibodies (Fig. 1*D*). As a result of this analysis, we conclude that SDF-1–expressing cells surrounding the ducts and adjacent small blood vessels have an expression pattern similar to that of SMA-positive myofibroblasts. SDF-1–expressing cells also surround large blood vessels (Fig. 1*B*, arrowhead). These observations indicate that SDF-1 is expressed in endothelial cells of the islet microvasculature and in stromal myofibroblasts within the periductal and perivascular interstitium. These findings suggest the existence of a paracrine signaling network between the islet microvascular endothelial cells and the vascular smooth muscle cells that produce SDF-1 and the islet endocrine and ductal epithelial cells that express CXCR4, the SDF-1 receptor.

Characteristics of RIP-SDF-1 mice. To investigate the functions of SDF-1 in pancreatic β-cells in vivo, we examined 4-week-old transgenic mice that express SDF-1 in pancreatic β-cells (25). The β-cells in the RIP-SDF-1 mouse pancreas expressed SDF-1 as previously reported and as expected (25) (supplemental Fig. 1*A*). In wild-type mouse islets, we observed only occasional SDF-1–expressing cells, already identified as the microvascular endothelial cells in the previous analysis. RIP-SDF-1 mice showed no obvious differences from age-matched wild-type C57Bl/6 mice in baseline metabolic parameters between 9 and 10 weeks of age (Table 1).

SDF-1 expression in pancreatic β-cells activates Akt and confers resistance against streptozotocin-induced diabetes. We next examined Akt phosphorylation states in RIP-SDF-1 mouse islets. Immunohistochemical analysis with anti–phospho-Ser^{473} Akt antibody demonstrated that cells containing activated Akt are present in the peripheral areas adjacent to the islets and in periductal regions in the wild-type mouse pancreas. However, in RIP-SDF-1 mice, Akt phosphorylation was enhanced in β-cells and in cells located in the periphery of the islet (Fig. 2*A* and supplemental Fig. 1*B*).

To determine whether β-cell survival is promoted by the SDF-1/CXCR4 axis, RIP-SDF-1 mice were injected with streptozotocin (STZ), known to induce diabetes by apoptosis of β-cells. STZ administration induced diabetes in wild-type mice, whereas transgenic mice maintained blood glucose levels substantially lower than those of wild-type mice after 2 weeks (Fig. 2*B*). Consistent with the differences in the glucose levels, the reduction of body weight of RIP-SDF-1 mice was also less than that of wild-type mice after STZ injection (Fig. 2*C*). Therefore, SDF-1 overexpression in the β-cells protected the transgenic mice from STZ-induced diabetes, possibly through the activation of Akt within β-cells.

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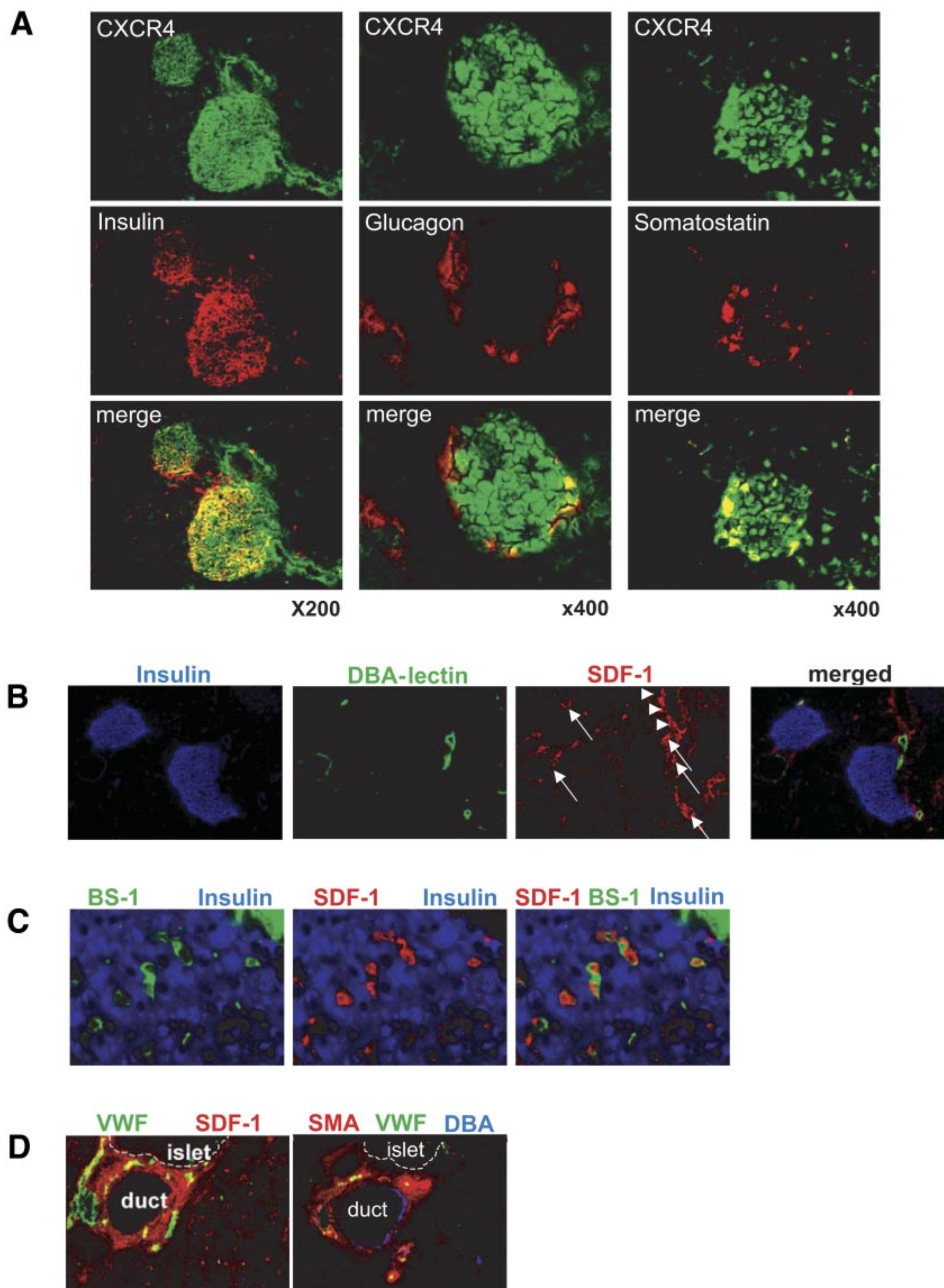

FIG. 1. CXCR4 and SDF-1 expression in 10-week-old mouse pancreas and islets. The majority of SDF-1 expression in the pancreas is in cells within the interstitium surrounding the ducts (identified by their staining with DBA) and blood vessels (arrows) of the exocrine parenchyma. *A*: CXCR4: Insulin, glucagon, and somatostatin antisera (red) and CXCR4 antiserum (green). *B*: SDF-1: Insulin (blue), SDF-1 (red), and DBA (green). Arrows and arrowheads indicate SDF-1 surrounding ducts and vessels, respectively (×200). *C*: SDF-1 in islets: SDF-1 (red), BS-1 lectin (green), and insulin (blue) (×400). *D*: SDF-1 in periductal regions: *left*, SDF-1 (red) and von Willebrand Factor (green); *right*, α-SMA (red), von Willebrand Factor (green), and DBA (blue). (Please see http://dx.doi.org/10.2337/db07-0291 for a high-quality digital representation of this figure.)

Islet morphology after STZ administration. To further understand the underlying mechanism of the resistance to STZ observed in the RIP-SDF-1 transgenic mice, islet morphologies were examined 6 h and 2 weeks after STZ administration. Pancreas sections were stained with DAPI, a specific nuclear stain. Numerous condensed and brightly glowing pyknotic nuclei characteristic of cells undergoing apoptosis were observed only in the islets 6 h after STZ administration. These findings indicate destruction of β-cells by apoptosis (Fig. 3*A*). However, the numbers of pyknotic nuclei were reduced in the islets of RIP-SDF-1 mice compared with those of wild-

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TABLE 1
Metabolic characteristics of RIP-SDF-1 mice

	Fed		Fasting	
	Wild type	RIP-SDF-1	Wild type	RIP-SDF-1
Body weight (g)	19.2 ± 0.31	20.0 ± 0.28	18.7 ± 0.20	18.3 ± 0.34
Glucose (mg/dl)	124 ± 8.14	119 ± 10.7	121 ± 6.66	105 ± 3.54
Insulin (ng/ml)	386 ± 19.5	411 ± 40.5	368 ± 13.7	335 ± 10.6

Each measurement was compared between wild-type and RIP-SDF-1 mice at 10 weeks of age by Student's t test, and no significant differences were observed. Values are means ± SEM of n = 7–9 female mice.

type mice. Notably, apoptotic cells were observed more frequently in the cores than in the peripheral regions of the transgenic mouse islets. Anti-insulin antibody and DAPI costaining showed that more surviving cells with normal nuclei located in peripheral areas of the transgenic mouse islets were insulin-expressing endocrine cells than in wild-type mouse islets (Fig. 3*B*). We also detected apoptotic cells by transferase-mediated dUTP nick-end labeling (TUNEL) staining and counted the TUNEL-positive β-cells in each islet of the wild-type and transgenic mice (Fig. 3*C* and *D*). Consistent with the findings in Fig. 3*A* and *B*, most of the TUNEL-positive β-cells were located in the core region of the islets, and β-cells located in the periphery of the islets were negative in the TUNEL assay. The TUNEL-positive β-cell ratios per islet in the wild-type and RIP-SDF-1 mice were 75.0 ± 2.78 and 26.9 ± 7.48%, respectively, showing a statistically significant difference ($P < 0.0001$). We next examined the expression of activated Akt (phospho-Akt) in the islets in response to STZ. Akt activation in the islets of wild-type and RIP-SDF-1 transgenic mice 6 h after the administration of STZ showed the appearance of phospho-Akt in the α-cells located at the periphery of the islets (Fig. 3*E*). In RIP-SDF-1 and not wild-type islets, phospho-Akt appeared in β-cells adjacent to the α-cells at the islet periphery.

At 2 weeks after STZ injection, small endocrine cell clusters, the majority of which were predominantly glucagon-expressing α-cells, were observed in the wild-type mouse pancreas (Fig. 3*F*). However, in the transgenic mice, a large proportion of β-cells were retained in the endocrine cell clusters. These preserved β-cells may represent STZ-resistant β-cells that retain their ability to secrete insulin in response to glucose, indicating that

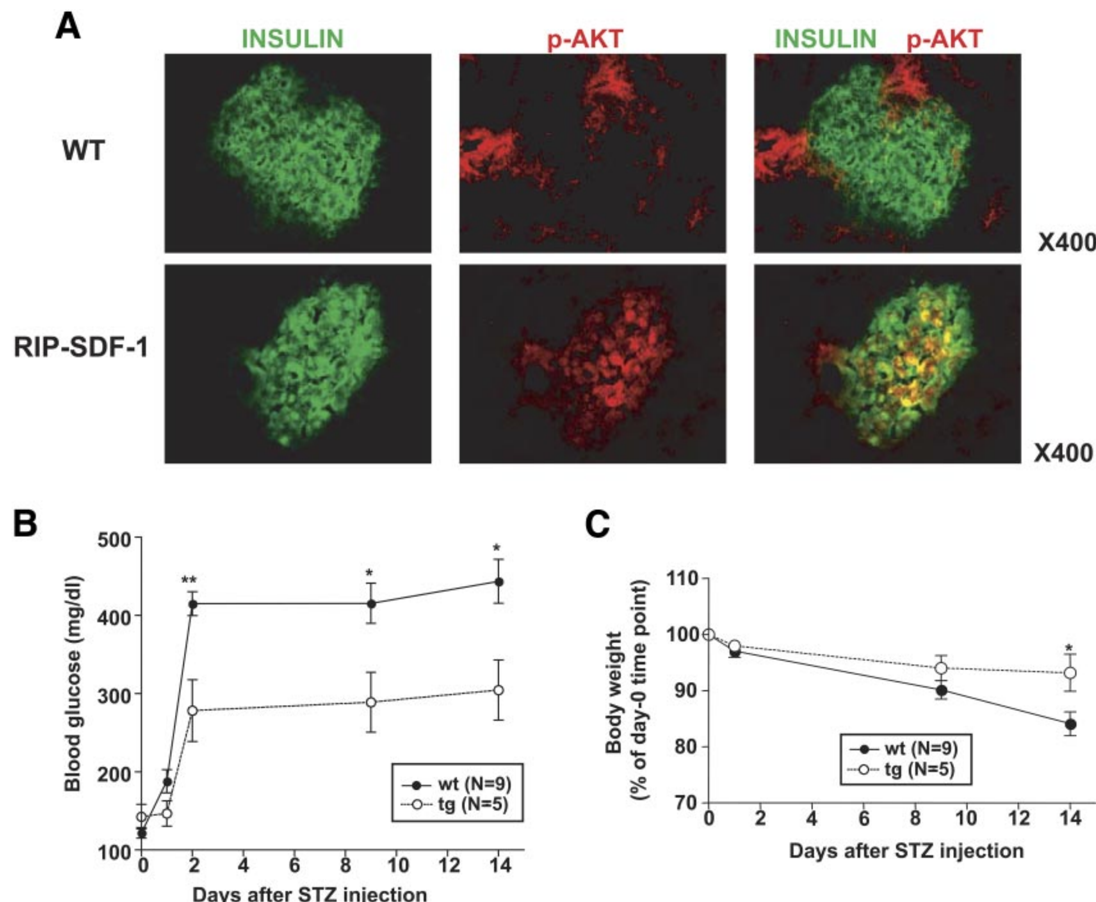

FIG. 2. Enhanced AKT phosphorylation in islets and resistance to STZ-induced diabetes in RIP-SDF-1 mice. *A*: Pancreas sections from 4-week-old wild-type (WT) and RIP-SDF-1 mice. Insulin (green) and phospho-Ser^{473} Akt (p-Akt) (red). *B*: Plasma glucose after STZ administration in wild-type (wt) and RIP-SDF-1 (tg) mice. *C*: Body weights after STZ administration in wild-type (wt) and RIP-SDF-1 (tg) mice (*P < 0.05; **P < 0.01). (Please see http://dx.doi.org/10.2337/db07-0291 for a high-quality digital representation of this figure.)

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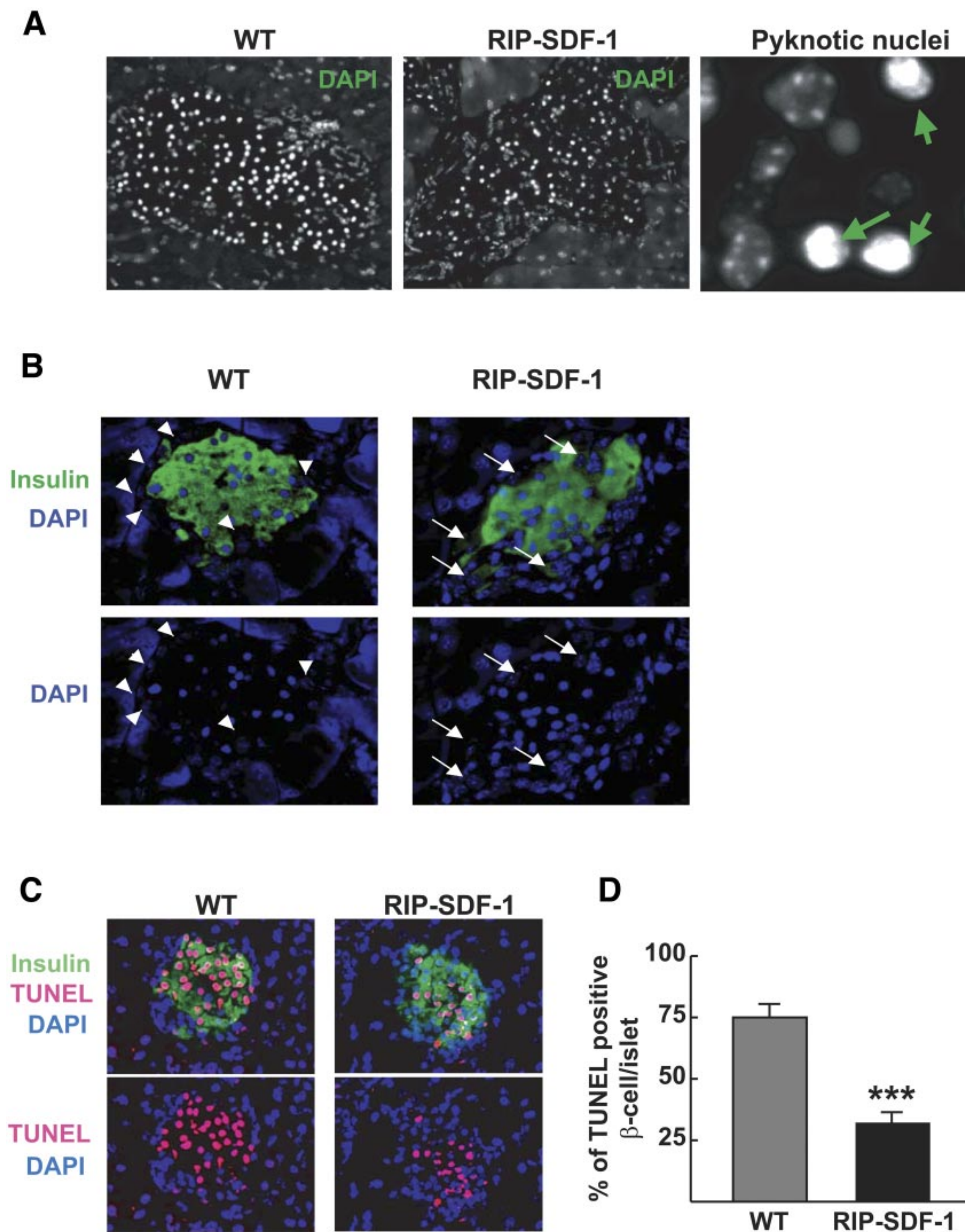

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SDF-1 expression in the islet β-cells can protect them from apoptosis induced by STZ.

CXCR4 antagonists induce apoptosis in MIN6 cells. To further explore signal transduction mechanisms of the SDF-1/CXCR4 axis in islet endocrine cells, studies were carried out in MIN6 and INS-1 cells, clonal β-cell lines in which it is feasible to conduct such studies. Immunostaining and RT-PCR of MIN6 cells showed coexpression of SDF-1 and CXCR4 in MIN6 cells in culture (Fig. 4*A* and *B*). In contrast, quantitative RT-PCR revealed that INS-1 cells express considerably lower levels of SDF-1 mRNA than MIN6 cells. Both cell lines expressed similar levels of CXCR4 mRNA (supplemental Fig. 2). These observations directed the use of SDF-1 inhibitors in MIN6 cells and SDF-1 agonists in INS-1 cells. To explore the possible functions of SDF-1 in MIN6 cells, CXCR4 antagonists were used to elucidate the SDF-1 functions. A small molecule CXCR4 antagonist, AMD3100, dose-dependently inhibited the growth of MIN6 cells as measured by DNA content (Fig. 4*C*). Next, we examined whether apoptosis contributed to the growth inhibition manifested by the CXCR4 inhibitors. The levels of fragmented DNA, a measure of apoptosis, were increased in MIN6 cells treated with AMD3100, as well as with STZ, a known inducer of apoptosis (Fig. 5*A*). Induction of apoptosis by the CXCR4 inhibitors also was examined with the TUNEL assay. Both AMD3100 and 12G5, a monoclonal inhibitory antibody to CXCR4, increased the numbers of TUNEL-positive MIN6

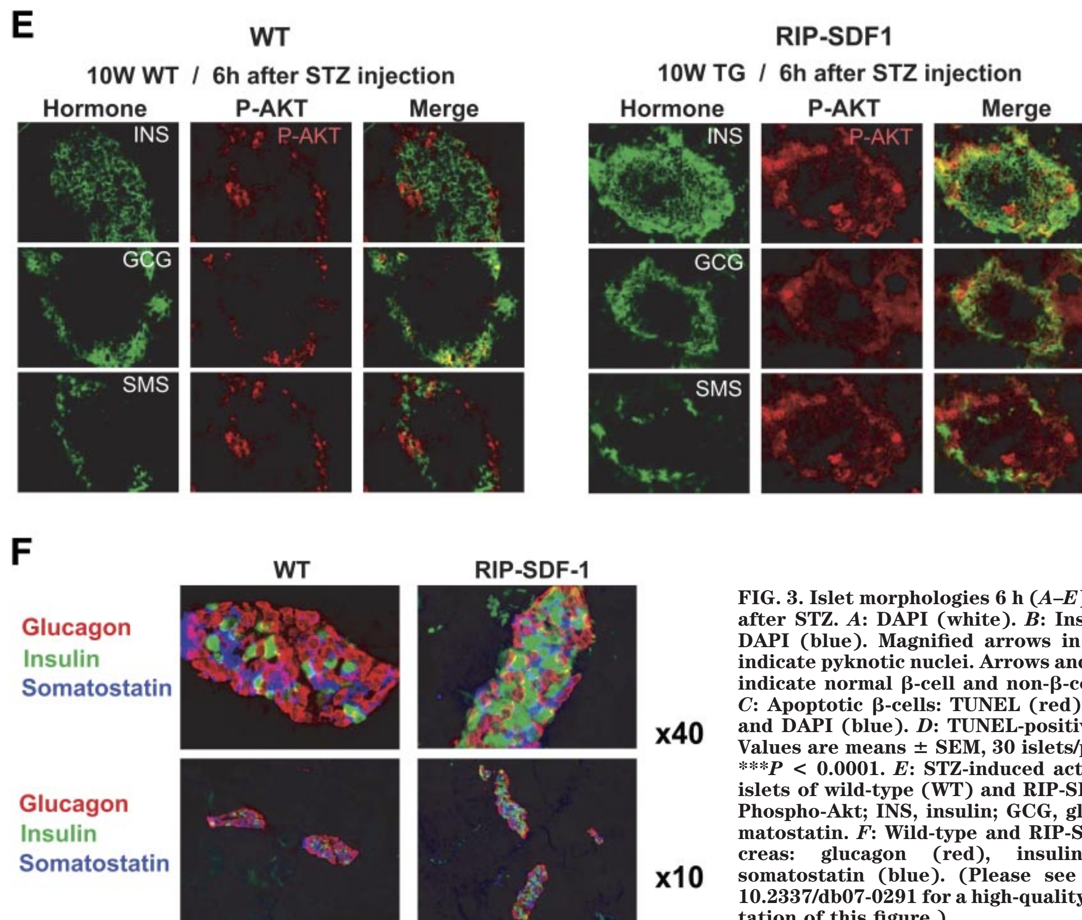

FIG. 3. Islet morphologies 6 h (*A–E*) and 2 weeks (*F*) after STZ. *A*: DAPI (white). *B*: Insulin (green) and DAPI (blue). Magnified arrows in *A* (*right panel*) indicate pyknotic nuclei. Arrows and arrowheads in *B* indicate normal β-cell and non-β-cells, respectively. *C*: Apoptotic β-cells: TUNEL (red), insulin (green), and DAPI (blue). *D*: TUNEL-positive β-cells (islet). Values are means ± SEM, 30 islets/pancreas ($n = 3$); $***P < 0.0001$. *E*: STZ-induced activation of Akt in islets of wild-type (WT) and RIP-SDF-1 mice. P-Akt, Phospho-Akt; INS, insulin; GCG, glucagon; SMS, somatostatin. *F*: Wild-type and RIP-SDF-1 mouse pancreas: glucagon (red), insulin (green), and somatostatin (blue). (Please see http://dx.doi.org/10.2337/db07-0291 for a high-quality digital representation of this figure.)

cells (Fig. 5*B* and *C*). Background apoptosis rates were lower in the AMD3100 experiments conducted in the presence of serum compared with the 12G5 experiments conducted in the absence of serum.

In addition, AMD3100 enhanced the activation of the proapoptosis enzyme caspase-3 in the MIN6 cells, as did STZ (Fig. 5*D*). Because the production of reactive oxygen species (ROS) by mitochondria is one of the major triggers of caspase-3 activation and apoptosis, we also measured the intracellular levels of ROS using the fluorescent indicator CMH_2DCFDA. Treatment of MIN6 cells with both CXCR4 inhibitors increased amounts of ROS though less potently than STZ treatment (Fig. 5*E*). These results suggest that the apoptosis induced by CXCR4 inhibitors may be mediated by mitochondrial dysfunction. To further explore the mechanism of induction of apoptosis by the inhibitors, expression levels of anti- and proapoptotic Bcl-2 family proteins were examined using immunoblot analysis. The expression levels of Bcl-2, an antiapoptotic protein, were clearly decreased in AMD3100-treated MIN6 cells, whereas the levels of the proapoptotic proteins Bcl-xL, Bax, and Bad remained unchanged (Fig. 6*A* and *B*). The amount of phosphorylated Bad was also reduced in AMD-treated cells. Phosphorylated Bad is required to prevent the heterodimerization and inactivation of antiapoptotic Bcl-2 and Bad (27). These observations suggest that the inhibition of SDF-1/CXCR4 signaling in MIN6 cells dysregulates the functions of the Bcl-2 family network, leading to mitochondrial dysfunction and apoptosis.

Suppression of Akt phosphorylation by the CXCR4 antagonist AMD3100. Because Akt is widely recognized to have an important role in the survival of islet β-cells, its phosphorylation status in the AMD3100-treated MIN6 cells was examined with an anti–phospho-Ser^{473} Akt antibody. Akt phosphorylation at Ser^{473}, critical for its activation, was reduced in the AMD3100-treated cells, while the phosphorylation of Erk was unchanged (Fig. 6*C*). To examine the effects of directly adding SDF-1 to β-cells on the activation of Akt, the clonal β-cell line INS-1 was used. SDF-1 dose-dependently activated Akt, as indicated by increased AKT phosphorylation detected on Western immunoblots (Fig. 6*D*, *left*). The phosphorylation of Akt induced by SDF-1 was inhibited by the CXCR4 antagonist AMD3100 (Fig. 6*D*, *right*).

These findings support the observations of the dysregulation of Bcl-2 family proteins described above because Akt is believed to regulate Bcl-2 expression through the activation of cAMP-responsive element binding protein (CREB) (28) and to directly phosphorylate the anti-apoptotic protein Bad at Ser^{136} (29) (Fig. 6*E*). These observations in MIN6 and INS-1 cells suggest that SDF-1 signaling through CXCR4 promotes β-cell survival through the activation of Akt.

Antiapoptotic actions of SDF-1 mediated by the activation of Akt. To further explore whether the antiapoptotic functions of SDF-1 are mediated by the activation of Akt, we investigated the effects of SDF-1 on Akt activation in the prevention of apoptosis in INS-1 cells and in primary pancreatic islet cells. The activation of caspase-3 was measured to evaluate the extent of apoptosis during either glucose deprivation or treatment with thapsigargin. Glucose deprivation induces apoptosis in β-cells (30,31). Thapsigargin inhibits endoplasmic reticulum Ca^{2+} ATPase, produces endoplasmic reticulum stress,

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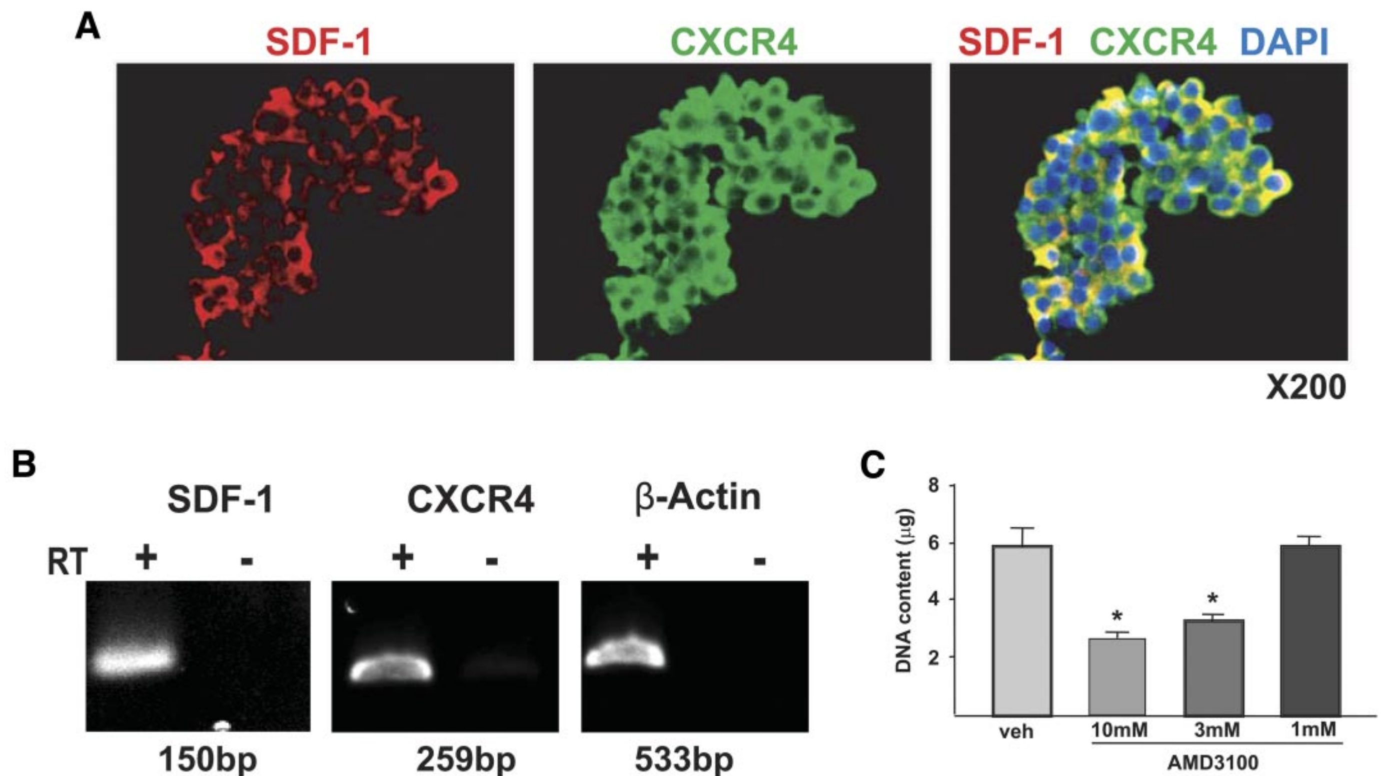

FIG. 4. Expression of SDF-1 and CXCR4 in MIN6 cells and induction of apoptosis by the CXCR4 antagonist AMD3100. *A*: SDF-1 (red), CXCR4 (green), and DAPI (blue). *B*: RT-PCR of SDF-1, CXCR4, and β-actin. *C*: DNA content in cells cultured with CXCR4 antagonist AMD3100 for 6 days (mean ± SEM, $n = 3$; $*P < 0.05$). veh, vehicle. (Please see http://dx.doi.org/10.2337/db07-0291 for a high-quality digital representation of this figure.)

and induces apoptosis in INS-1 cells (32,33). Glucose deprivation induced caspase-3 activity by 80%, and this effect was completely reversed by the concurrent incubation with SDF-1 (Fig. 7*A*, *left*). Thapsigargin induced caspase activity fourfold, and SDF-1 coincubation attenuated the proapoptotic effect of thapsigargin to activate caspase-3 (Fig. 7*A*, *right*). To investigate the role of Akt in anti-apoptotic effects of SDF-1, we measured caspase-3 activity under conditions in which endogenous Akt activity was inhibited or its expression reduced. SH-5, a phosphatidylinositol analog that prevents membrane recruitment of Akt and subsequent activation by membrane-associated phosphoinositide–dependent kinase, is a specific inhibitor for Akt (34,35). SH-5 decreases phosphorylation of Akt without affecting total Akt expression levels.

Coincubation of SDF-1 and the Akt inhibitor SH-5 reversed the anti-apoptotic effect of SDF-1 in the setting of both glucose deprivation–induced (Fig. 7*A*) and thapsigargin-induced apoptosis (Fig. 7*B*). To substantiate the findings in clonal β-cells, we examined isolated mouse islets ex vivo. We found that inhibition of Akt by SH-5 prevented the anti-apoptotic actions of SDF-1 on apoptosis induced by either thapsigargin or STZ (supplemental Fig. 3). Thus, the anti-apoptotic actions of SDF-1 appear to require activation of Akt in mouse islets and clonal β-cells. We also used a small interfering RNA (siRNA) knockdown strategy to reduce the expression of Akt in INS-1 cells. Quantitative RT-PCR and immunoblot analyses confirmed that both mRNA and protein levels of Akt were decreased by 80% by the introduction of Akt siRNA (Fig. 7*C*). Reduction of Akt expression with Akt siRNA reversed the anti-apoptotic effects of SDF-1 in the context of glucose deprivation– and thapsigargin-induced apoptosis (Fig. 7*B*). These findings indicate that SDF-1 exerts strong anti-apoptotic effects on INS-1 β-cells, and such effects are dependent on the activation of Akt.

DISCUSSION

In our study, we found the expression of SDF-1 in the endothelial and vascular smooth muscle cells of the islet microvasculature and expression of its receptor CXCR4 in islet endocrine cells. Earlier reports indicate that the islet microvasculature has an important role in supplying nutrients and oxygen to the islets and in the functioning of endocrine cells (36). Islet endothelial cells have unique protein expression patterns (37) and produce several growth factors (36). Hepatocyte growth factor–containing conditioned medium prepared from islet-derived endothelial cells stimulates pancreatic endocrine cell proliferation, and additional paracrine factors produced by the endothelial cells also may regulate β-cell mass (38,39). We also observed the expression of SDF-1 throughout the pancreas in cells residing within the periductal and perivascular interstitium. These mesenchymal-like cells are variously referred to as myofibroblasts, stellate cells, pericytes, and stromal cells. The origins and functions of these cells remain ill defined. These cells also express the neural stem cell marker nestin (40,41).

We demonstrated that SDF-1 promotes pancreatic β-cell survival in RIP-SDF-1 transgenic mice and in MIN6 and INS-1 clonal β-cells and does so through the activation of Akt. Our findings suggest the existence of paracrine signaling between endothelial and/or vascular smooth muscle and stromal cells and islet endocrine cells mediated by the SDF-1/CXCR4 axis. SDF-1 is involved in the development

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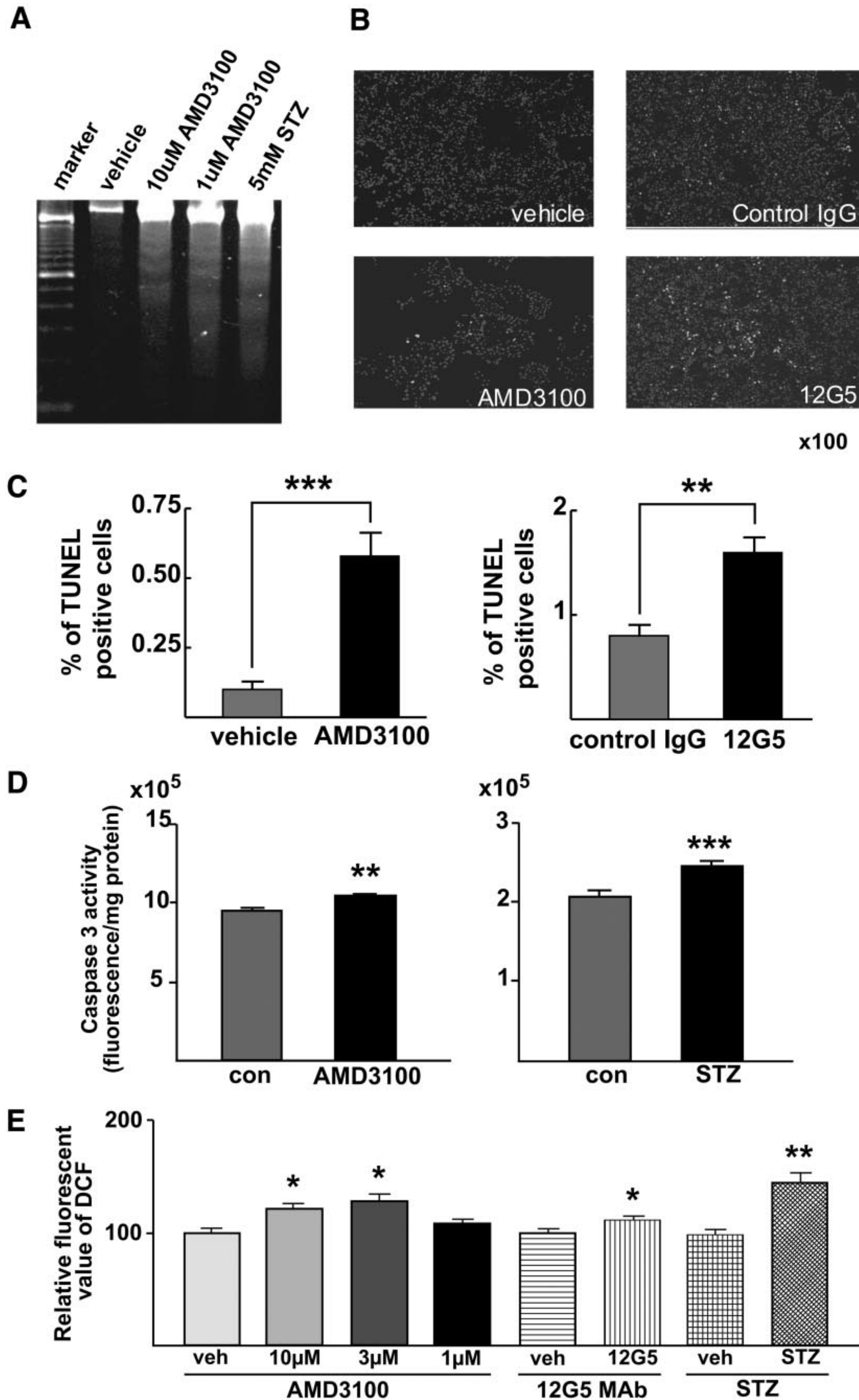

FIG. 5. Inhibition of CXCR4 signaling increases apoptosis in MIN6 cells. ***A*: DNA fragmentation after AMD3100. *B*: Apoptosis (TUNEL) after AMD3100 (*left panel*), control IgG, or monoclonal antibody 12G5 (*right panel*) for 48 h. TUNEL-positive cells, white; DAPI, gray. *C*: Percentage of TUNEL-positive cells (mean ± SEM of 15 fields, $n = 3$; ** $P < 0.01$; ***$P < 0.001$). *D*: Caspase-3 activities after AMD3100 and STZ (mean ± SEM, $n = 3$; **$P < 0.01$; ***$P < 0.001$). con, control. *E*: ROS production by AMD3100, 12G5 MAb, and STZ (mean ± SEM, $n = 3$; *$P < 0.05$; **$P < 0.01$). veh, vehicle.**

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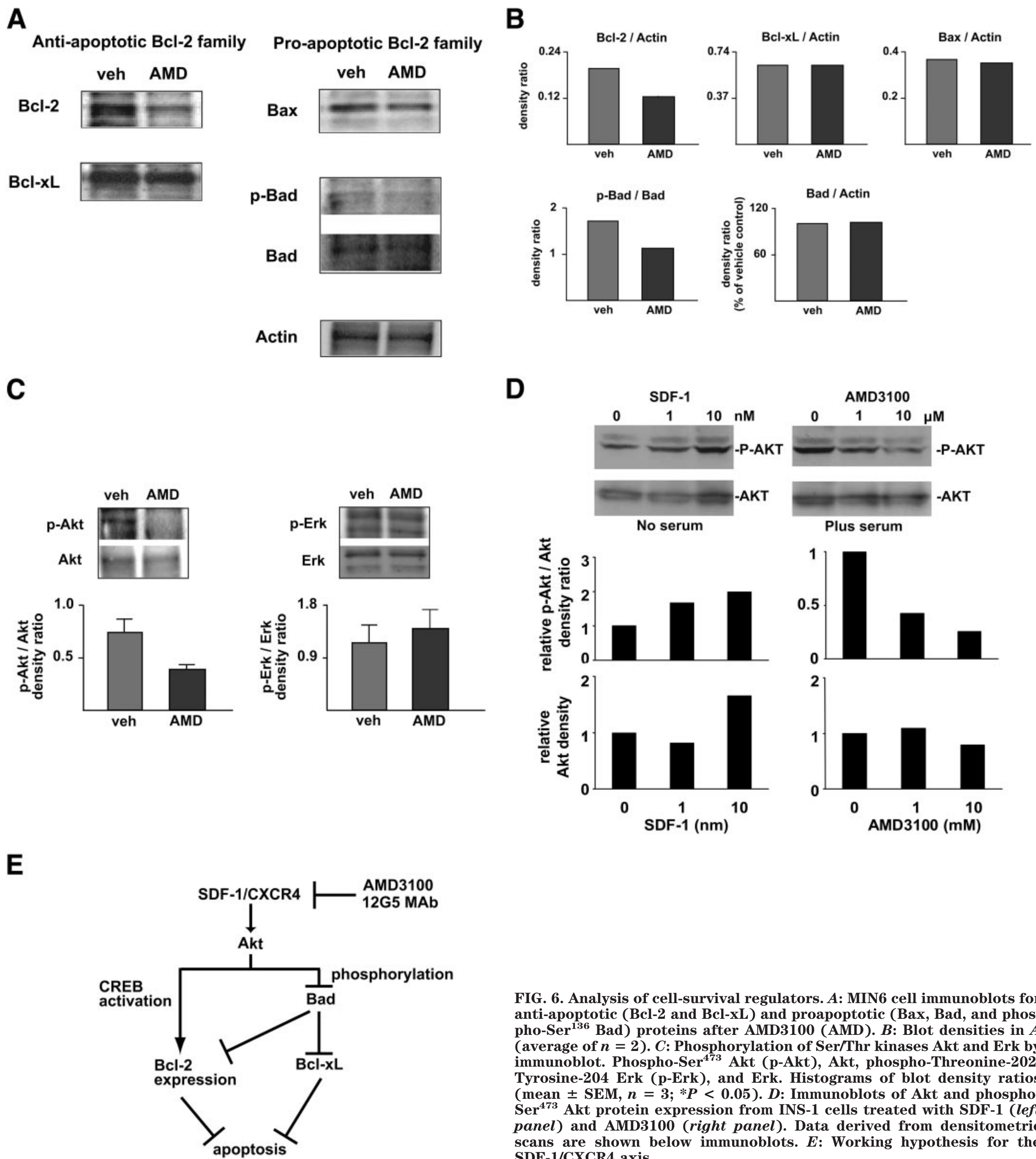

FIG. 6. Analysis of cell-survival regulators. *A*: MIN6 cell immunoblots for anti-apoptotic (Bcl-2 and Bcl-xL) and proapoptotic (Bax, Bad, and phospho-Ser^{136} Bad) proteins after AMD3100 (AMD). *B*: Blot densities in *A* (average of $n = 2$). *C*: Phosphorylation of Ser/Thr kinases Akt and Erk by immunoblot. Phospho-Ser^{473} Akt (p-Akt), Akt, phospho-Threonine-202/Tyrosine-204 Erk (p-Erk), and Erk. Histograms of blot density ratios (mean ± SEM, $n = 3$; *$P < 0.05$). *D*: Immunoblots of Akt and phospho-Ser^{473} Akt protein expression from INS-1 cells treated with SDF-1 (*left panel*) and AMD3100 (*right panel*). Data derived from densitometric scans are shown below immunoblots. *E*: Working hypothesis for the SDF-1/CXCR4 axis.

of hematopoietic stem cells by promoting stem cell proliferation and survival (7). We find that the SDF-1/CXCR4 axis also supports the survival of islet endocrine cells. The treatment of MIN6 cells with the CXCR4 inhibitor AMD3100 inhibits Bcl-2 expression and Bad phosphorylation and is accompanied by a decrement in Akt phosphorylation. These observations agree with others describing the regulation of Bcl-2 family members by Akt (28,29). In earlier studies of Akt knockout mice and transgenic mice constitutively expressing active Akt in β-cells, Akt was shown to be a major regulator of β-cell mass (16–18). Akt overexpression in β-cells leads to islet hyperplasia and resistance to STZ-induced apoptosis (16,17). Furthermore, the enhancement of β-cell mass may be attributed to β-cell survival rather than to the proliferation of existing β-cells (16). IGF-1/insulin and incretin hormones such as GLP-1 regulate β-cell proliferation and survival. Almost all of these factors stimulate Akt in β-cells in accord with our

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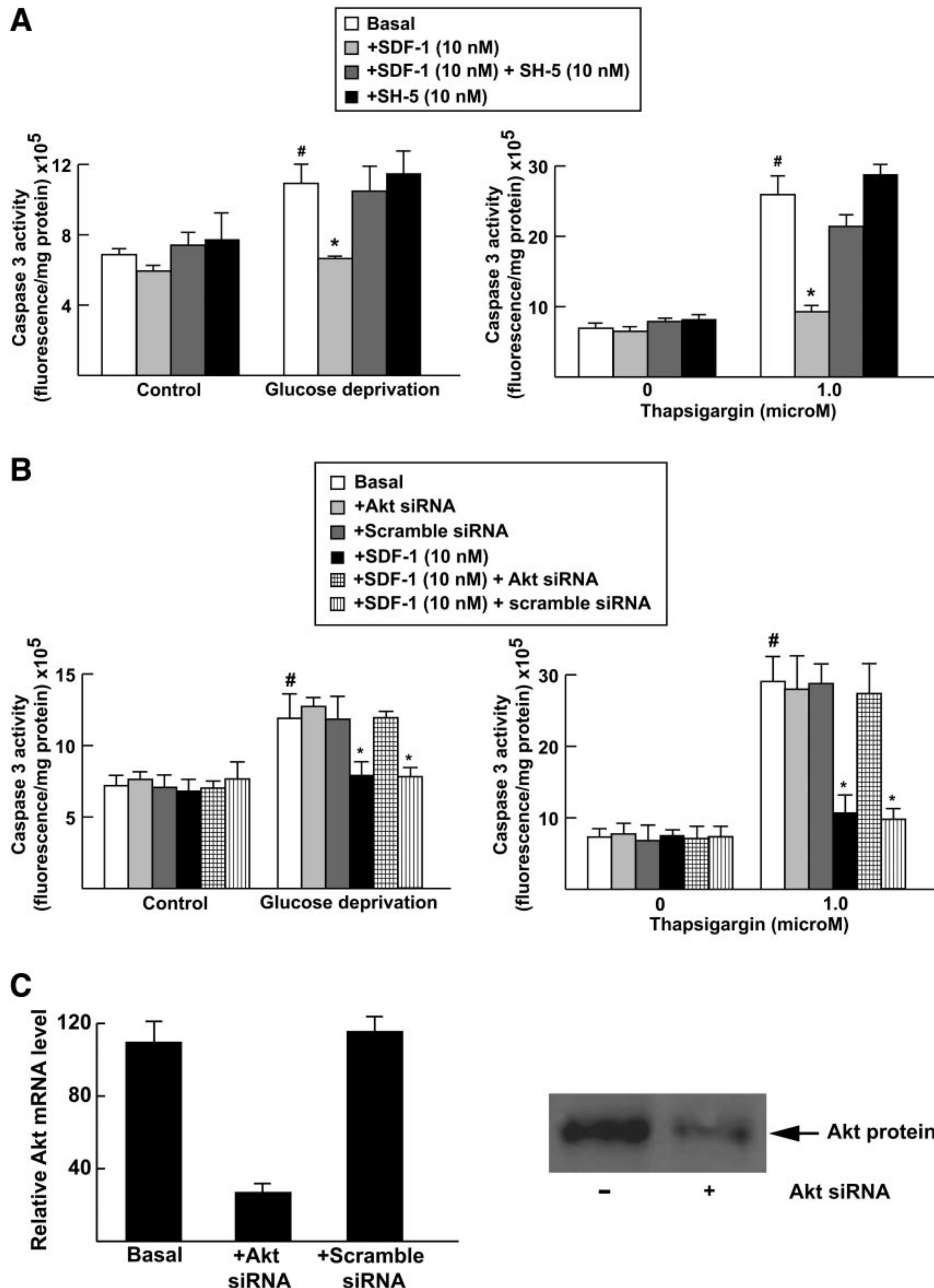

FIG. 7. Activation of Akt by SDF-1 inhibits apoptosis in INS-1 cells. ***A*: Akt inhibitor SH-5 blocks SDF-1 reversal of caspase-3 activation by glucose deprivation or thapsigargin. *B*: Akt siRNA inhibits caspase-3 inhibition by SDF-1. Akt or scramble siRNA transfected into INS-1 cells (mean ± SD, $n = 3$; #$P < 0.05$). *C*: Quantitative RT-PCR of Akt mRNA and immunoblot of Akt after siRNA transfection.**

findings on SDF-1/CXCR4 signaling, suggesting that Akt might be a common downstream target for these hormones. The mechanism of Akt activation by incretin hormones remains controversial (42), although cAMP signaling is associated with the IRS-2/Akt pathway through the activation of CREB (43,44). SDF-1/CXCR4 is reported to activate Akt by ligand interactions with G-protein–coupled receptors coupled to PI-3 kinase-γ. In our experiments, the phosphorylation of the serine/threonine kinase Erk, which is a downstream target of IRS-1 as well as Akt, was not changed. It is possible that SDF-1/CXCR4 can enhance Akt activity through PI-3 kinase-γ activation in pancreatic β-cells, bypassing the IRS-1 pathway.

No significant differences in blood glucose and insulin

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levels were observed in RIP-SDF-1 mice at 10 weeks of age despite the circumstance that these mice constitutively express SDF-1 in their β-cells and that they display substantial differences in their susceptibility to injury by STZ. This difference may be explained by indirect activation of Akt by SDF-1 overexpression in β-cells. It is possible that the activation of Akt by SDF-1 overexpression may be moderated by negative regulators of the PI-3 kinase/Akt pathway. For example, sustained activation of Akt may cause dedifferentiation of β-cells and suppression of glucose-responsive insulin secretion (45). This possibility is also supported by our observations that RIP-SDF-1 mice are only partially resistant to STZ-induced diabetes.

We observed β-cells with normal-appearing, nonapoptotic nuclei at the periphery of the islets of RIP-SDF-1 mouse islets 6 h after administration of STZ. Because SDF-1 and CXCR4 were expressed throughout the β-cell population in RIP-SDF-1 mice, the preferential distribution of surviving β-cells in peripheral compared with central regions of the islets was a surprising finding. The relative enhanced survival of β-cells in this pattern suggests that SDF-1/CXCR4 signaling cooperates with additional local signals to promote β-cell survival. Multiple explanations may account for these observations. One possibility is the differences in the accessibility of STZ to the β-cells between the core and the periphery of the islets because the blood flows from the core to the periphery of the islets (46). A second possibility relates to differences in cell types that reside in the core compared with the periphery of the islets. In this regard, it is worth commenting on the enhanced expression of phospho-Akt in the peripheral α-cells of the islets 6 h after administration of STZ (Fig. 5*E*) and the abundance of α-cells in the islets observed 2 weeks after the admininstration of STZ to the wild-type mice (Fig. 5*F*). At 2 weeks, most of the endocrine cells are glucagon-expressing α-cells, with only a few insulin-expressing β-cells present. These findings are reminiscent of those of Thyssen et al. (47), who found a marked hyperplasia of the α-cells following the administration of STZ to neonatal rats. These observations suggest the possibility that α-cells receive signals from STZ-damaged β-cells and support the idea that α-cells are involved in the β-cell regeneration that occurs following STZ-induced injury (47). It seems plausible that the SDF-1 expressed ectopically in the β-cells of the RIP-SDF-1 transgenic mice synergizes with signals from the α-cells to promote survival of the more peripherally located β-cells. It is tempting to speculate further that the cellular response to STZ may in some way involve a recapitulation of the ontogeny of islet growth and differentiation. The earliest endocrine cells that appear in the developing pancreatic anlage of the embryonic pancreas express glucagon (48).

Many factors have been reported to regulate β-cell mass, although it remains controversial whether they work alone or with multiple factors to regulate β-cell survival and proliferation. We propose that the SDF-1/CXCR4 axis is one of the components of a complex signaling network that can act in synergy with other factors to regulate β-cell mass. The SDF-1/CXCR4 signaling pathway provides a strategic opportunity to activate a component of the insulin signaling pathway at a point downstream of multiple effectors via the activation of Akt. One might speculate that SDF-1 agonists may be of eventual therapeutic utility in insulin-resistant states, including type 2 diabetes. SDF-1 agonists may act to bypass dysregulated insulin signaling components and activate Akt in β-cells. This may then facilitate compensatory hyperplasia of β-cells by prolonging cell survival and enhancing β-cell mass. Modulators of SDF-1/CXCR4 signaling, such as small molecule agonists, may represent promising drug candidates for future treatments of diabetes.

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