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

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Running title: SDF-1 promotes pancreatic beta-cell survival

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ABSTRACT

OBJECTIVE: Diabetes is caused by a deficiency of pancreatic beta cells that produce insulin. Approaches to enhance beta-cell mass by increasing proliferation and survival are desirable. We determined whether stromal cell-derived factor-1 (SDF-1/CXCL12) and its receptor CXCR4 are important for the survival of beta cells.

RESEARCH DESIGN AND METHODS: Mouse pancreata and clonal beta 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.

RESULTS: CXCR4 is expressed in beta 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 beta cells (RIP-SDF-1 mice) are resistant to streptozotocin-induced beta-cell apoptosis and diabetes. In Min6 beta 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 pro-apoptotic protein Bad. Active phosphorylated prosurvival kinase Akt is increased both in the beta cells of RIP-SDF-1 mice, and in INS-1 cells treated with SDF-1, sensitive to AMD3100. Inhibition of AKT expression by siRNA attenuates the ameliorative effects of SDF-1 on caspase-dependent apoptosis induced by thapsigargin or glucose deprivation in INS-1 beta 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 beta-cell survival via the activation of Akt suggesting that SDF-1 agonists may prove beneficial for the treatment of diabetes.

KEY WORDS: insulin/diabetes/SDF-1/apoptosis/islet
A contributing factor to the causation of diabetes mellitus is an insufficient mass of beta cells required to provide insulin in the amounts needed to maintain glucose homeostasis. The mass of beta cells is determined by the relative rates of beta-cell formation, by neogenesis and/or replication, and the rates of apoptosis. An understanding of the factors that control beta-cell growth and survival could provide new rational approaches for the treatment of diabetes.

Stromal cell-derived factor-1 (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 one of over 40 chemokines for which there are 18 known receptors. The SDF-1 receptor, CXCR4, is highly specific for SDF-1; the SDF-1/CXCR4 ligand-receptor pair is unique 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 that of chemotraction 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 IFN-gamma 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 SDF-1/CXCR4 axis for differentiated beta-cell functions in normal pancreatic islets remains unclear.

Several reports indicate that the serine/threonine kinase Akt/PKB, a downstream mediator of insulin, IGF-1, and GLP-1 signaling, is essential for the survival and proliferation of islet beta 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 beta-cell mass. The incretin hormones GLP-1 and GIP enhance Akt activity in islet beta cells (19,22) but GLP-1 and GIP receptor null mice have normal islet development (23). Therefore, the regulation of Akt activity and the proliferation of beta cells by insulin/IGF-1 and incretin hormones may be primarily directed towards the maintenance of beta-cell mass in the adult pancreas. SDF-1 also
SDF-1 promotes pancreatic beta-cell survival

stimulates Akt activity via the coupling of PI-3 kinase gamma to Gi (24). However, it is not yet known whether SDF-1 regulates Akt activity in adult differentiated beta cells.

Taking the earlier studies into consideration, we hypothesized that the survival of pancreatic beta 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 beta-cell survival through the activation of Akt in cultured clonal beta cells, mouse islets, and transgenic mice.

RESEARCH DESIGN AND METHODS

Transgenic mice

The RIP-SDF-1 transgenic mouse 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, due to published observations that a subset of RIP-SDF-1 mice develop small lymphocytic infiltrates consisting of dendritic, plasma, 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 Inc., Otsu, Japan) recombinant Taq DNA polymerase and primers RIP7 (5'-CAACCCTGACTATCTTCCCAG-3') and SDF-1 reverse (5'-CTTGTTTAAGCTTTCTCCAGGTAC-3').

Cell culture

MIN6 cells were cultured in DMEM medium containing 15% fetal bovine serum (FBS). INS-1 cells were cultured in RPMI with 10% FBS. The treatment of MIN6 cells with the 12G5 monoclonal antibody was conducted in serum free medium.

An expanded version of the “RESEARCH DESIGN AND METHODS” section is available in the online appendix "ONLINE ONLY SUPPLEMENTAL RESEARCH DESIGN AND METHODS” (http://diabetes.diabetesjournals.org).

RESULTS

Pancreatic expression of SDF-1 and CXCR4

To determine the sites of expression of SDF-1 and its receptor CXCR4, immunohistochemical analysis 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 (Figure 1A). All three major endocrine cells types of the islets, alpha, beta, and delta cells, express CXCR4. Only a minority of alpha cells co-express glucagon and CXCR4. These observations are in agreement with the 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, that is expressed in cells throughout 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 (identified by their staining with DBA) and blood vessels (arrows) of the exocrine parenchyma.
SDF-1 promotes pancreatic beta-cell survival

(Figure 1B). By co-staining 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 (Figure 1C). Pancreatic ducts and surrounding periductal interstitial and vascular tissues also were analyzed by immunostaining with DBA to identify ductal epithelium, von Willebrand factor (VWF) to indicate vascular endothelium, anti-SMA to mark vascular smooth muscle and myofibroblasts, and anti-SDF-1 antibodies (Figure 1D). 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 (Figure 1B, 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 beta cells in vivo, we examined 4-week old transgenic mice that express SDF-1 in pancreatic beta cells (RIP-SDF-1 mice) (25). The beta cells in the RIP-SDF-1 mouse pancreas expressed SDF-1 as reported previously and expected (25) (Supplemental Figure 1A, which can be found in an online appendix). In wild-type mouse islets we observed only occasional SDF-1 expressing cells, already identified as the microvascular endothelial cells in the previous analysis. The 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 beta 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-Ser473 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 beta cells as well as in cells located in the periphery of the islet (Figure 2A and Supplemental Figure 1B).

To determine whether beta-cell survival is promoted by the SDF-1/CXCR4 axis, RIP-SDF-1 mice were treated with streptozotocin (STZ), known to induce diabetes by apoptosis of beta cells. STZ administration induced diabetes in the wild-type mice whereas transgenic mice maintained their blood glucose levels substantially lower than those of wild-type mice after 2 weeks (Figure 2B). 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 the STZ injection (Figure 2C). Therefore, SDF-1 over-expression in the beta cells protected the transgenic mice from STZ-induced diabetes, possibly through the activation of Akt within beta cells.
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 the 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 treatment. These findings indicate destruction of beta cells by apoptosis (Figure 3A). However, the numbers of pyknotic nuclei were reduced in the islets of RIP-SDF-1 mice compared to those of wild-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 co-staining showed that surviving cells with normal nuclei located in peripheral areas of the transgenic mouse islets were insulin-expressing endocrine cells whereas non-beta endocrine cells appeared to survive in the peripheral regions of the wild-type mouse islets (Figure 3B). We also detected apoptotic cells by TUNEL staining and counted the TUNEL positive beta cells in each islet of the wild-type and transgenic mice (Figures 3C and D). Consistent with the findings in Figures 3A and B, most of the TUNEL positive beta cells were located in the core region of the islets, and beta cells located in the periphery of the islets were negative in the TUNEL assay. The TUNEL positive beta-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 (WT) and RIP-SDF-1 transgenic mice 6 hours after the administration of STZ showed the appearance of phospho-Akt predominantly in the alpha cells located at the periphery of the islets (Figure 3E). In the RIP-SDF-1, and not wild-type islets, phospho-Akt appeared in beta cells adjacent to the alpha cells at the islet periphery.

At two weeks after STZ injection, small endocrine cell clusters, the majority of which were predominantly glucagon-expressing alpha cells, were observed in the wild-type mouse pancreas (Figure 3F). However, in the transgenic mice, a large proportion of beta cells were retained in the endocrine cell clusters. These preserved beta cells may represent STZ-resistant beta cells that retain their ability to secrete insulin in response to glucose and indicate that SDF-1 expression in the islet beta 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 beta-cell lines in which it is feasible to conduct such studies. Immunostaining and RT-PCR of MIN6 cells showed co-expression of SDF-1 and CXCR4 in MIN6 cells in culture (Figures 4A 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 Figure 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 employed 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 (Figure 4C). 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 (Figure 5A). 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 cells (Figures 5B and C). Background apoptosis rates were lower in the AMD3100 experiments conducted in the presence of serum as compared to 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 (Figure 5D). 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 CMH2DCFDA. Treatment of MIN6 cells with both CXCR4 inhibitors increased amounts of ROS, although less potently than with STZ treatment (Figure 5E). These results suggest that the apoptosis induced by CXCR4 inhibitors may be mediated by mitochondrial dysfunction. To further explore the mechanism of the induction of apoptosis by the inhibitors, the expression levels of anti- and pro-apoptotic 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 (Figures 6A and B). The amount of phosphorylated Bad was also reduced in AMD treated cells.

**Suppression of Akt phosphorylation by the CXCR4 antagonist AMD3100**

Because Akt is widely recognized to have an important role in the survival of islet beta cells, its phosphorylation status in the AMD3100 treated MIN6 cells was examined with an anti-phospho-Serine 473 Akt antibody. Akt phosphorylation at serine 473, critical for its activation, was reduced in the AMD3100 treated cells, while the phosphorylation of Erk was unchanged (Figure 6C). To examine the effects of directly adding SDF-1 to beta cells on the activation of Akt, the clonal beta cell line INS-1 was employed. SDF-1 dose-dependently activated Akt as indicated by increased AKT phosphorylation detected on Western immunoblots (Figure 6D, left). The phosphorylation of Akt induced by SDF-1 was inhibited by the CXCR4 antagonist AMD3100 (Figure 6D, 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 CREB (28) and to directly phosphorylate the anti-apoptotic protein Bad at serine 136 (29) (Figure 6E). These observations in MIN6 and INS-1 cells suggest that SDF-1 signaling through CXCR4 promotes beta-cell survival through the activation of Akt.
Antiapoptotic actions of SDF-1 mediated by the activation of Akt

To further explore whether the anti-apoptotic 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 beta cells (30, 31). Thapsigargin inhibits endoplasmic reticulum Ca\(^{2+}\) ATPase, produces ER stress, 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 (Figure 7A, left). Thapsigargin induced caspase activity by 4-fold, and SDF-1 co-incubation attenuated the pro-apoptotic effect of thapsigargin to activate caspase-3 (Figure 7A, 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 was reduced. SH-5, a phosphatidylinositol analog that prevents membrane recruitment of Akt and subsequent activation by membrane associated PDK, is a specific inhibitor for Akt (34, 35). SH-5 decreases phosphorylation of Akt without affecting total Akt expression levels. Co-incubation 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 (Figure 7A) and thapsigargin-induced apoptosis (Figure 7B). To substantiate the findings in clonal beta 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 Figure 3). Thus, the antiapoptotic actions of SDF-1 appear to require activation of Akt in mouse islets as well as in clonal beta cells. We also employed an 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 (Figure 7C). 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 (Figure 7B). These findings indicate that SDF-1 exerts strong anti-apoptotic effects on INS-1 beta 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 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). HGF-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 beta-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,
SDF-1 promotes pancreatic beta-cell survival

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 beta-cell survival in RIP-SDF-1 transgenic mice and in MIN6 and INS-1 clonal beta 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 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 beta cells, Akt was shown to be a major regulator of beta-cell mass (16-18). Akt overexpression in beta cells leads to islet hyperplasia and resistance to STZ-induced apoptosis (16, 17). Furthermore, the enhancement of beta-cell mass may be attributed to beta-cell survival rather than to the proliferation of existing beta cells (16). IGF-1/Insulin and incretin hormones such as GLP-1 regulate beta-cell proliferation and survival. Almost all of these factors stimulate Akt in beta cells in accord with our 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 (22), although cAMP signaling is associated with the IRS-2/Akt pathway through the activation of CREB (53, 54). SDF-1/CXCR4 is reported to activate Akt by ligand interactions with G-protein coupled receptors coupled to PI-3 kinase gamma. 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 gamma activation in pancreatic beta cells, bypassing the IRS-1 pathway.

No significant differences in blood glucose and insulin 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 beta 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 the beta 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 beta cells and suppression of glucose-responsive insulin secretion (44, 45). This possibility is also supported by our observations that RIP-SDF-1 mice are only partially resistant to STZ-induced diabetes.

We observed beta cells with normal appearing, non-apoptotic nuclei at the periphery of the islets of RIP-SDF-1 mouse islets 6 h after the administration of STZ. Because SDF-1 and CXCR4 were expressed throughout the beta-cell population in RIP-SDF-1 mice, the preferential distribution of surviving beta cells in peripheral as compared to central regions of the islets was a surprising finding. The relative enhanced survival of beta cells in this pattern suggests that SDF-1/CXCR4 signaling cooperates with
additional local signals to promote beta-cell survival. Multiple explanations may account for these observations. One possibility is the differences in the accessibility of STZ to the beta 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 to the periphery of the islets. In this regard it is worth commenting on the enhanced expression of phospho-Akt in the peripheral alpha cells of the islets 6 hours after the administration of STZ (Figure 5E) and the abundance of alpha cells in the islets observed two weeks after the administration of STZ to the wild-type mice (Figure 5F). At two weeks most of the endocrine cells are glucagon-expressing alpha cells, with only a few insulin-expressing beta cells present. These findings are reminiscent of those of Thyssen and colleagues (47) who found a marked hyperplasia of the alpha cells following the administration of STZ to neonatal rats. These observations suggest the possibility that alpha cells receive signals from STZ-damaged beta cells and support the idea that alpha cells are involved in the beta-cell regeneration that occurs following STZ-induced injury (47).

It seems plausible that the SDF-1 expressed ectopically in the beta cells of the RIP-SDF-1 transgenic mice synergizes with signals from the alpha cells to promote survival of the more peripherally-located beta 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 beta-cell mass, although it remains controversial as to whether they work alone or with multiple factors to regulate beta-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 beta-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 beta cells. This may then facilitate compensatory hyperplasia of beta cells by prolonging cell survival and enhancing beta-cell mass. Modulators of SDF-1/CXCR4 signaling, such as small molecule CXCR4 signaling, may represent promising drug candidates for future treatments of diabetes.

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SDF-1 promotes pancreatic beta-cell survival


via CREB-mediated induction of IRS2. Genes Dev 17:1575-80


Table 1

Metabolic characteristics of RIP-SDF-1 mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Body Weight (g)</th>
<th>Glucose (mg/dl)</th>
<th>Insulin (ng/ml)</th>
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<td><strong>Fed</strong></td>
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<tr>
<td>Wild-type (n=9)</td>
<td>19.2±0.31</td>
<td>124±8.14</td>
<td>386±19.5</td>
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<tr>
<td>RIP-SDF-1 (n=8)</td>
<td>20.0±0.28</td>
<td>119±10.7</td>
<td>411±40.5</td>
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<tr>
<td><strong>Fasting</strong></td>
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<tr>
<td>Wild-type (n=8)</td>
<td>18.7±0.20</td>
<td>121±6.66</td>
<td>368±13.7</td>
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<tr>
<td>RIP-SDF-1 (n=7)</td>
<td>18.3±0.34</td>
<td>105±3.54</td>
<td>335±10.6</td>
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</table>

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

Figure 1. CXCR4 and SDF-1 expression in 10-week old mouse pancreas and islets. 
(A) CXCR4: Insulin, glucagon, somatostatin antisera (red) and CXCR4 antiserum (green). 
(B) SDF-1: Insulin (blue), SDF-1 (red), and DBA (green). Arrows, arrowheads indicate SDF-1 surrounding ducts, vessels, respectively (x200). (C) SDF-1 in islets: SDF-1 (red), BS-1 lectin (green), insulin (blue) (x400). (D) SDF-1 in periductal regions: Left, SDF-1 (red) and VWF (green); Right, alpha SMA (red), VWF (green), DBA (blue).

Figure 2. Enhanced AKT phosphorylation in islets and resistance to streptozotocin-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 in wild-type (solid circles, n=9) and RIP-SDF-1 (open circles, n=5) mice. (C) Body weights after STZ (*P<0.05, **P<0.01).

Figure 3. Islet morphologies 6h (A-E) and 2 weeks (F) after STZ. (A) DAPI (white), (B) Insulin (green), DAPI (blue). Right panel in (A), magnified arrows indicate pyknotic nuclei. Arrows, arrowhead in (B) indicate normal beta, non-beta cells, respectively. (C) Apoptotic beta cells: TUNEL (red), insulin (green), DAPI (blue). (D) TUNEL-positive beta cells (islet). Mean ±SEM, 30 islets/pancreas (n=3), ***P<0.0001. (E) STZ-induced activation of Akt in islets of WT and RIP-SDF-1 mice: phospho-Akt (P-Akt), insulin (INS), glucagon (GCG), somatostatin (SMS). (F) Wild-type and RIP-SDF-1 mouse pancreas: glucagon (red), insulin (green), somatostatin (blue).

Figure 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), DAPI (blue). (B) RT-PCR of SDF-1, CXCR4, beta-actin. (C) DNA content in cells cultured with CXCR4 antagonist AMD3100 for 6d (Mean±SEM, n=3, * P<0.05).

Figure 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 48hr. TUNEL-positive cells (green), DAPI (blue). (C) Percentage 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). (E) ROS production by AMD3100, 12G5 Mab, and STZ (Mean±SEM (n=3), *P<0.05, **P<0.01).

Figure 6. Analysis of cell-survival regulators. (A) MIN6 cell immunoblots for anti-apoptotic (Bcl-2 and Bcl-xL) and pro-apoptotic (Bax, Bad, and phospho-Serine-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-Serine-473 Akt (p-Akt), Akt, phospho-Threonine-202/Tyrosine-204, Erk (p-Erk), and Erk. Histograms of
SDF-1 promotes pancreatic beta-cell survival

 blot density ratios (Mean±SEM, n=3, *P<0.05). (D) Immunoblots of Akt and p-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.

**Figure 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 ± S.D, n=3, *P< 0.05). (C) QPCR of Akt mRNA and immunoblot of Akt after siRNA transfection.
SDF-1 promotes pancreatic beta-cell survival

Figure 1, Yano et al.
A. INSULIN p-AKT INSULIN p-AKT X400

WT

RIP-SDF-1 X400

B. Blood glucose (mg/dl)

Days after STZ injection

wt (N=9) tg (N=5)

C. Body weight (% of day-0 time point)

Days after STZ injection

wt (N=9) tg (N=5)

Figure 2, Yano et al.
SDF-1 promotes pancreatic beta-cell survival

Figure 3, Yano et al.
SDF-1 promotes pancreatic beta-cell survival

Figure 3E & F, Yano et al.
SDF-1 promotes pancreatic beta-cell survival

A. 

B. 

C. 

Figure 4, Yano et al.
SDF-1 promotes pancreatic beta-cell survival

Figure 5, Yano et al.
A. Anti-apoptotic Bcl-2 family

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B. Pro-apoptotic Bcl-2 family

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Figure 6, Yano et al.
SDF-1 promotes pancreatic beta-cell survival

C.

D.

E.

Figure 6, Yano et al.
SDF-1 promotes pancreatic beta-cell survival

A.

B.

C.

Figure 7, Yano et al.