Inhibition of Gluconeogenesis in Primary Hepatocytes by Stromal Cell-derived Factor-1 (SDF-1) through a c-Src/Akt-dependent Signaling Pathway

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Hepatic gluconeogenesis is elevated in diabetes and a major contributor to hyperglycemia. Stromal cell-derived factor-1 (SDF-1) is a chemokine and an activator of Akt. In this study, we tested the hypothesis that SDF-1 suppresses hepatic gluconeogenesis through Akt. Our results from isolated primary hepatocytes show that SDF-1α and SDF-1β inhibited glucose production via gluconeogenesis and reduced transcript levels of key gluconeogenic genes glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK). Additionally, SDF-1α and SDF-1β both inhibited activation of the PEPCK promoter. In examining the mechanism by which SDF-1 inhibits gluconeogenesis, we found that SDF-1α promoted phosphorylation of Akt, FoxO1, and c-Src, but did not activate insulin receptor substrate-1-like insulin. Blockade of Akt activation by LY294002, FoxO1 translocation by constitutively nuclear FoxO1 mutant, or c-Src activation by the chemical inhibitor PP2, respectively, blunted SDF-1α suppression of gluconeogenesis. Finally, our results show that knocking down the level of SDF-1 receptor CXCR4 mRNA blocked SDF-1α suppression of gluconeogenesis. Together, our results demonstrate that SDF-1 is capable of inhibiting gluconeogenesis in primary hepatocytes through a signaling pathway distinct from the insulin signaling.

Hepatic gluconeogenesis is a major contributor to hyperglycemia in both types I and II diabetes (T1DM and T2DM) (1). Gluconeogenesis becomes unrestrained in diabetes due to either deficient insulin secretion in T1DM or deficient insulin action in T2DM (1). Therefore, the key to treat diabetes is to provide insulin to patients with T1DM and reverse or bypass the deficient insulin signaling pathway in patients with T2DM. Identification of agents that can inhibit hepatic gluconeogenesis through a signaling pathway distinct from insulin may provide new avenues to curtail the elevated gluconeogenesis caused by insulin resistance in T2DM.

Hepatic gluconeogenesis plays an essential role in maintaining the blood glucose level normal during the fasting or non-feeding phase, and is inhibited by insulin when nutrients are abundant in the blood such as shortly after food ingestion (1). It is well established that insulin suppresses hepatic gluconeogenesis through activation of Akt (2, 3). Akt subsequently phosphorylates FoxO1, a key transcription factor in regulation of gluconeogenic gene expression (4). The phosphorylated FoxO1 will be translocated to the cytoplasm from the nucleus (5). As a result, gluconeogenesis is inhibited. In T2DM, suppression of hepatic gluconeogenesis by the Akt-dependent insulin signaling is reduced or lost due to insulin resistance plus a relatively insufficient insulin production (1). Because stromal cell-derived factor-1 (SDF-1) is a known Akt activator and its receptor is expressed in liver (6), it is possible that SDF-1 can bypass the blunted insulin signaling and consequently inhibit hepatic gluconeogenesis.

SDF-1, also named CXC chemokine ligand 12 (CXCL12), belongs to the family of CXC chemokines (7, 8). There are at least two SDF-1 isoforms (α, β) due to alternative splicing. SDF-1 was initially discovered in bone marrow stromal cells, and now has been found in various immune and non-immune tissues and organs, including lymph node, thymus, thyroid gland, appendix, bone marrow, uterus, lung, salivary gland, heart, skeletal muscle, and liver (9–12). As the specific receptor for SDF-1, CXCR4 is also widely expressed in both immune cells (T cells, B cells, and NK cells) and non-immune cells (myocytes, epithelial, neurons, dendritic cells, and hepatocytes) (11, 13, 14). The wide spectrum distribution of the SDF-1/CXCR4 system is consistent with its diverse functions.

As a chemoattractant, SDF-1/CXCR4 attracts various kinds of cells such as hemopoietic progenitor cells, T lymphocytes, pre-B-cells, monocytes, and dendritic cells (8). SDF-1/CXCR4 is essential for normal hematopoietic progenitor cell movement and adherence within the bone marrow microenvironment (8). Roles for SDF-1/CXCR4 have been indicated in vascular remodeling by recruiting smooth muscle cells (15), bone

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3 The abbreviations used are: SDF-1, stromal cell-derived factor-1; LDH, lactate dehydrogenase; IRS, insulin receptor substrate; NOD, non-obese diabetic; siRNA, small interfering RNA; PI, phosphatidylinositol; GFP, green fluorescent protein; PEPCK, phosphoenolpyruvate carboxykinase.
remodeling by regulating osteoclastogenesis (16), regulation of pituitary function (8), and neuronal generation by promoting neuronal migration and axonal pathfinding (17). As a result, deletion of either the SDF-1 or CXCR4 genes leads to a similar embryonic lethal phenotype, which is characterized by deficient B-lympho- and myelopoesis, deficient vasculogenesis, deficient myogenesis, and abnormal development of the heart and the central nervous system (13, 18).

In addition to its roles in the physiological events described above, SDF-1/CXCR4 has been implicated in inflammatory diseases (19), development and metastasis of cancers (20, 21), and atherosclerosis (22). Therefore, SDF-1/CXCR4 has become a potential therapeutic target for treatment of a variety of diseases such as human immunodeficiency virus infection, cancer metastasis, leukemia, rheumatoid arthritis, and stroke (23, 24).

Of our interest, differential roles for SDF-1/CXCR4 have been implicated in the development of diabetes. Elevated expression of SDF-1 in thymocytes may play a role in the development of autoimmune in non-obese diabetic (NOD) mouse, a T1DM model (25). Neutralization of SDF-1 function in NOD mice with antisera against SDF-1 reduces insulin and significantly delays the onset of diabetes (26). These results suggest that SDF/CXCR4 is a mediator of the autoimmune in NOD mice.

In contrast, transgenic overexpression of SDF-1 in pancreas significantly increases the survival of β-cells and render normal mice more resistance to streptozotocin-induced β-cell inflammation and diabetes probably through promoting the survival and migration of progenitor cells in the pancreas (27, 28). Furthermore, blockade of SDF-1 function with an antagonist of CXCR4 (AMD3100) leads to programmed cell death of insulin producing MIN-6 cells (28). These reports support the notion that SDF-1/CXCR4 directly protects pancreatic β-cells in normal subjects. The protective role is associated with activation of Akt (28). In this study, we have tested the hypothesis that SDF-1/CXCR4 inhibits hepatic gluconeogenesis through Akt.

**MATERIALS AND METHODS**

**Antibodies, Reagents, and Constructs**—Recombinant SDF-1α, SDF-1β, and macrophage inflammatory protein-1α were from PeproTech Inc. (Rocky Hill, NJ). Antibodies against total/phospho-Akt (tyrosine 473 and 308), total and phospho-FoxO1 (serine 256), and total/phospho-Src (tyrosine 416) were from Cell Signaling Technology. Antibody against phospho-IRS-1 (tyrosine 272), human insulin, Nα,O'-dibutyryladenosine 3',5'-cyclic monophosphate sodium (cAMP), and dexamethasone were from Sigma. Chemical inhibitors for Src tyrosine kinases PP2 and the negative control PP3 and the PI 3-kinase inhibitor LY294002 (catalog number 440202) were purchased from Calbiochem. The siRNA against CXCR4 (catalog number 161237) and related scrambled siRNA (catalog number 4635) were from Thermo Fisher Scientific Inc. Lipofectamine™ 2000 Transfection Reagents were from Invitrogen. The lactate dehydrogenase (LDH) assay kit was from Roche Applied Science. The phosphono-3-carboxykinase 1 (PEPCK) promoter construct, the constitutively nuclear form of FoxO1 encoded by adenovirus, and the adeno viral vector encoding GFP were kind gifts from Drs. Jianhua Shao, Domenico Accili, and Christopher Newgard, respectively (29–31).

**Isolation and Culture of Hepatocytes**—Primary hepatocytes were isolated from C57BL/6 male mice (8–10 weeks old) and cultured as previously described (32–37). In brief, under anesthesia with pentobarbital (intraperitoneal, 50 mg/kg body weight), isolated liver was perfused with Hanks’ balanced solution containing 0.5 mM EGTA, 0.004 N NaOH, 10 mM HEPES (Invitrogen) at 5 ml/min for 8 min, followed by continuous perfusion with serum-free Williams’ Medium E containing collagenase (Worthington, type II, 50 units/ml) (Invitrogen) supplemented with 10 mM HEPES for 12 min. Hepatocytes were harvested and purified with Percoll (Sigma) as previously described (32–37). The viability of hepatocytes was examined with trypan blue exclusion. Only cell isolates with viability over 95% were used. Hepatocytes were inoculated into collagen-coated plates (5 × 10^5 cells/well in 6-well plates and 1.25 × 10^5 cell per well in 24-well plates) in Williams’ Medium E with 10% fetal bovine serum, and were incubated for 24 h before experimentation. All mice used in the present study for isolation of hepatocytes were fed a normal chow diet under using regular schedule and were not fasted. All studies were approved by The Hamner Institutes for Health Sciences Animal Care and Use Committee and complied with guidelines from the United States National Institutes of Health. Hepa1c1c7 mouse hepatoma cells (ATCC) were maintained in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum.

**Measurement of Glucose Production**—Glucose production was assayed as described before (36, 37). Briefly, hepatocytes were pre-treated with SDF-1α, SDF-1β, or insulin at concentrations as noted for 1 h, followed by stimulation with cAMP/dexamethasone (10 μM/50 nM) in glucose-free Dulbecco’s modified Eagle’s medium containing 2 mM sodium lactate. For inhibition assay, cells were pre-treated with chemical inhibitors at the indicated concentrations at 37 °C for 30 min. The culture media were subsequently collected for measuring glucose and LDH. Levels of LDH were measured with a LDH assay kit (Roche). Cells were lysed with the lysis buffer for protein determination using the Bio-Rad DC protein assay kit (Bio-Rad). Glucose concentrations were determined with an YSI glucose analyzer (YSI, Yellow Springs, OH) and normalized to protein concentrations. Glucose production via gluconeogenesis was calculated as previously described (24). Specifically, glucose production in the presence of the gluconeogenic substrate (sodium lactate) was considered as the total glucose production and glucose production in the absence of sodium lactate was defined as glycogenolysis. Glucose production via gluconeogenesis = total glucose production – glycogenolysis.

**Immunoblotting Blotting**—Cells were lysed in lysis buffer (20 mM Tris-HCl, pH 7.5, 137 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 2 μg/ml leupeptin, and 10 μg/ml aprotinin), supplemented with 1 mM phenylmethylsulfonyl fluoride before use (38). Cell lysates (15 μg/lane) were resolved in 4–20% Tris glycine gels (Invitrogen) and transferred to nitrocellulose membranes (Bio-Rad). The presence of proteins was detected by immunoblotting with primary antibodies as indicated and alkaline phosphatase-conjugated sec-
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RESULTS

SDF-1 Suppresses Hepatic Gluconeogenesis in Primary Hepatocytes—It has previously been shown that SDF-1 can activate Akt in hepatocytes (12). Because Akt is a central mediator of insulin suppression of hepatic gluconeogenesis, we tested the hypothesis that SDF-1 plays an inhibitory role in hepatic gluconeogenesis. The glucagon/cAMP/PKA-dependent signaling is the primary stimulatory pathway of gluconeogenesis, and glucocorticoids play a prerequisite role in gluconeogenesis (1, 39). Thus, glucose production via gluconeogenesis in primary hepatocytes was stimulated with a membrane-permeable cAMP and dexamethasone in the presence or absence of SDF-1 or insulin, and quantified as detailed under “Materials and Methods.” As expected, glucose production via gluconeogenesis was promoted by cAMP/dexamethasem, but suppressed by insulin (Fig. 1A). The presence of either SDF-1α or SDF-1β inhibited cAMP/dexamethasone-induced glucose production via gluconeogenesis in a dose-dependent manner (Fig. 1A). To examine whether this inhibition by SDF-1 was due to cytotoxicity, levels of LDH in the cell culture media were quantified. Clearly, SDF-1 did not cause increased release of LDH, suggesting no detectable toxicity (Fig. 1B). In contrast, 1% Triton X-100 caused a significant release of LDH. These results support the hypothesis that SDF-1 inhibits gluconeogenesis in primary hepatocytes.

To further test this hypothesis, the effect of SDF-1 on expression of key gluconeogenic genes G6Pase and PEPCK was examined. Levels of G6Pase and PEPCK transcripts were elevated by treatment with cAMP/dexamethasome, but the elevations were significantly reduced by both SDF-1α and SDF-1β in a dose-dependent manner (Fig. 2A). Similarly, the activity of the PEPCK promoter measured by the luciferase reporter gene was
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FIGURE 3. SDF-1 inhibits glucose production through phosphorylation of Akt. A, primary hepatocytes were treated with SDF-1α, SDF-1β, or insulin at noted concentrations for 15 min. Subsequently, levels of phospho-Akt (at serine 473 or serine 308) and total Akt were evaluated by immunoblotting with specific antibodies. B, PI 3-kinase inhibitor LY294002 prevented SDF-1β phosphorylation of Akt. Primary hepatocytes were pretreated with LY294002 for 30 min followed by SDF-1β treatment for 15 min. Akt phosphorylation at serine 473 was detected by immunoblotting with specific antisera. C, SDF-1β suppression of glucose production was prevented by LY294002. Primary hepatocytes were treated with 1 μM LY294002 for 1 h prior to the treatment with cAMP/dexamethasome in the presence or absence of SDF-1β for 2.5 h. Glucose production was subsequently evaluated as detailed under "Materials and Methods." D, SDF-1β reduction in levels of key gluconeogenic gene transcripts was prevented by LY294002. Primary hepatocytes were pre-treated with 1 μM LY294002 for 1 h, and then treated with cAMP/dexamethasome for 4 h. Levels of PEPCK and G6Pase transcripts were measured by real-time PCR. Results represent three independent experiments. *, p < 0.05; **, p < 0.01; and ***, p < 0.001 in comparison to the first lane.

Glucose homeostasis, we examined whether or not SDF-1 activates Akt, which is necessary for insulin suppression of hepatic gluconeogenesis (1). Activation of Akt is usually evaluated by measuring phosphorylation levels at either Ser473 or Thr308, or both sites (40). Thus, we measured phosphorylation levels at these two sites by immunoblotting with specific antisera. As shown in Fig. 3A, both SDF-1α and -β stimulated phosphorylation at Ser473 significantly in a dose-dependent manner. SDF-1α and -β also promoted phosphorylation at Thr308 although SDF-1α-induced phosphorylation at Thr308 appeared to be less than that induced by SDF-1β. As a positive control, insulin stimulated Akt phosphorylation at both sites as expected. To determine the possible role of PI 3-kinase, a key component of insulin signaling and a known activator of Akt (1), primary hepatocytes were pre-treated with the PI 3-kinase inhibitor LY294002 prior to treatment with SDF-1β. As shown in Fig. 3B, SDF-1β-induced Akt phosphorylation was prevented by LY294002 in a dose-dependent manner. We next evaluated the effect of LY294002 on SDF-1- or insulin-mediated suppression of gluconeogenesis. In the absence of LY294002, the cAMP/dexamethasome–induced gluconeogenesis via gluconeogenesis was blunted by either SDF-1β or the positive control insulin as expected (Fig. 3C). However, the presence of LY294002 prevented SDF-1β suppression of glucose production via gluconeogenesis. As anticipated, insulin-

stimulated by cAMP/dexamethasome, but blunted by insulin, or SDF-1 (α and β) (Fig. 2B). The effect of SDF-1 appears to be weaker than equal molar concentrations of insulin. Together, these results further support the hypothesis that SDF-1 inhibits gluconeogenesis in isolated hepatocytes.

SDF-1 Stimulates Phosphorylation of Akt and FoxO1—To investigate the mechanism by which SDF-1 suppresses hepatic
mediated suppression of glucose production via gluconeogenesis was also blocked by LY294002.

To further examine the role of Akt activation in SDF-1 inhibition of gluconeogenesis, we evaluated the effect of the mutant FoxO1 on transcript levels of G6Pase and PEPCK. As shown in Fig. 4D, overexpression of the mutant FoxO1 reversed SDF-1 β reductions of both G6Pase and PEPCK mRNAs. The insulin-mediated decreases in G6Pase and PEPCK mRNA levels were also prevented by the mutant FoxO1 as predicted. However, overexpression of GFP (a control) had no effect. Together, these results indicate that FoxO1 is a target of SDF-1 in suppression of gluconeogenesis.

SDF-1 Suppresses Gluconeogenesis through a Signaling Pathway Distinct from Insulin—Tyrosine phosphorylation of insulin receptor substrate (IRS) proteins such as IRS-1 is necessary for activation of the insulin signaling pathway (1). To examine whether or not SDF-1 activates Akt in the same way as insulin, we measured levels of IRS-1 tyrosine phosphorylation. As shown in Fig. 5A, neither SDF-1α nor SDF-1β initiated any significant tyrosine phosphorylation of IRS-1, whereas insulin robustly stimulated IRS-1 tyrosine phosphorylation as expected. The negative control, macrophage inflammatory protein-1α, also did not promote any IRS-1 tyrosine phosphorylation.

It is known that the SDF-1 receptor CXCR4 is a Gα protein-coupled receptor, which is known to be able to activate c-Src (44, 45). It is also known that c-Src is an activator of Akt (46, 47). Therefore, we studied the effect of SDF-1 on activation of c-Src. Phosphorylations of c-Src at different sites may lead to either inhibition or activation of c-Src (48, 49). Activation of c-Src is usually evaluated by measuring phosphorylation levels at Tyr416. Therefore, we examined the effect of SDF-1 on c-Src phosphorylation at this site. Treatment of primary hepatocytes
Sodium lactate (2 mM) was added to some cells as a substrate of gluconeogenesis. Glucose production via SDF-1 reverse transcriptase-PCR using the specific alts and Methods. Levels of mary hepatocytes by reverse transfection for 36 h as detailed under “Materi-siRNA against CXCR4 (siCXCR4) or a scramble siRNA was introduced into pri-sDF-1/H9252
FIGURE 6. SDF-1 suppresses hepatic gluconeogenesis through c-Src.

A. cAMP/Dex
SDF-1α (nM) 100 100 100 100 100 100
Insulin (nM) 1 50
PP2 (nM) 25 100
PP3 (nM) 100

B. Glucose production via gluconeogenesis (%)
P<0.01
Insulin (nM) 1 50
PP2 (nM) 25 100
PP3 (nM) 100

C. PEPCK transcripts.
SDF-1α/Dex
SDF-1β/Dex
Insulin/Dex
PP2/Dex
PP3/Dex

D. PEPCK mRNA levels.
P<0.05
SDF-1α/Dex
SDF-1β/Dex
Insulin/Dex
PP2/Dex
PP3/Dex

FIGURE 5. SDF-1 suppresses hepatic gluconeogenesis through c-Src. Primary hepatocytes were treated with SDF-1 (α or β), macrophage inflammatory protein-1α (MIP; negative control), or insulin (positive control) for 15 min. Levels of phospho-IRS-1 (at tyrosine 612) and total IRS (A), and phospho-c-Src (at tyrosine 416, activating) and total c-Src (B) were determined by immunoblotting with specific antibodies. NS, nonspecific bands. C, primary hepatocytes were pretreated with PP2 or PP3 (negative control) for 30 min, and then treated with cAMP (10 μM)/dexa-methasome (Dex) (50 μM) in the continuous presence or absence of SDF-1β for 3 h. Sodium lactate (2 mM) was added to some cells as a substrate of gluconeogenesis. Glucose production via gluconeogenesis was quantified, calculated, and normalized to protein levels as detailed under “Materials and Methods.” Results represent mean ± S.E. of two independent experiments performed in triplicate. D, levels of PEPCK gene transcripts from these same cells were determined by real-time reverse transcriptase-PCR using specific TaqMan PCR probes. Results represent mean ± S.E. from two independent experiments performed in duplicate. *, P < 0.05 in comparison to the first lane. NS, non specific.

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Knocking Down the Level of SDF-1 Receptor CXCR4 Transcripts Prevents SDF-1 Suppression of Hepatic Gluconeogenesis—Because CXCR4 is the specific receptor of SDF-1 (44), we examined the role of CXCR4 in SDF-1 suppression of hepatic gluconeogenesis. The specific siRNA was introduced into primary hepatocytes to knock down the transcript level of the CXCR4 gene (Fig. 6A). Cells were then treated with cAMP/dexamethasome in the presence or absence of SDF-1, followed by measurements of glucose production via gluconeogenesis and levels of PEPCK transcripts. As shown in Fig. 6, B–D, the cAMP/dexamethasome-induced glucose production via gluconeogenesis and elevation of PEPCK transcript levels were significantly reduced by either SDF-1α or SDF-1β as predicted. In the presence of the siRNA against the CXCR4 gene, the inhibition of glucose production by either SDF-1α or SDF-1β was largely reversed (Fig. 6, B and C). Similarly, the SDF-1β-mediated reduction in PEPCK transcript levels was also significantly reversed by the specific siRNA (Fig. 6D). The control siRNA (scramble) had no effect. Together, these results show that with either SDF-1α or -β stimulated c-Src phosphorylation at Tyr416 (Fig. 5B), suggesting that SDF-1 can activate c-Src.

Next, the role of c-Src in SDF-1 suppression of gluconeogenesis was evaluated. As shown in Fig. 5C, glucose production via gluconeogene-sis was stimulated by cAMP/dexamethasome and inhibited by SDF-1β in a dose-dependent manner. Application of the c-Src inhibitor, PP2, significantly reversed the effect of SDF-1β in a dose-dependent manner. Application of PP3, a non-functional analogue of PP2, had no effect. Insulin also inhibited glucose production via gluconeogene-sis. Importantly, blockade of c-Src with PP2 did not affect the insulin effect at all.

To further investigate the role of c-Src in SDF-1 suppression of gluconeogenesis, we examined the effect of PP2 on levels of PEPCK transcripts. The mRNA level of the PEPCK gene was increased by cAMP/dexamethasome and the increase was significantly reduced by SDF-1β (Fig. 5D). SDF-1β-mediated reduction in levels of PEPCK transcripts was largely prevented by PP2 in a dose-dependent manner. Again, PP3 showed no effect. It is noteworthy that the insulin-mediated decrease in the PEPCK mRNA level was not reversed by PP2 (Fig. 5D). These results indicate that SDF-1 inhibits gluconeogenesis through c-Src, whereas insulin suppression of gluconeogenesis is independent of c-Src.

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*SDF-1* inhibits hepatic gluconeogenesis through its receptor CXCR4.

**DISCUSSION**

The predominant inhibitor of gluconeogenesis is insulin. That is why hepatic gluconeogenesis becomes a major contributor to hyperglycemia when the insulin function is faulted due to either absolute deficient insulin production in T1DM or deficient insulin action plus a relatively insufficient insulin production in T2DM (50). In this study, we have found that *SDF-1* can inhibit hepatic gluconeogenesis through a signaling pathway that is distinct from the insulin signaling.

There are many similarities between *SDF-1* and insulin in suppression of gluconeogenesis although *SDF-1* appears less potent in comparison to equal molar concentrations of insulin. Both *SDF-1* and insulin promote Akt phosphorylation, which is an integral component of insulin suppression of gluconeogenesis. Blockade of Akt activation by the PI 3-kinase inhibitor LY294002 prevents both insulin- and *SDF-1*-mediated inhibition of gluconeogenesis. Furthermore, introduction of the constitutively nuclear form of FoxO1, a downstream effector of Akt, reverses the inhibitory effect of both *SDF-1* and insulin. Besides, under various conditions, both *SDF-1* and insulin can activate other intracellular signaling pathways such as mitogen-activated protein kinases (ERK1/2 and p38), PKCs, NF-κB, and Akt (12, 51–53). Thus, it appears that the roles of *SDF-1* and insulin may be interchangeable.

Importantly, the signaling pathways of *SDF-1* and insulin are not exactly the same. The insulin activation of Akt and suppression of gluconeogenesis depend upon sequential activations of IRS1 and PI 3-kinase (50). Nevertheless, *SDF-1* does not activate IRS1. Instead, *SDF-1* activates c-Src, a known activator of Akt (46, 47). Blockade of c-Src activation blunts the effect of *SDF-1* in gluconeogenesis, whereas having no effect on insulin suppression of gluconeogenesis. Therefore, *SDF-1* may be used to bypass the blunted insulin signaling in subjects with insulin resistance.

Our results clearly show that *SDF-1* suppression of gluconeogenesis is mediated by the specific receptor CXCR4. Because CXCR4 is obviously expressed in hepatocytes (14), it is anticipated that administration of CXCR4 agonist such as *SDF-1* is able to inhibit gluconeogenesis. Additionally, it is established that CXCR4 is required for skeletal muscle development (18, 54). However, it is unclear whether or not a significant amount of CXCR4 is expressed in myotubes. Thus, it is hard to predict the effect of CXCR4 agonist on glucose metabolism in skeletal muscles, another major organ involved in metabolism.

Our results demonstrate that *SDF-1* is able to inhibit hepatic gluconeogenesis in isolated hepatocytes at concentrations at or higher than 1 nM. It is currently unknown whether or not the focal concentrations of *SDF-1* around hepatocytes in liver can reach 1 nM or higher. But it is unlikely that plasma levels of *SDF-1* fluctuate with food intakes like insulin. Therefore, *SDF-1/CXCR4* does not appear to play a significant role in regulation of hepatic gluconeogenesis under the physiological condition. Furthermore, it is unknown whether or not concentrations of *SDF-1* in the liver or blood are altered in T2DM. Thus, it is unclear whether or not *SDF-1* plays any role in the development of T2DM.

There is evidence that *SDF-1/CXCR4* may be involved in the development of T1DM. It has been shown in a T1DM mouse model (NOD) that *SDF-1* plays a role in the development of the autoimmune by promoting thymocyte migration (25, 26). Blockade of *SDF-1* function with antisera can reduce insulinitis and delays the onset of diabetes in the NOD mice (26). Conversely, overexpression of *SDF-1* in pancreatic β-cells can prevent streptozotocin-induced inflammation and onset of diabetes in normal animals. Therefore, application of *SDF-1* may be beneficial in preserving pancreatic β-cells in subjects with T2DM. Besides, it is also known that *SDF-1/CXCR4* promotes vasculogenesis and neurogenesis (15, 17). It is possible that application of *SDF-1* may prevent or slow vascular and neuronal degenerations that are frequently seen in diabetes. Here we show that *SDF-1* can inhibit hepatic gluconeogenesis at concentrations that are not toxic to hepatocytes through a signaling pathway that is different from the insulin signaling. Therefore, *SDF-1* may be potentially valuable in treatment of diabetes with insulin resistance by preserving β-cells, preventing or reversing degenerations of vasculatures and neurons, in addition to reduction of blood glucose levels through inhibition of hepatic gluconeogenesis.

In summary, we have identified a new inhibitor of hepatic gluconeogenesis in *SDF-1/CXCR4*. Importantly, *SDF-1* suppresses gluconeogenesis through a signaling pathway distinct from the insulin signaling. Therefore, the *SDF-1/CXCR4* system may be a potential target for modulating hepatic gluconeogenesis and blood glucose levels in diabetes.

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