Lai *et al. Stem Cell Research & Therapy* (2025) 16:133
https://doi.org/10.1186/s13287-025-04225-5

Stem Cell Research & Therapy

RESEARCH Open Access

Adipose stromal cells increase insulin sensitivity and decrease liver gluconeogenesis in a mouse model of type 1 diabetes mellitus

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Abstract

Background Diabetic ketoacidosis (DKA) is a serious complication of hyperglycemic emergency caused by insulin deficiency through accelerated liver gluconeogenesis and glycogenolysis. DKA is most common in type 1 diabetes (T1D). Transplantation of islet cells and pancreas is an alternative to insulin injection for treating T1D. However, this alternative is only suitable for some patients. This study investigated the effects and mechanisms of adipose stromal vascular fraction (SVF) cells on liver gluconeogenesis and insulin sensitivity in an insulin-dependent T1D animal model.

Methods SVF cells were obtained from wild-type inguinal adipose tissue and transplanted into the peritoneal cavity of type I diabetic Akita (*Ins2*^{Akita}) mice.

Results We found that transplantation of 5×10^6 SVF cells from wild-type adipose tissue significantly downregulated proinflammatory genes of *TNF-α*, *IL-1β*, *IL-33*, *iNOS*, and *DPP4* in the liver and upregulated anti-inflammatory factors IL-10 and FOXP3 in blood serum and liver tissue 7 days after injection. Moreover, we found that the expression levels of *G6pc* and *Pck1* were significantly decreased in the Akita mice livers. Furthermore, the intraperitoneal insulin tolerance test assay showed that diabetic Akita mice significantly had increased insulin sensitivity, reduced fasting blood glucose, and restored glucose-responsive C-peptide expression compared with the control Akita group. This result was noted 14 days after administration of 5×10^6 or 1×10^7 SVF cells from wild-type adipose tissue into diabetic Akita mice.

Conclusions Together, these findings suggest that adipose tissue-derived SVF cells could suppress liver inflammation, regulate liver gluconeogenesis, and improve insulin sensitivity in an animal model with T1D. Therefore, adipose SVF cells may be novel cellular therapeutic alternatives to maintain steady liver gluconeogenesis in T1D.

Keywords Adipose tissue, SVF, Gluconeogenesis, IL-10, T1D

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Background

Diabetes mellitus (DM) is a metabolic disorder characterized by an increase in blood glucose levels. Globally, the incidence rate of DM has dramatically increased in all countries, especially those in Africa, Southeast Asia, and the Western Pacific. For 2045, the International Diabetes Federation has warned that there will be an increase of up to 783 million people living with diabetes worldwide [1]. Therefore, it is important to prevent diabetes prevalence and monitor treatment strategies.

DM involves complex chronic-inflammation cascades that result in cellular dysfunction through several underlying processes such as hyperglycemia, insulin resistance, hyperinsulinemia, hyperlipidemia, and hyperhomocysteinemia [2, 3]. Diabetic ketoacidosis (DKA) is a form of diabetic emergency that is characterized mainly by the triad of hyperglycemia, ketosis, and anion-gap metabolic acidosis; therefore, its treatment should be immediate [4]. DKA is a life-threatening complication of DM. Abdominal pain, deep-gasping breathing, increased urination, vomiting, weakness, confusion, and loss of consciousness are the signs and symptoms of DKA [5]. Importantly, DKA usually occurs most commonly in type 1 diabetes (T1D). It may be the initial presentation in approximately 25–40% of patients with T1D.

DKA occurs because the human body lacks insulin, and elevated glucagon levels result in increased blood glucose release by liver gluconeogenesis and glycogenolysis [6]. However, gluconeogenesis or glycogenolysis regulates hepatic glucose production (HGP). Gluconeogenesis generates glucose from noncarbohydrate substrates. Glycogenolysis produces glucose through glycogen breakdown. Both processes contribute to HGP during the fasting period [7]. Hepatic gluconeogenesis is the process of glycogen generation from noncarbohydrate precursors such as lactate, pyruvate, glycerol, and glycogenic amino acids [8, 9]. Gluconeogenesis requires four critical enzymes as part of the reverse reaction of glycolysis. Pyruvate carboxylase transforms from glycolysis into oxaloacetate through decarboxylation catalyzed by phosphoenolpyruvate carboxykinase (PEPCK) to generate phosphoenolpyruvate. Fructose-1,6-bisphosphatase facilitates the conversion of fructose-1,6-bisphosphate to fructose-6-phosphate, while glucose-6-phosphatase (G6Pase) dephosphorylates glucose-6-phosphate (G6P) into glucose [7, 8, 10]. The major contributor to the high blood glucose levels observed in DM is the increased production of hepatic glucose due to enhanced gluconeogenesis [11]. Therefore, inhibition of gluconeogenesis may be the most direct route to decrease glucose production in the liver.

Over the past years, cellular therapy has become more conspicuous in the treatment of metabolic/endocrine-related diseases, degenerative disease neurological disorders, pulmonary dysfunctions, reproductive disorders, skin burns, cardiovascular conditions, and cancers [12, 13]. It is necessary to restore insulin production and glucose-dependent insulin secretion control in order to effectively cure diabetes. Insulin replacement therapy is the current standard of care for T1D; however, it is associated with substantial limitations: the risk of hypoglycemia and inability to prevent long-term complications [14]. Hence, stem cell therapy has emerged as a promising therapeutic approach for T1D because it can restore function of β cells and achieve long-term glycemic control [15]. Despite the fact that the precise therapeutic mechanism of stem cell therapies has not yet been clear, it has been shown that stem cells reduce hepatic fibrosis, accelerate liver regeneration, and rehabilitate liver function in vivo [16–18]. However, in clinical applications, their sustained efficacy is still in uncertain. This uncertainty may be due to several factors such as a paucity of donors, organ tissue rejection, surgical complications, reduced graft mass, and high medical costs [19]. Therefore, adipose tissue is considered an abundant source of stem cells. The stromal vascular fraction (SVF) cells are an appealing therapeutic option given that their harvesting methods are safe, fast, and they are available in large quantities from fat tissue. The adipose tissue-derived stromal vascular fraction (SVF) is a heterogeneous cell population with repair, regenerative, and immunomodulatory properties. It contains not only mesenchymal stem cells (CD45-CD31-CD29 + CD44+), but also fibroblasts, endothelial cells (CD45-CD31+), leukocytes, and pericytes [18, 20]. In a previous study using a non-alcoholic steatohepatitis mouse model, no significant changes were observed in the frequency of different cell types in the adipose tissue-derived SVFs [21]. Therefore, most experiments investigating the efficacy of SVF treatment in various models have used cell number as a method of standardization.

Few studies have investigated the role of SVF in liver gluconeogenesis, although various applications of SVF cells have been reported [20, 22]. Strategies that target the stem-like autoimmune progenitor pool present an opportunity for innovative and potent immunotherapeutic interventions in the context of T1D [23]. Therefore, in order to address this important and clinically relevant issue, we investigated the effects of adipose tissue-derived SVFs on liver gluconeogenesis in a mouse model with T1D.

Methods

Animals

The Akita strain is a monogenic model for phenotypes associated with type 1 diabetes. Six to eight weeks of pathogen-free Akita (Akita mutation mutant) (C57BL/6J background) have been purchased from Jackson

Laboratory (Bar Harbor, ME) and kept at the Animal Center of Kaohsiung Veterans General Hospital. They were house in individually ventilated cages (IVC) systems with ambient lighting control to provide 12 h light/12 h dark cycles. At 3–4 weeks, the mice received genotyping by PCR for the identification genotype. The Akita mutation causes a single amino acid substitution in the insulin 2 gene that causes incorrect folding of the insulin protein [24]. Heterozygous male mice for this mutation have a progressive loss of β-cell function and develop insulin-dependent diabetes, including hyperglycemia hypoinsulinemia, polydipsia, and polyuria, as early as 4 weeks. All animal experimental procedures were designed, performed, and approved by the Institutional Animal Care and Use Committee (IACUC) of Kaohsiung Veterans General Hospital (IACUC-2408-2607-23112-NSTC). This study has been reported in line with the ARRIVE guidelines 2.0.

Isolation of SVF cells from wild-type mice adipose tissue and treatment

Stromal vascular fraction cells isolated from bilateral inguinal adipose tissue from WT mice at 12 to 16 weeks of age. The adipose tissue were minced into small pieces and then digested with 2 mg/ml collagenase 8 (Sigma-Aldrich, Cat# C2139) in HBSS for 15 min in a 37 °C water bath. Subsequently, cells were then filtered through 100 μm cell strainers and centrifuged at 1700 rpm for 10 min. After centrifugation, the cell pellets were collected and retrieved as SVF cells for the following experiments. Counting the number of SVF cells by Cellometer (Nexcelom Bioscience) and used for this study. Wild type mice received 1 ml PBS intraperitoneal injection (total $n = 24$). The Akita mice were received once by injection of 5×10^6 or 1×10^7 SVF cells from wild-type adipose tissue or PBS control into peritoneal cavity. Akita mice were randomly divided into three groups (total $n = 36$): Group I received 1 ml PBS intraperitoneal injection as controls; Group II received 5×10^6 SVF cells intraperitoneal injection; Group III received 1×10^7 SVF cells intraperitoneal injection. The mice were euthanized by CO_2 gas overdose inhalation before SVFs harvesting. Otherwise, they received intraperitoneal administration of mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg) at 7 or 14 days after injection. No mortality occurred outside of planned euthanasia or humane endpoints. At the end of the study, the animals were sacrificed and then harvested liver tissue and blood for analyze.

RNA isolation and quantitative real-time polymerase chain reaction (RT-QPCR)

Total RNA was extracted from mouse liver tissue using the RNA Miniprep Purification Kits (GeneMark) and processed according to the manufacturer's instructions. Total RNA was reverse-transcribed to cDNA as the PCR template using the RT kit (Invitrogen, Carlsbad, CA, Lot# 2234812). The gene expression level was determined using primer pairs (Coralville, Iowa) by real-time PCR. For the real-time PCR assay, 200 ng of the cDNA template was added to 25 μl of the mixture containing 12.5 μl of 2X Fast SYBR Green Master Mix (Applied Biosystems, Cat# 4385612), 1.25 μl of each sense and antisense primer (25 μM) and 8 μl of sterile water. The amplification and detection were performed with an ABI Prism step-one plus sequence detection system (Applied Biosystems, New Jersey, USA). The results were analyzed using a comparative critical threshold (Ct) method in which the amount of target gene was normalized to the amount of endogenous control.

Western blotting analysis

Harvested liver tissues were homogenized in tissue extraction buffer (Sigma), containing a proteinase / phosphatase inhibitor cocktail (Roche). The homogenized samples were subjected to 10% or 8% SDS-PAGE at 50 to 100 V for 2 h and then transferred onto the PVDF membrane. After blocking and washing, the membranes were incubated with primary antibodies including DPP4 (GeneTex), iNOS, FOXP3, phosphor-JNK, phosphor-ERK, and β-actin (Cell signaling). Subsequently, the membranes were washed with TBST buffer followed by incubation with horseradish peroxidase (HRP)-labeled secondary antibodies (Jackson Immuno Research, West Baltimore Pike, PA). The blots were developed in the ImmobilonTW Western Chemiluminescence HRP substrate (Millipore, Corporation, Billerica, MA) and visualized by an enhanced chemiluminescence method.

Enzyme-linked immunosorbent assay (ELISA)

The mouse ELISA kit (eBioscience) was used for TNF-α and IL-10 assay. Blood was centrifuged at 3000 rpm 4 °C for 10 min and then collected serum for use. The ELISA plates were coated with 100 μl capture antibody at 4 °C overnight. After washing, 200 μl of assay dilution buffer was added to block at room temperature for 1 h. Samples and serial dilutions of standards were added and incubated at room temperature for 2 h. After incubation with the detection antibody, avidin-HRP was added and incubated at room temperature for 30 min. Then, the substrate, 3,3′,5,5′-tetramethylbenzidine (TMB), was added and incubated for 20 min. Finally, 100 μl of stop solution to stop the reaction and then subjected plate to absorbance measurement at 450 nm/570 nm by ELISA reader (BioTek). C-peptide was measured using ELISA (mouse C-peptide Elisa kit; #90050; Crystal Chem) according to the manufacturer's protocol.

Intraperitoneal insulin tolerance test (IPITT)

Mice were tested in the morning after a fasting period for 16 h. Rapid insulin (0.75 U/kg body weight; Novo Nordisk A/S) was injected intraperitoneally at time zero and tail-blood samples were collected and measured using a glucose meter (Accu-check performa; Roche, Switzerland) at 15, 30, 45, 60, 75, 90 and 120 min after insulin injection.

Statistical analysis

Data were analyzed using the unpaired *t* test for comparisons between two groups or by one-way analysis of variance (ANOVA) followed by Tukey's Multiple comparison test for the comparisons between multiple groups. All values in the figures and texts were expressed as mean ± standard error of the mean, and *p* values less than 0.05 are considered statistically significant.

Results

Transplantation of SVF cells from the adipose tissue of wild-type mice into the peritoneal cavity of Akita mice decreases hyperglycemia-induced inflammation gene expression in the liver

Previous studies have indicated that liver inflammation is an important cause of glucose imbalance in DM [25]. Therefore, we used the heterozygous spontaneous mutation of *Ins2* to induce T1D in Akita mice for experimentation. In order to investigate the effects of adipose tissue-derived SVF cells on T1D, we purified SVF cells from the inguinal adipose tissues of wild-type mice and transplanted with a different cell number of 5×10^6 or 1×10^7 of SVF cells into the peritoneal cavity of Akita mice. Previously, we have checked CD8, CD11b, and PDGFRα cells in SVFs across different experiments and found that the ratios of cells numbers of those cells in different SVFs are almost the same [26]. Therefore, we can standardize those cells with total cell numbers. The liver tissue were harvested and evaluated 7 days after cell injection. Our real-time polymerase chain reaction (qPCR) data showed that mRNA expression levels of proinflammatory genes i.e., *TNF-α*, *IL-1β*, *IL-33*, *CCL2*, *iNOS*, and *DPP4* were significantly increased in Akita mice compared with wild-type mice (Fig. 1). Interestingly, transplantation of 5×10^6 or 1×10^7 SVF cells from the adipose tissue of wild-type mice into the peritoneal cavity of Akita mice significantly downregulated proinflammatory genes of *TNF-α*, *IL-1β*, *IL-33*, and *iNOS* and *DPP4* mRNA expression levels in the liver (Fig. 1). Taken together, these results suggest that Akita mice exhibited an increase in proinflammatory gene expression, whereas transplantation of adipose tissue-derived SVF cells from wild-type mice reduced proinflammatory gene expression in the liver of a mouse model with T1D.

Transplantation of SVF cells from wild-type mice into the peritoneal cavity of Akita mice reduces the protein expression levels of TNF-α, iNOS, and DPP4 in the liver

To determine the effects of adipose SVF cells from wild-type mice on the proinflammatory protein expression levels of TNF-α, iNOS, and DPP4 in the liver of Akita mice, we examined these protein expression levels by enzyme-linked immunosorbent assay (ELISA) and immunoblotting at 7 days after cell transplantation. As shown in our results, Akita mice exhibited a higher expression level of TNF-α compared with wild-type mice (5.09 ± 4.39 vs. 65.57 ± 1.1 pg/ml) (Fig. 2a). Transplantation of 5×10^6 or 1×10^7 SVF cells from the adipose tissue of wild-type mice into the peritoneal cavity of Akita mice significantly decreased the TNF-α expression level (41.48 ± 2.19 vs. 65.57 ± 1.1; 50.66 ± 1.1 vs. 65.57 ± 1.1 pg/ml) in the liver (Fig. 2a). Moreover, immunoblotting demonstrated that the protein expression levels of DPP4 and iNOS were significantly increased in Akita mice compared with those in wild-type mice. After transplantation of 5×10^6 adipose tissue-derived SVF cells from wild-type mice into the peritoneal cavity of Akita mice, DPP4 and iNOS protein expression levels in the liver tissue significantly decreased. Meanwhile, the same transplantation of 1×10^7 adipose tissue-derived SVF cells significantly decreased protein expression levels of DPP4 (Fig. 2). Altogether, these data suggest that Akita mice exhibited heightened levels of proinflammatory markers, such as TNF-α, DPP4, and iNOS, in their liver tissue compared with wild-type mice. Therefore, transplantation of SVF cells from the adipose tissue of wild-type mice into Akita mice is capable of suppressing inflammation in the liver.

Transplantation of SVF cells from wild-type mice into akita mice induces IL-10 expression in the liver and blood

IL-10 is known to inhibit proinflammatory cytokines and antigen-presenting cells, promote tissue repair mechanisms, and play an important role in restricting excessive inflammatory responses [27, 28]. We investigated IL-10 expression levels in liver and blood of Akita mice after transplantation of 5×10^6 or 1×10^7 SVF cells from wild-type mice for 7 days by qPCR and ELISA, respectively. This step was performed to determine whether IL-10 is involved in the mechanism of SVF cell-induced reduction of liver inflammation in Akita mice. Our data showed that the expression level of *IL-10* mRNA was significantly lower in Akita mice than those in wild-type mice (Fig. 3a). Transplantation of 5×10^6 or 1×10^7 SVF cells from wild-type mice into the peritoneal cavity of Akita mice significantly increased *IL-10* mRNA expression in the liver (Fig. 3a). Similarly, transplantation of 5×10^6 or 1×10^7 SVF cells from the adipose tissue of wild-type mice into the peritoneal cavity of Akita mice significantly increased blood IL-10 levels (30.8 ± 11.7 vs. 13.2 ± 2.5;

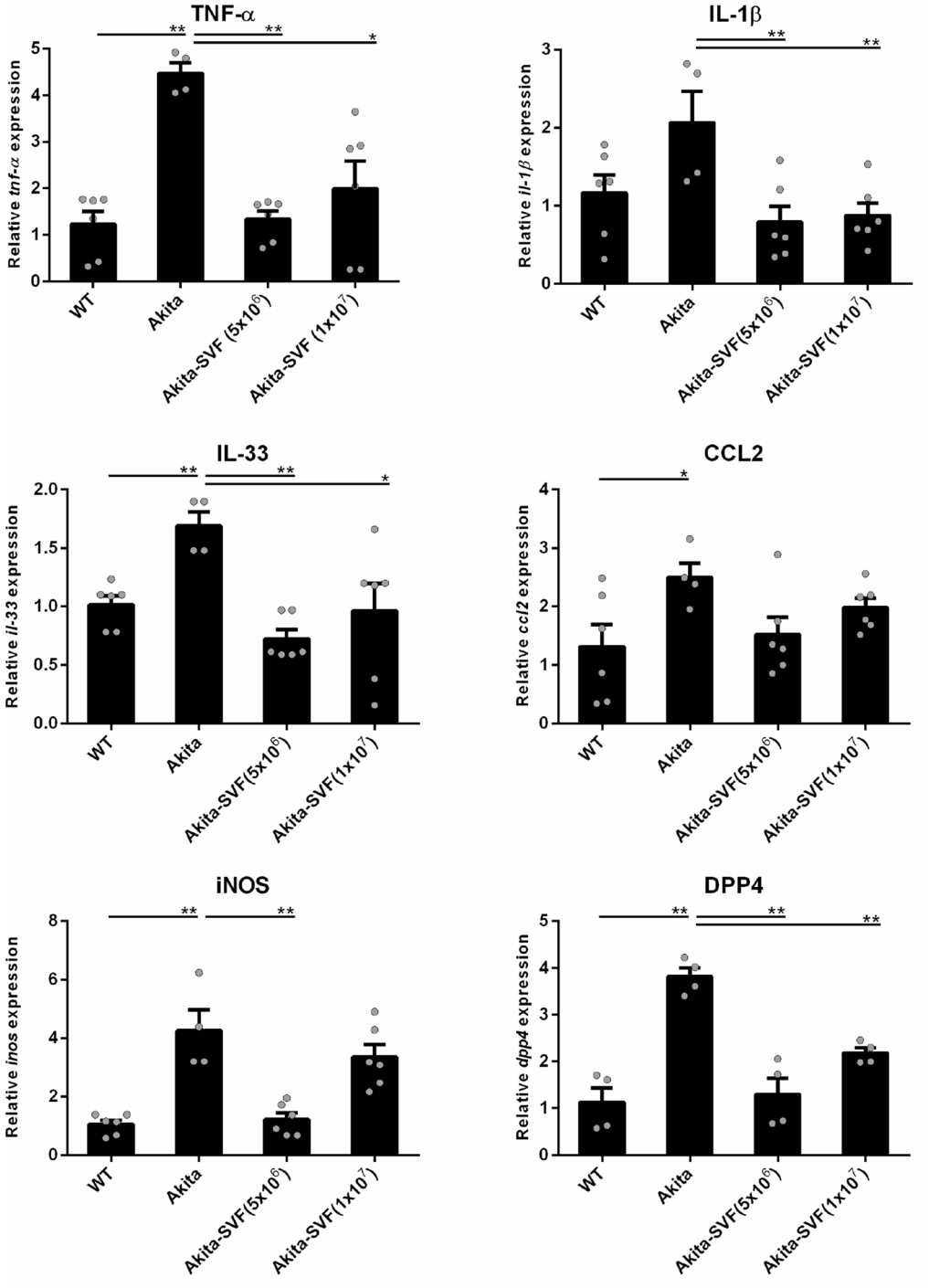

Fig. 1 Therapeutic effects of transplantation of 5×10^6 or 1×10^7 adipose tissue-derived stromal vascular fraction (SVF) cells from adipose tissue of wild-type mice in the liver in an Akita mouse model with type 1 diabetes. Adipose tissue-derived SVF cells were harvested from the adipose tissue of wild-type mice and then transplanted with a cell number of 5×10^6 or 1×10^7 into the peritoneal cavity of Akita mice. Subsequently, the liver was excised and the mRNA expression level of *TNF-α*, *IL-1β*, *IL-33*, *CCL2*, *iNOS*, and *DPP4* were determined by RT-qPCR after cell transplantation on day 7. *$p<0.05$; **indicates $p<0.01$ as compared with control Akita; $n=5$/group

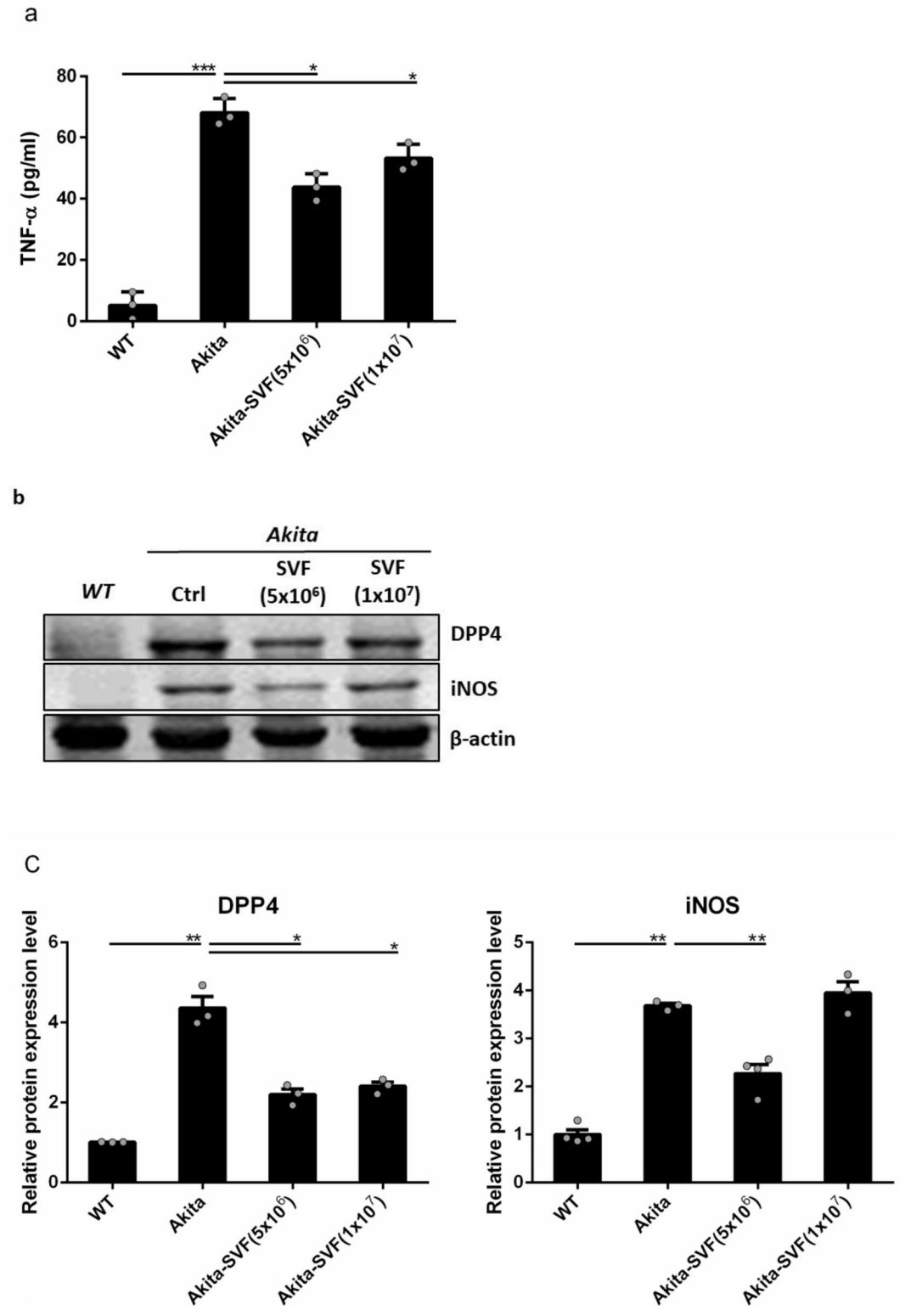

Fig. 2 Effects of transplantation of stromal vascular fraction (SVF) cells from wild-type adipose tissue into diabetic Akita mice on the protein expression levels of TNF-α, DPP4, and iNOS in the liver. Adipose tissue-derived SVF cells were harvested from the adipose tissue of wild-type mice and then transplanted with a cell number of 5×10^6 or 1×10^7 into the peritoneal cavity of Akita mice. Next, the liver tissue was collected and its protein extracted after cell transplantation for 7 days. The protein extract was then subjected to enzyme-linked immunosorbent assay to detect TNF-α expression (**a**) or western blotting analysis using antibodies against DPP4, iNOS and β-actin (**b**). (**c**) Densitometric analyses of (**b**) $n = 3$. *indicates $p < 0.05$; **indicates $p < 0.01$ as compared to control Akita

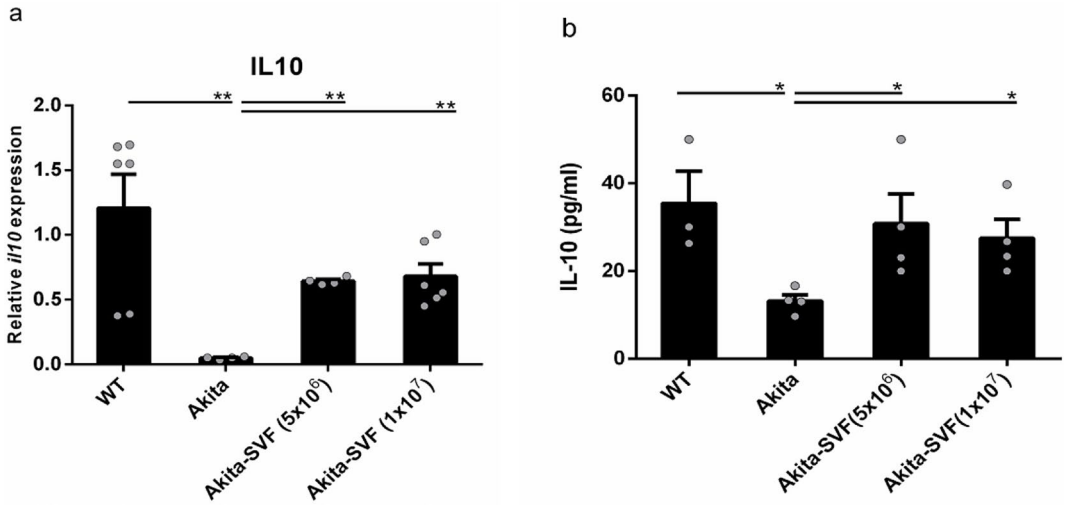

Fig. 3 Effects of transplantation of stromal vascular fraction (SVF) cells from wild-type adipose tissue into diabetic Akita mice on *IL-10* mRNA expression in the liver and blood IL-10 levels. Adipose tissue-derived SVF cells were extracted from the adipose tissue of wild-type mice and then transplanted with a cell number of 5×10^6 or 1×10^7 into the peritoneal cavity of Akita mice. One week later, liver tissue was harvested and mRNA expression levels were determined by real-time polymerase chain reaction. Circulating blood was collected at 7 days after cell transplantation and then subjected to enzyme-linked immunosorbent assay to detect IL-10 levels. *indicates $p<0.05$; **indicates $p<0.01$

27.4 ± 7.5 vs. 13.2 ± 2.5 pg/ml) at 7 days after cell transplantation (Fig. 3b).

Transplantation of SVF cells from wild-type mice into akita mice induces anti-inflammatory factor Treg expression in the liver

Induction of IL-10 has been shown to be an important mediator of Treg suppression and promoter of Treg differentiation [29]. Therefore, after proving that our transplantation experiments upregulated IL-10 expression, we performed the same procedure to check for promotion of Treg differentiation in the liver. Based on our results, the expression level of *Foxp3* (specification factor of Treg cells) was significantly lower in Akita mice than that in wild-type mice. Transplantation of 5×10^6 or 1×10^7 SVF cells from wild-type mice adipose tissue into the peritoneal cavity of Akita mice significantly increased *Foxp3* expression level (Fig. 4a) compared with their control Akita counterparts. Subsequently, we observed that transplantation of 5×10^6 or 1×10^7 SVF cells from wild-type adipose tissues significantly increased the protein expression level of FOXP3 and p-ERK in Akita mice at 7 days after cell transplantation (Fig. 4b and c). Collectively, these data suggest that Akita mice exhibited a decrease in Treg expression, whereas transplantation of 5×10^6 or 1×10^7 adipose tissue-derived SVF cells from wild-type mice enhanced anti-inflammatory mediators, such as IL-10 and Foxp3 expression in the liver to mitigate hyperglycemia-induced inflammation.

Transplantation of SVF cells from wild-type mice into akita mice suppresses the hepatic gluconeogenesis gene *G6pc* and *Pck1* expression

As indicated by Toda et al., IL-10 signaling is necessary for the normal suppression of gluconeogenic gene expression. Also, physiological concentrations of IL-10 and insulin can suppress glucose production in primary hepatocytes [30]. Inhibition of IL-10 promotes increased expression of inflammatory cytokines, worsens insulin signaling, and activates glucogenic and lipogenic pathways [31]. The gluconeogenic enzymes PEPCK and the catalytic subunit of G6Pase are key factors in hepatic gluconeogenesis [10]. As demonstrated in Fig. 5, the gluconeogenesis process of *G6pc* and *Pck1* mRNA expression level in the liver significantly increased in Akita mice that that with wild-type mice by qPCR. Intriguingly, transplantation of 5×10^6 or 1×10^7 SVF cells from wild-type adipose tissue into diabetic Akita mice significantly reduced the gluconeogenic genes of *G6pc* and *Pck1* expression in the liver compared with the control Akita mice group. These data suggest that Akita mice exhibited an increase in the expression level of gluconeogenic genes such as *G6pc* and *Pck1* in the liver. Transplantation of 5×10^6 or 1×10^7 SVF cells from wild-type adipose tissue attenuates liver gluconeogenesis to become more stable in this mouse model with T1D.

Transplantation of SVF cells from wild-type mice into akita mice improves insulin sensitivity and regulates blood glucose homeostasis

In this context, transplantation of adipose tissue-derived SVF cells from wild-type mice adipose tissue decreases

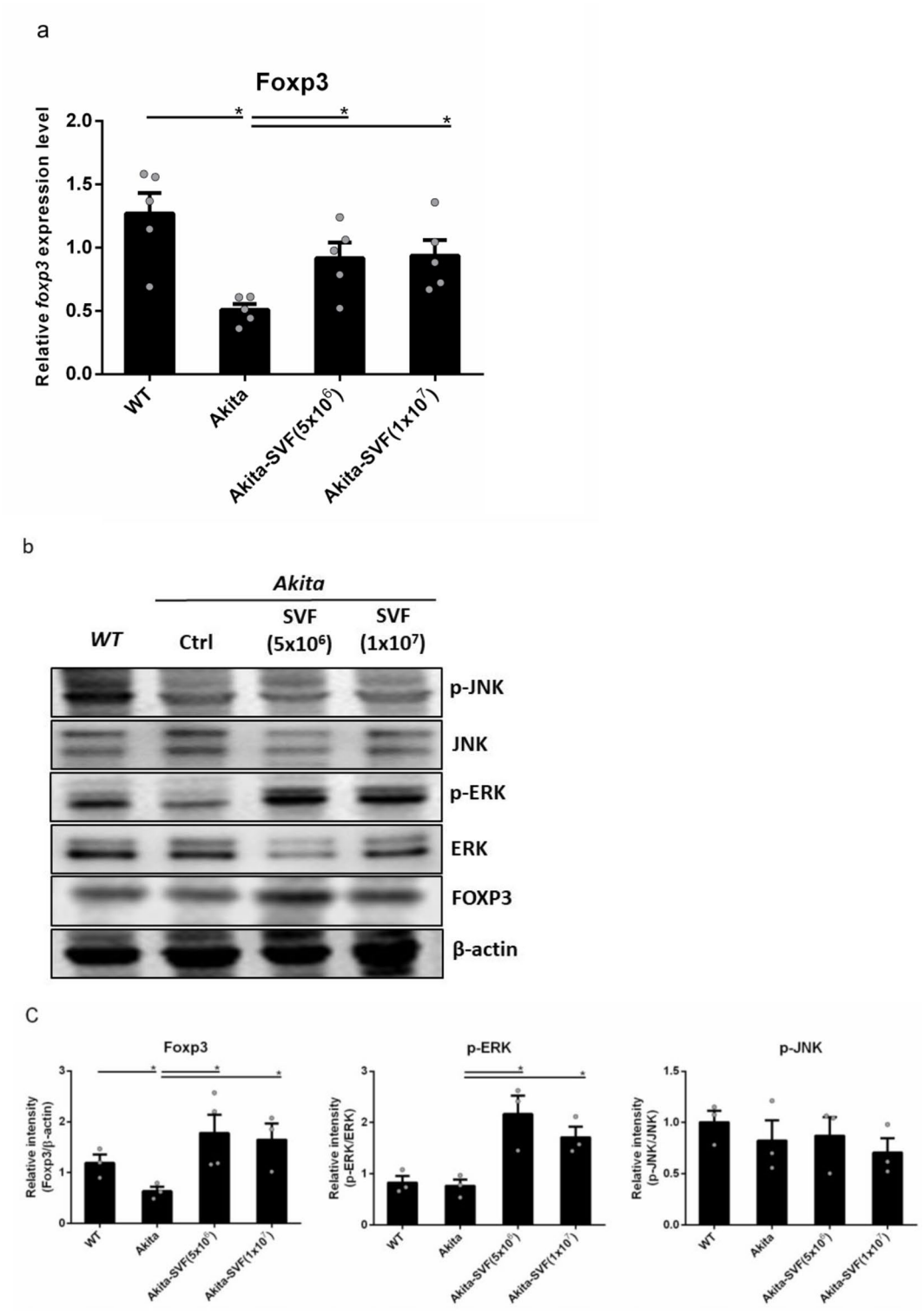

Fig. 4 Transplantation of stromal vascular fraction (SVF) cells from wild-type adipose tissue into diabetic Akita mice increased Foxp3 expression in the liver. Adipose tissue-derived SVF cells were harvested from the adipose tissue of wild-type mice and then transplanted with a cell number of 5×10^6 or 1×10^7 into the peritoneal cavity of Akita mice. Subsequently, liver tissue was harvested and Foxp3 expression level was elevated at 7 days after cell injection by (**a**) qPCR and analyzed with western blotting (**b**) using antibodies against Foxp3, p-ERK, ERK, p-JNK, JNK and β-actin. (**c**) The ratios of Foxp3/β-actin, p-ERK/ERK and p-JNK/JNK were also calculated. $n = 3$. *indicates $p < 0.05$; **indicates $p < 0.01$

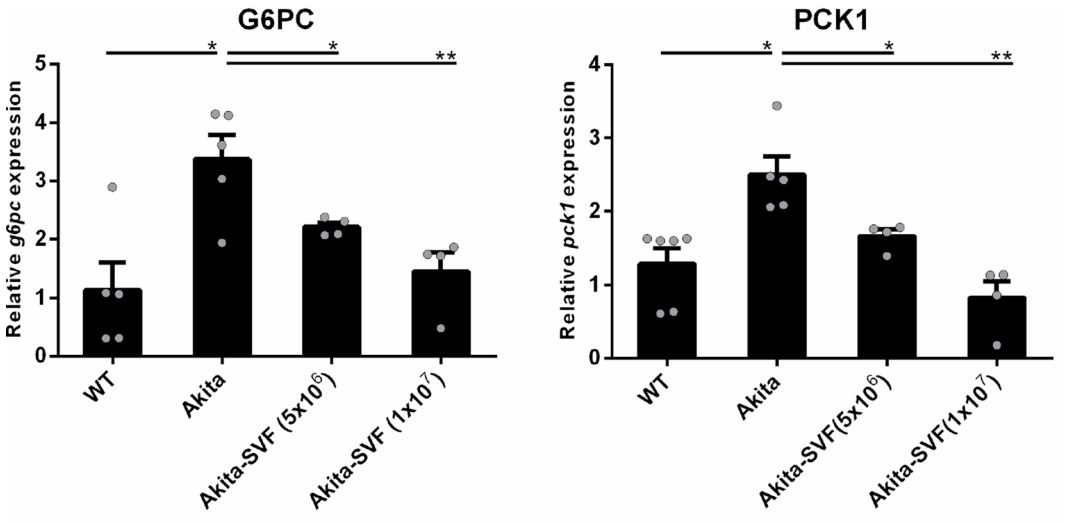

Fig. 5 Transplantation of stromal vascular fraction (SVF) cells from wild-type adipose tissue into diabetic Akita mice decreased *G6pc* and *Pck1* expression in the liver. Adipose tissue-derived SVF cells with a cell number of 5×10^6 or 1×10^7 were harvested from the adipose tissue of wild-type mice and then transplanted into the peritoneal cavity of Akita mice. One week later, the expressions of *G6pc* and *Pck1* mRNA in the liver were evaluated by RT-qPCR. *indicates $p<0.05$; **indicates $p<0.01$ as compared to control Akita; $n=5$/group

liver gluconeogenesis in Akita mice with diabetes. Next, we determined the effects of functional alteration on transplantation of 5×10^6 or 1×10^7 SVF cells from wild-type mice adipose tissue into the peritoneal cavity of Akita mice. After 16 h of fasting, blood glucose levels were significantly higher in Akita mice than those in wild-type mice (493.2 ± 65.5 vs. 88 ± 16.3 mg/dL). Importantly, transplantation of 5×10^6 or 1×10^7 adipose tissue-derived SVF cells from wild-type mice into diabetic Akita mice significantly decreased the fasting blood glucose levels at 14 days compared with control Akita mice (270.6 ± 39.3 vs. 493.2 ± 65.5; 346.7 ± 56.1 vs. 493.2 ± 65.5 mg/dL) (Fig. 6a). Furthermore, in order to evaluate the effects of SVF cells on diabetic Akita mice with reduced insulin sensitivity, the intraperitoneal insulin tolerance test assay was performed in Akita mice after transplantation of 5×10^6 or 1×10^7 adipose tissue-derived SVF cells from wild-type mice. We found that Akita mice exhibited a significant decrease in insulin sensitivity compared with wild-type mice. Transplantation of 5×10^6 or 1×10^7 adipose tissue-derived SVF cells from wild-type mice into diabetic Akita mice significantly increased insulin sensitivity compared with control Akita group on day 14 (Fig. 6b). Moreover, we also examined the levels of C-peptide in the blood serum 14 days after cell transplantation. The glucose-responsive level of the C-peptide in Akita mice significantly decreased compared with its wild-type counterpart (4.7 ± 6.3 vs. 1,080.4 ± 314.3 pg/ml). Transplantation of 5×10^6 or 1×10^7 adipose tissue-derived SVF cells from wild-type mice significantly increased the C-peptide level compared with control diabetic Akita mice (298.8 ± 123.9 vs. 4.7 ± 6.3; 340.5 ± 159.5 vs. 4.7 ± 6.3 pg/ml) (Fig. 6c). These data suggest that Akita mice presented high expression levels of fasting blood glucose and decreased insulin sensitivity. At the same time, transplantation of 5×10^6 or 1×10^7 adipose tissue-derived SVF cells from wild-type mice improves insulin sensitivity in diabetic Akita mice and modulates hyperglycemia-induced glucose imbalance.

Discussion

T1D is considered an autoimmune disease that selectively attacks insulin-producing pancreatic β cells resulting in insulin production and secretion [32]. Without insulin, glucose homeostasis and energy metabolism balance are completely disrupted. In order for blood glucose homeostasis to be maintained, HGP is regulated by glycogenolysis or gluconeogenesis [7]. Accelerated imbalance hepatic gluconeogenesis of DKA is a medical emergency due to insulin deficiency and is the leading cause of death in patients with T1D [33]. In this study, we demonstrated that transplantation of adipose tissue-derived SVF cells from wild-type mice into the peritoneal cavity of diabetic Akita mice significantly inhibited the most important enzymes *G6pc* and *Pck1* in the gluconeogenesis process and regulated fasting blood glucose levels (Fig. 6a). These data suggested that enhanced gluconeogenesis-induced glucose production in the liver is the major contributor to the high blood glucose levels in DM, and inhibiting gluconeogenesis directly decreases glucose production in the liver after cell transplantation. However, Norlin et al. demonstrated that the AMPK activator O304 improves glucose uptake and utilization in the skeletal muscle even in the absence of insulin and alleviates glycogen accumulation caused by hyperglycemia in both the skeletal muscle and heart in vivo of T2D [34]. Furthermore, the

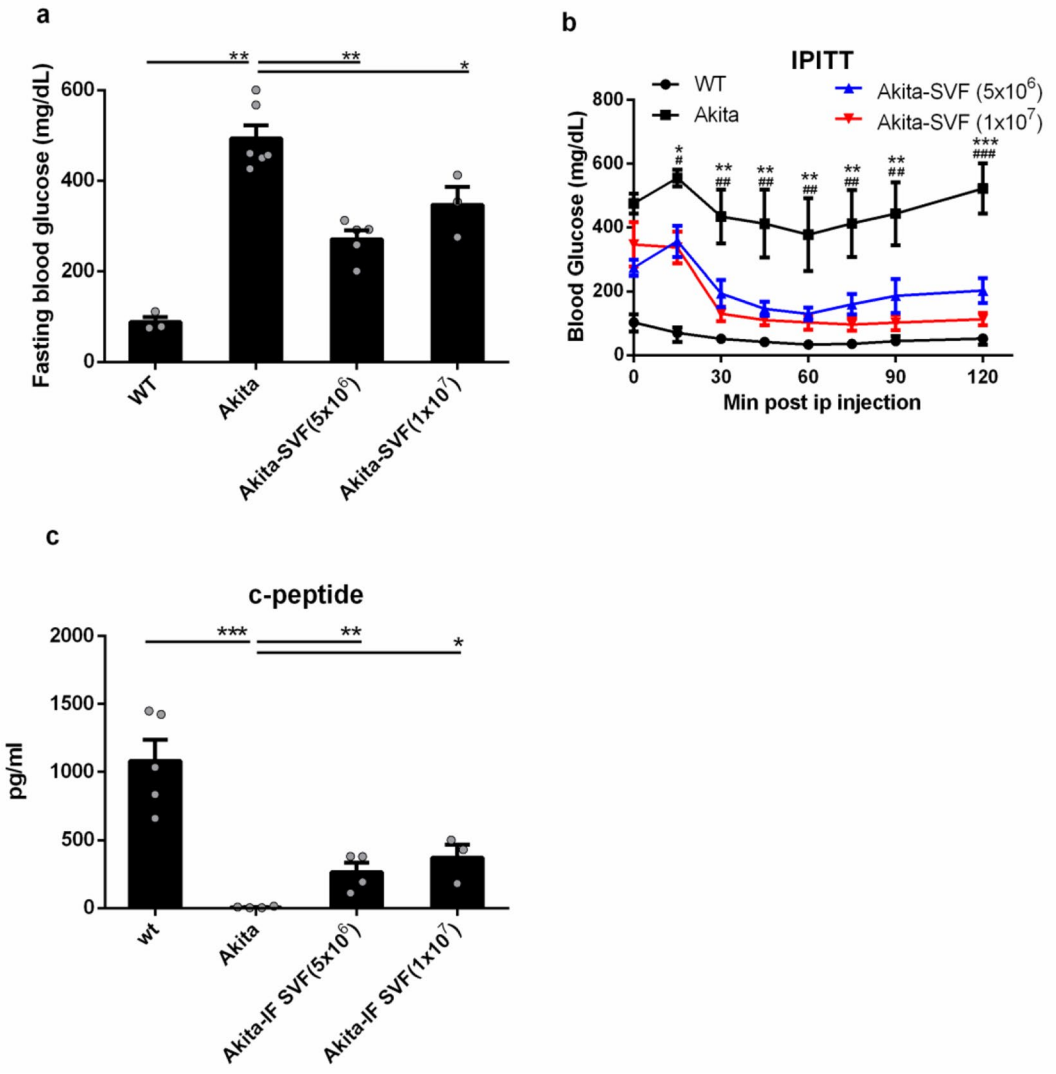

Fig. 6 Effects of transplantation of stromal vascular fraction (SVF) cells from the adipose tissue of wild-type mice into diabetic Akita mice on blood glucose, insulin sensitivity, and C-peptide levels. Adipose tissue-derived SVF cells were harvested from the adipose tissue of wild-type mice and then transplanted with a cell number of 5×10^6 or 1×10^7 into the peritoneal cavity of Akita mice for 2 weeks. Consecutively, after a 16-h fasting period, mice were administered insulin (0.75 mIU/g body weight) for 15 min. Blood glucose levels were measured (**a**) before and (**b**) every 15 min up to 2 h after insulin administration using a glucose meter. * and # summarize the significance between Akita-SVF(5×10^6) and Akita, Akita-SVF(1×10^7) and Akita, respectively. (**c**) The levels of C-peptide in serum were detected by enzyme-linked immunosorbent assay. $n=4$/group. *indicates $p<0.05$; **indicates $p<0.01$; *** indicates $p<0.001$

skeletal muscles, adipocytes, cardiovascular cells, and pancreatic beta cells exhibit an intricate relationship in glucose homeostasis and systemic insulin resistance. This is an intriguing topic worthy of further investigation on the transplantation effect of SVF cells.

Toda et al. demonstrated that IL-10 derived from adipose macrophages suppresses hepatic gluconeogenesis and reduces HGP in cooperation with insulin [30]. In contrast, inhibition of IL-10 leads to increased expression of inflammatory cytokine, deteriorated insulin signaling, and activated glucogenic and lipogenic pathways [31]. Endogenous IL-10 is a protective factor against diet-induced insulin resistance in the liver [35]. We demonstrated previously that adipose tissue-derived SVF plasma reverses insulin resistance by increasing the expression of M2 cytokines in adipose tissue from type 2 diabetic mice [22]. We demonstrated in this study that adipose tissue-derived SVF cells from wild-type mice significantly increased IL-10 expression in the liver and blood circulation in an animal model with T1D. Note that there is a higher level of expression of IL-10 in human adipose tissue-derived SVF cells than those in adipose tissue-derived mesenchymal stem cells to inhibit inflammatory cell infiltration and induce regeneration in mice liver cirrhosis [18]. The therapeutic function of SVF cells depends on not only the local effects but also

the indirect effects of immune regulatory factors or cytokine secretion through multiple mechanisms. SVF cells have immunomodulatory functions and can inhibit the production of proinflammatory cytokines by secreting the anti-inflammatory factor IL-10 or directly regulating the activity of macrophages and T cells [36]. Furthermore, some studies [37, 38] have indicated that SVF cells increase insulin sensitivity by improving insulin signaling pathways (such as IGF-1 and HGF). SVF cells also improve insulin secretion and glucose regulation by promoting the regeneration and functional recovery of pancreatic β-cells [39]. According to these studies, adipose tissue-derived SVF cells could be a novel strategy to increase anti-inflammatory factor IL-10 and improve hepatic gluconeogenesis balance in hyperglycemia.

For several decades, T1D was believed to be a chronic autoimmune disease due to an immune attack that decreases insulin secretion of islets β cells in the pancreas [40–42]. Regulatory T cells (Tregs) also exhibit dysfunction in this autoimmune disorder. Both $CD4^+$ and $CD8^+$ T cells are implicated in the development of T1D because they target several beta cell autoantigens and related peptide epitopes [43–46]. Moreover, a previous study showed that plasma DPP4 levels and DPP4 mRNA expression in rat tissues increased progressively during the development of streptozotocin-induced T1D [47]. During this process, the control and regulation of local inflammatory cytokine production are likely to be critical factors in determining the outcome of autoimmune progression [46]. Therefore, several immunotherapeutic clinical trials have been completed recently in human T1D, such as monoclonal anti-CD3 (teplizumab and otelixizumab) and anti-CD20 antibodies (rituximab) as well as anticytokines of IL-1β (canakinumab and anakinra) and TNF-α (etanercept). Targeting these factors is likely to preserve the remaining β-cell function, but curative treatments can only be realistically achieved by simultaneously trying to replace the lost part of the β-cell mass (lost during the autoimmune process) [46]. Thus far, immunotherapy alone has proven insufficient to achieve lasting preservation of β-cell function, which indicates the need to combine other strategies with β-cell therapy [32]. However, in this study, we demonstrated that transplantation of adipose SVF cells could also significantly reduce liver inflammation-related expression of *TNF-α*, *IL-33*, *iNOS*, and *DPP4*, as well as increase systemic anti-inflammatory factor IL-10 and liver Treg expression in diabetic Akita mice. As previously mentioned, certain studies demonstrated that mesenchymal stem cells (MSCs) could improve diabetic hyperglycemia and increase the number of cells of pancreatic islet β cells in mice and rats [48, 49]. Ezquer et al. demonstrated that injecting murine bone marrow multipotent mesenchymal stromal cells into STZ-treated mice increases insulin levels and significantly reduces hyperglycemia, with these effects lasting for at least 2 months [50]. Fluorescence tracing confirmed that MSCs do not directly into pancreatic progenitors but resulted in secondary lymphoid organs after their graft in vivo [50, 51]. However, it restored both systemic and local regulatory T-cell balance, increased the production of the anti-inflammatory factor IL-13, and reduced the levels of the proinflammatory factors IL-1β, IL-18, TNF-α, and MCP-1 [51]. Mice also showed an increase in the levels of epidermal growth factor (EGF), a pancreatic trophic factor involved in cell survival, and total insulin. These data imply that the reduction of inflammation is responsible for at least some of the beneficial effects of MSC treatment. Although MSCs are emerging as the most promising source for allogeneic cell therapy [52], their therapeutic use in T1D clinical trials remains highly controversial [53]. Intriguingly, we demonstrated that transplantation of adipose tissue-derived SVF cells into Akita mice not only showed a high anti-inflammatory property but also increased insulin sensitivity. It also induced the expression of glucose-response c-peptides (Fig. 6). In this context, this evidence suggests that stem cells in adipose tissue-derived SVF cells may promote the repair ability or regulate immune mediators in damaged, injured, or dysfunctional cells to modulate T1D. This may be due to the multifaceted nature of adipose tissue-derived SVF cells, which contain pluripotency stem cells that differentiate into insulin-positive cells and maintain survival of β cells in the pancreas via modulation of immunogenicity. Further study is needed to clarify their roles more elaborately.

Previous studies have also reported that fibroblast growth factor 21 (FGF21) plays a crucial role in regulating liver gluconeogenesis, and the liver is the only major organ that produces circulating FGF21 in T1D [54, 55]. Glucose transport activity in the target tissue stimulated by FGF21 is accomplished mainly by the upregulation of GLUT1 transcription [56]. The basal level of FGF21 was significantly lower in T1D patients than that in healthy controls [57]. Wente et al. demonstrated that continuous administration of FGF21 increased the activity of pancreatic β cells by activating extracellular signal-regulated kinase 1/2 (ERK1/2) and protein kinase B in animal models with diabetes [58]. As demonstrated in this study (Fig. 4), transplantation of adipose tissue-derived SVF cells into Akita mice could significantly increase phosphorylated ERK expression compared with control Akita mice. Although the role of FGF21 in this study was not explored, it may be another reason why adipose SVF cells transplanted could reverse the biological effects in the liver.

However, transplantation of adipose tissue-derived SVF cells from wild-type mice does not completely reverse the effects of hyperglycemia-induced liver dysfunction in

this study. Therefore, other mechanisms were involved in our experiment. Similarly, the number of adipose tissue-derived SVF cells was transplanted in a dose-dependent manner in this mouse model. Kim et al. indicated that cell number density is a crucial factor in inducing intracellular reactive oxygen species (ROS), leading to senescence of stem cells derived from human bone marrow [59]. Other studies also demonstrated that low Treg cell number density had high growth rates of viability and FOXP3 expression associated with expression of inhibitory molecules, lower intracellular oxygen, extracellular nutrient concentrations, and extracellular lactate accumulation on human Treg production in vitro [60]. Environmental factors such as ROS effects should be considered in cell transplantation medical efficacy of T1D treatment in the future.

Furthermore, another limitation of this study is that we focused on the biological effect within 2 weeks by transplanting adipose tissue-derived SVF cells into Akita mice for early intervention in T1D, and we did not extend the observation period beyond this time frame. Because SVF treatments often mediate short term effects, the longevity of the observed benefits remains an important question. In future studies, we intend to extend the observation period to determine whether the beneficial effects persist beyond 14 days. This may provide a clearer understanding of the long-term efficacy of SVF treatment. Additionally, we used cell number as a method of standardization, but we did not examine the frequency of different cell types in the adipose-derived SVFs in our model.

Conclusion

Transplantation of adipose tissue-derived SVF cells from wild-type mice into peritoneal cavity of diabetic Akita mice decreased the mRNA expression of proinflammatory-related genes; reduced the protein expression of TNF-α, DPP4, and iNOS; and increased the expression of IL-10 and Foxp3 in the liver. This transplantation also reduced the expression of the liver gluconeogenesis genes *G6pc* and *Pck1* and increased C-peptide levels. This study presents that such transplantation inhibited hepatic gluconeogenesis, improved insulin sensitivity, maintained blood glucose stability, and regulated the immune response in a T1D mouse model. Furthermore, adipose tissue-derived SVF cells are easy to harvest and purify. This research provided a novel clinical approach to cell therapy that is simpler, more cost-effective, safer, and scalable than insulin replacement therapy. This novel approach would reduce the occurrence of severe complications associated with T1D, such as acute DKA.

Abbreviations

DM	Diabetes mellitus
DKA	Diabetic ketoacidosis
SVFs	Stromal vascular fractions
HGP	Hepatic glucose production
PC	Pyruvate carboxylase
PEPCK	Phosphoenolpyruvate carboxykinase
G6Pase	Glucose 6-phosphatase
TNF	Tumor Necrosis Factor-α
IL-1β	Interleukin-1β
IL-33	Interleukin-33
CCL2	Monocyte chemoattractant protein-1
iNOS	Inducible nitric oxide synthase
DPP-4	Dipeptidyl peptidase-4
IL-10	Interleukin-10
Foxp3	Forkhead box protein P3
MSC	Mesenchymal stem cells
FGF21	Fibroblast growth factor 21

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13287-025-04225-5.

Supplementary Material 1

Supplementary Material 2

Acknowledgements

Not applicable.

Author contributions

Yen-Ju Lee & Hsiao-Chi Lai: Investigation, Validation, Data curation, Writing-Original draft preparation. Pei-Hsuan Chen & Chia-Hua Tang: Methodology. Lee-Wei Chen: Project administration, Conceptualization, Visualization, Supervision, Writing-Reviewing and Editing.

Funding

This work was supported by KAFGH-ZY-A-112017, MOST 110-2314-B-A49A-540-MY3 and KSVGH112-082.

Data availability

All relevant data and material to reproduce the findings are available within the paper.

Declarations

Ethics approval and consent to participate

(1) Title of the approved project: The effect of adipose stromal vascular fractions on type 1 diabetes- induced complications by regulating PPAR-γ; (2) Name of the institutional approval committee or unit: Institutional Animal Care and Use Committee of Kaohsiung Veterans General Hospital; (3) Approval number: IACUC-2408-2607-23112-NSTC (4) Date of approval: 15th Jan 2024. The authors declare that they have not use AI-generated work in this manuscript.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 2 April 2024 / Accepted: 11 February 2025
Published online: 11 March 2025

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