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Soluble T-cadherin secretion from endothelial cells is regulated via insulin/PI3K/Akt signalling

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ABSTRACT

Aim and objective: Our recent report showed that soluble T-cadherin promotes pancreatic beta-cell proliferation. However, how and where the secretion of soluble T-cadherin is regulated remain unclear.
Methods and results: Soluble T-cadherin levels significantly increased in leptin receptor-deficient *db/db* mice with hypoinsulinaemia or in wild-type mice treated with insulin receptor blockade by S961. Similar results were observed in human subjects; Diabetic ketoacidosis patients at the time of hospitalization had increased plasma soluble T-cadherin levels, which decreased after insulin infusion therapy. Patients with recurrent ovarian cancer who were administered a phosphatidylinositol-3 kinase (PI3K)-alpha inhibitor (a new anticancer drug) had increased plasma soluble T-cadherin and plasma C-peptide levels. Endothelial cell-specific T-cadherin knockout mice, but not skeletal muscle- or cardiac muscle-specific T-cadherin knockout mice, showed a 26 % reduction in plasma soluble T-cadherin levels and a significant increase in blood glucose levels in streptozocin-induced diabetes. The secretion of soluble T-cadherin from human endothelial cells was approximately 20 % decreased by insulin and this decrease was canceled by blockade of insulin receptor/Akt signalling, not Erk signalling.
Conclusion: We conclude that insulin regulates soluble T-cadherin levels and soluble T-cadherin secretion from endothelial cells is positively regulated by insulin/insulin receptor/Akt signalling.

1. Introduction

Over the past several decades, the incidence of diabetes mellitus and its complications has increased worldwide, and diabetes mellitus has become an urgent health problem. The major characteristic of the pathophysiology of diabetes is the gradual (sometimes rapid) loss of the function and mass of pancreatic beta-cells [1]. The supplementation of insulin, glucagon-like polypeptide-1 receptor agonists and oral hypoglycaemic agents are the major treatment methods for diabetes [1,2]. Although bariatric surgery can result in disease remission and the cessation of medications, this does not mean that the number of beta-cells in the pancreas has returned to normal. In addition, insulin injection therapy is the only therapy controlling type 1 diabetes, expecting an alternative fundamental therapy [3]. Promoting pancreatic beta-cell proliferation is considered one of the key mechanisms of managing diabetes [4,5]. There have been many studies investigating the factors involved in beta-cell proliferation [6–9], but a substantial strategy for the clinical translation of these factors is lacking.

T-cadherin is classified as a member of the classical cadherin family, although it is a unique cadherin with a glycosylphosphatidylinositol (GPI) anchor on its C-terminus [10,11]. We reported that T-cadherin is expressed in the heart, muscle, aorta, renal pericytes, and mesenchymal stem-like cells and that it specifically accumulates adiponectin, an adipose tissue-specific secretory factor [12–16]. Additionally, our previous

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studies showed that adiponectin binds to T-cadherin and enhances the biogenesis and secretion of small extracellular vesicles (exosomes) in T-cadherin-expressing cells [17–19].

Recently, we developed new monoclonal antibodies against T-cadherin and the ELISA to identify soluble forms of T-cadherin (soluble T-cadherin) in human and mouse plasma [20,21]. We next showed that increased levels of soluble T-cadherin in plasma contributed to pancreatic beta-cell proliferation under conditions of high-fat diet (HFD)-induced obesity and significantly alleviated streptozocin (STZ)-induced diabetes in systemic T-cadherin knockout (KO) mice [22]. RNA sequencing of isolated murine islets indicated that notch signalling was involved in beta-cell proliferation and that supplementation of recombinant soluble T-cadherin to isolated murine islets significantly increased notch signalling gene expression and the cell cycle [22]. We concluded that soluble T-cadherin is an endocrine factor involved in pancreatic beta-cell proliferation that acts by upregulating Notch signalling [22,23].

Here, we investigated the regulatory mechanisms of soluble T-cadherin secretion by evaluating the concentration of soluble T-cadherin under several diabetic conditions in mice and humans. We also investigated the source of soluble T-cadherin in tissues from three newly constructed tissue-specific T-cadherin KO mice. This study aimed to clarify the significance of soluble T-cadherin on glucose/insulin metabolism by investigating the source tissue/cell and the regulation of secretion.

2. Materials and methods

2.1. Chemicals and reagents

Streptozocin (STZ) (#S0130), dapagliflozin (#SML2804), and bovine insulin (#I0516) were purchased from Sigma-Aldrich. The insulin receptor antagonist S961 (#S6922), Akt inhibitor MK-2206 (#S1078), and Erk1/2 inhibitor SCH772984 (#S7101) were purchased from Selleck. For western blotting, we used precast polyacrylamide gels (Bio-Rad, #5671095J10) and these antibodies; phosphorylated Akt (Cell Signaling Technology, #4060S), total Akt (Cell Signaling Technology, #9272S), phosphorylated Erk1/2 (Cell Signaling Technology, #4370S), and total Erk1/2 (Cell Signaling Technology, #9102S). An osmotic pump, Alzet 1007D was used for continuous infusion. Blood glucose concentrations were measured using monitoring kits (Sanwa Kagaku Kenkyusho). Plasma insulin concentrations were determined using an ELISA kit (Morinaga Institute of Biological Science, #M1104). The plasma concentrations of soluble T-cadherin were measured using a human T-cadherin ELISA kit (Immuno-Biological Laboratories) [20].

2.2. Animals and diets

Cdh13 (T-cadherin) exon $2^{flox/flox}$ mice were transferred from Boston Children's Hospital under a material transfer agreement [22]. We used the Cre/loxP system to conditionally delete the *Cdh13* (T-cadherin) gene. We crossed T-cadherin exon $2^{flox/flox}$ mice with VE-cadherin-CreERT2 mice [24]. These tamoxifen-inducible Cre mice were administered 200 mg/kg 4-hydroxytamoxifen for five consecutive days to generate endothelial cell-specific T-cadherin KO mice. We crossed T-cadherin exon $2^{flox/flox}$ mice with muscle creatin kinase-Cre mice [25] or α-myosin heavy chain-Cre mice [26] to generate skeletal muscle- or cardiac muscle-specific T-cadherin KO mice. We purchased seven-week-old *db/db* mice from Japan Clea. Eight-week-old male mice were used for all experiments. Mice were fed normal chow (Oriental Yeast) except for an experiment using a high-fat (60 kcal%) diet (HFD) (Research Diets, #D12492) from 8 weeks of age for 6 weeks. All animal experiments were performed according the principles of the Guide for the Care and Use of Experimental Animals of Osaka University Graduate School of Medicine and were approved by the Animal Care Committee of Osaka University (approval no. 03-056-018).

2.3. Streptozocin treatment

Streptozocin (STZ) dissolved in 0.1 mol/L citrate buffer was intraperitoneally injected into 8-week-old male mice at 60 mg/kg for 5 consecutive days. The administration of dapagliflozin was conducted using a stock solution of dapagliflozin (125 mg/mL in 100 % ethanol) diluted to a final concentration of 0.02 mg/mL in distilled water. Then, the dapagliflozin solution was added to the drinking water. The water drinking bottles were changed twice a week [27].

2.4. S961 treatment

Eight-week-old male WT mice were anaesthetized and implanted Alzet pump with normal saline (control) or the insulin receptor antagonist S961 at a dose of 1.43 nmol/day for seven days [28].

2.5. Human blood specimens

We obtained blood specimens from two patients who were diagnosed with diabetic ketoacidosis and transported to the emergency room at Osaka University Medical Hospital (Case 1 and 2) and two patients who were newly administered CYH33, a selective PI3K-alpha inhibitor [29], for recurrent ovarian clear cell adenocarcinoma in the Department of Obstetrics and Gynaecology at Osaka University Medical Hospital (Case 3 and 4). This study followed the principles of the Declaration of Helsinki and was approved by the Human Ethics Committees of Osaka University (no. 885 for Case 1 and 2, no. 23290 for Case 3 and 4). Informed consent was obtained from the patients or their relatives after the purpose of the study was explained.

Case 1: A 48-year-old man who had been diagnosed with type 1 diabetes mellitus (the year of diagnosis was unknown) and had been receiving multiple daily insulin injections. He had also been diagnosed with chronic kidney disease. He had not visited the hospital for the last four months. He was transported to the emergency room and diagnosed with diabetic ketoacidosis. Blood specimens were collected on the day of hospitalization and 14 days after hospitalization.

Case 2: A 79-year-old woman who had been diagnosed with type 2 diabetes (approximately 13 years before the onset of ketoacidosis) and had been receiving basal-supported oral therapy using insulin degludec and sitagliptin. She had not visited the hospital for the last three months. She was transported to the emergency room and diagnosed with diabetic ketoacidosis. Blood specimens were collected on the day of hospitalization and three days after hospitalization.

Case 3: A 69-year-old woman who had been diagnosed with recurrent ovarian clear cell adenocarcinoma with lung metastasis was treated with CYH33. The day before the administration of CYH33, blood specimens were collected, and the patient was given metformin and empagliflozin to prevent adverse effects of CYH33 (increased plasma glucose). One day after the administration of CYH33, blood specimens were collected.

Case 4: A 60-year-old woman who had been diagnosed with recurrent ovarian clear cell adenocarcinoma with multiple lymph nodes, adrenal, and L4 vertebral metastases was treated with CYH33. Similar to Patient 3, the day before the administration of CYH33, blood specimens were collected, and she was given metformin and empagliflozin. One day after the administration of CYH33, blood specimens were collected.

2.6. Measurement of soluble T-cadherin in the supernatants of cell lines

Human umbilical vein endothelial cells (HUVECs, Takara Bio, #C-12208) were cultured in standard media supplemented with 2 % FBS and growth factors (Kurabo, #KE-2170S) in 24-well collagen-coated plates for 48 h. After discarding the standard media, the experimental media without FBS and growth factors containing 0 or 100 nM bovine

insulin, 0 or 1 μM S961, 0 or 5 μM Akt inhibitor MK-2206, and 0 or 1 μM Erk1/2 inhibitor SCH772984 were added to the wells and incubated for 24 h. After incubation, the supernatants were collected, and the concentrations of soluble T-cadherin were measured using an ELISA kit (described above). Although several hours or overnight serum starvation will be performed before supplementation of the reagents, the viability of HUVECs was impaired by the starvation. Thus we added the reagents just after discarding the standard media.

2.7. Insulin signalling assays

We cultured HUVECs in standard media (described above) in 12-well collagen-coated plates for 48 h. After discarding the standard media, the experimental media without FBS and growth factors containing 0 or 100 nM insulin, 0 or 1 μM S961, 0 or 5 μM of the Akt inhibitor MK-2206, and 0 or 1 μM of the Erk1/2 inhibitor SCH772984 were added to the wells and incubated for 15 min. After incubation, the supernatants were discarded, and the cells were lysed with RIPA buffer containing protease and phosphatase inhibitors.

2.8. Western blotting

We performed Western blot analyses as previously reported [17]. Three micrograms of whole-cell lysates were loaded onto 4–20 % gradient SDS–PAGE gels and transferred to nitrocellulose membranes. The membranes were incubated in the blocking buffer for detecting phosphorylation of proteins, Blocking One-P (Nacalai), for 1 h. After washing, the membranes were incubated with primary antibodies overnight at 4 °C and followed by incubation with secondary antibodies for 1 h at room temperature. The signals were developed by Chemi-Lumi One Super (Nacalai) and detected with ChemiDoc Touch (Bio-Rad).

2.9. Statistical analyses

We performed the statistical analyses using JMP Pro 15 (SAS Institute). *p* values less than 0.05 were considered to indicate statistical significance. The results are expressed as the mean ± SEM from at least three independent biological experiments unless otherwise specified. The methods used for the statistical tests and sample sizes are provided

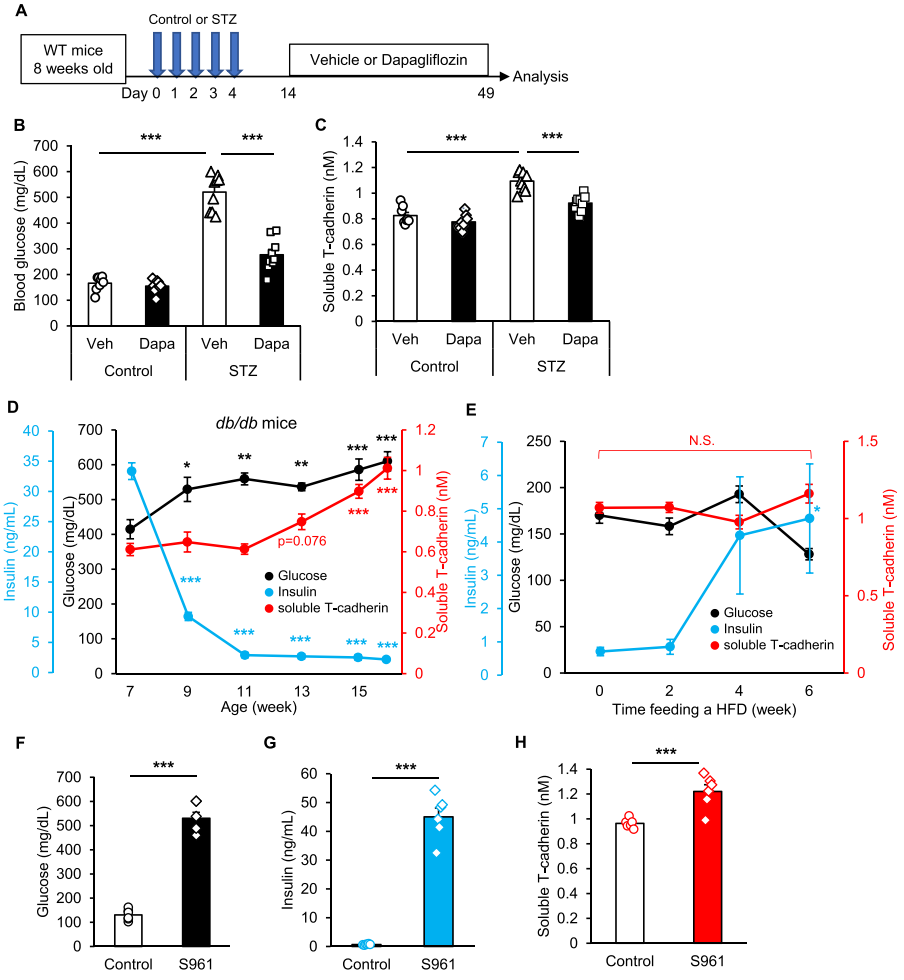

Fig. 1. Soluble T-cadherin levels are increased in STZ-induced diabetic mice and *db/db* mice and by the administration of S961.
A) Protocol for the administration of STZ and dapagliflozin. Two weeks after the initial injection of STZ, the oral administration of dapagliflozin was initiated.
B) Blood glucose levels 35 days after the initial administration of dapagliflozin in STZ-induced diabetic mice (n = 8–9. ***p < 0.001; Tukey–Kramer test).
C) Soluble T-cadherin levels 35 days after the initial administration of dapagliflozin in STZ-induced diabetic mice (n = 8–9. ***p < 0.001; Tukey–Kramer test).
D) Plasma glucose, insulin, and soluble T-cadherin levels in *db/db* mice (n = 8. *p < 0.05, **p < 0.01, ***p < 0.001; Dunnett test).
E) Plasma glucose, insulin, and soluble T-cadherin levels in WT mice fed a high-fat diet for six weeks (n = 6. *p < 0.05; Dunnett test).
F) Blood glucose levels in WT mice administered saline or S961 for seven days (n = 6. ***p < 0.001; unpaired *t*-test).
G) Plasma insulin levels in WT mice administered saline or S961 for seven days (n = 6. ***p < 0.001; unpaired *t*-test).
H) Plasma soluble T-cadherin levels in WT mice administered saline or S961 for seven days (n = 6. ***p < 0.001; unpaired *t*-test).
Data are shown as the mean ± sem.

in the figure legends.

3. Results

3.1. Insulin depletion or insulin signalling deficiency increases plasma soluble T-cadherin levels in mice

We previously reported that after the administration of STZ, the plasma concentration of soluble T-cadherin increased as blood glucose increased [22]. Therefore, we speculated that plasma soluble T-cadherin levels increase in response to insulin deficiency or high glucose concentrations. First, WT mice that were injected with multiple doses of STZ for 5 days were orally administered dapagliflozin (Fig. 1A). Consistent with our previous report, both blood glucose and plasma soluble T-cadherin levels were significantly increased after the administration of STZ, and the increase in soluble T-cadherin induced by STZ treatment was significantly decreased by the administration of dapagliflozin [27] (Fig. 1B and C).

Second, we examined the responses to these treatments in *db/db* mice, which is an obese diabetic mouse model. Plasma soluble T-cadherin levels were significantly increased in *db/db* mice after 11 weeks of age, plasma insulin levels were markedly reduced (Fig. 1D). This finding contrasted with that in HFD-induced obese mice. In this mouse model, insulin levels were consistently increased but soluble T-cadherin was not increased. These findings suggest that a lack of plasma insulin but not insulin resistance increases plasma soluble T-cadherin (Fig. 1E).

Third, we evaluated soluble T-cadherin under conditions in which insulin signalling was inhibited. WT mice administered S961, an insulin receptor inhibitor, by continuous infusion for one week [28,30]. At the end of S961 treatment, plasma soluble T-cadherin levels were significantly increased, and plasma insulin levels were also increased by S961 administration (Fig. 1F-H), suggesting that deficient insulin signalling is likely important for the increase in plasma soluble T-cadherin.

3.2. Plasma soluble T-cadherin levels are increased in diabetic ketoacidosis patients and ovarial cancer patients administered PI3K-alpha inhibitor

Furthermore, we evaluated human patients who were transported to the emergency room due to diabetic ketoacidosis.

Case 1 was a 48-year-old man who had been diagnosed with type 1 diabetes mellitus and had received multiple daily insulin injections. He had not visited the hospital for the last four months. He was transported to the emergency room and diagnosed with diabetic ketoacidosis. On the day of hospitalization, plasma glucose was severely elevated (927 mg/dL), and soluble T-cadherin was also markedly elevated (3.74 nM). Ten days after continuous insulin infusion therapy, his plasma glucose level decreased (189 mg/dL), and his soluble T-cadherin level decreased (1.35 nM).

Case 2 was a 79-year-old woman who had been diagnosed with type 2 diabetes and dementia and received basal-supported oral therapy using insulin degludec. She had not visited the hospital for the last three months. She was transported to the emergency room and diagnosed with diabetic ketoacidosis. On the day of hospitalization, plasma glucose was severely elevated (835 mg/dL), and soluble T-cadherin was also elevated (1.33 nM). Three days after continuous insulin infusion therapy, her plasma glucose level decreased (258 mg/dL), and her soluble T-cadherin level decreased (1.04 nM).

In these two patients, soluble T-cadherin was elevated at the point of insulin depletion due to severe diabetic ketoacidosis and decreased after insulin infusion therapy was administered (Fig. 2A).

We next examined patients who had been diagnosed with recurrent ovarian cancer with metastasis and were newly treated with CYH33. CYH33 is a selective phosphatidylinositol-3 kinase (PI3K)-alpha inhibitor that inhibits insulin/PI3K/Akt signalling [29].

Case 3 was a 69-year-old woman who had been diagnosed with recurrent ovarian cancer with lung metastasis was treated with CYH33. The previous day before starting the administration of CYH33, metformin and empagliflozin treatments were initiated to prevent an adverse increase in plasma glucose levels. One day after the start of CYH33 treatment, her plasma glucose was mildly elevated (from 97 to 133 mg/dL), and her plasma C-peptide was markedly elevated (from 1.3 to 9.1 ng/mL). The soluble T-cadherin concentration increased from 1.36 to 1.52 nM.

Case 4 was a 60-year-old woman who had been diagnosed with recurrent ovarian cancer with multiple lymph node, adrenal, and L4 vertebral metastases and was treated with CYH33. The previous day before starting the administration of CYH33, metformin and

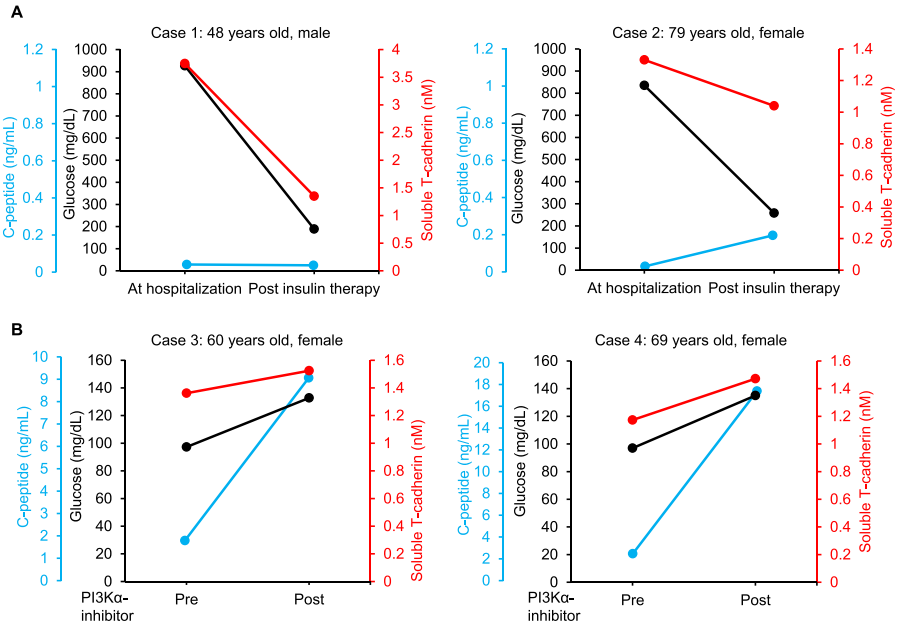

Fig. 2. Soluble T-cadherin in human patients diagnosed with diabetic ketoacidosis or administered a PI3K-alpha inhibitor.
A) Plasma glucose, C-peptide, and soluble T-cadherin levels in two patients diagnosed with diabetic ketoacidosis.
B) Plasma glucose, C-peptide, and soluble T-cadherin levels in two patients treated with CYH33(PI3K-alpha inhibitor) due to recurrent ovarian cancer.

empagliflozin treatments were initiated. One day after the start of CYH33 treatment, her plasma glucose was mildly elevated (from 97 to 135 mg/dL), and her plasma C-peptide was markedly elevated (from 3.6 to 17.5 ng/mL). The soluble T-cadherin concentration increased from 1.17 to 1.47 nM.

In these two cases, soluble T-cadherin was elevated after the pharmacological inhibition of the insulin receptor by CYH33 (Fig. 2B).

These findings suggest that the depletion of insulin or the deficiency of insulin signalling leads to an increase in plasma soluble T-cadherin levels.

3.3. Endothelial cell is one of the sources of plasma-soluble T-cadherin

We next evaluated which tissues produced soluble T-cadherin. Among the T-cadherin-expressing tissues or cells, we focused on skeletal muscle cells, cardiac muscle cells, and endothelial cells, which are considered important for glucose metabolism in response to insulin and for sensing whole-body insulin "demand".

We crossed VE-cadherin-CreERT2- [24], muscle creatine kinase (MCK)-Cre- [24], and α-myosin heavy chain (αMyHC)-Cre mice [26] with T-cadherin$^{flox/flox}$ mice (control) [22] and generated endothelial cell-, skeletal muscle-, and cardiac muscle-specific T-cadherin-KO mice, respectively. The concentration of plasma soluble T-cadherin was significantly reduced in endothelial cell-specific KO mice (26 % reduction, Fig. 3A) and skeletal muscle-specific KO mice (10 % reduction, Fig. 3B). This concentration was not significantly changed in cardiac muscle-specific KO mice (Fig. 3C). Next, we administered multiple low doses of STZ to these mice at eight weeks of age [6]. In a previous study performed under similar conditions, we reported that conventional T-cadherin KO mice had higher blood glucose levels than control mice after STZ treatment and that supplementation with soluble T-cadherin via liver-specific expression significantly reduced blood glucose and pancreatic insulin levels to almost equal levels as those in control mice [22]. Similar to the findings in conventional T-cadherin KO mice, blood glucose levels in endothelial cell-specific KO mice were significantly greater than those in control mice (Fig. 3D), although they were not changed in skeletal muscle-specific KO mice (Fig. 3E). These results suggest that endothelial cells are important sources of plasma-soluble T-cadherin, which may be related to the amelioration of STZ-induced diabetes.

3.4. The secretion of soluble T-cadherin from endothelial cells is negatively regulated by insulin-Akt signalling

To clarify how soluble T-cadherin secretion is regulated by insulin, we employed primary human umbilical vein endothelial cells (HUVECs) and measured soluble T-cadherin in the supernatants. Soluble T-cadherin was significantly reduced as the concentration of insulin in the media increased (Fig. 4A), whereas was not statistically affected by the concentration of glucose in the media without insulin (Sup. Fig. 1),

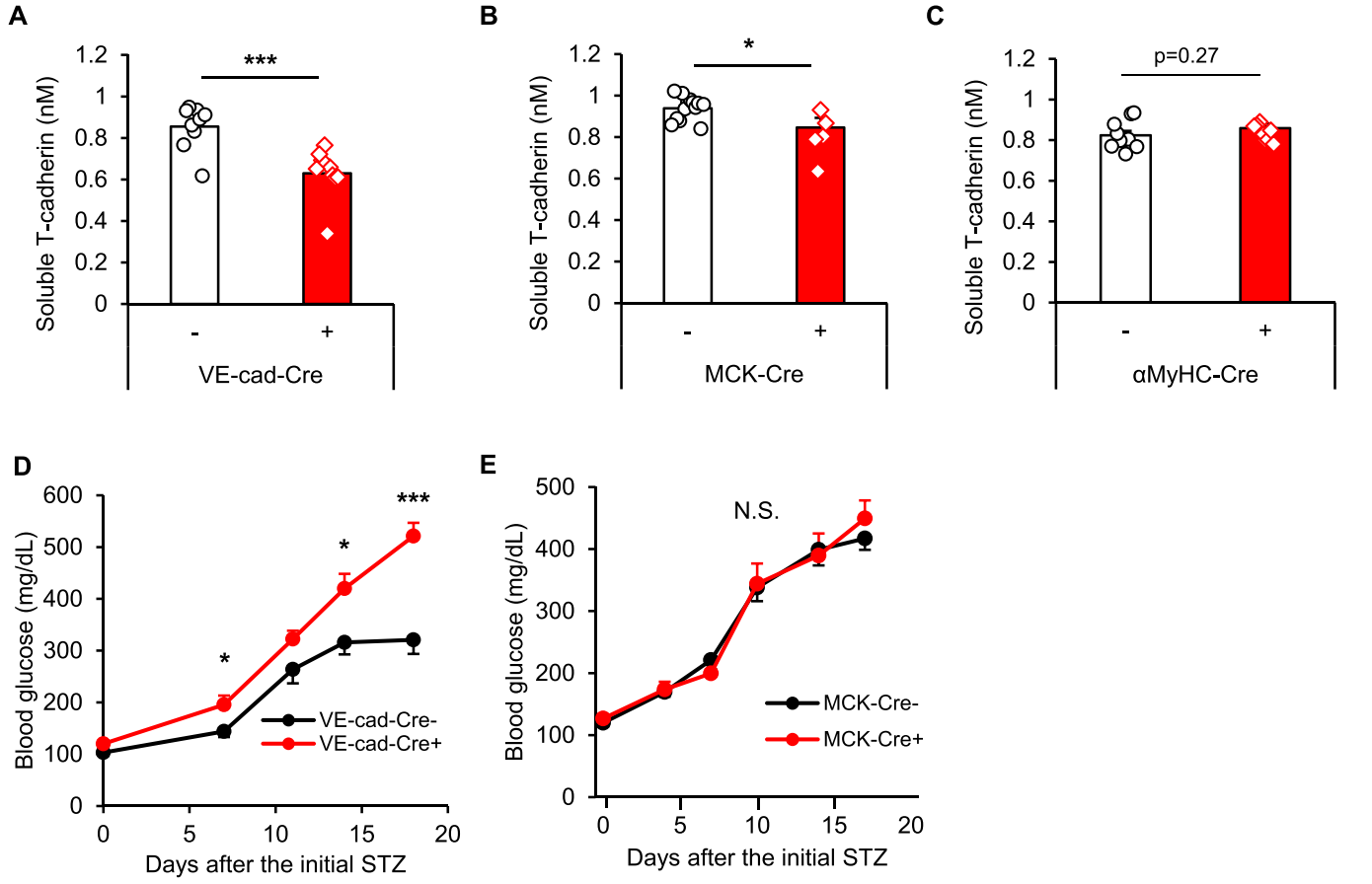

Fig. 3. Endothelial cell-specific T-cadherin KO mice exhibit decreased plasma soluble T-cadherin levels and worsened STZ-induced diabetes. A) Plasma soluble T-cadherin levels in endothelial cell-specific T-cadherin KO mice (n = 9).
B) Plasma soluble T-cadherin levels in skeletal muscle-specific T-cadherin KO mice (n = 13 Cre-, n = 7 Cre+).
C) Plasma soluble T-cadherin levels in cardiac muscle-specific T-cadherin KO mice (n = 10).
D) Blood glucose levels in endothelial cell-specific T-cadherin KO mice administered multiple doses of STZ (n = 9).
E) Blood glucose levels in skeletal muscle-specific T-cadherin KO mice administered multiple doses of STZ (n = 13 Cre-, n = 7 Cre+).
*p < 0.05, ***p < 0.001; unpaired *t*-test. The data are shown as the means ± sems.

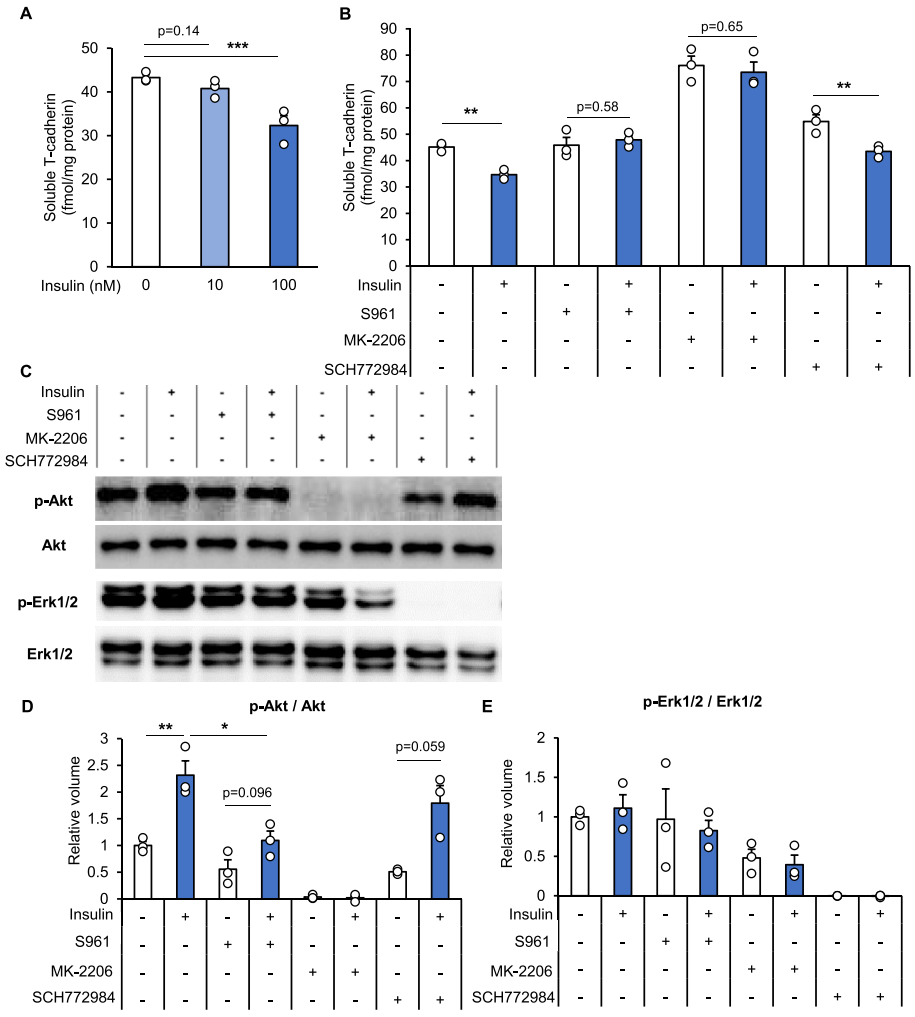

Fig. 4. Soluble T-cadherin in the supernatant of human endothelial cells is regulated by insulin/Akt signalling.
A) Soluble T-cadherin in the supernatants of HUVECs treated with 0, 10, or 100 nM bovine insulin.
B) Soluble T-cadherin in the supernatants of HUVECs treated with 0 or 100 nM bovine insulin, 0 or 1 μM S961, 0 or 5 μM MK-2206 and 0 or 1 μM SCH772984.
C) Western blotting showing the activity of Akt and Erk1/2 signalling in HUVEC lysates.
D) The activity of *p*-Akt/Akt in the HUVEC lysate. The relative signal volume was calculated.
E) The activity of *p*-Erk1/2/Erk1/2 in HUVEC lysates. The relative signal volume was calculated.
n = 3 in each group. *p < 0.05, **p < 0.01, ***p < 0.001; unpaired *t*-test. The data are shown as the means ± sems.

concordant with the observed results in the db/db mice (Fig. 1D). The reduction in soluble T-cadherin by insulin was eliminated with the addition of S961, an insulin receptor inhibitor, or MK-2206, an Akt inhibitor [31] (Fig. 4B). In contrast, SCH772984, an Erk1/2 inhibitor [32], did not influence the reduction in soluble T-cadherin (Fig. 4B). Next, we examined the activity of Akt and Erk1/2 signalling in the lysates of HUVECs. The phosphorylation of Akt (*p*-Akt) by insulin was significantly decreased by S961 but not by MK-2206, although this effect was not significantly reduced by SCH772984 (Fig. 4C and D). However, phosphorylated Erk1/2 signalling (*p*-Erk1/2) was not significantly elevated by insulin or decreased by S961 (Fig. 4C and E). These results suggest that the amount of soluble T-cadherin secreted from endothelial cells is negatively related to the activity of Akt signalling but not Erk1/2 signalling, consistent with the changes in plasma soluble T-cadherin caused by insulin signalling in mice and humans.

4. Discussion

In this study, we demonstrated that soluble T-cadherin in plasma was increased under conditions of insulin insufficiency or the inhibition of insulin/Akt signalling in mice. Soluble T-cadherin was also increased under conditions of severe insulin insufficiency in diabetes patients or pharmacological insulin signal blockade in patients. Among T-cadherin-expressing tissues/cells, the endothelial cells secrete soluble T-cadherin in response to the downregulation of insulin/Akt signalling.

GPI-anchored T-cadherin specifically binds to high-molecular-weight adiponectin, promoting the biogenesis and secretion of extracellular vesicles [16,17]. Moreover, soluble T-cadherin is a monomeric protein in plasma [20] and can improve glucose metabolism under diabetic conditions by promoting beta-cell proliferation through the upregulation of Notch signalling [22]. However, how and where soluble T-cadherin secretion is regulated remains to be determined.

First, we speculated that blood glucose levels might regulate soluble T-cadherin levels based on the results obtained in STZ-induced hyperglycaemic mice and dapagliflozin treatment (Fig. 1A and B). However, soluble T-cadherin levels were not elevated in hyperglycaemic conditions resulting from HFD-induced obesity. Furthermore, in *db/db* mice, which develop obesity and hyperglycaemia with later loss of pancreatic beta-cells and insulin deficiency, soluble T-cadherin levels were significantly elevated after the depletion of plasma insulin (Fig. 1D).

Moreover, the administration of STZ or S961 resulted in severe insulin insufficiency or pharmacological insulin receptor blockade, both of which led to an increase in soluble T-cadherin in plasma accompanied by severe hyperglycaemia. Therefore, we considered that insulin "demand" but not insulin resistance with hyperinsulinaemia might accelerate soluble T-cadherin secretion. This mechanistic hypothesis is consistent with our original report that soluble T-cadherin levels were elevated under STZ conditions but not under HFD conditions [22].

We determined that endothelial cells are one of the important contributors to plasma soluble T-cadherin levels among T-cadherin-expressing tissues/cells, and even a lack of T-cadherin in endothelial cells significantly affected glucose levels after sequential STZ treatments compared to those in the control group. Furthermore, the secretion of soluble T-cadherin from cultured endothelial cells in response to insulin decreased, and this decrease was dependent on insulin/Akt signalling. The molecules or mechanisms downstream of insulin/Akt signalling may be related to the secretion of T-cadherin (e.g. membrane shedding, truncated isoforms, and/or reaction efficacy of GPI transamidase).

We showed that plasma soluble T-cadherin levels were significantly reduced by 26 % and that endothelial cell-specific T-cadherin KO mice exhibited significantly increased blood glucose levels. We have shown that the expression of T-cadherin in the pancreas is restricted to endothelial cells [22], which may play an important role in the local concentration of soluble T-cadherin in the pancreas, such as in a paracrine manner. Moreover, endothelial cells transport circulating insulin to target tissues outside the vasculature, in addition to receiving insulin and signalling within the cell [33]. Delayed transendothelial transport of insulin signalling results in hyperphagia and insulin resistance in mice [33]. Insulin is also important for endothelial nitric oxide synthase (eNOS) production in endothelial cells, which maintains vascular homeostasis [34]. Endothelial cells in the whole body may sense insulin demand and provide feedback to pancreatic beta cells by secreting soluble T-cadherin.

We revealed that Akt activation attenuated soluble T-cadherin secretion from endothelial cells. However, the molecular mechanism through which Akt signalling regulates soluble T-cadherin secretion is still unclear. Insulin signalling is known to be downregulated in diet-induced obese mice. However, plasma soluble T-cadherin levels were not significantly different between HFD-induced obese mice and normal mice. Thus, the energy status of the catabolic state due to an insulin receptor signalling blockade, but not the anabolic state of HFD-induced obese mice, may accelerate soluble T-cadherin secretion.

There are several limitations in this study. It is uncertain that insulin is the only factor regulating systemic soluble T-cadherin levels. The data from human patients are limited and still not adequate to statistically determine the relationship between the concentration of soluble T-cadherin and insulin levels. Although our tissue-specific T-cadherin KO mice exhibited a total of 40 % reduction in soluble T-cadherin, the tissue sources of the remaining plasma soluble T-cadherin and their regulation have not been identified.

In conclusion, soluble T-cadherin secreted from endothelial cells is regulated by insulin/PI3K/Akt signalling.

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Data and code availability

- ●We submitted all the raw datasets to DRYAD with DOI, except for the clinical data of the patients, due to ethical restrictions.
- ●This paper does not report the original code.
- ●Any additional information required to reanalyse the data reported in this paper is available from the lead contact upon request.

CRediT authorship contribution statement

Tomonori Okita: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Shunbun Kita:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Shiro Fukuda:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Funding acquisition. **Yuta Kondo:** Investigation, Resources. **Taka-aki Sakaue:** Resources, Investigation. **Masahito Iioka:** Methodology. **Keita Fukuoka:** Resources, Investigation. **Keitaro Kawada:** Resources, Investigation. **Hirofumi Nagao:** Resources, Investigation. **Yoshinari Obata:** Resources. **Yuya Fujishima:** Resources, Investigation, Funding acquisition. **Takeshi Ebihara:** Resources. **Hisatake Matsumoto:** Resources. **Satoshi Nakagawa:** Resources. **Tadashi Kimura:** Resources. **Hitoshi Nishizawa:** Writing – review & editing, Funding acquisition. **Iichiro Shimomura:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2024.150403.

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